

# AHNS 2010 RESEARCH WORKSHOP

on The Biology, Prevention and Treatment of Head & Neck Cancer

October 28-30, 2010 Hyatt Regency Crystal City • Arlington, VA

# FINAL PROGRAM

**Presented by The American Head & Neck Society** 11300 W. Olympic Blvd., Suite 600, Los Angeles, CA 90064 *Phone*: 310-437-0559 *Fax*: 310-437-0585

www.ahns.info



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### WORKSHOP HISTORY

Originally entitled the "International Head and Neck Oncology Research Conference," the first conference was held in Rosslyn, Virginia in 1980. By the third conference, it was decided to schedule the workshop every four years, and to change the name of the conference to reflect its broad research scope.

**1st Research Workshop** 1980 • Rossyln, Virginia

**2nd Research Workshop** 1987 • Arlington, Virginia

**3rd Research Workshop** 1990 • Las Vegas, Nevada

**4th Research Workshop** 1994 • Rossyln, Virginia

**5th Research Workshop** 1998 • Rossyln, Virginia

6th Research Workshop 2002 • Rossyln, Virginia

**7th Research Workshop** 2006 • Chicago, Illinois

### 2010 RESEARCH WORKSHOP CORPORATE SUPPORTERS

The American Head & Neck Society gratefully acknowledges generous unrestricted educational grants in support of the AHNS 2010 Research Workshop by the following companies:

# Platinum Level

Bristol-Myers Squibb

### Gold Level

Genetech/Roche

# Silver Level

Varian

### Exhibitors

Fanconi Anemia Research Fund, Inc. KLS Martin Lifecell Xclear, Inc.







# **GENERAL INFORMATION**

# ON-SITE REGISTRATION HOURS

Location: Regency Foyer

Wednesday, Oct. 27	2:00pm - 6:00pm
Thursday, Oct. 28	6:30am - 5:00pm
Friday, Oct. 29	7:30am - 4:30pm
Saturday, Oct. 30	7:30am - 12:00pm

# SPEAKER READY ROOM HOURS

#### Location: Tidewater

Wednesday, Oct. 27	2:00pm - 6:00pn
Thursday, Oct. 28	6:00am - 6:00pn
Friday, Oct. 29	6:00am - 6:00pn
Saturday, Oct. 30	6:00am - 10:30an

# **EXHIBIT AREA HOURS**

Location: Regency Foyer

**Thursday, October 28** Hall Open 10:30am - 6:30pm

Friday, October 29Hall Open10:00am - 3:30pm

# POSTER SCHEDULE & VIEWING HOURS

 $Location: Independence \ Center \ A$ 

### Thursday, October 28

Authors Hang Posters 10:30am - 1:00pm Posters On Display 1:00pm - 6:30pm

### Friday, October 29

Posters On Display	8:00am - 4:30pm
Poster Session	4:30pm - 6:00pm
Authors Teardown	
Posters	6:00pm - 8:00pm

WHO:	The American Head & Neck Society
WHAT:	Research Workshop on the Biology, Prevention and Treatment of Head & Neck Cancer
WHEN:	October 28-30, 2010
WHERE:	<b>Hyatt Regency Crystal City</b> 2799 Jefferson Davis Highway Arlington, VA 22202
CONTACT:	<b>The American Head and Neck Society (AHNS)</b> 11300 W. Olympic Blvd., Suite 600 Los Angeles, CA 90064 <i>Phone:</i> +1-310-437-0559 <i>Fax:</i> +1-310-437-0585

# Research Workshop Educational Objectives

www.ahns.info

Email: registration@ahns.info

At the conclusion of this activity, participants will be able to:

- 1. Discuss the role of surgery, radiation therapy, chemoradiation, and combined modality therapy in the treatment of head and neck cancer and the effect of each treatment modality on clinical and functional outcomes.
- 2. Understand the clinical uses of new novel molecular agents in the management of head and neck cancer.
- 3. Be familiar with the advantages and disadvantages of ultrasound, PET and PET-CT in the evaluation of patients with head and neck cancer.
- 4. Understand the impact of treatment on functional outcome of head and neck cancer patients.
- 5. Describe significant advances in the field of head and neck cancer research that have occurred since the last research workshop was conducted.

# Research Workshop CME Credit Claim Process

Please use the worksheet on page 6 to track the number of CME hours you attend for each activity. After the meeting, an email will be sent to attendees with an on-line link to the survey and claim form.

AHNS has instituted a new process for claiming CME credits and printing certificates. All attendees wishing to receive a CME certificate for activities attended at the AHNS 2010 Research Workshop must first complete an on-line meeting evaluation form. Attendees will have access to the on-line meeting evaluation and credit claim form via a link on the AHNS website after the meeting.

Please allow 4-6 weeks for processing before your certificate arrives.

# HISTORY OF THE SOCIETY

On May 13, 1998, The American Head and Neck Society (AHNS) became the single largest organization in North America for the advancement of research and education in head and neck oncology. The merger of two societies, the American Society for Head and Neck Surgery and the Society of Head and Neck Surgeons, formed the American Head and Neck Society.

The contributions made by the two societies forming the AHNS are significant in the history of surgery in the United States. Dr. Hayes Martin conceived the Society of Head and Neck Surgeons in 1954, a surgeon considered by many to be the "father of modern head and neck tumor surgery." The purpose of the society was to exchange and advance the scientific knowledge relevant to the surgery of head and neck tumors (exclusive of brain surgery) with an emphasis on cancer of the head and neck. Two years later, The American Society for Head and Neck Surgery was organized with the goal to "facilitate and advance knowledge relevant to surgical treatment of diseases of the head and neck, including reconstruction and rehabilitation; promote advancement of the highest professional and ethical standards as they pertain to the practice of major head and neck surgery; and to honor those who have made major contributions in the field of head and neck surgery, or have aided in its advancement".

The new Society remains dedicated to the common goals of its parental organizations.

### **Mission Statement**

The purpose of this society is to promote and advance the knowledge of prevention, diagnosis, treatment and rehabilitation of neoplasms and other diseases of the head and neck, to promote and advance research in diseases of the head and neck, and to promote and advance the highest professional and ethical standards.

### Why Join the AHNS?

The American Head and Neck Society is an organization of physicians, scientists and allied health professionals dedicated to improving the understanding of Head and Neck Cancer and the care of patients afflicted with that disease. Membership is open to a wide variety of interested individuals in several categories that differ both in terms of responsibility and level of involvement in the society.

For more information about AHNS membership and to apply on-line, please visit www.ahns.info/membercentral or call +1 310-437-0559, ext. 110.



# **RESEARCH WORKSHOP PROGRAM COMMITTEE**

Carol R. Bradford, MD Program Chair Jennifer R. Grandis, MD Program Co-Chair Carol M. Bier-Laning, MD Ruud H. Brakenhoff, MD Joseph A. Califano, MD Andre L. Carvalho, MD Amy Y. Chen, MD Ezra W. Cohen, MD Terry A. Day, MD James A. Fagin, MD Robert L. Ferris, MD, PhD Neil D. Gross, MD Quynh-Thu Le, MD Mark E. Prince, MD James Rocco, MD, PhD Duane A. Sewell, MD Richard J. Shaw, BDS, FDS, MBChB, FRCS Erich M. Sturgis, MD Theodoros N. Teknos, MD Marilene B. Wang, MD Johan Wennerberg, MD, PhD Richard J. Wong, MD Wendell G. Yarbrough, MD Bevan Yueh, MD

# **PROGRAM LEADERS**

### **PROGRAM CHAIR**

Carol R. Bradford, MD



Dr. Bradford assumed leadership of the Department of Otorhinolaryngology at the University of Michigan on January 1, 2009. Dr. Bradford continues to serve as the Co-Director of the Head and Neck Oncology Program and Head and Neck Cancer Destination Program. She previously served as the director of the Head and Neck Surgery Division,

Associate Chair of the Department of Otolaryngology for clinical programs and education, and Residency Program Director.

She has long been a part of the U-M community. Dr. Bradford began her academic career at the University, and earned her B.S., M.S. and M.D. degrees here. Following her residency at U-M, she joined the faculty in 1992.

Dr. Bradford specializes in head and neck cancer surgery. She was the recipient of the Physician of the Year Award from Castle Connolly in 2009. She is President-Elect of the American Head and Neck Society. She also serves as the lead surgeon of an annual Head and Neck Surgery Mission Trip to Honduras.

Her translational research efforts are directed towards the study of biomarkers that predict outcome in head and neck cancer patients and the design of novel therapies to overcome resistance to treatment.

She has pursed several leadership training opportunities including the prestigious Executive Leadership in Academic Medicine Program for Women at Drexel University, the University of Michigan Healthcare Leadership Institute, and the Global Institute for Leadership Development.

Dr. Bradford is the honored recipient of several mentorship awards including first ever "ALOT" award given for mentorship by graduating senior residents in Otolaryngology, the Jeanne Cady Solis Award for American Medical Women's Association Mentorship, and the Token of Appreciation Award by Medical Students (TAMS).

### **PROGRAM CO-CHAIR**

Jennifer R. Grandis, MD



Dr. Jennifer R. Grandis was born and raised in Pittsburgh. She earned her BA in art history and biology from Swarthmore College and her medical degree with high honors from the University of Pittsburgh School of Medicine. She completed her internship in General Surgery and her residency in Otolaryngology at the University of

Pittsburgh. During her training she began to focus her studies on the biology of head and neck tumor growth. She has dedicated her research career to the study of the critical genetic alterations that characterize these cancers, with the ultimate goal of improving patient treatment and survival. Her laboratory was among the first to validate the epidermal growth factor receptor (EGFR) and Signal Transducers and Activators of Transcription (STATs) as therapeutic targets in head and neck cancer (HNC), thus paving the way for studies in other cancers. EGFR serves as a central integration point for coordination of a broad array of cellular signals. She demonstrated that G-protein-coupled receptors (GPCRs) "transactivate" EGFR contributing to HNC progression. Each of these basic science observations has been translated into clinical trials. She developed an antisense gene therapy approach targeting EGFR and completed a phase I study that found a high clinical response rate (35%), which correlated with decreased EGFR expression in the post-treatment biopsies, suggesting that modulation of the target protein may identify a subset of patients who will respond to therapy. A phase II trial will be implemented in 2010. She completed a pharmacodynamic study comparing the effects of an EGFR inhibitor, alone or in combination with a GPCR inhibitor, on biomarker expression profiles in HNC patients. She developed a transcription factor decoy approach to block STAT3 and demonstrated antitumor efficacy in preclinical HNC models. A phase 0 trial of this first in human STAT3 inhibitor was recently completed and future studies will optimize this approach to enable systemic administration. Dr. Grandis' contributions to cancer research have been recognized in many ways, including her receipt of the prestigious American Cancer Society Clinical Professorship, her election to the American Society of Clinical Investigation and the Association of American Physicians. At the University of Pittsburgh, she has received the University of Pittsburgh Cancer Institute Scientific Leadership Award, the Endowed Chair in Head and Neck Cancer Surgical Research, and the Chancellor's Distinguished Research Award.

# **KEYNOTE LECTURERS**

### William Pao, PhD



Dr. Pao is a physician-scientist with a special interest in thoracic oncology. Dr. Pao's research focuses on identification of genes involved in the pathogenesis of lung tumors and stratification of tumors into clinically relevant molecular subsets. Using information derived from these experiments, Dr. Pao seeks to improve treatment for patients with non-small

cell lung cancer. His research has yielded important insights into mechanisms of lung tumor sensitivity and resistance to inhibitors of the epidermal growth factor receptor.

Dr. Pao received his MD and PhD degrees from Yale University, where he studied the development and function of murine gammadelta T cells in the laboratory of Dr. Adrian Hayday. Following his clinical training in medical oncology at MSKCC, he received postdoctoral training in the laboratory of Dr. Harold Varmus. He ran an independent laboratory in MSKCC's Human Oncology and Pathogenesis Program (2005-2009) and was recruited to Vanderbilt in 2009.

Throughout his career, Dr. Pao has authored and co-authored more than 80 research articles and reviews in peer-reviewed journals such as Nature, the Journal of Clinical Investigation, the Proceedings of the National Academy of Sciences, Public Library of Science (PLoS), Medicine, the Journal of Clinical Oncology, and Cancer Research. He is a senior editor at Cancer Research, a journal editor for PLoS Medicine, has served as an editor for Journal of Clinical Oncology, and as a Medical Committee Research Member on Joan's Legacy: Uniting Against Lung Cancer Foundation. He has received an ASCO Young Investigator Award, the American College of Chest Physicians (AACP) CHEST Foundation and The LUNGevity Foundation Clinical Research Award in Lung Cancer, a Clinical Scientist Development Award from the Doris Duke Charitable Foundation, a V Foundation grant, the Barbara Parisi Lung Cancer Research Grant and the Hope Now Award from the Joan's Legacy Foundation, and an SU2C Innovative Grant Award. Dr. Pao has served or currently serves as PI on NCI K08 and R01 grants, and a project leader on NCI P01 and U54 grants. He is actively involved in numerous professional associations including the American Association for Cancer Research (AACR) and the American Society of Clinical Oncology (ASCO).

### Levi A. Garraway MD, PhD



Dr. Levi Garraway is an Assistant Professor of Medicine in the Department of Medical Oncology at the Dana-Farber Cancer Institute, Harvard Medical School. Dr. Garraway is also a faculty member of Dana-Farber's Center for Cancer Genome Discovery and an Associate Member of the Broad Institute, where he leads several large

cancer genome analyses and functional genomics efforts in the Broad Cancer Program.

Dr. Garraway received his A.B. in Biochemical Sciences from Harvard College in 1990, and his M.D. and Ph.D. degrees from Harvard Medical School in 1999. Thereafter, he completed his internship and residency in Internal Medicine at the Massachusetts General Hospital, where he also served as Medical Chief Resident in 2003. He received fellowship training in Medical Oncology at the Dana-Farber Cancer Institute. Dr. Garraway leads a 16member investigative team in cancer genomics at Dana-Farber and the Broad Institute. His research has informed several gene targets and "druggable" pathways relevant to the genesis and therapeutic vulnerabilities of melanoma and other malignancies. Dr. Garraway's group developed OncoMap, a platform for systematic cancer mutation profiling that has inspired multiple personalized cancer medicine efforts across the U.S. and beyond.

Dr. Garraway has been the recipient of several awards and honors, including the Minority Scholar Award from the American Association of Cancer Research, the Partners in Excellence Award from the Massachusetts General Hospital, and the Career Award in the Biomedical Sciences from the Burroughs-Wellcome Fund. In the fall of 2007, Dr. Garraway was awarded one of the first prestigious New Innovator Awards from the National Institutes of Health, worth \$1.5 million over five years. The Innovator Awards were given to the top 29 scientists from over 2200 applicants nationwide. In 2009, Dr. Garraway was inducted into the American Society for Clinical Investigation.

# **AHNS ACCREDITATION**

The American Head & Neck Society (AHNS) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to sponsor Continuing Medical Education for physicians. The AHNS designates this live activity for a maximum of 18.5 AMA PRA Category  $1 Credit(s)^{TM}$ . Physicians should only claim credit commensurate with the extent of their participation in the activity.

# CME WORKSHEET

This is not your CME credit form. Please use the worksheet below to track the number of CME hours you attend for each activity. Fill in the number of hours you attended each activity in the chart below to track your CME credits.

THURSDAY, OCTO	DBER 28, 2010		
Time	Activity	Credits Available	Hours Attended
7:45am - 8:10am	Welcome and Overview of Workshop	0	
8:10am - 9:00am	KEYNOTE LECTURE: "Realizing the Potential of Personalized Medicine in Solid Tumors"	.75	
9.00am - 10.30am	PANEL: Molecular Pathogenesis/Markers of Tumor Progression	15	
	ABSTRACTS: Cell Cycle Regulation/Apoptosis/Autophagy	1.7	
11:00am - 12:30pm	PANEL: Genetics/Epigenetics	1.5	
1	ABSTRACTS: Gene Therapy		
1.30pm - 3:00pm	NCI Head and Neck Steering Committee-Progress to Date and Future Goals Regarding the Integration of Translational Research Into Clinical Trial Development	15	
1.90pm - 9.00pm	PANEL: Carcinogenesis/Prevention	1.9	
2.20	ABSTRACTS: Immunology/Immunomodulation	1.5	
5:50pm - 5:00pm	ABSTRACTS: Angiogenesis/Tumor Microenvironment	1.5	
6:00pm - 8:00pm	Grant Writing Session 🚱 (Additional Fee)	2	
	Total Credits Available for Thursday, October 28, 2010:	8.75	
FRIDAY, OCTOBER	29, 2010		
8:30am - 10:00am	PANEL: Role of HPV in HNC-I	15	
0.90am - 10.00am	PANEL: Cell Adhesion/Recognition/Signaling	1.9	
10:30am - 12:00pm	Role of HPV in HNC-II	1.5	
10.90am 12.00pm	EGFR Targeting and Beyond: New Therapies and Therapeutic Targets	1.9	
1:00pm - 2:30pm	PANEL: Imaging Techniques (PET/MRI/Ultrasound) and Their Role in the Diagnosis and Assessment of Response to Therapy in Head and Neck Cancer	1.5	
	PANEL: Radiation Biology and Response to Therapy		
3.00pm - 4.30pm	PANEL: Proteomics/Genomics	15	
stoopin tisopin	ABSTRACTS: Therapies-Clinical	210	
	Total Credits Available for Friday, October 29, 2010:	6	
SATURDAY, OCTO	BER 30, 2010		
8:00am - 8:50am	KEYNOTE LECTURE: "Sequencing the Cancer Genome"	.75	
9.00am - 10.30am	PANEL: Functional Outcomes of Head and Neck Cancer Treatment and Survivorship	15	
9.00am 10.90am	ABSTRACTS: Correlative Studies in Conjunction with Clinical Trials: Potentials and Pitfalls	1.5	
10:30am - 12:00pm	PANEL: Invasion/Metastasis: Models and Applications	1.5	
10.00	PANEL: Salivary Malignancies: Basic and Translational Science		
12:00pm - 12:15pm	Closing Comments/Meeting Summary/Future Directions	0	
	Total Credits Available for Saturday, October 30, 2010:	3.75	
	TOTAL CREDITS AVAILABLE	18.5	

### To receive your CME credit:

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#### You are encouraged to...

- 1. Document (on this form) any concerns about commercially-biased presentations/ materials during educational sessions,
- 2. Make suggestions about how bias might have been avoided/minimized, and
- 3. Immediately take your completed form to the AHNS staff at the Registration Desk.

Your feedback will be shared with a member of the CME Compliance Committee, who will make the faculty aware of the concerns and/or suggestions.

# **COMMERCIAL BIAS**

The AHNS CME Compliance Committee has defined "bias" as an existing predisposition that may interfere with objectivity in judgment. Bias may be minimized through prior declaration of any source of conflict of interest, reference to evidence-based literature and expert opinions, and/or an independent peer-review process.

If an educational presentation certified for CME includes bias of any commercial interests<sup>\*</sup>, please provide the following details: \*Commercial interest is defined by the ACCME as an entity producing, marketing, re-selling, or distributing health care goods or services consumed by, or used on, patients.

Presentation:

**Commercial Bias by:** (ie faculty name, company rep)

**Promotion via:** (eg handouts, slides, what they said, actions)

### **COMMERCIAL BIAS ABOUT:**

(check all that apply)

(eg session name, etc)

Patient treatment/management recommendations were not based on strongest levels of evidence available.

Emphasis was placed on one drug or device versus competing therapies, and no evidence was provided to support its increased safety and/or efficacy.

Trade/brand names were used.

Trade names versus generics were used for all therapies discussed.

The activity was funded by industry and I perceived a bias toward the grantors.

The faculty member had a disclosure and I perceived a bias toward the companies with which he/she has relationships.

Other (please describe): \_

#### Suggestions for avoiding or minimizing bias:

#### EXTRA COPIES ARE AVAILABLE AT THE AHNS DESK

Please return this form to the AHNS Desk or mail it to:

AHNS CME, 11300 W. Olympic Blvd, Suite 600, Los Angeles, CA 90064

# **HOTEL FLOORPLAN**

### HYATT REGENCY CRYSTAL CITY



ALL MEETING AREAS, RESTOOMS AND PHONES ARE WHEELCHAIR ACCESSIBLE EXCEPT THE CONFERENCE THEATRE.

# **SCIENTIFIC PROGRAM**

### THURSDAY, OCTOBER 28, 2010

7:45am - 8:10am 8:10am - 9:00am



**KEYNOTE LECTURE:** "Genotype-Driven Cancer Medicine" William Pao, PhD, Associate Professor of Medicine; Ingram Associate Professor of Cancer Research; Assistant Director, Personalized Cancer Medicine, Vanderbilt University, Nashville, TN Introduction by: Carol R. Bradford, MD

Welcome and Overview of Workshop - Carol R. Bradford, MD & Jennifer R. Grandis, MD

Regency E-F Regency E-F

9:00am - 10:30am

**Concurrent Sessions** 

PANEL: Molecular Pathogenesis/Markers of Tumor Progression Co-Chairs: Nisha J. D'Silva, BDS, MSD, PhD & Wayne M. Koch, MD Location: Regency E-F

*Description:* This session will address genomic predictors of premalignancy progression, biomarkers of HNC progression, molecular markers of response to treatment and margin analyses and HNC prognosis.

- 9:00am Epigenomic Salivary Markers for HNC Detection Joseph A. Califano, MD
- 9:15am Molecular Biomarkers in HNC Progression Nisha J. D'Silva, BDS, MSD, PhD
- 9:30am Molecular Signatures of Response to Targeted Therapy - Christine H. Chung, MD
- 9:45am Margin Analyses and HNC Prognosis Wayne M. Koch, MD
- 10:00am S001: Prediction of Local Relapse in Head and Neck Cancer by Immunostaining of the Surgical Margins Using Biomarkers Identified in a Proteomics Screen <u>RH Brakenhoff, PhD</u>, BM Schaaij-Visser, PhD, CR Leemans, PhD, MD, E Bloemena, PhD, MD, M Slijper, PhD; ORL-HNS/Pathology, VU University Medical Center, Amsterdam and Netherlands Proteomics Centre, Utrecht, The Netherlands
- 10:08am S002: Reference Levels of Salivary Epigenetic Biomarkers in a Normal Cohort Inform Their Use in the Early Detection of OSCC and Surveillance of Recurrent OSCC <u>April M. Matthews, MBChB, MRCS,</u> <u>MBA</u>, Lakis Liloglou, BSc, PhD, E. A. Field, MDS, DDSci, FDSRCS, Richard J. Shaw, MD, MBChBHons, BDS, FRCSOMFS, RCPSGla, Janet M. Risk, BSc, PhD; University of Liverpool School of Dental Sciences, University Hospital Aintree.
- 10:16am **S003: Activated c-Src Contributes to SCCHN Tumor Growth, Invasion, and Resistance to Epidermal Growth Factor Receptor Targeting** <u>Guoqing He<sup>1</sup></u>, Sufi M. Thomas<sup>1</sup>, Sonali Joyce<sup>1</sup>, Jennifer R. Grandis<sup>1,2</sup>; Department of Otolaryngology1 and Pharmacology<sup>2</sup>, University of Pittsburgh and University of Pittsburgh Cancer Center, Pittsburgh, PA.

#### 10:24am Discussion

At the conclusion of this session, participants will be able to:

- Assess the value of molecular vs. histologic margin analysis for adequacy of surgical resection.
- + List 3 biomarkers that predict HNC progression.
- + List potential predictors of response to EGFR targeted therapy.
- Apply molecular marker detection for cancer surveillance.

ABSTRACTS: Cell Cycle Regulation/Apoptosis/Autophagy Co-Chairs: James W. Rocco, MD, PhD & Wendell G. Yarbrough, MD Location: Regency C-D

*Description*: Abstracts relevant to this session will be selected from the scored abstracts. In addition, Drs Rocco, Yarbrough and Yu have expertise in this area and will present related work from their respective laboratories.

- 9:00am Novel Protein Regulator of Tumor Suppressor ARF and NF-KB - Wendell G. Yarbrough, MD
- 9:08am Targeting Bcl2 Family Members in HNSCC James W. Rocco, MD, PhD
- 9:16am Role of PUMA in EGFR Targeted Therapy Jian Yu, PhD
- 9:24am Discussion
- 9:30am S004: Cross-Talk Between TGF-beta and NF-kappaB Signaling Pathways is Mediated Through TAK1 and Smad7 in Head and Neck Cancer <u>Christian Freudlsperger, MD DDS</u>, Jeffrey R. Burnett, BS, Yansong Bian, MD, PhD, Zhong Chen, MD, PhD, Carter Van Waes, MD, PhD; Department of Oral and Maxillofacial Surgery, Tuebingen University Hospital, Tuebingen, Germany; Tumor Biology Section, Head and Neck Surgery Branch, NIDCD, NIH, Bethesda, MD.
- 9:38am **S005: Apoptosis in Oral Squamous Cell Carcinoma** <u>Cláudia M. Coutinho-Camillo, PhD</u>, Silvia V. Lourenço, PhD, Ines N. Nishimoto, PhD, Luiz P. Kowalski, MD, PhD, Fernando A. Soares, MD, PhD; <sup>1</sup>Department of Anatomic Pathology, Hospital A.C. Camargo, São Paulo; <sup>2</sup>Department of General Pathology, Dental School, University of São Paulo, São Paulo; <sup>3</sup>Department of Otorhinolaryngology and Head and Neck Surgery, Hospital A.C. Camargo, São Paulo
- 9:46am S006: Targeting Focal Adhesion Kinase (FAK) in Anaplastic Thyroid Carcinoma Yunyun Chen, PhD, Maria Gule, MD, Ying Henderson, MD, PhD, <u>Stephen Y. Lai.</u> <u>MD PhD</u>: University of Texas M. D. Anderson Cancer Center, Houston, TX
- 9:54am Discussion
- 10:00am S007: Oral Cancer Progression Induced by Mutant p53 in Response to Chemotherapy Sergio Acin, PhD, Olga Mejia, BS, <u>Carlos Caulin, PhD</u>; Department of Head and Neck Surgery, The University of Texas M. D. Anderson Cancer Center, Houston, TX

Continued on next page...

#### 10:30am - 11:00am Coffee Break with the Exhibitors

11:00am - 12:30pm Concurrent Sessions

#### **PANEL:** Genetics/Epigenetics

Co-Chairs: Richard J. Shaw, MD & Maria J. Worsham, PhD Location: Regency E-F

*Description*: This sessions aims to give an overview of the global and gene specific platforms available in the genetic and epigenetic analysis of head and neck cancers, to offer some insight in specific areas of interest and how these might be developed into clinically useful biomarkers.

- 11:00am Molecular Modeling of HNC Diagnosis and Prognosis Maria J. Worsham, PhD
- 11:15am Next Generation (Massively Parallel) Sequencing and Personalized Medicine for Head & Neck Cancer Ross Sibson, PhD
- 11:30am Patterns of Somatic Gene Alterations Karl T. Kelsey, MD, MOH
- 11:45am DNA Methylation Assays in Head and Neck Cancer Richard J. Shaw, MD
- 12:00pm S010: Dysregulation of the PI3K/Akt/mTOR Pathway via Mutation and Copy Number Alteration in Oral Cavity Squamous Cell Carcinoma <u>Luc G. Morris, MD</u>, Ian Ganly, MD, PhD, Barry S. Taylor, PhD, Bhuvanesh Singh, MD, PhD, Agnes Viale, PhD, Adriana Heguy, PhD, Timothy A. Chan, MD, PhD; Memorial Sloan-Kettering Cancer Center
- 12:08pm S011: Global DNA Methylation Patterns are Prognostic of Clinical Outcome in HNSCC <u>Roberto A.</u> <u>Lleras</u>, Leslie R. Adrien, Thomas M. Harris, PhD, Nicolas F. Schlecht, PhD, Geoffrey Childs, PhD, Michael Prystowsky, MD, PhD, Richard V. Smith, MD, Thomas J. Belbin, PhD; Albert Einstein College of Medicine, Bronx, New York, USA; Montefiore Medical Center, Bronx, NY, USA

#### 10:08am S008: Surgical Trauma Stimulates Growth by Downregulation of Proapoptotic Genes in HNSCC Xenografts <u>Gustaf Lindgren, MD</u><sup>1</sup>, Åke Borg, Johan Vallon-Christersson<sup>2</sup>, Lars Ekblad, Elisabeth Kjellén<sup>2</sup>, Johan Wennerberg<sup>1</sup>; <sup>1</sup>Department of Otorhinolaryngology, Head and

Neck Surgery and <sup>2</sup>Department of Otorninolaryngology, Heda and Neck Surgery and <sup>2</sup>Department of Oncology, Lund University Hospital, Lund, Sweden

10:16am S009: Human Beta-Defensin 3 Promotes NF-kappaB Mediated CCR7 Expression and Anti-Apoptotic Signals in Squamous Cell Carcinoma of the Head and Neck <u>Yvonne K. Mburu</u>, Koji Abe, MD, Laura K. Ferris, MD, PhD, Saumendra N. Sarkar, PhD, Robert L. Ferris, MD, PhD; University of Pittsburgh School of Medicine, University of Pittsburgh Cancer Institute, University of Pittsburgh Medical Center

#### 10:24am Discussion

At the conclusion of this session, participants will be able to:

- List recent advances in the field of apoptosis/ autophagy and cell cycle regulation as they related to HNSCC.
- Understand how EGFR targeting is dependent upon the proapoptotic PUMA protein in HNSCC.
- Describe novel regulation of the ARF and NF-KB molecules in HNSCC.
- Understand the complex apoptotic pathways at work in HNSCC.

**Regency Foyer** 

#### ABSTRACTS: Developmental Therapeutics for Head & Neck Cancer - Co-Chairs: Hamid Ghandehari, BS, PhD & Richard J. Wong, MD Location: Regency C-D

*Description:* The session's goal is to review novel therapeutic agents and approaches in the management of head and neck cancer. Agents may include viral, antibody, small molecule, pharmacologic, polymer, and other therapeutic agents.

11:00am Herpes Viral Targeting of HNC - Richard J. Wong, MD

- 11:08am Engineering Polymers for HNC Gene Therapy Hamid Ghandehari, BS, PhD
- 11:16am **S013: Targeting Raf Kinase for the Treatment of Head and Neck Cancer** - Arti Yadav, MS, Bhavna Kumar, MS, Theodoros N Teknos, MD, <u>Pawan Kumar, MS PhD</u>; Department of Otolaryngology - Head and Neck Surgery, The Ohio State University Comprehensive Cancer Center, Columbus, OH
- 11:24am Discussion
- 11:30am S014: Oral Resveratrol Treatment Inhibits Skeletal Muscle and Cardiac Atrophy by Inhibiting NF-kB Activity in Vivo - <u>Scott Shadfar, MD</u>, Marion E. Couch, MD, PhD, Kibwei A. McKinney, MD, Xiaoying Yin, MD, Jessica E. Rodriguez, PhD, Monte S. Willis, MD, PhD; University of North Carolina.
- 11:38am S015: Enhancement of Cetuximab Therapy with Anti-CD137 Antibody in the Treatment of Head and Neck Squamous Cell Carcinoma - Holbrook E. Kohrt, MD, <u>Peder Lund, BS</u>, A. Dimitrios Colevas, MD, Kipp Weiskopf, BS, Ronald Levy, MD, John B. Sunwoo, MD; Stanford University School of Medicine

### **AHNS 2010 RESEARCH WORKSHOP**

 12:16pm S012: Delected in Colorectal Cancer Gene Promoter Hypermethylation Can Be Used as a Single Marker for Cancer Progression in Salivary Rinse Juliana L. Schussel, DDS, MSc, Kavita M. Pattani, MD, Zhe Zhang, MS, Chad Glazer, MD, Steven Goodman, MD, PhD, David Sidransky, MD, Miriam Robbins, DDS, MPH, A. Ross Kerr, DDS, MSD, David Sirois, DMD, PhD, Joseph A. Califano, MD; Depart. Otolaryngol HN Surg and Oncology, Johns Hopkins MI, Baltimore, MD; Depart. Oral Pathology, University of Sao Paulo, SP, Brazil; Depart. Oral Medicine NY University, NY; Milton J. Dance HN Center, Greater Baltimore Med Center, Baltimore, MD

#### 12:24pm Discussion

At the conclusion of this session, participants will be able to:

- Gain an understanding of the genetic and epigenetic techniques available at a genome wide scale.
- The approaches and scale of Next Generation Methods & The likely impact of Next Generation Methods in terms of discovery and application.
- Gain an understanding of the genetic and epigenetic techniques available at a single gene scale.
- Appreciate the process by which a genetic or epigenetic aberration might be demonstrated to be tumour specific and functionally relevant i.e. a driver rather than a passenger.
- Insight into how these aberrations might be ultimately developed into clinically relevant predictive, prognostic or diagnostic biomarkers; or into productive drug targets.

- 11:46am
   S016: Biphenyl Isoxazolidine, a Novel Small Molecule

   ESX Mimic, Inhibits Cell Invasion, Migration, and

   Proliferation in Head and Neck Squamous Cell Carcinoma

   Manchao Zhang, PhD,

   Christopher E. Taylor, BS, Theodoros

   N. Teknos, MD, Anna K. Mapp, PhD, Quintin Pan, PhD; The

   Ohio State University Medical Center; Arthur G. James Cancer

   Hospital and Richard J. Solove Research Institute, The Ohio State

   University Comprehensive Cancer Center; University of Michigan
- 11:54am Discussion
- 12:00pm S017: Development of a STAT3 Decoy for Systemic Administration in Head and Neck Cancer - <u>Malabika</u> <u>Sen<sup>1</sup></u>, Sufi. M. Thomas<sup>1</sup>, Srinivas Rapireddy<sup>5</sup>, Joanne Yeh<sup>4</sup>, Haibin Shi<sup>4</sup>, Changyou Li<sup>3</sup>, Daniel. E. Johnson<sup>3</sup>, Danith Ly<sup>5</sup> and Jennifer R. Grandis<sup>1,2</sup>; Department of Otolaryngology<sup>1</sup>, Pharmacology<sup>2</sup>, Medicine<sup>3</sup>, University of Pittsburgh and University of Pittsburgh School of Medicine, PA, Department of Structural Biology<sup>4</sup>, University of Pittsburgh Medical School, Pittsburgh, PA, Department of Chemistry<sup>5</sup>, Carnegie Mellon University, Pittsburgh, PA
- 12:08pm S018: Synthetic HSP90 Inhibitor, PF-04928473 and Pro-Drug PF-04929113, Demonstrates Potent Anti-Tumor Activity in Head and Neck Cancer by Inhibiting Multiple Proliferative and Pro-Survival Signaling Pathways - Jay Friedman, PhD, <u>Stephanie</u> <u>Contag. BA</u>, Michael Hu, MD, Christian Freudlsperger, DMD, Vishnu Kannabiran, BS, Pattatheyil Arun, MD, James Mitchell, PhD, Jamie Skillings, MD, Julie Kan, PhD, Zhong Chen, MD, PhD, Carter Van Waes, MD, PhD; Tumor Biology Section, Head and Neck Surgery Branch, NIDCD and Radiation Biology Branch, NCI, National Institutes of Health, Bethesda, MD; Pfizer Inc, San Diego, CA
- 12:16pm **S019: Novel Oncolytic Virus Therapy in Squamous Cell Carcinoma** - <u>Old Matthew, MD</u>, Jun-Ge Yu, MD, Theodoros Teknos, MD, Balveen Kaur, PhD; The Ohio State University

#### 12:24pm Discussion

At the conclusion of this session, participants will be able to:

• Gain an understanding of how novel agents (viral, antibody, small molecule, pharmacologic, polymer, other) may be designed for the therapy of head and neck cancer.

### **SOCIAL EVENTS**

#### Welcome Reception

Thursday, October 28, 2010, 5:00pm - 6:30pm

Please join us in the Exhibit Area as we welcome you to the Research Workshop on the Biology, Prevention and Treatment of Head and Neck Cancer. Sponsored by our exhibitors, this event will feature cocktails and light hors d'oeuvres. Event is free to all registered workshop attendees and registered accompanying persons.

AHNS acknowledges our Platinum Level Donor in support of this event: **Bristol-Myers Squibb** 

#### Poster Session & Social Event Reception

Friday, October 29, 2010, 4:30pm - 8:00pm

On Friday, we begin in Independence A for our poster session with the authors for some starter cocktails. Then we move back downstairs into Potomac I-IV for a more relaxed setting of music, more food, more cocktails, and finish with enough time to dine separately with your friends and colleagues. Event is free to all registered workshop attendees and registered accompanying persons.

AHNS acknowledges a generous educational grant from our Gold Level Donor: **Genetech/Roche** 

# **SCIENTIFIC PROGRAM**

12:30pm - 1:30pm Lunch on your own

1:30pm - 3:00pm Concurrent Sessions

#### **PANEL:** NCI Head and Neck Steering Committee - Progress to Date and Future Goals Regarding the Integration of Translational Research Into Clinical Trial Development

Co-Chairs: John A. Ridge, MD, PhD & Andy Trotti, MD Location: Regency E-F

*Description:* Dr. Schuller served as Co-chair for the NCI H&N Steering Committee 2008-2010, along with Drs Forastiere and Trotti. Dr Trotti is Co-chair of the RTOG H&N Commmittee, and served as Co-chair for the NCI H&N Steering Commmittee, along with Drs Forastiere and Schuller, 2008-2010. Dr Burtness is Chair of the ECOG H&N Committee. Dr Ridge served as a NCI H&N Task Force Co-chair for previously untreated/locally advanced (PULA) H&N cancers, 2008-2010.

1:30pm Genesis of Steering Committee - David E. Schuller, MD

- 1:50pm Interactions of Steering Committee with RTOG Andy Trotti, MD
- 2:10pm Interactions of Steering Committee with ECOG Barbara A. Burtness, MD
- 2:30pm Role of Head and Neck Society in Steering Committee - John A. Ridge, MD, PhD

#### 2:50pm Discussion

At the conclusion of this session, participants will be able to:

- Gain an understanding of the structure and process of the NCI H&N Steering Committee implemented in 2008.
- Gain an understanding of which studies were approved, and how NCI has supported development and conduct of translational research in these approved clinical trials over the last 3 years.
- Appreciate the strengths and weaknesses of the new NCI H&N governance structure as assessed by key investigator and cooperative group leadership.

#### **PANEL:** Carcinogenesis/Prevention

Co-Chairs: Frank G. Ondrey, MD, PhD & Dong Moon Shin, MD Location: Regency C-D

*Description*: This panel will explore contemporary concepts in head and neck cancer clinical prevention and emerging molecular and dietary epidemiologic evidence of associated risks.

- 1:30pm Chemoprevention with Green Tea Dong Moon Shin, MD
- 1:45pm **PPAR Gamma Strategies and Oral Cancer Prevention** - Frank G. Ondrey, MD, PhD
- 2:00pm EGFR Targeting for Chemoprevention Scott M. Lippman, MD
- 2:15pm Q&A
- 2:20pm S020A: Detection of Head and Neck Squamous Cell Carcinoma and Cervical Lymph Node Metastasis in a Mouse Model Using Activatable Near-Infrared Fluorescence Probes - <u>S Keereweer, MD</u>, JDF Kerrebijn, MD, PhD, IM Mol, PBAA Van Driel, EL Kaijzel, PhD, JSD Mieog, MD, AL Vahrmeijer, MD PhD, RJ Baatenburg de Jong, MD, PhD, CWGM Lowik, PhD; Erasmus Medical Center Rotterdam, Leiden University Medical Center
- 2:28pm S020B: Detection of Merkel Cell Virus and Correlation With Microscopic Disease in the Sentinel Lymph Nodes of Patients With Merkel Cell Carinoma - <u>Myriam</u> Loyo, William H Westra, Juliana Schussel, Sewon Kang, Mariana Brait, Joseph A Califano, David Sidransky, Janis M Taube; The Johns Hopkins Medical Institutions
- 2:36pm **S020C: In Vivo Detection of Cancer Precursor DNA** Lesions - Wilbur K Mills, Ilangovan Ramachandran, PhD, Antonio M Reis, MD, <u>Lurdes Queimado, MD, PhD</u>; Departments of Dermatology and Otorhinolaryngology, University of Oklahoma Health Sciences Center
- 2:44pm Discussion

At the conclusion of this session, participants will be able to:

- Understand the natural history of carcinogenesis and molecular changes during the head and neck cancer progression.
- Understand and update the current concepts of chemoprevention, biomarkers and epidemiology of the head and neck cancer.

**Regency** Foyer

3:00pm - 3:30pm Coffee Break with the Exhibitors

3:30pm - 5:00pm Concurrent Sessions

#### **ABSTRACTS:** Immunology/Immunomodulation

Co-Chairs: Robert L. Ferris, MD, PhD & John H. Lee, MD Location: Regency E-F

Description: The current status of work in progress will be presented, relevant to immunology, immunosppression and therapeutic immunomodulation in patients with head and neck cancer. Invited talks from leaders in the field will be followed by proferred abstract presentations submitted by attendees.

- 3:30pm Immune Activation by EGFR-Specific Antibodies in Head and Neck Cancer - Robert L. Ferris, MD, PhD
- 3:38pm Mechanisms of Immune Mediated Clearance for HPV+ HNSCC John H. Lee, MD
- 3:46pm MAGE-A3/HPV Peptide Vaccines Duane A. Sewell, MD
- 3:54pm Tadalafil Induced Modulation of Immune Response in HNSCC Joseph A. Califano, MD
- 4:02pm Therapeutic Strategies for Decreasing the Role of Regulatory T Cells (TREG) in Tumor Escape - Theresa L. Whiteside, PhD
- 4:10pm STAT3 Signaling Can Regulate Myeloid Derived Suppressor Cell Function in Head & Neck Cancer Patients - Young Kim, MD, PhD
- 4:18pm Combination Immunomodulation in Head and Neck Cancer Sara Pai, MD, PhD
- 4:26pm Discussion
- 4:31pm S021: Use of Phosphodiesterase Type 5 Inhibitors for Immune Therapy of Head and Neck Squamous Cell Carcinoma - Donald Weed, MD, Felix Roth, PhD, <u>Bjorn Herman, MD</u>, Zoukaa Sargi, MD, Carmen Gomez, MD, Paolo Serafini, PhD; University of Miami Miller School of Medicine
- 4:39pm S022: Cetuximab Promotes Dendritic Cell Maturation and Cross-Priming of EGFR-Specific T Cells in Head and Neck Cancer Patients -Steve C. Lee, MD, PhD, Pedro A. Andrade Filho, MD, H. Carter Davidson, MD, PhD, Andres Lopez-Albaitero, MD, Raghvendra Srivastava, PhD, Hirak der-Terossian, MD, Varun Reddy, Sandra P Gibson, BS, William Gooding, BS, Soldano Ferrone, MD, PhD, Robert L. Ferris, MD, PhD; University of Pittsburgh, Loma Linda University
- 4:47pm S023: Inducible Nitric Oxide Synthase (iNOS) Controls STAT-3-Mediated Tumor / Host Cross-Talk Required for Accumulation and CD8+ T-Cell Suppressive Activity of Myeloid-Derived Suppressor Cells (MDSC) in Cutaneous Melanoma - Falguni Parikh, MS, Padmini Jayaraman, PhD, Esther Lopez-Rivera, PhD, David Cannan, BS, <u>Andrew G</u> Sikora, MD, PhD; Mount Sinai School of Medicine, Department of Otolaryngology - Head and Neck Surgery

4:55pm Discussion

At the conclusion of this session, participants will be able to:

- Understand the mechanisms of immunosuppression in head and neck cancer patients.
- Describe the clinically available immunotherapeutic agents and possible mechanisms of action.

#### 5:00pm - 6:30pm **Opening Reception in Exhibit Area**

6:00pm - 8:00pm

**ABSTRACTS:** Angiogenesis/Tumor Microenvironment Co-Chairs: Ezra E.W. Cohen, MD & Seungwon Kim, MD Location: Regency C-D

Description: This session will review the latest developments in antiangiogenic therapy in HNC. We will also review the role of stem cells in the pathogenesis of HNC.

- 3:30pm Concurrent Targeting of VEGFR and EGFR in HNC - Seungwon Kim, MD
- 3:45pm Role of Angiogenesis Inhibitors in HNC Treatment - Ezra E.W. Cohen, MD
- 4:00pm Endothelial and Head and Neck Cancer Stem Cell Cross Talk - Jacques E. Nor, DDS, MS, PbD
- 4:15pm HNC Stem Cells Mark E. Prince, MD
- 4:30pm S024: Best Resident Basic Science Paper A C57BL/6 Syngeneic Mouse Model of **Oral Cavity Squamous Cell Carcinoma** Nancy Judd, MD, Joshua Brotman, Clint Allen, MD, Ashley Winkler, Ravindra Uppaluri, MD,PhD; Washington University School of Medicine in St Louis
- S025: Monitoring the Response of Patient 4:38pm Tumor-Derived HNSCC Xenografts to Antivascular Therapy Using MRI Jaimee M. Lockwood, BS, Steve G. Turowski, MS, Mukund Seshadri, DDS, PhD; Roswell Park Cancer Institute, Buffalo, NY
- 4:46pm S026: BFGF Expression Correlates with pMTOR/eIF4E/p4EBP1 Over Expression in Head and Neck Squamous Cell Carcinoma Surgical Margins - Lilantha Herman-Ferdinandez, MD, DOHNS, Cheryl <u>Clark,PhD</u>, Tara Moore-Medlin, Olexandr Ekshyyan, PhD, Fleurette Abreo, MD, Gloria Caldito, PhD, Cherie-Ann Nathan, MD, PhD; Department of Otolaryngology and Head and Neck Surgery-LSUHSC Shreveport, LA

#### 4:54pm Discussion

- At the conclusion of this session, participants will be able to:
- · Understand the current role of angiogenesis inhibitors in the treatment of HNSC.
- Understand rationale for combining antiangiogenic therapy with anti-EGFR therapy.
- Understand role of stem cell in regulating the microenvironment of HNC

#### **Regency Foyer**

#### **GRANT WRITING WORKSHOP with Mock Study Session** (Additional Fee) Regency C-D Description: The goal is to provide information on developing a hypothesis, do's and don'ts of the research plan, have round table mock study sessions by successful grant writers and provide useful hints in career development for a surgeon. 6:00pm Workshop Introduction - Cherie-Ann O. Nathan, MD 6:10pm Choosing the Right Grant and Grant Writing Pearls - Jennifer R. Grandis, MD 6:40pm R21 Perseverance and Persistence - Robert L. Ferris, MD 6:50pm The Stepping Stone to a Successful Surgeon Scientist: Writing a K08 - Stephen Y. Lai, MD, PhD 7:00pm Mock Study Session Workshop: R01, K08, Core Grant - Thomas E. Carey, PhD, Robert L. Ferris, MD, Jennifer R. Grandis, MD, Wayne M. Koch, MD, Duane A. Sewell, MD. & Scott E. Strome, MD

At the conclusion of this session, participants will be able to:

- Be knowledgeable in grantsmanship and grant writing issues.
- · Understand the review process and how to revise unfunded grants for resubmission.

### FRIDAY, OCTOBER 29, 2010

8:30am - 10:00am Concurrent Sessions

#### **PANEL:** Role of HPV in HNC, Part I

Co-Chairs: Duane A. Sewell, MD & Erich M. Sturgis, MD Location: Regency E-F

*Description:* This session is designed to review existing data and/or emergind data regarding the role of human papillomavirus in the etiology, prognosis, and/or management of head and neck cancers. Invited and peerreviewed presentations representing these concepts will be presented.

- 8:30am Sexual Behaviors and Oral HPV Infection Gypsyamber D'Souza, PhD
- 8:45am HPV16 DNA in Peripheral Blood as a Marker of Disease Extent and Recurrence - Erich M. Sturgis, MD
- 9:00am Modulating CD4+CD35+ Tregs in an Animal Model of HPV Associated Head and Neck Cancer Elizabeth Field, MD
- 9:15am Q&A
- 9:20am S027: Low Dose of Cyclophosphamide Enhances Antitumor Effects of Therapeutic HPV DNA Vaccine Sofia Lyford-Pike, MD, Shiwen Peng, MD, PhD, Chien-Fu Hung, PhD, T.-C. Wu, MD, PhD, Sara I Pai, MD, PhD; <sup>1</sup>Department of Otolaryngology-Head and Neck Surgery, <sup>2</sup>Pathology, <sup>3</sup>Oncology, <sup>4</sup>Obstetrics and Gynecology, <sup>5</sup>Molecular Microbiology and Immunology, Johns Hopkins Medical Institutions, Baltimore, Maryland
- 9:28am S028: HPV+ Cancer Cell Immune Response During Treatment is Attenuated by Enhanced Lactate Production Cathy Zhuang, MD, PhD, Dan Vermeer, BS, <u>Yuh-Seog Jung,</u> <u>MD, PhD</u>, Allison Haugrud, BS, Hyun-Joo Ahn, MD, PhD, John H. Lee, MD, FACS, Keith Miskimins, PhD; Sanford Cancer Research Center, University of South Dakota, Sioux Falls, SD
- 9:36am S029: Second Primary Cancers in Patients with an Index Head and Neck Cancer: Subsite-Specific Risks and Trends Over Time - <u>Luc G Morris, MD</u>, Andrew G Sikora, MD, PhD, Richard B Hayes, DDS, MPH, PhD, Snehal G Patel, MD, MS, Ian Ganly, MD, PhD; Memorial Sloan-Kettering Cancer Center, Mount Sinai School of Medicine, New York University School of Medicine
- 9:44am S030: Best Resident Clinical Paper: Matted Nodes Are a Poor Prognostic Factor in Oropharyngeal Squamous Cell Carcinoma Independent of HPV and EGFR Status <u>Matthew E Spector, MD</u>, K Kelly Gallagher, MD, Mohannad Ibrahim, MD, Eric J Chanowski, BS, Emily Light, MPH, Jeffrey S Moyer, MD, Mark E Prince, MD, Gregory T Wolf, MD, Carol R Bradford, MD, Jonathan B McHugh, MD, Kitrina Cordell, DDS MS, Thomas Carey, PhD, Francis P Worden, MD, Avraham Eisbruch, MD, Douglas B Chepeha, MD, MSPH; University of Michigan.

#### 9:54am Discussion

At the conclusion of this session, participants will be able to:

- Discuss the utility of HPV16 DNA in peripheral blood as a potential marker of disease extent.
- + Discuss how Tregs might be modulated in HPV associated cancers.

10:00am - 10:30am Coffee Break with the Exhibitors

#### **PANEL:** Cell Signaling and Targeted Therapies

Co-Chairs: Cherie-Ann O. Nathan, MD & Carter Van Waes, MD, PhD Location: Regency C-D

*Description*: The signal network is being elucidated in head and neck cancer. Emerging mediators and targets for therapy will be highlighted.

- 8:30am Signal Networks and Targeting HSP90 for Therapy Carter Van Waes, MD, PhD
- 8:45am EGFR Degradation and Cisplatin Sensitivity Mukesh K. Nyati, MD
- 9:00am Growth Factor-Induced Resistance to Targeted Therapy - Mark J. Jameson, MD, PhD
- 9:15am MTOR Signaling as a Therapeutic Target Cherie-Ann O. Nathan, MD
- 9:30am Protein Kinase CK2 as an Emerging Target Zhong Chen, MD, PhD
- 9:45am Discussion
- At the conclusion of this session, participants will be able to:
- List 3 signal pathways/targets under preclinical or clinical investigation.
- Describe 3 pathways/targets that mediate resistance to current EGFR targeted therapies.
- Describe potentially useful biomarkers for selection and monitoring of patient response.

**Regency** Foyer

#### 10:30am - 12:00pm Concurrent Sessions

#### PANEL: Role of HPV in HNC, Part II

Co-Chairs: Carole Fakhry, MD & Erich M. Sturgis, MD Location: Regency E-F

*Description:* This session is designed to review existing data and/ or emerging data regarding the role of human papillomavirus in the etiology, prognosis, and/or management of head and neck cancers. Invited and peer-reviewed presentations representing these concepts will be presented.

- 10:30am Therapeutic Vaccine for HPV16 Positive HNC and Oral HPV Infection in HIV-Infected Men and Women Carole Fakhry, MD, MPH
- 10:45am Genomic Instability Caused by HPV16 E6 and E7 Dennis McCance, MD
- 11:00am Role of microRNAs in HPV-Associated Head and Neck Cancer - Saleem A. Khan, PhD
- 11:15am Q&A
- 11:20am S031: Human Papillomavirus Modulates Cancer Stem Cell Function in Head and Neck Squamous Cell Carcinoma - Manchao Zhang, PhD, Hongpeng Liu, BS, Theodoros N Teknos, MD, <u>Quintin Pan, PhD</u>; Department of Otolaryngology-Head and Neck Surgery, The Ohio State University Medical Center; Arthur G. James Cancer Hospital and Richard J. Solove Research Institute, The Ohio State University Comprehensive Cancer Center.
- 11:28am S032: Oral Prevalence and Clearance of High-Risk Human Papilloma Virus (HR-HPV) in Healthy People in San Patrignano, a Rehabilitation Community for Substance Abusers - D B Pugliese, MD, G Bruzzesi, C Montaldo, MD, M. Landi, MD, A. Mastinu, MD, L. Porcu, MD, V Torri, MD, <u>L D Locati, MD</u>, L Licitra, MD; Centro Medico San Patrignano, University of Caglairi, Fondazione ANDI Onlus, Mario Negli Institute, Fond. IRCCS Itituto Nazionale Tumori
- 11:36am **S033: Human Papillomavirus (HPV) and Oropharynx Cancer (OPC) in the TAX 324 Trial** - <u>Marshall Posner, MD</u>, Jochen Lorch, MD, Olga Goloubeva, PhD, Ming Tan, PhD, Lisa Schumaker, Nicholas Sarlis, MD, Robert Haddad, MD, Kevin Cullen, MD; Dana-Farber Cancer Institute; Greenebaum Cancer Center; Sanofi-Aventis
- 11:44am S034: HPV Detection in Head and Neck Squamous Cell Carcinoma: A Comparison of Testing Methods Samantha J Davis, BS, Emily Light, MS, Martin P Graham, BS, Heather M Walline, MS, Jay Stoerker, PhD, Mark E Prince, MD, Gregory T Wolf, MD, Douglas B Chepeha, MD, Jeffrey S Moyer, MD, Carol R Bradford, MD, Avraham Eisbruch, MD, Thomas E Carey, PhD, Jonathan B McHugh, MD; University of Michigan - Department of Otorhinolaryngology, Department of Pathology, & Cancer Center, Ann Arbor, MI; Sequenom, Ann Arbor, MI
- 11:54am Discussion

At the conclusion of this session, participants will be able to:

- Discuss the utility of a therapeutic HPV16 vaccine in HIV-infected patients with HPV16 positive head & neck cancer.
- Discuss how Tregs might be modulated in HPV associated cancers.
- Discuss the role of microRNAs in HPV-associated head and neck cancers.

#### **PANEL:** EGFR Targeting and Beyond: New Therapies and Therapeutic Targets - Co-Chairs: Barbara A. Burtness, MD & Neil D. Gross, MD

Location: Regency C-D

*Description:* Drugs that target the Epidermal Growth Factor Receptor (EGFR) have been utilized extensively to treat head and neck squamous cell carcinoma. Yet, the ideal clinical application of EGFR inhibitors, and similar targeted molecular therapies, has yet to be determined. This session will examine contemporary research strategies that utilize these agents, particularly in the setting of clinical trials.

- 10:30am Application of Targeted Therapies to Aggressive Cutaneous HNSCC - Neil D. Gross, MD
- 10:45am SRC Family Kinases as Targets in HNC Ann Marie Egloff, PhD, MPH
- 11:00am Molecular Targeting Studies in ECOG Barbara A. Burtness, MD
- 11:15am **Use of Novel Therapies in RTOG Studies** Stuart J. Wong, MD
- 11:30am S035: Novel Mechanisms of IGF-IR/EGFR Cross-Talk that Counteracts the Antitumor Action of the Human Anti-IGF-IR Antibody IMC-A12 in Head and Neck Cancer - <u>Dong Hoon Shin, Postdoctoral Fellow,</u> Ho-Young Lee, Professor; The University of Texas MD Anderson Cancer Center
- 11:38am S036: Evaluation of EGFR Gene Amplification Status, MRNA, Protein, and Phosphoprotein Levels Expression in Head and Neck Cancer Patient Tissues - <u>S E Wheeler</u>, R Seethala, MD, D Siwak, PhD, K Cieply, C Sherer, G Mills, MD, PhD, J R Grandis, MD, AM Egloff, PhD; University of Pittsburgh, Department of Otolaryngology; University of Pittsburgh Medical Center, Department of Pathology; University of Texas M.D. Anderson Cancer Center, Department of Systems Biology
- 11:46am S037: Autocrine EGFR Ligand Production Determines the Response to Cetuximab in Head and Neck Squamous Cell Carcinoma Cell Lines - <u>Lars Ekblad, PhD</u>, Goro Oshima, MD, Elisabeth Kjellén, MD, PhD, Hiroyuki Mineta, MD, PhD, Anders Johnsson, MD, PhD, Johan Wennerberg, MD, PhD; Lund University, Hamamatsu University School of Medicine

#### 11:54am Discussion

At the conclusion of this session, participants will be able to:

- Discuss current therapeutic strategies for aggressive cutaneous squamous cell carcinoma of the head and neck.
- + Discuss novel drug targets in squamous cell carcinoma.
- Discuss the use of EGFR and similar targeted agents in current and future cooperative group clinical trials.

# **SCIENTIFIC PROGRAM**

12:00pm - 1:00pm Lunch on your own

1:00pm - 2:30pm Concurrent Sessions

PANEL: Imaging Techniques (PET/MRI/Ultrasound) and Their Role in the Diagnosis and Assessment of Response to Therapy in Head and Neck Cancer - Co-Chairs: Suresh K. Mukherji, MD & David L. Schwartz, MD

Location: Regency E-F

*Description*: Panelists will review the current role of advanced functional imaging techniques in the management of head and neck cancer. The emerging contribution of novel imaging of tumor proliferation, blood supply, and metabolism to current treatment strategies and experimental targeted therapies will be emphasized.

- 1:00pm Imaging Thymidine Update during HNC Treatment Yusuf Menda, MD
- 1:20pm **CT-Perfusion as a Means to Assess Treatment Response** - Suresh K. Mukherji, MD
- 1:40pm MR Spectroscopy in Head and Neck Cancer Harish Poptani, PhD
- 2:00pm **Functional Image Guidance for Advanced IMRT** David L. Schwartz, MD

#### 2:20pm Q&A

- At the conclusion of this session, participants will be able to:
- List current functional imaging modalities available for localizing and biologically characterizing head and neck cancer in patients.
- Apply functional imaging techniques to relevant clinical cases and research questions in head and neck oncology.
- Identify applications of functional imaging towards advanced radiotherapy planning and development of clinical therapy trials for head and neck cancer.

#### PANEL: Radiation Biology and Response to Therapy Co-Chairs: Avraham Eisbruch, MD & Quynh-Thu Le, MD Location: Regency C-D

Description: Panelists will discuss indivualizing treatment of head and neck cancer patients in combination with radiation via (1) targeting the tumor microenvironment, (2) optimal sequencing and integration of radiation with inhitibitors of EGFR receptor pathways, (3) indidualized design of radiation delivery with intensity modulated radiation therapy (IMRT) to minimize late toxicity and (4) improve salivary gland function after radiation with manipulation of growth factor pathways.

- 1:00pm Where Are We in Targeting Hypoxia and Tumor Microenvironment in HNSCC - Quynh-Thu Le, MD
- 1:15pm Integration of EGFR Inhibitors with Radiochemotherapy - Mukesh K. Nyati, PhD
- 1:30pm Individualizing IMRT Treatment in HNC Avraham Eisbruch, MD
- 1:45pm Mechanisms of IGF-Mediated Rescue of Radiation Induced Salivary Gland Dysfunction Kirsten H. Limesand, PhD
- 2:00pm S038: Predictive Biomarkers for Combined Chemotherapy with 5-Fluorouracil and Cisplatin in Oro-and Hypopharyngeal Cancers <u>Y Hasegawa, MD, PhD</u>, M Goto, DDS, PhD, N Hanai, MD, T Ozawa, MD, H Hirakawa, MD; Aichi Cancer Center.
- 2:08pm S039: Mechanisms of Acquired Resistance to Anti-Angiogenic Therapy in Preclinical Models of Head and Neck Squamous Cell Carcinoma (HNSCC) <u>Rekha Gyanchandani, MS</u>, Seungwon Kim, MD, Jennifer R Grandis, MD; Department of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; Department of Otolaryngology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.
- 2:16pm **S040: Cisplatin Enhances an Immune Mediated Clearance of HPV Positive Head and Neck Cancer** <u>William C Spanos, MD</u>, Denise Schwabauer, MS, Daniel Vermeer, BS, John H Lee, MD; Sanford Research/USD, Sioux Falls, SD

#### 2:24pm Discussion

At the conclusion of this session, participants will be able to:

- Discuss the available biomarkers for selecting patients with tumor hypoxia and the targeting strategies for the tumor microenvirnonment.
- Understand the optimal way to sequencing EGFR inhibitors with radiation and chemotherapy.
- List organ tolerance for normal tissue in head and neck cancer when using IMRT and how best to protect these structures.
- Discuss new strategies for rescuing salivary function after irradiation in patients.

2:30pm - 3:00pm Coffee Break with the Exhibitors

**Regency** Foyer

#### 3:00pm - 4:30pm Concurrent Sessions

**PANEL:** Proteomics/Genomics Co-Chairs: Ole Brinkmann, MD & Joseph A. Califano, MD Location: Regency E-F

*Description:* High throughput analytic techniques have allowed for application of sensitive molecular assays for detection of analytes specific to malignancy, as well as analysis of complex pathways involved in carcinogenesis and therapeutic response. This session will explore the use of proteomic and genomic analyses in assessment of disease burden and therapeutic response in head and neck squamous cell carcinoma, as well as biomarkers for the detection of head and neck cancers.

- 3:00pm Redox Modulated Metabolic and Signaling Networks as Molecular Predictors of Response to Radiation Therapy in HNC Cristina M. Furdui, PhD
- 3:15pm Salivary Soluble Markers for Head and Neck Cancer - Elizabeth J. Franzmann, MD
- 3:30pm On-Chip Immunoassays of Tumor Specific Autoantibodies for Head and Neck Cancer Detection - Ho-Sheng Lin, MD
- 3:45pm Epigenetic Artifact in Head and Neck Cancer Cell Lines - Joseph A. Califano, MD
- 4:00pm Salivary Genomics and Proteomics for Oral Cancer Detection - Ole Brinkmann, DMD, PhD
- 4:15pm Discussion

At the conclusion of this session, participants will be able to:

- Understand the potential use of saliva based assays for assessment of disease burden in head and neck cancer patients.
- Understand the potential use of serum and saliva based assays for early detection of head and neck cancer in high risk population.
- Understand the impact of metabolic signaling networks and their impact on response to radiation in head and neck cancer.
- Understand the artifact and epigenetic alterations induced by adherent cell culture of head and neck cancer.

#### **ABSTRACTS:** Therapies-Clinical

Co-Chairs: Arlene A. Forastiere, MD & Gregory T. Wolf, MD Location: Regency C-D

*Description*: This session will provide an overview of research within four Head and Neck SPOREs spanning premalignancy to advanced disease. The session will focus on novel therapies, targets or multi-disciplinary approaches.

Hot Topics From the HN SPORES:

- 3:00pm Inter-SPORE Trials and Collaborations from the Pittsburgh HN SPORE Jennifer R. Grandis, MD
- 3:10pm Ligand-Specific Therapeutic Nanoparticles in Head and Neck Cancer - Dong Moon Shin, MD
- 3:20pm Targeted Therapy for Larynx Preservation Gregory T. Wolf, MD
- 3:30pm Gene Expression Profiles and Oral Cancer Risk Scott M. Lippman, MD
- 3:40pm Q&A
- 3:50pm **S042: Targeting HER2 In The Treatment of Salivary Ductal Carcinoma** -<u>Matthew Pierce, BS, BA</u>, Merrill Kies, MD, Randal Weber, MD, FACS, Michael Kupferman, MD; The University of Texas MD Anderson Cancer Center
- 3:58pm **S043: Understanding Resistance: EGFR Amplification, HRAS** and PIK3CA Hotspot Mutations in Head and Neck Squamous Cell Carcinoma - <u>M. De Herdt, Msc</u>, R. Baatenburg de Jong, Prof, PhD, MD, E. Zwarthoff, Prof, PhD, E. Berns, PhD; ErasmusMC, Rotterdam, The Netherlands
- 4:06pm Discussion
- 4:10pm S044: Phase II Study of Erlotinib in Combination with Docetaxel and Radiation in Locally Advanced Squamous Cell Cancer of the Head and Neck (SCCHN) - <u>P Savvides</u>, M Yao, R Rezaee, J Bokar, P Fu, J Wasman, N Sarlis, A Dowlati, M Machtay, P Lavertu; CASE Comprehensive Cancer Center, University Hospital of Cleveland Medical Center, Cleveland, OH Sanofi-Aventis US, Inc Bridgewater, NJ

4:18pm **S045: Effect of Radiotherapy and Chemotherapy on the Risk** of Mucositis During IMRT for Oropharyngeal Cancer - <u>Giuseppe</u> <u>Sanguineti</u>, MariaPia Sormani, PhD, G. Brandon Gunn, MD, Francesco Ricchetti, MD, Arlene Forastiere, MD; Johns Hopkins University, University of Texas Medical Branch, University of Genoa

#### 4:26pm Discussion

At the conclusion of this session, participants will be able to:

- Understand the spectrum of translational research within the SPORES.
- Understand areas of current research interest as related to novel therapeutics, and opportunities for expanded participation.
- Understand approaches to combining targeted therapies with cytotoxics and radiotherapy in multidisciplinary disease management.

Independence Ballroom A Potomac I-IV

4:30pm - 6:00pmPoster Session with Wine and Cheese6:00pm - 8:00pmSocial Event Reception

# SCIENTIFIC PROGRAM

### SATURDAY, OCTOBER 30, 2010

8:00am - 8:50am



9:00am - 10:30am **Con** 

**Concurrent Sessions** 

PANEL: So You're A Head and Neck Cancer Survivor: What's Next? Chair: Bevan Yueh, MD, MPH Location: Regency E-F

Description: This panel will explore issues of survivorship and symptom management in head and neck cancer survivors. Panelists will discuss areas such as depression, substance abuse, acupuncture, and sexual intimacy in the setting of HPV related cancers.

9:00am What Works in a Smoking Cessation Program Elba C. Díaz Toro, MD

9:12am Relapsed Smoking Cessation Vani N. Simmons, PhD

- 9:24am Important Aspects of an Effective Smoking Cessation Program Janice A. Blalock, PhD
- 9:36am Prevention and Predictors of Post-Treatment Depression William J. Burke, MD
- 9:48am Acupuncture in Symptom Management Weidong Lu, MB

10:00am A Role for Behavioral Changes after Diagnosis with HPV-Associated HNSCC Carole Fakhry, MD, MPH

10:12am Discussion

At the conclusion of this session, participants will be able to:

- Understand the multidimensional facets of smoking cessation.
- Appreciate the potential advantages of preventing the emergence of depression during treatment for head and neck cancer.
- Appreciate potential benefits of acupuncture in managing functional outcomes such as dysphagia, xerostomia and pain for patients with head and neck cancer.

KEYNOTE LECTURE: "Sequencing the Cancer Genome"

Levi A. Garraway, MD, Department of Biological Chemistry and Molecular Pharmacology, Dana-Farber Cancer Institute/Brigham and Women's Hospital Introduction by: Jennifer R. Grandis, MD

> **ABSTRACTS:** Correlative Studies in Conjunction with Clinical Trials: Potentials and Pitfalls Co-Chairs: Terry A. Day, MD & Johan P. Wennerberg, MD, PhD Location: Regency C-D

Description: This session will present peer-reviewed abstracts in relation to the topic.

- 9:00am **S046: Polymorphisms in ERBB Family Growth Factor Receptors and Ligands and Risk of Head and Neck Squamous Cell Carcinoma (HNSCC)** - <u>Ann Marie Egloff, PhD MPH</u>, Brenda Diergaarde, PhD, Marjorie Romkes, PhD, Joel Weissfeld, MD MPH, Jennifer Grandis, MD, Christopher Fung, BS; Departments of Otolaryngology and Epidemiology and Center for Clinical Pharmacology, University of Pittsburgh, Pittsburgh, PA
- 9:08am S047: Oropharyngeal Squamous Cell Carcinoma and HPV-associated Cancers in Women: An Epidemiological Evaluation of Association - Hadi Seikaly, MD, FRCSC, <u>Vincent L Biron,</u> <u>MD, PhD</u>, David W Cote, MD, MPH, CCFP, Jeffrey Harris, MD, FRCSC; University of Alberta
- 9:16am **S048: Tumor Infiltrating Lymphocytes (TIL) and Human Papillomavirus-16 Associated Oropharynx Cancer** - <u>Derrick Wansom, BA</u>, Emily Light, MS, Dafydd Thomas, MD, Francis Worden, MD, Mark Prince, MD, Susan Urba, MD, Douglas Chepeha, MD, Bhavna Kumar, MS, Kitrina Cordell, DDS, Avraham Eisbruch, MD, Jeremy Taylor, PhD, Jeffrey Moyer, MD, Carol Bradford, MD, Nisha D'Silva, DDS, Thomas Carey, PhD, Jonathan McHugh, MD, Gregory Wolf, MD; UM Head and Neck SPORE Program, University of Michigan, Ann Arbor, MI
- 9:24am **S049: Imaging and Spectroscopic Modalities for Noninvasive Evaluation of Oral** Lesions - <u>Richard A Schwarz, PhD</u>, Darren M Roblyer, PhD, Wen Gao, PhD, Vanda M Stepanek, MD, PhD, Tao T Le, BS, Vijayashree S Bhattar, BS, Jessica K Wu, BS, Nadarajah Vigneswaran, BDS, DMD, DrMedDent, Ann M Gillenwater, MD, FACS, Rebecca Richards-Kortum, PhD; Rice University, Houston, TX, Beckman Laser Institute, Irvine, CA, University of Texas M. D. Anderson Cancer Center, Houston, TX, University of Texas Dental Branch at Houston, Houston, TX
- 9:32am Discussion
- 9:40am **S050: Best Prevention & Detection Paper: Vitamin or Mineral Supplement Intake and the Risk of Head and Neck Cancer: Pooled Analysis in the INHANCE Consortium** <u>Qian Li, PhD</u><sup>1</sup>, Mia Hashibe, PhD<sup>2</sup>, Paolo Boffetta, PhD<sup>3</sup>, Erich M Sturgis, PhD<sup>4</sup> International Agency for Research on Cancer; <sup>1</sup>International Agency for Research on Cancer, Lyon, France; <sup>2</sup>University of Utah School of Medicine, Salt Lake City; <sup>3</sup>International Prevention Research Institute, Lyon, France; <sup>4</sup>University of Texas Anderson Cancer Center, Houston
- 9:48am **S051: Investigation of the Prognostic Characteristics of Head and Neck Squamous Cell Carcinomas (HNSCCA) in the Young Population -** <u>Konstantinos Kourelis, MD</u>, Terrance Tsue, MD, FACS, Douglas Girod, MD, FACS, Kevin Sykes, MPH, Yelizaveta Shnayder, MD, FACS; Department of Otolaryngology, Kansas University Medical Center.
- 9:56am Discussion
- 10:00am **S052: Alternative Tobacco Product Use in Rural African American Men** <u>William R. Carroll.</u> <u>MD</u>, Herman R Foushee, PhD, Isabel Scarinci, PhD, MPH; University of Alabama – Birmingham
- 10:08am **S053: Venous Thromboembolism in Head and Neck Cancer Patients after Surgery** <u>Leo</u> <u>Thai. BS</u>, Neil Gross, MD, William Stott, BS, Mark Wax, MD, Peter Andersen, MD; Oregon Health & Science University
- 10:16am S054: A Ten-Year Clinicopathological Study of 141 Cases of Ameloblastoma Satheesh Kumar Poolakkad Sankaran, MDS Trainee, Anita Balan, MDS, Ajith, MDS, Deepti Simon, MDS; 1Department of Oral Medicine and Radiology, Government Dental College, Thiruvananthapuram, Kerala, India.
- 10:24am Discussion



Regency E-F

#### 10:30am - 12:00pm Concurrent Sessions

#### **PANEL:** Invasion/Metastasis: Models and Applications Co-Chairs: Thomas E. Carey, PhD & Ernst-Jan M. Speel, PhD Location: Regency E-F

*Description:* This panel will discuss current issues in Head and neck cancer metastasis and invasion using model systems and clinical data. The session will include current quality standards for cell lines, early metastatic behavior of HPV-induced cancers, the role of cancer stem cells in invasion and metastasis, physiologic studies of lymphatic metastasis, and the role of receptor crosstalk in HNSCC metastasis.

- 10:30am Tumor Cell Lines: Current Quality Standards Thomas E. Carey, PhD
- 10:45am Early Metastatic Behavior of HPV-Induced Oropharyngeal Cancer - Ernst-Jan M. Speel, PhD
- 11:00am Physiologic Studies of Lymphatic Metastases Vincente Resto, MD, PhD
- 11:15am Receptor Crosstalk in HNSC Metastasis Steven A. Rosenzweig, PhD
- 11:30am Stem cells in Invasive, Migratory and Experimental Metastasis - Samantha J. Davis, MD
- 11:45am Global Methylation and Gene Expression Differences in HPV positive and HPV Negative Squamous Cell Carcinomas - Maureen A. Sartor, PhD
- At the conclusion of this session, participants will be able to:
- Understand the importance of proper cell line documentation and use for research.
- Appreciate the current level of understanding of biological behavior of HPV-induced oropharyngeal cancers.
- Understand the physiology of lymphatic metastasis.

Adjourn

12:15pm

- + Understand the complexity of receptor signaling in the metastatic process.
- Appreciate the role of cancer stem cell in invasion and metastasis.
- Awareness of the strong differences in methylation and gene expression in HPV+ and HPV- cancer cells.

12:00pm - 12:15pm	Closing Comments/Meeting Summary/Future Directions
	Carol Bradford, MD & Jennifer Grandis, MD

**PANEL:** Salivary Malignancies: Basic and Translational Science Co-Chairs: Patrick Ha, MD & Christopher A. Moskaluk, MD, PhD Location: Regency C-D

*Description:* This session will appreciate the complex mechanisms underlying salivary gland malignancies. We will broadly highlight several areas of novel research.

- 10:30am Resources for Salivary Gland Tumor Research Yasaman Shirazi, PhD
- 10:40am Epigenetic Alterations in Salivary Gland Tumors Patrick Ha, MD
- 10:55am Expression Analyses and Modeling of Salivary Cancers - Wendell G. Yarbrough, MD
- 11:10am Phosphoproteomic Analysis of Adenoid Cystic Carcinoma - Christopher A. Moskaluk, MD, PhD
- 11:25am Dissecting the Receptor Tyrosine Kinase Signaling Pathways for Potential Therapies in Adenoid Cystic Carcinoma of the Salivary Glands - Osamu Tetsu, MD

#### 11:40am Discussion

At the conclusion of this session, participants will be able to:

- Appreciate the goal of research directed at understanding the mechanisms underlying salivary gland malignancies.
- Understand the array-based expression modeling of salivary gland cancers.
- Understand the concept of epigenetics and the role it plays in cancer.
- Understand the role of kinome analysis in adenoid cystic carcinoma and how this may translate into targeted therapies.

Regency E-F

# **FACULTY LISTING**

Janice A. Blalock, PhD - University of Texas MD Anderson Cancer Center, Houston, TX Carol R. Bradford, MD - University of Michigan, Ann Arbor, MI Ole T. Brinkmann, DMD - UCLA School of Dentistry, Los Angeles, CA William J. Burke, MD - University of Nebraska Medical Center, Omaha, NE Barbara A. Burtness, MD - Fox Chase Cancer Center, Philadelphia, PA Joseph A. Califano, MD - Johns Hopkins Medicine, Baltimore, MD **Thomas E. Carey, PhD** - University of Michigan, Ann Arbor, MI Zhong Chen, MD - National Institute on Deafness and Other Communication Disorders, Bethesda, MD Amy Y. Chen, MD, MPH - Emory University, Atlanta, GA Christine H. Chung, MD - Johns Hopkins University, Baltimore, MD Ezra E.W. Cohen, MD - University of Chicago, Chicago, IL Samantha J. Davis, MD - University of Michigan School of Medicine, Ann Arbor, MI Terry A. Day, MD - Medical University of South Carolina, Charleston, SC Elba C. Díaz Toro, DMD - Puerto Rico Comprehensive Cancer Center at the University of Puerto Rico, San Juan, Puerto Rico Nisha D'Silva, BDS, MSD, PhD - University of Michigan School of Dentistry, Ann Arbor, MI Gypsyamber D'Souza, PhD, MPH, MS - Johns Hopkins University, Baltimore, MD Ann Marie Egloff, PhD, MPH - University of Pittsburgh, Pittsburgh, PA Avraham Eisbruch, MD - University of Michigan Health System, Ann Arbor, MI Carole Fakhry, MD, MPH - Johns Hopkins University, Baltimore, MD Robert L. Ferris, MD, PhD - Eye & Ear Institute, Pittsburgh, PA Elizabeth Field, MD - University of Iowa, Iowa City, IA Arlene A. Forastiere, MD - Johns Hopkins Oncology Center, Baltimore, MD Elizabeth J. Franzmann, MD - University of Miami Miller School of Medicine, Miami, FL Christina M. Furdui, PhD - Wake Forest University Baptist Medical Center, Winston-Salem, NC Levi A. Garraway, MD - Dana-Farber Cancer Institute/Brigham and Women's Hospital, Boston, MA Hamid Ghandehari, BS, PhD - University of Utah, Salt Lake City, UT Jennifer R. Grandis, MD - University of Pittsburgh, Pittsburgh, PA Neil D. Gross, MD - Oregon Health & Science Univ, Portland, OR Patrick K. Ha, MD - John Hopkins Medicine, Baltimore, MD Mark J. Jameson, MD, PhD - University of Virginia Health System, Charlottesville, VA Karl T. Kelsey, MD, MOH - Brigham and Women's Hospital, Boston, MA Saleem A. Khan, PhD - University of Pittsburgh School of Medicine, Pittsburgh, PA Seungwon Kim, MD - Eye & Ear Hospital, Pittsburgh, PA Young Kim, MD, PhD - Johns Hopkins Hospital, Baltimore, MD Wayne M. Koch, MD - Johns Hopkins University, Baltimore, MD Stephen Y. Lai, MD, PhD - MD Anderson Cancer Center, Houston, TX Quynh-Thu Le, MD - Stanford University, Stanford, CA John H. Lee, MD - Sanford Cancer Research Ctr, Sioux Falls, SD Kirsten H. Limesand, PhD - The University of Arizona, Tucson, AZ Ho-Sheng Lin, MD - Wayne State University, Detroit, MI Scott M. Lippman, MD - MD Anderson Cancer Center, Houston, TX

Weidong Lu, MB - Harvard University, Boston, MA Dennis McCance, MD - Queen's University, Belfast, Northern Ireland, United Kingdom Yusuf Menda, MD - University of Iowa, Iowa City, IA Christopher A. Moskaluk, MD, PhD - University of Virginia Health System, Charlottesville, VA Suresh K. Mukherji, MD - University of Michigan, Ann Arbor, MI Cherie-Ann O. Nathan, MD - Louisiana State University Health Science Center, Shreveport, LA Jacques E. Nor, DDS, MS, PhD - University of Michigan, Ann Arbor, MI Mukesh K. Nyati, PhD - University of Michigan Health System, Ann Arbor, MI Frank G. Ondrey, MD, PHD - University of Minnesota, Minneapolis, MN Sara I. Pai, MD, PhD - The Johns Hopkins Medical Institutions, Baltimore, MD William Pao, PhD - Vanderbilt University, Nashville, TN Harish Poptani, MD - University Pennsylvania Medicine, Philadelphia, PA Mark E. Prince, MD - University of Michigan, Ann Arbor, MI Vicente Resto, MD, PhD - The University of Texas Medical Branch, Galveston, TX John A. Ridge, MD, PhD - Fox Chase Cancer Center, Philadelphia, PA James W. Rocco, MD, PhD - Massachusetts Eye & Ear Infirmary, Boston, MA Steven A. Rosenzweig, MD - Medical University of South Carolina, Charleston, SC Maureen A. Sartor, PhD - University of Michigan, Ann Arbor, MI David E. Schuller, MD - Ohio State University, Columbus, OH David L. Schwartz, MD - North Shore - Long Island Jewish Health System, New Hyde Park, NY Duane A. Sewell, MD - University of Maryland, Baltimore, MD Richard J. Shaw, BDS, FDS, MBChB, FRCS - University of Liverpool, Liverpool, England, United Kingdom Dong Moon Shin, MD - Emory Clinic, Atlanta, GA Yasaman Shirazi, PhD - Center for Integrative Biology and Infectious Diseases, Bethesda, MD Ross Sibson, PhD - Clatterbridge Centre for Oncology NHS Foundation Trust, Wirral, England, United Kingdom Vani N. Simmons, PhD - H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL Ernst-Jan M. Speel, PhD - Maastricht University Medical Center, Maastricht, The Netherlands Scott E. Strome, MD - Unviersity of Maryland, Baltimore, MD Erich M. Sturgis, MD - MD Anderson Cancer Ctr, Houston, TX Osamu Tetsu, MD, PhD - University of California, San Francisco, San Francisco, CA Andrea (Andy) Trotti, III, MD - University of South Florida, Tampa, FL Carter Van Waes, MD, PhD - National Institute on Deafness and Other Communication Disorders, Bethesda, MD Johan Wennerberg, MD, PhD - University Hospital, Lund, Sweden Theresa L. Whiteside, PhD - University Pittsburgh - School of Medicine, Pittsburgh, PA Gregory T. Wolf, MD - University of Michigan, Ann Arbor, MI Richard J. Wong, MD - Memorial Sloan-Kettering Cancer Center, New York, NY Stuart J. Wong, MD - Medical College of Wisconsin, Milwaukee, WI Maria J. Worsham, PhD - Henry Ford Health System, Detroit, MI Wendell G. Yarbrough, MD - Vanderbilt University Medical Center, Nashville, TN Jian Yu, PhD - University of Pittsburgh, Pittsburgh, PA Bevan Yueh, MD, MPH - University of Minnesota, Minneapolis, MN

# **FACULTY & PRESENTER DISCLOSURES**

The following faculty and presenters provided information indicating they have a financial relationship with a proprietary entity producing health care goods or services, with the exemption of non-profit or government organizations and non-health care related companies. (Financial relationships can include such things as grants or research support, employee, consultant, major stockholder, member of speaker's bureau, etc.)

NAME	COMMERCIAL INTEREST	WHAT WAS RECEIVED	FOR WHAT ROLE
Ruud H. Brakenhoff, MD*	Merck	Consultant Fee	Advisory Board
	Interna	Research Grant	Principal Investigator
William J. Burke, MD	Forest Research Institute	Research Grant	Scientific Research
Barbara Burtness, MD	GenMab Agensys PER NCCN IMEDEX IMER GlaxoSmithKline Merck Serono Pfizer YM Biosciences Amgen Roche Genentech	Consultant Fee Consultant Fee Honorarium Honorarium Honorarium Consultant Fee Consultant Fee Consultant Fee Consultant Fee Consultant Fee Consultant Fee Consultant Fee Consultant Fee	Safety Monitoring Board Safety Monitoring Board Speaker Speaker Speaker Consultant Consultant Consultant Consultant Consultant Consultant Consultant Consultant Consultant Consultant Consultant Consultant
Thomas E. Carey, PhD	Sequenom	Honorarium	Advisory Board
Ezra E.W. Cohen, MD*	Amgen	Consultant Fee	Consultant
	BMS	Honorarium	Speaker
	Primal Life	Consultant Fee	Consultant
	sanofi-aventis	Honorarium	Speaker
	Vicus	Consultant Fee	Consultant
Stephanie Contag, MD	Pfizer, Inc.	Research Grant	Clinical Research Training Program
	Pfizer, Inc.	Intellectual Property Rights	Research Study
Terry A. Day, MD*	Eli Lilly	Nothing	Speaker's Bureau
	Head & Neck Cancer Alliance	Nothing	Board of Directors
	Merck	Consultant Fee	Consultant
Gypsyamber D'Souza, PhD, MPH, MS	Merck	Consultant Fee	Advisory Board
	Merck	Research Grant	Scientific Research
Robert L. Ferris, MD, PhD*	Amgen	Research Grant	Scientific Research
	Bristol Myers Squibb	Research Grant	Scientific Research
Elizabeth J. Franzmann, MD	Roche	Ownership Interest	Consultant
Levi A. Garraway, MD	Foundation Medicine	Consultant Fee	Consultant
	Foundation Medicine	Ownership Interest	Advisory Board
	Novartis	Research Grant	Scientific Research
	Novartis	Consultant Fee	Consultant
Hamid Ghandehari, BS, PhD	Theratarget	Ownership Interest	Co-Owner
Neil D. Gross, MD*	Bristol Myers	Honorarium	Speaker
	Sanofi Aventis	Honorarium	Speaker
Quynh-Thu Le, MD	Amgen	Consultant Fee	Consultant
	GSK	Consultant Fee	Consultant
	GSK	Research Grant	Scientific Research
	Varni	Research Grant	Scientific Research
Ho-Sheng Lin, MD	Inspire Medical	Consultant Fee	Consultant
	Life Cell	Consultant Fee	Consultant
Yusuf Menda, MD	Siemens Medical Solutions	Research Grant	Scientific Research
Suresh K. Mukherji, MD	Philips	Consultant Fee	Consultant

### **AHNS 2010 RESEARCH WORKSHOP**

NAME	COMMERCIAL INTEREST	WHAT WAS RECEIVED	FOR WHAT ROLE
William Pao, PhD	Astra Zeneca	Consultant Fee	Consultant
	BMS	Consultant Fee	Consultant
	Molecular MD	Ownership Interest	Scientific Research
	Molecular MD	Consultant Fee	Consultant
	Xcovery	Research Grant	Scientific Research
Marshall Posner, MD	sanofi-aventis	Research Grant	Scientific Research
Panos Savvides, MD	Eli Lilly	Honorarium	Speaking and/or Teaching
	sanofi-aventis	Honorarium	Speaking and/or Teaching
	sanofi-aventis	Consultant Fee	Consultant
Richard A. Schwarz, PhD	Remicalm, LLC	Ownership Interest	Advisory Board
	Remicalm, LLC	Royalty	Licensed Patents
Richard J. Shaw, BDS, FDS, MBChB, FRCS*	GlaxoSmithKline	Consultant Fee	Consultant/Advisory Board
Scott E. Strome, MD	Gliknik	Ownership Interest	Advisory Board
	Mayo Clinic	Honorarium	Scientific Research
Derrick Wansom, MD	IRX Therapeutics, Inc.	Consulting Fee	Consultant

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Seungwon Kim, MD Young Kim, MD, PhD Wayne M. Koch, MD Konstantinos Kourelis, MD Pawan Kumar, MS PhD Stephen Y. Lai, MD, PhD John H. Lee, MD Steve C. Lee, MD PhD Qian Li, PhD Roberto A. Lleras, MD Kirsten H. Limesand, PhD Gustaf Lindgren, MD Scott M. Lippman, MD L.D. Locati. MD Jaimee M. Lockwood, BS Weidong Lu, MB Sofia Lyford-Pike, MD Old Matthew, MD April M. Matthews, MBChB, MRCS, MBA Yvonne K. Mburu, MD Dennis McCance, MD Luc G.T. Morris, MD Christopher A. Moskaluk, MD, PhD Cherie-Ann O. Nathan, MD Jacques E. Nor, DDS, MS, PhD Mukesh K. Nyati, PhD Frank G. Ondrey, MD, PHD Sara I. Pai, MD, PhD Quintin Pan, PhD Matthew Pierce, B,S BA Harish Poptani, MD Mark E. Prince, MD\* Vicente Resto, MD, PhD John A. Ridge, MD, PhD James W. Rocco, MD, PhD\* Steven A. Rosenzweig, MD Giuseppe Sanguineti, MD

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#### **\$001:** PREDICTION OF LOCAL RELAPSE IN HEAD AND NECK CANCER BY IMMUNOSTAINING OF THE SURGICAL MARGINS USING BIOMARKERS IDENTIFIED IN A

**PROTEOMICS SCREEN** - <u>RH Brakenhoff, PhD</u>, BM Schaaij-Visser, PhD, CR Leemans, PhD, MD, E Bloemena, PhD, MD, M Slijper, PhD; ORL-HNS/Pathology, VU University Medical Center, Amsterdam and Netherlands Proteomics Centre, Utrecht, The Netherlands

Squamous cell carcinomas in the head and neck (HNSCC) frequently develop within precursor fields of genetically altered cells that can reach dimensions of multiple centimeters. These fields are sometimes macroscopically visible as leukoplakia and erythroplakia lesions. Using genetic analyses we showed that in approximately 25% of surgically treated HNSCC patients the fields remain behind in the surgical margins. Recently we also showed that the presence of a genetically defined field is a risk factor for local relapse. Under the microscope these fields may be recognized as dysplasia, but it is unclear whether its presence and grading in mild, moderate and severe dysplasia is associated with the risk for malignant transformation. To detect these fields in surgically treated patients, the resection margins should be genetically analyzed. This is laborious and not easily implemented in routine daily practice. We therefore performed a proteomics screen to identify protein biomarkers that could predict local relapse using a simple immunostaining procedure. The proteomes of genetically characterized normal, precursor and tumor tissues of eight patients were compared by twodimensional difference in-gel electrophoresis (2D-DIGE) and proteins with significantly differential expression levels were identified by mass spectrometry (LC-FT-ICR-MS/MS). Forty proteins showed a highly significant differential level of expression (FDR-corrected p<0.05). Most discriminative markers suited for immunostaining were keratin 4 and cornulin. The prognostic value of these two candidate protein biomarkers was evaluated by immunohistochemical analysis of 222 surgical margins taken from the specimen of 46 HNSCC patients who developed local relapse or remained disease-free. Low expression in the surgical margins of keratin 4 (p=0.002, RR=3.8), cornulin (p=0.025, RR=2.7), and their combination (p=0.0005, RR=8.8) showed a highly significant association with the development of local relapse. Immunostaining for mutated p53 also showed outcome associations. Dysplasia grading had no prognostic relevance. Immunohistochemical assessment of keratin 4 and cornulin expression as well as mutant p53 in surgical margins of HNSCC patients outperforms histopathological grading in predicting the risk for local relapse. These markers can be used to initiate more frequent and lifelong surveillance of patients at high risk for local relapse, and enable selection for adjuvant treatment or prevention trials.

**S002:** REFERENCE LEVELS OF SALIVARY EPIGENETIC BIOMARKERS IN A NORMAL COHORT INFORM THEIR USE IN THE EARLY DETECTION OF OSCC AND SURVEILLANCE OF RECURRENT OSCC - <u>April M Matthews</u>, <u>MBChB, MRCS, MBA</u>, Lakis Liloglou, BSc, PhD, E A Field, MDS, DDSci, FDSRCS, Richard J Shaw, MD, MBChBHons, BDS, FRCSOMFS, RCPSGla, Janet M Risk, BSc, PhD; University of Liverpool School of Dental Sciences, University Hospital Aintree

Introduction: Aberrant methylation of tumour suppressor genes plays a significant role in the biology of cancer. Reproducible concordance of epigenetic profiles between matched tumour and saliva samples, together with ease of collection and patient acceptance of this noninvasive method, identifies saliva as an attractive longitudinal sampling method for screening of OSCC in high risk groups. This may aid earlier detection and treatment resulting in improved prognosis. The levels of gene promoter methylation in normal saliva is unknown. This study aims to define a reference level of methylation biomarkers in normal saliva which can be used in future studies of OSCC and oral dysplasia. Method: Saliva was collected from 74 normal patients attending Liverpool Dental Hospital emergency department. DNA was extracted, bisulphite treated and interrogated for a panel of epigenetic biomarkers using quantitative methylation specific PCR. A panel of 5 genes was selected on the basis of tumour specificity from studies on tumour/normal matched tissue pairs performed by this research group. Results: All samples tested showed methylation levels less than 0.5% for cyclin A1, cytoglobin and TMEFF2 gene promoters. A further increase in the sensitivity of the assay is possible for these 3 genes. Only 2/74 samples demonstrated p16 methylation levels > 0.5% while 2/64 showed >0.5% methylation in ADAMTS9 promoter. Preliminary data from 30 saliva from dysplasia patients indicate that these biomarker cut-offs will allow the identification of lesions at increased risk of progression. Conclusion: A cut-off at least as low as 0.5% methylation has been established in saliva samples for 3 gene promoters which are often highly methylated in cancer tissue. These data can now be used to assess the sensitivity and specificity of this panel of genes in differentiating oral cancer from normal samples. Two additional biomarkers are under further investigation, although clinical follow-up of the 4 individuals with methylation greater than 0.5% is not possible due to the limitations imposed by anonymous collection.

#### **S003:** ACTIVATED C-SRC CONTRIBUTES TO SCCHN TUMOR GROWTH, INVASION, AND RESISTANCE TO EPIDERMAL GROWTH FACTOR RECEPTOR TARGETING <u>Guoqing He<sup>1</sup></u>, Sufi M Thomas1, Sonali Joyce<sup>1</sup>, Jennifer R Grandis<sup>1,2</sup>; Department of Otolaryngology<sup>1</sup> and Pharmacology<sup>2</sup>, University of Pittsburgh and University of Pittsburgh Cancer Center, Pittsburgh, PA

Abstract Purpose: Squamous cell carcinoma of the head and neck (SCCHN) are highly invasive tumors that are difficult to treat with conventional modalities. Single agent molecular therapeutics targeting the epidermal growth factor receptor (EGFR) also continues to be a challenge. Increased understanding of invasion mechanisms and resistance to EGFR tyrosine kinase inhibitors (TKIs) are critical in the development of new therapeutics for SCCHN. We previously demonstrated that c-Src is activated in SCCHN and mediates invasion downstream of EGFR. The role of c-Src activation in tumor progression and response to therapy remains incompletely understood. In this study, we investigated the effects of c-Src activation on SCCHN proliferation, invasion and response to the EGFR TKI erlotinib. Experimental Design: The functional consequences of c-Src expression were examined using representative SCCHN cells engineered to express dominant-active (DA) or dominant-negative (DN) mutants of c-Src. Cells were subjected to proliferation and invasion assays. These cells were then tested for the effects of erlotinib on growth and invasion. Results: Expression of DA c-Src increased the growth rate of SCCHN cells. Activation of c-Src significantly increased cell invasion through Matrigel transwell chambers. In addition DA c-Src-expressing cells were associated with resistance to growth inhibition induced by erlotinib. DN c-Src-expressing cells had no phosphorylation of c-Src on EGF stimulation and demonstrated lower growth rates compared to the control cells transfected with the vector backbone (vector control). In addition the cells were less invasive in response to EGF in vitro. Conclusions: These results suggest that activated c-Src signaling enhances tumor growth, invasion, and may contribute to resistance to epidermal growth factor receptor inhibitors. Targeting c-Src in combination with EGFR TKIs may be a feasible therapeutic approach for SCCHN.

#### **\$004:** CROSS-TALK BETWEEN TGF-BETA AND NF-KAPPAB SIGNALING PATHWAYS IS MEDIATED THROUGH TAK1 AND SMAD7 IN HEAD AND NECK CANCER - <u>Christian</u>

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TGF-beta signaling plays a dual role in epithelial malignancies, including head and neck squamous cell carcinoma (HNSCC). Inactivation of TGFbeta signaling promotes de novo development of epithelial cancer, while activation of residual TGF-beta signaling in established cancer clearly favors the progression to a more malignant phenotype. The mechanism responsible for these double-edged effects of TGF-beta signaling is less well characterized. Since aberrant activation of transcription factor Nuclear Factor-kappaB (NF-kappaB) promotes the malignant phenotype similar to TGF-beta-mediated effects in late-staged tumors, we examined whether TGF-beta cross-activates NF-kappaB. Here, we show TGFbeta1 treatment induced NF-kappaB activation in HNSCC lines through sequential activation of IKK and phosphorylation of IkappaBalpha, the inhibitor of NF-kappaB. This NF-kappaB activation was mediated through TGF-beta-activated kinase 1 (TAK1), since knocking down TAK1 using siRNA decreased TGF-beta1-induced NF-kappaB-dependent reporter gene activity. Furthermore, TAK1 knockdown decreased degradation of IkappaBalpha and nuclear translocation of the transactivating NF-kappaB subunit p65. Consequently, p65 DNA binding activity and transcription of NF-kappaB downstream genes were attenuated. Furthermore, TGF-beta-induced NF-kappaB/p65 activation, promotes increased SMAD7 expression, which in turn preferentially suppresses TGF-beta canonical over non-canonical TAK1-NF-kappaB signaling. Celastrol, used in traditional Chinese medicine, decreased TGF-beta1-induced phosphorylation of TAK1 and p65, and suppressed basal, TGF-beta1and TNFalpha-induced NF-kappaB reporter gene activity. In addition, Celastrol reduced HNSCC cell proliferation (IC50 =  $1.2-1.3\mu$ M) and increased sub-G0 DNA fragmentation indicating induction of apoptosis. In conclusion, we identified cross-talk between TGF-beta and NF-kappaB pathways, where TGF-beta signaling leads to NF-kappaB activation through a sequential activation of TAK1 and IKK activities, and promotes malignant phenotype of HNSCC. Furthermore, induction of NF-kappaB further promotes attenuation of canonical TGF-beta signaling through increased Smad7 expression. With Celastrol being a potent suppressor of TAK1 mediated NF-kappaB activation, we present a potential therapeutic strategy targeting this pro-oncogenic TGF-beta pathway.

#### **S005:** APOPTOSIS IN ORAL SQUAMOUS CELL CARCINOMA

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Apoptosis is a genetically programmed form of cell death and aberrations of the apoptotic mechanisms that cause excessive or deficient programmed cell death have been linked to a wide array of pathologic conditions. Using a Tissue Microarray (TMA) comprising 229 cases of oral squamous cell carcinoma (OSCC), we have analyzed the immuno-expression of Bcl-2, Bcl-x, Bcl-2, related protein A1, BAG-1, Bak, Bax, Bim/ Bod, Bim-Long, Bad, Bid, PUMA, caspases-3, 6, 7, 8, 9 and 10, Apaf1, cytochrome c, Smac/DIABLO, survivin and p53 in order to determine possible correlations between the expression of these proteins and clinicopathologic features of OSCC. The results were quantitatively analyzed using an automated imaging system (ACIS III). All proteins tested were present in the OSCC samples studied. Our results showed that high expression of Bak, Bax, Bcl-xL, Bcl-2-related protein, PUMA, Apaf1, caspases-3, 9 and 10 and survivin was associated with absence of vascular invasion. Increased expression of Bim/Bod and BAG-1 was associated with the presence of perineural infiltration. Increased expression of Bax was associated with tumors occurring in oral tongue and increased expression of the Bcl-2-related protein, caspases-3 and 9, survivin and PUMA was associated with tumors occurring in the floor of mouth. An increase in Bid, Bim-Long, caspases-6 and 7 and survivin expression was associated with moderately to well-differentiated tumors. An increase in Bcl-2-related protein and caspase-3 was associated with early tumor stages whereas an increase in caspase-6 expression was associated with advanced tumor stages. Increased expression of caspase-3 was also associated with the presence of lymph node metastasis. Increased expression of PUMA and caspase-7 was also associated with recurrence of the tumor. Bcl-x, Bcl-2, Bad, Smac/DIABLO, cytochrome c and p53 proteins were not significantly associated to any of the clinicopathological characteristics analyzed. Overall survival was statistically different between the patients that presented low and high expression of Bak and Bcl-2-related protein. Disease-free survival was statistically different between the patients that presented low and high expression of caspase-7, PUMA and Bcl-x. Multivariate Cox analysis demonstrated that caspase-7, PUMA and Bim-Long could be independent prognostic factors for OSCC patients. Supported by FAPESP grants 98/14335-2 and 07/50608-4.

#### **S006:** TARGETING FOCAL ADHESION KINASE (FAK) IN ANAPLASTIC THYROID CARCINOMA - Yunyun Chen, PhD, Maria Gule, MD, Ying Henderson, MD, PhD, <u>Stephen Y Lai, MD PhD</u>; University of Texas M. D. Anderson Cancer Center, Houston, TX

Introduction: Anaplastic thyroid carcinoma (ATC) is one of the most lethal human malignancies with a median survival of six months. Given the limited current therapeutic options, development of more effective treatment is critical. We investigated the functional role of focal adhesion kinase (FAK) in ATC and assessed potential anti-tumor effects of targeting FAK directly. We also examined the potential contribution of signal transducer and activator of transcription 3 (STAT3) to resistance against targeted therapies directed against the Src-FAK complex. Methods: ATC cell lines (Hth104, Hth83, SW1735, C643 and K18) were grown in tissue culture and as orthotopic xenograft tumors. Activation of FAK and associated downstream signaling intermediates activation was determined by immunoblotting. Functional effects of FAK inhibition by siRNA were assessed in assays measuring anchorage-independent colony formation, apoptosis, migration and invasion. Results: ATC cells lines and orthotopic murine xenograft tumors demonstrated increased FAK activation as measured by phosphorylation at Tyr-397 and Tyr-861, as compared to normal thyroid cells (NThy) and normal thyroid tissue. Downregulation of FAK expression by siRNA led to inhibition of FAK and downstream signaling intermediates, including Paxillin and p130Cas. Decreased FAK activation resulted in lower colony formation in the methylcellulose growth-death assay and increased apoptosis. ATC cells also demonstrated decreased migration and invasion following treatment with FAK siRNA. Given previous studies that demonstrated decreased FAK activation through Src kinase inhibition, we assessed Src kinase inhibition in ATC and found decreased FAK activation was coupled with increased activation of STAT3, a potential mechanism for resistance against Src inhibition. Conclusions: Specific targeting of FAK may represent an effective treatment strategy for ATC. Decreased FAK activation led to reduced ATC cell survival and invasion. Increased

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STAT3 activation resulted from Src inhibition, which may facilitate resistance to Src inhibition. Targeted therapy against FAK directly rather than the Src-FAK complex may be more effective in ATC.

#### **\$007:** ORAL CANCER PROGRESSION INDUCED BY MUTANT P53 IN RESPONSE TO CHEMOTHERAPY - Sergio Acin, PhD, Olga Mejia, BS, <u>Carlos Caulin, PhD</u>; Department of Head and Neck Surgery, The University of Texas M. D. Anderson Cancer Center,

Advanced oral cancers exhibit high frequency of relapse, and the estimated 5-year survival rates are <30%, as recurrent tumors tend to be aggressive and refractory to therapy, largely limited to chemotherapy and radiation. Therefore, understanding mechanisms involved in resistance to therapy is critical for designing novel and more efficient treatments for patients with advanced oral cancer. Oral cancers arise through the accumulation of genetic alterations that involve activation of oncogenes and/or inactivation of tumor suppressor genes. The p53 gene is frequently mutated in oral cancers, and potential p53 gain-of-function mutations are associated with poor prognosis and lack of response to chemotherapy. We generated a mouse model for sporadic oral cancer and demonstrated that the p53 gain-of-function mutation p53R172H (equivalent to human p53R175H) contributes to oral tumor initiation, accelerates tumor growth and promotes progression to carcinoma. We found that mutant p53R172H may exert these oncogenic effects by attenuating the DNA damage response induced in the oral tumors to prevent the accumulation of genetic alterations and tumor progression. In addition, using cell lines derived from the oral tumors that developed in these mice, we observed that p53R172H induces resistance to DNA damage induced by the chemotherapeutic drug doxorubicin. Upon injection into nude mice, cells that survived exposure to doxorubicin developed tumors that grew faster than tumors generated by the parental cells that were never exposed to doxorubicin. Spectral karyotyping revealed that cells that survived to doxorubicin acquired increased number of translocations compared to the parental cells. These results indicate that oral tumor cells that express the p53R172H gain-of-function mutation may acquire increased tumorigenic potential and genomic instability upon exposure to chemotherapy. Together, these observations indicate that mutant p53 modulates the DNA damage response and induces tumor progression in response to DNA damage, and suggest that p53 profiling may predict the response of oral cancers to chemotherapy. Identifying mechanisms involved in resistance to chemotherapy induced by mutant p53 will help to design novel therapies to treat patients with advanced oral cancers.

# **S008:** SURGICAL TRAUMA STIMULATES GROWTH BY DOWNREGULATION OF PROAPOPTOTIC GENES IN

HNSCC XENOGRAFTS - <u>Gustaf Lindgren, MD</u><sup>1</sup>, Åke Borg<sup>2</sup>, Johan Vallon-Christersson<sup>2</sup>, Lars Ekblad<sup>2</sup>, Elisabeth Kjellén<sup>2</sup>, Johan Wennerberg<sup>1</sup>; <sup>1</sup>Department of Otorhinolaryngology, Head and Neck surgery and <sup>2</sup>Department of Oncology, Lund University Hospital, Lund, Sweden

Background: Several studies on the use of erythropoietin (EPO) to treat anemia in patients undergoing cancer treatment has shown adverse effects on tumour control and survival. Experimental studies indicate that this could be linked to an interaction with wound healing processes and not an effect on tumour cells per se. Surgical trauma stimulates proliferation through wound healing. Many mechanisms in wound healing, e.g. paracrine growth factor signalling, angiogenesis and DNA-replication initiation are also disturbed in tumorigenesis. We have previously studied whether erythropoietin in combination with surgical trauma stimulates growth of squamous cell carcinoma of the H&N (HNSCC) and in an earlier study we could demonstrate an increased growth in xenografted tumours undergoing surgical trauma when exposed to EPO. Materials and Methods: In the present study human HNSCC xenotransplanted to nude mice treated with EPO. The tumours were transected in a standardised procedure to mimic surgical trauma and the change in gene expression of the tumours was investigated with c-DNA microarray. Results: There was little change in the expression of genes involved in angiogenesis and hypoxia but a consistent downregulation of the expression of genes involved in apoptosis, including BCL2L13, AIFM1, BCLAF1 and Caspase 1. This suggests that the increased tumour survival during erythropoietin treatment is due to inhibition of apoptosis, an effect that becomes significant during tissue damage. Conclusion: This is the first time that the apoptosis inhibiting effect of erythropoietin is correlated to surgical trauma. Interaction with the surrounding stroma can prove to be an important mechanism for the transduction of the EPOtumour signalling. Our findings have important clinical considerations since this model of subtotal surgery can be applicable to study the kinetics of minimal residual disease after surgery.

**S009:** HUMAN BETA-DEFENSIN 3 PROMOTES NF-KAPPAB MEDIATED CCR7 EXPRESSION AND ANTI-APOPTOTIC SIGNALS IN SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK - <u>Yvonne K Mburu</u>, Koji Abe, MD, Laura K Ferris, MD, PhD, Saumendra N Sarkar, PhD, Robert L Ferris, MD, PhD; University of Pittsburgh School of Medicine, University of Pittsburgh Cancer Institute, University of Pittsburgh Medical Center

The microenvironment of aerodigestive cancers contains tumor promoting inflammatory signals often involved in innate immunity. The epithelial malignancy, squamous cell carcinoma of the head and neck (SCCHN), is characterized by secretion of inflammatory mediators that can promote tumorigenesis and lymph node metastasis. Human betadefensin 3 (hBD3) is one such antimicrobial mediator of innate immunity produced by squamous epithelial cells in response to tissue damage and inflammation. Here, we hypothesized that the observed overexpression of hBD3 in SCCHN may have a tumor-promoting effect or contribute to nodal metastasis, which has previously been linked to chemokine receptor 7 (CCR7) overexpression. Indeed, treatment of non-metastatic SCCHN cells with hBD3 induced surface CCR7 expression and migration toward its ligand, CCL19. The hBD3-induced CCR7 upregulation in SCCHN cells was significantly reduced by inhibition of nuclear factor (NF)kappaB, an inflammatory transcription factor known to influence CCR7 expression. Moreover, hBD3 stimulation provided anti-apoptotic signals to SCCHN cells, as evidenced by tumor resistance to cisplatin-induced cell death, which was regulated by phosphoinositide-3-kinase (PI3K)/ Akt activation. Interestingly, the observed hBD3-mediated effects were not dependent on G-protein coupled receptors or toll-like receptors, as has been previously published, but hBD3 was internalized through endocytosis, allowing intracellular signal transduction. Our findings suggest that hBD3 represents a novel, NF-kappaB-regulated mediator of CCR7 expression and anti-apoptotic pathways, which may be exploited by developing SCCHN tumors to enhance their survival and metastasis.

#### **SOIO:** DYSREGULATION OF THE PI3K/AKT/MTOR PATHWAY VIA MUTATION AND COPY NUMBER ALTERATION IN ORAL CAVITY SQUAMOUS CELL

**CARCINOMA** - <u>Luc G Morris, MD</u>, Ian Ganly, MD, PhD, Barry S Taylor, PhD, Bhuvanesh Singh, MD, PhD, Agnes Viale, PhD, Adriana Heguy, PhD, Timothy A Chan, MD, PhD; Memorial Sloan-Kettering Cancer Center

Introduction: The phosphatidylinositol 3-kinase (PI3K) signaling pathway is integral to cell growth, proliferation and survival, and is upregulated in multiple human cancers. Several oncogenes and tumor suppressors within

this pathway are altered by mutation, amplification or deletion, and are currently being investigated as therapeutic targets in ongoing clinical trials for head and neck and other solid cancers. The pattern of dysregulation has not been characterized in oral cavity squamous cell carcinoma (OCSCC). Our objective was to comprehensively analyze the pattern of mutational and copy number alteration in this pathway in OCSCC. Methods: After IRB approval, DNA was extracted from 32 microdissected frozen OCSCC samples and matched normal tissues. High-throughput sequencing of the 23 component genes of the PI3K pathway was performed. Three integrated sequence assemblies were screened for non-polymorphism coding mutations, which were independently validated on PCR. Array comparative genomic hybridization (aCGH) was then performed on the Agilent 1M platform. The RAE computational framework was used for segmentation and identification of regions of statistically significant copy number alteration (CNA), which were then validated with qPCR. Immunohistochemistry of downstream proteins was used to confirm pathway activation. Results: Among the 23 component genes in the PI3K pathway, activating mutations were identified in PIK3CA in 6.3% of samples. Copy number gain was present in 15 genes (including PIK3CA in 45.2%), and loss in 4 genes (including PTEN in 12.9%). Altogether, 72% of tumor samples contained either activating mutations or CNAs within the PI3K pathway. Tumors with PIK3CA mutation or EGFR amplification had a significantly higher rate of copy number alterations in the PI3K pathway. Conclusions: In the first comprehensive mutational and copy number analysis of the 23 component genes of the PI3K pathway in cancer, we report a low frequency of somatic mutations, and high frequency of copy number alteration, in OCSCC. In contrast to colorectal, breast, ovarian, and brain cancers, copy number alteration, not mutation, appears to be the main source of pathway activation. These findings may have relevance to therapeutics targeting tumors in which the PI3K pathway is dysregulated, or in which PI3K pathway activation mediates resistance to EGFR inhibition.

# **S011:** GLOBAL DNA METHYLATION PATTERNS ARE PROGNOSTIC OF CLINICAL OUTCOME IN HNSCC - <u>Roberto</u>

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Head and neck squamous cell carcinomas (HNSCCs) account for almost 6% of all malignancies in the United States, yet the mortality rate has changed little over the last 10 years due to the frequency of tumor recurrence and lymph node metastasis. As with other cancers, hypermethylation of promoter CpG islands is associated with the inappropriate silencing of critical genes. More recently, DNA methylation of promoter regions have also been shown to be a potentially prognostic tool useful in predicting clinical outcomes in cancer patients. In this study, we tested the hypothesis that the pattern of global DNA methylation in a HNSCC clinical specimen contains information that can be used as a prognostic biomarker and a molecular classifier of this disease. We performed global DNA methylation profiling of 120 primary tumor samples from patients with oral cavity (n=64) or oropharyngeal (n=56) squamous cell carcinoma in order to identify possible predictors of patient clinical outcome. The methylation status of 27,578 CpG loci were evaluated using the Illumina Infinium Human Methylation 27K beadchip. Global methylation data was analyzed with a forward feature selection algorithm using a Support Vector Machine (SVM) implemented in Java as a training learner, and signatures were subsequently cross validated using a new instance of the SVM evaluating for predictive performance. Using this approach with oropharyngeal cancer patients,

we identified a panel of CpG loci which predicted nodal metastasis at diagnosis with an accuracy above 95% and a second signature that predicted aggressive disease within 24 months of diagnosis with an accuracy above 90%. Corresponding signatures for oral cavity cancer patients yielded independent CpG signatures with accuracies above 88%. These results demonstrate the utility of DNA methylation for accurate prediction of clinical outcomes. These studies have laid the groundwork for a population-based study to examine DNA methylation patterns in human malignancies and to identify associations between specific epigenetic signatures and clinical parameters.

#### **S012:** DELECTED IN COLORECTAL CANCER GENE PROMOTER HYPERMETHYLATION CAN BE USED AS A SINGLE MARKER FOR CANCER PROGRESSION IN

SALIVARY RINSE - Juliana L Schussel, DDS, MSc, Kavita M Pattani, MD, Zhe Zhang, MS, Chad Glazer, MD, Steven Goodman, MD, PhD, David Sidransky, MD, Miriam Robbins, DDS, MPH, A. Ross Kerr, DDS, MSD, David Sirois, DMD, PhD, Joseph A Califano, MD; Depart. Otolaryngol HN Surg and Oncology, Johns Hopkins MI, Baltimore, MD; Depart. Oral Pathology, University of Sao Paulo, SP, Brazil; Depart. Oral Medicine NY University, NY; Milton J Dance HN Center, Greater Baltimore Med Center, Baltimore, MD

Aberrant promoter hypermethylation has been recently proposed as a means for detection of HNSCC in salivary rinses. Here we evaluate the ability of a previously reported 7-gene methylation panel status to correlate with premalignant and malignant oral lesions. We used a large prospective cohort of salivary rinses obtained from patients with benign, dysplastic, and cancer diagnoses to determine promoter hypermethylation in highrisk patients. Clinical risk assessment was performed and correlated with histological diagnosis and biomarker status. Quantitative methylationspecific PCR (Q-MSP) was performed analyzing methylation status of 7 genes (CCNA1, MGMT, MINT31, TIMP3, P16, DAPK, DCC) in salivary rinses of 191 patients. Logistic regression and receiver operating characteristic (ROC) analyses were used to examine the association of methylation status with histologic diagnosis and to estimate classification accuracy, respectively. On univariate analysis, diagnosis of dysplasia/ cancer was associated with age (OR=1.3, 95%CI= (1.01-1.6, p=0.014) and 7-gene panel methylation (OR=2.2, 95%CI=(1.3-4.0), p=0.006); DCC methylation was also strongly associated (OR=3.3, 95%CI=(1.7-6.6), p=0.004). On multivariable modeling, histologic diagnosis was independently associated with 7 gene panel (OR=2.0, 95%CI=(1.1-3.6), p=0.027) or DCC (OR=2.8, 95%CI=(1.4-5.7), p=0.004) methylation. A subset analyzed (n=161) without prior biopsy proven malignancy received clinical risk classification based on lesion examination. On univariate analysis, DCC (OR=2.6, 95%CI=(1.1-6.1), p=0.026) and clinical risk classification (OR=2.5, 95%CI=(1.3-5.1), p=0.008) were associated with diagnosis of dysplasia/cancer, and remained significant on multivariate analysis (DCC: OR=2.5, 95%CI= (1.1-6.0), p=0.037, risk classification: OR=2.5, 95%CI=(1.2-5.0), p=0.012). Clinical risk classification identified dysplasia/cancer with a sensitivity of 56% (95%CI=41-71%) and specificity of 66% (95%CI= 57-75%). The sensitivity of clinical risk classification combined with 7-gene panel methylation improved to 71% (95%CI=56-83%) and with DCC methylation to 69% (95%CI=54-81%). The 7-gene panel methylation, as well DCC as a single marker, was independently associated with histologic diagnosis in salivary rinses. The results shows the potential ability of these biomarkers to predict risk for presence of oral premalignancy and malignancy using a non invasive approach and can improve the efficiency of clinical risk classification. These findings demonstrated an easy and efficient method for oral screening and prevention.

**S013:** TARGETING RAF KINASE FOR THE TREATMENT OF HEAD AND NECK CANCER - Arti Yadav, MS, Bhavna Kumar, MS, Theodoros N Teknos, MD, <u>Pawan Kumar, MS PhD</u>; Department of Otolaryngology - Head and Neck Surgery, The Ohio State University Comprehensive Cancer Center, Columbus, OH

Head and neck squamous cell carcinoma (HNSCC) is the sixth most frequent cancer worldwide and five-year survival rate (<50%) is among the lowest of the major cancers. Treatment of advanced head and neck tumors remains a challenging clinical problem, due to the persisting high rate of local and distant failure. Currently, many advanced stage head and neck tumors are being treated with concurrent chemoradiation therapy but this treatment regimen is often associated with significant toxicity. Recent research efforts have attempted to develop targeted tumor-specific therapies to minimize normal tissue toxicity but maximize therapeutic benefits. One such potential target for HNSCC is Raf kinase. EGFR, which is overexpressed in 80-90% of HNSCC, uses the Ras-Raf-MAPK signaling pathway to mediate enhanced tumor cell survival and promote tumor progression. We have recently demonstrated that patients with HNSCC show markedly higher Bcl-2 expression in tumor-associated endothelial cells and this enhanced Bcl-2 expression is directly associated with tumor progression and metastasis. In addition, Bcl-2 modulates radio-resistance by activating the Raf-MEK-ERK signaling cascade. Therefore, we hypothesize that targeting of Raf kinase in advanced HNSCC with sorafenib could reverse the resistant phenotype in tumor cells and tumor-associated endothelial cells thereby enhancing the therapeutic efficacy and decreasing the adverse effects of chemo-radiation treatment. We used both in vitro and in vivo models to test the efficacy of sorafenib either as a single agent or in combination with chemo-radiation treatment. Sorafenib significantly enhanced the anti-proliferative effects of chemo-radiation treatment and it also reversed the chemo and radioresistance in endothelial and tumor cells by down-regulating DNA repair proteins. This combination treatment also significantly inhibited endothelial cell tube formation on Matrigel, tumor cell migration and tumor cell invasion. In a SCID mouse xenograft model, sorafenib significantly enhanced the anti-tumor and anti-angiogenic effects of chemo-radiation treatment. In addition, sorafenib treatment was equally effective as a maintenance regimen. Taken together, our results suggest a potentially novel strategy to use sorafenib to overcome the resistance in tumor cells and endothelial cells lining the tumor blood vessels, thereby enhancing the effectiveness of the chemo-radiation therapy.

#### **S014:** ORAL RESVERATROL TREATMENT INHIBITS SKELETAL MUSCLE AND CARDIAC ATROPHY BY INHIBITING NF-KB ACTIVITY IN VIVO - <u>Scott Shadfar, MD</u>, Marion E Couch, MD, PhD, Kibwei A McKinney, MD, Xiaoying Yin, MD, Jessica E Rodriguez, PhD, Monte S Willis, MD, PhD; University of North Carolina

Background: Emerging evidence suggests that nuclear factor – kappa B (NF-\_B) plays an integral role in the signaling pathways coupled to skeletal muscle loss and cardiovascular catabolism in various clinical conditions, including cachexia. Resveratrol, a natural phytoalexin found in the skin of grapes, recently has been shown to have beneficial effects on a variety of disease processes, including the inhibition of NF-\_B. We therefore investigated whether resveratrol could therapeutically protect against skeletal muscle and cardiac atrophy induced in a murine model of adenocarcinoma-induced cachexia. Methods: Mice were treated daily with oral resveratrol (100-500 mg/kg) after inoculation of tumor cells in the right flank. Daily total body weights, food intake, final tumor weights and carcass weights were measured. Magnetic resonance body composition analysis assessing lean body and fat mass was also completed in addition to echocardiography evaluating heart size and function. RNA isolation with semi quantitative real time PCR determination of MuRF1 expression was conducted. Nuclear extractions from gastrocnemius and heart muscles were used in an ELISA-based assay quantifying NF-\_B (p65) activity. Results: Daily oral administration of resveratrol (100-500 mg/ kg) significantly inhibited both skeletal muscle and cardiac atrophy after 17 days of tumor challenge. No evidence of hepatic or renal toxicity from oral resveratrol was identified (100-500 mg/kg). Resveratrol significantly inhibited weight loss at the level of total body weight, hind limb weight, and the weight of the quadriceps and heart. Echocardiographic evaluation of the heart further determined that resveratrol protected against cardiac atrophy, evidenced by maintenance of LV heart wall thickness. Nuclear NF-\_B (p65) activity from heart and gastrocnemius demonstrated that resveratrol significantly inhibited the increased NF-\_B activity induced by the tumor. We found that resveratrol significantly inhibited NF-\_B regulated MuRF1 at doses that inhibited tumor-induced cardiac atrophy. Conclusions: Overall, our results indicate that oral resveratrol therapy provides clinical benefit in a cachexia model through the inhibition of NF-\_B. MuRF1 inhibition also suggests a specific mechanism by which resveratrol protects against cardiac atrophy. These findings may potentially have application in the treatment of head and neck cachexia and diseases with similar pathophysiologies such as heart failure.

**\$015:** ENHANCEMENT OF CETUXIMAB THERAPY WITH ANTI-CD137 ANTIBODY IN THE TREATMENT OF HEAD AND NECK SQUAMOUS CELL CARCINOMA - Holbrook E Kohrt, MD, <u>Peder Lund, BS</u>, A. Dimitrios Colevas, MD, Kipp Weiskopf, BS, Ronald Levy, MD, John B Sunwoo, MD; Stanford University School of Medicine

Monoclonal anti-EGFR antibody (cetuximab) is the only approved biologic therapy for head and neck squamous cell carcinoma (HNSCC). However, the objective response rate to cetuximab is only approximately 10%. One mechanism of its action is thought to be antibody-dependent cell-mediated cytotoxicity (ADCC), in which leukocytes expressing Fc receptors, such as NK cells, engage antibodies bound to target cells, triggering the release of cytolytic granules and effector cytokines that result in target cell lysis. Thus, enhancing NK cell ADCC represents a possible approach for improving the efficacy of mAb therapy. CD137 is a member of the TNFR family expressed on activated NK cells, T cells, and macrophages, and our group has previously demonstrated its immunomodulatory activity against lymphoma. Here, we report that the CD56dim population of NK cells isolated from healthy donors upregulate CD137 when exposed to HNSCC tumor cell lines in the presence of cetuximab but not control antibody. This increased expression of CD137 is coincident with a down-regulation of Fc\_ receptor CD16, indicating that ADCC activation results in CD137 upregulation. This observation is clinically relevant because we also observed that peripheral blood NK cells, examined from HNSCC patients post-infusion of cetuximab, demonstrate increased expression of CD137 relative to NK cells examined from the same patients pre-infusion. The functional significance of this CD137 expression is demonstrated by co-culture experiments, in which we added anti-CD137 antibody in combination with cetuximab to NK cells exposed to HNSCC cells. The addition of anti-CD137 antibody results in an increase in HNSCC cell apoptotic cell death, indicating that anti-CD137 antibody enhances ADCC mediated cytotoxicity by NK cells. Because anti-CD137 is currently in human clinical trials, these observations have the potential for rapid translation to the clinic.

#### **S016:** BIPHENYL ISOXAZOLIDINE, A NOVEL SMALL MOLECULE ESX MIMIC, INHIBITS CELL INVASION, MIGRATION, AND PROLIFERATION IN HEAD AND NECK SQUAMOUS CELL CARCINOMA - <u>Manchao Zhang, PhD</u>, Christopher E Taylor, BS, Theodoros N Teknos, MD, Anna K Mapp, PhD,

Christopher E Taylor, BS, Theodoros IN Teknos, MLD, Anna K Mapp, PhD, Quintin Pan, PhD; The Ohio State University Medical Center; Arthur G. James Cancer Hospital and Richard J. Solove Research Institute, The Ohio State University Comprehensive Cancer Center; University of Michigan

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer with an annual incidence of approximately 400,000 cases worldwide. Clinical management of HNSCC has evolved over the past several decades; however, the long-term survival of HNSSC patients has remained static. Elevated levels of epidermal growth factor receptor (EGFR) was reported in over 90% of HNSCC and associated with poor clinical response and prognosis. It is clear from numerous clinical trials that a majority of HNSCC patients with elevated EGFR levels do not respond to EGFR inhibitors. Inherent resistance to EGFR inhibitors may be due to nuclear EGFR-dependent actions and/or activation of other EGFR family members, ErbB2 and ErbB3. Epithelial specific Ets transcription factor (ESX) is well recognized to regulate ErbB2 expression. In silico analysis revealed multiple ESX binding sites in the promoter regions of EGFR and ErbB3. Thus, inhibition of ESX may be an attractive therapeutic strategy to target multiple EGFR members simultaneously resulting in an increase in efficacy of and prevention of resistance to EGFR inhibitors. We synthesized a small molecule, biphenyl isoxazolidine, designed to mimic ESX and thus serve as a competitive inhibitor for the binding interaction between ESX and transcriptional co-activators required for gene transcription. EGFR, ErbB2, and ErbB3 mRNA expression and protein levels were decreased following biphenyl isoxazolidine treatment in SCC25 and CAL27 HNSCC cells. Biphenyl isoxazolidine inhibited cell proliferation with an IC50 of 49 umol/L for SCC25 and 50 umol/L for CAL27. Cell migration and invasion was significantly blocked with a sub-threshold concentration (12.5 umol/L; no effect on cell proliferation) of biphenyl isoxazolidine; 86% and 73% inhibition for migration and 55% and 56% inhibition for invasion in SCC25 and CAL27, respectively. Moreover, biphenyl isoxazolidine enhanced the potency of gefitinib, an EGFR kinase inhibitor, by 6-fold and lapatinib, a dual EGFR/ErbB2 kinase inhibitor, by 2-fold in CAL27. Our results provide evidence that inhibition of ESX to reduce EGFR, ErbB2, and ErbB3 expression may be a novel approach to enhance the response rate of HNSCC patients to EGFR inhibitors.

# **S017:** DEVELOPMENT OF A STAT3 DECOY FOR SYSTEMIC ADMINISTRATION IN HEAD AND NECK CANCER - <u>Malabika</u>

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Signal Transducer and Activator of Transcription-3 (STAT3) is upregulated in numerous human cancers including head and neck squamous cell carcinoma (HNSCC). We developed a transcription factor decoy targeting STAT3 that exhibits antitumor efficacy in several preclinical cancer models and we recently completed pharmacodynamic testing in a phase 0 human trial with intratumoral inoculation in HNSCC patients. The clinical use of STAT3 decoy has been limited by rapid degradation in serum leading to a requirement for local administration. To enable systemic delivery, chemically modified STAT3 decoy such as tetra-nucleotide single stranded DNA sequences (DN4), 18-atom hexa-ethyleneglycol spacers (DS18) linking one end and 18-atom hexa-ethyleneglycol spacers linking the two ends (cyclic STAT3 decoy) of the parent STAT3 decoy were made and tested. Cyclic STAT3 decoy showed increased half-life of up to 12 h, DN4 up to 4 h and DS18 up to 31/2 h compared with the parent STAT3 decoy. The modified decoys demonstrated increased thermal stability compared to the parent STAT3 decoy, inhibited head and neck cancer cell proliferation, showed comparable uptake and bound to recombinant STAT3 protein as avidly as the parent decoy. DS18-modified STAT3 decoy demonstrated downmodulation of STAT3 target genes in HNSCC cell lines. Furthermore, a single intravenous injection of DS18 resulted in downmodulation of STAT3 target genes in HNSCC xenograft tumors suggesting that a chemically modified STAT3 decoy can be delivered systemically and lead to decreased STAT3 signaling in the tumor. These results suggest that chemical modifications of a transcription factor decoy oligonucleotide targeting STAT3 may lead to a STAT3-selective inhibitor that can be systemically administered for cancer therapy.

**S018:** SYNTHETIC HSP90 INHIBITOR, PF-04928473 AND PRO-DRUG PF-04929113, DEMONSTRATES POTENT ANTI-TUMOR ACTIVITY IN HEAD AND NECK CANCER BY INHIBITING MULTIPLE PROLIFERATIVE AND PRO-SURVIVAL SIGNALING PATHWAYS - Jay Friedman, PhD, <u>Stephanie</u> <u>Contag. BA</u>, Michael Hu, MD, Christian Freudlsperger, DMD, Vishnu Kannabiran, BS, Pattatheyil Arun, MD, James Mitchell, PhD, Jamie Skillings, MD, Julie Kan, PhD, Zhong Chen, MD, PhD, Carter Van Waes, MD, PhD; Tumor Biology Section, Head and Neck Surgery Branch, NIDCD and Radiation Biology Branch, NCI, National Institutes of Health, Bethesda, MD; Pfizer Inc, San Diego, CA

Purpose: To examine the biological activity of a small molecule inhibitor of Hsp90, PF-04928473, and the orally available pro-drug PF-04929113 in head and neck squamous cell carcinoma (HNSCC). Experimental Design: Hsp90 protein expression in human HNSCC tumor specimens was determined using a tissue array. Hsp90 mRNA and protein expression were compared between normal human keratinocytes (HEKA) and HNSCC cell lines (UM-SCC). The effect of PF-04928473 on cell proliferation was determined by MTT assay. Cell cycle and death were measured by flow cytometry. The effect on signaling molecules and cytokine production were determined using Western blot, reporter gene, and Luminex assays. Combination with radiation was examined using the clonogenic survival assay. The effect of PF-04929113 on tumor growth and Hsp90 client proteins were examined in a xenograft mouse model and by immunohistochemistry (IHC). Results: Hsp90 is overexpressed in human HNSCC specimens and cell lines when compared to controls. PF-04928473 inhibited UM-SCC cell growth, induced cell death and modulated cell cycle. Combining radiation and PF-04928473 increased radiosensitivity. PF-04928473 caused down-regulation of c-MET receptor, EGFR receptor, IKK\_, IKK\_, and BCLXL, as well as up-regulation of c-PARP, p53, PUMA, and p21 expression. The drug inhibited p-AKT, p-ERK, p-p65, and p-STAT3, decreased NF-\_B, AP-1, STAT3, BCLXL, and IL8 reporter activities, and blocked IL8, IL6, and VEGF production. PF-04929113 significantly inhibited tumor growth. Tumor specimens revealed that PF-04929113 induced p53, PUMA, and TUNEL, and decreased c-MET, SRC, p-STAT3, p-AKT, p-ERK, Ki67 and CD31 protein expression. Conclusion: PF-04928473 and PF-04929113 exhibit effective anti-tumor activity through modulation of proliferation, apoptosis, signaling pathways, and inflammatory and angiogeneic cytokine production.

#### **S019:** NOVEL ONCOLYTIC VIRUS THERAPY IN

**SQUAMOUS CELL CARCINOMA** - <u>Old Matthew, MD</u>, Jun-Ge Yu, MD, Theodoros Teknos, MD, Balveen Kaur, PhD; The Ohio State University

Background: Oncolytic herpes simplex viruses (HSV OVs) have the potential to make a significant impact in the targeted treatment of cancer patients, particularly in a field where the overall 5-year survival rate for oral cavity and oropharyngeal squamous cell carcinomas (SCC) is still less than 56%. Current OVs have been tested in vitro, in animal models, and in limited clinical trials. While the results in vitro and in animal models have shown good efficacy, results from clinical trials have been inconclusive with respect to OV effectiveness. Tumor angiogenesis has been implicated in the failure of OV therapy in clinical trials. In addition, physiologic levels of copper have been discovered to inhibit HSV OV activity. Hypothesis: The efficacy of HSV OVs in the treatment of SCC will be enhanced with the addition of Tetrathiomolybdate (TM), a copper chelator with antiangiogenic properties that is FDA approved for Wilson's disease. Methods: We tested HSV OVs coupled with TM in SCC cell lines with and without physiologic levels of copper. Results: We demonstrated the inhibitory effect of physiologic levels of copper in HSV OV therapy. We showed that TM rescues HSV OV activity in the presence of physiologic levels of copper and increases the efficacy of HSV OVs after pre-treatment with TM in head and neck SCC cell lines. Conclusion: TM is a powerful antiangiogenic and antineoplastic agent in SCC cell lines. TM improves the efficacy of HSV OV therapy in SCC cell lines models. Broader studies are needed to further establish the safety and improved efficacy of HSV OV therapy with the addition of TM prior to translating this novel therapy to phase one clinical trials.

#### **SO20A:** DETECTION OF HEAD AND NECK SQUAMOUS CELL CARCINOMA AND CERVICAL LYMPH NODE METASTASIS IN A MOUSE MODEL USING ACTIVATABLE NEAR-INFRARED FLUORESCENCE PROBES - <u>S Keereweer, MD</u>, JDF Kerrebijn, MD, PhD, IM Mol, PBAA Van Driel, EL Kaijzel, PhD, JSD Mieog, MD, AL Vahrmeijer, MD PhD, RJ Baatenburg de Jong, MD, PhD, CWGM Lowik, PhD; Erasmus Medical Center Rotterdam, Leiden University Medical Center

Introduction: Intraoperative assessment of tumor-free margins in head and neck cancer surgery is critical to completely remove the primary tumor and improve prognosis. Currently, the surgeon is confined to visual appearance and palpation of the tumor. Optical imaging has the potential to traverse the gap between radiology and surgery by providing real-time visualization of tumor tissue, warranting image-guided surgery. Recently, new probes have been designed that only become fluorescent after contact with targeted proteases. This is the first study that describes the use of activatable near-infrared (NIR) fluorescence probes for optical imaging of head and neck squamous cell carcinoma (HNSCC) and cervical lymph node metastasis. Methods: A HNSCC xenograft mouse model was developed using 6 x 104 luciferase-bearing OSC-19 human HNSCC cells that were injected into the tongue of 10 Balb/C nu/nu mice. Tumor progression was followed by bioluminescence imaging (BLI) and by visual inspection of the tongue. At day 21, mice were randomly allocated to administration of one of two activatable NIR fluorescence probes: ProSense680<sup>™</sup> (VisEn; activated by cathepsins) and MMPSense680<sup>™</sup> (VisEn; activated by matrix metalloproteinases). Fluorescence imaging (FLI) of the mouse and organs was performed. Two control groups, each containing 5 Balb/C nu/nu mice without tumor, were also injected with the activatable probes. Results: Seven days after injection of OSC-19 cells, tongue tumors had developed in all 10 mice. After 11 days, all mice had developed cervical lymph node metastasis (Figure 1A). The primary tumor and cervical lymph node metastases were successfully detected by the use

of both activatable NIRF probes (Figure 1B). The difference between the FLI signal intensity of the tumor versus the control mice was statistically significant for both probes. Conclusions: This preliminary study shows the establishment of a HNSCC mouse model, in which tumor growth and regional metastases could be assessed by BLI. Moreover, this is the first time that visualization of HNSCC and cervical lymph node metastases was achieved by FLI using activatable NIR fluorescence probes. The technique of image-guided surgery has potential to be translated into the clinic in order to improve the complete removal of HNSCC.

#### **S020B:** DETECTION OF MERKEL CELL VIRUS AND CORRELATION WITH MICROSCOPIC DISEASE IN THE SENTINEL LYMPH NODES OF PATIENTS WITH MERKEL CELL CARINOMA - <u>Myriam Loyo</u>, William H Westra, Juliana Schussel, Sewon Kang, Mariana Brait, Joseph A Califano, David Sidransky, Janis M Taube; The Johns Hopkins Medical Institutions

Introduction: Merkel Cell Carcinoma (MCC) patients without macroscopic nodal disease often undergo sentinel lymph node (SNL) biopsy, and approximately 30% subsequently demonstrate microscopic metastatic disease. The recurrence rate for patients with histopathologic evidence of nodal disease is approximately 3x higher when compared to node-negative patients. As survival can be dramatically improved with adjuvant nodal treatment, the accurate detection of nodal disease is critical. Recently, the Merkel Cell Virus (MCV) was discovered in association with MCC and is detectable in approximately 90% of cases. The purpose of this study was to determine whether MCV could be detected in the SLN of patients with MCC and to correlate this finding with the histopathologic nodal disease. Materials and Methods: Twenty-five cases of MCC with SLN procedure were identified in the surgical pathology files of The Johns Hopkins Hospital. For each case, following microdissection and DNA extraction, quantitative PCR was used to detect regions LT3 and LT1 of the MCV, and the viral load was calculated. Seventeen cases had matched primary tumors, which were also tested for MCV. Results: MCV was detected in 16/17 (94%) of the primary tumors, with an average viral load of 2.83 viral copies/ genome (range 2.53-3.09). MCC was present in 5 /25 (20%) SLNs by histopathology, and MCV was detected in 11/25 (44%) SLN by PCR. The average viral load in the SLN was 3.04 viral copies per genome (range 2.89-3.64). 16/25 (64%) SLN showed a correlation between the MCC histologic and MCV PCR results (n=3, both positive; n=13, both negative). 2/25 (8%) of the samples were positive by histopathology and PCR negative. Of note, 7/25 (28%) samples had detectable virus without demonstrable microscopic disease. Discussion/Conclusion: The survival of patients with histopathologically positive SLN can be improved by as much as 50% with adjuvant nodal treatment. In our study, we were able to detect patients who were SLN positive for MCV by PCR, even if negative by routine histopathology. The application of molecular techniques to detect sub-histologic disease in sentinel lymph nodes of patients with MCC may identify an additional subset of patients who would benefit from adjuvant nodal treatment.

#### **S020C:** IN VIVO DETECTION OF CANCER PRECURSOR

**DNA LESIONS** - Wilbur K Mills, Ilangovan Ramachandran, PhD, Antonio M Reis, MD, <u>Lurdes Queimado, MD, PhD</u>; Departments of Dermatology and Otorhinolaryngology, University of Oklahoma Health Sciences Center

Background: Tobacco smoking causes many types of cancer. Smoking, UV radiation, and other genotoxins cause DNA damage and induce unique cancer p53 "mutational fingerprints". However, a direct correlation between DNA damage and specific mutations is lacking. Recently, we have developed a novel primer-anchored PCR based damage-detection assay (PADDA) to detect in vivo DNA damage. To determine whether PADDA can identify in vivo nucleotide lesions that act as precursors to neoplastic mutations, we investigated the origin of two distinct types of mutations: (a) spontaneous mutations in the yeast CAN1 gene, and (b) the specific tumor signature mutation previously reported on codon 122 of the p53 tumor suppressor gene (Trp53) in UVB radiation induced mouse skin tumors. Methods: For UVB studies, the dorsal skin of wild-type (WT) and repair defective (Xpc-/- Trp53+/-) mice was shaved and exposed to a single dose of UVB radiation. PADDA was used to map base damage on the transcribed (TS) and non-transcribed strand (NTS) of the yeast CAN1 and the mouse Trp53 genes. The position of the identified nucleotide damage was compared with the published mutation distribution for each gene. Association between nucleotide damage detection and mutation was determined by Fisher's Exact Test. Results: Several hotspots for DNA damage were identified in CAN1. A comparison between identified DNA damage and literature reported CAN1 mutations revealed that persistent endogenous nucleotide damage co-localizes with spontaneous mutations. Twenty-four hours after UVBradiation, significant levels of unrepaired nucleotide lesions persisted in genomic DNA of Xpc-/- Trp53+/- compared with WT mice. Most of the damage affecting Trp53 codon 122 in Xpc-/- Trp53+/- mice was localized in the NTS and strictly localized to the two nucleotides of Trp53 codon 122 that were previously found mutated in 64% of all tumors arising in this genotype. Conclusions: We demonstrate for the first time a significant correlation between the persistence of precisely localized nucleotide lesions and the later establishment of mutations at those specific nucleotides. Given the mutagenic potential of those lesions, the application of PADDA for the identification of tobacco-induced DNA lesions might have major implications for early detection of cancer precursor lesions.

#### **S021:** USE OF PHOSPHODIESTERASE TYPE 5 INHIBITORS FOR IMMUNE THERAPY OF HEAD AND NECK SQUAMOUS CELL CARCINOMA - Donald Weed, MD, Felix Roth, PhD, <u>Bjorn.</u> <u>Herman, MD</u>, Zoukaa Sargi, MD, Carmen Gomez, MD, Paolo Serafini, PhD; University of Miami Miller School of Medicine

Objective: Immunotherapy is a compelling area of research for head and neck squamous cell carcinoma (HNSCC), but its efficacy is dramatically compromised by the immunosuppressive environment associated with this disease. Two major immunosuppressive cell populations, myeloid derived suppressor cells (MDSC) and regulatory T-cells (Treg), associated with HNSCC are inhibited by phosphodiesterase type 5 (PDE5) inhibitors. PDE5 inhibition enhances intra-tumoral T-cell infiltration and activation, reduces tumor outgrowth, and primes spontaneous anti-tumor response in murine models. In vitro T-cell proliferation occurs when sildenafil is added to anergic PBMCs from HNSCC patients. We are evaluating whether preoperative tadalafil (Cialis, a specific PDE5 inhibitor) administration can modulate anti-tumor immune response in HNSCC patients. Design: This is a randomized double blind placebo controlled study. Setting: Tertiary Care Medical Center. Patients: Patients with HNSCC of the oral cavity or oropharynx, whose initial treatment was definitive surgical resection. Intervention: Patients were treated with tadalafil (10mg or 20mg daily) or placebo for 20 days before surgery. Main Outcome Measures: Blood and tumor specimens were examined by FACS and immunofluorescence before and after tadalafil administration to delineate the degree of immunosuppression and the presence of an anti-tumor immune response. Results: Fourteen patients have enrolled in the trial with analysis of immunologic parameters available for eight. The study remains blinded and correlations between immunologic parameters and tadalafil treatment are not yet available. Approximately 15% of patients enrolled thus far have been in the placebo group, 42% in the 10mg/day and 42% in the 20mg/day dose groups. Significant

improvement of immunological parameters has been found in 37.5% of patients analyzed, a moderate improvement in 37.5%, and no improvement in 25%. An interim un-blinded analysis of the clinical trial correlating immunological parameters with treatment group will be presented. Conclusion: Although this is an ongoing clinical trial, the data collected seems to indicate tadalafil possesses immune-modulatory properties in HNSCC patients, reversing tolerogenic mechanisms and boosting antitumor immunity.

#### **S022:** CETUXIMAB PROMOTES DENDRITIC CELL MATURATION AND CROSS-PRIMING OF EGFR-SPECIFIC T CELLS IN HEAD AND NECK CANCER PATIENTS - <u>Steve</u> <u>C Lee, MD, PhD</u>, Pedro A Andrade Filho, MD, H. Carter Davidson, MD, PhD, Andres Lopez-Albaitero, MD, Raghvendra Srivastava, PhD, Hirak der-Terossian, MD, Varun Reddy, Sandra P Gibson, BS, William Gooding, BS, Soldano Ferrone, MD, PhD, Robert L Ferris, MD, PhD; University of Pittsburgh, Loma Linda University

Tumor antigen (TA)-specific monoclonal antibodies (mAb) are clinically effective for the treatment of some hematological malignancies and solid tumors. However, the clinical efficacy only occurs in a subset of cancer patients. These findings emphasize the need to characterize their mechanism(s) of action, since this information is essential to enhancing clinical responders and to selecting the patients most likely to benefit. To investigate this question, we studied the epidermal growth factor receptor (EGFR) specific mAb cetuximab, a clinically effective, FDA-approved mAb which is increasingly used in patients with colorectal (CRC) and head and neck squamous carcinoma (SCCHN). Using in vitro and in vivo xenograft models, cetuximab antitumor activity is strongly influenced by certain polymorphisms in Fc? receptors (Fc?R) expressed by lymphocytes. However, in CRC published results are conflicting, and we found no such predictive value for cetuximab-treated SCCHN patients. These findings have prompted us to investigate other mechanisms of action to explain the clinical activity in cancer patients. Here, we show that cetuximab improves dendritic cell (DC) mediated activation of cytotoxic T lymphocytes (CTL), termed cross-presentation, and characterize them mechanism and tumor antigen specificity of this effect in vitro and in cetuximab-treated patients. We demonstrate that cetuximab induces expression of DC maturation markers and enhances antigen presentation machinery (APM) components, such as TAP1/2, which are associated with optimal DC cross-presentation. This effect is mediated by the interaction of cetuximab with EGFR on the tumor cell and Fc?R IIIa on NK cells. DC matured with cetuximab activated NK cells also cross-primed both EGFR- and MAGE-3 specific CTL. These data are relevant in vivo, since cetuximab-treated, HLA-A\*0201+ SCCHN patients displayed significantly higher circulating EGFR853-861-specific T cells (p<0.005), compared to cetuximab-naïve SCCHN patients or healthy controls. We conclude that cetuximab administration leads to expansion of EGFR-specific T cells during oncologic therapy, suggesting a novel potential marker of clinical efficacy of therapeutic mAbs.

#### **S023:** INDUCIBLE NITRIC OXIDE SYNTHASE (INOS) CONTROLS STAT-3-MEDIATED TUMOR / HOST CROSS-TALK REQUIRED FOR ACCUMULATION AND CD8+ T-CELL SUPPRESSIVE ACTIVITY OF MYELOID-DERIVED SUPPRESSOR CELLS (MDSC) IN CUTANEOUS MELANOMA Falguni Parikh, MS, Padmini Jayaraman, PhD, Esther Lopez-Rivera, PhD, David Cannan, BS, <u>Andrew G Sikora, MD, PhD</u>; Mount Sinai School of Medicine, Department of Otolaryngology - Head and Neck Surgery

Cutaneous melanoma is among the cancers of fastest-increasing incidence in the U.S., and commonly presents on sun-exposed areas of the head and neck. While immunotherapy, such as anti-tumor vaccination, can

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provide sporadic benefit to patients with advanced melanoma, no reliablyeffective immunotherapeutic approach has yet emerged. One barrier to immunotherapy of solid tumors is tumor-mediated immunosuppression, including the induction of myeloid-derived suppressor cells (MDSC). MDSC are immature myeloid cells which infiltrate solid tumors, including cutaneous melanoma and squamous cell carcinoma, and potently inhibit anti-tumor T lymphocyte responses. The accumulation and suppressive activity of MDSC are driven by release of tumor-derived soluble factors, such as vascular endothelial growth factor (VEGF), and enforced expression of the transcription factor STAT3 by MDSC. Since our previous data suggest a role for inducible nitric oxide synthase (iNOS) in modulating the accumulation and intratumoral trafficking of MDSC, and iNOS is kown to promote VEGF release, we examined the effect of iNOS inhibition on tumor-MDSC cross-talk in the MT-RET murine melanoma model. Myeloid cells derived from bone-marrow of wild-type C57BL/6 mice accumulated significantly greater numbers of Gr-1+CD11b+ MDSC when co-cultured ex-vivo with MT-RET cells in a transwell system, or in the presence of MT-RET-cultured supernatants. The iNOS selective reversible competitive inhibitor N6-(1-iminoethyl)-L-lysine-dihydrochloride (L-NIL) reduced both the number and per-cell T cell suppressive activity of MDSC in co-culture or in the presence of MT-RET-conditioned supernatants. MT-RET tumors were determined by ELISA to secrete VEGF into the culture medium in vitro, and L-NIL markedly reduced VEGF concentrations in both MT-RET culture and MT-RET / myeloid cell co-culture. On day 3 of co-culture, levels of activated phospho-STAT3 in MDSC co-cultured with MT-RET cells were decreased by L-NIL treatment. Treatment of RET tumor bearing mice with L-NIL downregulated serum VEGF levels, reduced MDSC numbers in bone marrow, spleen and tumor, and abolished the ability of purified Gr1+Cd11b+ MDSC to suppress CD8+ T cell proliferation, thus confirming the relevance of these findings in vivo. These data suggest that iNOS controls tumor-myeloid cell crosstalk required for MDSC-mediated accumulation and function, and that targeted iNOS inhibition is a promising strategy for reversing MDSC-mediated immunosuppression.

#### **S025:** MONITORING THE RESPONSE OF PATIENT TUMOR-DERIVED HNSCC XENOGRAFTS TO ANTIVASCULAR THERAPY USING MRL. Jaimage M. Lach

ANTIVASCULAR THERAPY USING MRI - <u>Jaimee M Lockwood,</u> <u>BS</u>, Steve G Turowski, MS, Mukund Seshadri, DDS, PhD; Roswell Park Cancer Institute, Buffalo, NY

We have recently characterized the angiogenic profiles of human head and neck squamous cell carcinoma (HNSCC) xenografts established by transplantation of surgical tumor tissue using non-invasive magnetic resonance imaging (MRI). Here, we examined the vascular response of patient-tumor derived HNSCC xenografts to a tumor-vascular disrupting agent (tumor-VDA), 5,6-dimethylxanthenone-4-acetic acid (DMXAA) using two different contrast-enhanced MRI (CE-MRI) techniques. Studies were carried out using two patient tumor-derived HNSCC xenografts, SCC18243 and SCC17089, established in athymic nude mice. In the first study, early changes in vascularity of SCC18243 xenografts were examined at 2h post therapy (22 mg/kg DMXAA, i.p.) using dynamic contrast-enhanced MRI with the low molecular weight contrast agent, Gd-DTPA. Change in normalized T1 signal intensity post contrast was used to calculate tumor enhancement (E) as a measure of vascular function at both time points. In the second study, the relative blood volume (rBV) of SCC17089 xenografts was measured at baseline prior to VDA therapy and 24 h post therapy (25 mg/kg DMXAA, i.p) using T1-weighted CE MRI with a large molecular weight contrast agent, albumin-GdDTPA. Consistent with previous observations, a significant increase in E (p=0.001) was observed with Gd-DTPA enhanced MRI 2h post therapy suggestive of early changes in vascular permeability following treatment. Albumin-GdDTPA enhanced MRI revealed a significant reduction (p=0.001) in rBV 24h post VDA therapy compared to baseline pretreatment values. These results demonstrate the usefulness of contrast-enhanced MRI in monitoring antivascular therapy in vivo. Although the tumor-VDA DMXAA has been shown to exhibit antivascular activity in other tumor models, these results demonstrate, for the first time, potent vascular disruptive activity of DMXAA in patient tumor-derived HNSCC xenografts.

#### **\$026:** BFGF EXPRESSION CORRELATES WITH PMTOR/ EIF4E/P4EBP1 OVER EXPRESSION IN HEAD AND NECK **\$QUAMOUS CELL CARCINOMA SURGICAL MARGINS** Lilantha Herman-Ferdinandez, MD, DOHNS, <u>Cheryl Clark, PhD</u>, Tara Moore-Medlin, Olexandr Ekshyyan, PhD, Fleurette Abreo, MD, Gloria Caldito, PhD, Cherie-Ann Nathan, MD, PhD; Department of Otolaryngology and Head and Neck Surgery-LSUHSC Shreveport, LA

Objective: Angiogenesis facilitates tumor growth, invasion and metastasis while fulfilling nutritional requirements of the tumor. Basic fibroblast growth factor (bFGF) has been identified as a potent angiogenic factor regulated by MTOR and downstream components eIF4E and 4EBP1. Previous studies demonstrated MTOR inhibitors and antisense eIF4E downregulate bFGF. Correlating bFGF and pMTOR, eIE4E and p4EBP1 overexpression in HNSCC surgical margins would suggest MTOR pathway inhibition is a viable molecular target. Design: Data on 357 margins from 73 cancer patients were analyzed to determine association among pMTOR, p4EBP1, eIF4E and b-FGF. Each margin was given a score of 1 for overexpression of a biomarker and 0 otherwise. Chi-square or Fisher test was used to determine significant association between any two biomarkers. Conditional associations were determined between two biomarkers given the value of a biomarker (1 or 0). Results: Among the margins, 28% (103), 19% (71), 50% (175) and 46% (41) had overexpression of pMTOR, p4EBP1, eIF4E, and bFGF, respectively. Pair wise correlations among the biomarkers are highly significant (p<.01). The observed significant conditional pair wise correlations are: between p4EBP1 and bFGF when pMTOR=1(n=30) or 0(n=59, p=.02). The nonsignificant p-values for some of the conditional pair wise associations may be due to the relatively small available sample sizes used to calculate these associations. Conclusions: The results suggest a significant correlation between bFGF and pMTOR, p4EBP1 and eIF4E overexpression in HNSCC surgical margins. bFGF expression in HNSCC surgical margins makes it a prime target for molecular therapy and due to the strong correlation of expression of bFGF with the over expression of pMTOR, p4EBP1 and eIF4E, anti angiogenic MTOR inhibitors and antisense eIF4E could be tested as multi targeted molecular therapeutic agents. Clinical Significance of Study: If established anti-angiogenics decrease the levels bFGF in patient tissue and serum samples the change could be considered predictive of response to therapy. Support: This work was supported by NIH RO1-CA102363 to CO Nathan.

# **S027:** LOW DOSE OF CYCLOPHOSPHAMIDE ENHANCES ANTITUMOR EFFECTS OF THERAPEUTIC HPV DNA

**VACCINE** - <u>Sofia Lyford-Pike, MD</u>, Shiwen Peng, MD, PhD, Chien-Fu Hung, PhD, T.-C. Wu, MD, PhD, Sara I Pai, MD, PhD; 1Department of Otolaryngology-Head and Neck Surgery, 2Pathology, 3Oncology, 4Obstetrics and Gynecology, 5Molecular Microbiology and Immunology, Johns Hopkins Medical Institutions, Baltimore, Maryland

Abstract: Human papillomavirus (HPV) is etiologically associated with 20 to 25% of HNSCC, and 60-70% of those tumors which localize to the oropharynx. HPV DNA vaccines have been developed to enhance

specific T cell responses against the HPV viral oncoproteins, E6 and E7. However, we have found high frequencies of inhibitory regulatory T cells (T Regs) within HNSCC, which may inhibit the potency of vaccine induced anti-tumor effects. Low dose cyclophosphamide has been shown to have immuno-stimulating activity, partially through the decrease of T Regs in cancers. In the current study, we hypothesized that a combination of low dose cyclophosphamide can enhance antitumor effects of therapeutic HPV DNA vaccines in a HPV-16 pre-clinical model. Methods: HPV tumor bearing C57BL/6 mice were treated with 50 mg/kg cyclophosphamide intraperitoneally. One day later, the mice were vaccinated with pNGVL4a-CRT/HPV16 E7(detox) by electroporation. These mice were treated with the same regimen every 7 days for a total of 3 vaccinations. Tumor volumes were monitored twice a week and the survival was recorded. 7 days after last vaccination, mice were sacrificed and HPV-16 E7-specific CD8+ T cell responses were analyzed with IFN-\_ intracellular staining and flow cytometry. Regulatory T cells were analyzed by CD4, Foxp3 and CD25 staining. Results: We found that the combination of low dose cyclophosphamide and therapeutic HPV DNA vaccine generated an enhanced antitumor effect and significantly improved the survival of HPVtumor-bearing mice. Depletion of regulatory T cells by cyclophosphamide and preferential accumulation of E7-specific CD8+ T cells in the tumor contributed to this enhanced antitumor effect. Conclusion: Combination of low dose cyclophosphamide with CRT/E7 DNA vaccine enhanced antitumor effects compared to individual modality therapy. This data provides support for the combination of cyclophosphamide and therapeutic HPV DNA vaccines to treat HPV-positive HNSCC patients.

#### **S028:** HPV+ CANCER CELL IMMUNE RESPONSE DURING TREATMENT IS ATTENUATED BY ENHANCED LACTATE PRODUCTION - Cathy Zhuang, MD, PhD, Dan Vermeer, BS, <u>Yuh-Seog</u> <u>Jung, MD, PhD</u>, Allison Haugrud, BS, Hyun-Joo Ahn, MD, PhD, John H. Lee, MD, FACS, Keith Miskimins, PhD; Sanford Cancer Research Center, University of South Dakota, Sioux Falls, SD

Normal cellular metabolism is altered in cancer cells, shifting away from the TCA cycle towards glycolysis, increasing glucose consumption and lactate production. This key characteristic change in metabolism is termed the Warburg effect. Importantly, HPV 16 E7 oncoprotein alters the function pyruvate kinase type M2, increasing glucose consumption and lactate production. Clinically, increased lactate production in head and neck cancers is associated with a decreased response to therapy. Cancers with high lactate production have a poor five year survival, approximately 40% worse than similar tumors with low lactate production. Lactate has also recently been shown to disrupt functions of key immune cells (CD8 and DC's) in vitro. We have recently shown that an immune response is required to clear HPV+ head and neck squamous cell carcinomas (HNSCC) in vivo. In this project we test the hypothesis that lactate within the tumor microenvironment inhibits immune mediated clearance of HPV+ cancers. We show that human and mouse HPV+HNSCC's have enhanced lactate production. Inhibition of lactate with either Dichloroactetate (DCA) or oxamate decreases tumor cell growth in colony forming assays. DCA-mediated lactate inhibition in vivo was well tolerated, decreased tumor lactate levels, increased tumor pH and resulted in enhanced immune mediated clearance during treatment with cisplatin and radiation therapy. Furthermore, over-expression and shRNAmediated knock down of lactate dehydrogenase (LDH) confirmed the role of LDH and lactate production in this response. These findings suggest that altered metabolism not only helps a tumor grow away from a blood supply but inhibits immune clearance during therapy. In addition, the data suggest a role for lactate production in the initial development of tolerance to the virally infected cells.

**S029:** SECOND PRIMARY CANCERS IN PATIENTS WITH AN INDEX HEAD AND NECK CANCER: SUBSITE-SPECIFIC RISKS AND TRENDS OVER TIME - <u>Luc G Morris, MD</u>, Andrew G Sikora, MD, PhD, Richard B Hayes, DDS, MPH, PhD, Snehal G Patel, MD, MS, Ian Ganly, MD, PhD; Memorial Sloan-Kettering Cancer Center, Mount Sinai School of Medicine, New York University School of Medicine

Background: Patients with head and neck squamous cell carcinoma (HNSCC) are at significantly elevated risk of second primary malignancies (SPM), most commonly within the head and neck, lung and esophagus (HNLE). Our objectives were to identify additional sites in which SPM risk is meaningfully elevated, to identify subsite-specific differences in SPM risk and distribution, and to describe trends in risk over the past three decades. Methods: Population-based cohort study of 75,087 patients with HNSCC in the SEER program. Excess SPM risk was quantified using standardized incidence ratios, excess absolute risk, and number needed to follow. Trends in SPM risk were analyzed using joinpoint log-linear regression. Results: In HNSCC patients, the excess absolute risk (EAR) of second primary cancers was 167.7 per 10,000 person-years at risk. Over one year, 60 patients need to be followed to observe 1 additional SPM attributable to HNSCC. Lung cancers were the most common (EAR=75.2), followed by head & neck (EAR=59.8), then esophagus (EAR=14.2). The 4th most common SPM was colorectal cancer (EAR=4.3). Other SPM sites with lower levels of elevated risk included the bladder, cervix, stomach, pancreas and liver. The burden of SPMs was highest for hypopharyngeal SCC, and lowest for laryngeal SCC patients. The most common SPM site for oral cavity and oropharynx SCC patients was the head & neck. For laryngeal and hypopharyngeal SCC patients, the lung was the most common SPM site. Since 1975, SPM risk has been stable among patients with index oral cavity, laryngeal or hypopharyngeal cancers. In 1991, SPM risk among patients with oropharyngeal SCC began to fall substantially (annual percentage change= -4.6%, p=.03), and the oropharynx now carries the lowest SPM risk of any HNSCC subsite. Conclusions: HNSCC patients experience elevated risk of SPMs in the HNLE sites and several other tobacco and HPV-associated sites. The most common sites of elevated SPM risk are lung, head & neck, esophagus and colorectum. SPM risk levels and distribution differ by head and neck subsite. SPM risk has fallen dramatically for oropharyngeal cancer patients in the HPV era.

#### **\$030:** MATTED NODES ARE A POOR PROGNOSTIC FACTOR IN OROPHARYNGEAL SQUAMOUS CELL CARCINOMA INDEPENDENT OF HPV AND EGFR STATUS Matthew E Spector, MD, K Kelly Gallagher, MD, Mohannad Ibrahim, MD, Eric J Chanowski, BS, Emily Light, MPH, Jeffrey S Moyer, MD, Mark E Prince, MD, Gregory T Wolf, MD, Carol R Bradford, MD, Jonathan B McHugh, MD, Kitrina Cordell, DDS MS, Thomas Carey, PhD, Francis P Worden, MD, Avraham Eisbruch, MD, Douglas B Chepeha, MD, MSPH; University of Michigan

Objective: To determine if patients with matted nodes from oropharyngeal squamous cell carcinoma have a poor prognosis independent of HPV and EGFR status. Patients and Methods: 78 previously untreated patients, M:F 70:8, who underwent weekly carboplatin, taxol and concomitant intensity modulated radiation therapy for advanced stage (III, IV) oropharyngeal squamous cell carcinoma between 2003 and 2007. CT scans were reviewed regarding node characteristics. For the purpose of this study, matted nodes were defined as three continuous nodes with loss of intervening fat plane that is replaced with extracapsular spread. Marker status was determined by pathologic review from a tissue microarray in 73 of the 78 patients. The major variables under study were: nodal stage, matted node status,

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HPV status, EGFR intensity, tobacco exposure (never, ever, current), pattern of recurrence, and cause of death. Results: Overall survival for the entire cohort was 74.4% (58/78). The overall survival of patients who initially presented with matted nodes was 37.5% (6/16); this was poorer than the overall survival of patients with non-matted nodes which was 83.9% (52/62), p <0.0005. Matted nodes were a poor prognostic factor independent of HPV, EGFR, and smoking status. The most common cause of death in patients with matted nodes was lung metastasis in 8/10 patients. Of the patients who developed lung metastasis, 87.5% (7/8) did so within two years. Patients who did not have matted nodes were more likely to develop a local recurrence (50% [5/10]) and only one died from distant disease. 71.2% (52/73) of the cohort was HPV positive. Matted nodes were less likely to occur in HPV positive patients (15.4%, 8/52) than HPV negative patients (38.1%, 8/21). In the HPV positive group, 9/52 patients died of disease. 6 of these 9 patients died of distant metastasis and 5 of the 6 patients that died of distant metastasis had matted nodes. Conclusion: Matted nodes are a poor prognostic factor independent of HPV and EGFR status. In addition, matted nodes accounted for the majority of disease specific deaths in the HPV positive cohort, identifying a group of patients who develop distant metastasis that would benefit from systemic therapy.

#### **\$031:** HUMAN PAPILLOMAVIRUS MODULATES CANCER STEM CELL FUNCTION IN HEAD AND NECK SQUAMOUS CELL CARCINOMA - Manchao Zhang, PhD, Hongpeng Liu, BS,

Theodoros N Teknos, MD, <u>Quintin Pan, PhD</u>; Department of Otolaryngology-Head and Neck Surgery, The Ohio State University Medical Center; Arthur G. James Cancer Hospital and Richard J. Solove Research Institute, The Ohio State University Comprehensive Cancer Center

The pathogenesis of HNSCC is changing due to the recognition of HPV infection as a major risk factor. HPV-positive HNSCC is associated with improved clinical outcome compared to HPV-negative HNSCC; however, the mechanism of this observation remains to be elucidated. Cancer stem cells (CSCs) are a small sub-set of cancer cells within the tumor with the exclusive capacity to divide and expand the CSC pool or to differentiate into heterogeneous non-tumorigenic cells that constitute the bulk of the tumor. There is emerging evidence that CSCs are refractory to chemotherapy and radiation suggesting that CSCs may be responsible for disease relapse and progression. The role of HPV on CSC function is currently unknown. In this study, the effects of HPV on CSC number, self-renewal capacity, and cis-platinum sensitivity was examined. HPV-positive HNSCC cell lines (SCC2, UMSCC47, and SCC90) have a 93% increase (p<0.005) in the number of ALDH+ CSCs than HPV-negative HNSCC cell lines (SCC15, SCC25, and CAL27). Tumorsphere formation, an in vitro assay for selfrenewal, is enhanced (34%, p<0.05) in ALDH+/HPV-positive compared to ALDH+/HPV-negative HNSCC cells. Cis-platinum treatment of HPV-negative HNSCC cell lines resulted in a dramatic enrichment of ALDH+ CSCs; 300%, 130%, and 190% enrichment for SCC15, SCC25, and CAL27, respectively. In contrast, a reduction in ALDH+ CSCs was observed in HPV-positive HNSCC cell lines following cis-platinum; 30%, 39%, and 70% inhibition in SCC2, UMSCC47, and SCC90, respectively. Similarly, cis-platinum had limited effect on tumorsphere formation in HPV-negative HNSCC cell lines but completely abrogated tumorsphere formation in HPV-positive HNSCC cell lines. Our data indicate that CSC phenotype is modulated by HPV resulting in an unexpected increase in the number of CSCs, possibly through enhanced self-renewal, but importantly, elevated sensitivity to cis-platinum treatment. Ectopic expression of E6/E7 in human tonsillar epithelial cells (HTEC) resulted in a 330% increase in ALDH+ CSCs and enhanced tumorsphere formation compared to control-HTECs. These observations indicate that E6/E7 is sufficient to recapitulate the CSC phenotype observed in HPV-positive HNSCC. Taken together, our results demonstrate that HPV alters CSC function and provides an intriguing mechanism to explain the divergent clinical response between HPV-positive and HPV-negative HNSCC.

**\$032:** ORAL PREVALENCE AND CLEARANCE OF HIGH-RISK HUMAN PAPILLOMA VIRUS (HR-HPV) IN HEALTHY PEOPLE IN SAN PATRIGNANO, A REHABILITATION COMMUNITY FOR SUBSTANCE ABUSERS - D B Pugliese, MD, G Bruzzesi, C Montaldo, MD, M Landi, MD, A Mastinu, MD, L Porcu, MD, V Torri, MD, <u>L D Locati, MD</u>, L Licitra, MD; Centro Medico San Patrignano, University of Caglairi, Fondazione ANDI Onlus, Mario Negli Institute, Fond. IRCCS Itituto Nazionale Tumori

Background: Few data are available about the prevalence of HR-HPV oral infection in healthy people [4% for HPV16 was reported in a casecontrol study from a US series (D'Souza, NEJM 2007)]. Some factors seem to contribute to increase the risk of infection (e.g. poor oral care, marijuana and tobacco exposure, alcohol abuse). San Patrignano is a drug-free rehabilitation community designed to cure substance abusers. At entrance, a program of oral care is established, since May 2007, a strict smoke-free policy has been adopted. This community-based population is considered at "high-risk" for HPV due to their behavioral habits. Our aim was to measure the prevalence of HR-HPV oral infection in this selected population. Methods: From March 2007 to April 2008 all subjects who agreed to follow the oral care program were evaluated. A brushing of the base of the tongue, bilateral tonsils and glossotonsillar sulcus was performed at baseline, at 6 and at 12 months. All baseline samples were collected before the smoke-free policy was adopted while the other samples were collected after this date. HPV DNA analysis was carried out by semi-nested PCR while HPV genotype was analyzed by capillary sequencing method. The logistic regression model was used to determine if demographic, behavioural characteristics and immunological status were correlated to the prevalence of HR-HPV and to estimate the relative risk; a p-value less than 0.05 was considered statistically significant. Results. 194 (148 M/46 F) subjects were analyzed. 25 (13%) were HR-HPV positive (8 HPV31, 5 HPV16, 5 HPV18, 3 HPV26, 2 HPV33, 2 HPV35) at baseline, 1 sample was still positive at 6 months which turned out negative at 12 months. HR-HPV infection was not statistically associated to age, gender, HIV status, AIDS, HCV, number of years resident in the community, use of marijuana/hashish, number of years of drug consumption, tobacco and alcohol exposure, sex with drug addicts and condom use. Conclusions: In this "high-risk" population the baseline oral prevalence of HR-HPV was 13%. Intensive efforts towards lifestyle behaviour changes were made. After one year 100% clearance of HPV was observed. Supported by Fondazione ANDI (Associazione Nazionale Dentisti Italiani) onlus.

#### **S033:** HUMAN PAPILLOMAVIRUS (HPV)AND OROPHARYNX CANCER (OPC) IN THE TAX 324 TRIAL

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Background: HPV related OPC (HPVOPC) has a different biology than environmentally related OPC (EROPC). It is important to obtain and study long-term efficacy and outcomes of different types therapy from protocol driven trials in these two diseases as we design new treatments. Methods: Tumor tissues from patients (pts) with OPC treated on TAX324, a prospectively randomized, international, Phase III trial of Sequential Therapy (ST) for locally advanced head and neck cancer (HNC) were studied by PCR for HPV16 status. Five-year minimum follow up data for survival (OS), progression free survival (PFS), and sites of failure through December, 2008 were compared among HPVOPC and EROPC. Results: There are 264 evaluable pts with OPC; 111 (42%) had evaluable biopsies. Fifty-six (50%) were HPV+ (HPVOPC) and 55 (50%) were HPV- (EROPC). There were not enough evaluable pts to compare differences between treatment arms. Median follow-up period (range) for HPVOPC and EROPC pts was 83(77-93) and 82 (68-86) months, respectively. OS was significantly better for HPVOPC vs EROPC pts, 79% versus 31% (HR = 0.20, 0.10-0.38, p<.0001). PFS was also significantly better 73% vs 29% (p = <.0001). There were 7 (13%) local regional failures (LRF) and 3 (5%) distant metastases (DM) among HPVOPC pts compared to 23 (42%) and 6 (11%), respectively among EROPC (p < .006 for LRF). There were 5 (9%) and 12 (22%) deaths from unrelated causes among HPVOPC and EROPC, respectively (p = .07). Conclusions: The long term OS and PFS obtained with ST in HPVOPC are unprecedented and durable. The improvement in OS in HPVOPC is primarily due to better LRC. Contributions from reduced non-cancer deaths and DM play an additional role. These data support developing different therapeutic approaches for HPVOPC and EORPC. ST is very effective in HPVOPC. We can aim to reduce long-term morbidity in HPVOPC, by reducing radiotherapy dose after ST; and we might approach HPV- disease with more aggressive ST and CRT.

# **\$034:** HPV DETECTION IN HEAD AND NECK SQUAMOUS CELL CARCINOMA: A COMPARISON OF TESTING

**METHODS** - <u>Samantha J Davis, BS</u>, Emily Light, MS, Martin P Graham, BS, Heather M Walline, MS, Jay Stoerker, PhD, Mark E Prince, MD, Gregory T Wolf, MD, Douglas B Chepeha, MD, Jeffrey S Moyer, MD, Carol R Bradford, MD, Avraham Eisbruch, MD, Thomas E Carey, PhD, Jonathan B McHugh, MD; University of Michigan - Department of Otorhinolaryngology, Department of Pathology, & Cancer Center, Ann Arbor, MI; Sequenom, Ann Arbor, MI

Objective: Human papillomavirus (HPV) plays an etiologic role in a large proportion of head and neck squamous cell carcinomas (HNSCC). Currently, there is no consensus on how to test head and neck cancer patients for HPV. The imminent development of less aggressive treatments for HPV-positive HNSCC makes optimizing these techniques now more critical than ever. Design: Immunohistochemistry for p16 expression (p16) and in situ hybridization for HPV (ISH; Vendana) were performed on tissue microarrays. HPV status of tumor DNA was assessed by RT-PCR-mass spectroscopy (PCR; Sequenom). Performance characteristics among the three tests were evaluated. Subjects: 82 consented patients with HNSCC. Results: Two-thirds (50/82) of tumors were HPV-positive by PCR. Of these, 70% (35/50) were positive by all assays. Of the remaining 15 PCR-positive cases, 11 were ISH-negative/ p16-positive and 1 was ISH-positive/p16-negative. Three cases were ISHnegative/p16-negative; one had <1 viral copy/cell, and two contained HPV types not represented in the ISH assay. Two cases were ISH-positive/ p16-positive but PCR-negative. There was a significant difference in HPV detection by PCR vs. ISH (p=0.0027) and ISH vs. p16 (p=0.0002) but not PCR vs. p16. Using PCR results as the reference, p16 had a sensitivity of 92% and a specificity of 75%. The sensitivity and specificity of ISH were 72% and 94%, respectively. The positive predictive value of ISH was higher than that of p16 (95% vs. 85%), but the negative predictive value was lower (68% vs. 86%). Conclusions: Currently, most HPV testing is based on PCR technology. In this comparison, RT-PCR-MS detected HPV in 14 more cases than ISH but 4 fewer than p16. Discrepancies between RT-PCR-MS and ISH results may be partially accounted for by differences in the HPV types detected. IHC for p16 is a robust method of detecting active HPV infection, but p16 is occasionally upregulated due

to other tumor suppressor pathway aberrations. RT-PCR-MS determines HPV type and can detect HPV even when transcriptionally inactive and/or present in low quantities. However, ISH and p16 may be useful in confirming PCR results. At present, we use all three assays to most accurately assess HPV status in our head and neck cancer patients.

#### **\$035:** NOVEL MECHANISMS OF IGF-IR/EGFR CROSS-TALK THAT COUNTERACTS THE ANTITUMOR ACTION OF THE HUMAN ANTI-IGF-IR ANTIBODY IMC-A12 IN HEAD AND NECK CANCER - <u>Dong Hoon Shin, Postdoctoral Fellow</u>, Ho-Young Lee, Professor; The University of Texas MD Anderson Cancer Center

Background: The insulin-like growth factor-I receptor (IGF-IR) axis, which has been linked to cell proliferation, survival, angiogenesis, and invasion, is frequently deregulated in head and neck squamous cell carcinoma (HNSCC) and thus is emerging as a promising target for therapy for the disease. However, the mechanisms mediating resistance to the IGF-IR targeting agents are poorly understood. Methods: The effects of IMC-A12 on viability/proliferation and apoptosis of a panel of HNSCC cells cultured in normal tissue culture plates or poly(HEMA)coated plates were examined by 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT), 3-(4,5-dimethylthiazol-2-yl)-5(3carboxymethonyphenol)-2-(4-sulfophenyl-2H-tetrazolium (MTS), and western blot analyses. The effects of IMC-A12 on anchorage-independent cell proliferation were analyzed by soft agar assay. The antitumor effects of IMC-A12, either alone or in combination with inhibitors targeting EGFR, were evaluated using xenograft or orthotopic tongue tumor models of representative IMC-A12-sensitive and -resistant HNSCC cells. The transcriptional, translational, and posttranslational regulations of proteins involved in IMC-A12-mediated EGFR activation were measured by real-time polymerase chain reaction, western blotting, and metabolic labeling with 35S-methionine, immunohistochemical analysis. Results: We found that: (a) treatment with IMC-A12 led to activation of EGFR and Akt and (b) activated EGFR/Akt pathway induced mTOR-mediated increases in EGFR and Akt1 protein expressions in HNSCC cells. Cotargeting EGFR abolished resistance to IMC-A12 and synergistically induced apoptosis in HNSCC cells in vitro and in vivo. Most HNSCC tissues with EGFR overexpression had associated high levels of IGF-IR expression. Conclusions: Our data suggest that IMC-A12-induced sequential activation of EGFR, and PI3K/Akt/mTOR followed by enhanced synthesis of EGFR and Akt proteins counteract the antitumor action of the anti-IGF-IR humanized monoclonal antibody, IMC-A12 in HNSCC. Our results indicate the need for integration of molecularly targeted agents blocking EGFR into treatment regimens with IGF-IR Ab for patients with HNSCC. Supported by the National Institutes of Health Grants R01 CA109520 (to H-Y. Lee), CA100816 (to H-Y. Lee) and 5P50CA097007 (to S. M. Lippman)

#### **\$036:** EVALUATION OF EGFR GENE AMPLIFICATION STATUS, MRNA, PROTEIN, AND PHOSPHOPROTEIN LEVELS EXPRESSION IN HEAD AND NECK CANCER PATIENT TISSUES - <u>S E Wheeler</u>, R Seethala, MD, D Siwak, PhD, K Cieply, C Sherer, G Mills, MD, PhD, J R Grandis, MD, AM Egloff, PhD; University of Pittsburgh, Department of Otolaryngology; University of Pittsburgh Medical Center, Department of Pathology; University of Texas M.D. Anderson Cancer Center, Department of Systems Biology

Background: EGFR has been reported to be overexpressed in Head and Neck Squamous Cell Carcinoma (HNSCC), and high levels of EGFR protein and EGFR gene amplification have been independently reported to be associated with poor prognosis. To our knowledge, tumor levels of EGFR site-specific phosphorylation (Y992, Y1068) have not
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been reported as prognostic indicators for HNSCC. Methods: Tumor cores from 58 HNSCC surgical patients were arrayed in triplicate on two tissue microarrays and evaluated for EGFR gene amplification by dual-color FISH and for EGFR protein level by immunohistochemical (IHC) staining. EGFR protein and phosphoprotein (EGFR P-Y1068 and P-Y992) tumor levels were measured using reverse-phase protein array (RPPA) and EGFR mRNA quantified by RT-PCR in corresponding fresh-frozen tumor specimens. Results: EGFR gene amplification was present in 23% of tumors, and these tumors expressed significantly higher levels of EGFR protein by IHC than tumors without EGFR gene amplification (P<0.0001). EGFR protein was present at elevated levels in 36% of tumors without EGFR gene amplification. Patients with tumor EGFR gene amplification tended to have reduced progression-free survival (PFS) (P=0.12). EGFR protein and EGFR P-Y992 levels measured by RPPA were not associated with PFS. High tumor levels of EGFR P-Y1068 were significantly associated with reduced PFS (P=0.001). Conclusions: Similar to previous findings, we find that patients with tumor EGFR gene amplification tend to have reduced PFS. Tumors with EGFR gene amplification have significantly higher EGFR protein levels as assessed by IHC. Increased EGFR protein levels in tumors are not always associated with gene amplification; this may be an important mechanism for increased EGFR expression. Site-specific EGFR phosphorylation at Y992 and Y1068 were differentially associated with PFS, indicating that signaling pathways associated with these sites may play different roles in disease progression.

#### **\$037:** AUTOCRINE EGFR LIGAND PRODUCTION DETERMINES THE RESPONSE TO CETUXIMAB IN HEAD AND NECK SQUAMOUS CELL CARCINOMA CELL LINES Lars Ekblad, PhD, Goro Oshima, MD, Elisabeth Kjellén, MD, PhD, Hiroyuki Mineta, MD, PhD, Anders Johnsson, MD, PhD, Johan Wennerberg, MD,

PhD; Lund University, Hamamatsu University School of Medicine

Cetuximab is a chimeric mouse-human monoclonal antibody which inhibits EGFR signaling. Though interfering with a signaling pathway essential for carcinogenesis and tumor cell survival, cetuximab has not provided clinically impressive improvements of treatment efficacy in studies on head and neck squamous cell carcinoma (HNSCC). To improve the treatment efficacy of cetuximab there is a need for good predictive strategies. In this study we compared the production of all seven EGFR ligands, in eight HNSCC cell lines, with the response to cetuximab in vitro. The cells were treated with saturating concentrations of cetuximab and 48 hrs later the concentrations of TGF-alpha, EGF, HB-EGF, amphiregulin and betacellulin were determined in the growth medium. The expression of epiregulin and epigen mRNA was measured by qRT-PCR. The growth inhibiting effect of cetuximab was measured with the SRB assay and Emax and EC50 values were calculated. Four different ligands were found to be expressed by the cell lines: TGF-alpha, amphiregulin, epiregulin and epigen. The most frequently produced ligand was TGF-alpha and there was an overall correlation between the autocrine activity of TGF-alpha and the effect (Emax) of cetuximab. However, one cell line, the only one expressing epigen, did not have any detectable autocrine activity of TGF-alpha but was effectively inhibited by cetuximab. We also found a 1000-fold difference in the sensitivity (EC50) to cetuximab. This could be explained by the amount and species of the produced EGFR ligands. In conclusion, our work shows a relationship between the degree of autocrine EGFR ligand production and the effect of cetuximab, implicating that these ligands might predict the treatment response in HNSCC patients. The main correlation was between TGF-alpha production and treatment effect, but also other ligands were important and it might therefore be necessary to measure several or all

EGFR ligands. The results also suggested that the optimal therapeutic dose might be determined by the autocrine amounts of the different ligands produced by the tumor cells. The research was supported by the Swedish Cancer Society and Stiftelsen Laryngfonden.

#### **S038:** PREDICTIVE BIOMARKERS FOR COMBINED CHEMOTHERAPY WITH 5-FLUOROURACIL AND CISPLATIN IN ORO- AND HYPOPHARYNGEAL CANCERS -<u>Y Hasegawa, MD, PhD</u>, M Goto, DDS, PhD, N Hanai, MD, T Ozawa, MD, H Hirakawa, MD; Aichi Cancer Center

Background: In the present study, we planned to analyze chemosensitivity in more detail at the gene level. We investigated the relation between the clinical efficacy of combination chemotherapy and gene expression. Methods: Patients and tissue samples: Sixty-four tumor specimens from patients undergoing radical treatment for squamous cell carcinomas of the oro- and hypopharynx in Stage II, III or IV, were included in the study. Informed consent in writing for the experimental use of tissue samples was obtained from all patients. Treatment Schedule: All patients were administered induction chemotherapy with a combination of 5-FU and cisplatin before definitive therapy. This chemotherapy was used in order to select patients for organ preservation based on the response and decrease in late salvage surgery rate. After induction chemotheray, responders received definitive radiotherapy or concurrent chemoradiotherapy. Real-Time RT-PCR quantification: Using biopsy specimens, we analyzed their gene expression profiles with the following 26 markers, which we thought were likely predictors of the response to anti-cancer agents: TS, DPD, OPRT, TP, COX2, MDR1, MRP1, VEGF, EGFR, HER2, PIK3CA, PTEN, p53, Rb1, Bcl2, BclX, BAX, GSTπ, ERCC1, XPA, E2F1, ENT1, Rev3, B-tubulin, Survivin and p16. The mRNA levels of each gene were measured by real-time RT-PCR based on TaqMan chemistry and quantitated. The amount of each mRNA in the tissue, standardized to the GAPDH mRNA, was expressed as follows:  $\Delta CT = [CT \text{ target}]$ - CT GAPDH ]. Results: Clinical T factor was a statistically significant variable in uni- and multivariate analysis. Moreover, responses of primary lesions for chemotherapy were significantly correlated with the mRNA expression level of MDR1, VEGF, ERCC1, XPA, Survivin and p53 in a univariate logistic regression analysis. Meanwhile, using a multivariate analysis with these factors, the expression of XPA and Survivin were demonstrated to be independent predictors for chemotherapy. Conclusions: The nucleotide excision repair pathway (NER) is the major pathway for repair of DNA damage. mRNA expressions of XPA and ERCC1 related to NER were associated with response to chemotherapy. Genes belonging to NER could be attractive markers for therapy in head and neck squamous cell carcinomas.

**\$039:** MECHANISMS OF ACQUIRED RESISTANCE TO ANTI-ANGIOGENIC THERAPY IN PRECLINICAL MODELS OF HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC) - <u>Rekha Gyanchandani, MS</u>, Seungwon Kim, MD, Jennifer R Grandis, MD; Department of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; Department of Otolaryngology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

Purpose: The Vascular Endothelial Growth Factor (VEGF) is ubiquitously expressed and targeted with FDA-approved agent bevacizumab in many types of cancer including breast, colon, lung and renal carcinoma. However, its efficacy in most cases is limited, with emergence of drug resistance within a few months of therapy. In head and neck cancers, anti-angiogenic drugs such as bevacizumab are still under clinical investigation. Most of the patients in these clinical trials show stable disease initially, but experience tumor re-growth. These incomplete drug responses suggest the presence of alternative signaling mechanisms that emerge in the setting of VEGF blockade. Due to lack of adequate models for studying drug resistance, very little is known about these compensatory mechanisms that counter the suppression of angiogenesis. Experimental Design: In this study, we established HNSCC xenograft models of acquired resistance to long-term bevacizumab treatment and evaluated the angiogenic profile of HNSCC cells from the isogenic pair of bevacizumab-sensitive and resistant tumors. Results: In response to bevacizumab, the sensitive tumors showed 79% growth inhibition compared to vehicle treated controls. In contrast, the resistant tumors from the isogenic pair showed 22% tumor inhibition. HNSCC cells from xenografts that acquire resistance to bevacizumab show up-regulation of pro-angiogenic placental growth factor (PlGF) and down-regulation of anti-angiogenic proteins (such as TIMP-1, TIMP-2, IP-10, IL-12, IFN-g). Notably, there is a marked reduction of IL-8 production by HNSCC cells from resistant tumors and lowering of VEGF levels. Conclusions: Our results indicate that long-term anti-VEGF therapy resulted in HNSCC xenografts that are less responsive to bevacizumab treatment compared to their isogenic parental counterparts. Also, the resistant tumors acquire an altered cytokine profile, which might contribute to anti-VEGF resistance. These findings will be important for designing novel combinatorial approaches to overcome this resistance phenotype.

#### **\$040:** CISPLATIN ENHANCES AN IMMUNE MEDIATED CLEARANCE OF HPV POSITIVE HEAD AND NECK CANCER <u>William C Spanos, MD</u>, Denise Schwabauer, MS, Daniel Vermeer, BS, John H Lee, MD; Sanford Research/USD, Sioux Falls, SD

Human papilloma virus (HPV) cancers have improved survival following therapy compared to their HPV negative counterparts. Our preliminary data suggests that treatment with cisplatin and radiation induces an immune response that aids in clearing HPV positive cancers. We have examined cellular signaling present on head and neck epithelial cells that may aid in the immune response. CD40 receptor, a membrane glycoprotein, is found on dendritic cells as well as epithelial cells lining the oropharynx and nasopharynx. The interaction of CD40 receptor to CD40 ligand (CD40L) is important in driving the immune system to a T cell response. An activating CD40 antibody (CD40L) has been used in the treatment of solid tumors (melanoma and colorectal). Using a mouse model of HPV positive head and neck cancer we have investigated the role of CD40 in head and neck cancer. Mouse tonsil epithelial cells (MTECs) transformed with HPV16 E6E7 and H-Ras (oncogenes) as well as human HPV positive and negative head and neck squamous cell cancer (HNSCC) cell lines had similar growth and clonogenic survival when treated with the activating antibody CD40L compared to IgG control. This suggests that receptor activation on the epithelial cells does not induce cell death. HPV positive tumors were implanted into CD40 knockout, RAG-1 and wild-type mice. Interestingly CD40 knockout mice grew with similar velocity to immunodeficient RAG-1 mice (no B and T cells) with 0% survival despite treatment with cisplatin and radiation, while 80% of wild-type mice cleared their tumors. CD40 receptor activation as an adjuvant to cisplatin/radiation treatment of HPV positive tumors in immune competent C57BL mice improved survival by 20% compared to IgG cisplatin/radiation control. Complete clearance with tumor free survival occurred in 10% of mice treated with CD40 ligand alone. Cisplatin treatment increases total CD40L in vitro in both mouse and human cell lines suggesting a possible mechanism of action. CD40 receptor is a important component of the immune mediated clearance of HPV positive HNSCC in mice. Augmentation of the immune response using CD40 ligand may allow for improved treatment of HPV and other tumor virus related cancers.

# **S042:** TARGETING HER2 IN THE TREATMENT OF SALIVARY DUCTAL CARCINOMA - Matthew Pierce, BS, BA,

Merrill Kies, MD, Randal Weber, MD, FACS, Michael Kupferman, MD; The University of Texas MD Anderson Cancer Center

Background: Salivary Ductal Carcinoma (SDC) is a high grade malignancy of the major salivary glands, with a clinical course characterized by loco-regional recurrence, distant metastasis and a high mortality rate. Due to its histological and molecular resemblance to intraductal carcinoma of the breast, molecular therapies that target HER2 have been proposed. In this study, we reviewed our experience of trastuzumab therapy, a monoclonal antibody selective for HER2, for patients with HER2+ SDC. Methods: All patients treated at MDACC with trastuzumab for SDC from 1997-2007 were reviewed. Statistical analysis was used to investigate relationships between clinical\pathologic characteristics and treatment response. Results: Sixteen patients were identified, with a male predominance (15) and an average age of 59 years. The majority of tumors arose in the parotid gland (94%). Histologically, 13 (81%) had high grade tumors, and 13 had perineural invasion. Twelve (75%) had stage IV on original presentation. HER2/neu positivity was seen in all tested tumors (15/16). Four (25%) were treated with trastuzumab for primary disease whereas 13 (81%) were treated for recurrent disease. On average, trastuzumab therapy was initiated 24 months after completion of treatment for the primary disease, with an average treatment duration of 11 months. Average time from initiation of trastuzumab therapy to death was 23 months and 13 patients (81%) received combination chemotherapy along with trastuzumab. Seven (44%) of the 16 patients showed stabilization of disease after an average of 21 months of treatment, and at 1 & 2 years, 9 (56%) and 5 (31%) were alive with disease, respectively. Conclusions: Outcomes of patients with SDC are poor, with most succumbing to their disease within 3 years of diagnosis. This retrospective study suggests that the addition of trastuzumab, as either monotherapy or in combination with cytotoxic agents, can lead to disease stabilization in a significant percentage of patients. Further studies are necessary to identify HER2 positive patients who would most benefit from this treatment strategy.

### **S043:** UNDERSTANDING RESISTANCE: EGFR AMPLIFICATION, HRAS AND PIK3CA HOTSPOT MUTATIONS IN HEAD AND NECK SQUAMOUS CELL CARCINOMA - <u>M. De Herdt, Msc</u>, R. Baatenburg de Jong, Prof, PhD,

MD, E. Zwarthoff, Prof, PhD, E. Berns, PhD; ErasmusMC, Rotterdam, The Netherlands

Introduction: Head and neck cancer is a heterogeneous disease with a poor prognosis. For biomarker based and patient tailored treatment relevant molecular targets need to be identified. Objective: To study the prevalence of EGFR amplification and hotspot mutations in BRAF, HRAS, KRAS, NRAS, PIK3CA and FGFR3 in retrospectively collected head and neck squamous cell carcinomas (HNSCCs), relate the outcome with prognosis and identify putative markers for targeted therapies. Patients & Methods: EGFR amplification and hotspot mutations in BRAF, HRAS, KRAS, NRAS, PIK3CA and FGFR3 were evaluated in 58 primary HNSCCs of different localizations, by means of Southern blot analysis (1) and specifically designed multiplex mutation assays (2,3) respectively. The results obtained were correlated with patient and tumor characteristics. Results: The EGFR pathway was altered in 10/58 (17%) HNSCCs. The EGFR gene was amplified in 3/58 (5%) HNSCCs. PIK3CA mutation E545K was identified in 5/58 HNSCCs (9%). Two HRAS codon 13 mutations (G13R and G13V) were found in 2/58 HNSCC (4%). One tumor (2%) had mutations in both HRAS (Q61L) and PIK3CA (E542K). All other alterations were mutually exclusive. Eight out of 11 alterations were found in patients with tumors of the oral cavity (p = 0.033). Patients with hotspot mutations tend to have a poorer prognosis, although the numbers are too small for firm conclusions. Conclusion: Based on our data and in silico data analysis we conclude that although uncommon, HRAS and PIK3CA mutations may aid in the stratification of patients predicted to be insensitive to treatment with EGFR inhibitors, since both RAS and PIK3CA signaling cascades are downstream of this receptor tyrosine kinase. Nevertheless, patients with PIK3CA positive tumors might benefit from treatment with PIK3CA inhibitors. Prospective studies are needed to confirm our results. 1. L. Speleman et al., Head Neck 29, 341 (2007). 2. J.M. van Oers et al., Clin Cancer Res 11, 7743 (2005). 3.I. Lurkin et al., PLoS One 5, e8802.

# **S044:** PHASE II STUDY OF ERLOTINIB IN COMBINATION WITH DOCETAXEL AND RADIATION IN LOCALLY ADVANCED SQUAMOUS CELL CANCER OF THE HEAD

AND NECK (SCCHN) - <u>P Savvides</u>, M Yao, R Rezaee, J Bokar, P Fu, J Wasman, N Sarlis, A Dowlati, M Machtay, P Lavertu; CASE Comprehensive Cancer Center, University Hospital of Cleveland Medical Center, Cleveland, OH Sanofi-Aventis US, Inc Bridgewater, NJ

Background: EGFR is highly expressed in SCCHN, representing an established therapeutic target. Erlotinib (E) is an EGFR tyrosine kinase inhibitor that may potentiate the efficacy of concurrent radiation (RT) and docetaxel (D). Maximum tolerated dose (MTD) of the combination was established in the phase I evaluation of the combination (Savvides P et al; AHNS 2008: S048). This phase II trial represents the attempt to establish the efficacy and toxicities of the combination in patients (pts) with locally advanced SCCHN. Methods: Pts with previously untreated stage III-IVb SCCHN receive once-daily RT (70.2Gy, 1.8Gy/day) or IMRT, weekly D (20 mg/m2/week for the duration of RT) and daily E (150 mg) for two weeks prior to, during concurrent chemoradiation (CRT) and for up to two years following CRT. A total of 48 pts will enroll. Correlative studies include detection of HPV in tumor biopsies, E pharmacokinetics and E and D pharmacogenetics. Results: Twenty pts (15 males), mean age 59 years (range 36-75), with stage III (n= 2) or IV (n=18) have enrolled. Primary site: pharynx (n=13), larynx (n=5) and oral cavity (n=2). 14 pts have completed CRT and are evaluable for response. 1 pt not evaluable after denying RT completion (total of 61.2 Gy to the primary site, remaining without evidence of disease 30 months after enrollment); 2 completed CRT but not restaged yet and 3 are still undergoing CRT. After a median followup of 13 months (range: 1 - 30), 2 pts died after recurrent disease and 12 pts remain in complete response (CR) (9 after CRT, 1 patient underwent neck dissection for residual disease and 2 patients underwent salvage surgery of the primary site and neck dissection). Four pts, in CR, are currently receiving adjuvant erlotinib. In the remaining 5 pts, adjuvant E was discontinued based on pt decision (n=4) or because of multiple surgeries for vascular and cardiac valve disease (n=1). Among those evaluable for response, the complete response rate is 86% with 95% confidence interval (0.6, 0.96). Conclusions: For patients with locally advanced SCCHN, preliminary data suggest that the addition of E to concurrent RT with D is feasible, safe and active. Supported in part by Genentech, Sanofi-Aventis, NIH grants P30 CA43703 and M01 RR-000080. Clinicaltrials.gov identifier: NCT00720304.

# **S045:** EFFECT OF RADIOTHERAPY AND CHEMOTHERAPY ON THE RISK OF MUCOSITIS DURING IMRT FOR

**OROPHARYNGEAL CANCER** - <u>Giuseppe Sanguineti</u>, MariaPia Sormani, PhD, G. Brandon Gunn, MD, Francesco Ricchetti, MD, Arlene Forastiere, MD; Johns Hopkins University, University of Texas Medical Branch, University of Genoa

Purpose: To define the roles of radiotherapy and chemotherapy on the risk of grade 3+ mucositis during IMRT for oropharyngeal cancer. Materials and Methods: 140 consecutive patients treated with IMRT for oropharyngeal cancer at two Institutions in non-overlapping treatment era were selected for the present analysis. All patients were treated with a dose painting approach, 3 dose levels and comprehensive bilateral neck treatment under the supervision of the same RadOnc (GS). 71 pts received platinum-based concomitant chemotherapy (conc CHT). Patients were seen weekly during treatment and mucositis was scored prospectively with blinding to dosimetric data. Peak acute toxicity > confluent mucositis during the course of IMRT was considered the endpoint. A number of clinical and dosimetric factors, including the DVH of the oral mucosa (OM), were considered. For each patient, based on the actual elapsed treatment time, the OM-DVH was rebinned to calculate the DVH per week (wDVH). Uniand multivariate logistic regression were run to identify predictors of grade 3+ mucositis during IMRT. Results: Overall 112 patients (80%) developed the endpoint. The region that best discriminated between patients with/without the mucositis defined endpoint was found at 10.1 Gy/w (V10.1) and 21cc (D21), along the x- and y-axis of the absolute cumulative OM-wDVH, respectively. On multivariate analysis D21 (OR=1.017, 95%CI: 1.009-1.024, p<0.001) was the only independent predictor of grade 3+ mucositis. However, V10.1 and D21 were highly correlated (rho=0.885, p<0.001). Conc CHT showed a trend (OR: 3.095, 95%CI: 0.903-6.698, p=0.079), that became statistically significant when the two patients who received only 1 cycle of CHT were pooled with the patients who did not receive CHT (OR=2.830, 95%CI: 1.032-7.757, p=0.043). No interaction was detected between dosimetric factors and conc CHT. From the model it can be estimated that conc CHT increased the dose to 21 cc of OM by  $\approx$  61.2 cGy per week. Conclusions: Radiotherapy and chemotherapy act independently in determining acute mucosal toxicity; conc CHT increases the risk of mucosal grade 3+ tox  $\approx$ 3 times over RT alone and it is equivalent to an extra ≈4.3 Gy to 21 cc of OM over a 7-week course.

**\$046:** POLYMORPHISMS IN ERBB FAMILY GROWTH FACTOR RECEPTORS AND LIGANDS AND RISK OF HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC) - <u>Ann</u> <u>Marie Egloff, PhD MPH</u>, Brenda Diergaarde, PhD, Marjorie Romkes, PhD, Joel Weissfeld, MD MPH, Jennifer Grandis, MD, Christopher Fung, BS; Departments of Otolaryngology and Epidemiology and Center for Clinical Pharmacology, University of Pittsburgh, Pittsburgh, Pennsylvania

Background: The epidermal growth factor receptor (EGFR), a member of the ErbB family of growth factors receptors, is overexpressed in the majority of HNSCC and has been implicated in HNSCC development and progression. EGFR forms homodimers or heterodimers with other ErbB family receptors when activated. The EGFR intron 1 CA repeat polymorphism has been previously reported to be associated with risk of HNSCC. We hypothesized that functionally relevant polymorphisms in ErbB family members and/or ligands would modulate risk for HNSCC and conducted a large case-control study to test this hypothesis. Methods: Our study population consisted of 581 Caucasian HNSCC patients and 603 cancer-free Caucasian control subjects enrolled in the University of Pittsburgh Head and Neck SPORE Project 1. Controls were frequency-matched to cases by age (10-year categories) and sex. Putative functional SNPs in EGFR, ERBB2, ERBB3, ERBB4, AREG, TGFA and HBEGF with minor allele frequencies  $\geq 10\%$ were selected based upon published literature and public databases. In addition, tag SNPs were selected for EGFR (pair-wise correlation r2>0.70). Genotyping was conducted using Sequenom's MassARRAY system. Deviation from Hardy-Weinberg equilibrium was evaluated using chi-square tests. Associations between alleles and genotypes and HNSCC risk were examined using a Pearson Chi-square test and a Cochran-Armitage trend test, respectively. Per (minor) allele odds ratios and corresponding 95% confidence intervals were also calculated for each SNP. Results: Four SNPs in EGFR (rs12535536, rs1253871, rs6593206 and rs6970262) and two in ERBB4 (rs7589006 and rs13025469) were statistically significantly associated (P<0.05) with HNSCC risk. The minor alleles of rs12535536 and rs1253871 in EGFR and rs7589006 and rs13025469 in ERBB4 were associated with an increased risk of developing HNSCC; minor alleles of the other two significant EGFR SNPs were associated with a decreased risk. Conclusions: These results suggest that common variants in EGFR and ERBB4 may be associated with risk of HNSCC.

#### **\$047:** OROPHARYNGEAL SQUAMOUS CELL CARCINOMA AND HPV-ASSOCIATED CANCERS IN WOMEN: AN EPIDEMIOLOGICAL EVALUATION OF ASSOCIATION - Hadi Seikaly, MD, FRCSC, <u>Vincent L Biron, MD, PhD</u>, David W Cote, MD, MPH, CCFP, Jeffrey Harris, MD, FRCSC; University of Alberta

Objective: Human papilloma virus (HPV) is a well-established etiological factor in oropharyngeal squamous cell carcinoma (OPSCC) and malignencies of the female genital tract. To date there are no studies examining the epidimiological association between the two disease processes. The objective of this study is to evaluate the association between OPSCC and HPV-related precancerous or cancerous lesions of the female genital tract. Design: Retrospective, epidemiological study. Methods: Demographic, survival and pathologic data on all female patients diagnosed with OPSCC in Alberta between 1998 and 2008 was collected. A review of pathology reports in these patients was undertaken to identify HPV-related vulvar, vaginal and cervical precancerous or cancerous lesions. Standardized incidence ratios (SIRs) of HPV-related lesions in oropharyngeal cancer patients was calculated using control data obtained from provincial cancer surveillance. Disease specific survival of patients with oropharyngeal cancer only versus oropharyngeal and HPV-related lesions was also compared. Setting: Tertiary care centers in Alberta. Patients: All female patients with diagnosed with OPSCC in Alberta between 1998 and 2008. Main Outcome Measures: SIRs and survival of patients with cervical cancer and OPSCC. Results: Our results show an elevated incidence of HPV-related cancerous lesions in women with OPSCC in comparison to the control population with an SIR of 29.4 (95% CI 12.05-74.98). Distinct survival trends were also identified between patients with both OPSCC and HPV-related genital lesions versus OPSCC only. Conclusions: Women with oropharyngeal cancer have a significantly elevated risk of developing HPV-related genital cancers which suggests frequent HPV co-infection of oropharyngeal and genital tissues in this patient population. This has significant implications for screening and councelling of this patient population.

#### **S048:** TUMOR INFILTRATING LYMPHOCYTES (TIL) AND HUMAN PAPILLOMAVIRUS-16 ASSOCIATED

**OROPHARYNX CANCER** - <u>Derrick Wansom, BA</u>, Emily Light, MS, Dafydd Thomas, MD, Francis Worden, MD, Mark Prince, MD, Susan Urba, MD, Douglas Chepeha, MD, Bhavna Kumar, MS, Kitrina Cordell, DDS, Avraham Eisbruch, MD, Jeremy Taylor, PhD, Jeffrey Moyer, MD, Carol Bradford, MD, Nisha D'Silva, DDS, Thomas Carey, PhD, Jonathan McHugh, MD, Gregory Wolf, MD; UM Head and Neck SPORE Program, University of Michigan, Ann Arbor, MI

Human Papillomavirus-16 (HPV-16) associated squamous carcinoma of the oropharynx has a favorable prognosis. Patients with HPV-16 positive cancers have elevated peripheral blood CD8+ T lymphocyte levels that correlate with response to chemotherapy and survival. TILs were assessed in pretreatment biopsies from a prospective patient cohort to determine if TIL subsets differed by HPV status, clinical factors, patient outcome or peripheral blood T cell levels. Methods: Measured were CD8, CD4, and Treg (FoxP3) lymphocytes by immunohistochemistry in a tissue microarray created from patients (n=46) with advanced oropharynx cancer. Correlations with peripheral blood levels, HPV status, expression of EGFR, p53 and clinical outcome were determined. Patients were treated with a single course of neoadjuvant chemotherapy (cisplatin, 5-fluorouracil) followed by either surgery (non-responders) or chemoradiation (cisplatin 100 mg/ m2 every 3 weeks x 3; 70 Gy, 2 Gy daily x 7 wks) for responders. Median follow up was 6.6 years. Results: HPV-16 positive patients had improved survival (p=0.016). Degree of T cell infiltration did not differ by HPV status but was significantly related to response to chemoradiation, disease specific (DSS) and overall survival (OS). Higher infiltration by CD8, CD4 and FoxP3 subsets was significantly associated with lower T stage and survival. Even after adjusting for HPV status, CD8, FoxP3 and total T cells were significantly associated with DSS (p=0.0236; 0.0040; 0.0197) and OS (p=0.0137; 0.0158; 0.0115, respectively). Less T cell infiltration (p=0.0130) and CD4 in particular (p=0.0792) were associated with higher EGFR expression. FoxP3 infiltration correlated significantly and directly with CD4 and CD8 infiltration but not with peripheral blood levels. Conclusions: Improved outcomes are associated with increased TILs independent of HPV status and suggest the local immune response to HPV-16 may be related in part to factors such as tumor size, EGFR expression, smoking history, performance status or innate immunity. Assessment of TILs in tissue microarrays is difficult due to small size of sample cores and variation in tumor representation in cores. Further study in whole tumor sections and functional analysis of individual subsets may be necessary to detect differences in local immunity in HPV-16 related cancers. P50 CA097248, R01 DE13346, NIDCD T32 DC005356.

**S049:** IMAGING AND SPECTROSCOPIC MODALITIES FOR NONINVASIVE EVALUATION OF ORAL LESIONS - <u>Richard</u> <u>A Schwarz, PhD</u>, Darren M Roblyer, PhD, Wen Gao, PhD, Vanda M Stepanek, MD, PhD, Tao T Le, BS, Vijayashree S Bhattar, BS, Jessica K Wu, BS, Nadarajah Vigneswaran, BDS, DMD, DrMedDent, Ann M Gillenwater, MD, FACS, Rebecca Richards-Kortum, PhD; Rice University, Houston, TX, Beckman Laser Institute, Irvine, CA, University of Texas M. D. Anderson Cancer Center, Houston, TX, University of Texas Dental Branch at Houston, Houston, TX

Background: Fluorescence imaging and point spectroscopy are two adjunctive techniques for noninvasive early detection of oral cancer. Both methods detect alterations in tissue autofluorescence, scattering, and absorption due to changes in the concentration of native fluorophores such as collagen, elastin, keratin, NADH, and FAD, changes in hemoglobin

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concentration and oxygenation, changes in epithelial thickness, and changes in nuclear morphology. Objective: Determine whether combining widefield fluorescence imaging with depth-sensitive point spectroscopy improves the ability to distinguish healthy oral tissue from dysplastic and cancerous tissue over each method alone. Evaluate the potential of depth-sensitive point spectroscopy to reduce false positive results associated with benign lesions by targeting the epithelium and minimizing the signal from the stroma. Methods: 46 sites in 29 oral cancer patients were measured using both widefield fluorescence imaging and depthsensitive spectroscopy. Classification algorithms were applied to generate diagnostic predictions based on imaging alone, spectroscopy alone, and the combination of both methods. Histopathology was used as the gold standard. Separately, 26 sites clinically identified as benign lesions in 13 subjects were measured using depth-sensitive spectroscopy. Diagnostic performance was examined in specific types of benign lesions. Expert clinical impression or histopathology (where available) was used as the gold standard. Results: For sites measured using both spectroscopy and imaging, area under the receiver operating characteristic curve was 0.925 for imaging alone, 0.835 for spectroscopy alone, and 0.930 for imaging and spectroscopy combined. Spectroscopy displayed a lower area under the curve but higher specificity at a preestablished threshold value than imaging. Among the clinically benign lesions measured using depthsensitive spectroscopy, those classified correctly as true negative included sites described as mucositis, submucous fibrosis, keratosis, pigmented lesion, inflammation, and some lichen planus sites. Sites misclassified as false positive included pemphigus vulgaris, geographic tongue, and some lichen planus sites. One lichen planus site was correctly classified as true positive, confirmed by histopathology. Conclusion: The overall performance of noninvasive optical diagnostic methods may be enhanced through the use of platforms that combine imaging and spectroscopic modalities. Depth-sensitive spectroscopy may assist in distinguishing some types of benign lesions from dysplasia and cancer.

#### **\$050:** VITAMIN OR MINERAL SUPPLEMENT INTAKE AND THE RISK OF HEAD AND NECK CANCER: POOLED ANALYSIS IN THE INHANCE CONSORTIUM - <u>Qian Li.</u>

<u>PhD<sup>1</sup></u>, Mia Hashibe, PhD<sup>2</sup>, Paolo Boffetta, PhD<sup>3</sup>, Erich M Sturgis, PhD<sup>4</sup> International Agency for Research on Cancer; <sup>1</sup>International Agency for Research on Cancer, Lyon, France; <sup>2</sup>University of Utah School of Medicine, Salt Lake City; <sup>3</sup>International Prevention Research institute, Lyon, France; <sup>4</sup>University of Texas Anderson Cancer Center, Houston

Background: Head and neck cancer (HNC) is a significant cause of morbidity and mortality, with over half a million cases worldwide each year. While high fruit and vegetable intake is thought to be protective against HNC, it is unclear whether vitamin and mineral supplement intake is associated with decreased HNC risk. Methods: We analyzed individual-level pooled data from 12 case-control studies (7,002 HNC cases and 8,383 controls) participating in the International Head and Neck Cancer Epidemiology (INHANCE) consortium. Odds ratios (OR) and 95% confidence intervals (CIs) for use of any vitamins, multivitamins, vitamin A, vitamin C, vitamin E, calcium, beta-carotene, iron, selenium, and zinc supplement were assessed. All ORs were adjusted for age, sex, race, study center, education, smoking, alcohol drinking and fruit/vegetable intake. Results: A decreased risk of HNC was observed with ever use of vitamin C (OR=0.76, 95%CI=0.59-0.96) and with ever use of calcium supplements (OR=0.64, 95%CI=0.42-0.97). There did not appear to be any associations between HNC risk and ever use of any vitamins, multiple vitamins, vitamin A, vitamin E, beta-carotene, iron, selenium or zinc. More than 10-years of vitamin C intake was associated with decreased HNC

risk (OR=0.72, 95%CI=0.54-0.97). The frequency (>=7 tablets per week) and cumulative intake (>365 tablets) of calcium supplement were associated with decreasing HNC risk. An inverse association was observed between any vitamin E use and oral cavity cancer, (OR=0.73, 95%CI=0.56-0.96), but not other HNC subsites. Conclusions: This is the first report of inverse associations between both vitamin C and calcium use and HNC in a very large sample size. The next steps will include adjustment on micronutrients from dietary intake.

#### **\$051:** INVESTIGATION OF THE PROGNOSTIC CHARACTERISTICS OF HEAD AND NECK SQUAMOUS CELL CARCINOMAS (HNSCCA) IN THE YOUNG POPULATION Konstanting Koundle, MD, Targara Tura, MD, F

**POPULATION** - <u>Konstantinos Kourelis, MD</u>, Terrance Tsue, MD, FACS, Douglas Girod, MD, FACS, Kevin Sykes, MPH, Yelizaveta Shnayder, MD, FACS; Department of Otolaryngology, Kansas University Medical Center

Background: The unusual occurrence of HNSCCA in particularly young patients (age<40 years), who could not have sustained a prolonged exposure to the classical risk factors, merits special consideration, with regard to the biologic and clinical behaviour of these tumors. Patients & Methods: The study population comprises 69 patients with HNSCCA aging between 17 and 40 years, who have been evaluated at a tertiary care center. This was compared to an equally-sized group, including HNSCCA patients older than 40 years. The two groups were matched for sex, tumor site, grade, TNM stage at diagnosis, and exposure to tobacco and alcohol. The mean follow-up period for the "young" group was 28 months (range: 1-142m), whereas for the "old" was 36 months (range: 3-96m). Recurrence type and frequency, as well as disease-free, disease-specific, and overall survival were determined. Results: Young women with HNSCCA demonstrated an increased risk of recurrence, when compared with females over 40 years (P=0.019). Young patients had a worse prognosis after regional recurrence in the neck, both in terms of disease-specific (P=0.023) and overall survival (P=0.004). As regards to survival analysis by tumor site, young patients with early tongue cancer tended to have the shortest survival, but this finding was not statistically significant (P=0.056). Conclusions: HNSCCA in the young population shows particular features in its natural course, so that it may be considered a distinct disease entity from its counterpart in the older population. The atypically aggressive behaviour of these neoplasms warrants modifications in the treatment management of patients in the younger age group.

**S052:** ALTERNATIVE TOBACCO PRODUCT USE IN RURAL AFRICAN AMERICAN MEN - <u>William R Carroll, MD</u>, Herman R Foushee, PhD, Isabel Scarinci, PhD, MPH; University of Alabama – Birmingham

Introduction: Tobacco-related disease is a primary source of morbidity and mortality for African American men. Recent studies suggest that alternative tobacco products may have supplanted cigarettes as the most common products used by young African Americans. Effective cessation and prevention strategies require accurate data on specific product use. The purpose of this project was to determine prevalence of tobacco product use among young African American men of the Black Belt region of Alabama. The Black Belt is named for the rich dark soil that supported the agricultural industry of an earlier era. These counties are predominantly African American, among the poorest in the US, and are characterized by striking health disparities. The data will ultimately inform development of relevant cessation and/or prevention strategies. Methods: Trained interviewers verbally administered surveys to African American men aged 19-30 in five representative counties of the Black Belt region. Participants were stratified by income and education level. Prevalence rates for current and ever-use of nine different tobacco products were determined. Results: Four hundred fifteen participants met eligibility criteria and completed the survey. Cigarettes were the most common product ever (54%) and currently (39.9%) used. Of concern, 74% of those who ever used cigarettes remained current users. Marijuana and blunts were the products used next most commonly. Current minicigars use was restricted to 35 respondents (8.9%). Other products, bidis / kreteks, smokeless tobacco, and pipes were used very uncommonly in this sample. Conclusion: In contrast to the reported trend of alternative products supplanting cigarettes among young African Americans, cigarette use remains the dominant source of tobacco exposure among young African American men of the Black Belt counties of Alabama. Effective intervention efforts must target cigarette cessation and prevention in this vulnerable population.

#### **\$053:** VENOUS THROMBOEMBOLISM IN HEAD AND NECK

**CANCER PATIENTS AFTER SURGERY** - <u>Leo Thai, BS</u>, Neil Gross, MD, William Stott, BS, Mark Wax, MD, Peter Andersen, MD; Oregon Health & Science University

Purpose/Objective(s): Cancer patients undergoing major surgery are considered high risk for venous thromboembolism (VTE). The risk of VTE in head and neck cancer patients undergoing surgical ablation is unknown. The purpose of this study was to report the incidence of VTE in head and neck cancer patients after extensive resection and microvascular reconstruction and to explore the impact of VTE on survival. Materials/Methods: Single-institution, retrospective cohort study performed between 2007 -2009; 134 head and neck cancer patients underwent a total of 139 procedures. All patients underwent extensive cancer resection and simultaneous microvascular free tissue transfer reconstruction. Demographic, clinical and surgical data were abstracted for all cases. The primary endpoint was identification of confirmed (pulmonary embolism, PE; deep venous thrombosis, DVT) or suspicious (acute respiratory failure, sudden cardiac arrest, leg edema without imaging) cases of VTE within 30 days of surgery. Associations between covariates and VTE were assessed using multivariable logistic regression analysis and Kaplan -Meier survival analysis. Results: Most patients were male (59%) with squamous cell carcinoma (76%). The mean patient age was 65.4 years. A total of 8 (5.8%) confirmed or suspicious VTE were identified; 1 PE, 1 DVT, 1 acute respiratory failure, 1 sudden cardiac arrest and 4 cases of leg edema. In the multivariable analysis, the strongest predictors of VTE were a prior history of VTE (p=0.004; odds ratio [OR], 25.11; 95% confidence interval [CI], 1.13-556.40), red cell transfusion (p=0.009; OR, 1.80; CI, 1.16-2.80), high BMI (p=0.015, OR, 1.29, CI, 1.05-1.58), and older age (p=0.046; OR, 1.10; CI, 1.00-1.19). In multivariate models, confirmed VTE and suspicious VTE were significant predictors of decreased 2-year survival (hazard ratio=2.87, 95% CI, 1.39-17.08, p = 0.019) and (HR=1.79, 95% CI, 1.11-6.50, p = 0.019), respectively, when stratified against non-VTE patients. Conclusion: The incidence of VTE in head and neck cancer patients after major surgery involving microvascular reconstruction, ranged from 1.4% (confirmed) to 5.8% (confirmed and suspicious). These results suggest that the incidence of VTE may be underestimated in head and neck cancer patients after extensive resection and microvascular reconstruction. Prospective studies are needed to define the true extent of this problem.

#### **\$054:** A TEN-YEAR CLINICOPATHOLOGICAL STUDY OF 141 CASES OF AMELOBLASTOMA - <u>Satheesh Kumar Poolakkad</u> <u>Sankaran, MDS Trainee</u>, Anita Balan, MDS, Ajith, MDS, Deepti Simon, MDS; Department of Oral Medicine and Radiology, Government Dental College, Thiruvananthapuram, Kerala, India

Objective: To Evaluate and compare the Relative Frequency, Distribution, Sites of presentations, recurrence rate, surgical outcome, Radiologic variation and Histological variations of 141 cases of ameloblastoma reported to the department of Oral medicine and Radiology, Government Dental college, Trivandrum from the year 2000-2009. Methods: A retrospective analysis was done on 3222 Biopsy cases reported to Oral Medicine and Radiology Department, Government dental college, Trivandrum dating from January 2000 to may 2009 and the Biopsy records were evaluated from the Department of Oral medicine and Radiology and from the Department of Oral and maxillofacial surgery. Results: Out of 3222 biopsy cases reported to Oral Medicine and Radiology Department, Government dental college, Trivandrum, 1082 cases were diagnosed as odontogenic cysts and tumors and tumor like lesions of the jaw. Out of 1082 case, higher frequency were the, cyst of inflammatory origin (periapical and radicular cyst)n=589,54.4%, Ameloblastoma(n=141, 13.03%), Dentigerous cyst (n=98, 9.05%), keratinizing Odontogenic tumor(n=74,6%), Fibrous dysplasia(n=25, 2%) N=25,females=19(75%),males=6(24% ), Dentigerous cyst showing ameloblastoma transformation(n=24, 2%)Eruption cyst(n=55,5%),osteoma(n=22, 2%),Central ossifying fibroma(n=20, 1.8%) N=20,females=13(65%),males=7(35%),odontom e (n=16, 1.4%), Adenomatoid odontogenic tumor(n=6, 0.5%), Central giant cell granuloma(n=9, 0.8%), central odontogenic fibroma (n=6, 0.5%),cementoblastoma(n=8, 0.8%),Pindborgs tumor(n=5,0.4%),Benign fibrous histiocytoma(n=3, 0.2%), and other rarities which accounted for only less than 0.2% were juvenile ossifying fibroma, Ameloblastic fibrodontoma, chondrosarcoma, central odontogenic myxoma, Plasma cell myeloma. The various histological types of ameloblastoma include follicular (n=47, 33%), acanthomatous (n=32, 22%), plexiform (n=26, 19%), follicular with acanthomatous change(n=24,18%), granular cell ameloblastoma(n=2,1%), desmoplastic(n=3,2%), unicystic ameloblastoma (n=4,3%), basal cell ameloblastoma (n=3,2%). Radiologic variation were assessed (multilocular pattern n=93,66%, unilocular pattern n=48,34%).sex predilections (females n=77,55%, males n=64,44%), site of presentation(anterior maxilla n=3 2%, posterior maxilla n=14,10% anterior mandible n=37, 26%, posterior mandible n=87,62%). Conclusion: The early detection and understanding relative frequencies, recurrence rates and sites of presentation of odontogenic cysts, tumors and tumor like lesions of jaw are essential for the early diagnosis and management of these destructive lesions.

### ANGIOGENESIS/TUMOR MICROENVIRONMENT

**P001:** LOCALLY DELIVERED CURCUMIN PREVENTS ORAL TUMOR FORMATION IN CARCINOGENESIS-INDUCED MODEL - Cheryl Clark, PhD, Tara Moore-Medlin, Andrew Nida, Adam Master, Jeffrey Phillips, MD, <u>Lilantha H Ferdinandez</u>, MD, Cherie-Ann O Nathan, MD, FACS; LSUHSC-Shreveport and Feist-Weiller Cancer Center

Objective: Second primary tumors as a result of field cancerization are a significant problem amongst patients with risk factors for head and neck cancer, indicating a need for chemopreventive agents among tobacco users. This is the first study evaluating the naturally occurring bioactive food compound curcumin on carcinogen-induced oral cancer. Curcumin's low bioavailability has slowed its transition to clinical trials. We hypothesize curcumin has great chemopreventive potential in HNSCC where local application and mucosal absorption could bypass bioavailability problems. Design: An oral-specific chemical carcinogenesis model that mimics tobacco-induced carcinogenesis in humans was created by delivering 50µg/ mL 4NQO in the drinking water to wild-type C57Bl/6 mice for 12 weeks. Mice were then treated daily with either vehicle (control), or 15mg curcumin by local delivery, gavage, or combined local and gavage daily for 28 days (16 weeks time point), and followed up to 22 weeks, at which time mice were sacrificed, tongues harvested and inspected for tumor formation. Results: The number of dysplastic lesions was significantly less in mice treated with both local and gavage curcumin,  $\chi^2(1, N=9) = 6.56$ , p = 0.0105. The proportion of exophytic tumors in the local delivery curcumin group (2/10) was statistically significant from the control group (8/10, p=0.0073), but not gavage delivery (6/10, p=0.33) or combined delivery (6/9, p=0.51), highlighting the importance of correct dosing and local effects. Conclusions: This is the first oral carcinogen-induced curcumin chemoprevention study. Curcumin appears to inhibit oral tumor growth and blocks tumor progression when applied locally. Local curcumin delivery may offer the greatest benefit for chemoprevention in HNSCC where efficient mucosal absorption via the rich blood supply bypasses first-pass hepatic metabolism that overcomes the bioavailability problems of systemically delivered curcumin. Clinical Significance of Study: This preferential inhibition of tumor formation in HNSCC by prolonged local contact could be useful in the design of clinical trials with curcumin. Support: This work was supported by the Feist-Weiller Cancer Center.

#### **P002:** OVEREXPRESSION OF SIP1 AND DOWN-REGULATION OF E-CADHERIN PREDICT DELAYED NECK METASTASIS IN STAGE I/II ORAL TONGUE SQUAMOUS CELL CARCINOMA AFTER PARTIAL GLOSSECTOMY - <u>Koji</u> <u>Sakamoto, MD</u>, Yorihisa Imanishi, MD, Toshiki Tomita, MD, Hiroyuki Ozawa, MD, Takamasa Tagawa, MD, Masayuki Shimoda, MD, Seiichi Shinden, MD, Masato Fujii, MD, Katsushi Shibata, MD, PhD, Kaoru Ogawa, MD; Department of Otorhinolaryngology, Saiseikai Utsunomiya Hospital

Twenty to 30% of patients with Stage I/II oral tongue carcinoma develop delayed neck metastasis (DNM) after partial glossectomy within 2 years and sometimes result in unfavorable course. Clinicopathologic and molecular factors have been investigated to predict DNM but still unclear. Recently E-cadherin and its repressor are thought to have a key role in tumor progression and metastasis, the mechanism of which is called epithelial to mesenchymal transition (EMT). This study was aimed to examine the role of EMT in DNM and to determine the factors predictive of DNM. Quantitative real-time RT-PCR was carried out to evaluate mRNA expression of E-cadherin and its repressors (snail, SIP1 and twist) in 7 human head and neck squamoous carcinoma cell lines, which revealed significant inverse correlation between SIP1 and E-cadherin. Next, we performed immunohistochemical staining to evaluate protein expression of E-cadherin and its repressors in the specimens of the primary lesions of 37 stage I/II tongue carcinoma patients who had initially undergone only partial glossectomy without prophylactic neck dissection. Univariate analysis revealed loss of E-cadherin and overexpression of SIP1 are significantly correlated with DNM, although no inverse correlation between E-cadherin and its repressors was found. Regarding other clinicopathologic factors, mode of invasion 3/4, vascular invasion and muscular invasion were also significantly correlated with DNM. Multivariate analysis (including clinicopathologic and the molecular factors) elucidated that overexpression of SIP1, loss of E-cadherin and vascular invasion are independently correlated with DNM. These results suggest that DNM in stage I/II oral tongue carcinoma is closely relating to EMT and especially SIP1 and E-cadherin are considered as the useful markers to predict DNM.

#### **P003:** THE UNIQUE ROLE OF STAT PROTEINS IN ENDOTHELIAL CELLS MEDIATING ANGIOGENESIS - Jonah D Klein, BS, Seungwon Kim, MD; Department of Otolaryngology, University of Pittsburgh

Signal transducers and activators of transcription (STATs) play important regulatory roles in tumor cells. There are several family members including STAT1 and STAT3. STAT3 is downstream of growth pathways including epidermal growth factor receptor (EGFR) and interleukin-6 (IL6) and has proven to be a promising therapeutic target in head and neck cancer. As STAT3 targeting mechanisms are being developed in preclinical models, it is interesting to understand how these agents would affect endothelial cells and angiogenesis. The two primary targeting agents used in our studies are a STAT3 transcription factor decoy and siRNA. Biochemical approaches were taken to study the signaling of STAT proteins in endothelial cells while phenotypic changes, including proliferation, migration, apoptotic staining, and tubule formation, were also assessed. Our studies indicate that STAT1 and STAT3 play important roles in endothelial cell physiology, yet their signaling pattern, and target gene expression is unique from head and neck tumor cells. The STAT3 decoy inhibits growth, induces apoptosis, decreases migration, and decreases tubule formation of endothelial cells when compared to a mutant control. When employing the use of STAT3 siRNA similar results were observed, but there was less of an inhibitory effect on tubule formation. Therefore, due to the STAT3 decoy's ability to also bind STAT1, we assessed the role that STAT1 plays in endothelial cells and found that STAT1 plays a crucial role in growth, migration and tubule formation. Contrary to the antagonistic role of STAT1 and STAT3 in tumor cells, these proteins in endothelial cells appear to be playing a compensatory role. To better understand the differences between the STAT proteins in endothelial cells and tumor cells we undertook studies addressing target gene expression and found that the target genes of STAT proteins in endothelial cells are unique from tumor cells. Future studies include better understanding the mechanisms by which STAT1 and STAT3 mediate endothelial cell physiology. But as evidenced here, the STAT3 decoy appears to have an anti-angiogenic effect.

**P004:** PROGNOSTIC SIGNIFICANCE OF EXPRESSION OF MTA1 AND S100A4 IN TONSILLAR SQUAMOUS CELL CARCINOMA - Jun-Ook Park, MD, Min-Sik Kim, MD, PhD, Kwang-Jae Cho, MD,l PhD, Young-Hoon Joo, MD; Seoul St. Mary's Hospital, The Catholic Universijty of Korea, Seoul, Korea

Background and Objectives: The status of cervical lymph node metastasis had a great influence on the prognosis of the patients with oropharyngeal cancer. We evaluated the expression pattern of MTA1 and S100A4 by immunohistochemical stain and its effect on lymph node metastasis in

squamous cell carcinoma of tonsil by studying the relationship of the clinicopathologic features. Subjects and Methods: Forty-two patients with squamous cell carcinoma of the tonsil were classified as their tumor stages, lymph node metastasis and their paraffin-embedded surgical specimens were investigated by immunohistochemical analysis using antibodies for MTA1 and S100A4. Results: The expression rate of MTA1 was 61.9%. S100A4 was not stained in tumor cells of tonsillar squamous cell carcinoma, but stained in adjacent lymphocytes, macrophage, fibroblast, endothelial cells in immunohistochemical stain analysis. There was significant correlation between the expression of MTA1 and lymph node metastasis(p=0.012). There were no significant correlation between the expression of MTA1 and T stage, neural invasion, vascular invasion, depth of invasion. Conclusion: The overexpression of MTA1 is related the metastasis of tonsillar cancer and will be a useful marker for predicting the lymph node metastasis. S100A4 is overexpressed in adjacent stromal cells around tonsillar cancer cells, and the extracelluar S100A4 may has a role in tumor metastasis and progression .

### **P005:** HIGH RISK HPV AND CERVICAL LYMPH NODE METASTASIS IN ORAL AND OROPHARYNGEAL CANCER

<u>Young-Hoon Joo, MD</u>, Min-Sik Kim, MD, PhD; The Catholic University of Korea, Seoul, Korea

Objective: To determine the role of high risk human papillomavirus (HPV) in lymph node metastasis and the depth of invasion in oral and oropharyngeal cancer. Study Design: Retrospective analysis. Setting: Otolaryngology department in a university hospital. Subjects and Methods: The study included 156 subjects with squamous cell carcinoma of the oral cavity and oropharynx. High risk HPV in situ hybridization was performed to detect HPV infection. Results: The positive rate of high risk HPV in situ hybridization was 15.4% (24/156). There was a significant difference in the fraction positive high risk HPV between oral (6.7%) and oropharygeal (26.9%) cancer (p=0.001). Significant correlations were found between positive high risk HPV and cervical lymph node metastasis, tumor depth of invasion (p=0.012, p=0.003, respectively). There was statistical significant association between high risk HPV positivity and the disease-specific survival in patients with postoperative radiotherapy (p=0.043). Conclusion: High-risk HPV infection is significantly related to cervical lymph node metastasis and depth of invasion in oral and oropharyngeal cancer patients.

#### **P006:** LAMININ/INTEGRIN BINDING OF HEAD AND NECK CANCER CELLS UNDER LOW SHEAR STRESS FLOW RATES DIFFERS FROM THEIR INTERACTION UNDER STATIC, NON-FLOWING CONDITIONS - <u>Susan M Fennewald, PhD</u>, Vicente A Resto, MD, PhD; University of Texas Medical Branch at Galveston

Background: Cancer metastasis to the sentinel lymph node is an important step in the progression of head and neck cancer. Cancer cells interact with surrounding cells and extracellular matrix elements as they move and flow in the lymph system and within the lymph node. Flow rates and the associated shear stresses which result are much less than those present in blood flow, but are not insignificant. One of the extracellular matrix elements encountered in the lymph system is laminin which binds to the integrin proteins present on many cells. Integrins are divalentcation dependent heterodimeric proteins composed of alpha and beta subunits that can be present in either high or low affinity conformations. Objectives: To determine which laminin heterodimers mediate binding of head and neck squamous carcinoma cells (HNSCC) to laminin under flowing conditions characteristic of the lymph system. Results: The head and neck squamous carcinoma cells contain at least 6 different alpha ( $\alpha$ 1,  $\alpha$ 2, $\alpha$ 3, $\alpha$ , $\alpha$ 6,  $\alpha$ 9) and 3 beta ( $\beta$ 1,  $\beta$ 3,  $\beta$ 4) integrin subunits. As expected, they are able to bind to laminin under static, non-flowing conditions. We now show that they are also capable of binding strongly to laminin under conditions of low shear stress corresponding to lymph flow. The binding to laminin under static, Ca<sup>++</sup>-dependent conditions can be inhibited by antibodies to either the  $\alpha$ 6 or  $\beta$ 4 subunits indicating that the binding under these conditions is via the  $\alpha$ 6 $\beta$ 4 heterodimer which is known to be a laminin receptor. However, under low shear stress these same antibodies fail to inhibit binding. The  $\alpha$ 6 and  $\beta$ 4 antibodies also fail to inhibit binding. The  $\alpha$ 6 and  $\beta$ 4 antibodies also fail to inhibit binding under static conditions where the integrins have been activated by Mn<sup>++</sup> and are in a high-affinity state or where the  $\beta$ 1 integrins are activated by antibody binding. Conclusion: The condition of flow present in the lymph system may serve to activate the integrin receptors and facilitate binding of cancer cells to various elements of the lymph system during metastasis.

#### **P007:** THE EFFECT OF WOUND FLUID ON GROWTH OF SQUAMOUS CELL CARCINOMA AS XENOGRAFT IN VIVO Johan Wennerberg, MD, PhD, Gustaf Lindgren, MD, Lars Ekblad, PhD,

Elisabeth Kjellén, MD, PhD; Dept ORL, H&N Surgery and Dept of Oncology, University Hospital, Lund University, Lund, Sweden

Background: The process of wound healing and the process of tumor growth are related and show many similarities. Both comprise remodelling of the extracellular matrix, neoangiogenesis, cell proliferation and cell migration. During wound healing chemotactic factors, cytokines and growth factors are produced, and it is clinically known that less than radical surgery, as well as diagnostic procedures can stimulate tumour re-growth and implantation metastasis. Material and Methods: Human wound fluid (HWF) was collected from patients operated with thyroidectomy or parotidectomy for benign H&N diseases. Human head and neck squamous cell carcinoma (HNSCC) cell-lines were cultivated under standardized conditions and supplemented with either HWF, human serum (HS) or fetal calf serum (FCS). Cells were then inoculated s.c. on nude mice (Balb/C) in numbers varying from 0.05 to 5.0x 10(6) cells and the resulting tumor growth was recorded. Results: When the cells were incubated with wound fluid 24h prior to injection the take rate was significantly increased. Injection of cells cultured in fetal calf serum (FCS) and resuspended in wound fluid immediately before injection resulted in no increase in take rate. If the tumour cells were both cultured in wound fluid 24 h prior to injection and then resuspended in wound fluid just before injection the take rate increased to. In an optimal setting HWF lowered the number of cells needed for tumor take with a factor of 4 compared to FCS. This stimulatory effect could be antagonized if the animals were pre-treated with the epidermal growth factor receptor blocking monoclonal antibody cetuximab. Conclusion: Local recurrence of HNSCC after surgery is an everyday clinical problem. Our findings point at local wound-healing as a contributory factor and also indicate possible ways to counteract this effect.

#### **P008:** MOLECULAR MEDIATORS OF LYMPHATIC

**METASTASIS** - <u>Tove M Goldson, MD, PhD</u>, Vicente A Resto, MD PhD; University of Texas Medical Branch

Objective: Lymph node metastasis characterizes more aggressive tumors and predicts resistance to therapy. The interactions responsible for cancer cell lodgment within lymph nodes remain unknown. In order to establish interactions, cells that enter lymph nodes via afferent lymphatic vessels must first overcome shear stress in the microenvironment. Our goal is to understand how cancer cells overcome these forces. Our laboratory has demonstrated that head and neck squamous cell carcinoma (HNSCC) cell lines bind to lymphocytes via L-selectin under low shear stress levels found in the lymphovascular compartment.

HNSCC do not express known L-selectin ligands; however, the ligand(s) demonstrate the canonical characteristics of known L-selectin ligands, including N-glycosylation. This study aims to identify the L-selectin ligand(s) expressed by HNSCC. Methods: Putative L-selectin ligands from HNSCC were isolated using affinity chromatography. Their identities were determined using MALDI-TOF mass spectrometry and protein sequencing. Their extracellular expression was confirmed using flow cytometry and immunoprecipitation. Antibodies were used to evaluate their ability to support L-selectin binding under lymphodynamic shear stress. To identify possible splice variants, mRNA was evaluated by rtPCR and northern blot. Site-directed mutations of N-glycosylated residues were generated to confirm its role in L-selectin binding. Results: Several putative ligands were isolated. The extracellular expression of nucleolin was confirmed by flow cytometry. The ability of HNSCC to bind L-selectin under shear stress was inhibited by antibodies to nucleolin. A portion of the nucleolin expressed by HNSCC is N-glycosylated. HNSCC cell lines that express mutant nucleolin are being generated to characterize the role of N-glycosylated nucleolin in L-selectin binding under shear stress. Nucleolin mRNA gave several discreet bands that may indicate splice variants. Sequencing is underway to identify the possible isoforms. Conclusions: The identification of the novel ligand(s) responsible for HNSCC binding to lymphocytes via L-selectin will provide insight into the mechanisms by which cancer cells form adhesive interactions in the lymph node, may provide an early marker for the metastatic phenotype, and may provide new therapeutic targets. Additionally, analysis of their signaling pathways may help us understand how tumor cells grow in the lymph node microenvironment and induce immune tolerance.

### **P009:** IN VIVO INVASION OF HEAD AND NECK SQUAMOUS CELL CARCINOMA CELLS DOES NOT

**REQUIRE MACROPHAGES** - Tatiana Smirnova, PhD, <u>Alfred</u> <u>Adomako, PhD</u>, Nico Van Rooijen, PhD, Michael B Prystowsky, MD, PhD, Jeffrey E Segall, PhD; Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, New York; Free University Medical Center, Amsterdam, The Netherlands

Invasion of tumor cells into the local stroma is an important component in cancer progression. In vivo analysis using a breast cancer model showed that macrophages contribute to local invasion. In this article we report studies of the in vivo invasion of head and neck squamous cell carcinoma (HNSCC) cells in response to applied gradients of a growth factor (EGF) and a chemokine (CXCL12), utilizing orthotopic floor-ofmouth models and direct observation of invading cells. Analysis of the invading cells indicated that over 75% of them were tumor cells, about 15% macrophages, and fewer than 10% were unidentified. Surprisingly, although macrophages invaded together with tumor cells, macrophage contributions were not required for HNSCC invasion. CXCL12-induced in vivo invasion of HNSCC cells was also observed and found to occur via a unidirectional transactivation of EGFR by CXCR4. Inhibition of TACE using TAPI-2 selectively inhibited CXCL12-induced invasion but not EGF-induced invasion, consistent with CXCL12 activation of EGFR via release of EGFR ligands. This project identified a significant difference between HNSCC and breast cancer invasion. We proved that unlike breast cancer invasion, macrophages are not required for HNSCC invasion. To our knowledge, we were also the first to provide in vivo evidence of CXCL-12-mediated transactivation of EGFR. This finding suggests HNSCC patients could benefit from anti-EGFR therapies in combination with anti-CXCR4 (CXCL-12 receptor) therapies.

#### **P010:** BETA-1 INTEGRIN IS ESSENTIAL FOR GROWTH, ANOIKIS AVOIDANCE, AND ADHESION TO LAMININ IN HEAD AND NECK SQUAMOUS CELL CARCINOMA CELL

LINES - <u>Peter Szaniszlo, PhD</u>, Carla Kantara, Susan M Fennewald, PhD, Tove M Goldson, MD, PhD, Xinrong Tao, PhD, Lisa A Elferink, PhD, Vicente A Resto, MD, PhD; Departments of Otolaryngology, Neuroscience and Cell Biology, Sealy Center for Cancer Cell Biology, University of Texas Medical Branch, Galveston, Texas

Background and Objective: A major determinant of the lethal progression of Head and Neck Squamous Cell Carcinoma (HNSCC) is the spreading of cancer cells to regional lymph nodes. Among other steps, this metastatic spread requires tumor cell proliferation, survival in the lymph circulation, and attachment to cells in the lymph node under low sheer stress conditions, processes that are regulated by adhesion molecules. The adhesion molecule family of integrins plays an important role in cell-cell and cell-matrix interactions requisite for cancer cell adhesion, growth, migration and invasion. In this study we have investigated the role of integrins in HNSCC progression and metastatic potential. Methods: To determine the contribution of different integrin species to cell-cell interactions under low sheer stress conditions we used a circular parallel plate flow chamber apparatus and specific anti-integrin antibodies. We created beta-1 integrin (ITGB1) knockdown (KD) clones from established HNSCC cell lines using targeted shRNAs and studied their proliferation, survival and adhesion capabilities in vitro, as well as their tumorigenic and metastatic potential in vivo using an orthotopic mouse model of HNSCC. Results: Laminin is one of the major surface factors HNSCC cells attach to under low sheer stress conditions. Beta-1 integrin is expressed in HNSCC cells at high levels and regulates their laminin binding capabilities as determined both by antibody blocking and ITGB1-KD experiments. ITGB1-KD cells have lower proliferation rate and diminished anoikis avoidance capabilities compared to wild type HNSCC cells. Knockdown of the ITGB1 inhibits tumor growth and metastasis formation in an orthotopic mouse model of HNSCC. Conclusions: Our results suggest that ITGB1 plays an essential role in proliferation, survival, and attachment capabilities of squamous carcinoma cells and could provide a potential target in the treatment of Head and Neck Cancers.

**P011:** MODELING THE CROSSTALK BETWEEN HEAD AND NECK CARCINOMA CELLS AND STROMAL FIBROBLASTS THAT DRIVES MALIGNANT INVASION - Kati Rasanen, PhD, Meenhard Herlyn, DVM, DSC, Anil Rustgi, MD, <u>Devraj Basu, MD, PhD</u>; The University of Pennsylvania, The Wistar Institute, The Philadelphia Veteran's Administration Medical Center

Epithelial to mesenchymal transition (EMT) is a developmental gene regulatory program that promotes migration and phenotypic conversion of epithelial cells. A related process is proposed to occur in carcinoma cells as a key event driving malignant invasion and metastasis. There is an ongoing need to develop in vitro models for investigating mechanisms of such invasion by carcinomas at their stromal interfaces. For this purpose, we have identified human head and neck squamous cell carcinoma (HNSCC) cell lines in which EMT is occurring dynamically and on a continual basis. Scratch assays and video microscopy demonstrated that a mesenchymallike subpopulation within these cell lines possesses enhanced migratory capacity relative to the epithelial subset. This mesenchymal-like subset also showed greater invasive potential when grown as collagen-embedded spheroids and in Boyden chamber assays. HNSCC interactions with stroma were modeled by incorporating human fibroblasts in conventional 2D co-culture experiments and using organotypic 3D reconstructs, which provide a discrete invasive interface with collagen-embedded fibroblasts.

Exposure to stromal fibroblasts increased differentiation of the epithelial subset toward mesenchymal-like phenotype in co-cultures and enhanced invasion of the HNSCC cells in 3D reconstructs. Gel contraction assays demonstrated that the mesenchymal-like carcinoma subpopulation was more effective than the epithelial subset in driving the fibroblast activation that further promotes EMT. ShRNA-mediated silencing in HNSCC cells of connective tissue growth factor (CTGF), a secreted protein overexpressed by the mesenchymal-like subpopulation, directly reduced the size of the same subpopulation. Loss of CTGF in co-cultured HNSCCs also indirectly limited their mesenchymal differentiation and invasiveness by reducing fibroblast activation, which disrupts a positive feedback loop by diminishing fibroblast-derived CTGF. These results define a system for modeling the acquisition of invasive properties by subpopulations of malignant cells within HNSCCs, via active crosstalk with stromal fibroblasts. They further suggest that tumor-derived CTGF is a central mediator in the network of crosstalk between HNSCC cells and fibroblasts that drives malignant invasion

### **P012:** METRONOMIC REGIMEN ENHANCES THE ANTI-TUMOR AND ANTI-ANGIOGENIC EFFECTS OF THE BH3-

MIMETIC DRUG AT101 - <u>Atsushi Imai, MD</u>, Benjamin D Zeitlin, PhD, Fernanda Visioli, DDS, Zhihong Dong, MD, PhD, Sudha Krishnamurthy, DDS, Frank Worden, MD, Shaomeng Wang, PhD, Jacques E Nör, DDS, PhD; University of Michigan Ann Arbor, MI; University of the Pacific Arthur A. Dugoni School of Dentistry, San Francisco, CA

Background: Rescent studies have shown that Bcl-2 functions as a proangiogenic signaling molecule in addition to its well-known effect as an inhibitor of apoptosis. The discovery of AT101, a BH3-mimetic drug that is effective and well tolerated when administered orally, suggested the possibility of using a molecularly targeted drug in a metronomic regimen ( i.e. low dose, high frequency). Methods: We generated xenograft human head and neck squamous cell carcinomas (HNSCC) with humanized vasculature in immunodefficient mice. Mice received either daily 10 mg/kg AT101 (metronomic) or weekly 70 mg/kg AT101 (bolus) for three weeks in combination with weekly 5 mg/kg taxotere. The effect of single drug AT101 and combination AT101/taxotere on angiogenesis and on the survival of primary human endothelial cells and HNSCC were also evaluated *in vitro*. Results: Metronomic AT101 in combination with taxotere increased mouse survival (P = .002), decreased tumor mitotic index (P < .001) and decreased microvessel density (P < .001), as compared to bolus deliverly of AT101 combined with taxotere. The potentiation of the anti-tumor effect in the metronomic AT101 group was achieved using the same total amount of drug and without changes in systemic toxicities. In vitro, combination of AT101 and taxotere showed additive toxicity for endothelial cells and additive or synergistic for HNSCC. Notably, sub-apoptotic concentrations of AT101 potently inhibited the angiogenic potential of endothelial cells in vitro. Conclusion: These data unveiled the benefit of metronomic delivery of a molecularly targeted drug, and suggest that patients with head and neck cancer may benefit from continuous administration of low dose BH3-mimetic drug.

#### **P013:** SNAIL MOBILIZES THE MEMBRANE-ANCHORED COLLAGENASES, MT1-MMP AND MT2-MMP, TO DRIVE CANCER CELL INVASION PROGRAMS - <u>Ichiro Ota, MD PhD</u>,

Noritomo Okamoto, MD, Katsunari Yane, MD, PhD, Hiroshi Hosoi, MD, PhD; Dept of Otolaryngology, Nara Medical University

The metastatic spread of tumor cells to distant organs via hematogeneous routes represents the most important cause of morbidity and mortality in cancer. The initiation of the invasive phenotype has been linked to the

aberrant expression of zinc-finger transcriptional repressors, like Snail, which act by triggering an epithelial-mesenchymal cell-like transformation (EMT-like) via the regulation of largely undefined, downstream effectors. Using live chick embryos as a platform for monitoring human cancer cell i) transmigration across basement membrane as well as stromal barriers, ii) induction of neo-angiogenesis and iii) metastasis, we demonstrate that each of these activities are regulated by the membrane-anchored metalloproteinases, MT1-MMP and MT2-MMP. Importantly, the pro-invasive, angiogenic and metastatic activities of MT1-MMP and MT2-MMP are unique relative to all other metalloproteinase family members and cannot be mimicked by the secreted MMPs, MMP-1, MMP-2, MMP-3, MMP-7, MMP-9 or MMP-11. As MT1-MMP and MT2-MMP were found to be both sufficient and necessary for regulating key determinants of the cancer cell phenotype, the role played by these enzymes during Snail induction of a cancer cell EMT-like program was assessed. Tumor cell populations incapable of expressing an invasive, angiogenic or metastatic phenotype were rendered competent for each of these activities following induction of Snail expression. Coincident with the Snail-mediated acquisition of this aggressive pattern of cell behavior, recipient cells up-regulate expression of MT1-MMP and MT2-MMP. siRNA-specific silencing of the MT-MMPs, however, ablated completely the ability of Snail to drive cancer cell invasion, angiogenesis or metastasis. Taken together, these data demonstrate that MT1-MMP and MT2-MMP cooperatively function as direct-acting, pro-invasive factors that confer Snail-triggered cancer cells with the ability to penetrate connective tissue barriers, induce angiogenesis and initiate the metastatic process in vivo. These findings suggested that Snail-targeted therapy could be effective to cancer invasion and metastasis.

# **BASIC SCIENCE**

P014: THYMIDYLATE SYNTHASE EXPRESSION AS A PREDICTOR OF CLINICAL RESPONSE TO 5-FU-BASED CHEMOTHERAPY IN PATIENTS WITH MAXILLARY SINUS SCC - <u>Ryuji Yasumatsu, MD</u>, Torahiko Nakashima, MD, Moriyasu Yamauchi, MD, Shizuo Komune, MD; Kyushu University

Objective: It is important to preserve organs and function and improve the cure rate for the patients with maxillary sinus SCC. Many studies indicated that the TS, which is the target enzyme of 5-FU, is an indicator for response to 5-FU-based chemotherapy. Methods: We constitutively expressed an antisense TS cDNA in the HNSCC cell line. We examined the effects of TS expression on 5-FU cytotoxicity and the value of immunohistochemical TS expression as a predictive indicator for 5-FU efficacy in patients with maxillary sinus SCC.Results: Antisense TS transfection increases the cytotoxicity of 5-FU in vitro. Maxillary sinus SCC patients with negative TS expression had significantly better response rates for 5-FU based chemotherapy compared with those with positive TS expression in immunohistochemical findings. Conclusions: These results indicate that TS expression plays an important role in the sensitivity to 5-FU chemotherapy, and TS expression affects the chemotherapeutic effect of 5-FU in patients with maxillary sinus SCC. The assessment of TS expression level might be useful both in the management and in the treatment of maxillary sinus SCC.

#### **P015:** RECURRENT COPY NUMBER GAINS OF ACVR1 AND CORRESPONDING TRANSCRIPT OVEREXPRESSION ARE ASSOCIATED WITH SURVIVAL IN HEAD AND NECK SQUAMOUS CELL CARCINOMAS - Eliane Ambrosio, PhD, Sandra

Drigo Linde, PhD, Nadia Aparecida Bérgamo, PhD, Fabíola Encinas Rosa, PhD, Fernanda Bernardi Bertonha, Msc, Francine Blumental de Abreu, Luis Paulo Kowalski, PhD, MD, <u>Silvia Regina Rogatto, PhD</u>; AC Camargo Cancer Treatment and Research Center, São Paulo, Brazil; Department of Urology, Faculty of Medicine, Sao Paulo State University - UNESP, Botucatu, SP, Brazil

Aims: Molecular and cytogenetic studies may lead to the discovery of new molecular markers and pathways involved in the pathogenesis of head and neck carcinomas, which may translate into novel clinical therapeutic approaches focusing on target molecular therapies. In this study, recurrent focal gains at 2q24 overlapping the ACVR1 gene in head and neck carcinomas are reported. Methods and Results: Twenty-eight samples were evaluated by FISH using the probes RP11-546J1 (2q24) and RP11-21P18 (internal control). Significant gains at 2q24 were detected in most cases at frequencies varying from 3%-35%. ACVR1 gains and amplifications were associated with longer overall survival (P=0.022). Subsequent ACVR1 mRNA expression analysis in 80 cases revealed overexpression in 46% (37 out of 80) of these tumors, confirming the chromosomal amplification observed by FISH. In laryngeal carcinomas, overexpression of ACVR1 mRNA levels was associated longer overall survival (P=0.016). Multivariate analysis revealed that ACVR1 is an independent prognostic marker in laryngeal carcinomas (P=0.031, HR=0.316, 95% CI=0.111-0.902). Conclusions: These findings indicate that genomic gains at 2q24 were translated in ACVR1 overexpression, which is associated with longer overall survival in laryngeal carcinomas. To our knowledge, this is the first report indicating the relevance of ACVR1 expression in head and neck cancers.

#### **P016: ESTABLISHMENT OF A HEAD AND NECK SQUAMOUS CELL CARCINOMA CELL LINE EXPRESSING HIGH-RISK HUMAN PAPILLOMAVIRUS-16** - <u>Alice Tang, BA</u>, Martin P Graham, BS, Samantha J Davis, BS, John H Owen, BS, Jung Je Park, MD, PhD, Heather M Walline, MA, Jay Stoerker, PhD, Jonathan McHugh, MD, Douglas B Chepeha, MD, Carol R Bradford, MD, Thomas E Carey, PhD, Mark E Prince, MD; Department of Otolaryngology: Head & Neck

Surgery, University of Michigan, Ann Arbor, MI Objectives: There are few head and neck squamous cell carcinoma (HNSCC) cell lines that are positive for human papillomavirus (HPV). High-risk HPV have an etiologic role in the development of certain HNSCCs. These tumors are distinct in behavior from HPV-negative tumors, and HPV+ HNSCC cell lines can help distinguish the basis for these differences. We established and characterized a new HPV-16 cell line from a tumor of the oral cavity. Methods: The tumor specimen was dissociated and cultured. In situ hybridization (ISH) for several HR-HPV subtypes and immunohistochemistry (IHC) for relevant HNSCC biomarkers were performed using tumor sections. Cultured cells were passaged several times in monolayer and suspension-promoting conditions. HPV type was determined by real-time competitive PCR and matrix-assisted laser desorption/ionization-time of flight mass spectroscopy separation of products on a matrix-loaded silicon chip array. Primers designed to amplify the E6 region distinguishing 15 discrete HR-HPV types were used. Expression of E6, E7 and p53 were assessed by reverse-transcriptase PCR. Fluorescent-activated cell sorting for cancer stem-cell (CSC) markers was used to isolate subpopulations of cells that were implanted into NOD/SCID mice to evaluate tumorigenic potential. Results: The UMSCC-104 cell line was derived from a recurrent tumor of the anterior floor of mouth from a 70 year-old male. Cells double

in 48 hours and can grow in suspension as cellular aggregates. The tissue sections were negative for HR-HPV subtypes by ISH, however more sensitive measures were able to detect HPV-16 DNA and E6/E7 expression in UMSCC-104. IHC revealed strong staining for p16 and EGFR, and weak staining for p53. Sequencing of cDNA showed that p53 was wild type. Flow cytometry analysis revealed low but significant ALDH+ (0.6%) and CD44+ (1.39%) CSC subpopulations. Heterotransplantation of ALDH+ and ALDH+ cells into NOD/SCID mice resulted in tumor growth from the ALDH+ cells after 6 weeks. Conclusions: HPV+ tumors of the oral cavity are rare, and UMSCC-104 is a new HPV-16 cell line that aid in studying HR-HPV in HNSCC. Continued observation of the heterotransplanted ALDH- population for tumor production and the ALDH+ for distant metastasis is underway.

#### **P017:** PHOTODYNAMIC THERAPY DOWNREGULATES MATRIX METALLOPROTEINASES IN ORAL CARCINOMA Mina Le, MD, Beverly Wuertz, BA, Merrill A Biel, MD, PhD, Frank G

Ondrey, MD, PhD; University of Minnesota

Upregulation of matrix metalloproteinases (MMPs), particularly MMP-2 and MMP-9, is a common feature in head-and-neck malignancies and represents an opportunity for intervention at the interface between carcinoma in situ and microinvasive cancer. These enzymes potentiate invasion by degrading the extracellular matrix, among other functions. Photodynamic therapy (PDT) is an emerging anticancer modality in which a photosensitizing agent is preferentially taken up by malignant tissue and then activated with a laser, resulting in the formation of reactive oxygen species which kill tumor. We hypothesized that photodynamic therapy would downregulate MMP-2 and MMP-9 in cultures of oral squamous cell carcinoma (cell line CA-9-22) and leukoplakia (cell line MSK-Leuk1). Cells were cultured to 50-90% confluence, photosensitized with 0.5-1 mg/mL methylene blue, and treated with a 665 nm laser at fluence 6.3 J/cm^2. Twenty-four hours after PDT, cells were harvested for isolation of RNA, which was converted to cDNA for qPCR. Cell supernatants were analyzed by ELISA and gelatin zymography to confirm the PCR findings at a protein and functional level. MMP-9 gene expression was found to be significantly decreased in carcinoma and in leukoplakia, as well as in HPV-transformed oral keratinocytes, following the administration of PDT (p<0.05). MMP-2 and -9 enzymatic activity also decreased in all cell lines after PDT. This work demonstrates that methylene-blue-mediated PDT can downregulate proteins vital at the interface of invasion of head-and-neck cancer. Additionally, these results illuminate one aspect of the mechanism of PDT and suggest that MMP-2 and MMP-9 may even serve as biomarkers for the efficacy of photodynamic therapy.

# **P018:** TARGETED RADIATION CAUSES MAXILLARY BONE DAMAGE IN A RAT MODEL INVESTIGATING

**OSTEORADIONECROSIS** - <u>Bob B Armin</u>, MD, Akishige Hokugo, DDS, PhD, Ichiro Nishimura, DDS, DMSc, DMD, Matthew Tamplen, BS, John Beumer III, DDS, MS, Elliot Abemayor, MD, PhD, Vishad Nabili, MD 1) Division of Head and Neck Surgery, David Geffen School of Medicine at UCLA. 2) The Weintraub Center for Reconstructive Biotechnology, UCLA School of Dentistry

Objectives: 1. Assess bone damage with targeted radiotherapy to the rat maxilla. 2. Compare findings to recently developed rat mandibular model of osteoradionecrosis (ORN). Introduction: Mandibular ORN continues to be a complication risk from radiation treatment for head and neck cancer. The incidence of maxillary ORN, especially in Western countries, appears lower than mandibular ORN. Whether the maxilla is protected from ORN compared to the mandible remains to be studied. We set out

to investigate whether radiogenic bone damage can be produced in the rat maxilla by modifying a post radiation dental extraction model already created for mandibular ORN. Methods: Ten male Sprague-Dawley rats were divided into two groups. The experimental group had brachytherapy to the left maxilla (n = 6) while the control group had placement of a brachytherapy catheter without irradiation (n = 4). The 2nd left molar was extracted one week after radiation and the maxilla was harvested 3 weeks following dental extraction. Presence of alopecia and bony mucosal covering of the dental extraction site was documented. The ratio of bone volume to total volume (BV/TV) of the dental extraction socket was measured using a standard method with micro CT. Results: The radiated group demonstrated alopecia overlying the radiation side. All rats had mucosal coverage of the extraction site while only the radiated rats demonstrated scarring of the mucosa. The median BV/TV was 0.21 for the radiated group and 0.49 for the control group (p=0.01). Conclusions: Similar to the mandible, the maxilla is susceptible to radiogenic bone injury as demonstrated by the significant decrease in bone volume of the radiated dental extraction socket. Comparing our results to the previous rat mandibular study of ORN, a similar diminution of bone volume is seen; yet all radiated maxillae demonstrate bony mucosal coverage with scarring while the radiated mandibles demonstrated bony exposure. This finding is similar to the clinical presentation of maxillary ORN, which in general follows a more benign course. Our results may further suggest that the selective targeting of tumors more common in Western countries may influence the lower incidence of maxillary ORN seen in our clinical practice.

#### **P019:** INCREASED TUMORIGENICITY AND CHEMOTHERAPEUTIC RESISTANCE IN HEAD AND NECK CANCER STEM CELLS Arely WWW MD Myore Venue PhD Sare

**CANCER STEM CELLS** - <u>Arthur W Wu, MD</u>, Mysore Veena, PhD, Saroj Basak, PhD, Eri Srivatsan, PhD, Marilene B Wang, MD; UCLA Division of Head and Neck Surgery, Greater Los Angeles Veterans Administration Hospital Department of Surgery

Background: Head and Neck Squamous Cell Carcinoma (HNSCC) is a tumor made up of a heterogeneous population of cancer cells. CD44 is a cell surface marker highly expressed in putative cancer stem cells. Here, we demonstrate that fluorescence activated cell sorting (FACS) for the CD44 cell surface marker isolates a population of HNSCC cells with increased growth rate, colony forming ability, and chemoresistance. Methods: FACS was used to isolate CD44High and CD44Low cells from the HNSCC cell line SCC1 (U. of Michigan), derived from an aggressive oral HNSCC. CD44High, CD44Low and unsorted cells were compared with respect to cell growth using the MTT assay for 8 days. They were also seeded into agar and grown for 6 weeks to determine colony forming potential and tumorogenicity. Sorted and unsorted cells were also compared for their sensitivity to cisplatin. Live cells were measured using the MTT assay and compared to untreated cells at the same time point in order to calculate relative growth rates. Additionally, SCC1 cells were analyzed using FACS with and without cisplatin treatment to determine changes in the population of CD44High cells. Results: CD44High SCC1 cells grew the fastest in culture with CD44Low cells growing significantly slower. Unsorted cells had a similar growth rate compared to CD44+ cells, which is not surprising given the fact that CD44+ cells made up roughly 85% of the cell population in this cell line. CD44+ cells grown in soft agar demonstrated the presence of significantly more colonies and a larger average colony size after 6 weeks in culture compared to unsorted and CD44- cells. CD44+ SCC1 cells also were less sensitive to cisplatin with little difference in cell growth between treated and untreated cells. Using FACS to analyze unsorted cells after treatment with cisplatin, we observed enrichment in the CD44+ cell population. Conclusion: The cell

surface marker CD44 is expressed in a subset of HNSCC cells that have higher growth rates, tumorogenicity, and resistance to chemotherapy. These HNSCC stem cells may be the reason for resistance and recurrence. Understanding the mechanism behind their specific phenotype will be valuable in advancing treatments for HNSCC.

#### **P020:** BIOMARKERS IN LARYNGEAL DYSPLASIA - A SYSTEMATIC REVIEW AND META-ANALYSIS - <u>Paul Nankivell.</u> <u>Mr</u>, Matthew Weller, Mr, Christopher McConkey, Mr, Vinidh Paleri, Mr, Hisham Mehanna, Mr; Institute of Head and Neck Studies and Education (InHANSE)

Introduction: Laryngeal dysplasia is a pre-malignant condition. Currently, prediction of which cases will progress from dysplasia to cancer is poorly achieved. Histological grading is known to suffer from wide inter and intra-rater variability between pathologists and the natural history of the condition is poorly understood. The differential expression of certain biomarkers in dysplastic and cancerous lesions may help improve predictive ability. Aims: The aim was to perform a systematic review and meta-analysis on predictive and prognostic biomarkers in laryngeal dysplasia. Material: A systematic review and meta-analysis of published literature from 1966 to March 2010. Methods: Longitudinal, observational, case-controlled or randomised controlled trials of histologically confirmed laryngeal dysplasia cases were included. Both prospective and retrospective studies were included. Quality and confounding assessment was performed using the MINORS tool. Risk ratios and 95% confidence intervals (CI) were calculated for each biomarker. Where possible, data from individual studies was pooled and a meta-analysis performed. Results: The search protocol yielded 286 results. 9 studies met the inclusion criteria. 13 different biomarkers were assessed, with some studies examining multiple biomarkers. The commonest biomarkers assessed were p53 (5 studies), Ki67, and cyclin D1 (2 studies each). Risk ratios for ranged from 0.60 (95% CI 0.10, 3.75) to 6.60 (95% CI 0.43, 102.01). Cortacin appeared to have statistically significant predictive ability, with a risk ratio of 84.55 (5.30, 1348.56). A meta-analysis of studies using p53 failed to show this biomarker having a significant predictive ability. Data for the other biomarkers will be presented. Conclusions: Currently there is no good evidence for the use of biomarkers in predicting the future behaviour of laryngeal dysplastic lesions. Only three studies (one on p53 and one on cortacin/cyclin D1 and one on Ki67) showed statistically significant results. Better reporting of studies using the REMARK consensus guidelines should help to improve this in the future.

**P021:** DYNAMIC MOLECULAR INTERACTION BETWEEN PKCEPSILON AND RHOA IN LIVE CELLS - <u>Tizhi Su, PhD</u>, Samuel Straight, PhD, Liwei Bao, MD, Greg Cavey, PhD, Theodoros N Teknos, MD, Quintin Pan, PhD; Arthur G. James Cancer Hospital and Richard J. Solove Research Institute, The Ohio State University Comprehensive Cancer Center; University of Michigan Medical School; Van Andel Research Institute

Accumulating evidence has clearly defined the importance of PKCepsilon and small Rho GTPases in tumor metastasis. Previous work from our laboratory demonstrated that PKCepsilon signals through RhoA to modulate cell invasion and motility in head and neck squamous cell carcinoma. However, the molecular mechanism of PKCepsilon-regulated RhoA activation remains to be elucidated. Recombinant PKCepsilon directly phosphorylated recombinant RhoA. Phosphopeptide mapping using liquid chromatography-mass spectroscopy identified T127 and S188 as confident RhoA phosphorylation sites and T19 and S26 as tentative RhoA phosphorylation sites. Interestingly, recombinant PKCepsilon bound to recombinant RhoA in the absence of PKC activators and ATP.

This observation suggests that the association between PKCepsilon and RhoA does not require an active PKCepsilon conformation and perhaps these two proteins are pre-assembled in the cell prior to an activation signal. The pre-assembled kinase-substrate complex is an emerging concept in kinase biology and may be critical for sub-cellular trafficking of the phosphorylated substrate. We used time-lapse fluorescence microscopy and fluorescence resonance energy transfer (FRET) microscopy to examine the dynamic spatial and temporal interaction between PKCepsilon and RhoA in live cells. Activation of PKCepsilon resulted in a dramatic coordinated translocation of PKCepsilon and RhoA from the cytoplasm to the cell membrane using time-lapse fluorescence microscopy. FRET analysis revealed that the molecular interaction between PKCepsilon and RhoA is a biphasic event; an initial peak at the cytoplasm 90 seconds post-activation and a gradual prolonged increase at the cell membrane starting at 120 seconds post-activation to the end of the time-course (900 seconds). These results provide evidence that the PKCepsilon-RhoA complex is assembled in the cytoplasm and is subsequently translocated to the cell membrane in tandem. Taken together, our work demonstrates, for the first time, that PKCepsilon phosphorylates and intimately modulates the cell membrane translocation of RhoA.

#### **P022:** STRESS-RELATED NEUROHORMONAL MEDIATORS INFLUENCE THE HUMAN ORAL CANCER CELLS BEHAVIOR - Daniel G. Bernahé. PhD. Adriano C. Tamae, Glauco I.

**BEHAVIOR** - <u>Daniel G Bernabé, PhD</u>, Adriano C Tamae, Glauco I Miyahara, PhD, Éder R Biasoli, PhD, Sandra P Oliveira, PhD; Oral Oncology Center and Department of Basic Sciences, School of Dentistry of AraÇatuba, UNESP - Univ Estadual Paulista, AraÇatuba, SP, Brazil

Little is known about the interference of stress-related neurohormonal factors in the oral squamous cell carcinoma (OSCC). Interleukin-6 (IL-6) is a cytokine that plays an important role in progression of oral cancer. In this study, human OSCC cell lines SCC9 and SCC25 were used to evaluate the effects of stress-related mediators norepinephrine (NE), isoproterenol, and cortisol on IL-6 expression and secretion and cell proliferation. IL-6 expression was evaluated by real time PCR, and protein production was assessed by ELISA. The effect of hormones on cell proliferation was examined by MTT. Expression of B-adrenergic receptors (B-ARs) was studied by real time PCR in the cell lines, as well as in 20 samples of OSCC, 17 samples of leukoplakia, and 15 samples of normal oral mucosa. Real time PCR studies revealed constitutive ß1 and ß2-adrenergic receptors (ß-ARs) expression in the SCC9 and SCC25 cells. The results showed that NE and isoproterenol significantly enhanced IL-6 mRNA expression and protein production in supernatants of SCC9 and SCC25 cells. Physiological stress levels of NE and isoproterenol (10  $\mu$ M) at 1 hour elicited the most robust IL-6 increase. In these conditions, NE induced an increase of 501.5%  $\pm$  34.8% and 237.7%  $\pm$  37.6% in IL-6 mRNA expression in SCC9 (p<0.001) and SCC25 cells (p<0.05), respectively. Regarding IL-6 secretion, 10 µM NE induced a 5-fold increase at 1 hour, 3.7-fold increase at 6 hours and 3.2-fold at 24 hours in SCC9 cells. These effects were blocked by the B-adrenergic antagonist propranolol, supporting a role for B-ARs in IL-6 secretion. The effects of cortisol varied according to the cell line and hormone concentration. Pharmacological concentrations of cortisol (1000 nM) inhibited IL-6 production by SCC9 and SCC25 cells. Cortisol dose that simulates stress conditions (10 nM) tended to increase IL-6 expression in SCC9 cells. NE (10 µM, at 6 hours) and cortisol (1000 nM, at 48 hours) stimulated increase proliferation of SCC9 cells. All OSCC samples expressed B1 and ß2-ARs. Quantitatively, ß1-AR was more expressed in OSCC samples, whilst ß2-AR expression was lower in leukoplakia. These findings suggest that stress hormones can affect OSCC progression.

#### **P023:** CANCER STEM CELL EXPRESSION PATTERNS IN RECURRENT HEAD AND NECK SQUAMOUS CELL CARCINOMA - <u>Marcus M Monroe, MD</u>, Neil D Gross, MD, Wong H Melissa, PhD; Oregon Health and Science University

Objective: Cancer stem cells have recently been discovered to be an important subpopulation of cancer cells with increased tumorigenic, treatment resistance and metastatic potential. In head and neck squamous cell carcinoma (HNSCC) the ideal markers to identify these cells and to define their clinical significance are only just being elucidated. The objective of this study is to correlate expression patterns of subsets of cancer stem cell markers known to be important in epithelial malignancies across various stages of HNSCC. Methods: 40 paraffin-embedded samples of patients with head and neck squamous cell carcinoma undergoing potentially curative resection as well as site-matched non-tumor and normal controls were immunostained with antibodies raised against known cancer stem cell markers (CD44, CD133, CD166, CD24, LGR5, CD117). Expression patterns were evaluated using an automated open source image quantification program and correlated to clinical and histopathologic parameters. Results: Expression of cancer stem cell markers increased in advanced stage and recurrent HNSCC as compared with early stage disease. CD44 expression was noted in 13.7% of tumor cells in early stage HNSCC compared with 21.9% in advanced/ recurrent HNSCC. CD 133 and CD166 expression increased from 4.5% and 4% in early stage disease to 16.6% and 14.7% in advanced/recurrent disease respectively. A smaller subset of tumor cells co-expressed multiple cancer stem cell markers and similarly the percentage of co-expressing cells increased with advanced stage/recurrence. CD44 and CD166 coexpressing cells increased from 1.5% in early stage to 2.8% in advanced stage/recurrent disease. CD44 and CD133 co-expressing cells increased from 1.8% to 6.8 (p=0.05). Conclusion: In this study we describe the expression patterns of a subset of cancer stem cell markers (CD44, CD133, CD166, CD24, LGR5, CD117) in early stage, late stage, and recurrent HNSCC, site-matched adjacent controls and normal samples. Variable expression of cancer stem cell markers was seen across disease stage and in recurrence, highlighting the potential importance of these cells in defining HNSCC progression and treatment resistance.

#### **P024:** ARRAY COMPARATIVE GENOMIC HYBRIDIZATION TO DETECT GENOMIC ALTERATIONS IN HEAD AND NECK SQUAMOUS CELL CARCINOMA - <u>Min-Sik Kim, MD, PhD</u>, Young-Hoon Joo, MD, Kwang-Jae Cho, MD, PhD, Jun-Ook Park, MD; Seoul St. Mary's Hospital, The Catholic Universijty of Korea, Seoul, Korea

Objective: Although genetic alteration in head and neck squamous cell carcinoma (HNSCC) has been observed, its clinical significance has not been completely investigated. This study was aim to investigate genomic aberration across whole genome that predict the clinical outcome of head and neck squamous cell carcinoma (HNSCC). Materials and Methods: Surgical specimens are from 38 patients who underwent surgical resection due to primary squamous cell carcinoma of head and neck. One-megabase resolution array comparative genomic hybridization was applied. Tumor and normal tissues were microdissected and the extracted DNA was used for hybridization. The recurrent genomic alterations were analyzed for their association with clinicopathological features. Result: 38 samples were from tumors of the oral cavity, sinonasal cavity, larynx, hypopharynx, and oropharynx. 20 amplicons, 5 homozygous deletions, and 38 minimally altered regions common to HNSCC were identified. Among them, chromosomal profile of DNA copy number was characterized by frequently detected, gains at 6q12(35/38, 92.10%) and losses at 6q16.1(34.21%), 21q21.1(36.84%). Genomic loss on 4q13.2 was found to be significantly associated with disease recurrence. Also, loss of 6q16.1

was found to be associated with early nodal metastasis. Conclusion: This study demonstrated that high resolution array CGH could define more novel regions possibly associated with the poor prognosis of HNSCC. These results will give a clue for further studies to elucidate HNSCC pathogenesis or to develop biomarkers for predicting the prognosis or treatment response.

#### **P025:** IN VIVO NEAR-IR IMAGING WITH QUANTUM DOTS ENTRAPPED IN PLGA NANOSPHERES - <u>Kwang Jae Cho</u><sup>1</sup>, MD Your June Lo<sup>2</sup>, PhD Doug HS and MD Your Helt Pack MD MD.

MD, Yong-kyu Lee<sup>2</sup>, PhD, Dong Il Sun<sup>1</sup>, MD, Young Hak Park<sup>1</sup>, MD, Min Sik Kim<sup>1</sup>, MD; <sup>1</sup>Department of Otolaryngology-Head & Neck Surgery, The Catholic University of Korea, Seoul, Korea; <sup>2</sup>Department of Chemical and Biological Engineering, Chungju National University, Chungju, Korea

Introduction: Quantum dots (Nanometer-scale semiconductor nanocrystals, QDs) have attracted significant attentions during the last decades because they can dramatically improve the use of fluorescent markers in biological imaging. Purpose: In this study, we synthesized water-soluble and biocompatible QDs nanospheres and investigated its availability for in vivo imaging. Material and Methods: Luminescent Near-IR CdTe/CdSe QDs were synthesized and encapsulated in PLGA nanospheres to prepare water-soluble and biocompatible QDs nanospheres. QDs were encapsulated with PLGA nanospheres by a solid dispersion method and optimized to have high fluorescence intensity for in vivo imaging detection. The resultant PLGA nanospheres with QDs were characterized by various analytical techniques such as UV-Vis measurement, light scattering, fluorescence spectroscopy, transmission electron microscopy (TEM) and atomic forced microscopy (AFM). Finally, we also evaluated toxicity and body distribution of QDs loaded in PLGA nanospheres in vitro and in vivo, respectively. Results: The QDs loaded in PLGA nanospheres were spherical and showed a diameter range of 100-150 nm in size. The QD nanospheres increased their stability against photooxidation and photobleaching, which have the high potential for applications in biomedical imaging and detection. We have also attained noninvasive in vivo imaging with light photons represents an intriguing avenue for obtaining biological information by the use of Near-IR light. Conclusions: Our results demonstrate the potential applications of QDs loaded in PLGA nanospheres for the detection of disease including tumors using in vivo imaging method.

#### **P026:** A CRITICAL ROLE OF C-CBL INTERACTING PROTEIN OF 85 KDA IN THE DEVELOPMENT AND PROGRESSION OF HEAD AND NECK SQUAMOUS CELL CARCINOMAS VIA THE RAS-ERK PATHWAY - <u>Takabiro</u> <u>Wakasaki, MD</u>, Muneyuki Masuda, PhD, MD, Hiroaki Niiro, PhD, MD, Shizuo Komune, PhD, MD; Department of Otorhinolaryngology, Graduate School of Medical Sciences, Kyushu University

Activation of the transforming growth factor (TGF)-alpha epidermal growth factor receptor (EGFR)-mediated signaling pathway is a common mechanism for dysregulated growth of head and neck squamous cell carcinoma (HNSCC). c-Cbl interacting protein of 85 kDa (CIN85) is an adaptor protein which facilitates EGFR internalization. Little is known, however, about a role of CIN85 in EGFR signaling as well as its relevance to tumor development and progression of HNSCC. Here we demonstrate that CIN85 is highly expressed in HNSCC tumor samples compared to adjacent normal tissues and this overexpression is significantly correlated with advanced clinical stage. The experiments using CIN85overexpressing and knockdown HNSCC cell lines showed that CIN85 promotes HNSCC growth and facilitates EGFR internalization without apparently affecting phosphorylation of EGFR. Moreover, CIN85 promoted TGF-alpha-induced activation of Ras and phosphorylation of downstream molecules such as c-Raf, MEK, and ERK, leading to expression of c-Myc that is critical for sustained proliferation of HNSCC. Taken together, these findings suggest that CIN85 not only controls EGFR internalization but also promotes the EGFR-mediated tumor development and progression, and thus CIN85 may serve as a potential therapeutic target in a subset of HNSCC.

#### **P027:** PROGRESSIVE TUMOR FORMATION IN MICE WITH CONDITIONAL DELETION OF TGF-BETA SIGNALING IN HEAD AND NECK EPITHELIA IS ASSOCIATED WITH ACTIVATION OF THE PI3K/AKT PATHWAY - <u>Yansong Bian</u>, <u>MD</u>, PhD, Carter Van Waes, MD, PhD, Ashok Kulkarni, PhD; National Institute on Deafness and Other Communication Disorders

The precise role of transforming growth factor (TGF)-\_ signaling in head and neck squamous cell carcinoma (SCC) has not been fully understood. The results from our previous study indicated that defects in the TGF-\_ signaling pathways are common in human HNSCCs. Here, we report generation of an inducible head- and neck-specific knockout mouse model by crossing TGF-\_ receptor I (Tgfbr1) floxed mice with K14-CreERtam mice. By applying tamoxifen (TM) to the mouse oral cavity to induce Cre expression, we were able to conditionally delete Tgfbr1 in the mouse head and neck epithelia. On tumor induction with 7, 12-dimethylbenz(\_) anthracene (DMBA), 45% of Tgfbr1 conditional knockout (cKO) mice (n=42) developed squamous cell carcinomas (SCCs) in the head and neck area starting from 16 weeks after treatment. However, no tumors were observed in the control littermates. A molecular analysis revealed an enhanced proliferation and loss of apoptosis in the basal layer of the head and neck epithelia of Tgfbr1 cKO mice after DMBA treatment. The most notable finding of our study is that the phosphoinositide 3-kinase (PI3K)/Akt pathway was activated in SCCs that developed in the Tgfbr1 cKO mice on inactivation of TGF-\_ signaling through Smad2/3 and DMBA treatment. These observations suggest that activation of Smadindependent pathways may contribute cooperatively with inactivation of Smad-dependent pathways to promote head and neck carcinogenesis in these mice. Our results revealed the critical role of the TGF-\_ signaling pathway and its crosstalk with the PI3K/Akt pathway in suppressing head and neck carcinogenesis.

#### **P028:** MET SIGNALING AS A THERAPEUTIC TARGET FOR HEAD AND NECK CANCERS - Xinrong Tao, PhD, Peter Szaniszlo, PhD, Susan Fennewald, PhD, Vicente Resto, MD, PhD, <u>Lisa A Elferink, PhD</u>; University of Texas Medical Branch

Objective: Head and Neck Squamous Cell Carcinoma (HNSCC) is characterized by a high degree of metastasis to cervical lymph nodes, correlating with an aggressive phenotype and poor patient prognosis. Increased signaling via Hepatocyte Growth Factor (HGF) and its tyrosine kinase receptor MET is a common event in HNSCC that could enhance tumor cell migration and metastasis and as such, may represent important therapeutic targets. The purpose of this study is to test the hypothesis that MET signaling is involved in the progression and spread of HNSCC. Methods: We used a lentiviral system for RNA interference to target the MET oncogene in established HNSCC cell lines, which like many primary and metastatic tumors express high levels of endogenous MET protein. The effect of MET silencing on in vitro cell proliferation, migration and survival was examined. Results: We show that silencing Met expression by RNA interference 1) impaired activation of downstream MAPK signaling, 2) reduced the capacity of Met knockdown cells to grow in an anchorage independent manner and 3) resulted in the suppression of HGF-induced cell motility. Using an orthotopic mouse model for HNSCC, we are currently examining the in vivo effect of Met

knockdown on primary tumor growth, incidence, metastasis and survival. Conclusions: Our findings indicate an important role for Met signaling for HNSCC growth and dissemination, and provide a strong rationale for targeting the MET/HGF signaling axis to treat Head and Neck Cancers.

### **P029:** DELTANP63 VERSATILELY REGULATES A BROAD NF-KAPPAB GENE PROGRAM AND PROMOTES SQUAMOUS CELL PROLIFERATION, MIGRATION AND

**INFLAMMATION** - <u>Xinping Yang, MD</u>, Hai Lu, MD, PhD, Bin Yan, PhD, Rose-Anne Romano, PhD, Jay Friedman, PhD, Praveen Duggal, MD, Clint Allen, MD, Ryan Chuang, Reza Ehsanian, Satrajit Sinha, PhD, Carter Van Waes, MD, PhD, Zhong Chen, MD, PhD; Tumor Biology Section, Head and Neck Surgery Branch, National Institute on Deafness and Other Communication Disorders, NIH

Head and neck squamous cell carcinoma (HNSCC) and many epithelial malignancies exhibit increased proliferation, invasion and inflammation, concomitant with aberrant nuclear activation of TP53 and NFkappaB family members deltaNp63, c-REL and RELA. However, the mechanisms of crosstalk by which these transcription factors coordinate gene expression and the malignant phenotype remains elusive. Here we demonstrate that deltaNp63 regulates a cohort of genes involved in cell growth, survival, adhesion and inflammation, which substantially overlaps with the NF-kappaB transcriptome. deltaNp63, c-REL and/or RELA are recruited and together exhibit novel binding to p63 or NF-kappaB sites of target gene promoters. Overexpressed \_\_p63- or TNF-\_-induced NF-kappaB and IL-8 reporter activation depend upon RELA or c-REL regulatory binding sites. Modulation of deltaNp63 significantly altered proliferation, survival, and migration by HNSCC cells in vitro. Consistent with the above, \_Np63 co-localizes with markers of proliferation (Ki67), adhesion (\_4 integrin), and inflammatory cell infiltration in HNSCC specimens. Overexpression of \_Np63\_ in transgenic mice leads to epidermal hyperplasia and skin inflammation concomitant with increased c-REL and target cytokines, similar to HNSCC. Our study reveals deltaNp63 as a master transcription factor that in coordination with NF-kappa\_, orchestrates a broad gene program promoting epidermal hyperplasia, inflammation, and the malignant phenotype of HNSCC.

#### **P030:** EVALUATION OF MULTIPLE HEAD AND NECK CANCER CELL LINES FOR MULTI-CELLULAR SPHEROID FORMATION - <u>Alice Tang, BA</u>, Dang Vu-Phan, Carol R Bradford, MD, Thomas E Carey, PhD, Mark E Prince, MD Department of Otolaryngology: Head & Neck Surgery, University of Michigan, Ann Arbor, MI

Objectives: Three-dimensional cultures offer an alternative to monolayer cultures to recapitulate the in vivo microenvironment. Few studies have investigated spheroids from head and neck squamous cell carcinoma (HNSCC) cell lines, yet this model is becoming increasingly important in other malignancies. Our aim was to determine if cell lines are able to form spheroids and if spheroids demonstrate cancer stem cell (CSC) characteristics. Methods: Six cell lines were evaluated for spheroid formation: UMSCC-6, -29, -47, -38, -74B and UDSCC-2. The conditions tested include serum-free media with or without supplements (B27, bFGF, EGF) and commercially available ultra-low attachment plates (Corning) or agarose-coated plates. Limiting dilution experiments for UMSCC-29, -47, and -74B were performed to determine minimum cell number threshold for spheroid formation and self-renewal capacity of single cells. Spheroids were placed in adherent conditions in serumcontaining media to establish if cells could differentiate. Two cell lines underwent fluorescent-activated cell sorting for ALDH to determine if CSCs (ALDH+) were enriched when maintained in suspension. Results: Five cell lines, UMSCC-6, -29, -47, -38 and -74B were able to form

compact spheroids, while UDSCC-2 formed loose cellular aggregates. Cell lines had similar spheroid formation efficiency with or without media supplements, contrary to other malignancies where B27, bFGF, and EGF greatly promoted spheroid growth. No differences were seen between the low-attachment plates used. When cells were seeded at densities of 80,000, 50,000, 10,000 cells/mL, spheroids formed. However, <2500 cells/mL and single cells failed to produce spheroids after 30 days of culture. Preliminary data showed UMSCC-29 in suspension enriched CSC from 4.71% to 8.88% ALDH+, while UMSCC-38 showed no difference (2.87% vs 2.74%). After cells were sorted into ALDH+ and ALDH- subpopulations, no difference in spheroid formation was observed. Conclusions: Head and neck cancer cell lines that are the cornerstone of basic science research have the ability to generate and be maintained as multi-cellular tumor spheroids. Because this system more closely mimics physiologic conditions than monolayer cultures, this model should be incorporated into routine investigations. Further studies are planned to test conditions that would enrich CSCs in spheroids and validate if spheroids exhibit distinctive CSC behavior.

**P031:** MIR-107 REGULATES PKCEPSILON AND INHIBITS THE TUMORIGENICITY OF HEAD AND NECK SQUAMOUS CELL CARCINOMA CELLS - <u>Jharna Datta, PhD</u>, Mozaffarul Islam, PhD, Theodoros N Teknos, MD, Quintin Pan, PhD; Department of Otolaryngology-Head and Neck Surgery, The Ohio State University Medical Center; Arthur G. James Cancer Hospital and Richard J. Solove Research Institute, The Ohio State University Comprehensive Cancer Center

Head and neck squamous cell carcinoma (HNSCC) is the sixth most prevalent cancer worldwide with about 600,000 new cases diagnosed in the last year. The 5-year survival rate of HNSCC has remained stagnant despite advances in the clinical management of this disease. Understanding the molecular pathways that lead to aggressive HNSCC is crucial to identify new "druggable" targets for anti-cancer drug development. Protein kinase Cepsilon (PKCepsilon) is elevated in HNSCC and regulates cell invasion and motility thorough activation of Rho GTPases. Our laboratory and others provide evidence that PKCepsilon phosphorylates and modulates a cascade of signal transduction pathways, including Akt, EGFR, Rho GTPases, and Stat3. Thus, targeting PKCepsilon may be an attractive therapeutic approach to dampen multiple hyper-active signaling pathways in concert. At this time, the molecular mechanism of PKCepsilon dysregulation in HNSCC remains to be elucidated. In silico analysis with TargetScan, MiRBase, and miRanda identified numerous putative miR binding sites in the 3'-UTR region of PKCepsilon. We screened a panel of miRs using qPCR and miR-107 was identified to have an inverse association with PKCepsilon in HNSCC cell lines (SCC15, SCC25, and CAL27) with high endogenous PKCepsilon. Ectopic expression of miR-107 in SCC15, SCC25, and CAL27 cells significantly reduced PKCepsilon mRNA expression and protein levels. The expression of a luciferase reporter construct containing the 3'-UTR of PKCepsilon was down-regulated by miR-107. Deletion of the three cognate miR-107 binding sites on the 3'-UTR of PKCepsilon completely abolished the regulation by miR-107. These observations confirm PKCepsilon as a validated target of miR-107. Ectopic expression of miR-107 in CAL27 cells inhibited cell proliferation (35% inhibition, p<0.05), DNA replication (60% inhibition, p<0.005), clonogenic survival (55% inhibition, p<0.005), invasion (80% inhibition, p<0.005), and migration (55% inhibition, p<0.005). Our data indicate that miR-107 decreases PKCepsilon levels and dramatically reduces the tumorigenicity of HNSCC cells. Further work on the potential application of miR-107 as a therapeutic drug to suppress PKCepsilon in HNSCC is warranted.

**P032:** P38 DOWNREGULATES E-CADHERIN IN HNSCC IN A SNAIL INDEPENDENT MANNER - Guanyu Wang, MD, PhD, Jie Luo, MS, David Hu, MD, Qahera Munaim, BS, Steven Dubinett, MD, <u>Maie</u> <u>St. John, MD, PhD</u>; University of California, Los Angeles

Objectives: Understanding the molecular mechanisms that mediate HNSCC metastasis may enable identification of novel therapeutic targets. We recently reported the role of the E-cadherin transcriptional repressor, Snail, in the inflammation-induced promotion of EMT in HNSCC. Herein we demonstrate that inflammatory mediators also upregulate p38, thus further defining the cycle by which inflammation promotes tumor progression. Methods: A molecular biology study. Real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR), Western blot analysis, and 3-D spheroid culture were used to determine how inflammation affects p38 and EMT. Results: Treatment of HNSCC cells with the p38 inhibitor, SB203580, caused the upregulation of E-cadherin without affecting Snail levels. ShRNAmediated knockdown of Snail did not affect the capacity of SB203580 to upregulate E-cadherin. SB203580 treated HNSCC cells were able to form compact spheroids in three- dimensional culture in comparison to the invasive appearance of untreated HNSCC cells. An inverse relationship between p38 and E-cadherin was also demonstrated by immunoflourescence. We show for the first time in HNSCC that inflammatory mediators upregulate p38; p38 does not affect Snail expression or the normal repression of E-cadherin transcription. Conclusion: Our results suggest that two pathways - Snail and p38 – act independently and converge on E-cadherin expression: one at the transcriptional level and one at the protein level to ensure the rapid down-regulation of E-cadherin and a robust EMT. Our findings support previous work demonstrating that p38 is required in gastrulation to downregulate E-cadherin. This newly defined pathway for transcriptional regulation of E-cadherin in HNSCC has important implications for targeted chemoprevention and therapy.

#### **P033:** INSIGHTS INTO HEAD AND NECK CANCER FROM PROTEOMIC AND TRANSCRIPTOMIC ANALYSIS OF P53 GAIN OF FUNCTION MUTATIONS - Anna Behrendt, BS, Nikolina Vlatkovic, PhD, Carlos P Rubbi, PhD, Rosalind E Jenkins, PhD, Kevin Park, PhD, Bryony H Lloyd, PhD, Ross Sibson, PhD, Terence M Jones, MD,

Park, PhD, Bryony H Lloyd, PhD, Ross Sibson, PhD, Terence M Jones, MD, <u>Mark T Boyd, PhD</u>: University of Liverpool and University Hospital Aintree, Liverpool UK Background: In addition to loss of function p53 mutation can result in oncogenic gain-of-function (GOF). p53 GOF mutants likely act

through altered transcriptional activity and/or novel protein-protein interactions. Recent reports indicate that p53 GOF mutants promote TGFB-dependent metastatic potential via inhibition of p63 function and also promote invasion through increased integrin (a5ß1) and EGFR recycling, again linked with suppression of transcriptionally active TAp63. To characterise the mechanism/s of action of p53 GOF mutant in head and neck cancers, proteomic and transcriptomic analyses were performed in laryngeal squamous cell carcinoma (LSCC) cells expressing two GOF mutants representing two distinct mutational classes: structural p53-R175H and contact p53-R273H. Methods: Tandem affinity chromatography, mass spectrometry, immunoprecipitation, AffymetrixTM-exon-array analyses, live-cell and Boyden chamber motility assays. Results: p53-R273H-expressing (but not p53-R175H) LSCC cells display increased motility in live cell imaging and in Boyden chambers. Exon-array analyses reveal multiple differentially regulated pathways in R273H-expressing cells. The most significantly altered pathways determine cell adhesion and extra cellular matrix re-modelling (P<0.000001). These cells express

predominantly dominant-negative deltaNp63 and thus the R273H mutant appears to act via an alternative pathway in LSCC cells, there being no TAp63 activity to suppress. A number of known regulators of motility/invasion/metastasis have been identified in this analysis and are currently undergoing validation and selected genes will be subjected to functional examination. In addition we have identified several proteins in p53-R175H complexes including Hsp70, which have been confirmed by immunoprecipitation to selectively interact with the structural p53-R175H mutant and not with the p53-R273H contact mutant. Results from these studies will also be presented. Conclusions: We have identified proteins that co-purify selectively with mutant p53-R175H and also genes that are differentially expressed in cells expressing mutant p53-R273H. Our results suggest that in LSCC cells, p53 GOF mutants may act via different pathways than those previously identified in other cancers and moreover it appears there may be differences in the modes of action of structural and DNA-contact mutants. Functional analysis of these pathways should provide insights into the mechanism/s of action of two classes of p53 GOF mutants in LSCC and may lead to the identification of novel therapeutic targets.

#### **P034:** CYTOLOGY BASED COMPARISON OF DIFFERENTIAL GENE EXPRESSION BETWEEN ORAL CANCER AND NON CANCEROUS MUCOSAL LESIONS Antonia Kolokythas, DDS, Joel Schwartz, DMD MS, Guy Adami, PhD; University of Illinois at Chicago

Introduction: Oral squamous cell carcinoma is among the top ten epithelial malignancies in the United States and worldwide. Histologic confirmation with biopsy remains the gold standard for diagnosis of the initial disease as well as evaluation of suspicious lesions during the follow up period. Several limitations in obtaining accurate diagnosis are often encountered including inability to confirm the presence of malignancy. A non-invasive method of tissue sampling and analysis beyond microscopic examination is thus highly desirable. RNA analysis for identification of genes that are shown to be differentially expressed in squamous cell carcinoma can be used perhaps more reliably, than traditional methods, for detection of cancer. We aim to demonstrate that RNA can be accurately isolated from epithelial cells obtained from brush cytology and gene expression analysis can be accomplished in a reproducible fashion. Materials and Methods: Brush cytology samples were obtained from oral squamous cell carcinomas of 12 male patients with biopsy proven disease as well as from non-malignant lesions of age and gender matched controls. Results: High quality mRNA was isolated from the epithelial cells obtained from the cancer as well as the control group that allowed for gene expression analysis. mRNA from brush cytology samples obtained from the cancer group demonstrated statistically significant enrichment of KRT17 gene, among others, compared to the control group. KRT17 has been demonstrated to be highly enriched in surgically derived tissue from oral cancers compared to controls. Conclusions: We were able to use epithelial cells obtained non-invasively and obtain high quality mRNA for accurate gene expression analysis. Furthermore we were able to demonstrate statistically significant difference in expression of KRT17, between samples from malignant lesions obtained from cancer patients and those from non-malignant lesions of the non cancer- control group. Efforts are underway to identify additional OSCC markers detectable by this assay. **P035:** TKTL1 CONTRIBUTES TO CELL PROLIFERATION IN MINIMALLY TRANSFORMED ORAL KERATINOCYTES VIA INCREASED AEROBIC GLYCOLYSIS, ELEVATED ROS PRODUCTION AND HIF1A ACCUMULATION - <u>Wenyue Sun</u>, <u>PhD</u>, Yan Liu, PhD, Chunbo Shao, PhD, David Zaboli, MD, Patrick Ha, MD, Joseph Califano, MD; Johns Hopkins Medical Institutions and Milton J. Dance Head and Neck Center, Greater Baltimore Medical Center, Baltimore, Maryland

Transketolase 1 (TKTL1) encodes a member of the pentose phosphate pathway, which allows for glucose conversion to ribose for nucleic acid synthesis as well as glucose degradation to lactate controlled by transketolase enzyme reactions. Aberrant expression of TKTL1 has been reported in head and neck squamous cell carcinomas (HNSCC), yet its functional significance in HNSCC development remains to be defined. Previously, we reported that TKTL1 is activated by promoter hypomethylation and contributes to HNSCC carcinogenesis through increased aerobic glycolysis and Hypoxia inducible factor 1 alpha subunit (HIF1\_) stabilization. It is suggested that expression of HIF1\_ is an early event in oral cancer carcinogenesis. In the present study, to define the function of TKTL1 in HNSCC initiation, we overexpressed TKTL1 in human minimally transformed oral keratinocyte OKF6 cells. Our results showed that overexpression of TKTL1 promoted cell proliferation in OKF6 cells that is reversible by a glycolytic inhibitor 2-deoxyglucose. We also showed increased lactate production (an aerobic glycolytic metabolite) and HIF1\_ expression in TKTL1-expressing OKF6 cells. By comparison of the expression profiles between TKTL1- and vectorexpressing OKF6 cells, n-Myc (NMYC), hexokinase 3 (HK3) and lactate dehydrogenase (LDHA) were found overexpressed in TKTL1-expressing OKF6 cells. In addition, we found that the production of reactive oxygen species (ROS) was significantly increased in TKTL1-expressing OKF6 cells in comparison with that in the vector-expressing cells. Collectively, our study suggests that TKTL1 may contribute to HNSCC initiation through elevated aerobic glycolytic metabolites and the resultant HIF1\_ accumulation. It is likely that TKTL1 upregulates MYC expression, that in turn unregulates HK3 and LDHA expression, thereby elevating lactate production as well as HIF1\_ accumulation and growth promotion. Since it is known that ROS regulates HIF1\_ mechanically, TKTL1 might also increase HIF1\_ accumulation through increased ROS production.

#### **P036:** DYSREGULATION OF MICRORNA-34A EXPRESSION IN HEAD AND NECK SQUAMOUS CELL CARCINOMA PROMOTES TUMOR GROWTH AND TUMOR

**ANGIOGENESIS** - Bhavna Kumar, MS, Arti Yadav, MS, Theodoros N Teknos, MD, <u>Pawan Kumar, MS, PhD</u>; Department of Otolaryngology - Head and Neck Surgery, The Ohio State University Comprehensive Cancer Center, Columbus, OH

Head and Neck squamous cell carcinoma (HNSCC) results from deregulation of multiple signaling pathways leading to tumor cells acquiring proliferative, invasive, angiogenic and metastatic properties. Interactions between neoplastic cells and the surrounding microenvironment also play a major role in tumor progression. Recent studies have demonstrated that microRNA's (miRs) play a critical role in cancer development where they can act as oncogenes or as oncosuppressors. A microRNA array analysis of a panel of ten HNSCC cell lines reveled that miR-34a is significantly downregulated in all these cell lines as compared to normal human oral keratinocyes (HOK). In addition, miR-34a expression was also downregulated in highly angiogenic endothelial cells (endothelial cells overexpressing Bcl-2; EC-Bcl-2) as compared to normal human endothelial cells. However, little is known about the role of miR-34a in HNSCC tumor growth and tumor angiogenesis. In this study, we examined if dysregulation of miR-34a in HNSCC promotes tumor growth and tumor angiogenesis. The study was performed using a panel of established human head & neck cancer cell lines. miRs expression in tumor and endothelial cells was examined by miR-arrays and further validated by real time PCR. Lipofectamine-2000 was used to transfect miR-34a in HNSCC cell lines and human endothelial cells. Cell-proliferation assay was performed by MTT and colony formation assay. Tumor cell and endothelial cell motility was assessed by scratch assay. Expression of miR-34a downstream mediators was measured by Western blot. Ectopic expression of miR34a significantly inhibited HNSCC cell proliferation. In addition, enhanced expression of miR34a in HNSCC cells significantly inhibited tumor cell colony formation and tumor cell migration. Similar to tumor cells, miR-34a significantly inhibited EC-Bcl-2 proliferation and migration by down regulating c-met, Bcl-2 expression and upregulating phosphor p38 MAPK expression. We further examined the effect of miR-34a on tumor growth and tumor angiogenesis using a SCID mouse xenograft model. Ectopic expression of miR-34a significantly inhibited tumor growth and tumor angiogenesis. Taken together, these findings suggest that dysregulation of miR-34a expression is common in HNSCC and modulation of miR34a activity might represent a novel therapeutic strategy for the treatment of head and neck cancers.

#### **P037:** IDENTIFICATION AND CHARACTERIZATION OF A METASTATIC HEAD AND NECK SQUAMOUS CELL CARCINOMA LINE - <u>John Henry J Owen</u>, Samantha J Davis, Alice Tang, Martin P Graham, Michael J Czerwinski, Silvana Papagerakis, Carol R Bradford, Thomas E Carey, Mark E Prince; University of Michigan

Objectives: Developing a library of immortalized cell lines can provide a useful tool for studying the diversity of primary tumors. A highly aggressive HNSCC of the oral cavity was diagnosed in a twenty-six-yearold pregnant woman with a low risk factor profile. The patient developed bilateral lung metastasis and unfortunately died despite aggressive therapy. The tumor was cultured to establish a unique cell line with high invasive potential. Cancer stem cell characteristics were studied both in vitro and in vivo. Methods: Cells from primary tumor, HN-111, were analyzed for expression of the surface marker CD44 by flow cytometry to evaluate and isolate the cancer stem cell (CSC) population. CD44  $^{\rm high}$  and CD44  $^{\rm low}$ fractions were injected into the tail veins of immunodeficient mice, with the lungs harvested at six months to evaluate for metastases. Cells from the primary tumor were serially passaged and genotyped as a unique cell line, UMSCC-103. The newly established cell line was evaluated for the presence of cancer stem cells using CD44 and ALDH expression. Preparations of the primary tumor, murine metastases, and cultured cells were immunohistochemically evaluated. Results: HN-111 contained a CD44<sup>high</sup> population of 17.5%. Using a lung colonization model, cells within this population formed in distant metastases in 2/2 mice, with one mouse developing a large abdominal tumor. CD44<sup>low</sup> injections failed to form metastasis. CD44<sup>high</sup> cells constituted 16.1% of UMSCC-103. The proportion of CD44<sup>high</sup> cells was unchanged from the primary tumor to the cell line. The cell line CD44<sup>high</sup> subpopulation of cells contained 19.14% ALDH positive cells. Cells within the CD44<sup>high</sup> fraction were able to form distant metastases while CD44<sup>low</sup> cells were not. The primary tumor, resultant cell line and metastasis originating from the CSC were histologically similar. Conclusion: The original cancer and the resultant cell line are highly similar in their appearance, behavior and cancer stem cell content. The development of cell lines from patients with unique characteristics, in this case young age, lack of risk factors, pregnant and with a high metastatic potential, will greatly increase our ability to study and understand this deadly disease.

#### **P038:** C-MYC AND TGFBETA COOPERATION LEADS TO SPONTANEOUS HEAD AND NECK TUMOR FORMATION IN THE MOUSE - <u>Vivian F Wu, MD, MPH</u>, Xiao-Yan Wang, PhD, Molh Wilson Smith David Savar, MD, Xiao Jing Wang, MD, PhD, Pacali

Molly Wilson-Smith, David Sauer, MD, Xiao-Jing Wang, MD, PhD, Rosalie C Sears, PhD; Departments of Otolaryngology-Head and Neck Surgery, Pathology and Molecular and Medical Genetics, Oregon Health and Science University; Department of Pathology, University of Colorado Denver Health Sciences Center

Background: c-Myc protein is highly expressed in approximately 70% of human tumors, including head and neck squamous cell carcinoma (HNSCC). Phosphorylation at conserved sites Serine62 and Threonine58 regulate c-Myc protein turnover. We predict that aberrant phosphorylation of c-Myc is a major mechanism for c-Myc overexpression in cancer and that cooperating factors, such as TGFB, may enhance the oncogenic potential of deregulated c-Myc. Methods: We have developed a conditional, inducible head and neck specific system that consists of three mouse lines: K5-CrePR1 (where Cre recombinase is activated by application of a subpharmacologic dose of RU486 via oral gavage), TGFBRII<sup>#/#</sup> and Rosa flox stop (RFS)-c-Myc<sup>WT</sup> or the phosphorylation mutant RFS-c-Myc<sup>T58A</sup>. These lines were cross-bred to generate compound mice that allow constitutive expression of c-MycWTor<sup>T58A</sup> and homozygous deletion of TGFBRII. Results: Strong cooperation was observed between induced c-MycWT expression and TGFBRII loss, leading to development of HNSCC to near complete penetrance (mean survival 5.5mo). Interestingly, cooperation was severely dampened between c-Myc<sup>T58A</sup> and TGFßRII loss, (2 cases of oral tongue SCC have developed in 11mo). Control c-Myc<sup>WTorT58A</sup> and no Cre animals have not generated tumors. SCCs confirmed by Hematoxylin/Eosin stains include hard palate, hypopharynx, oral tongue (2), anterior FOM, nasal cavity (4), and pinna (3). Immunofluorescence indicates overexpression of total c-Myc oncoprotein in tumor samples with increased pS62, decreased pT58 and inflammatory markers in tumor compared to adjacent tissue. Conclusions: Our data suggests that the pro-tumorigenic environment created by deletion of TGFBRII facilitates c-Myc oncogenic activity. It is also possible that TGFß enhances the deregulated pS62 form of c-Myc allowing more permissive access to Myc target genes. Understanding the role of c-Myc phosphorylation patterns and the effects of TGFß will provide better strategies for creating targeted therapies for HNSCC.

### **P039:** A C57BL/6 SYNGENEIC MOUSE MODEL OF ORAL CAVITY SQUAMOUS CELL CARCINOMA - <u>Nancy Judd, MD</u>.

Joshua Brotman, Clint Allen, MD, Ashley Winkler, Ravindra Uppaluri, MD, PhD; Washington University School of Medicine in St Louis

Objectives: Despite the clinical incidence of and broad research efforts in oral cavity squamous cell carcinoma (OSCC), there are few tractable pre-clinical syngeneic models of this disease. We thus developed several new murine C57BL/6 oral cancer cell lines that (a) serve as robust models as they parallel human OSCC, (b) enable detailed study of immune responses to OSCC to model pre-clinical immunotherapy studies in C57BL/6 mice where a broad range of gene targeted mice are available. Methods: We generated primary OSCCs by serial application of 7,12-dimethylbenz[a]anthracene on oral mucosa. Four cell lines (OSCC1, OSCC2, OSCC7 and OSCC10) were derived from independent primary tumors and were transplanted to the flanks of C57BL/6 mice to study growth phenotypes and anti-tumor immune responses. In vitro, these cell lines were characterized by assessing known signaling cascades and by monitoring invasiveness. Results: All cell lines formed progressively growing tumors when transplanted into wildtype mice. Analyses of immune responses in tumor bearing mice showed a large infiltrate of Gr1+/CD11b+ myeloid derived suppressor phenotype cells in both the tumor microenvironment and also in tumor bearing draining lymph

nodes. We then chose to compare OSCC1 and OSCC2 as the former displayed indolent growth and the latter was more aggressive with lymph node metastatic capacity. Interrogation of several canonical tumorigenic signaling pathway components—including STAT3, AKT, NF\_\_, c-MET, TGF\_ and MAPK— revealed that Extracellular Receptor Signaling Kinase 1 and 2 (ERK1/2) were selectively overexpressed in OSCC2. Treatment with U0126, a specific MEK1/2 inhibitor, resulted in significant inhibition of in vitro migration and Matrigel invasion of OSCC2. Conclusions: We have established 4 new C57BL/6 OSCC cell lines using chemical carcinogenesis that will allow us to assess both the natural host immune responses and intracellular pathways contributing to tumor growth and metastasis. This approach represents a tractable preclinical model that may be useful for the in vivo study of small-molecule as well as immune-based pharmacotherapies.

# **P040:** CCND1 AMPLIFICATION AND PROTEIN OVEREXPRESSION IN ORAL SQUAMOUS CELL

**CARCINOMA OF YOUNG PATIENTS** - Estela Kaminagakura, DDS, PhD, Isabela Cunha, MD, PhD, Fernando Soares, MD, PhD, Inês Nishimoto, PhD, <u>Luiz Paulo Kowalski, MD, PhD</u>; A.C. Camargo Hospital, São Paulo, Brazil

Objectives: to evaluate correlation with CCND1 amplification and protein overexpression with clinicopathological features and clinical outcomes in patients younger than 41 years with oral squamous cell carcinoma (SCC). Methods: 87 oral SCC from young patients were evaluated using the Tissue Microarray (TMA) technique, immunohistochemistry and fluorescence in situ hybridization (FISH). These cases were compared with 112 oral cancer patients older than 50 years (controls). Results: Cyclin D1 overexpression was observed in 47.7% of tumors in young group and in 32.8% of controls (p=0.03). In the young group, the CCND1 amplification and overexpression were higher than control patients and the differences were statistically significant. In the young group, protein overexpression decreased the disease free survival (DFS); in control patients, the cyclin D1 overexpression decreased DFS and overall survival (OS). In both groups, the amplification had no influence on prognosis. The protein overexpression is an indicator of worse DFS in both groups.

#### **P041:** SCCRO 5, A NOVEL ONCOGENE AND POTENTIAL THERAPEUTIC TARGET IN HNSCC - <u>C Bommeljé, MD</u>, V Weeda, MD, Y Ramanathan, PhD, B Singh, MD, PhD; Memorial Sloan Kettering Cancer Center

Objective: Protein degradation by the ubiquitin-proteasome pathway plays an important role in many biological steps including cell cycle progression and immune responses. Post-translational modification of cullins by neddylation, which takes place in the nucleus, is a key regulatory mechanism of ubiquitination. We have previously identified SCCRO1/ DCUN1D1 and found that it is oncogenically activated by amplification at 3q and regulates ubiquitin ligase activity through cullin neddylation. Our in silico analysis shows that SCCRO5/DCUN1D5 is a family member of SCCRO1/DCUN1D1 and is located on 11q22, a region known to be amplified in aerodigestive squamous cell carcinoma (SCC). The aim of this study is to elucidate the role of SCCRO5/DCUN1D5 in oral SCC by 1) differential gene expression in oral SCC, 2) clinical correlation and 3) in vitro assessment of its biochemical activity. Methods: Gene expression analysis was performed on a total of 36 oral SCC, tumor relative to its matched normal tissue, by real time-PCR. We assessed binding of the SCCRO5/DCUN1D5 protein with neddylation pathway components by GST-pull down assays. Soft agar assays with SCCRO5/ DCUN1D5 and mutants were done. To assess functional activity, in vitro

neddylation reactions were performed and Nedd8 conjugation to cullins was monitored by western blotting under single turnover conditions. Results: Expression of SCCRO5/DCUN1D5 was upregulated in 42% of OSCC and overexpression correlated with disease free survival. SCCRO5/DCUN1D5 binds to components of the neddylation pathway including all cullins, CAND1, UCB12 both in vitro using GST-pull downs and in HeLa transfected with immunoprecipitation for HAtagged SCCRO5/DCUN1D5. Some mutants were not able to do so. In addition, we found that SCCRO5/DCUN1D5 increases the efficiency of cullin neddylation in a binding dependent manner. And SCCRO5 promotes cell growth more than its mutants. Conclusions: Our molecular work demonstrates that SCCRO5/DCUN1D5 functions in promoting cullin neddylation. It suggests that like SCCRO1/DCUN1D1, SCCRO5/DCUN1D5 also functions as an E3 for cullin neddylation. Collectively, these data suggest that SCCRO5/DCUN1D5 is active in oral carcinogenesis and is a potential novel oncogene that may represent a novel target for therapeutic approaches and may be used in clinic to aid prognostication.

#### **P042:** Moved to S020C

#### **P043:** AN EVALUATION OF DNA METHYLATION CHANGES IN THE MYB PROMOTER IN SALIVARY GLAND ADENOID CYSTIC CARCINOMA - <u>Chunbo Shao, MD, PhD</u>, Weiliang Bai, MD, Joseph Califano, MD, Patrick Ha, MD; Johns Hopkins Medical Institute

Purpose: Epigenetic changes have been associated with cancer-specific expression differences in human malignancies, including salivary adenoid cystic carcinoma (ACC). It is widely accepted that promoter methylation can regulate the transcription of genes, including tumor suppressor genes (TSGs) and oncogenes. The transcription factor gene MYB has been identified recently as an oncogene candidate in several human malignacies, including ACC of salivary gland, where a fusion construct has been identified. There are nine closely arranged CpG islands close to the transcription starting site of MYB gene. We wanted to know if the abnormal overexpression level of MYB was due to promoter hypomethylation in ACC compared to normal salivary gland tissue. Experimental Design: We performed CpG islands prediction analysis using Methprimer. The criteria for CpG islands are: 1) an Observed/ Expected CpG ratio over 0.6, 2) the percentage of G plus C over 50%, and 3) a window size of at least 100 bp. Within the -5 kb to +5 kb region relative to the transcription starting site of MYB, there are total of nine consecutive, closely arranged, CpG islands, all located in the -1 kb to + 2 kb region. Bisulfite genomic sequencing analysis was performed in each of the nine CpG islands. In total, 12 primary ACC tumors and 12 normal salivary gland tissues were studied. Results: With bisulfite genomic sequencing, our data showed that there was no detectable differential methylation in any of the nine CpG islands of MYB comparing ACC to normal salivary gland tissues. Conclusions: There are no differential methylation changes in the CpG island promoter regions of the MYB gene in salivary gland ACC. The overexpression of MYB in ACC was not due to promoter demethylation and must be explained by other mechanisms.

### **P044:** REGULATION OF THE EGFR SIGNALING AXIS BY

MICRORNA IN HNSCC - Xiaoli Wu, MD, <u>Mihir K Bhayani, MD,</u> Milena S Nicoloso, MD, Yunyun Chen, PhD, George A Calin, MD PhD, Stephen Y Lai, MD PhD University of Texas M. D. Anderson Cancer Center

Introduction: Recent advances in treatment for head and neck squamous cell carcinoma (HNSCC) include specific targeted therapy against the epidermal growth factor receptor (EGFR), which is overexpressed in many

HNSCC cell lines and specimens. However, clinical response to EGFRspecific treatment has not been as dramatic as preclinical studies may have suggested. This discordance suggests that additional regulatory mechanisms may be present and alternative approaches to inhibiting EGFR may be more effective. The current study examined the role of microRNA (miRNA) in the regulation of the EGFR signal transduction pathway. Methods: Candidate miRNA that bind the EGFR gene were identified. Expression levels of the miRNA were assessed by quantitative real-time polymerase chain reaction (qRT-PCR) in HNSCC and transformed oral keratinocyte cell lines. The effects of miRNA on EGFR and signaling molecule expression levels were assessed by immunoblotting. A reporter vector containing the luciferase gene fused to the 3'-untranslated region (UTR) of various genes was employed to assess miRNA effects on transcription. Functional studies were performed to assess effects of candidate miRNA on HNSCC cell line viability and apoptosis. Results: We identified novel microRNA (miRNA) that regulate expression of EGFR. miR-ETC (EGFR Targeting Candidate) and its complementary star sequence (miR-ETC\*) had decreased expression levels in HNSCC cells as compared to transformed oral keratinocyte cells. Expression of miR-ETC and miR-ETC\* in HNSCC cells decreased EGFR expression, but only miR-ETC\* expression decreased Akt (protein kinase B) and mTOR (mammalian target of rapamycin) levels. Decreased expression of these signal transduction molecules are associated with increased apoptosis and decreased cellular proliferation. Expression of miR-ETC\* in HNSCC cells resulted in decreased transcriptional activity as measured with luciferase reporter genes containing the 3'-UTR of either EGFR, Akt1 or mTOR. Additionally, site-directed mutagenesis of binding sites within the 3'-UTR of EGFR demonstrated specific interaction with miR-ETC\*. Conclusions: Our findings demonstrate the coordinate regulation of EGFR-mediated signal transduction at multiple levels. Functional effects of miR-ETC\* expression in HNSCC were related to decreased expression of not only EGFR, but also Akt1 and mTOR. Thus, miR-ETC\* may represent a novel targeted therapy for HNSCC.

**P045:** WNT INHIBITORY FACTOR 1 DOWN-REGULATION CORRELATES WITH WNT PATHWAY ACTIVATION IN SALIVARY GLAND TUMORS - Ilangovan Ramachandran, PhD, Wilbur K Mills, Antonio M Reis, MD, <u>Lurdes Queimado, MD, PhD</u>; Departments of Otorhinolaryngology and Dermatology, The University of Oklahoma Health Sciences Center

Background: Aberrant activation of the Wnt/ß-catenin signaling pathway leads to several human cancers. We have previously shown that Wnt inhibitory factor 1 (WIF1) is rearranged in some salivary gland pleomorphic adenomas and down-regulated in several salivary gland tumor cell lines. Down-regulation of WIF1 also occurs in some tobacco-related neoplasms and correlates with an increase in ß-catenin. Over-expression of ß-catenin, a downstream mediator of the Wnt pathway, has been reported in salivary gland tumors. However, mutations in the intracellular components of the Wnt pathway are rare in these tumors, suggesting that alterations in the Wnt extracellular components may play a major role on Wnt activation. Aims: To determine the frequency of alterations in WIF1 expression in salivary gland tumors and if changes in WIF1 expression correlate with activation of the Wnt pathway. Methods: The expression of WIF1 and active ß-catenin was analyzed in a large cohort of salivary gland tumors by immunohistochemistry. Multivariable logistic regression was performed to establish an association between two or more variables. Results: We demonstrated that WIF1 expression is high in normal salivary gland epithelium, but significantly down-regulated in salivary gland cancer. In normal salivary gland tissues, ß-catenin expression was localized almost exclusively to the cell membrane. However, in salivary gland tumors, we observed a decrease in membranar ß-catenin, which was associated with an

increased cytoplasmic and nuclear ß-catenin. Conclusions: We demonstrate that WIF1 is frequently down-regulated in malignant salivary gland tumors. A strong correlation was observed between WIF1 down-regulation and increased cytoplasmic and nuclear beta-catenin localization. Our data suggest that WIF1 down-regulation leads to activation of Wnt pathway and therefore, WIF1 might be used as a potential candidate for the treatment of salivary gland cancer.

### **CELL CYCLE REGULATION/APOPTOSIS/AUTOPHAGY**

P046: CLINICOPATHOLOGIC IMPLICATIONS OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA (PPARGAMMA) EXPRESSION IN SQUAMOUS CELL CARCINOMA (SCC) OF THE LARYNX - <u>Konstantinos Kourelis,</u> <u>MD</u>, Yelizaveta Shnayder, MD, FACS, Terrance Tsue, MD, FACS, Douglas Girod, MD, FACS; Department of Otolaryngology, Kansas University Medical Center

Background: Laryngeal SCCs emerge at the end of a multi-stage, cellalterating sequence which initially involves the metaplastic change of the respiratory epithelial layer into squamous, followed by carcinogenesis, that is, erruption of premalignant lesions and eventually malignant tumors. The latter lesions keep acquiring aggressive traits, a process known as tumor progression. The entire chain of histopathologic events is dominated by the function of gene-regulatory molecular factors, which have been excessively studied. The importance of a relatively novel but promising regulator, the nuclear receptor PPARgamma, is investigated in the present study, with regard to carcinogenesis evolution and prognosis of laryngeal SCCs. Patients and Methods: Tissue sections from normal, premalignant, and neoplastic specimens of 59 patients with laryngeal cancer, were analyzed by immunohistochemistry for PPARgamma expression. The patients were followed-up for 5 years. Results: PPARgamma expression was significantly higher in metaplastic squamous foci, in comparison with normal respiratory epithelium (P<0.001). The molecular factor had been steadily down-regulated in the course of tumor progression from high to poor differentiation (P=0.001). This decline of expression was also accompanied by shorter survival, but the association did not reach significance (P=0.364). Conclusions: PPARgamma is implicated both in the early cellular modifications, as well as in the final aberrations related to the gradual transformation of normal laryngeal epithelium into poorly-differentiated SCCs.

#### **P047:** AN APPROACH TO ANALYZE HPV/ EARLY RESPONSE GENE CORRELATIONS IN OROPHARYNGEAL CANCER: PRELIMINARY DATA WITH NF KAPPA B P65

Daniel Schneider, MD, <u>Ryan R Fader, BA</u>, Beverly Wuertz, BS, Frank G Ondrey, MD; University of Minnesota

HPV is an emerging risk factor for oropharyngeal cancers and the pathobiology responsible for the etiology of this disease is under active investigation. In the present study we investigated whether HPV DNA and NF kappa B p65 RNA could be extracted from paraffin embedded specimens and whether there were any associations between HPV positivity and NF kappa B p65 expression. Tumor specimens were obtained from thirty nine post-resection patients from the University of Minnesota and paraffinized. Genomic DNA/RNA was isolated from patient tumor samples via Roche High Pure RNA Paraffin Kit and confirmed with photospectrometry. HPV status analysis was completed via PCR of genomic DNA using primer sets for general HPV presence (GP5+/6+ and CPIG/IIG primers), followed by agarose gel electrophoresis. Relative NF kappa B p65 mRNA expression was analyzed via qRT-PCR (Roche Universal Probe Library) for both p65 and beta-2 microglobulin of isolated RNA. We found that 6 of 39 specimens were positive for HPV+ DNA by CPIG/IIG primers and that 5 of these specimens were also positive for GP5+/6+. This represents a 15.4% rate of HPV+ samples in our cohort. Of the 6 HPV+ specimens, 5 out of 6 of these specimens were also positive for NF kappa B p65. Of the 33 specimens that were negative for HPV only 10 of these specimens were positive for NF kappa B p65. By contingency table analysis there was a significant association with oropharyngeal tumors that are HPV+ tumors and detectable levels of NF kappa B p65 (p=0.0236). This study demonstrates that both viral DNA and tumor RNA can be extracted from microtome sectioned tumor bank specimens, with qPCR being able to be performed as well. This will allow standard paraffinized specimens to be utilized for q PCR analysis of HPV associated oropharyngeal carcinogenesis. We conclude the high percentage of NF kappa B p65+ RNA in the HPV + specimens warrants further investigation as a downstream mechanism of HPV associated carcinogenesis.

### **CORRELATIVE STUDIES**

**P048:** CLINICOEPIDEMIOLOGICAL STUDY OF THYROID SWELLINGS WITH SPECIAL REFERENCE TO ROLE OF SERUM TSH IN THYROID NEOPLASMS - <u>Sangita Bhandary</u>, Dilip Karmacharya, Nirmal Baral, BP Koirala; Institute of Health Sciences

Introduction: Nepal lies in the area of endemic iodine deficiency resulting in high incidence of patients presenting with thyroid swellings in ENT OPD. TSH level is a noble predictor of type of thyroid disorder specially in cases of thyroid neoplasms and malignancies. Objectives: 1. To study descriptive analysis of patients presenting with thyroid enlargement 2. To study and correlate biochemical parameters of above cases with special references to serum TSH level. Materials and Methods: Prospective study of 90 patients presenting with thyroid swellings due to various causes were included in the study. Detail clinical examination, biochemistry and histopathological examination was done in all cases. Of the 90 cases 58 patients underwent some or the other form of thyroid surgery. The clinical profile of the patients, the thyroid hormone status and histopathological findings were recorded and data analysed with the main objective of correlating serum TSH level with various types of thyroid swellings. Results: 90 patients with thyroid swellings were included in the study over the period of one year. M:F ratio was 5.25:1. Most of them presented with solitary thyroid nodule (70%). Malignancy was more common in patients having serum TSH level in the range of 1.8 -6.2 milli Unit/L (41.6%). Conclusion: Thyroid neoplasms are common disease in eastern Nepal. Biochemical parameters and FNAC are useful predictors of type of diseases and neoplasms especially in developing countries.

#### **P049:** INCIDENCE AND MORTALITY OF PATIENTS WITH HEAD AND NECK SQUAMOUS CELL CARCINOMA, A FUNCTION OF ABO BLOOD GROUPING? A

**RETROSPECTIVE STUDY** - Stewart I Adam, MD, <u>Keith M Wilson</u>, <u>MD</u>, Stephen M Overholser, MD, Jareen Meinzen-Derr, PhD; Department of Otolaryngology-Head and Neck Surgery, University of Cincinnati (UC) Neuroscience Institute and UC College of Medicine, Cincinnati, OH

Background: Although evidence shows a correlation between ABO blood groups and some types of cancer, this association has not been investigated relative to the incidence and prognosis of head and neck cancers. Our retrospective study examines if any relationship exists between ABO blood groups and patients with various types of head and neck cancers. Methods: Of 582 patients who underwent treatment for head and neck

carcinomas (1997-2002), blood type data were available for 455 patients with oral cavity, oropharyngeal, hypopharyngeal, and laryngeal squamous cell carcinoma; information on tumor status was substantiated by surgical pathologic reports. Blood group characteristics of the patients were compared with healthy blood donors from the Hoxworth Blood Center during the same 5-year period. Results: Among all patients, no significant correlation was observed between blood type and incidence of squamous cell carcinoma or survival rates. However, cancer rates in patients with type A blood were significantly higher in both whites and African Americans. In terms of increased cancer incidence and mortality rates by blood group, no significant association was shown for patients with laryngeal or hypopharyngeal cancers whereas an association was observed in patients with oropharyngeal and oral cavity cancers and blood type A. As expected, patients with higher stage cancer had lower 5-year survival rates, no matter what the age or blood type (p < .0001). Conclusions: Continuing epidemiologic studies and more in-depth biochemical analysis of the association between blood types and head and neck cancers may decipher the underlying mechanisms for these findings, and ultimately improve prevention and treatment strategies.

#### **P050:** DETECTION OF EPSTEIN-BARR VIRUS IN ORAL SQUAMOUS CELL CARCINOMA IN SÃO PAULO - BRAZIL. CORRELATION WITH CLINICOPATHOLOGIC VARIABLES, RISK FACTORS AND SURVIVAL - <u>Glauco I Miyahara, PhD</u>, Lívia T Arêde, MSc, Adriana Demathé, MSc, José F Garcia, PhD, Maria Lúcia M

I Arêde, MSc, Adriana Demathe, MSc, José F Garcia, PhD, Maria Lúcia M Sundefeld, PhD, Neivio J Mattar, MSc, João C Deolino, MSc, Éder R Biasoli, PhD; Oncology Centre - Univ Estadual Paulista, Brazil; Araçatuba Pathology Institute, Brazil

The Epstein-Barr virus (EBV) has a double-stranded DNA and is part of human herpesvirus family. It is the most potent virus immortalizing cell known and is associated with head and neck cancer and has been studied for its possible relation with oral squamous cell carcinoma (SCC). The aim of this study was to detect the presence of EBV in 57 patients with OSCC in São Paulo State - Brazil and correlate its presence with clinicopathologic variables, risk factors and patients' survival. Nested PCR technique was used in paraffin embedded samples of 20 samples of mouth floor SCC, 25 tongue and 12 oropharyngeal. The virus was found in 10% of mouth floor samples, 12% of tongue and 0% of oropharynx. No statistically significant difference was observed among the HPV DNA presence and gender, race, age, risk factors, histological grade, clinical staging and patients' survival. There was no statistically significant difference between the EBV presence and anatomical localization, gender, race, age, risk factors, clinical staging, histological grade and patients' survival. The result suggests that EBV did not have participation in carcinogenesis or oral SCC. Keywords: Human Herpesvirus , Oral squamous cell carcinoma, Polymerase chain reaction.

#### **P051:** P16INK4 ANTIGEN IMMUNOEXPRESSION IN SQUAMOUS CELL CARCINOMA OF THE OROPHARYNX IN THE VA POPULATION - <u>Chafeek Tomeh, MD</u>, Lauren Cooper, MD, Merry E Sebelik, MD, Nadeem Zafar, MD, Benjamin Oberman, BA; VA Memphis, TN

Objective: To study immunoexpression of p16ink4 antigen, a surrogate marker for high-risk HPV infection, in oropharyngeal carcinoma amongst veterans. Design: Presence of p16ink4 expression was studied by immunohistochemistry on paraffin embedded tissue from 21 consecutive cases of oropharyngeal squamous cell carcinoma (OC). p16ink4 expression status was correlated with clinical data derived from a retrospective chart review. tting: A tertiary level Veterans Administration Medical Center with a mid-south urban referral base. Patients: Veterans who were treated for OC between 1/1/2000 and 12/31/2008, had post-treatment follow-up for a minimum of 2 years, and for whom paraffin-embedded tissue was available for analysis were included in this study. Eighty five (85) patients meeting these criteria were identified, of whom 21 eligible patients have been processed thus far. Main Outcome Measure: Prevalence of p16ink4 expression in OC, with sub-site analysis by tumor location, stage, age at diagnosis, race, gender, treatment modalities and outcome, degree of alcohol and tobacco use. Results: p16ink4 expression was observed in 12/21 (57.1%) samples. Amongst these, 7/12 (60%) were strongly positive (over 90% of cells), with cytoplasmic and nuclear staining. Of note, amongst p16ink4 (-) tumors, 77.8% were derived from African-American patients, whereas p16ink4 (+) tumors were more evenly distributed between African-American and Caucasian patients, suggesting racial disparity in p16ink4 antigen expression in OC in this cohort. Conclusion: Preliminary data indicate that OC amongst mid-south veterans has a strong association with HPV-related expression of p16ink4. Published data indicate that while HPV(+) OC are diagnosed at a more advanced stage than HPV (-) cancers1, HPV (+) status is strongly associated with positive therapeutic response and survival compared to the HPV (-) tumors2. Routine diagnostic workup to establish the implication of HPV (p16ink4 status) appears as a very useful triage strategy for OC. The current study, when completed, will help establish the prevalence of HPV amongst this cohort of cases with OC, in a tertiary referral setting, with potential therapeutic implications. 1Pathogenesis and Prevention of Head and Neck Cancer (1st ed.). New York: Springer 2010 (ISBN 978-1-4419):1471-2. 2Journal of the National Cancer Institute 2008; 100 (4): 261-269.

# **P052:** A RETROSPECTIVE STUDY ON SINO NASAL MALIGNANCES IN SOUTH INDIAN POPULATION - <u>Vishnu</u>

<u>V Reddy Martha, Prof</u>, Nagalakshmi K, Research Scholar, Kaiser Jamil, Prof, Kumudhachalam P, Prof; Department of Otolaryngology, Government ENT Hospital, Koti, Hyderabad

Aims: Sino nasal malignancies (SNM) are more aggressive form of tumors which are challenging to diagnose, treat, and follow clinically. Current study is a retrospective study on SNM and its prevalence reported in Andhra region of south India. Materials & Methods: This study group includes 124 patients of Sino nasal malignancies among 2429 of head and neck cancers (HNC) diagnosed from ENT hospital, Hyderabad, A.P in the last 10 years, and excluded other head and neck cancers. Patient evaluation and diagnosis included a complete physical examination with nasal endoscopy, imaging and biopsy. Results: In the present study we found 5.1% of SNM among HNC.81.45% cases were squamous cell carcinomas, 3.22% were adenocarcinomas, 4.83% were adenoid cystic carcinoma, Basal cell carcinoma were 1.61%, nerve sheath tumors 0.8%, 4.03% mucoepidemoid carcinoma, malignant melanomas were 3.22% and 0.8% were mucoidadeno carcinoma. Males (66.12%) were at double the risk than females (33.87%). It was also seen that among the anatomical distribution Nose cases were found to be highest (52.41%) when compared to those occurring at maxilla (45.96%) and ethmoid regions (1.61%). And the age factor may also influence the susceptibility of the disease because we found 35.48 % of the cases between the age group of 51-60 years. Conclusion: Sino nasal malignancies are rare form of cancers. The study helps to know the status of SNM disease in this region and it also provides the information on various kinds of SNM and its ratios among both males and females. We found the male cases were found to be higher than females so we predict that the habitual risk includes smoking, and alcohol consumption might be the possible reasons for this inproportionality. Study has to be extended on the risk factors and on diagnostic methods to detect SNM at early stages.

**P053:** THE IMPACT OF CLINICAL VS. PATHOLOGICAL STAGING IN ORAL CAVITY CARCINOMA - A MULTI-INSTITUTIONAL ANALYSIS OF SURVIVAL - Hadi Seikaly, MD, FRCSC, <u>Vincent L Biron, MD, PhD</u>, O'Connell A Daniel, MD, MSc, FRCSC, Dort C Joseph, MD, MSc, FRCSC, T. Wayne Matthews, MD, FRCSC, Jeffrey R Harris, MD, FRCSC; University of Alberta and University of Calgary

Objectives: To evaluate any disparity in clinical versus pathological TNM staging in oral cavity squamous cell carcinoma (OCSCC) patients and any impact of this on survival. Design: Demographic, survival, staging, and pathologic data on all (460) patients undergoing surgical treatment for OCSCC in Alberta between 1998 and 2006 was collected. Clinical TNM vs pathological TNM staging was compared. Patients were stratified as pathologically down-staged, up-staged or same-staged. Main Outcome Measures: Survival differences between groups were analyzed using Kaplan-Meier and Cox regression models. Results: Tumor staging overall was unchanged in 82.7% of patients, with pathological up-staging and down-staging in 14.3% and 2.7% of cases respectively. Two and five year disease specific survivals for all groups were calculated as follows. Group1 - Early stage OCSCC that underwent no stage change: 87% and 79%. Group 2 - Early stage OCSCC upstaged: 65% and 47%. Group 3 - Advanced stage OCSCC downstaged: 82% and 72%. Advanced stage OCSCC who underwent no stage change: 54% and 43%. Conclusions: Some disparity exists in clinical vs. pathological staging in OCSCC. Patients that are pathologically down-staged or up-staged have a comparable disease specific survival to patients who undergo no stage change. To ensure appropriate adjuvant treatment modalities are utilized in patients who are subsequently upstaged to advanced stage OCSCC, surgical resection should be utilized in all cases of OCSCC.

#### **P054:** HPV INCIDENCE AND CHROMOSOMAL CHANGES IN KOREAN OROPHARYNGEAL CARCINOMA - <u>Yub-Seeg Jung.</u> <u>MD</u>, PhD, Won See Park, MD, PhD, Jong-Il Kim, MD, PhD, Junsun Ryu,

MD, PhD, Won Sco Park, MD, PhD, Jong-I Kim, MD, PhD, Junsan Kya, MD, PhD, Moon Kyung Choi, John H. Lee, MD, FACS; National Cancer Center, Goyang, Gyeonggi Province, Korea

Recent data from Europe and the US suggest oncogenic human papillomavirus (HPV)-related oropharyngeal squamous cell carcinoma (OSCC) exhibits unique biologic and epidemiologic patterns, compared to HPV(-) cancers mainly related to smoking. Although HPV-related carcinogenesis always starts with the viral DNA persistence and expression, intrinsic gene rearrangements of host cells are also likely needed. Previous reports have suggested that HPV(+) cancer tends to exhibit less genomic changes because viral oncogenes cause some of the changes necessary for cancer progression. However, persistent expression of the HPV oncogenes has been associated with genomic instability. Little work has examined these questions in the Korean population. The aim of this study was to assess the incidence and clinical characteristics of HPVrelated cancers in Korean OSCC patients, and genome-wide copy number variation (CNV) patterns in relation to the presence of HPV as well as its clinical implications. 51 OSCCs patients were enrolled. Clinicopathologic registry was constructed retrospectively. HPV was tested by detection and typing PCR, and DNA chip. Array-based comparative genomic hybridization with 4,363 genomic probes was used for genome-wide CNV study. Bioinformatic analyses were followed about CNV profile differences between HPV(+) and HPV(-) OSCC specimens. 78.4 % (40 cases) of the studied cases exhibited HPV DNA positivity. Among these, 90% was HPV 16. HPV(+) cancers occurred significantly more in younger age (61.1 vs. 67.6 years), female (15.4 vs. 8.3 %) and nonsmoker (28.2 vs. 8.3 %) group. Basaloid feature was shown more in HPV(+) patients (12.8 vs. 0 %). Genome-wide CNV study revealed less gain (88.3

vs.77.1 probes on average) and loss spot (74.3 vs. 58.2) in the HPV(+) group. Patterns like multiple large CNV segments and deep CNV gain or loss were detected significantly less in HPV(+) group. Our data suggest HPV(+) OSCCs exhibits more stable genomic integrity compared to exclusively smoking-related HPV(-) group.

### IMMUNOLOGY/ IMMUNOMODULATION

**P055:** CD4+FOXP3+CD39+ REGULATORY T CELLS SUPPRESS POLYFUNCTIONAL CYTOKINE EXPRESSION IN EFFECTOR T CELLS OF PATIENTS WITH HEAD AND NECK SQUAMOUS CELL CARCINOMA - <u>Patrick J Schuler, MD</u>, Magis Mandapathil, MD, Bastian Schilling, MD, Anasthasios Kotsakis, MD, Jonas T Johnson, MD, Sven Brandau, PhD, Stephan Lang, MD, Thomas K Hoffmann, MD, Theresa L Whiteside, PhD; University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA and University of Duisburg-Essen, Essen, Germany

Background: The frequency and function of regulatory T cells (Treg) are increased in the peripheral blood and the tumor site of patients with head and neck squamous cell carcinoma (HNSCC). The production of immunosuppressive adenosine by Treg may be responsible for inhibition of expression in activated effector T cells. Methods: A method for isolation of CD4+CD39+ Treg from peripheral blood mononuclear cells (PBMC) of 10 patients with HNSCC and 10 normal donors (NC) was developed using antibody-coated beads. The method was based on surface expression of CD39 ectonucleotidase on the surface of human Treg. The frequency of Treg (CD4+FOXP3+CD39+) in PBMC and polyfunctional cytokine expression (PCE) by autologous effector T cells were determined by multicolor flow cytometry. Single-cell analysis measured expression of MIP-1ß, IL-2, TNF-a, IFN-g and CD107a in activated effector cells prior to and after Treg depletion from the PBMC by magnetic beads. Blocking of adenosine production by Treg using the CD39 inhibitor (ARL67156) or CD73 inhibitor (aß-methylene ADP) and blocking of adenosine binding to A2a receptors on T effector cells were used to restore PCE in these cells. Results: PCE was significantly higher in activated effector cells obtained from NC compared to cancer patients' T cells (p=0.009). The frequency of Treg in PBMC of patients was negatively correlated to PCE in effector T cells. Treg depletion or treatment with the inhibitors of adenosine production or binding to effector cells resulted in reexpression of cytokines in effector T cells of HNSCC patients (p=0.009). Conclusions: Depletion of Treg from PBMC or inhibition of their enzymatic ectonucleotidase activity re-establishes PCE in effector T cells of HNSCC patients, suggesting that these strategies could become useful in improving antitumor immune responses in patients with cancer.

### **P056:** ISOLATION AND CHARACTERIZATION OF ADENOSINE-PRODUCING HUMAN CD4+CD39+ REGULATORY T CELLS BY MAGNETIC IMMUNOBEADS

<u>Patrick J Schuler, MD</u>, Jonas T Johnson, MD, Sven Brandau, PhD, Stephan Lang, MD, Thomas K Hoffmann, MD, Theresa L Whiteside, PhD; University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA and University of Duisburg-Essen, Essen, Germany

Background: Human regulatory T cells (Treg) express cell surfaceassociated ectonucleotidases CD39 and CD73, which cleave ATP to AMP and AMP to immunosuppressive adenosine. CD39 is expressed on Treg but not on effector T cells and may be a new marker suitable for Treg isolation from human blood or tissues. Methods: To establish a method for positive selection of human CD4+CD39+ Treg, negative isolation of CD4+ cells by a commercially available biotin-conjugated antibody cocktail against CD8, CD14, CD16, CD19, CD36, CD56, CD123, TCR-gamma/delta, and Glycophorin-A was performed. Next, Treg cells were isolated by biotinconjugated CD39 antibodies in combination with monoclonal anti-biotin magnetic beads. Peripheral blood mononuclear cells (PBMC) obtained from 10 patients with head and neck cancer (HNC) and 5 healthy individuals (NC) were separated using an AutoMACS. Multiparameter flow cytometry was used to phenotype T cells for Treg-associated markers (CD39, CD73, FOXP3, TGF-B, IL-10, CTLA-4). Treg functions were measured in CSFEbased proliferation assays using autologous CD4+CD25neg responder cells (RC). Results: The CD4+ CD39+ T cell population isolated by immunobeads had >95% viability and >85 % purity. The mean recovery  $\pm$ SD was  $71 \pm 5\%$ . The CD4+CD39+ T cells were positive for the following markers: FOXP3= 80 ± 7%; CD73=50 ± 4%; CTLA-4=30 ± 2%; TGF-ß =  $70 \pm 10\%$ . The isolated CD4+CD39+ cells contained two phenotypically and functionally distinct subpopulations: CD25+FOXP3bright cells which effectively suppressed proliferation of CD4+CD25neg RC and CD25negFOXP3dim cells which had minimal suppressor function. RC proliferation was increased upon blocking of adenosine A2a receptors on RC with a specific inhibitor, ZM241385. The removal of CD4+CD39+ Treg from PBMC of HNC patients, restored polyfunctional cytokine expression in T cells, suggesting that adenosine production was the main mechanism of suppression. Conclusions: This method of human Treg separation based on positive selection of T cells expressing the surface marker (CD39) with enzymatic activity is more advantageous than negative selection by CD127. It gives higher yields of adenosine-producing Treg, including CD39+CD25negFOXP3dim cells which could represent a precursor subset, and it makes it possible to study the role of adenosineproducing Treg in various human diseases such as cancer, graft-versus-hostdisease and allergy.

#### **P057:** THE ROLE OF REGULATORY T CELLS IN CETUXIMAB IMMUNOTHERAPY - <u>Steve C Lee, MD, PhD</u>, Pedro A Andrade Filho, MD, Marta E Szajnik, MD, Magis Mandapathil, MD, Seldene Fernene MD, PhD Therae Wikissid, PhD Rehet L Fernie MD

Soldano Ferrone, MD, PhD, Theresa Whiteside, PhD, Robert L Ferris, MD, PhD; University of Pittsburgh, Loma Linda University

Therapeutic monoclonal antibodies (mAb) have demonstrated clinical activity, but the variables responsible for their antitumor mechanisms and the factors responsible for patient heterogeneity have not been determined. Cetuximab, an epidermal growth factor receptor (EGFR) specific mAb, induces antibody dependent cellular cytotoxicity (ADCC) in vitro and in murine cancer models, a phenomenon that appears to be clinically relevant, since clinical response correlates with polymorphisms in mAb-binding Fc?R expressed by natural killer (NK) cells. Head and neck cancer (HNC) patients have been shown to possess IL-10 and TGF-? secreting regulatory T cells (Treg), which may suppress cellular immunity. While these cytokines may inhibit various NK cell activities, little information is available regarding the role of Treg cells on ADCC. We investigated the effect of Treg on cetuximab-mediated NK lytic activity in vitro, and correlated the levels of Treg cell in cetuximab treated HNC patients. Purified CD4+CD25+ T cells cultured in the presence of rapamycin were found to suppress cetuximab-mediated ADCC by NK cells, which was mimicked by the addition of exogenous TGF-beta or IL-10. Treg- mediated NK cell suppression was significantly blocked by incubation with neutralizing Abs to IL-10 or TGF-?, suggesting a major mechanism of this suppressive effect. Cetuximab treated HNC patients were found to possess elevated levels of Treg, but these cells were significantly less frequent in clinical responses, as compared to nonresponders to cetuximab therapy. Thus, Treg potently suppress cetuximab-mediated NK cell ADCC via an IL10/TGF-beta mediated mechanism, and this effect appears to have clinical significance.

**P058:** COMBINATORIAL IMMUNOTHERAPY USING CETUXIMAB ENHANCED ANTI-TUMOR ACTIVITY OF EGFR-SPECIFIC T CELLS AGAINST HEAD AND NECK CANCER CELLS. *Badra A Andrada Elha MD*. *Andras Lanaz Alhait* 

**CANCER CELLS** - <u>Pedro A Andrade Filho, MD</u>, Andres Lopez-Albaitero, MD, Athanassios Argiris, MD, Robert L Ferris, MD, PhD; University of Pittsburgh

Convincing clinical evidence indicates that the epidermal growth factor receptor (EGFR)-specific monoclonal antibody (mAb), cetuximab, is effective for advanced head and neck squamous cell carcinoma (HNC). However, not all patients respond to cetuximab, and clinical responses are not correlated with level of EGFR expression on tumor cells. This indicates that tumor escape from cetuximab activity may occur, and in vitro, cetuximab-treated tumors internalize EGFR to avoid surface cetuximab binding. However, EGFR internalization and degradation may lead to increased levels of EGFR-derived peptides for recognition by EGFR-specific T cells. Indeed, EGFR protein levels were reduced after cetuximab treatment in SCCHN lines, approximately 40% below control mAb treated tumor cells. We found that EGFR853-861 specific CTL are elevated in the circulation of HNC patients as compared to healthy controls (p<0.001), a phenomenon enhanced in the setting of cetuximabtrated HNC patients (p<0.005). We also show that HNC lines treated with cetuximab were enhanced by EGFR853-861 specific CTL recognition (p<0.05), indicating that EFR degradation in the presence of cetuximab treatmnt lead to enhanced processing and presentation of EGFR peptides to cytotoxic T lymphocytes (CTL). Thus, T cell based immunotherapy in combination with cetuximab treatment may provide a mechanism of countracting tumor cell escape from cetuximab therapy.

#### **P059:** ANALYSIS OF TUMOR-INFILTRATING

LYMPHOCYTES IN A SYNGENEIC ORAL CANCER MODE <u>L Clint T Allen, MD</u>, Nancy P Judd, MD, Ravindra Uppaluri, MD, PhD; Washington University in St. Louis

Objectives: Tumor-infiltrating lymphocytes may play a role in promoting a pro-tumor microenvironment in oral cavity squamous cell carcinoma. We developed syngeneic transplantable oral cavity squamous cell carcinoma cell lines, which allowed us to study the natural host immune response to oral cavity cancer progression in immunocompetent mice. Methods: OSCC-2, a syngeneic oral cavity squamous cell carcinoma cell line, was transplanted into the oral cavities or flanks of C57BL/6 mice. Regional lymph nodes, spleens and bone marrow were harvested from tumor bearing and non-tumor bearing mice as well as tumors from the latter. Multiparameter flow cytometric analysis was performed using primary antibodies to detect cell surface makers designed to differentiate subsets of tumor-infiltrating lymphocytes. Results: When injected into wild type C57BL/6 mice, OSCC-2 demonstrated highly aggressive local growth and regional metastatic potential. Harvested primary tumors were found to have early and robust recruitment of both CD11b+Gr1+ myeloid derived suppressor cell (MDSC) and CD11b+F4/80+ tumor-associated macrophage (TAM) populations. Conversely, low levels of CD4+ and CD8+ T-cells were detected. Compared to non-tumor bearing mice, expansion of MDSC and TAM populations was observed in the spleen, but not the marrow, of tumor bearing mice. Similarly, when compared to non-tumor bearing mice, regional lymph nodes in tumor bearing mice with regional metastases demonstrated significant expansion of both MDSC and TAM populations. Surface marker analysis of TAM populations in primary tumors and tumor bearing lymph nodes reveals a proportion to be polarized toward a type II, or pro-tumor, phenotype. Conclusions: Primary oral cavity tumors as well as tumor bearing regional lymph nodes demonstrate robust recruitment of MDSC and TAM populations. Functional studies and cytokine expression profiles of these

tumor-infiltrating lymphocytes may provide further understanding of their role in locoregional progression of oral cavity malignancies.

#### **P060:** DIFFERENCE IN IMMUNE MEDIATED RESPONSES AGAINST SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK (SCCHN) WITH CETUXIMAB AND PANITUMUMAB, TWO EGFR-SPECIFIC ANTIBODIES DIFFERING IN IGG ISOTYPE - <u>Andres Lopez Albaitero, Resident</u>, Steve Lee, Fellow, Pedro Andrade, Post Doctoral Fellow, Raghvendra Srivastava, Post Doctoral Fellow, Robert L Ferris, Attending Physician; Department of Otolaryngology University of Pittsburgh

Cetuximab and panitumumab are EGFR-specific monoclonal antibodies (mAb) that are FDA-approved and clinically effective in cancer patients. Although they cross-compete for EGFR binding, cetuximab is an IgG1 isotype mAb and panitumumab is an IgG2 isotype, suggesting that immune cell activation may differ. We have previously shown that cetuximab has an immune mediated effect in vitro, however, whether these effects are also observed using panitumumab treatment is not known. We found that cetuximab, but not panitumumab, mediates efficient antibody dependent cellular cytotoxicity (ADCC), generation of EGFR-specific T cell responses, as well as stimulation of dendritic cell (DC) maturation. These differences may affect their potential clinical applications in relation to combinatorial approaches with conventional or immune-based therapies.

### **OUTCOMES RESEARCH**

# **P061:** CORTACTIN IS ASSOCIATED WITH PERINEURAL INVASION IN THE DEEP FRONT AREA OF LARYNGEAL

**CARCINOMAS** - Eliane Papa Ambrosio, PhD, FabÍola Encinas Rosa, PhD, Maria Aparecida Custódio Domingues, PhD MD, Rolando André Rios Villacis, Renata de Almeida Coudry, PhD MD, José Vicente Tagliarini, PhD MD, Fernando Augusto Soares, PhD MD, Luis Paulo Kowalski, PhD MD, <u>Silvia Regina Rogatto, PhD</u> AC Camargo Cancer Treatment and Research Center, São Paulo, Brazil; 7Department of Urology, Faculty of Medicine, UNESP - São Paulo State University, Botucatu, SP, Brazil

Cortactin gene (CTTN), mapped at 11q13, has been associated with an aggressive clinical course in many cancers, due to its function of invasiveness. The purpose of this study was to evaluate CTTN protein and their prognostic value in the deep front and superficial areas of laryngeal squamous cell carcinomas (LSCC). The transcript expression levels were evaluated in a subset of cases. Overexpression of CTTN cytoplasmatic protein (80% of cases in both deep front and superficial areas) and transcript (30% of samples) was detected in a significant number of cases. In more than 20% of cases, observation verified membrane immunostaining in deep front and superficial areas. Patients presenting perineural invasion were significantly associated with N stage and recurrence (P=0.0058 and P=0.0037, respectively). Higher protein expression levels was correlated with perineural invasion (P=0.004) in front cells suggesting that this area should be considered a prognostic tool in laryngeal carcinomas. Although the majority of cases presented moderate to strong CTTN expression on the tumor surface, two sets of cases revealed a differential expression pattern in the deep front. A group of cases with absent to weak expression of CTTN in deep front showed good prognosis parameters and a second group, with moderate to strong expression of CTTN, showed an association with unfavorable prognosis, suggesting an association with worse outcome. Taken together, these results suggest that the deep front might be considered a grading system in laryngeal carcinomas and that cortactin is a putative marker of worse outcome in the deep front of laryngeal carcinomas.

#### **P062:** MASTICATION AND TONGUE FUNCTION IN PATIENTS TREATED FOR MALIGNANCIES IN TONGUE AND/OR FLOOR OF MOUTH; A ONE YEAR PROSPECTIVE

**STUDY** - <u>Caroline M. Speksnijder, PT MSc</u>, A. van der Bilt, PhD, M.A.W. Merkx, MD, PhD, R. Koole, MD, PhD; University Medical Center Utrecht & Radboud University Nijmegen Medical Centre

Background: People confronted with oral cancer run a high risk of deteriorated oral function. Reduced tongue function may affect mastication, deglutition, and speech. Reduced masticatory function may affect quality of life and food choice. An altered food choice may result in lower intakes for key nutrients and weight loss. Methods: Dental state, bite force, masticatory performance, tongue sensation, tongue mobility, and maximum force of the tongue were determined in a group of 45 patients with a squamous cell carcinoma of tongue and/or floor of mouth and 60 healthy persons matched on age. Twenty-three patients had surgery and 22 had surgery and radiotherapy. Patients were measured maximal 4 weeks before surgery, shortly (4-6 weeks) after surgery, shortly (4-6 weeks) after radiotherapy, 6 and 12 months after surgery. Healthy persons were measured once. Results: Surgical intervention had a large negative impact on oral function. Radiotherapy further worsened dental state, bite force, and masticatory performance. However, radiotherapy did not significantly influence sensation, mobility, or maximum force of the tongue. Nevertheless, patients treated by surgery and radiotherapy had a significant worse dental state, bite force, masticatory performance, tongue sensation, and tongue mobility than patients treated by surgery only. The tongue force in patients of both groups increased significantly in the first half year after surgery. However, this increase disappeared in the next half year. Conclusion: Objective determination of oral function one year after surgery showed that treatment of malignancies in tongue and/or floor of mouth had a significant impact on mastication and tongue function in all patients.

#### **P063:** FREE FLAP RECONSTRUCTION OF IATROGENIC

MAXILLARY DEFECTS - <u>Richard K McHugh, MD, PhD</u>, Paul Kim, MD, Charles Stewart IV, MD; Loma Linda University Medical Center

Objective: To review reconstructive options with an emphasis on free tissue transfer for patients with therapeutic partial and total maxillary defects. Design: Retrospective review of cases. Setting: Single tertiary referral center. Two surgeons (PDK and CES) performing reconstructions. Patients: Thirty-three consecutive patients underwent surgical resection of malignant tumors resulting in partial or total maxillary defects between February 2005 and October 2009. Squamous cell carcinoma in 25 patients represented the most common pathology, while there were three with carcinoma ex pleomorphic adenoma, two with basal cell carcinoma, one osteosarcoma, one melanoma, and one adenoid cystic carcinoma. Intervention: Free tissue transfer reconstruction included: 12 anterolateral thigh, 4 fibula, 1 myocutaneous radial forearm, 1 osteocutaneous radial forearm, and 15 flaps based on the subscapular system. Main Outcome Measures: Reconstructive separation of the oral and sinonasal cavities, swallowing, details of free tissue transfer including type of defect and anastomotic vessels, speech ability, and complications. Results: Separation of oral and sinonasal cavities was maintained in all cases, although one case required a return to the OR for repair of palatal dehiscence. One might expect greater usage of osteocutaneous flaps in larger maxillary defects, however we found that osteocutaneous or myocutaneous tissues were utilized for all sizes of maxillary defects. The facial artery, common facial vein, and the external jugular vein were the most commonly used anastomotic vessels. Orbital rim reconstruction utilized titanium mesh, several cases in combination with hydroxyapatite. Two free tissue transfers failed requiring a second free tissue transfer. Conclusions:

Free tissue transfer as the reconstructive basis of therapeutic maxillary defects has been shown to be effective in restoring general facial contour and function in a single-stage operation. The usage of varied myocutaneous or osteocutaneous flaps for all sizes of maxillary defects represents the broad utility of these flaps. The type and design of flap appears related to the type of complex defects extending beyond the maxilla. Free tissue transfer remains a mainstay of cosmetic and functional reconstruction for complex palate and maxillary defects.

#### **P064:** SYSTEMATIC REVIEW OF PROGNOSTIC BIOMARKERS IN OROPHARYNGEAL SQUAMOUS CELL CARCINOMA - <u>I W Rainsbury</u>, W Ahmed, H Mehanna; Institute of Head and Neck Studies and Education (InHANSE)

Background: There is a growing volume of literature investigating the significance of prognostic biomarkers in Oropharyngeal Squamous Cell Carcinoma (OPSCC). Human Papilloma Virus (HPV) has recently been the subject of particular interest, but a number of others are also thought to influence prognosis. Methods: We performed an electronic literature search of the Cochrane, MEDLINE, Zetoc and National Cancer Trials databases. Inclusion criteria were: abstracts reporting on patients with OPSCC; follow-up studies of prognostic significance of  $\geq 1$  biomarker; minimum dataset includes rates of survival, response to treatment or recurrence. Exclusion criteria were: <10 patients with OPSCC; distant metastases or recurrent disease included; in vitro studies; oropharynx not specifically reported; inadequate survival data. The two reviewers independently selected papers, assessed their quality using an objective scoring method for prognostic studies, and extracted data. Differences of opinion were resolved by discussion. Meta-analysis of Relative Risk (RR) was performed on groups of two or more biomarkers for Overall Survival (OS) and Disease-Specific Survival (DSS). Results: 3052 studies were identified, of which 59 satisfied the inclusion and exclusion criteria. Two biomarkers, HPV and p53, were suitable for meta-analysis. HPV positive tumours showed a clear OS and DSS benefit (combined RR, 95% Confidence Interval (CI) = 0.45, 0.32-0.64; 0.35, 0.20-0.60 respectively). Raised p53 showed no overall survival benefit (combined RR, 95% CI = 0.74, 0.40-1.39). Raised HIF1\_, c-met, CD31, COX2, ki-67, nuclear Cyclin D1, nuclear survivin, and PLK also conferred OS benefit; as did reduced Bcl-2, CD3, and p16; and lack of either allelic amplification at 11q13, or loss at 16q loci. Increased MVP, EGFR and VEGF; and decreased p16 were associated with better DSS. However, none of these biomarkers had been sufficiently widely investigated for meta-analysis to be performed. Conclusion: Only HPV and p53 have been studied in enough detail to allow calculation of pooled results for OS and DSS in OPSCC. There are several other promising candidates for prognostic biomarkers in this disease, but larger studies are needed to confirm their significance.

#### P065: EXTRACAPSULAR EXTENSION IN OROPHARYNGEAL SQUAMOUS CELL CARCINOMA NODAL METASTASES IS A POOR PREDICTOR OF DISEASE RECURRENCE AND PATIENT OUTCOME - <u>James S Lewis Jr.</u> <u>MD</u>, Danielle H Carpenter, MD, Bruce H Haughey, MB, ChB, Qin Zhang, BS, Wade L Thorstad, MD; Washington University in St. Louis

Background: Extracapsular extension (ECE) in squamous cell carcinoma (SCC) nodal metastases usually predicts worse outcome. However, there are no histologic grading criteria for ECE, and there have been almost no studies on oropharyngeal SCC alone. In the HPV era, this study quantifies the extent of ECE in oropharyngeal SCC utilizing a clear, novel grading system and examines the impact on outcomes. Design: Surgically treated oropharyngeal SCC cases with nodal metastases were identified from clinical databases. All received post op radiation. Lymph nodes were graded as 0 (tumor within substance of node), 1 (tumor filling subcapsular sinus with thickened capsule but no irregular peripheral extension), 2 (tumor ≤1mm beyond capsule), 3 (tumor >1mm beyond capsule), or 4 (no residual nodal tissue or architecture). p16 immunohistochemistry was performed. Results: 63 of 101 (62%) had definitive ECE, with scores 2 or higher. 13 were grade 0, 25 grade 1, 7 grade 2, 19 grade 3, and 37 grade 4. 90.7% of cases were p16 positive. ECE grades did not correlate with nodal size (p=0.28) or p16 status (p=0.28)= 0.8). In follow up, 10 patients (9.8%) had disease recurrence with one (0.9%) local, 4 (3.9%) regional, and 7 (6.9%) distant failures. Only 3 of 64 (4.7%) grade 0-3 cases recurred while 7 of 37 (18.9%) with grade 4 recurred (p = 0.0347). In univariate analysis, survival was no different for grade 0 or 1 (no grade 1 patients recurred) while grade 4 ECE was associated with shorter overall, disease specific, and disease free survival (p=0.001, 0.001 and 0.002 respectively). Six of the 7 patients developing distant metastases had grade 4 ECE (p = 0.0092). All but one of these was p16 positive. However, grade 4 ECE correlated with higher T-stage (T1/2 versus T3/4; p = 0.0153) and in multivariate analysis was not associated with statistically poorer overall (p = 0.1375), disease specific (p = 0.0897), or disease free survival (p = 0.2005). Conclusion: The impact of ECE in nodal metastases is limited in oropharyngeal SCC. Only ECE 4 associates with disease recurrence but not independently of T stage and other variables.

#### **P066:** THYROID LOBECTOMY FOR TREATMENT OF WELL DIFFERENTIATED INTRATHYROID MALIGNANCY - <u>Iain J</u> <u>Nixon, MD</u>, Iain Ganly, MD, PhD, Snehal G Patel, MD, Frank L Palmer, BA, Monica M Whitcher, BA, Ashok R Shaha, MD, Jatin P Shah, MD, PhD Memorial Sloan Kettering Cancer Center

Objectives: The objective of our study is to report our experience in the management of well differentiated intrathyroid cancers by either lobectomy or total thyroidectomy. Patients and Methods: A retrospective analysis identified 884 patients with intrathyroid cancer treated with surgery between 1986 and 2005. The male to female ratio was 1:3.8 (196 men and 724 women). The median age was 46 years (range 4-91). The primary surgical procedure was total thyroidectomy in 504 (57%) and thyroid lobectomy in 380 (43%) patients. 18 patients treated with initial lobectomy were taken back for immediate completion thyroidectomy based on the results of the surgical pathology. Results: With a median follow up of 97 months (range 13-290 months) the overall survival, disease specific survival and recurrence free survival for all patients at 10 years were 92%, 100% and 98% respectively. Univariate analysis showed no significant difference in overall survival by extent of surgical resection, with 10 year overall survival rates of 93% for thyroid lobectomy versus 91% for total thyroidectomy (p=0.56). Age and gender were found to be significant on univariate analysis. Multivariate analysis of male gender, age >45, T stage and surgical procedure showed that age over 45y and male gender adversely affected overall survival, whereas both T stage and type of surgery were not. Comparing the lobectomy and total thyroidectomy groups there was a significant difference in the 10 year rates local recurrence 4% and 0% respectively p=0.01. There was no significant difference in regional recurrence (0.3% versus 0.8%, p=0.14) or distant recurrence (0.3% versus 0.6%, p=0.42). Of the 380 patients treated with initial lobectomy, 35 (9%) had removal of the contralateral lobe; 18 (5%) had immediate and 17 (4%) delayed completion. Conclusion: Patients presenting with a single focus of well differentiated thyroid cancer without evidence of spread beyond the gland or nodular disease in the contralateral lobe can be safely managed by thyroid lobectomy alone.

#### **P067:** BONE IMPACTED FIBULAR FREE FLAP IN HEAD AND NECK RECONSTRUCTION: A HISTORICAL CASE CONTROLLED STUDY - Hadi Seikaly, M,D FRCSC, <u>Brittany Barber</u>, <u>BSc</u>, Peter T Dziegielewski, MD, Johan Wolfaardt, BDS, Mdent, PhD, Daniel O'Connell, MD, MSc, FRCSC, Jeffrey R Harris, MD, FRCSC University of Alberta, Institute for Reconstructive Sciences in Medicine

Background: The bone impacted fibular free-flap (BIFFF) was recently developed to improve the success of dental implantation and prosthedontic fitting in head and neck reconstruction. While a previous study has described the technique and demonstrated its efficacy, the impact on local complication rates and success of dental implantation has not been assessed. Objective: To compare the local complication types and rates as well as dental implant-related complications of the traditional and BIFFF used in mandibular and mid-face reconstruction. Furthermore, to determine predicitve factors of such complications. Design: Retrospective historical cohort study. Setting: University of Alberta tertiray care head and neck cancer treatment center. Patients: Patients diagnosed with head and neck cancers undergoing surgical ablation and reconsturction with a fibular free flap. Traditional fibular free flaps were used from 2000-2005 and the BIFFF was employed from 2005-2009. Interventions: The use of a fibular free flap for mandibular or mid-face bony contouring following tumor extirpation. Main Outcome Measure(s): Short and long term local complications directly related to the fibular site of reconstruction. Complications considered to be significant were those necessitating surgical intervention. Secondly, peri-operative variables associated with increased complication rates were to be identifed with logistic regression analysis. Results: 116 cases meeting inclusion criteria were identified. 27 complications were found with 18 occuring in the BIFFF reconstructions (22%) and 9 in the traditional fibular flaps (26%). This difference was statistically insignificant (p > 0.05). The most common complication was hardware exposure (n=22). Multivariate logistic regression analysis found that midface reconstruction was a statistically significant predictor of complications (p = 0.008). All other variable did not demonstrate statistical significance. The success rate of dental implants at 1 and 3 years was 61% and 23% for the traditional flaps and 100% and 83% for the BIFFF. These differences were statistically significant (p < 0.001). Conclusions: The BIFFF is a safe means of reconstruction with comparable local complication rates to traditional fibular free-flaps for mandible and maxillary defects. However, the success rate of dental implantation is superior with the BIFFF.

### **P068:** THE IMPACT OF MICROSCOPIC EXTRA THYROID EXTENSION ON OUTCOME IN PATIENTS WITH CLINICAL T1 AND T2 WELL DIFFERENTIATED THYROID CANCER

Iain J Nixon, MD, <u>Ian Ganly, MD, PhD</u>, Snehal G Patel, MD, Frank L Palmer, BA, Monica M Whitcher, BA, Ashok R Shaha, MD Jatin P Shah, MD; Memorial Sloan Kettering Cancer Center

Objective: The objective of our study was to report the impact of microscopic thyroid extension (ETE) on outcome in patients with cT1 or cT2 well differentiated thyroid cancer. We also wanted to determine the effect of extent of surgery and radioiodine on outcome in patients with microscopic ETE. Patients and Methods: Nine hundred and eighty-four patients (54%) who had thyroid surgery for cT1/T2 N0 disease were identified from an institutional database of 1810 patients treated between 1986-2005 at Memorial Sloan Kettering Cancer Center. Survival and recurrence outcomes were analyzed using the Kaplan-Meier method. Univariate analysis was carried out by the log rank test and multivariate analysis by cox regression. Results: With a median disease specific follow up of 50 months, disease specific survival

(DSS) and recurrence free survival (RFS) at 5 years within the entire group were 100% and 99% respectively. The 5 year local recurrence free survival (LRFS), regional recurrence free survival (RRFS) and distant recurrence free survival (DRFS) were 100%, 99% and 99% respectively. In comparison of the pT1/pT2 and pT3 groups, no significant difference in 5 years DSS or RFS was found (100% versus 100% and 99% versus 98%, p=0.187 respectively). In the group of 112 patients who had total thyroidectomy or lobectomy, there were no disease specific deaths and only 3 recurrences (all in the lateral neck). Other than male gender, no other variable, including extent of thyroid surgery and administration of post operative radioactive iodine were significant predictors of outcome on univariate or multivariable analysis. Conclusion: Outcomes in patients thought to have well differentiated clinically intrathyroid cancers are excellent and not significantly affected by the discovery of ETE on histopathological analysis. The extent of surgical resection and administration of post operative radioactive iodine in these low risk lesions does not have an impact on survival or recurrence.

# **P069:** COMPARISON OF THE PROGNOSTIC ABILITY OF THE WHO AND BINARY ORAL DYSPLASIA GRADING

**SYSTEMS** - <u>Paul Nankivell</u>, Mr, Hazel Williams, Dr, Paul Matthews, Dr, David Snead, Dr, Christopher McConkey, Mr, Hisham Mehanna, Mr Institute of Head and Neck Studies and Education (InHANSE)

Introduction: Histological grading of oral dysplasia remains the gold standard for predicting progression to cancer. This is despite the method being known to suffer from wide inter-rater variability. A newer binary grading system has been proposed with the aim of reducing this interrater variability, which has been confirmed in previous studies by our group. For a grading system to be useful clinically however, it needs to be able to predict future clinical outcome; in this case the risk of progression to malignancy. The prognostic ability of the binary system has not yet been independently validated. Aims: To independently assess the prognostic ability of the WHO and binary grading systems in a cohort of oral dysplasia cases. Methods: 43 cases of oral dysplasia with a minimum of 3 years clinical follow-up were graded. 3 pathologists (2 head and neck specialists) blinded to the initial grade and clinical outcome independently assessed each slide. The slides were graded using the WHO and binary systems after assessment of cytological and architectural features. A survival analysis was performed using a Kaplan Meier technique. The endpoint was transformation to malignancy, thereby giving a progression-free survival curve. Results: There was a statistically significant difference between the progression-free survival rates of cases graded into high and low risk using the binary system. Some low risk cases still progressed however. The different WHO grades also appear to have different progression-free survival rates, however this was not significant. When the WHO system is itself divided into a binary system, (mild dysplasia versus everything else), a statistically significant difference between the categories is seen. This difference is larger than that seen for the binary system and no low risk cases progressed to malignancy. Conclusion: The binary system does appear to be able to distinguish between cases at higher and lower risk of progression to malignancy. However, some low risk cases still progressed, which was not the case for the WHO system. This would suggest the WHO system may actually be more useful in a clinical setting if used as a binary system.

### **P070:** HPV STATUS IN YOUNG NON-SMOKING HNSCC

**PATIENTS** - <u>H.S. van Monsjou, MD</u>, E.S. Jordanova, PhD, M.L.F. van Velthusen, MD, PhD, C.J.M. Melief, MD, PhD, M.W.M. van den Brekel, MD, PhD, A.J.M. Balm, MD, PhD; Department of Head and Neck Surgery and Oncology (H.S.V.M,M.W.V.D.B,A.J.B), Pathology (M.L.V.V.) Netherlands Cancer Institute, Amsterdam, The Netherlands; Department of Immunohematology (C.J.M.), Pathology (E.S.J.) Leiden University Medical Center, Leiden

Purpose: The number of human papillomavirus (HPV) induced HNSCC is increasing significantly for the last decades. Patients with HPV positive carcinoma tend to be younger, non-smokers and non-drinkers1. In a cross section of HNSCC patients, HPV-associated SCC's are mainly located in the tonsil, and are characterized by p16 ink4A accumulation as well as reduced pRb expression and over-expression of wild-type p53 2. A group of young HNSCC patients were selected to test the presumed overrepresentation of HPV markers. Patients and Method: Paraffin embedded, archival biopsy material from 45 cases of HNSCC patients younger than 40 years were analyzed. The group consisted of 22 oral cavity, 18 oropharyngeal, 3 hypopharyngeal and 2 laryngeal squamous cell carcinomas. HPV subtypes were identified by the INNO-LiPA prototype research genotyping assay, a highly sensitive reverse hybridization assay. P16 ink4A expression was tested by immunohistochemistry analysis using monoclonal antibodies. Results: Fourteen (31%) of all 45 HNSCC unequivocally exhibited HPV 16 integration, and 55% of the oropharyngeal tumors were HPV 16 positive. Immunohistochemically p16ink4a overexpression was found positive in 80 % of the HPV positive tumors. Within the oropharyngeal SCC's seven out of 11 (64%) HPV positive cases were tonsillar carcinomas, in the HPV negative cases it was 3 out of seven (42%). Fifty percent of the HPV positive oropharyngeal carcinomas had a history of tobacco use and 70% used alcohol. In the HPV negative oropharyngeal carcinoma's only one case (14%) did not smoke. Conclusions: Although HPV positive tumors seem to be overrepresented in this group of young patients, in comparison to other epidemiological studies in the Netherlands, young patients seem not to represent a distinct molecular and clinical disease entity. Furthermore, HPV16 infection was not exclusively detected in non-smoking and non-drinking young HNSCC patients. References: <sup>1</sup>Fakhry C, Gillison ML: Clinical implications of Human Papillomavirus in Head and Neck Cancers. Journal of Clinical Oncology 24:2606-2609,2006. <sup>2</sup>Hafkamp HC, Manni JJ: A subset of Head and Neck Squamous Cell Carcinomas exhibits integrations of HPV16/18 DNA and overexpression of p16 ink4A and p53 in the absence of mutations in p53 exons 5-8. International Journal of Cancer 107: 394-400,2003

#### **P071:** SEMI-AUTOMATIC TUMOR VOLUME MEASUREMENTS IN HEAD AND NECK CANCER USING DYNAMIC CONTRAST ENHENCED MR-IMAGING - <u>Wouter</u> <u>L Lodder, MD</u>, Kenneth G Gilbuijs, PhD, Charlotte A Lange, MD, Alfons J

Balm, MD, PhD, Michiel W van den Brekel, MD, PhD; Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital

Introduction: Tumor volume is a significant prognostic factor in the treatment of head and neck cancer. Unfortunately it is not routinely measured because of the workload attached. In prior studies, semiautomatic volumetric measurement of tumor extent at contrast enhanced (CE)-MRI provided a more accurate and reproducible assessment of the extent of breast cancer compared to manual assessment1. Objective: To assess the feasibility of semi-automatic volumetric measurement of head and neck cancer using dynamic contrast-enhanced MRI (DCE MRI). Methods: The study group consisted of consecutive 24 patients, seen between September 2009 and March 2010 and who received DCE-MRI prior to treatment. DCE-MRI was acquired using a Philips 3.0 T Achieva scanner. Fast volumetric dynamic imaging was performed in the first 45 s after Gd-DTPA contrast injection using T1-weighted FFE sequences at voxel size of 1.3x.1.3x2.5 mm3 and sampling rate of 1.5 s. A workstation previously developed for semi-automated segmentation of cancers on DCE MRI was employed to segment the head and neck cancers1. The required user interaction is limited to indicating the location of the tumor in the MR images. Subsequently, the tumor is segmented in 3D using constrained volume growing and producing a volumetric measurement of the extent. Linear regression analysis was used to assess the precision of the volumetric measurements compared to that obtained by manual delineation of the tumor. Results: The automatically derived tumor volumes ranged from 0,65 to 42,3 (median 12,8, SD 12,2). The success rate was 21/24=88%: in three cases no visual agreement was reached with the automated method. The pearson's correlation coefficient was 0,89. The semi-automated method was approximately 5-10 times faster to produce the result than the manual method. Conclusion: Semi-automated volumetric assessment of the volume of head-and-neck cancers provides potential for increased precision and routine use compared with manual assessment of tumor extent. Reference: 1Alderliesten T., Schlief A.T.E.F., Peterse J.L., Loo C.E., Teertstra H.J., Muller S.H., Gilhuijs K.G.A., Validation of Semi-Automatic Measurement of the Extent of Breast Tumors at Contrast-Enhanced MRI. Invest. Radiol., 2007; 42(1):42-49.

**P072:** MINIMALLY-INVASIVE PARATHYROIDECTOMY: AN ALGORITHM FOR POST-OPERATIVE CARE - Jamie Tibbo, MD, <u>FRCSC</u>, George Kurien, MD, Hadi Seikaly, MD, FRCSC, Jeffrey R Harris, MD, FRCSC; University of Alberta

Objective: To review the post-operative complications for a surgeons postoperative algorithm for Minimally Invasive Parathyroidectomy (MIP), to evaluate the safety of this approach. Design: Retrospective review. Setting: Tertiary Care Centre. Methods: We reviewed the medical records of 135 patients who underwent MIP for primary hyperparathyroidism by the senior surgeon from December 2001 - September 2009. All patients underwent our standard post-operative algorithm, which involves a baseline rapid-Parathyroid Hormone level assay, followed by intra-operative-PTH (ioPTH) levels post-isolation, and 5, 10, and 20 minutes post-excision. The patient is then discharged home the same day if they have a normal ioPTH level (20 minute post-excision), and are otherwise doing well. They are seen in follow-up in 4-6 weeks, and do not have serial calcium levels checked post-operatively. We identified post-operative complications, that were presumed to be a result of the surgery. Results: There were no major complications identified for any of the cases. We observed a low rate of minor complications as well. Minor complications included: recurrent or persistent hyperparathyroidism requiring surgery (2/135), recurrent or persistent hyperparathyroidism (not requiring surgery) with normal calcium levels (4/135), neck swelling or discomfort (3/135), neck cellulitis (2/135), and hypoparathyroidism (1/135). Conclusion: Based on our review, we believe our algorithm for the post-operative management of patients with primary hyperparathyroidism is safe with a low complication rate.

#### **P073:** BEAVERTAIL MODIFICATION OF THE RADIAL FOREARM FREE FLAP IN TOTAL ANTERIOR GLOSSECTOMY: TECHNIQUE AND FUNCTIONAL

**OUTCOMES** - Hadi Seikaly, MD, FRCSC, <u>Peter T Dziegielewski, MD</u>, Jana Rieger, PhD, Dan O'Connell, MD, MSc, FRCSC, Prabhjyot Singh, MD, Nabil Rizk, MD, Jeffrey R Harris, MD, FRCSC; University of Alberta

Objectives: (1) To describe the beavertail modification of the radial forearm-free flap in reconstruction of total anterior tongue defects. (2) To evaluate the swallowing, speech and quality of life outcomes in these

patients. Design: Retrospective review of prospectively collected data. Setting: Tertiary care academic hospital. Patients: 18 patients with advanced oral cancers. Interventions: All patients were treated with total anterior glossectomy with beavertail modified radial forearm freeflaps. All patients received adjuvant radiation therapy. Main Outcome Measure(s): The main outcome was swallowing ability as defined by complete independence of a feeding tube. Other swallowing outcomes included aspiration and pharyngeal residue scores as well as swallowing transit time as per video fluoroscopic swallowing studies. Speech was evaluated in terms of word and sentence intelligability. Quality of life was assessed via the EORTC-H&N35. Results: 15% of patients were tube feed dependent at 6 months post-operatively. However, by 1 year after surgery, all patients were consuming a full oral diet. All patients showed no significant deterioration in aspiration, or pharyngeal residue scores or swallowing transit tmes at 1 year post-operatively (p > 0.05). Mean single word intelligability was 62% postoperatively, while sentence intelligability was 75%. These values did not change significantly from the pre-operative time frame (p > 0.05). Quality of life scores also did not significantly deteriorate over the course of 1 year (p > 0.05). Conclusions: Total anterior glossectomy reconstructed with the beavertail modified radial forearm free-flap offers patients with advanced oral cancers good swallowing, speech and quality of life outcomes.

#### **P074:** FUNCTIONAL OUTCOMES AND QUALITY OF LIFE IN TOTAL GLOSSECTOMY WITH LARYNGEAL PRESERVATION AND FREE FLAP RECONSTRUCTION: CASE SERIES AND SYSTEMATIC REVIEW - Hadi Seikaly, MD, FRCSC, <u>Peter T Dziegielewski, MD</u>, Jana Rieger, PhD, Prabhjyot Singh, MD, Morgan Langille, MD, Arjun Joshi, MD, FRCSC, Jeffrey R Harris, MD, FRCSC; University of Alberta

Objectives: To investigate functional outcomes and quality of life in patients who have undergone total glossectomy with laryngeal preservation at one institution as well as in the literature. Design: Retrospective review and systematic review of the literature. Setting: Tertiary care academic hospital. Patients: 12 patients with advanced oral/ oropahryngeal cancers were treated with total glossectomy with laryngeal preservation. All tongues were reconstructed with anterolateral thigh free flaps. 28 similar patients were identified in the literature. Interventions: All patients were at our institution were treated with primary surgery and adjuvent radiation therapy. A systematic review of the literature revelaed that 50% of patients were treated with primary surgery, while 50% were treated with salvage surgery. Main Outcome Measure(s): The main outcome was swallowing ability as defined by complete independence of a feeding tube. Other swallowing outcomes included aspiration and pharyngeal residue score as well as swallowing transit time as per video fluoroscopic swallowing studies. Speech was evaluated in terms of word and sentence intelligability. Quality of life was assessed via the EORTC-H&N35. Functional outcomes measures varied between studies. The only measure that could be analyzed in a systematic review was dependency of tube feeding. Results: 50% of patients were tube feed dependent at 1 year post-operatively. All patients who could swallow did not show evidence of aspiration. Pharyngeal residue as well as swallowing transit times were significantly worse post-operatively (p < 0.05). Mean single word intelligability was 40% postoperatively, while sentence intelligability was 65%. Quality of life scores did not significantly change from pre- to post-operative states (p > 0.05). Systematic review demonstrated that 60% of patients undergoing total glossectomy with laryngeal preservation will be tube feed dependent at 1 year post-operatively. Multivariate logistic regression analysis demonstarted that younger patients with less advanced disease had a better chance of recovering swallowing function (p < 0.05).

Conclusions: Total glossectomy with laryngeal preservation and free-flap reconstruction can lead to intelligable speech with a stable quality of life. Swallowing ability is possible in younger patients with less advaced disease.

**P075:** CERVICAL METASTASES IN OROPHARYNGEAL CANCER: WHEN IS BILATERAL NECK TREATMENT INDICATED? - Hadi Seikaly, MD, FRCSC, <u>Peter T Dziegielewski, MD,</u> <u>FRCSC</u>, Daniel O'Connell, MD, FRCSC, MSc, Brittany Barber, BSc, Vince Biron, MD, PhD, Jeffrey Harris, MD, FRCSC; University of Alberta

Objective: Oropharyngeal carcinoma has a high propensity for cervical metastases. While the majority of these patients will require neck treatment, the role bilateral treatment remains controversial. The purpose of this study is to determine the rate and levels of bilateral cervical metastases in oropharyngeal cancer and to identifty factors predictive of contralateral spread of disease. Design: Retropsective review. Setting: The University of Alberta tertiary care head and neck cancer treatment center. Patients: 238 consecutive patients treated surgically for oropharyngeal squamous cell carcinoma from 1998-2008. Interventions: All patients were treated with primary surgical resection, including bilateral neck dissection and post-operative radiation therapy with or without chemotherapy. Neck dissection consisted included ipsilateral modifed radical neck dissection (levels I-V) and contralateral modefied selctive (levels I-IV) or modiefed radical neck dissection (levels I-IV). Main Outcome Measure(s): Presence of bilateral or contralateral positive neck nodes on final histological analysis. Results: 179 (75%) patients demonstrated ipsilateral, 55 (23%) showed bilateral and 10 (4%) had only contralateral cervical metastases. Patients with clincally N0 necks demonstratred a 5% rate of bilateral nodal involvement. Those with ipsilaterally positive necks, were found to have positive disease on the contralateral side in 20% of cases. The pattern of lymph node spread ipsilaterally was: level I (16%), level II (58%), level III (34%), level IV (17%), level V (10%). Contralaterally positibe lymph nodes were found in: level I ( 5%), level II (17%), level III (12%), level IV (7%), level V (1%). Multivariate logistic regression analysis identifed patients with clinically T3/T4 tumors or N1/2a/2b necks as being more likely to exhibit bilateral neck disease on final pathology (p < 0.05). Conclusions: The incidence of bilateral neck disease in oropharyngeal cancer is higher than once thought. Patients with oropharyngeal squamous cell carcinoma clincally staged T3/T4 or with ipsilaterally positive necks should receive bilateral neck treatment.

**P076:** CENTRAL LYMPH NODE METASTASES OF INDETERMINATE LESIONS: A PROSPECTIVE COHORT STUDY - Jeffrey R Harris, MD, FRCSC, <u>Peter T Dziegielewski, M</u>D, David W Cote, MD, FRCSC, MSc, Lakshmi Puttagunta, MD, FRCSC, Jack Slatnik, MD, FRCSC, Jamie Tibbo, MD, FRCSC, Hadi Seikaly, MD, FRCSC; University of Alberta

Objectives: To determine the incidence of disease spread to central compartment lymph nodes in hemithyroidectomy performed for indeterminate biopsies. Design: Prospective cohort study. Setting: Tertiary care academic center. Patients: 30 patients with indeterminate pathology on fine needle aspiration biopsy. Interventions: Diagnostic hemi-thyroidectomy with central lymph node dissection. Main Outcome Measure(s): The incidence of thryoid cancer in the thyroid and central lymph node compartment specimens. Results: 25 (83%) of patients demonstrated were found to have paillary thyroid carcinoma in their thyroid specimens. All other specimens demonstrated benign nodules. Of the 25 positive specimens 8 (32%) had positive spread of disease to the central lymph nodes. Of the 5 patients with benign nodules, 2 (40%) were found to have micrometastases to the central lymph nodes. Conclusions:

This study suggests that the incidence of disease spread to central lymph nodes on indeterminate biopsies is higher than once thought. Dissection of the central compartment should be considered when a diagnostic hemithyroidectomy is performed.

#### **P077:** COMPLICATIONS OF CENTRAL LYMPH NODE DISSECTION IN THYROIDECTOMY: A RANDOMIZED CONTROLLED TRIAL - PRELIMINARY RESULTS - Jeffrey R Harris, MD, FRCSC, Peter T Dziegielewski, MD, Robert Hart, MD, FRCSC, Lakshmi Puttagunta, MD, FRCSC, Jack Slatnik, MD, FRCSC, Elaine Fung, MD, Hadi Seikaly, MD FRCSC; University of Alberta

Objectives: To determine the complication rates of thyroidectomy with or without central lymph node dissection. Design: Mutli-centre double blinded randomized controlled trial. Setting: Tertiary care academic centers. Patients: 60 patients undergoing total thyroidectomy for indeterminate pathology on fine needle aspiration biopsy. Interventions: Patients were block randomized to either: (Arm 1) undergo or (Arm 2) not undergo central lymph node dissection in addition to total thyroidectomy. Main Outcome Measure(s): Short term hypo-calcemia was defined as the need for supplemental calcium for post-operative (first 24 hours) ionized calcium values of < 0.9 mmol/L or symptomatic hypo-calcemia. Long term hypocalcemia was deifned as the need for supplemental calcium at 1 month post-operaively. Long term vocal cord dysfunction was defined by the voice handi-cap index (VHI) as well as endoscopic movement of vocal cords. Results: 34 patients underwent central lymph node dissection and 26 did not. 8 (23.5%) patients in Arm 1 and 5 (19.2%) in Arm 2 demonstrated short-term hypocalcemia. This difference was statistically insignificant (p > 0.05). 1 patient in each group demonstrated long term hypocalcemia (p > 0.05). No patient had vocal cord dysfunction. Mean change in VHI scores were statistically indifferent between groups (p > 0.05). Conclusions: Preliminary results suggest that the addition of central lymph node dissection to total thyroidectomy, does not significantly increase the risk of hypocalcemia or vocal cord dysfunction.

**P078:** RAPID PROTOTYPE MODELING IN HEAD AND NECK ONCOLOGIC RECONSTRUCTION - Hadi Siekaly, MD, FRCSC, <u>Praby Singh, MD</u>, Jamie Tibbo, MD, Peter Dziegielewski, MD, Jeffrey Harris, MD; Division of Otolaryngology - Head & Neck Surgery, University of Alberta

Background: Rapid prototype modeling is emerging as both a teaching tool and surgical planning aid. As a surgical simulator, prototype models provide the benefit of assisting in the planning for the reconstruction of defects following surgical ablation of large head and neck tumors. Surgical learners also benefit from the models as it improves their understanding of complex head and neck reconstruction. 3D prototype model are made from, skeletal CT scans, followed by rendering software to create a prototype prosthesis with the aid of a 3D printer. This generates a surgical model that assists in surgical reconstruction of these defects. These models have not previously been evaluated as a teaching tool in a tertiary Head and Neck referral center. Objective: Assess the utility of medical models in surgical simulation and resident education. Methods: All patients under going bony reconstruction of the head and neck with the assistance of a medical model were reviewed. Staff Head & Neck surgeons, Head and Neck Fellows and Chief Residents from 2000-2009 were surveyed on the teaching utility and benefit to patient outcomes of the medical models. The validated surveys were based on usability standards from the International Standards Organization. Results: 194 patients underwent rapid prototyping from 2000-2009 (Craniofacial Reconstruction 13.4%, Frontal Sinus Reconstruction 4.1%, Mandibular Reconstruction 39.2%,

Maxillary Reconstruction 43.3%). One of the head and neck surgeons did not feel that the models had any teaching utility. All trainees surveyed felt that medical modeling significantly improved educational opportunities, made the reconstruction easier and improved patient outcomes. Conclusions: The medical model rapid prototype protocol is a significant advancement in Head & Neck Surgery. It had the unique advantage of being both a powerful educational tool to assist surgical learners while providing significantly improved patient outcomes.

#### **P079: TREATMENT AND PROGNOSIS OF MESENCHYMAL TUMORS OF THE SALIVARY GLANDS** - <u>Cara L Cunningham, BS</u>, Adel El-Naggar, MD, Randal S Weber, MD, Michael E Kupferman, MD; MD Anderson Cancer Center

Background: Mesenchymal tumors of the salivary glands are rare and represent 0.3% of salivary gland neoplasms. Because of the diverse nature of these tumors and the limited data available, we reviewed our experience as it pertains to contemporary treatment modalities and prognosis. Methods: A database of over 1500 patients treated at M.D. Anderson Cancer between 1990-2007 for malignant major salivary gland tumors was reviewed. Eighteen patients were identified who were treated for mesenchymal cell tumors. Demographic, clinical and histopathologic data were reviewed. Results: There were 15 males and 3 females with a median age at presentation of 49 years. Sixteen tumors originated in the parotid gland as well as one each from the submandibular and sublingual glands. Histological classification revealed 2 neurogenic, 3 myogenic, 2 lipomatous, 2 fibrous and 9 unclassified tumors. Molecular analysis for markers such as vimentin, keratin, desmin and SMA was required for establishing accurate diagnoses in 10/18 cases. Overall, 5 out of 18 cases were sarcoma ex pleomorphic adenomas. Seven were originally staged T12, six were stage T34 and five were unknown. All patients were treated with surgical resection, and adjuvant therapy was utilized in 13 patients. Recurrence developed in 7 patients (39%), and 4 developed distant metastases. Five years after completion of treatment for the primary tumor, 6 patients were lost to follow up and of the remaining 12, 5 were still alive. At 10 years, 8 were lost to follow up and of the remaining 10, only 2 were still alive. Conclusions: In our series, mesenchymal cell tumors of the salivary glands had a high rate of recurrence and were associated with a poor prognosis. Further insights into the molecular pathogenesis of these tumors may yield novel approaches to therapy.

#### **P080:** FUNCTIONAL AND COSMETIC OUTCOMES OF PATIENTS WITH MAXILLECTOMY DEFECTS RECONSTRUCTED WITH VASCULARIZED FREE TISSUE TRANSFER - Hadi Seikaly, MD, FRCSC, Jamie J Tibbo, MD, FRCSC, Jana Reiger, PhD, Jeffrey R Harris, MD, FRCSC; University of Alberta

Objectives: To assess the functional and cosmetic outcomes of patients with maxillectomy defects reconstructed with vascularized free tissue transfer. Methods: We analyzed prospectively collected data on 46 patients with maxillectomy defects reconstructed with vascularized free tissue transfer (mainly radial forearm and fibula free flaps). Functional outcomes after reconstruction was assessed using a comprehensive collection of outcomes parameters including: PERCI-SARS for assessment of velopharyngeal orofice area, nasometer for assessment of nasalance, and standardized recordings for assessment of speech inteligibility. Cosmetic analysis was performed using eight naïve viewers providing assessment via a 10 point Likert scale. Results: In most parameters measured for both functional and cosmetic outcomes, results were excellent for free tissue reconstruction. Post-op nasalance scores were slightly worse than pre-op (p=0.012), however, this was not of clinical significance since speech intelligibility scores were near perfect. Swallowing outcomes were excellent with only 1

patient having aspiration post-op, and most patients exhibiting excellent oral transit times. Conclusions: Patients and reconstructive surgeons should expect excellent functional and cosmetic results with reconstruction of maxillectomy defects with free tissue transfer. Our next step will be to compare free tissue transfer reconstruction with the gold-standard at most institutions; palatal obturator.

P081: SWEDISH NATIONAL QUALITY REGISTER OF HEAD & NECK CANCER (SHNC-QR) - A POPULATION BASED REGISTER OF H& N CANCER INCIDENCE, TREATMENT AND OUTCOME - Johan Wennerberg, MD, PhD, Martin Beran, MD, PhD, Erik Holmberg, Anders Westerborn, MD, Johan Reizenstein, MD, Gunnar Adell, MD, Eva Brun, MD, PhD, Lena Cederblad, MD, Lena Damber, Mats Engström, MD, PhD, Eva Hammerlid, MD, PhD, Anders Högmo, MD, PhD, Göran Laurell, MD, PhD, Magnus Niklasson, MD, PhD, Björn Zackrisson, MD, PhD; Depts of ORL/ H&N Surgery, Oncology and Oncologic Centers at the University Hospitals in Lund, Gothenburg, Örebro, Stockholm, Uppsala, Umeå, and Linköping, Sweden

Background: Registries with high coverage are invaluable for describing patterns of care and outcomes for a population of patients. We report preliminaray data from a new prospective, longitudinal, observational national register of head and neck carcinoma (HNC) in Sweden comprising all ENT and Oncology departments diagnosing and treating HNC. Methods: Patients with a new HNC or a SPT are eligible for SHNC-QR. Coverage is double-checked through the Swedish Cancer Registry which receives compulsory information from clinical and pathology departments. Data are entered via a web-based system. Histology, site, TNM, stage, personal data and performance, treatment, planned and completed, as well as follow-up are reported. Patients are followed from time of diagnosis and for a minimum of 5 years, or until death. Results: Data on 1195 pts have been enrolled during 2008 comprising 96% of patients with HNC in Sweden. Until April 2010 2486 new cases have been entered into the register. Oral cavity is the most prevalent site (29 %) followed by oropharynx (22 %) and larynx (15%). Curative treatment is intended in 89%. Treatment comprises surgery alone in 26%, surgery in combination with radiotherapy in 18 % and radiotheraphy alone in 47%. After 13 month of follow up 64% of the patients are tumour free. Conclusions: SHNC-QR is a comprehensive, population based, prospective register of HNC with participation of all treatment centers and supported by the Swedish National Health Board. It allows population based analysis of incidence, prevalence, treatment and outcomes. Within a few years this can with accuracy also be done by site/sub-site.

#### **P082:** DO MULTIDISCIPLINARY CONFERENCES REALLY CHANGE MANAGEMENT DECISIONS AND PATIENTS' OUTCOME. WHERE IS THE EVIDENCE? - Jennifer M Croke, MD, Samy El Sanad MD: The Ottawa Hamital Cancer Cantra

Samy El-Sayed, MD; The Ottawa Hospital Cancer Centre

Introduction: Multi-disciplinary teams (MDTs) are now considered to be an integral part in the care of cancer patients as their management has become exceedingly complex over recent decades. Moreover, it is currently considered standard in many tertiary centres to have regularly scheduled multi-disciplinary cancer conferences (MCCs) in which MDTs meet to discuss relevant diagnostic, pathological and therapeutic aspects of a cancer patient's care. MCCs have been legislated to become part of the cancer management strategy. Although it appears intuitive that MCCs are essential to patient management and overall care there is surprisingly little evidence in the literature to support their use. Objectives: 1. To evaluate the literature that exists and our own experience, for the use of MCCs. 2. To determine whether or not the evidence supports the notion that MCCs are beneficial to clinical decision making and patient outcomes. Methods: An Ovid MEDLINE search was conducted from 1950 to February 2010 and included all languages to determine the evidence for the impact of MDTs and MCCs on clinical decision making and patient outcomes. Additionally, we analyzed evidence from our own experience with MCCs at a Canadian tertiary care teaching hospital. Results: 426 potentially relevant abstracts were found and reduced to 19 abstracts that focused on the impact of MDTs/MCCs on clinical decision making and patient management. We reviewed these manuscripts and highlighted the evidence that exists for their use. The evidence that support the use of MDTs/MCCs is rather limited in scope and production of evidence. The majority of studies assess the impact of MCCs on clinical decision making rather than their impact on patient outcomes. Evidence from our own experience reveals that the majority of new patients are discussed in MCCs and that in 45% of cases that discussion will significantly change the individual physician's treatment decision. Conclusion: Today, MCCs are a fundamental element in the management of cancer patients. Studies show that MCCs lead to changes in decision making and overall patient care. However, a paucity of true evidence exists and therefore future prospective studies in this area are necessary to gauge their true effectiveness.

#### **P083:** SCAR LENGTH AND POST-OPERATIVE SCAR SATISFACTION: A RANDOMIZED CLINICAL TRIAL - Jeffrey Harris, MD, FRCSC, <u>Praby Singh, MD</u>, Jaymi Dumper, MD, O'Connell Dan, MSc, MD, Hadi Seikaly, MD, FRCSC; Division of Otolaryngology - Head and Neck Surgery - University of Alberta

Background: The optimal size of incision and hence scar length for thyroid surgery is of great interest to Head and Neck Surgeons. A longer incision size is felt by many to provide easier access during thyroidectomy but is offset by the possible cosmetic concerns to patients. Cosmetic advantages for small incisions have been frequently espoused but rarely critically evaluated. The purpose of this study was to determine if scar length in thyroidectomy affects patient's satisfaction with their scar. A secondary objective is to determine if there is any increase in post-operative complications or surgical times for the two incision groups. Objective: To compare the cosmetic and functional outcomes for thyroidectomy through either a 5 or 10cm incision. Methods: Patients were randomized to either a 5cm or 10cm incision at the time of surgery. Patients were then evaluated at 6 months and again after 12 months. 13 patients were randomized to the 10cm group, and 9 patients to the 5cm group. The scar was evaluated by patients using the "patient and observer scar assessment scale" and by the surgeon using the "Vancouver scar scale". RESULTS: There was no clinically significant difference between the two incision size groups for either scar assessment by the patient or the surgeon. Operative characteristics and post-operative complication rates were no different between the two groups. Conclusions: There is no difference in cosmetic or surgical outcomes to the patient undergoing thyroidectomy for benign disease with respect to a 5cm or 10 cm incision size. These results would suggest a limited cosmetic benefit for smaller incisions in thyroid surgery.

#### **P084:** ENDOSCOPIC MICRODEBRIDEMENT VERSUS TRACHEOTOMY IN TUMOR OBSTRUCTED AIRWAYS - Hadi Seikaly, MD, FRCSC, <u>Monty Ghosh, MD</u>, Jamie Tibbo, MD, FRCSC, Peter T Dziegielewski, MD, Jeffrey R Harris, MD FRCSC; University of Alberta

Background: Acute airway obstruction is an uncommon emergent presentation of laryngeal cancer that requires immediate surgical intervention. Historically tracheotomy has been the most effective method of securing the airway but this procedure is associated with prolonged hospital stay and stomal recurrence. Objective: to assess the effectiveness and compare the complication profile of tracheotomy vs microdebrider assisted (MA) securing of the airway in this patient population. Methods:

A retrospective review of all patients with laryngeal cancer admitted to the head and neck surgery service from 1999 - 2010 was performed. Results: 145 patients were treated for laryngeal cancer and 38 presented with obstruction disease. The air way was secured with the microdebrider in 11 patients and tracheotomy in the rest. The complication profiles of both groups were similar. Length of hospital stay was less in the MA group than patients who had received tracheotomies (p=0.02). One patient in the tracheotomy group had a stomal recurrence. Conclusion: Endoscopic microdebrider assisted securing of the airway is safe and effective in patients with obstructing laryngeal cancer.

#### **P085:** AUTOFLUORESCENCE VISUALIZATION TO IDENTIFY HIGH RISK LEUKOPLAKIAS - Maureen Sullivan, DDS, Mary Reid, PhD, <u>Vijayvel Jayaprakash, MBBS, PhD</u>, Nestor Rigual, MD, Thom Loree, MD, Jennifer Frustino, DDS, Timothy Johnson, MS, Mihai Merzianu, MD; Roswell Park Cancer Institute

Introduction: The risk of malignant transformation of oral leukoplakia is best predicted by the histologic findings. However, identifying the high-risk leukoplakias with conventional clinical examination (CE) remains a challenge. Methods: We evaluated 115 leukoplakias in 52 high-risk patients with CE followed by autofluorescence visualization (AFV) at a blue excitation wavelength of 405nm. On CE, leukoplakias were identified as 'suspicious' based on various clinical characteristics including erythema, firm consistency, ulceration, raised margins, pain, bleeding or proximity to previous malignant site. On AFV, lesions that appeared grey or dark were identified as 'suspicious'. The histopathology findings were correlated with the CE and AFV findings. Results: Based on histologic examination, 16(4%) leukoplakias were categorized as benign (BL; normal mucosa, simple hyperplasia and parakeratosis without atypia), 68(59%) were categorized as low risk leukoplakias (LRL; 37 mild dysplasia and 31 parakeratosis with hyperplasia or atypia), and 31(27%) were categorized as high risk leukoplakias [HRL;14 moderate dysplasia (MD), 3 severe dysplasia(SD), 5 in-situ carcinoma(CIS) and 9 invasive squamous cell carcinoma(SCC)]. Thirtythree leukoplakias were identified as 'suspicious' using AFV, but not by CE. Of these, 24 were LRLs (including 15 with mild dysplasia) and 6 were HRLs (1 MD, 2 SD, 2 CIS and 1 SCC). Twelve leukoplakias were identified as 'suspicious' by CE, but not with AFV. These included 4 BL and 8 LRL (including 2 mild dysplasia), but no HRL. Of the 68 LRLs, 59 were suspicious using AFV; but only 43 were suspicious using CE. Of the HRLs, all 31 were identified using AFV and 5 lesions(16%), including 1 SCC and 2 CIS would have been missed by CE alone. The sensitivity for LRL detection using CE and AFV was 63.2% and 86.7%, respectively. The sensitivity for HRL detection using CE and AFV was 80.6% and 100%, respectively. Conclusion: Addition of AFV to clinical evaluation can improve the ability to identify the subset of leukoplakias at a higher risk of malignant transformation. Although our study shows a clear increase in detection of high-risk leukoplakia and thus benefiting high-risk patients, cost-effectiveness studies are needed to identify its place in diagnostic armamentarium.

#### **P086:** COST-BENEFIT ANALYSIS OF WHOLE BODY PET-CT IN THE DIAGNOSTIC WORKUP OF ADVANCED HEAD AND NECK CANCER - Hadi Seikaly, MD, FRCSC, <u>George Kurien, MD</u>, Jia

Hu, MD, Jeffrey R Harris, MD, FRCSC; University of Alberta

Background: PET-CT is an imaging modality that combines an 18F-FDG labeled PET scan with a contrast-enhanced diagnostic CT scan in a single unit. It has the benefit of providing both anatomic and functional data. Objective: The objective of this study is to assess the cost-benefit of whole-body PET-CT as a diagnostic tool in head and neck cancer. Design: Cost-benefit economic analysis. Method: A retrospective cohort compared to a hypothetical cohort based on current literature, in a tertiary cancer centre. Patients with advanced stage (AJCC III-IVB) squamous cell head and neck cancer in the year 2003 were reviewed for the number and nature of diagnostic and treatment procedures. Costs of diagnostic tests and treatment (whether radical or palliative) were compared to a hypothetical cohort of patients using PET-CT as a sole diagnostic tool using current literature on test characteristics in detection of distant metastases. The main outcome measure was the cost of the diagnostic workup and the secondary outcome measure was the cost of the treatment as a result of the diagnostic workup. Results: The study determined that the cost of diagnostic PET-CT workup was \$722 per patient, while the traditional workup was \$450 per patient. In the traditional workup, all patients received CT scans of the neck, 92% received chest x-rays, and 31% received CT scans of the chest. The sensitivity of the traditional workup for lung metastases at 12 months was 14.3%. The average cost of curative surgery was \$81,290, with radiotherapy costing \$8,224, and chemotherapy costing \$1,158. Fifty surgeries were performed and 26 patients were treated nonsurgically. In this cohort (n=76), PET-CT would theoretically detect 3 more cases of metastatic disease based on improved sensitivity, and would have reduce the total cohort cost of treatment by \$198,526 by relegating these patients to palliation. Conclusions: While PET-CT is a more expensive test when used as a sole entity for diagnostic workup of head and neck cancer, the cost-savings lie in the superior detection of distant metastases and thereby cost-saving in avoidance of radical treatment.

#### **P087: OUTCOMES OF MICROVASCULAR FREE FLAP TRANSFER IN THE ELDERLY** - Hadi Seikaly, MD, FRCSC, <u>Jason A</u> <u>Vaz</u>, David W Côté, MD, MPH, Jeffrey R Harris, MD, FRCSC; Division of Otolaryngology - Head and Neck Surgery, University of Alberta

Background: Free tissue transfer reconstructions have become the standard of care after head and neck cancer resection because of their superior reliability, and functional outcomes. The reconstructive surgeon, however, is often faced with the dilemma of offering free flap reconstruction to the elderly, after surgical extirpation in the head and neck, due to the perception that these patient population tolerates these complex procedures poorly. Objective: The purpose of this study was to evaluate the outcomes of free flap reconstruction in the elderly. Design: A 30 month retrospective review of all microvascular free flap reconstruction cases in patients from a large head and neck oncology program in a tertiary care center. Setting: Academic research. Patients: A series of 280 free flap patients were reviewed. Patients were stratified into two age groups; 45-64.9yrs (n=178) and =65yrs (n=102). Main Outcome Measure: The functional and surgical outcomes of free flap reconstruction in the elderly. Results: Of the total 280 cases, 4 (1.4%) patients had primary free flap microvascular failure and 20 required a salvage procedure. The most common reasons for flap failure included hematoma and venous congestion. There was no significant difference in anastomotic failure (p=0.46) or flaps requiring salvage (p=0.46) between the two groups. The increased number of comorbidities (n=0.001) over a higher number of physiological system (0.005) in the elderly group did not adversely affect microvascular outcomes (p>0.05). In both groups, number of complicated recoveries and number of deaths within 30 days of surgery showed no significant difference. However, the length of postoperative inpatient stay was longer in the elderly group (26.9 days) compared to the younger group (21.6 days) (p=0.029). Conclusions: Microvascular flap success and overall postoperative course were similar in the elderly and younger patient cohorts. Reconstructive surgeons should not restrict the use of free flaps based on their patients' age.

#### **P088:** POSTERIOR TIBIAL ARTERY FREE FLAP IN RECONSTRUCTION OF THE HEAD & NECK - Jeffrey R Harris, MD, FRCSC, <u>Jason A Vaz</u>, David W Côté, MD, MPH, Hadi Seikaly, MD, FRCSC; Division of Otolaryngology - Head and Neck Surgery, University of Alberta

Background: The posterior tibial flap is a soft tissue flap that has been described in foot, arm and lower leg reconstruction. This flap is potentially an excellent flap option for reconstruction of the head and neck because it offers thin, pliable and well vascularized tissue. Moreover, a large skin paddle can be obtained with a long, pedicle. Objective: The purpose of this study was to report a series of patients who underwent resection of major head and neck carcinomas followed by reconstruction using the posterior tibial free flap. Design: A retrospective review of all microvascular free flap reconstruction cases in patients from a large head and neck oncology program in a tertiary care center from January 1st, 2007 to January 1st, 2010. Setting: Academic research. Patients: A consecutive series of 7 patients that had undergone reconstruction with a posterior tibial flap at the University of Alberta were reviewed. Main Outcome Measure: Microvascular and surgical outcomes of posterior tibial artery free flap reconstruction of the head and neck. Results: The posterior tibial flap was used to repair defects of the oral cavity (n=4) and oropharynx (n=3) following resection of either squamous cell carcinoma (n=6) or adenocarcinoma (n=1). On average, the free flap measured 12cm x 9.8cm with a pedicle length of 9cm. In all cases, the flap was elevated with at least 2 musculocutaneous perforators in an average of 71 minutes. In one case, the free flap was neurotized with the sural nerve and anastamosed to the lingual nerve. All free flaps survived without need for salvage surgery. The average postoperative inpatient stay was 18.7 days, and no patients died within 30 days of primary surgery. Conclusions: The posterior tibial flap is well suited for head and neck reconstruction and should be added to the reconstructive surgeon's armamentarium.

#### **P089:** TRANSORAL LASER MICROSURGICAL RESECTION OF ORAL CAVITY SQUAMOUS CELL CARCINOMA - <u>Douglas</u>

<u>H Cowan, MD</u>, Douglas A Girod, MD, FACS, Kevin J Sykes, MPH; Department of Otolaryngology-Head and Neck Surgery, University of Kansas Medical Center

Objectives: To review oncologic and functional outcomes and prognostic variables of patients who underwent transoral laser microsurgery (TLM) for squamous cell carcinoma (SCCA) of the oral cavity. Study Design: Retrospective single-center chart review. Methods: From 1999 to 2008, 65 patients with SCCA of the oral cavity were initially treated with transoral laser microsurgical resection. Overall survival, diseasespecific survival, local and locoregional control rates were analyzed using the Kaplan-Meier method. Functional outcomes including immediate and long-term speech and swallow ability were analyzed along with other treatment-related morbidity. Results: Of the 65 patients with SCCA of the oral cavity, 62% (n=40) were neoplasms involving the oral tongue alone, 22% (n=14) involved the floor of mouth, 9% (n=6) involved the hard palate and 8% (n=5) involved the buccal mucosa. Distribution of the pathological T stages are as follows; 60% of patients were T1 (n=39), 35% were T2 (n=23) and the remaining 5% (n=3) were T3/ T4. Ten patients (15%) belonged to disease stage III or IVa based on T stage or neck disease. Selective neck dissection was performed in 63% of patients (n=40); 28% of patients received postoperative radiotherapy (n=18). Mean follow-up for all patients was 42.5 months. The Kaplan-Meier overall survival estimate was 92% at 2 years and 78% at 5 years. The respective 2 and 5-year Kaplan-Meier disease-specific survival estimates were 96% and 88%. The 5 year Kaplan-Meier local and

locoregional control estimates were 97% and 92% respectively. Median hospital stay was 2 days. Tracheostomy was temporarily required for 2 patients. Three patients required PEG tube placement of which 2 were eventually removed. Outcomes were also reported based on tumor site, TNM staging and the use of neck dissection and/or adjuvant therapy. Conclusions: TLM with or without adjuvant post-operative therapy provided excellent local and locoregional control with minimal treatment-related morbidity. Transoral laser microsurgery appears to be a safe and effective treatment for SCCA of the oral cavity.

# **P090:** SALVAGE SURGERY FOLLOWING RADIATION FAILURE FOR LARYNGEAL CANCER IN ELDERLY

**PATIENTS** - <u>JJ Lusardi</u>, M A Varvares, MD; Saint Louis University School of Medicine

Objective: As conservation-directed therapy for laryngeal cancer has become the standard of treatment, salvage surgery has played a prominent role for management of recurrences. Few studies have stratified the survival rates from salvage surgery by age, particularly the elderly patient subset. The objective of this study is to report the one year survival rate of patients >80 years old who undergo salvage surgery for squamous cell carcinoma of the larynx. Design: Literature review and retrospective outcome analysis of cancer cases from the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) database. Patients: Patients > 80 years old with laryngeal cancer who underwent surgery following radiation therapy. Main Outcome Measure: Overall survival and cancer specific survival. Results: The one year overall survival of patients who matched the inclusion criteria (n=1,413) was 86.3% (95% Confidence Interval [CI] 85.4-87.2). The cancer specific survival at one year was 92.1% ([CI] 91.4-92.8). The five year overall survival and five year cancer specific survival were similar between the patient cohort extracted from SEER compared to patients undergoing salvage surgery of all ages from the literature (overall survival 48.1% vs 47%) (cancer specific survival 74.2% vs 71.4%). The patients from the literature used to calculate the overall survival (n=312) and cancer specific survival (n=111) did not differ significantly from the SEER cohort in terms of cancer stage or laryngeal site. Conclusion: Elderly patients (>80 years old) have a favorable one year prognosis in regards to overall survival and survival from cancer recurrence following salvage surgery. This subset of patients has similar survival outcomes compared to patients of all ages who undergo salvage surgery.

#### **P091:** COMPARISON OF PREOPERATIVE ULTRASONOGRAPHY, T99M-SESTAMIBI, AND SPECT IN LOCALIZATION OF PARATHYROID ADENOMAS FOR MINIMALLY-INVASIVE PARATHYROID SURGERY: A COST ANALYSIS - Jeffrey Harris, MD, FRCSC, Brittany Barber, BMSc, David Cote, MD, MPH, Hadi Seikaly, MD, FRCSC; University of Alberta

Objectives: To compare the accuracy of ultrasonography (US), T99m-Sestamibi scanning (T99mS), and SPECT/CT in localizing parathyroid adenomas for minimally-invasive parathyroid surgery. To compare the cost and efficiency of each, and determine an optimal diagnostic strategy. Design: A retrospective review. Method: Localization of parathyroid adenomas provided by US, T99mS, and SPECT was tested against intraoperative findings. Results were recorded as correct if they localized to the correct quadrant specified by the surgeon or non-localizing if they were not detected or localized incorrectly. Analysis of combination modalities was undertaken to suggest an optimal preoperative localization strategy for a minimally-invasive approach. Setting: University of Alberta tertiary care otolaryngology-head and neck surgery service. Patients: Patients diagnosed with parathyroid adenomas undergoing minimally-

invasive parathyroid surgery from 2003-2009. Interventions: Use of US, T99mS, and SPECT/CT individually and in combination to localize parathyroid adenomas preoperatively. Main Outcome Measure(s): Correct number and localization of parathyroid adenomas to 1 of 4 quadrants or an ectopic location, rate of non-localization of each modality, and the rate of localization of combined modalities. Results: Analysis revealed localization rates to correct quadrant for ultrasound, T99mS scanning, and SPECT/CT as 49.5%, 65.1%, 81.0% respectively. Combination imaging pairs of US-SPECT/CT and US-T99mS demonstrated difference in localization of 88.1% to 86.0% respectively. An ICER cost analysis of both combination strategies demonstrates that for every 100 patients, 88 are correctly localized with US-SPECT compared to 86 for US-T99mS with a difference of only \$2400 per additional correctly localized gland with SPECT/CT. Conclusions: Highest accuracy is obtained with ultrasound and SPECT/CT in localization of parathyroid adenomas. Cost analysis reveals US-SPECT/CT combination is more cost-efficient in preventing additional costs associated with nonlocalization, including increased operating room time and potential surgical complications for the patient.

#### **P092:** PEG SITE SEEDING FROM HEAD AND NECK SQUAMOUS CELL CARCINOMA: A CASE SERIES AND SYSTEMATIC REVIEW - <u>Andrew T Huang, MD</u>, Alexandros Georgolios, MD, Nicholas Hatch, BS, Evan Reiter, MD; Virginia

Georgolios, MD, Nicholas Hatch, BS, Evan Reiter, MD; Virginia Commonwealth University Goals: 1.) Describe the complication and clinical course of

percutaneous endoscopic gastrostomy (PEG) site seeding of head and neck squamous cell carcinoma (SCC). 2.) Review the literature of all documented cases of PEG site metastases from head and neck SCC and identify trends in therapy for future research. Methods: Retrospective review of six patients with head and neck SCC from a single institution with pathologically proven metastasis of disease to their PEG site. A PubMed literature search was carried out to identify all published cases of PEG site metastases. Review was limited to SCC's of the oral cavity, oropharynx, hypopharynx and larynx. Thirty-one papers were identified for a total of thirty-five reported cases. Data including patient age, sex, primary tumor subsite, time to PEG site metastasis, method of PEG implemented, primary and metastatic tumor treatment, and length of survival were analyzed. Data: The average age at diagnosis was 64 years. All patients had Stage IV disease. PEG tubes were all placed prior to initiation of primary therapy, and each head and neck subsite was equally represented. The average time to PEG site metastasis was 10.5 months. In review of the literature, PEG site metastasis occurred most commonly in Stage IV disease (78%). The oropharynx was the most common site of primary cancer (43%), followed by hypopharynx (27%), oral cavity (17%), and larynx (13%). Average time to PEG site metastasis was 8.05 months, and average length of survival was 12.1 months. All PEG's were placed by the Ponsky "pull" technique. Ten reports detailed treatment of PEG metastasis, with longest average survival in patients receiving chemoradiotherapy (24 months). Conclusion: The six cases of PEG site metastasis documented in this series represent the largest series in the literature. As all metastases appear to be associated with the "pull" technique of PEG placement, we recommend use of other methods for gastrostomy placement, especially in patients with oropharyngeal and hypopharyngeal primary tumors.

**P093:** CEREBRAL NETWORK ACTIVATION DURING VERBAL COMMUNICATION IN LARYNGECTOMEES: AN FMRI STUDY - Lucia Oriella Piccioni, MD, Salvatore Toma, MD, Fabrizio Ferrario, MD, Bussi Mario, MD; Department of Otorhinolaryngology, San Raffaele Scientific Institute and Vita-Salute University, Milan, Italy

Aim of the Study: We focused our attention: to identify larynx area in a somatotopic cerebral contest; to investigate brain modification after laryngeal surgery. Patients and Methods: From January to March 2009 we enrolled 18 patients, divided in three groups: group 1, 6 healthy volunteers; group 2, 6 patients underwent reconstructive subtotal laryngectomy; group 3, 6 patients treated with total laryngectomy. By using functional Magnetic Resonance Imaging we have studied our subjects during performation of a series of oral tasks with phonatory and articulatory elements. Results: During phonatory tasks the groups 2-3 showed the activation of the same areas described in the group 1 but we also observed an extension of these, proportional to laryngeal surgical damage. Moreover in the groups 2-3, we recorded the activation of cerebral areas which are silent in the healthy controls (basal ganglia, Rolandic operculum, cerebellum and the SMA). In the lip and tongue tasks we observed a pattern of activation comparable in the 3 groups. Conclusions: This study permitted the mapping of human motor cortical areas that control intrinsic laryngeal muscles. The same functional study performed in subtotal and total laryngectomees has lead to the identification of an expansion of the larynx area activation, if compared to healthy controls. This observation is suggestive for neuronal recruitment phenomena, proportional to the laryngeal injury degree. Moreover, we observed the functional recovery of brain regions considered evolutionary ancient and virtually quiescent in healthy human population. So we can suppose the existence of meaningful neuronal plasticity phenomena consequent to laryngeal surgical damage.

# QUALITY OF LIFE

**P094:** FIRST REPORT ON THE VALIDATION OF FIRST EVER SPEECH SPECIFIC QUESTIONNAIRE FOR ENGLISH SPEAKING HEAD AND NECK CANCER PATIENTS: THE SPEECH HANDICAP INDEX "SHI" - <u>Raghav Dwivedi, MRCS,</u> <u>DOHNS, MS</u>, Suzanne St. Rose, PhD, Edward Chisholm, MD, FRCS, Justin Roe, MSc, Cert, MRCSLT, Christopher Nutting, MD, FRCR, Peter Clarke, FRCS, Cyrus Kerawala, FRCS, FDSRCS, Peter Rhys-Evans, FRCS, Kevin Harrington, FRCR, PhD, Rehan Kazi, MS, FRCS, PhD; Head and Neck Unit, Royal Marsden Hospital and The Institute of Cancer Research, Fulham Road, London, UK

Background: Although there are many voice-specific scales, surprisingly there is no speech-specific questionnaire for English-speaking head and neck cancer (HNC) patients. The aim of this study is to validate the Speech Handicap Index (SHI) as the first speech-specific questionnaire in the English language. Methods: Sixty-three consecutive English-speaking patients in follow-up for oral or oropharyngeal cancers at The Royal Marsden Hospital, London, UK were recruited for this study. English version of Speech Handicap Index (SHI) consists of 30 well-constructed questions to evaluate the patient's speech and speech-related psycho-social functions. The University of Washington quality of life questionnaire (UWQOL-v4) was used as a gold standard for the validation process. A randomly selected subset of thirty-two patients was asked to complete both the questionnaires again after four weeks in order to assess testretest reliability. Internal consistency was assessed using Cronbach's alpha coefficient while test-retest reliability and construct validity were assessed using Spearman's rank correlation coefficient. Group validity was determined using the Mann-Whitney U-test. P-value <0.05 was taken as significant. Results: The internal consistency reliability for Total SHI and SHI speech domain as calculated by Cronbach's alpha coefficient

was 0.98 and 0.95, respectively. For SHI psycho-social domain alpha coefficient was 0.98. Test-retest reliability of Total SHI and SHI speech domain as calculated by Spearman's rank correlation coefficients were 0.92 and 0.88, respectively. For SHI psycho-social domain the coefficient was 0.89. The correlation coefficients between Total SHI score, the SHI speech domain, the SHI psycho-social domain and overall SHI speech assessment question, and speech domain of UWQOL were 0.72, 0.72, 0.71 and 0.68, respectively. The correlation coefficients between Total SHI score, the SHI speech domain, the SHI psycho-social domain and overall SHI score, the SHI speech domain, the SHI psycho-social domain and overall SHI score, the SHI speech domain, the SHI psycho-social domain and overall SHI speech assessment question, and social domain of the UWQOL were 0.44, 0.44, 0.43 and 0.35, respectively. Significant differences (P<0.05) were detected when patients were grouped according to T stage and tumor site. Conclusion: English version of the SHI is a precise, reliable and valid speech assessment tool for HNC patients. It can be a useful adjunct in the rehabilitation of HNC patents.

#### **P095:** A REPORT ON THE DEVELOPMENT AND VALIDATION OF FIRST EVER SPEECH SPECIFIC PERCEPTUAL SPEECH EVALUATION TOOL THE "LONDON SPEECH EVALUATION SCALE (LSE-SCALE)" FOR HEAD AND NECK CANCER PATIENTS - <u>Raghav Dwivedi, MRCS,</u> <u>DOHNS, MS</u>, Suzanne St. Rose, PhD, Edward Chisholm, MD, FRCS, Cyrus Kerawala, FRCS, FDSRCS, Peter Clarke, FRCS, Christopher Nutting, MD, FRCR, Peter Rhys-Evans, FRCS, Kevin Harrington, FRCR, PhD, Rehan Kazi, MS, FRCS, PhD; Head and Neck Unit, Royal Marsden Hospital and The Institute of Cancer Research, Fulham Road, London, UK

Background: Although there are many perceptual voice evaluation scales available but to date there is no scale available for speech evaluation in head and neck cancer (HNC) patients. The aim of this study was to develop and validate first ever speech-specific scale for perceptual evaluation of speech in HNC patients. Methods: Based on an extensive literature review and discussion in a multidisciplinary setting 5 speech parameters (intelligibility, articulation, speech rate, nasality and asthenia) and overall grade of speech impairment were selected and evaluated for the development and validation of this scale. Speech samples of 117 subjects (65 consecutive follow-up HNC patients and 52 healthy volunteers) were recorded on the Electroglottograph (EGG) equipment using a standard protocol consisting reciting specific words and a standard text passage. All samples were independently judged and rated by 3 experienced raters (speech and language therapists) and were re-rated 12 weeks apart for establishing test-retest reliability. Internal consistency reliability, intra-rater reliability and inter-rater reliability of different parameters of this scale and overall grade were determined using Cronbach's alpha and Spearman's rank correlation coefficients. Construct validity was tested using Spearman's rank correlation coefficient. Group validity was tested by Mann-Whitney U-test. Results: The Cronbach's alpha coefficients for internal consistency ranged from 0.87-0.90 for connected speech and 0.79-0.84 for words for all raters. The Spearman's correlation coefficients for intra-rater reliability of these parameters for connected speech they varied between 0.38-0.87 while for words the values ranged between 0.50-0.71. The Spearman's correlation coefficients for inter-rater reliability of connected speech were between 0.55-0.99, while for words they ranged between 0.40-0.57. For construct validity, the Spearman's correlation coefficient between overall grade of the connected speech and overall speech evaluation question of SHI and speech question of UWQOL were 0.47 and 0.41, respectively. The values of correlation coefficients for words ranged between 0.42- 0.55. Significant group differences were also observed. Conclusion: The LSE scale is a reliable and valid tool for speech evaluation in HNC patients. It can be a useful adjunct in the rehabilitation of HNC patients. This scale may also be used in the rehabilitation of neurological patients having speech problems.

#### **P096:** RELATIONSHIPS BETWEEN COGNITIVE AND SENSORY PAIN AND AFFECT IN PATIENTS UNDERGOING HEAD AND NECK CANCER TREATMENT - <u>Dena J Fischer</u>, DDS, <u>MSD</u>, MS, Young Ok Kim, DrPH, RN, CHES, Joel B Epstein, DMD, MSD, Lawrence E Feldman, MD, Diana J Wilkie, PhD, RN, FAAN; University of Illinois College of Dentistry, University of Illinois at Chicago College of Nursing, University of Illinois at Chicago College of Medicine

Objective: Disease- and treatment-related cancer pain is a multidimensional phenomenon. Cognitive dimension factors that may alter pain perception in cancer patients have not been well-studied. The study aim is to explore relationships between cognitive dimension variables and sensory pain characteristics, depression and anxiety in patients undergoing head and neck cancer (HNC) treatment. Methods: In a cross-sectional study, 108 consecutive patients (76% male, mean age 54.4±12.7 years) undergoing radiation treatment for HNC, reported cognitive pain variables: 1) satisfaction with pain levels (SAT); 2) expectation of pain levels (EXP); and 3) tendency to tell others about their pain (TELL). They also reported sensory pain characteristics (McGill Pain Questionnaire) and pain intensity (Pain Intensity Number Scale) as well as depression (Center for Epidemiologic Studies Depression Scale) and anxiety (State Trait Anxiety Inventory). Descriptive statistics and ttests or ANOVAs were performed with SPSS17 (Chicago, Illinois). Results: Subjects who were not satisfied with their pain levels (NotSAT) reported significantly higher pain intensity (p=.000), number of pain words chosen (NWC; p=.016), affective (p=.039) and sensory (p=.007) pain indices compared to those who were satisfied with their pain. Further, NotSAT subjects had significantly higher depression levels (p=.014) but not anxiety. Subjects in whom the pain was worse than expected (EXP-W) reported higher pain intensity (p=.000), number of pain sites (p=.048), total pain pattern (p=.046), affective (p=.000), sensory (p=.007) and total (p=.015) pain indices compared to those in whom pain was less than expected. EXP-W subjects reported higher levels of depression (p=.009) but not anxiety. Subjects who told others about their pain (TELL-Y) had lower levels of current pain (p=.015) compared to those who did not tell others about pain (TELL-N). TELL-Y reported lower pain indices levels and lower depression, though not significantly different than TELL-N. Conclusion: Pain intensity, sensory pain indices and depression levels consistently differed by some (SAT, EXP) but not all (TELL) of the cognitive variables. Patients who are not satisfied with their pain or have pain worse than expected are vulnerable to more severe pain. Clinicians should consider the cognitive dimension and the impact upon the pain experience in HNC patients undergoing treatment.

### **P097:** COST EFFECTIVENESS OF THE USE OF POST-OPERATIVE PTH AND CALCIUM TO DETERMINE EARLY DISCHARGE OF POST-THYROIDECTOMY PATIENTS

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Background: Studies and professional guidelines suggest that early postoperative calcium and parathormone(PTH) levels are reliable predictors of the incidence of hypocalcaemia after total thyroidectomy. This may have cost implications as patients can be discharged early from the hospital. Objective: To undertake an economic evaluation of the utility of post-operative PTH and calcium level in deciding early discharge after total thyroidectomy. Methods: We analysed 97 consecutive total and completion thyroidectomy operations. We calculated the costs of the current post-operative protocol which requires in patient monitoring of calcium for at least 2 days, compared with the use of a combination of PTH(> 1.1pmol/l) and adjusted calcium(>2mmol/l) on the first postoperative morning to decide discharge on the first post-operative day. Normal range for PTH in our lab is 1.1 - 4.2 pmol/L. At our hospital,

overnight stay for a patient costs £169(\$248), PTH assay £8.7(\$12.8 ) and calcium assay £4.3(\$6.3 ). Results: 97 consecutive patients, who underwent total and completion thyroidectomy. Using the current post-operative monitoring protocol, 36/97 patients required calcium supplementation remained for 3 nights and the rest for 2 nights, costing £38870/-(\$57,076.7) for 230 patient days. Using the PTH/Calcium combination proposed above, 44/97(45%) patients could be discharged on the first post-operative day, with a sensitivity of 0.69, specificity of 0.94, PPV of 0.95 and NPV of 0.64. 34 of the remaining 53 patients required calcium supplements and hence stayed for 3 nights and the other 19 remained for 2 nights. Therefore this protocol incurred a total cost of £31940/-(\$46,900), and therefore saved £6930/-(\$10,176), but potentially would have discharged 2 patients who needed calcium. Conclusions: A criteria of PTH level > 1.1 pmol/l and corrected serum calcium level > 2.0 mmol/l in early post-operative period in our study correctly predicted that 45% of patients can be safely discharged the next day with 6% false negative rate. It has a potential to reduce costs by 18%.

# **P098:** SURGICAL AND PROSTHETIC RECONSIDERATIONS

**IN MAXILLECTOMY PATIENTS** - <u>Bernd Lethaus, MD DMD</u>, Frans de Beer, DMD, Peter Kessler, PhD, MD, DMD; University Clinincs Maastricht, Dpt. Cranio-Maxillofacial Surgery

The purpose of this study was to establish and evaluate new possibilities for rehabilitation of patients with obturator prosthesis who had undergone partial or total maxillectomy due to tumour ablation surgery. Eleven patients with maxillary defects were reconstructed with a CAD/CAM designed prosthesis. Missing retention was gained by inserting implants in the remaining bone so that an expansion of the surgical defect to gain further retention could be avoided. All patients were treated successfully according to the above described treatment plan. The Obturator Functioning Scale (OFS) of the Memorial Sloan-Kettering Cancer Centre was applied to evaluate the functional quality of the obturator prosthesis and patient's satisfaction. It showed good results in all fields of functional outcome and social acceptance.

#### **P099:** TEMPOROMANDIBULAR JOINT RECONSTRUCTION AFTER MAJOR HEAD AND NECK EXTIRPATION SURGERY: A PROSPECTIVE COHORT ANALYSIS - Hadi Seikaly, MD, FRCSC, <u>Morgan Langille, MD, BSc</u>, Walter Dobrovolsky, DDS, FRCDC, Jeffery Harris, MD, FRCSC; University of Alberta, Edmonton, Canada

Objective: To assess the long term Quality of Life (QOL) and functional outcomes in patients who have received total temporomandibular (TMJ) extirpation and a novel reconstruction technique with a prosthetic TMJ in combination with osteocutaneous free flap. Design: Prospective cohort. Patients: Six patients having undergone mandibulectomy and TMJ resection were reviewed. Reconstruction was achieved with osteocutaneous free flap with Lorenz TMJ prosthesis. Main Outcome Measures: A speech-language pathologist collected the functional outcomes data using a standard protocol. The mandibular function was assessed with measurement of oral opening and occlusion. Swallowing function was assessed via modified barium swallows. We administered the European Organization for Research and Treatment of Cancer (EORTC) QLQ-H&N35 questionnaire. Results: We identified six patients who received TMJ replacement surgery between August 2004 and July 2009. Average age at time of surgery was 45 years (range 33-65). Average followup between the surgery and administration of the QOL questionnaire was 24 months (range 8-55). Barium swallow showed evidence of silent aspiration in one patient (17%). Two patients (33%) had slight difficulty with the oral stage of swallowing. The average scores for the EORTC QLQ-H&N35 showed satisfactory quality of life in speech, swallowing,

eating, salivary function and mouth opening. No patients required the use of a feeding tube. Conclusion: TMJ reconstruction with total joint prosthesis and free osteocutaneous flaps result in good long-term functional outcome and satisfactory quality of life.

#### **P100:** SUBMANDIBULAR GLAND TRANSFER: OUR 10-YEAR EXPERIENCE - Jamie J Tibbo, MD, FRCSC, Naresh R Jha, MBBS, FRCSC, Jeffrey R Harris, MD, FRCSC, David Williams, MD, FRCSC, Hadi Seikaly, MD, FRCSC; University of Alberta; Cross Cancer Institute

Objective: To review our 10-year experience with submandibular gland transfer (SGT). Background: Radiation therapy is commonly used in the treatment of head and neck malignancies. Xerostomia is a permanent devastating side-effect of head and neck irradiation. Ten years ago, our group described the submandibular gland transfer procedure which involves moving a submandibular gland outside the radiation field, forward into the submental space. The gland is mobilized by transecting the facial artery, with the gland receiving retrograde blood-flow. The gland is spared from radiation, and function is preserved. This method has been used in patients with laryngeal, oropharyngeal and nasopharyngeal cancer, as well as cancers with unknown primaries. Methods: Over the last 10 years we have performed over 250 submandibular gland transfer procedures. A total of 143 of these patients were enrolled in trials and compared with 22 control patients, for salivary flow and quality of life. We combined the data from 3 separate clinical trials for our analysis. Results: Patients who had SGT had significantly better saliva production, and consistency after 24 months follow-up, based on quality of life questionnaires (p<.0001 and p<.005, respectively). There was also significantly better baseline and stimulated saliva flow after 24 months follow-up (p = 0.48 and p = 0.008, respectively). SGT did not result in a significantly different perception of taste (p=0.846 at 24 months). Conclusion: Submandibular gland transfer has been used successfully in the prevention of radiation-induced xerostoma in our centre for 10 years, and has been shown to result in improved salivary flow and quality of life.

#### **P101:** EMPLOYMENT OF PATIENTS FOLLOWING TREATMENT OF ADVANCED HEAD AND NECK CANCER Hadi Seikaly, MD, <u>Raiyan Chowdhury, MD</u>, Sheehan Chowdhury, MD, Jeffery Harris, MD; University of Alberta

Background: The treatment of advanced head and neck cancer has evolved significantly over the last 20 years. The advent of new surgical techniques and organ preservation protocols have led to increased locoregional control while improving the patients functional outcomes and quality of life. Another measure of wellbeing is the employment status of the individual yet there is a paucity of literature on the subject. Objective: To evaluate the post treatment employment of patients with advanced head and neck cancer Methods: We retrospectively contacted all patients undergoing major head and neck cancer resection and reconstruction surgery at the University of Alberta from January 2005 to December 2007. Patients were questioned regarding their duration and type of employment and the degree to which they felt they could meet their duties. Results: A total of 232 patients were contacted and reviewed. 23% had died of their disease. 55% of patients that were alive were employed full-time and performing their duties adequately. 92% of the employed patients returned to work within the first year. 84% return to the pretreatment occupation. 21% of laryngectomy patients returned to any form of employment. The type of employment ranged from CEO of a major corporation to manual laborer. Conclusions: Return to meaningful and successful employment continues to be an arduous task for head and neck cancer patients especially after undergoing a laryngectomy.

#### **P102:** TOBACCO CESSATION AS A RESULT OF COMMUNITY BASED HEAD AND NECK CANCER SCREENING - <u>Edie R Hapner, PhD</u>, Justin C Wise, PhD, Kellie L Bauer, Med; Emory University, Georgia State University

The aim of this study was to examine the usefulness of a large-scale head and neck cancer screening for reducing at risk behaviors in an at risk population. The specific questions answered were: 1) Did a community head and neck cancer screening result in cessation or reduction of cigarette use?; 2) Were there differences in the rates of cessation or reduction of cigarette use in individuals who receive a positive screening versus a negative screening?; 3) Did background factors predict whether individuals stopped or reduced cigarette use? Six hundred and twenty participants, ages 18-75 (M = 43.06, SD = 13.02) years were screened for oral/laryngeal cancers during two NASCAR race weekend events. Of the 620 participants that were screened, 156 (25%) required further medical follow up. 179 were smokers, 251 were nonsmokers, and 148 were past smokers. Chi-square analysis indicated that a significantly higher proportion of smokers (13%) evidenced positive finders compared to nonsmokers (8%) and past smokers (6%),  $\chi^2$  (n = 578) = 28.25, p < .001. A significantly higher proportion of males (16%) evidenced positive findings compared to females (10%),  $\chi^2$  (n = 578) = 4.81, p < .05. ANOVA analysis followed by a Tukey post hoc analysis (p < .05) indicated that past smokers were significantly older (M = 46.99) than smokers (M = 40.40) and nonsmokers (M = 42.89), F (2, 575) = 10.92, p < .001,  $\eta$ 2 = .04. Kruskall Wallis analysis followed by a Dunn's Multiple Comparison post hoc test indicated that smokers were from a significantly lower SES background compared to nonsmokers,  $\chi 2$  (n = 578) = 18.13, p < .001. Seventy-five of the participants (47%) who self-identified as a smoker were contacted six months later. Participants reported smoking significantly less cigarettes per day (M = 13.78) at the six month follow up compared to the number of cigarettes (M = 19.43)smoked at the baseline time point, F (1, 72) = 31.86, p < .001,  $\eta 2 = .31$ . Forty-four (59%) participants reduced the number of cigarettes they smoked per day. Eleven participants reported quitting smoking.

### THERAPIES-CLINICAL

**P103:** MICROINVASIVE ACCESS TO THE VISCERAL AUTOFLAPS FOR MICROSURGICAL RECONSTRUCTION IN HEAD AND NECK CANCER PATIENTS - Igor Reshetov, Valery Chissov, Sergey Kravtsov, <u>Mikhail Ratushnyy</u> P.A. Hertzen; Moscow Cancer Research Institute, Moscow, Russia

Background: In the P.A. Hertzen Moscow Cancer Research Institute was developed a method of microinvasive abdominal access to form the visceral autoflaps in cancer patients. Methods: We have an experience of treatment 44 patients aged from 16 to 55 years with malignant local spreaded craniofacial (24) and oropharyngeal tumors (20). For plastic closing the large postoperative defect were used the abdominal organs. We chose para umbilical incision as the appropriate access to the abdominal cavity with minimal external trauma of the anterior abdominal wall. Using video assisted technique (video endoscopy system) aponevrosis was dissected along median centerline. Donor's organs (omentum, greater curve of the stomach, transverse colon) were delivered through the minilaparotomy wound on the anterior abdominal wall, then vessel's peduncle of free flap was exposed (right gastroomental vessels, vessels colicae media) and visceral autoflap was formed. Dissection away the transplant followed by the extracorporeal forming of the organs' anastomozis. In 3 cases was made an attempt to form the 1 gastroomental and 1 colon-omental autoflaps and in 1 case at adiposity during formation omental flap. After inspection the abdominal cavity usual upper median laparotomy was performed. The massive commissural process in the abdominal cavity caused the widening of the access. The plan of the operation among these 3 patients was fulfilled; the flaps were formed and transported on recipient's wound. Results: In 41 cases the operation was completely made through the minimal access (4 patients had abdominal operative intervention before). It was formed and prepared for autotransplantation 23 omental free flaps, 4 gastroomental and 14 colon-omental flaps. There were no intra- and postoperative abdominal complications. Based on the results of clinical and morphological data comparison there were no reliable feature of any structural and functional changes of gastric and omental flap mucous. The follow up period was up to 1 year. Conclusion: Microinvasive technology to form visceral autoflaps for head and neck reconstruction allows to minimize operative trauma and to shorten the period of postsurgical treatment. We recommend using this access when operating the weak cancer patients and young women to avoid additional undesirable scar on donor's site.

#### **P104:** MICROSURGICAL AUTOTRANSPLANTATION AS A COMPONENT OF REHABILITATION OF PATIENTS WITH TUMORS INVOLVING FACIAL SKELETON - Igor Reshetov, Andrey Polyakov, Sergey Kravtsov, Oleg Matorin, <u>Mikhail Ratushnyy</u> P.A. Hertzen; Moscow Cancer Research Institute

Objectives: expansion of surgical treatment radicalism and improvement of patients' functional and social rehabilitation, using the resources of microsurgical tissue autotransplantation method. Material and Methods: Surgical treatment of malignant tumors in maxillofacial zone was conducted for 272 patients. The fourth stage of tumorous process was in 60 % of cases in primary tumors group. Scull base and pachymeninx resection was made in 37 (14%) cases. For eliminating of 175 (65%) orofacial defects, 37 (14%) craniofacial defects, 54 (19%) oroorbitofacial, 5 (2%) isolated defects of lower jaw and for functional rehabilitation, autotransplantation of 300 spare flaps was conducted: visceral - 102; skin-muscular-bone (radial, iliac, scapular, fibular, rib-muscular, rib-scapular) - 141, 30 different skin-muscular flaps and 25 skin-fascia radial flaps. Visceral flaps were used for elimination of defects of oral cavity floor, oropharynx, skin-muscular-bone autoflaps for combined defects of facial skeleton and soft tissue. Results: Complications appeared in 67 (25 %) cases. Total flap necrosis was registered in 4,7 %. Natural nutrition and breathing was restored for 88,6 % patients. 93,2 % of patients were satisfied with the cosmetic results, 32 % returned to their job. Conclusions: Microsurgical reconstruction - a method of effective rehabilitation of patients with a head and neck cancer.

#### **P105:** MUCOSAL MELANOMA OF THE HEAD AND NECK

<u>Andrew G Shuman, MD</u>, Emily Light, MS, Stephen H Olsen, MD, Melissa A Pynnonen, MD, Jeremy M Taylor, PhD, Timothy M Johnson, MD, Carol R Bradford, MD; University of Michigan

Objectives: Mucosal melanoma of the head and neck is a rare but aggressive neoplasm with a poor prognosis. We sought to identify statistically-significant clinical and pathologic predictors of survival. Study Design: Retrospective case series (n=52). Methods: After IRB approval, a single institution's experience with mucosal melanoma of the head and neck was reviewed using a prospectively-collected database, for which patient characteristics and the clinical course were described and analyzed. Results: With a median follow-up of 82 months, the median overall survival was 47 months, with a 5-year overall survival of 37%. The median disease-free survival was
30 months, with a 5-year disease-free survival of 29%. Younger age (p=.023), lower T grade (p<.001), and lower AJCC stage (p<.001)correlated with better overall survival. Positive surgical margins correlated with poorer overall survival (p=.008), but patients who required re-excision to achieve negative margins had outcomes that were not significantly different from those whose margins were initially negative (p=.7). Gender, smoking history, and primary site did not affect disease-free or overall survival. Adjuvant radiation and/ or chemotherapy did not predict improved outcomes. The pathologic criteria that predicted improved overall survival were fewer mitoses (p=.038) and the absence of ulceration (p=.007); more superficial depth of invasion demonstrated a trend towards improved survival (p=.063). Conclusions: Our experience confirms the utility of current staging systems in predicting outcomes of mucosal melanoma of the head and neck, and stresses the importance of achieving negative surgical margins. Pathologically, fewer mitoses and the absence of ulceration predict better outcomes. Further studies will be necessary in order to change the paradigm of care for this rare and deadly disease.

### **P106:** THE EFFICACY OF SUPERSELECTIVE INTRA-ARTERIAL INFUSION FOR UNRESECTABLE CARCINOMA

**OF THE PARANASAL SINUSES** <u>- Akihiro Homma, MD, PhD</u>, Nobihiko Oridate, MD, PhD, Tomohiro Sakashita, MD, Fumiyuki Suzuki, MD, PhD, Jun Furusawa, MD, Shigenari Taki, MD, Naoya Inamura, MD, Takatsugu Mizumachi, MD, PhD, Satoshi Kano, MD, PhD, Daisuke Yoshida, MD, Rikiya Onimaru, MD, PhD, Satoshi Fukuda, MD, PhD; Department of Otolaryngology-Head & Neck Surgery, Hokkaido University Graduate School of Medicine

Background: Treatment outcomes for patients with unresectable paranasal sinus carcinoma have been reported poor. Purpose: To evaluate the efficacy of superselective intra-arterial cisplatin infusion with concomitant radiotherapy (RADPLAT) for patients with T4b paranasal sinus cancer. Patients and Methods: Between 1999 and 2007, 19 patients with untreated, T4b cancer of the paranasal sinus were given superselective intra-arterial infusion of cisplatin (100-120 mg/m2/week) with simultaneous intravenous infusion of thiosulfate to neutralize cisplatin toxicity and conventional external-beam radiotherapy (65-70Gy). Results: Thirteen patients had tumors arising in the maxillary sinus, and 6 in the ethmoid sinus. Fourteen patients had squamous cell carcinomas, 4 undifferentiated carcinomas, and one had adenoidcystic carcinoma. Lymph node involvement was present in 5 patients. Two patients with large tumors received induction chemotherapy prior to radiotherapy to avoid exposing the eyeball and/or the optic nerve of the unaffected side to radiation. With a median follow-up period of 7 years (range 3-11 years), the 5-year local progression-free rate and overall survival were 67.3 % and 63.2%, respectively. No patient died as a result of treatment toxicity or had a cerebrovascular accident. Osteonecrosis (n=1), brain necrosis (n=1) and ocular/visual problems (n=6) were observed as late adverse reactions. Six patients primarily experienced local relapse. Of these one patient was successfully salvaged by endonasal endoscopic resection. Neck disease and distant metastasis were found at the same time in one case, and distant metastasis was found in three patients without primary or neck recurrence. Conclusions: Although patient numbers were small, and this was a single institution experience, the present study findings were of a better outcome than previous reports from other centers. We confirmed the efficacy of RADPLAT, which can concentrate the attack of supradose cisplatin on unresectable paranasal sinus cancer, as well as preserving organs. Late adverse reactions should be monitored in future studies.

**P107:** VARIABILITY OF MALIGNANCY RATES FOR FOLLICULAR LESIONS AND NEOPLASMS: IMPACTS ON THERAPEUTIC MANAGEMENT - <u>Zachary Vandegriend, MD</u>, Ho-Sheng Lin, MD, FACS; Wayne State University

Fine-needle aspiration (FNA) of thyroid nodules has become the primary diagnostic tool for determining the need for thyroidectomy. Malignancy rates of biopsies for follicular lesions and neoplasms guide surgical management but cytologic diagnostic criteria remain somewhat controversial and vary among institutions. From 2002-2009, 222 charts from a single institution were reviewed. Of 28 biopsies diagnosed as "follicular lesion", 17 (60.7%) were malignant. Of the 13 biopsies diagnosed as "follicular neoplasm", 6 (46.1%) were malignant. These results are significantly higher than the average risk of malignancy cited by the American Thyroid Association (ATA) of 10% and 25% for follicular lesions and neoplasms respectively. The diagnosis of follicular lesions and neoplasms can vary greatly between institutions due to local practices and the lack of quantitative diagnostic criteria. This should be considered when determining the need for thyroidectomy. Further objective diagnostic criteria is necessary to decrease this variability among institutions.

## **P108:** ATORVASTATIN PREVENTS ISOPRENYLATION OF RHOC IN HEAD AND NECK SQUAMOUS CELL

**CARCINOMA** - <u>Mozaffarul Islam, PhD</u>, Smita Sharma, MS, Theodoros N Teknos, MD; Department of Otolaryngology-Head and Neck Surgery and Comprehensive Cancer Center, The Ohio State University, Columbus, OH

Introduction: Head and neck squamous cell carcinoma (HNSCC) accounts for more than 95% of all malignancies of head and neck cancer. Previous studies in our laboratory have shown that elevated RhoC expression correlates with lymph node metastasis in HNSCC patients. Statins are a class of drugs used to reduce cholesterol levels in patients by inhibiting HMG-CoA reductase. Interestingly, HMG-CoA reductase is also a key enzyme for the sysnthesis of long chain fatty acids (geranyls and prenyls), which are found to be attached to many cellular proteins. Importantly, the proper function and activity of RhoC also depends on prenylation. Given that RhoC is a major protein involved in metastasis, we hypothesized atorvastatin can reduce metastasis by inhibiting RhoC activity. In this study, we treated HNSCC lines with different concentrations of atorvastatin and observed a significant reduction in active RhoC expression, translocation of RhoC from cytosol to membrane, stress fiber formation and cell motility. This will be followed by a detailed functional study of RhoC activity in absence or in presence of atorvastatin so as to delineate its robustness as a potentially novel therapeutic target. Methods: Active RhoC was measured by GLISA, western blot analysis was performed on cytosol and membrane fractions using RhoC antibody. Stress fiber formation was analyzed with Texas Red Phalloidin. Cell motility assays were done by scratch method in presence or absence of atorvastatin, isoprenoid activator (GGPP), and geranylgeranylation transferase inhibitor (GGT1). Results: Atorvastatin decreased the activity of RhoC by 64% and 57% in UM-SCC-1 and -47 respectively. Western blot analysis revealed increased levels of RhoC in the cytosolic fraction of atorvastatin treated cells. Similarly, a remarkable decrease in stress fiber formation was detected in these cells. Cell motility decreased in a dose dependent manner ranging from 37%-79% in cells treated with 1uM-7uM atorvastatin. The scratch assays of untreated cells regained more than 90% of the gap after 24hrs. Conclusion: Our results show that atorvastatin can inhibit RhoC activity by preventing its geranylgeranylation resulting in the reduction of cell motility in HNSCC cells lines. Therefore atorvastatin has the potential to prevent head and neck cancer metastasis in patients.

#### **P109:** TREATMENT OUTCOME OF CONCURRENT CHEMORADIOTHERAPY WITH INTRAARTERIAL CDDP/ DOC AND ORAL S-1 FOR ADVANCED HEAD AND NECK

**CANCER** - <u>Torahiko Nakashima, MD</u>, Satoshi Toh, MD, Ryuji Yasumatsu, MD, Takeshi Kamitani, MD, Yoshiyuki Shioyama, MD, Shizuo Komune, MD; Department of Otorhinolaryngology and Clinical Radiology, Kyushu University, Japan

Background: Owing to the development of interventional radiological techniques, intra-arterial (IA) chemotherapy for treating head and neck cancer is nowadays widely used and has been studied and evaluated in many institutions. Protocols combining IA chemotherapy with concurrent radiotherapy demonstrated high organ preservation rates in locally advanced head and neck cancer. Multiple trials, particularly those using high dose cisplatin (RADPLAT) have been reported to show high response rate. However, the feasibility of the RADPLAT protocol is still controversial. In our institution, concurrent chemoradiotherapy (CRT) using oral S-1 has been ultilized for treating head and neck cancer to pursue the possibility of organ preservation. Objectives: To determine the effect and feasibility of the combination of intra-arterial (IA) cisplatin (CDDP) and docetaxel (DOC) and concurrent chemoradiotherapy with oral S-1 for advanced head and neck cancer. Methods: 27 patients with unresectable stage IV disease including carcinoma of the maxillary sinus (9 cases), oropharynx (13 cases), hypopharynx (1 case), larynx (1 case), oral cavity (1 cases), parotid gland (2 cases) were treated. Patients received intra-arterial infusion of CDDP (50-70mg/m2) and DOC (50-60mg/m2) from the femoral artery followed by TAR therapy (TS-1; orally, 65 mg/m2/day, twice a day. Vitamin A (Retinol Palmitate): 50,000U/day, intra-musculary on each day of radiation. Radiation; 1.5-2Gy/day: 5days/week). The intra-arterial infusion was repeated up to 3 times and the radiation was given up to 60-70Gy. Results: Complete response was achieved in 16 patients and partial response in 9, giving an overall response rate of 92%. The estimated 3-year disease free survival rate was 60%. The most common Grade 3 or 4 toxicities were anorexia (50%), stomatitis (72%) and leukopenia (81%), all of which were manageable. Conclusions: Concurrent chemoradiotherapy with intraarterial CDDP/DOC and oral S-1 was effective and tolerated. Although preliminary due to the short follow up duration, the response rate supports further and definitive evaluation of this combination for the treatment of highly advanced head and neck cancer.

#### **P110:** 3D BIOMODELING IN MANDIBULAR RECONSTRUCTION: A PROSPECTIVE TRIAL IN SURGICAL SIMULATION - <u>Peter T Dziegielewski, MD</u>, Jay Zhu, MD, Ben King,

BDes, Andrew Grosvenor, DT, MIMPT, Prabjhyot Singh, MD, Walter Dobrovolsky, DDS, FRCD, Jeffrey R Harris, MD, FRCSC, Hadi Seikaly, MD, FRCSC; University of Alberta

Objective: To assess the effectiveness of 3D biomodels as surgical simulators for mandibular reconstruction in the training of resident surgeons. Design: Prospective, cross-over training trial. Setting: Tertiary Care Teaching Hospital. Participants: Naïve junior residents being trained in Otolaryngology-Head and Neck Surgery or Plastic Surgery. Residents had participated in craniofacial operations, but had never been involved in a mandibular reconstruction or any case using 3D biomodels. Interventions: Participants bent and fixated a titanium mandibular reconstruction plate to a 3D biomodel with a standardized anterior hemi-mandibular defect. Participants were randomized to perform the task by free-hand or 3D biomodel-assisted means. 24-48 hours later, the opposite technique was performed. Prior to the task, the residents were shown an instructional video for the technique they were about to perform. Main Outcome Measure(s): The primary outcome was construct accuracy measured by: anterior mental projection (AMP) as well as inter-condylar splay (ICS) and inter-angular splay (IAS). Measures were compared to a complete 3D biomodel of the standardized mandible (control). Secondary outcomes included time of reconstruction as well as "usability" of each technique as per an International Standards Organization (ISO) based questionnaire. Results: 3D model-assisted mandibular reconstruction plates demonstrated statistically indifferent AMP, ICS and IAS compared to the control (p>0.05). Conversely, the free-hand technique lead to significantly prognathic (x=3mm; p=0.01) plates with narrowed ICS (x=8mm; p=0.03) and IAS (x=6mm; p=0.03). Furthermore, all accuracy measures were statistically different between techniques (p < 0.05). While, residents were on average 8.3 minutes faster in performing 3D biomodel assisted plate bending, the difference was not statistically (p=0.18). The usability questionnaire demonstrated that 90% of residents found the 3D model-assisted technique easier to learn and use and more accurate and efficient than the free-hand method. Moreover, the 3D biomodel-assisted method received higher ratings on 10-point Likert scales than the free-hand method in all domains including perceived comfort, accuracy, efficiency and ease of learning (p<0.05). Conclusions: 3D biomodels offer effective surgical simulation for mandibular reconstruction. The technique provides novice surgeons with a user-friendly means to achieve accurate reconstruction, with minimal training.

#### P111: THE USE OF SODIUM LAURYL SULFATE ASSOCIATED WITH CALCIUM HYDROXIDE (HCT10) INCREASE SALIVARY FLOW IN PATIENTS UNDERGOING RADIOTHERAPY - <u>Eder R Biasoli, PhD</u>, Tereza Semenoff, PhD, Alex Semenoff-Segundo, PhD, Daniel G Bernabe, PhD, Glauco I Miyahara, PhD, Euripedes O Marinho, PhD; Oral Oncology Centre - Univ Estadual Paulista - UNESP - São Paulo, University of Cuiabá - UNIC - Mato Grosso, Univ Federal Triangulo Mineiro - UFTM - Minas Gerais, Brazil

Objective: To evaluate salivary flow and the scintigraphic elimination of patients treated with a rinse of sodium lauryl sulphate associated with calcium hydroxide (HcT10) during or after radiation therapy in the cervicofacial area. Methods: Patients were divided into two groups: Group I (G1) was composed with post-irradiated patients (n = 20) and Group II (G2) was composed with patients who were undergoing radiotherapy at the start of this study (n = 23). Patients were evaluated by sialometry - one saliva collection before the start of mouthwash and 4 other every 14 days (C1-C5), and scintigraphy, the first one performed before the start of the mouthwash and the last one between 3 and 4 months later. Results: Two patients in G1 and eight patients in G2 did not perform the secondary scintigraphy. There was, from C4, a significant increase in the amount of saliva when compared to C1 (p < 0.05) for G1 and a decreased amount of saliva into G2 even with the use of HcT10 (p < 0.05). The results of scintigraphy demonstrated an augmentation in the parotid gland function in G1 (p < 0.05), without, however there are differences in the submandibular glands (p <0.05). Regarding the G2 to a decrease in light of all the salivary glands (p <0.05). Conclusion: HcT10 proved beneficial for the increase of saliva in G1, but was unable to keep the amount of saliva for the G2. Keywords: Neoplasm, Radiotherapy, Xerostomia, Sodium Dodecyl Sulfate, Calcium Hydroxide.

#### **P112:** TREATMENT RESULTS OF OSTEOSARCOMAS OF THE HEAD AND NECK AREA - <u>Arturo Madrid, MD</u>, Rocio Las

Heras, MD, Felipe Capdeville, MD, Marcelo Veloso, MD, Hans Harbst, MD, Bettina Müller, MD, Francisca Fernandez, MD; Instituto Nacional Del Cancer, Santiago, Chile. Clinica Alemana De Santiago, Chile

Objectives: Describe the therapeutic modality, complications and survival of a group of patients treated for osteosarcoma of the head

and neck area in an Oncologic Center. Methods: Clinical data of 13 patients carrying osteosarcoma of the head and neck area between September 1998 and September 2009 were reviewed. Data collected were: age and sex; grading of the tumor; therapeutic scheme; type of surgery and reconstruction; survival. Therapeutic scheme was designed by a multidisciplinary comitee. All patients were operated and reconstructed by the same team. Protocol consisted in surgery followed by chemotherapy in patients susceptible to be left with free margins; otherwise, chemotherapy was indicated prior and after surgery. Results: Average age was 30 (17-45), 7 patients were women and 6 men. Localization was 8 maxillar, 3 mandibular and 2 ethmoid. Eight tumors were graded G1, four G2 and one G3. Seven patients had neoadjuvant and all had adjuvant chemotherapy. Treatment for maxillary tumors was 4 radical and 3 partial maxillectomies, one patient had no surgery because of late diagnosis. Four of them were reconstructed with rectum abdomini free flaps. Treatment for mandibular tumors were mandibulectomies, 2 reconstructed with free peroneal flap and one with free trapezium flap. Ethmoid tumors were treated with a craniofacial resection. There were 4 complications: two surgical site infections, one partial atrophy of a rectum abdomini free flap and a partial wound dehiscence. Four patients had positive margins after resection; two died of recurrence and the other two are alive free of disease after 5 years of surgery. Nine patients had free margins, from whom one died of local recurrence, one from another cause and the rest are alive free of disease. Median follow up was 34 months (4 - 132) with a 3-year survival of 69.2%. Conclusions: Considering the low prevalence of this disease, this report is a large single-center experience. Our results are similar to published reports. We believe that patients with tumors susceptible to be resected with negative margins should go directly to surgery given de impossibility of surgical rescue as usually done in long bone tumors.

### **P113:** SINONASAL UNDIFFERENTIATED CARCINOMA: THE ALBERTA EXPERIENCE AND SYSTEMATIC REVIEW -

Hadi Seikaly, MD, FRCSC, <u>Caroline C Xu, MD</u>, Peter T Dziegielewski, MD, Lakshmi Puttagunta, MD, FRCPC, Khalida Nasim, MD, Jeffrey R Harris, MD, FRCSC; University of Alberta

Objective: To evaluate the survival outcomes of patients treated for sinonasal undifferentiated carcinoma (SNUC) in the literature and at our institution. Design: Retrospective chart review and systematic review of the literature. Setting: Tertiary care academic center. Patients: 20 patients treated for SNUC at the Cross Cancer Institute from 1986 to 2010. 140 additional patients were identified in the literature. Main Measured Outcomes: Disease free survival as well as patient demographic data. Results: For patients treated in Alberta, the 5-year overall disease-free survival was 5% with a median survival time of 9.1 months. Of 140 patients identified in the literature, 107 (76.4%) were males and 72.9% presented with advanced stage IV disease. The 5-year overall disease-free survival for these patients was 16% with a median survival time of 15.0 months. Cox regression analysis with disease stage as a covariate revealed no statistically significant difference in survival between single versus multi-modality treatment (p = 0.06) or between concurrent chemoradiation versus surgery-based treatments (p = 0.06). Conclusions: Treatment of SNUC remains challenging with dismal survival outcomes. There appears to be no statistically significant difference in overall disease-free survival between treatment modalities. As such, novel treatment modalities should be explored.

#### **P114:** PRELIMINARY RESULTS OF A PHASE II STUDY OF RADPLAT AND TARCEVA IN LOCALLY ADVANCED HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCCA) - Sini Kalapurakal, MD, Pratima Chalasani, MD, Kathy Robinson, BSN, RN, James Malone, MD, Cathy Clausen, MD, Bruce Shevlin, MD, <u>Krishna Rao<sup>\*</sup></u>, <u>MD, PhD</u>, K. Thomas Robbins<sup>\*</sup>, MD; Simmons Cancer Institute at SIU

Introduction: In HNSCCA, the highest rates for locoregional control and survival have been achieved when chemotherapy has been administered concomitantly with radiation therapy. More recently, biological therapy with EGF receptor antagonists have also shown efficacy in combination with radiation or chemotherapy in the treatment of locally advanced or metastatic HNSCCA. However, no substantial data exists on combining all three modalities concurrently. Methods: 21 patients were enrolled into a phase II trial that combined intra-arterial (IA) cisplatin (CDDP), radiation, and oral daily tarceva in patients with T3 or T4 N0-2 lesions (oral cavity, oropharynx, hypopharynx, and larynx). 9.5% of the patients were female. The average age of patients was 53.1 years. 9.5% of the patients were African Americans, while the rest were individuals of non-Hispanic European heritage. 14 % of the patients had stage III disease with the remainder (86%) presenting with stage IV disease. The total dose of radiation to the primary tumor and upper neck was 70 Gy. Chemotherapy with IA CDDP (150mg/m2) was given on days 1, 8, 15, and 22 concurrently with radiotherapy. During the 7 week treatment period, patients were given Tarceva, 150 mg/day. Primary endpoints of the study were the safety/efficacy of the combination and locoregional control. Secondary endpoints measured the level of function post-treatment. Results: Two patients were not evaluable. 73.6% of the remaining patients received the complete treatment. With a median follow-up of 1.7 years, the overall survival is 68.4%, and the relapse/ persistent disease rate stands at 21%. There were 28 serious adverse events (SAE), including one unrelated death. 18% of SAE were considered related to tarceva. Conclusion: Overall survival and locoregional control for this combination compares favorably with historical data with chemoradiation. The combination was well tolerated with nausea, vomiting, and diarrhea representing the most common SAE. \*Co-Senior Authors.

#### P115: IS GREATER TOXICITY ASSOCIATED WITH GREATER BENEFIT AFTER MULTIAGENT CONCURRENT CHEMOTHERAPY AND HYPERFRACTIONATED RADIATION (MACCRT) FOR LOCOREGIONALLY ADVANCED HEAD AND NECK SQUAMOUS CELL CARCINOMAS (HNSCC)? - <u>Cristina P Rodriguez, MD</u>, David J Adelstein, MD, Lisa A Rybicki, MS, Jerrold P Saxton, MD, John Greskovich, MD, Joseph Scharpf, MD, P. Daniel Knott, MD, Brian B Burkey, MD, Benjamin G Wood, MD, Denise I Ives, RN; Cleveland Clinic

Introduction: Although MACCRT has become a standard curativeintent treatment for loco-regionally advanced HNSCC, it results in considerable acute toxicity. This single institution retrospective review examines whether the degree of acute treatment related toxicity is associated with treatment outcomes. Methods: We reviewed the results of two separate phase II clinical trials of MACCRT conducted at our institution. Patients on both studies received cisplatin 20mg/m2/day and 5-fluorouracil 1000mg/m2/day as 96 hour continuous infusions during the 1st and 4th weeks of radiation therapy. Radiation was delivered in twice daily fractions of 120 cGy to a total dose of 66-72 Gy. Patients on the second trial also received gefitinib 250mg once daily beginning on day 1 of radiation and continuing for 2 years. Toxicities including nausea/ vomiting, mucositis/dysphagia, neutropenia, thrombocytopenia and unplanned hospitalization; and outcomes including overall survival (OS), freedom from recurrence (FFR), local and distant control were recorded. Cox analysis was used to assess associations among toxicities and outcomes. Results: These two trials enrolled 104 patients; 44 treated with MACCRT alone and 60 treated with MACCRT and gefitinib. Patients were predominately Caucasian (93%) and male (83%) with a median age of 57 (range 24-75) years. Fifteen (14%) patients had AJCC Stage III disease, and 89 (86%) had Stage IVA-IVB disease. Primary sites were in the oropharynx in 64 patients (62%), larynx 20 (19%), hypopharynx 14 (13%) and oral cavity 6 (6%). With a median follow-up of 59 (range 17-163) months, the 5 year Kaplan-Meier estimated local control without surgery was 82%, local control with surgery 95%, distant metastatic control 83%, FFR 71% and OS 65%. No correlation was found between nausea/ vomiting or mucosits/dysphagia and outcome. However, on multivariable analysis neutropenia was associated with superior freedom from recurrence (grade 3-4 vs. grade 0-2, HR=0.45 p=0.041), and improved overall survival (grade 2-4 vs. grade 0-1, HR=0.51, p=0.040). The need for hospitalization because of neutropenia and fever was also associated with an improved survival (HR=0.55 p=0.046). Conclusions: Our observations support an association between greater myelotoxicity and improved treatment efficacy.

#### P116: EVALUATION OF PET-BASED RADIOTHERAPY PLANNING OF PATIENTS WITH HEAD AND NECK SQUAMOUS CELL CANCER - <u>Maria Gebre-Medhin, MD, PhD</u>, Eva Brun, MD, PhD; Department of Oncology, Lund University Hospital, Lund, Sweden

Head and neck squamous cell cancer (HNSCC) affects around 1300 persons per year in Sweden, and is treated mainly with radiotherapy, surgery, or a combination of both of these treatment modalities. During radiotherapy planning, delineation of target volumes is normally based on computed tomography (CT) scans together with available clinical information. Functional imaging, particularly 18-F-fluorodeoxyglucose (FDG)-positron emisson tomography (PET), has during recent years become an increasingly important tool in the staging and evaluation of cancer patients, including HNSCC patients. FDG-PET has a high sensitivity and specificity for staging of lymph node involvement in HNSCC patients, and may provide additional information regarding tumor extension and the presence of distant metastases. Since 2008 HNSCC patients receiving radical radiotherapy at Lund University Hospital go through a FDG-PET as part of their radiotherapy planning. We are currently analyzing clinical data from 104 patients to evaluate the importance of this imaging modality. In particular, we are studying to what extent the addition of FDG-PET has led to reevaluation of tumor stage or tumor extension, or changes in treatment strategies. We are also evaluating whether the number of tumor recurrences have been affected compared to historical controls. The results of our analyses will be presented at the conference.

#### P117: FLAP RECONSTRUCTION FOR ORAL CAVITY AND OROPHARYNX DEFECTS AFTER TRANSORAL LASER MICROSURGERY (TLM) - Dan A Sdrulla, MD PhD, Bruce H Haughey, MBChB; Washington University

Objective: To retrospectively evaluate flap incidence and indications in head and neck cancer patients who underwent TLM. Methods: Retrospective analysis of computerized medical records of 319 patients who underwent TLM for oral cavity (120 patients) or oropharynx (199 patients) head and neck cancer. Results: Out of these patients, six patients (2%) required free flap reconstruction for the oral cavity surgical defect and an additional six patients (2%) for the oropharyngeal surgical defect. 92% of the patients had advanced disease at the time of the presentation (stage III or IV). Radial forearm fasciocutaneous flaps were used for eight patients, anterolateral thigh fasciocutaneous flaps were used in two patients and pectoralis major muscle miniflaps were used in two patients. Flap survival was 100%. Speech intelligibility was good in 67% of these patients. Only one patient maintained long-term G-tube dependence. Indications for oral cavity free flap reconstruction included half to two-thirds anterior glossectomy, hemiglossectomy with base of tongue resection, and hemiglossectomy with unilateral floor of mouth resection. Indications for oropharyngeal free flap reconstruction included full thickness soft palate resection, lateral extension of the pharyngeal wall resection to expose the carotid system, or extensive resections involving most of the unilateral pharyngeal wall, base of tongue and floor of mouth. No fistulas were observed postoperatively. Conclusion: Flap transfer following transoral laser microsurgery is rarely required, but allows successful reconstruction of large endoscopically generated surgical defects, allowing excellent wound healing and functional preservation.

#### **P118:** NEOADJUVANT INTRA-ARTERIAL CHEMOTHERAPY AND SURGICAL RESECTION FOR TREATMENT OF RECURRENT HEAD AND NECK CANCER: CLINICAL AND PATHOLOGICAL OUTCOMES - James P

<u>Malone, MD</u>, Justin Fischer, MD, Amit Date, MD, Krishna Rao, MD, PhD, Joel Tennenhouse, MD, Dean Collette, MD, Muthuswamy Dhiwakar, MD, K T Robbins, MD; Southern Illinois University School of Medicine, St. John' s Hospital

Objectives: (1) Determine feasibility, toxicity and disease control in patients with recurrent head and neck cancer treated with neoadjuvant, intra-arterial (IA) chemotherapy followed by surgical resection. (2) Assess pathologic disease response with the above treatment regimen. Methods: Nine patients with recurrent head and neck squamous cell carcinoma (HNSCC) were treated with 3 - 4 cycles of weekly IA cisplatin (150mg/ m2) followed by surgical resection. All patients had previous treatment with surgery and postoperative radiation or chemoradiation. Clinical treatment outcomes including treatment-related toxicities, disease status, and survival were assessed. Treatment-related toxicities were graded according to the National Cancer Institute Common Toxicity Criteria version 3.0. Pathology reports from tumor resection were reviewed to determine the extent of pathologic disease response at the primary site and cervical lymph nodes. Results: Five patients received 4 consecutive weekly cycles and 4 patients received 3 consecutive weekly cycles of IA cisplatin infusions. Two patients had Grade 3 toxicities: brachial plexus neuropathy, hypokalemia, deep venous thrombosis, catheterrelated infection and dysphagia. Two additional patients had Grade 4 toxicities: neutropenia, airway compromise, and pulmonary embolus. The brachial plexopathy was unique to the 3 patients with peristomal recurrence. Surgical salvage was performed at a median time of 4 weeks after completion of chemotherapy. At a median follow-up of 24.4 months, 3 patients are without evidence of disease, 1 patient is alive with stable disease, 4 patients are dead of disease, and 1 patient is dead of other causes. Residual tumor was identified at the primary tumor site in 7 patients after surgery. Two patients had a histologic complete response at the primary site. Tumor resection margins were positive in 3 patients and close in 2 patients. Residual nodal disease was present in only 1 out of 4 patients that underwent neck dissection. Conclusion: Neoadjuvant IA cisplatin followed by resection is feasible for the treatment of recurrent HNSCC after previous multimodality therapy. Treatmentrelated toxicities are acceptable and comparable to other neoadjuvant chemotherapy regimens. A complete pathologic response is noted in some patients following the IA chemotherapy. Additional studies with longterm follow-up are necessary to confirm the effectiveness of this regimen.

### **P119:** SURGICAL MANAGEMENT OF SINONASAL

HEMANGIOPERICYTOMAS: A CASE SERIES - <u>Fernando Gomez-</u> <u>Rivera, MD</u>, Samer Fakhri, MD, Ehab Hanna, MD, Michael E Kupferman, MD University of Texas; MD Anderson Cancer Center and University of Texas Health Science Center at Houston

Introduction: Hemangiopericytomas (HP) are low-grade sarcomas that are rarely found in the sinonasal tract. The diagnosis of this tumor and evaluation of its clinical course remain challenging for surgeons, sinonasal presentation is rare. Although endoscopic-based approaches are being increasingly utilized in the surgical management of sinonasal malignancies, the role of endoscopic management of HP has not been adequately studied. Methods: A retrospective chart-review of patients seen with HP at 2 institutions over a 19-year period was performed. Demographic, clinico-pathological, surgical and follow-up information was retrieved. Descriptive statistics were used. Results: From 1989-2008, 15 patients with a diagnosis of HP were identified and treated. Eleven patients were male, median age was 43. Patients had symptoms for 12 months (median) before diagnosis. Most common presenting symptoms were obstruction (79%), epistaxis (35%) and rhinorrhea (20%). Eleven (73%) had previous surgery, including 5 biopsies. Five cases were classified as T1, 2 T2 and 5 were T4a. The most common location was the nasal cavity 92%, followed by ethmoid 42%, sphenoid 28% and maxillary sinus 21%. Surgical resection was performed in 14/15 patients; 7 were treated endoscopically and 7 were managed with open approaches. Preoperative embolization was performed in 2 patients. The median intraoperative blood loss was 500 cc, median operative time was 165 minutes, 174 for endoscopic and 112 for open approaches. Four patients had postoperative radiation and one received postoperative chemotherapy. The recurrence rate was 26%, 4 local and one distant, with a median follow-up of 28 months. One local recurrence had intracranial extension into the middle fossa. Recurrences were treated with surgery in 2 cases, surgery and radiation in one and chemotherapy alone in one case. At last follow-up one patient died and 2 are alive with disease, the rest have no evidence of disease. Conclusion: Sinonasal hemagiopericytomas are uncommon neoplasms with unpredictable behavior, however most of them have a benign course. Surgery for these tumors can be challenging, and both endoscopic as well as open approaches may be necessary to treat these highly vascular tumors. The role of adjuvant therapy is unclear and should be offered in selected cases.

#### **P120:** SUSPICIOUS THYROID NODULES: DO RADIOLOGISTS' MANAGEMENT RECOMMENDATIONS ADHERE WITH ATA GUIDELINES? - Jeffrey R Harris, MD, FRCSC, Jason A Vaz, David W Côté, MD, MPH, Hadi Seikaly, MD, FRCSC; Division of Otolaryngology - Head and Neck Surgery, University of Alberta

Background: Patients with atypical or suspicious thyroid nodules are often investigated with various radiologic modalities and may proceed with diagnostic hemithyroidectomy in accordance with the American Thyroid Association (ATA) guidelines. Often, radiologists suggest pre-operative management for these patients based on the radiographic characteristics of their nodules. Objective: The objective of this study was to determine whether these recommendations are then followed by surgeons who use the ATA 2006 guidelines. Design: A retrospective review of all patients who underwent diagnostic hemithyroidectomy by the Head and Neck oncology service at the University of Alberta was performed. Demographic data, preoperative imaging reports and postoperative pathology data was collected. Setting: Tertiary care academic center. Patients: A consecutive series of 203 cases was reviewed. Main Outcome Measure: Adherence to radiologist's recommendations by otolaryngologists. Results: 131 (64.5%) patients had preoperative imaging reports containing radiologist recommendations. Of this group, 25.2% (n=33) patients were found to have malignant lesions after diagnostic hemithyroidectomy. Radiologist recommendations were followed by surgeons in 90.1% (n=118) of patients. In patients where recommendations were not followed, radiologists recommended performing needle biopsy (n=3), MRI (n=1), nuclear medicine testing (n=4), PTH assay (n=1), or detailed subsequent ultrasound followup (n=4). In all cases where radiologist recommendations were not followed, management guidelines recommended proceeding directly to diagnostic hemithyroidectomy. Conclusions: In 90% of cases, radiologist recommendations for management closely matched the ATA 2006 guidelines. 10% of the time, however, diagnostic hemithyroidectomy was clinically indicated instead of more conservative approach with further imaging as recommended by the radiology team.

#### **P121:** IS WEIGHT LOSS PREDICTIVE OF THE NEED FOR RE-PLANNING OF PATIENTS WITH HEAD AND NECK CANCER TREATED WITH IMRT RADIOTHERAPY? RESULTS OF A PROSPECTIVE STUDY - <u>Samy El-Sayed</u>, MD, <u>FRCR, FRCPC</u>, Joel Broomfield, MD, Jamie Bahm, MRT; University of Ottawa

Introduction: The Radio-therapeutic management of Head and neck cancer patients requires a lot of precision particularly in the era of IMRT. The use of sophisticated immobilization and daily verification is imperative. On the other hand many patients do require repeat immobilization shell, CT Simulation and re-planning of radiotherapy during their treatment due to change of body habitus after significant weight loss and tumour shrinkage among other reasons. So far the decision to re-plan is done based on daily observation by the treating therapists and is usually made late in the course of the treatment leading to rush re-planning, risk of delaying treatment or treating with suboptimal immobilization shell and setup. Objectives: 1. To evaluate the percentage of patient who do require re-planning in a population of newly diagnosed Head and Neck cancer patients. 2. To determine if the percentage of weight loss can be used as a predictor of the need for re-planning XRT. 3. If there is a correlation between early weight loss and the need for re-planning, can that be used to avert late re-planning? Methods: This is a prospective study of a cohort of 86 newly diagnosed patients with head and neck cancer treated with IMRT with the daily use of IGRT. Baseline as well as weekly weights were measured while on treatment. All patient s were evaluated by dietician initially and weekly during treatment. Patients were supported intensively with regular diet evaluation and feeding tubes as necessary. Percentage of weight loss was noted and correlated with patients who required re-planning. Results: Despite the intensive support, 30% of patients lost more than 10% of their body weight. 15% of patients have required re-planning. Weight loss was not the only factor to predict the need for re-planning as there was as many patients needing re-planning without weight loss. On the other hand, 60% of patients with significant weight loss have required replanning. Conclusion: It seems that early weight loss is highly predictive of the requirement for re-planning. We would recommend that any patient with more than 10% weight loss to have repeat immobilization, CTSIM and re-planning.

### TRANSLATIONAL RESEARCH

## **P122:** USE OF ADIPOSE DERIVED STEM CELLS IN HEAD & NECK RECONSTRUCTIVE SURGERY: FRIEND OR FOE?

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Introduction: Head and neck cancer (HNC) is the sixth most common malignancy, accounting for >400,000 new cases per year. Following surgical resection and radiation, patients exhibit impaired wound healing, scar contracture, and disfigurement of the head and neck region, often requiring reconstructive surgery. Autologous fat transplantation is one treatment option for post-irradiation wounds. Abdominal fat tissues, which contain adipocyte stem cells (ASC), are harvested and injected into the affected area. ACS's can improve and accelerate wound healing, tissue regeneration and cellular differentiation. However the safety and efficacy of ASC's and their potential interaction with HNC is unknown. The goal of this study is to investigate the interaction between human ASC's and HNC cell lines, CAL-27 and SCC-4, oral squamous cell carcinomas (OSCC). Methods: A wound-healing assay was used to measure HNC cell migration in the presence of ASC conditioned media (CM). 300,000 CAL-27 HNC cells or 200,000 SCC-4 HNC cells were cultured in 12 well plates in DMEM and 10% fetal bovine serum (FBS) and allowed to adhere for 24 hours. Cultures were incubated with ASC CM at the following percentages: 0%, 20%, or 50% CM following a 'scratch' wound and were cultured for an additional 6 hours for CAL-27 and 16 hours for SCC-4. Photographs of the CAL-27 cultures were taken at times 0 and 6 hours, and of the SCC-4 cultures at 0 and 16 hours. The pictures were analyzed using ImageJ (NIH, Bethesda, MD) to calculate percent gap closure. Results: ASC CM stimulated migration, causing a 10.89% gap closure, at 20% CM,  $\pm$  5%, and 14.29% gap closure, at 50% CM,  $\pm$  6%, for the CAL-27, compared to 0% CM (7%). ASC CM caused a 48.02% gap closure, at 20% CM,  $\pm$  9%, and 43.89% gap closure, at 50% CM, ± 9%, for the SCC-4 HNC cell line, compared to 0% CM (35.79%). Conclusions: These results demonstrate that ASC CM stimulates migration of HNC cells. Future studies will identify the ASC secreted factors that influence HNC cell migration. It remains to be determined if fat transplantation therapy is safe in HNC patients.

### **P123:** RESISTANCE/RESPONSE MOLECULAR SIGNATURE FOR ORAL TONGUE SQUAMOUS CELL CARCINOMA -

<u>Amritha Suresh, PhD</u>, Muhil Vannan, MSc, Dhanya Kumaran, MSc, Zeynep Gumus, PhD, Priya E Sivadas, MSc, Elango Murugaian, PhD, Vikram E Kekatpure, MS, MCh, Subramanian A Iyer, MS, MCh, FRCS, Kumarasamy Thangaraj, PhD, Moni A Kuriakose, MD, FRCS; Mazumdar Shaw Cancer Centre, Bangalore, India, Amrita Institute of Medical Sciences, Kochi, India, Weill Medical College of Cornell University, NYU, Centre for Cellular and Molecular Biology, Hyderabad, India

Worldwide incidence rate of oral tongue cancer is on the rise in contrast to the steady decline observed in the overall incidence of head and neck squamous cell carcinoma. Oral tongue cancer also has lower survival rates compared to the other head and neck sub-sites. Identifying molecular means of early diagnosis and accurate prediction of treatment outcome is the focus of current research. This study uses genome wide analysis to profile patients with oral tongue cancer showing resistant/response to treatment. It further attempts to validate the markers in tissues and saliva in an effort to evaluate their efficacy in diagnostics/prognostication. Microarray profiling of 20 primary and post-treatment tongue cancer samples was carried out using the Affymetrix system (HG U133 plus 2). Analysis was carried out primarily using AVADISTM, and GeneSpring 7.2 softwares. Significant genes identified by both approaches and with p<0.01 and fold change>2 were selected for further validation. Expression profiling provided subsets of genes differentially regulated in the responsive group (MMP1, MMP9, MMP10, EMP1, TNC, SP3, IGLA, CCL18) and in the resistant group (ABCG1, COL5A1, CTSC, HBB, CLCA4, JAG2, FJX1). Subsets of genes were validated in tissue samples by Quantitative PCR and immunohistochemistry, and further in saliva samples, and correlated with their clinical status to arrive at a select panel of candidate markers. A panel of four genes; COL5A1, HBB, IGLA and CTSC in combination could predict the treatment response of the patients with a specificity of 0.88 and sensitivity of 1. Profiling of HBB expression alone in primary tumors could predict resistance with 85% sensitivity. Validation in saliva samples revealed that the use of a panel of 7 markers (MMP1, FN1, IL8, IL1B, IgLA, ABCG1, and COL5A1) could detect patients with pre-malignant lesions and carcinoma of the tongue with a sensitivity of 0.7 and specificity of 0.88. Our study provides a specific panel of markers that is effective in predicting resistance/response in oral tongue cancer. The detection of a subset of differentially expressed markers obtained from tissue and saliva samples suggested that further exploration of such markers in saliva might prove to have important diagnostic and prognostic value.

#### **P124:** TOBACCO METABOLITES AS BIOMARKERS OF RISK IN HEAD AND NECK SQUAMOUS CELL CARCINOMA: A PRELIMINARY ANALYSIS - <u>Samir S Khariwala, MD</u>, Bevan Yueh, MD, Dorothy Hatsukami, PhD, Stephen Hecht, PhD; University of Minnesota

Background: Tobacco use is a well-known risk factor for the development of various cancers including head and neck squamous cell carcinoma (HNSCC). One of the most carcinogenic components of tobacco smoke is 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) which can be measured through its metabolite 4-(Methylnitrosamino)-1-(3-Pyridyl)-1-Butanol (NNAL). Tobacco smoke also contains polycyclic aromatic hydrocarbons (PAH) which are quantified via 1-hydroxypyrene (1-HOP). Recent data from a large Chinese cohort shows that elevated NNAL levels, when adjusted for levels of smoking, are associated with lung cancer risk. Objective: We seek to compare smokers with and without HNSCC with respect to urinary NNAL and 1-HOP. Here we report a preliminary analysis of the first 20 patients enrolled in a case-control study comparing urinary NNAL and 1-HOP levels in cases (smokers with HNSCC) and controls (smokers without HNSCC). Methods: Smokers with a new diagnosis or history of HNSCC were enrolled in our study. Subjects provided a sample of blood and urine as well as a tobacco use questionnaire. NNAL was quantified using gas chromatography-mass spectrometry while 1-HOP was quantified by high performance liquid chromatography. Subjects were matched based on volume and duration of use to controls contained in a University of Minnesota database. Results: Matching and subsequent analysis was performed in SAS ver. 9.2 (Cary, NC). In cases and controls, NNAL levels (mean +/- SE) were 1.53 +/- 0.32 and 2.19 +/-0.43, respectively and 1-HOP levels (mean +/- SE) were 1.53 +/- 0.25 and 1.02 +/- 0.18, respectively. Conditional logistic regression was performed. NNAL demonstrated and OR of 0.836 (95% CI = [0.537, 1.216], p=0.389) and 1-HOP demonstrated an OR of 2.631 (95% CI = [0.526, 22.469], p=0.2832). Conclusion: This preliminary analysis showed higher levels of 1-HOP, but not NNAL, in smokers with HNSCC compared to smokers without HNSCC with comparable smoking history. These findings did not reach significance. This data merits further exploration and confirmation via a larger series of patients. If confirmed, these findings may indicate that tobacco carcinogenesis in HNSCC occurs not through greater effective dose of NNK or PAH but rather through increased levels of DNA adduct formation or carcinogen-induced apoptosis.

#### P125: Moved to S020B

**P126:** GENE METHYLATION PROFILING OF HEAD AND NECK SQUAMOUS CELL CARCINOMA IN RELATION TO HPV-16 POSITIVITY - <u>Michael Moran, MBBCh, MRCS</u>, M Dellett, PhD, D J Sharpe, PhD, B Forcione, H A Colyer, BSc, J Jamison, R Shah, MBBS, P Maxwell, PhD, K Mills, PhD, J James, PhD, FDS, FRCPath, D J McCance, PhD; The Queen's University of Belfast

Objective: Oropharyngeal squamous cell carcinoma (OSCC) incidence has increased in recent years and this rise appears to be linked to increased incidence of human papillomavirus (HPV) related OSCC. It is of interest that patients with this disease entity have improved prognosis when compared with HPV negative cancers, however the exact mechanism is as yet unclear. Methylation is essential for regulation of gene expression and sustains expression patterns through mitosis. In cancer it has been observed that scattered CpG sites throughout the genome become hypomethylated, whilst the CpG islands in the promoter regions of some genes, in particular tumour suppressor genes, become hypermethylated. This is associated with decreased gene expression and can also lead to genomic instability. This study profiles methylation patterns of HPV-16 positive and negative OSCC and aims to determine differences between these two groups. Design: The global methylation patterns of OSCC samples were investigated using the Methylated CpG Island Amplification Microarray (MCAM) method and University Health Network microarrays containing 12192 CpG island clones. HPV-16 positivity was determined using the Roche linear array HPV genotyping test and/or immunohistochemistry testing for p16. P16 immunohistochemistry was performed as HPV-16 integration disrupts the retinoblastoma pathway and induces an overexpression of p16. Principal component analysis was carried out using the Partek Genomics Suite. Subjects: Paraffin embedded OSCC tumour samples from 30 patients (25 males and 5 females) were examined. The mean age at diagnosis of these patients was 59.5 years. Results: Of the 30 samples, 26 were HPV-16 negative and 4 were HPV-16 positive. Loess normalisation and analysis of variance tests between HPV-16 positive and negative samples were conducted. Statistically significant methylation patterns were found for 319 genes, with a p value of <0.05 and fold changes less than -1.5 and greater than 1.5. Conclusions: While the numbers in the study are small, preliminary results indicate variation in methylation patterns according to HPV-16 positivity, with both hypermethylation and hypomethylation differences demonstrated. Further investigation is needed to determine if these differences predict prognosis.

#### **P127:** Moved to S020A

**P128:** GENE EXPRESSION ANALYSIS DEMONSTRATES IMMUNE RESPONSE AS A PREDICTOR OF SENSITIVITY TO CHEMORADIATION IN SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK - <u>Jan Akervall, MD</u>, PhD, Bryan Thibodeau, PhD, Timothy Geddes, Sean Park, MD, PhD, Peter Chen, MD, Barbara Pruetz, George Wilson, PhD; Depts. of Otolaryngology, Radiation Oncology and BioBank, William Beaumont Hospital

Background: 15% of patients of patients with Squamous Cell Carcinoma of the Head and Neck (SCCHN) do not respond to chemoradiation, thus suffering unnecessary morbidity and delay to receive effective therapy. Previous studies have shown that biological properties of these malignancies might reflect sensitivity to chemoradiation, indicating that biological staging could be used to overcome current insufficiencies of TNM-classification for treatment decisions. Material & Methods: 12 patients with laryngeal and oropharyngeal SCCHN were selected for the study, 6 of which had a complete response to treatment and 6 who were non-responders. RNA from prospectively collected, pre-treatment tumor specimens were subjected to gene expression analysis using Affymetrix Human Exon 1.0ST arrays. Results: ANOVA (p=0.05) and a 2-fold cutoff were used to identify 262 altered genes, including 151 genes over-expressed in the complete responders compared to the non responders. The DAVID online classification program was then used to categorize these genes by Gene Ontology. Genes overexpressed in the complete responders included 38 genes that were associated with immune response, including leukocyte, lymphocyte, and T cell activation. These immune-related genes include several MHC genes as well as immunoglobulin genes (e.g CD2, CD3 D,E and G, HLA-F, HLA-DMB) indicating a possible increased presence of tumor-infiltrating lymphocytes TILs) in the complete responders. In addition, over-expression of chemokine ligands (CCL5, CL21, CCR7, CXCL10,11 and 13), interleukins (IL2RB and RG, IL7R and IL20RB), and interferon-related genes (IRF1,4 and 8, IFI6, 44 and 44L) in the complete responders may indicate increased ability to elicit an immune response against the tumor cells. Conclusions: We present a novel panel of genes, related to immune response, that suggest that lymphocyte count deficiencies are associated with aggressive SCCHNs. These data can potentially lead to an assay that can be used clinically to predict SCCHN patients that will benefit from chemoradiation and suggest that reinforcement of the immune system may improve the response of immunodeficient tumors to treatment. The immediate next step in this study is further validation of the gene expression data using RT-PCR and ELISA based assays, followed by analysis of a large non-selected cohort of SCCHN patients.

#### P129: ABERRANT DNA METHYLATION OF ZNF FAMILY NUCLEIC ACID BINDING PROTEIN GENES ON CHROMOSOME 19 IN OROPHARYNGEAL SQUAMOUS CELL CARCINOMA - <u>Thomas J Belbin, PhD</u>, Leslie R Adrien, MS, Richard V Smith, MD, Thomas M Harris, PhD, Roberto Lleras, MSc, Nicolas F Schlecht, MS, PhD, Geoffrey Childs, PhD, Michael B Prystowsky, MD, PhD; Albert Einstein College of Medicine/Montefiore Medical Center

Identification of epigenetically affected genes has become an important tool for understanding both normal and aberrant gene expression in cancer. Here we report a whole-genome analysis of DNA methylation profiles in fresh, frozen oropharyngeal squamous cell carcinoma (OPSCC) tissues and adjacent normal mucosa samples using the Illumina HumanMethylation27 BeadChip using patient genomic DNA. We initially compared whole-genome patterns of DNA methylation among 24 OPSCC primary tumors and 24 matched normal adjacent mucosal samples. From a survey of 27,578 CpG loci spanning more than 14,000 genes, we identified 958 CpG loci in which measurements of DNA methylation were altered in the primary tumors relative to the normal mucosal samples. These alterations were subsequently validated in an independent set of 21 OPSCC patients. A survey of these loci by chromosomal location revealed an abnormally high number of differentially methylated loci on chromosomes 19. Many of the loci on chromosome 19 are associated with genes belonging to the ZNF family of nucleic acid binding proteins. We have since confirmed a corresponding decrease in expression of several of these genes in the same patient tumor specimens. These observations are now being validated using bisulfite sequencing and Taqman real-time PCR. This study reports for the first time on the aberrant methylation and loss of expression of ZNF family proteins in oropharyngeal cancer. The aberrant methylation and transcriptional silencing of these potential tumor suppressor genes represent a new avenue of exploration for pathways affected in this disease. **P130:** QUANTITATIVE PROMOTER METHYLATION DIFFERENTIATES CARCINOMA EX PLEOMORPHIC ADENOMA FROM PLEOMORPHIC ADENOMA OF THE SALIVARY GLANDS - <u>Andrew Schache, MRCS, FDS</u>, Richard Shaw, MD, FRCS, FDS, Julia Woolgar, FRCPath, FDS, PhD, Janet Risk, PhD, Gillian Hall, MD, MFDS, Derek Lowe, MSc, CStat, Triantafillos Liloglou, PhD; University of Liverpool, United Kingdom; University Hospital Aintree, Liverpool, UK; University of Manchester, United Kingdom; Edge Hill University, Liverpool, United Kingdom

Carcinoma ex Pleomorphic Adenoma (Ca ex PSA) is a rare and poorly understood malignancy of salivary glands originating from Pleomorphic Adenoma (PSA). It accounts for 3-4% of salivary neoplasms and approximately 12% of all salivary malignancies. Tumour suppressor gene (TSG) promoter hypermethylation in Ca ex PSA and PSA may offer a potential biomarker for progression, basis for a diagnostic tool, or insight into molecular mechanisms responsible for malignant transformation. We evaluate a large archival series of Ca ex PSA cases alongside unrelated PSA using RT-PCR to quantify promoter methylation. 59 salivary neoplasms were analysed, 28 PSA (47%) and 31 Ca ex PSA (53%).

DNA was extracted from fixed tissue and bisulphite treated. Quantitative promoter methylation data was obtained for seven TSGs previously reported to be methylated in epithelial malignancies, including those of salivary origin. Methylation was observed in 20 of 31 Ca ex PSA samples (64.5%) and 2 of 28 (7.1%) PSA samples (p<0.001). RASSF1 was the single gene promoter for which methylation was shown to be a significant predictor of malignant disease (p<0.001). Upon aggregation of results, the presence of methylation in hTERT, WT1, RASSF1 or p16INK4A was associated with a significantly higher chance of malignancy (odds ratio 24, 95% C.I. 4.7-125, p>0.001). As a panel, the sensitivity for detecting malignancy was 64.5% (95% C.I. 45-81%) and specificity for excluding benign disease was 92.9% (95% C.I. 77-99%). Our analysis describes a comparison of the quantitative methylation profile in a series of rare salivary tumours and their benign precursor. RASSF1 promoter methylation is shown to be tumour-specific to Ca ex PSA samples. Additionally, the combined value of a panel of four gene promoters demonstrates that methylation at any single promoter site is a significantly positive predictive factor for malignancy. As genomic analysis becomes routine, promoter methylation has potential to be of diagnostic, prognostic and therapeutic importance in the management of the benign and malignant variants of this salivary tumour.

#### **P131:** A CLINICALLY RELEVANT BIOMARKER IN HPV-MEDIATED OROPHARYNGEAL SQUAMOUS CELL

**CARCINOMA** - <u>Andrew Schache, MRCS, FDS</u>, Richard Shaw, MD, FRCS, FDS, Janet Risk, PhD, Julia Woolgar, FRCPath, FDS, PhD, Derek Lowe, MSc, CStat, Triantafillos Liloglou, PhD; University of Liverpool, United Kingdom; University Hospital Aintree, Liverpool, UK; Edge Hill University, Liverpool, United Kingdom

The increasing incidence of Oropharyngeal Squamous Cell Carcinoma (OPSCC) has been well documented in recent decades. Recognition of the role of HPV-16 in the pathogenesis of a significant proportion of these tumours is similarly becoming more apparent, with evidence indicating a clear survival benefit irrespective of treatment modality. Accurate identification of HPV-16 mediated malignancy is a necessity to differentiate from coincidental or transient infection. Simple HPV DNA detection lacks specificity and therefore the establishment of a predictive biomarker for HPV +ve OPSCC holds significant benefit both in clinical practice and for adequate stratification in clinical trials. The carcinogenic effects of HPV 16 are believed to be linked to disruption of viral E2 gene expression and the consequent elaboration of E6 and E7 following their release from the control of E2. The significance of epigenetic changes in the HPV genome is explored through analysis of E2 promoter methylation using pyrosequencing. Additional correlation of epigenetic changes will be made with analysis of viral DNA integration and viral gene expression. From a series of 120 OPSCC cases, all HPV16 DNA positive tumours were analysed using p16 Immunohistochemistry (IHC), real time PCR for viral E2, E6 and E7 gene expression, and pyrosequencing to determine E2 promoter methylation. Viral DNA integration was measured by PCR. E2 promoter methylation varied between 10% and 90% amongst HPV positive samples. E2 methylation correlates well with p16 IHC and has merit for further investigation as a clinically relevant biomarker. HPV integration into the host genome was further shown in a significant proportion of cases. The role of epigenetic regulation of the HPV genome is discussed with particular reference to possible viral-host epigenetic interactions.

#### **P132:** STROMAL CARBONIC ANYHDRASE AS A PROGNOSTIC MARKER IN SURGICALLY TREATED ORAL CAVITY SQUAMOUS CELL CARCINOMA - Nigel Brockton, PhD,

Alexander Klimowicz, PhD, Anthony Magliocco, MD, Stephanie Petrillo, MSc, Harold Lau, MD, T. Wayne Matthews, MD, Luke Rudmik, MD, <u>Joseph C</u> <u>Dort, MD, FACS</u>: The University of Calgary - Faculty of Medicine

Introduction: Tumor hypoxia results from an imbalance between the supply and consumption of oxygen. Tumor stromal elements play an important role in various stages of malignancy including angiogenesis, invasion, and metastasis. Stromal hypoxia may alter the biology of oral cavity squamous cell carcinoma (OSCC). Carbonic Anhydrase-9 (CAIX) is a marker of tissue hypoxia and has been associated with reduced survival in Head and Neck SCC. Methods: Analysis of clinical and molecular data collected from patients with oral cavity squamous cell carcinoma (OSCC) treated between 1995 and 2005. Clinical data were obtained from the Alberta Cancer Registry and chart review. Tissue microarrays of the primary tumour, metastatic lymph nodes and adjacent normal lymph nodes were created from the paraffin-embedded specimens. The hypoxia marker evaluated was CAIX. Quantitative immunohistochemistry (HistoRx) was used to quantify CAIX. Primary outcomes were local recurrence rate and 5-year survival. Results: 104 patients with OSCC were identified. High expression of stromal CAIX is associated with reduced 5-year overall survival compared to low expression of CA-9, 35% vs. 79%, respectively (p<0.001). Discussion: Oral cavity tumor outcomes are variable and the TNM staging system is the current standard for prognostication. Tumor biology is variable and not well captured with current staging methods. We have demonstrated that stromal CAIX highly predicted reduced overall 5-year survival. Stromal CAIX is a potential molecular marker for oral cavity SCC.

#### P133: ESTABLISHMENT OF AN ORAL CAVITY SQUAMOUS CELL CARCINOMA LINE FROM A NEVER-SMOKER PATIENT - <u>Steven J Wang, MD</u>, Janyaporn Phuchareon, PhD, David W Eisele, MD, Osamu Tetsu, MD. PhD; University of California, San Francisco

The majority of patients who are diagnosed with oral cancer in the United States have an active or prior history of smoking. Nevertheless, a significant portion of oral cavity cancers, at least 10%, occur in patients who have never smoked. Recent reports in the literature suggest that oral cavity squamous cell carcinomas from non-smoking patients may differ in biological properties and behavior compared with those from smoking patients, highlighting the need for a better understanding of the molecular biology of these unique tumors. One important means to gain insight into the biological behavior of oral cavity cancer cells is through the establishment

of cancer cell lines which may be used to identify diagnostic biomarkers and to evaluate novel therapeutic strategies. To date, most oral cancer cell lines have been established from tumors derived from patients who were smokers, or whose smoking status is unknown. Here we report the successful establishment of an oral tongue squamous cell carcinoma line from a 44-year-old female never-smoker patient. Immunohistochemical analysis revealed that the tumor is p16 negative. To ensure that the new cell line is free of contamination from non-carcinoma cells, we performed DNA fingerprint analysis to confirm that the cell line is identical to the donor tumor tissue. To avoid the effects of extended passage number on selective pressure and genetic instability in the cell line, the genotype, karyotype, and phenotype of the new cell line is currently under investigation using cells with low passage numbers. We anticipate that new insights from this novel oral tongue squamous cell carcinoma line will provide us a representative and robust experimental model of oral cavity carcinoma in non-smokers.

#### **P134:** ROLE OF HYALURONAN SYNTHASES TO PROMOTE CD44-DEPENDENT HEAD AND NECK SQUAMOUS CELL CARCINOMA PROGRESSION - <u>Steven J Wang, MD</u>, Gabriel Wong, BS, Lilly Y Bourguignon, PhD; University of California, San Francisco

Background: CD44 is a transmembrane receptor found on many different benign and malignant cells. Hyaluronan (HA), a major component of the extracellular matrix, is the primary ligand for CD44 receptors. In cancer cells, HA interaction with CD44 promotes multiple signaling pathways that influence tumor cell progression behaviors in a variety of solid tumors. Increasing evidence indicates that HA and CD44 signaling play an important role in head and neck squamous cell carcinoma (HNSCC) progresion. HA is primarily synthesized by hyaluronan synthases, and the current study investigated the role of hyaluronan synthase to promote CD44-dependent HNSCC progression behaviors. Methods: Immunohistochemical analysis of hyaluronan and hyaluronan synthase expression in primary HNSCC tumor specimens was performed. Functional assays of hyaluronan synthase activity, HA production, and CD44-mediated tumor cell proliferation and migration behaviors were carried out on established HNSCC cell lines and primary tumor cell cultures. Results: Increased hyaluronan synthase expression in HNSCC clinical specimens was associated with poor clinicopathologic characteristics. In addition, increased hyaluronan synthase activity led to greater HA production and promotion of CD44-dependent in vitro tumor progression behaviors in HNSCC cells. Conclusions: Increased hyaluronan synthase activity is associated with CD44-dependent HNSCC progression.

#### **P135:** APPLICATION OF HIGH RESOLUTION IMAGING WITH A MINIATURIZED MICROENDOSCOPE TO ABLATIVE TRANSORAL ROBOTIC SURGERY IN THE OROPHARYNX: AN EX-VIVO STUDY - <u>Chan W Park, MD</u>, Gregg Goldstein, MD, Lauren Levy, Peter Vila, Vivek Gurudutt, MD, Marita Teng,

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Background: The use of transoral robotic surgery (TORS) is an emerging treatment modality for cancers of the oropharynx. TORS allows surgical resection of the primary site with very low morbidity and can even eliminate the need for radiation in certain patients. In this setting, obtaining negative pathological margins is critical, as positive margins will mandate adjuvant treatment of the primary site. Since intraoperative "frozen section" analysis of margins is expensive, timeconsuming, and cumbersome, we performed ex vivo evaluation of a, low cost, high resolution miniaturized endoscopic microscope suitable for "real-time" histological analysis and determination of margin status during TORS. Objective: The objective of this study was to compare images taken with the high resolution micro-endoscope (HRME) with standard histopathologic examination in surgically resected samples of known squamous cell carcinoma (SCCa) of the oropharynx and to determine initial compatibility of the HRME with transoral robotic surgery. Patients, Interventions, and Outcome Measurements: The tissue samples studied in this report were obtained by transoral robotic resection from patients with biopsy-proven SCCa of the oropharynx. A total of five patients had cancers of the oropharynx (2 isolated to base of tongue, 3 extending from base of tongue to pharyngeal wall and tonsil). Surgical specimens were imaged ex vivo with the HRME device, including areas with overt tumor, normal mucosa, and transitional areas. Images obtained were then compared with "gold standard" histopathology examination. Results: Each of the oropharyngeal subsites (tonsil, pharyngeal wall, and base of tongue) has unique imaging properties. Squamous mucosa was identified by bright nuclei surrounded by dark cytoplasm in an ordered pattern. Tissue that had SCCa had abundant irregular nuclei crowded together in a random pattern with scant cytoplasm surrounding each nuclei. Intermediate dysplasia tissue had an arrangement of nuclei and cytoplasm that was in between normal mucosa and carcinoma. Standard histopathologic examination of study samples confirmed the results obtained by the HRME. Conclusions: It was feasible to obtain highresolution histopathologic information using the HRME device for tumors of the oropharynx. Future integration of this device with transoral robotic surgery will allow real time histological information to guide margin determination during TORS.

#### **P136:** APRICOXIB UPREGULATES 15-PGDH AND PGT IN

**HNSCC** - Guanyu Wang, MD, PhD, Jie Luo, MS, Mariam Dohadwala, PhD, David Hu, MD, Francis Burrows, PhD, Sara Zaknoen, MD, Steven Dubinett, MD, <u>Maie St. John, MD PhD</u>; University of California, Los Angeles; Tragara Pharmaceuticals

Head and neck squamous cell carcinoma (HNSCC), is the sixth most common cancer in the world. Patients with HNSCC are at considerable risk of mortality, with more than 300,000 deaths attributable to the disease per year. The five-year survival rate for patients with advanced head and neck cancer remains approximately 15-20 percent, emphasizing the importance of new therapeutic strategies. In spite of extensive research on EGFR and COX-2 inhibitors, their combined use for HNSCC is still in its developmental stage. We evaluated the efficacy of the newer COX-2 inhibitor, apricoxib, in HNSCC cell lines. Here, we report that in comparison with celecoxib, apricoxib shows greater efficacy in vitro; and we define the mechanisms which may be responsible for this. Our studies show that apricoxib is effective in preventing tumor cell growth in 3-dimensional, and anchorage-independent growth assays; as well as decreasing the capacity for tumor cell migration. Treatment of HNSCC cells with apricoxib also causes greater up regulation of E-cadherin expression and down regulation of vimentin, as compared with celecoxib treatment. This has significant implications for targeted chemoprevention and anti-cancer therapy because E-cadherin expression has been implicated as a marker of sensitivity to EGFR TKI. In addition to PGE2 down regulation, indicating COX-2 inhibition, we also show that apricoxib treatment causes a marked up regulation of 15-PGDH (prostaglandin dehydrogenase) and PGT (prostaglandin transporter), both of which decrease PGE2. These effects are further amplified in 3-dimensional culture, indicating the import of the microenvironment in drug efficacy. This up regulation of 15-PGDH and PGT with apricoxib treatment may explain its increased efficacy over celecoxib in the assays we tested. As apricoxib affects other pathways of

PGE2 degradation and uptake, there is ultimately less PGE2 to drive metastasis and tumor progression. These newly defined mechanisms for the increased efficacy of apricoxib have important implications for targeted chemoprevention and therapy.

#### **P137:** NON-INVASIVE IN VIVO DIAGNOSTIC OF EPITHELIAL DYSPLASIA IN ORAL MUCOSA VIA TWO-PHOTON AUTOFLUORESCENCE SPECTROSCOPY - <u>Kert</u>

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Epithelial neoplasms account for more than 80% of all cancers and are associated with high mortally and morbidity. The five-year survival rate for individuals diagnosed with oral cancer at all stages of development is approximately 50%, but is significantly higher in the case of early detection. Thus efficacious efforts directed at improving these statistics must allow for the earliest possible detection of malignancies. Current clinical methods for lesion visualization and staging rely on white light inspection followed by biopsy. Single photon (1P) autofluorescence spectroscopy has proven to be a powerful diagnostic tool for the elucidation of the morphological and biochemical transformations associated with dysplasia, due in part to its sensitivity and non-invasive nature. Although this optical interrogation process is non-invasive, it is limited in its application due to its lack of specificity, photobleaching of intrinsic fluorophores, lack of depth discrimination and significant scattering and absorption in turbid environments such as mucosal tissue. Two photon (2P) microscopy circumvents many of the deficiencies of 1P with the particular advantage of depth discrimination and the generation of secondary endogenous signals such as second harmonic generation (SHG) optical signatures from collagen and DNA material. In this investigation, 2P spectroscopy was utilized to analyze the spectral components of endogenous fluorophores such as FAD and NADH in normal and dysplastic oral mucosa at 780nm, 800nm, 840nm and 890nm. Oral cancer was induced in the buccal pouch of Syrian Golden hamsters by tri-weekly topical application of 9,10-dimethyl-1,2-benzanthracene (DMBA). Investigated sites were immediately marked for biopsy, processed for histology and H&E staining, and graded by a pathologist. Both 2P autofluorescence images and SHG signals were analyzed for the keratin layer, superficial and basal epithelium, and submucosa. Results revealed layer-specific spectroscopic properties that 1P spectroscopy does not, providing insight into depth-resolved spectroscopic characteristics of normal and neoplastic tissue. Our results indicate that this technique shows excellent potential as a diagnostic tool for monitoring some of the early physiological changes associated with the progression from normal cells to dysplasia.

#### P138: PATHWAYS CONTRIBUTING TO ORAL SQUAMOUS CELL CARCINOMAS WITH RESPECT TO NODAL STATUS AND EXTRACAPSULAR SPREAD (ECS) AS IDENTIFIED BY ANALYSIS OF COPY NUMBER CHANGES DETECTED BY ARRAY COMPARATIVE GENOME HYBRIDIZATION (ACGH) - <u>Bryony H Lloyd, PhD</u>, Julia A Woolgar, BDS, FRCPath, FDS, RCS, Eng, PhD, Janet M Risk, PhD, Jag Dhanda, BDS, MBBS, Richard Shaw, MD, FCRSOMFS FDSRCSEng, PhD, David R Sibson, PhD University of Liverpool

Background: We and others have reported that ECS in the cervical lymph nodes is the most significant adverse prognostic indicator, proving rapidly fatal in >75% of cases despite radical surgery and adjuvant chemoradiotherapy (Shaw et al., Head Neck, 2009). We report an exploratory study applying aCGH as an initial screen to detect molecular pathways that are significantly perturbed according to clinical parameters including the presence of ECS. Methods: Samples representing T2 and T4 stage OSCC cases (n=65) having no nodal involvement, positive nodes or positive nodes plus ECS from a larger single centre series (n>200) were analysed by aCGH using 720K human genomic arrays. Copy number alterations were detected by circular binary segmentation. Genes within minimal, recurrent regions were mapped to canonical pathways to assess significance. Results: Samples could be grouped by principal component analysis according to stage and nodal status but not ECS. Multiple pathways were significantly altered according to node positive status. These included cytoskeletal remodelling: PKA in cytoskeleton reorganisation ( $p = 1.14 \times 10-4$ ), hedgehog signalling ( $p = 3.76 \times 10-3$ ), and TGF beta signalling ( $p = 4.04 \times 10-3$ ). Stages T2 and T4 had copy number differences indicative of significant deregulation of the canonical pathways: WNT signalling (p = 5.75 x10-5), TGF beta dependant induction of EMT (p=1.56 x10-4), actin cytoskeleton remodelling by Rho GTPases (2.55x10-4), and AMT/ATR regulation of the G1/S check point(p=1.26x10-3). Discussion: That samples with ECS could not be distinguished may be a result of their smaller numbers (n=22) or host factors like reduced host immune response underlying the ECS cases may be important. These may be manifest as expression changes associated with ECS and may be detected through the integration of expression array studies that we currently have underway.

### **P139:** THE ROLE OF FDG PETCT IN DIAGNOSIS AND MANAGEMENT OF OLFACTORY NEUROBLASTOMA

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Background: Olfactory neuroblastoma (ONB) is a rare tumor of neuroectodermal origin that arises from the olfactory epithelium in the roof of the nasal cavity. The stage is predictive of survival and local disease has an excellent prognosis. Surgical treatment for localized disease is successful, however there is also a role for postoperative radiotherapy. Radical radiotherapy can be used if surgery is not deemed to be appropriate and chemotherapy has a role for tumor spread beyond the nasal cavity and paranasal sinuses. The current standard imaging modality for assessment of these tumors is contrast enhanced computed tomography (CT), although magnetic resonance imaging (MRI) has a role also. This case series indicates a role for [F18]-2'-fluoro-2'-deoxy-D-glucose (FDG) positron-emission tomography combined with CT (PETCT) for radiological evaluation of ONB. Methods: A retrospective review of the medical records of all patients diagnosed with ONB in Northern Ireland between 1996 and 2009 was carried out. FDG PETCT images were analyzed where available and correlation made with clinical data. Eight patients (4 male, 4 female) were included, and four of these individuals had FDG PETCT scanning. Results: FDG PETCT is an effective imaging tool for assessment of newly diagnosed ONB, recurrence of disease and distant metastases. Findings on PETCT images correlated with the clinical picture in the cases studied. Increased FDG uptake was seen in all confirmed recurrent ONB. One case had FDG positive directed surgical management of recurrent disease on two occasions. The final FDG PETCT indicated no recurrence and the patient remains alive and disease free. The second patient presented with Kadish stage D disease, which was positive on FDG PETCT. Response to therapy assessment with FDG PETCT was negative and the patient died approximately 12 months after treatment. The third patient also had Kadish stage D disease which was also FDG positive, but

the patient rapidly deteriorated and died. The fourth patient demonstrates the use of PETCT in confirming disease remission. Conclusions: PETCT is an effective tool in the imaging of ONB. ONB tumors appear to be FDG positive and this characteristic highlights the perceived benefit of PET-CT over conventional CT imaging.

#### **P140:** EGF-SUBA ENHANCES THE CYTOTOXICITY OF RADIATION AND CISPLATIN IN LARYNGEAL SQUAMOUS CELL CARCINOMA (LSCC) CELLS - Mohammed A Aslam, Joseph M Backer, PhD, Anna Behrendt, BS, Vlatkovic Nikolina, PhD, Mark T Boyd, PhD, <u>Terence M Jones, MD</u>; University of Liverpool, University Hospital Aintree, Liverpool UK and Sibtech Inc. CT

Background: EGF-SubA is a novel cytotoxic drug that comprises a bacterial endotoxin (SubA) covalently bound to EGF to permit targeting of EGFR-expressing cells. The SubA moiety cleaves GRP78, a key component of the unfolded protein response which is induced as part of a survival response under conditions of stress (e.g. hypoxia/hypoglycaemia) commonly observed in solid tumours. Analysis of GRP78 expression on a TMA with 196 patient samples indicates that GRP78 is up-regulated in squamous cell carcinoma of the head and neck (SCCHN) and thus we hypothesize that EGF-SubA might prove a potent inhibitor of SCCHN cells. We have found that picomolar concentrations of EGF-SubA induce EGFR-dependent cytotoxicity in LSCC cells. We therefore examined whether inhibition of GRP78 by EGF-SubA potentiates the cytotoxic effects of gamma radiation and/or cisplatin in LSCC cells. Methods: LSCC cell lines (n=4) were examined by clonogenic assay to determine radiosensitivity, MTT for viability and by flow cytometry for cell cycle and apoptosis. Results: EGF-SubA acted as a radiosensitizing agent reducing the surviving fraction at 2Gy (SF2) by 30% in 3 lines and 10% in the remaining line. In all lines, EGF-SubA evoked at least an additive cytotoxic effect when used in combination with cisplatin whilst in some instances evidence of a possible synergistic effect was observed: e.g. 50pM EGF-SubA and 2µM cisplatin result in 60% and 58% of cells surviving when used alone whereas used in combination only 22% of cells survive. Flow cytometry demonstrated that cell death was substantially due to apoptosis. Conclusions: In vitro EGF-SubA alone is cytotoxic to LSCC cells at picomolar concentrations. Here we demonstrate that EGF-SubA, used as an adjuvant, enhances the cytotoxicity of gamma radiation and cisplatin. The potential of EGF-SubA as an adjuvant treatment for patients with LSCC warrants further investigation.

#### **P141:** MOLECULAR MARKERS TO DETECT OCCULT LYMPH NODE METASTASIS IN ORAL TONGUE SQUAMOUS CELL CARCINOMA - <u>Amritha Suresh, PhD</u>, Muhil Vannan, MSc,

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Metastasis in lymph nodes is the single most important prognostic marker of Head and Neck cancer; in case of tongue SCC, the 5-year survival rate improves drastically when patients are diagnosed at the pN0 stage. An accurate staging of clinically N0 necks is hence essential to enable appropriate treatment; currently management mostly includes elective neck dissection (END). Sentinel Lymph Node mapping in HNSCC is reported to accurately predict the status of the neck; however the intra operative pathological evaluation by frozen section analysis is highly insensitive; sensitivity being around 50% in tongue cancer. The primary objective of this study is to assess the efficacy of a set of biomarkers (CK14, eIF4E and DSG3) in detecting occult nodal metastasis in comparison with the current pathological evaluations. Forty four patients (126 nodes) with oral tongue cancer were analyzed by Quantitative PCR (QPCR) and their detection efficacies compared to the pathological evaluation by frozen sections, serial step sectioning (SSS) followed by H&E as well as cytokeratin immunohistochemistry (IHC) the latter being considered as the gold standard. Individually analyzed, the markers scored varied sensitivities (CK15: 0.6, eIF4E: 0.92 and DSG3: 0.88) and specificities (CK14: 0.9; eIF4E: 0.74; DSG3: 0.8). An evaluation using the two markers, CK14 and DSG3 further increased the efficacy (sensitivity: 0.88; specificity: 0.85) while a combined evaluation identified all the patients with macrometastasis, micrometastasis and isolated tumor cells (sensitivity: 1). The efficacy of detection using all the markers is equivalent to that of SSS with cytokeratin IHC and is positively superior to frozen section analysis. Immunohistochemical analysis of DSG3 in 36 patients (97 nodes) indicated a high specificity of detection of patients susceptible to recurrence (specificity: 0.9) as compared to cytokeratin (specificity: 0.7). Twenty-four percent of the biomarker positive patients were pathologically negative; one recurred (time to recurrence: 16 months), while the remaining patients are under follow up. It is hence evident that analysis with multiple markers can detect occult lymphatic metastasis with high sensitivity; a panel of thoroughly evaluated markers will help in accurately staging the pN0 neck with high sensitivity and specificity.

### **P142:** MET ACTIVATION AS A MECHANISM OF

**RESISTANCE TO EGFR INHIBITORS** - <u>Kelly M Quesnelle</u>, Sonali C Joyce, Ann M Egloff, PhD, Laura A Stabile, PhD, Kathleen Cieply, Sanja Dacic, MD, Jill M Siegfried, PhD, Jennifer R Grandis, MD; Departments of Otolaryngology, Pharmacology & Chemical Biology, and Pathology, University of Pittsburgh Medical School, Pittsburgh, Pennsylvania

Erlotinib, a reversible epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI), is currently in phase III clinical trials for use as a radiosensitizing agent in head and neck cancer. Despite widespread overexpression of EGFR in head and neck cancer, the monotherapy response rates to EGFR inhibitors are modest. In the present study, we have examined the Met receptor as a mechanism of growth in the context of EGFR blockade. We have shown a preclinical model of squamous cell carcimona of the head and neck (SCCHN) to be resistant to both erlotinib and the monoclonal antibody cetuximab in vivo as compared to isogenic parental cells. EGFR inhibitor-resistant SCCHN cells were found to have decreased levels of EGFR in the plasma membrane and increased phosphorylation of Met (pY1234/5) despite consistent Met copy numbers and protein levels. Resistant cells also have decreased levels of e-cadherin which is shown to correlate with increased Met expression in a patient cohort, suggesting an EMT transition as cells switch from EGFRdriven to Met-driven growth mechanisms. Further, we found a highly synergistic benefit of EGFR and Met co-targeting using erlotinib and the Met inhibitor SU-11274. Co-targeting of Met and EGFR is also required to decrease signaling through ERK1/2 in the presence of EGF and HGF. Taken together, these data suggest that combination targeting of EGFR and Met may prove beneficial in the context of EGFR inhibitor resistance.

#### **P143: DIFFERENTIAL PATHLENGTH SPECTROSCOPIC ANALYSIS OF PHOTODYNAMIC THERAPY** - <u>Baris Karakullukcu,</u> <u>MD</u>, Chad Kanick, PhD, Bing Tan, MD, PhD, Dominic Robinson, PhD; The Netherlands Cancer Institute, Erasmus University Medical Center

Rationale: mTHPC mediated photodynamic therapy (PDT) is an approved therapy for palliative treatment of head and neck cancers in Europe[17-21]. While superficial tumors are treated with surface illumination deeper tumors can be treated with interstitial PDT. There is a variation of clinical response with some tumors not fully responding. The response to PDT depends on the presence of three components; light, photosensitizer and oxygen. If any one is missing, there is no biological effect. Studies have shown that inter-and intra-subject differences in parameters such as tissue optical properties, uptake/synthesis of photosensitizer and tissue response to PDT can lead to wide variations in the light dose delivered during PDT. In pre-clinical models, we and others have shown that monitoring of PDT is possible non-invasively by using fluorescence and reflectance spectroscopy, in combination with state of the art light dosimetry. It is now necessary to translate these approaches to the clinic where the relationship between treatment parameters and delivered PDT dose can be significantly different. Main Study Parameters/ Endpoints: Spectrosopic quantitative analysis of oxygen saturation and mTHPC concentration in tumor tissue, light dosimetry, serum MTHPC concentration. These measurement parameters is linked to the clinical results. Results: With differential pathlength spectroscopy quantitive analysis of oxygen saturation and mTHPC concentration in human subjects becomes possible. These data can correlate to clinical results that will enable us to understand the variations in clinical respionse.

#### P144: INVESTIGATION OF MTBP, A POTENTIAL BIOMARKER AND DETERMINANT OF OUTCOME IN SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK (SCCHN) - Ashraf Y El-Fert, PhD, Anna Behrendt, BS, Carlos P Rubbi, PhD, Arpita Ray-Sinha, PhD, Terence M Jones, MD, Nikolina Vlatkovic, PhD, <u>Mark T Boyd, PhD</u>; University of Liverpool and University Hospital Aintree, Liverpool, UK

Background: MTBP is an MDM2 binding protein we identified in a yeast two-hybrid screen. Early experiments showed that MTBP can cause p53-independent G1 arrest when over-expressed in cells in culture. Subsequent studies showed that MTBP increases MDM2-mediated ubiquitylation and degradation of p53, whilst decreasing ubiquitylation of MDM2. These latter studies suggest an oncogenic role for MTBP up-regulation consistent with DNA microarray data showing that MTBP is over-expressed in a number of cancers, including those of the head and neck. However, in vivo studies of mice resulting suggest that MTBP might behave as a metastasis suppressor, a conclusion supported by in vitro studies of mouse cells. We report the first analysis of MTBP expression in human cancer and investigate how MTBP expression alters critical cell phenotypes. Methods: Immunohistochemical analysis of 200 samples using antibodies for p53, MDM2 and MTBP with statistical analysis of correlations between expression and clinical parameters. Gene targeting of Mtbp in mice. Live-cell imaging of laryngeal SCCHN cells with MTBP modulation by siRNA or cells expressing chimaeric MTBP-mCherry. Results: Using p53 and MDM2 expression to dichotomise patients into "wild-type" and "mutant" groups, we found that loss of MTBP expression is significantly associated with reduced survival (P=0.004, HR 2.775, 95% CI: 1.389-5.543) in p53 "mutant" patients. MTBP nuclear and cytoplasmic expression are differentially associated with tumour stage and with MDM2 expression. These observations are supported in vitro with increased MDM2 expression promoting a shift to cytoplasmic expression of MTBP and by studies showing dramatic real-time changes in sub-cellular MTBP distribution. Deletion of Mtbp in mice indicates it is an essential gene and down-regulation of MTBP increases co-ordinated cell migration (wound healing assays) and (random/chemo-kinetic) motility in SCCHN cells. Conclusions: Our results implicate loss of MTBP, in the context of mutant p53, as marker of a sub-population of poor outcome patients and provide evidence for a p53 status-sensitive role for MTBP in suppressing disease progression. In vitro data supports our conclusion that MTBP may act to suppress motility, contributing to reduced metastasis and therefore to increased patient survival.

#### **P145:** NUTLIN-3 RADIOSENSITISATION IN LARYNGEAL SQUAMOUS CELL CARCINOMA - Arvind K Arya, FRCS, Ashraf El-fert, PhD, Timothy Devling, PhD, Richard M Eccles, PhD, Mohammed A Aslam, Carlos P Rubbi, PhD, Nikolina Vlatkovic, PhD, John Fenwick, PhD, Bryony H Lloyd, PhD, David R Sibson, PhD, Terence M Iones, MD, Mark

Bryony H Lloyd, PhD, David R Sibson, PhD, <u>Terence M Jones, MD</u>, Mark T Boyd, PhD; University of Liverpool, UK: University Hospital Aintree, Liverpool, UK

Background: Primary radiotherapy (RT) is a mainstay of treatment for laryngeal squamous cell carcinoma (LSCC). Whilst the cure rates for early (T1) vocal cord tumours are high, RT proves ineffective in up to a third of T3 carcinomas. Moreover, RT is associated with debilitating early and late treatment-related toxicity, thus finding means to deescalate therapy, whilst retaining/augmenting therapeutic effectiveness is highly desirable. p53 is a key mediator of radiation responses; we therefore investigated whether Nutlin-3, a small molecule inhibitor of MDM2 (an essential negative regulator of p53), might radiosensitise LSCC cells. Methods: We performed clonogenic assays to measure radiosensitivity in a panel of LSCC cell lines (for which we determined p53 mutational status) in the presence and absence of Nutlin-3. Results: LSCC cells harbouring wild-type p53 were significantly radiosensitized by Nutlin-3 (P<0.0001, log rank scale) and displayed increased cell cycle arrest and significantly increased senescence (P<0.001) in the absence of increased apoptosis and thus our data suggest that senescence may mediate this increased radiosensitivity. Conclusion: This is the first study demonstrating Nutlin-3 as an effective radiosensitiser in LSCC cells which retain wild-type p53. The clinical application of Nutlin-3 might improve local recurrence rates or allow treatment de-escalation in these patients.

### **P146:** ANTI-LYMPHANGIOGENIC PROPERTIES OF MTOR INHIBITORS IN HNSCC EXPERIMENTAL MODELS

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Background: Tumor cell dissemination to regional lymph nodes via the lymphatic system represents the first step in head and neck squamous cell carcinoma (HNSCC) metastasis and is the most important poor prognostic factor for recurrence decreasing survival by 50%. Formation of tumor-associated lymphatic vessels plays an active role in the metastatic disease spread as evidenced by significant correlation between intratumoral lymphatic vessel density and lymph node metastasis. The anti-lymphangiogenic properties of mTOR inhibitors are not well understood and have not been analyzed in HNSCC. Methods: The antiproliferative effects of mTOR inhibitors (temsirolimus and sirolimus) on lymphatic endothelial cell (LEC) lines of mouse (SV-LEC) and human (HMEC) origin were evaluated using the CellTiter 96 Aqueous cell proliferation assay (Promega). Protein extracts were obtained from the LEC and HNSCC cells treated with mTOR inhibitors and western blots used to determine the effects of the treatment on the mTOR pathway factors, vascular endothelial growth factors and their receptors. Results: We found significant inhibition of lymphatic endothelial cell lines proliferation at all doses of mTOR inhibitors tested (1-1000 ng/ ml). The growth of SV-LEC and HMEC was inhibited by over 35% after 72h of treatment (P<0.05), indicating potent anti-lymphatic effects of mTOR inhibitors. Western blot analysis showed decreased proliferation was associated with mTOR signaling inhibition shown by a significant decrease in S6 ribosomal protein phosphorylation at Ser235/Ser236 and by a shift of the phosphorylated isoforms to nonphosphorylated "a" isoform of total 4EBP1. mTOR inhibition was also associated with decreased cyclin D1 expression. Furthermore, we found strong

inhibition of VEGFR-3 (Flt-4) expression in both lymphatic EC and SCC40 HNSCC cells after 100 ng/ml temsirolimus treatment. Conclusion: The results indicate mTOR inhibitors potently inhibit lymphatic proliferation by interfering with lymphatic growth factor receptor expression, an important mechanism in a disease where lymph node metastasis is the primary predictor of poor survival.

# **P147:** MODULATION OF HNSCC GROWTH USING THE NOVEL HISTONE DEACETYLASE INHIBITOR LBH589

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Although small molecule histone deacetylase inhibitors (HDACi) have shown promising results in multiple malignancies, their efficacy in HNSCC therapy has been poorly explored. Here, we investigate the novel HDACi LBH589 in HNSCC treatment. LBH589 is in phase I/II clinical trials for both hematological and solid tumors. Although it possesses similar toxicity to the FDA-approved HDACi Vorinistat, LBH589 has been shown by others to possess a 10-fold potency over Vorinostat. As seen in other cancer models, LBH589 induced G2/M cell cycle arrest and cell death using human HNSCC cell lines. Although HDACi have been perceived to work by inducing re-expression of silenced genes, our global gene expression microarray analysis of LBH589-treated versus non-treated cell lines revealed that down-regulation of genes required for DNA replication (e.g. MYC, MCM-3, MCM-4, MCM-5), chromosome segregation (e.g. NEK-6) and G2-M progression (e.g. PLK-1). This conclusion is supported by comparisons of gene expression of primary tumors and their adjacent normal tissue. Thus, downregulation of cell cycle related genes provides a more plausible explanation for the early cell cycle/mitotic arrest and cell death observed as opposed to only exploring upregulated genes upon HDACi treatment. We have models to explain this observation in light of the mechanism of action of HDACi. At moderate doses, LBH589-induced G0/G1 arrest was abrogated when combined with Taxol suggesting a synergistic effect when LBH589 was combined with Taxol. This suggests that LBH589 could be an important and effective treatment modality for HNSCC patients.

#### **P148:** TRANSCRIPTION FACTOR BORIS INDUCES SPECIFIC ACTIVATION OF THE NORMALLY REPRESSED MAGEA11 AND SBSN GENES DURING HEAD AND NECK TUMOROGENESIS - <u>Daria A Gaykalova, PhD</u>, Sheetal Bhan, PhD, Joseph A Califano, MD Johns Hopkins University

BORIS (Brother Of the Regulator of Imprinted Sites), paralog of insulator CTCF, is a multifunctional DNA binding protein. Unlike CTCF, BORIS has a restricted expression in testes, but not in any other normal tissues. BORIS has been shown to be highly expressed in different human cancer types, such as head and neck cancer, and tumor-derived cell lines. BORIS has also been shown to induce expression of several established and novel oncogenes involved in tumorogenesis. It has been demonstrated that BORIS can participate in activation of the highly conserved MAGEA11, a cancer-testis antigen (CTA), which is normally expressed only in testis. BORIS was shown to induce MAGEA11 in the head and neck, as well as lung cancers. The other target of the BORIS is SBSN, suprabasin, a protein that is normally expressed only in suprabasal layer of epidermis, and is directly activated by BORIS. Both these genes have full or partial BORIS/CTCF binding sites close to their promoter regions. We have demonstrated the effect of BORIS on regulation of these genes in a model cell line. We have also demonstrated that overexpression of BORIS can lead to eventual repression of the target genes via additional not direct mechanism of action. We have proposed that BORIS binds to the normally methylated and repressed promoters of MAGEA11 and SBSN to induce demethylation and derepression of these two genes. Additional molecular mechanisms of BORIS-dependent direct and indirect activation of the MAGEA11 and SBSN would be discussed. We proposed a model for BORIS-dependent activation of the two normally silenced genes MAGEA11 and SBSN in cancer. We predict that these two genes together with aberrant BORIS expression in tumors, like head and neck cancer be used for early diagnostics and gene-specific therapy.

#### **P149:** ELEVATED CDC42 ACTIVITY AS A RESISTANCE MECHANISM TO LAPATINIB AND AG1478 TREATMENT IN SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK -<u>E Ofo, Mr</u>, Jean-Pierre Jeannon, Mr, Peter Parker, Professor, Tony Ng, Professor; Richard Dimbleby Department of Cancer Research, Kings College London; Guy's & St Thomas' NHS Foundation Trust, London

Introduction: The Epidermal Growth Factor Receptor (EGFR) is over expressed in several solid tumours including squamous cell carcinoma of the head & neck (SCCHN). Drugs that directly block the action of EGFR are currently available. However, a major unanswered question is how best to select patients most likely to benefit from treatment, as response rates to EGFR targeted therapies in SCCHN, especially small molecule tyrosine kinase inhibitors (TKI) are very low (5-15%). Resistance to EGFR targeted therapy may stem from aberrant receptor trafficking. The Rho GTPase Cdc42 is known to affect EGFR down regulation by sequestering the E3 Ubiquitin Ligase c-Cbl, preventing it from catalyzing EGFR ubiquitination and it's subsequent degradation. The aim of our study is to determine the role of Cdc42 in mediating cancer cells response to ErbB receptor targeted therapy. Methods: Using multiphoton fluorescence resonance energy transfer (FRET) and fluorescence lifetime imaging microscopy (FLIM) we've analysed Raichu-Cdc42 activity in A431 and HN5 Squamous Carcinoma Cells following EGFR TKI treatment. FRET allows us to measure protein-protein interactions, and post-translational modifications that occur on a nanometre (nm) scale. FLIM not only allows detection of FRET, but also permits quantification of the strength and subcellular localisation of the defined protein-protein interaction within the 10nm range of our FRET probes. The Raichu-Cdc42 FRET probe is used as a biosensor of endogenous Cdc42 activity, and has been shown to provide quantification of the balance between Guanine Nucleotide Exchange Factors (GEFs) and GTPase Activating Proteins (GAPs) at the cell membrane. Results: Raichu-Cdc42 activity increases significantly following lapatinib or AG1478 treatment. Using a WASP-GST pulldown assay, we've also demonstrated that elevated endogenous active (GTP-bound) Cdc42 occurs with TKI treatment. Increased activity of downstream signalling proteins such as Cdc42 occurred despite complete blockade of EGFR C-terminal tyrosine phosphorylation using AG1478 and lapatinib, as confirmed by the absence of Phospho-EGFR (Tyr-1173) on western blotting. Conclusion: Our results suggest a positive feedback loop between EGFR and Cdc42 may occur in response to EGFR inhibition with TKI. This would have implications for receptor trafficking, which may account for resistance to EGFR targeted therapy in SCCHN.

**P150:** HPV-ASSOCIATED SURVIVAL AND RESPONSE TO THERAPY IN HEAD AND NECK CANCER - <u>Christian R Salazar</u>, <u>MPH</u>, Mphil, Robert D Burk, MD, Thomas J Belbin, PhD, Nicole Kawachi, MD, Janae Ostoloza, MD, Missak Haigentz, MD, Madhur Garg, MB, BS, Michael B Prystowsky, MD, PhD, Richard V Smith, MD, Nicolas F Schlecht, PhD; Columbia University, Albert Einstein College of Medicine, Montefiore Medical Center

Background: Recent evidence suggests that HPV-positive head and neck squamous cell carcinoma (HNSCC) cases have better survival than HPV-negative cases. However, it is unclear if similar patterns of survival exist across different tumor sites, and whether better prognosis can be explained by type of treatment subsequently received. To examine HPV-associated prognosis, we compared survival and tumor recurrence among HPV+ and HPV- cases presenting with primary HNSCC. Methods: We collected frozen tumor samples from 177 histologically confirmed HNSCC patients undergoing curative treatment in the Bronx, NYC. HPV detection and genotyping was performed by MY09/11-PCR and nested-PCR targeting E6/URR. Total RNA was extracted and tested by RT-PCR to verify HPV16-E6/E7 expression. Kaplan-Meier plots were generated and Cox proportional hazard models constructed to assess predictors of disease-specific survival and local/ regional recurrence (LRR) with clinical and demographic covariates. Results: The overall prevalence of HPV DNA by anatomic site varied significantly from 43.6% for oropharynx, 20.0% for larynx, 8.1% for lip/ oral cavity, and 26.7% for hypopharynx (p=0.0001). After adjustment by sex, age, tumor site, stage, smoking and alcohol consumption, overall survival was better for HPV+ cases as compared to HPVcases (HR=0.52, 95%CI: 0.26-1.07), and significantly better when assessed exclusively in the oropharynx (HR=0.23, 95%CI: 0.06-0.87) Administration of chemo-radiation therapy somewhat improved overall survival among HPV+ cases, though not significantly (HR HPVpositive=0.38, 95%CI: 0.05-2.80 vs. HR HPV-negative=0.43, 95%CI: 0.19-0.99). A protective association was also found for disease-specific survival (HR=0.46, 95%CI: 0.12-1.55) and LRR, after adjustment for chemo-radiation (HR=0.78, 95%CI: 0.24-2.46). Similar patterns of survival were observed when we examined E6+/E7+ compared to E6-/ E7- cases. Conclusions: Improved overall and disease-specific survival, as well as LRR, was observed with HPV+ status, with the strongest effect associated with the oropharynx. The difference in survival does not appear to be related to chemo-radiation therapy. Further research is necessary to investigate whether other treatment modalities modify the HPV-associated survival benefit.

**P151:** A NOVEL MODULAR POLYMER PLATFORM FOR THE TREATMENT OF HNSCC - *David Hu, MD*, *Guanyu Wang, MD, PhD, Linda Wang, BS, Ontario Lau, MD, David Elashoff PhD, Ben Wu, DDS, PhD, Maie St John, MD, PhD; University of California, Los Angeles* 

Objective: The management of HNSCC patients with advanced or recurrent disease poses a considerable challenge. The surgical demand in such a setting is for wider resection or, in some instances when the tumor is fixed to the underlying vital structures, to debulk large tumors. The ability to decrease mortality, and improve survival for these patients has been a longstanding goal. We have developed a novel polymer platform that has the following characteristics: is biocompatible; is ultimately biodegradable into biocompatible breakdown products; and can serve as a platform to deliver immunomodulators and chemotherapeutic agents so as to most effectively kill tumor cells in the proximity of the polymer application. This polymer wrap is designed to be applied intraoperatively to the surgical bed after removing or debulking the tumor. Herein, we evaluate the therapeutic efficacy of this novel modular polymer platform in the treatment of HNSCC in an animal model. Design: in vivo study. Setting: Academic research laboratory. Subjects: Animal Model: C3H/HeJ mice. Interventions: All mice underwent partial resection of their tumors. Animals were then randomized to receive implantation of (1) no polymer; (2) plain polymer; (3) plain polymer with local cisplatin injection; (4) cisplatin polymer. Main Outcome Measurements: Tumor size was measured until the mice were euthanized. At necropsy, the tumors were excised and weighed. Results: Our results using this novel polymer platform demonstrate a remarkable reduction in tumor growth. The cisplatin secreting polymer effectively reduced SCCVII/SF tumors in C3H/ HeJ mice by over 15-fold (90%) on day 12 (P < 0.001) as compared to control (surgical debulking only), plain polymer, and plain polymer + intratumoral cisplatin injection groups. Conclusion: We have obtained promising results from our pilot in vivo studies using a single layer polymer releasing cisplatin only. We are in the process of developing the optimal combination of the bilayers to improve the outcome for patients with advanced or recurrent HNSCC. Once this polymer platform is further optimized we will plan for the ultimate validation in the context of a prospective trial in patients with unresectable advanced or recurrent HNSCC.

P152: CHEMOPREVENTIVE EFFECTS OF A PPAR-? LIGAND - PIOGLITAZONE IN THE CARCINOGENESIS PROCESS OF THE UPPER AERODIGESTIVE TRACT INDUCED BY 4-NITROQUINOLINE-1-OXIDE IN SWISS MICE - Ricardo R Gama, Allan L Giovanini, Fernanda S Rosa, Daniel C Ogata, Andre L Vettore, PhD, Carolina Talini, Denise Feniman, Douglas Kamei, Celso F Junior, Allan Coco, Andre L Carvalho, MD, PhD; Universidade de Sao Paulo, Faculdade Evangélica do Paraná, Universidade Positivo, Universidade Federal Sao Paulo, Hospital Cancer Barretos

Introduction: Among 3% to 7% of patients with head and neck carcinoma will develop subsequent primary tumors of the upper aerodigestive tract (UADT) annually, so it is beyond doubt the importance in advancing in new chemopreventive strategies. In this research the potential chemopreventive effects of a PPAR- $\gamma$  ligand – pioglitazone were tested in an animal model of UADT carcinogenesis induced by 4-nitroquinoline-1-oxide (4-NQO) in Swiss mice. Methods: The animals were submitted to cancer induction with 50 µg/ml of 4-NQO diluted in water for 8 weeks. After this, animals were selected to receive chemoprevention with the adition of 100 ppm of pioglitazone in basal diet in postinitiation phase during 24 weeks or pioglitazone 100 ppm in initiation and postinitiation phases during 32 weeks, also there was a control group not receiving chemoprevention. Results: The great majority of animals developed invasive squamous cell carcinoma in more than one site of UADT or more frequently the association between premalignant and malignant lesions. The incidence for invasive oral carcinoma was similar between groups: control-72,9%, pioglitazone in postinitiation-76,1% and pioglitazone in initiation and postinitiation-62,5% (p=0,63). The incidence for invasive esophageal carcinoma was also similar among the groups: control-37,8%, pioglitazone in postinitiation-57,1% and pioglitazone in initiation and postinitiation-31,2% (p=0,22). Although the similarity in incidence for invasive gastric carcinoma between groups: control-13,5%, pioglitazone in postinitiation-9,5% and pioglitazone in initiation and postinitiation-18,7%, an agressive gastric carcinogenesis with metastatic spread was seen in pioglitazone groups that was not observed in control group. The cancer specific survival rates in the 24 weeks that succeeded the conclusion of the tumor induction phase was not statistically significant when comparing the groups with or without

chemoprevention (p=0,80). Conclusion: Based on the methodology of this study and the obtained results the use of different forms of chemoprevention with pioglitazone, brought no benefit in avoiding, retarding or blocking the carcinogenesis process in UADT initiated by 4-NQO. It seems that the action of pioglitazone in conjunction with 4-NQO might have created a new model of gastric carcinogenesis.

### **P153:** EXPRESSION PROFILE OF CYTOKINES AND GROWTH FACTORS SECRETED BY HEAD AND NECK

SQUAMOUS CELL CARCINOMAS - <u>Maria Athanassiou, PhD</u>, Omar Shkeir, Petros Papagerakis, DDS, PhD, Cezar Lapadatescu, MD, Carol R Bradford, MD, FACS, Thomas E Carey, PhD, Mark E Prince, MD, FACS, Silvana Papagerakis, MD, PhD; Otolaryngology Head Neck Surgery; Pediatric Dentistry; University of Michigan Ann Arbor

Objectives: Head and neck squamous carcinomas (HNSCC) have devastating mortality rates with morbidity mainly due to metastasis. Genetic or epigenetic alterations intrinsic to cancer cells, as well as the tumor microenvironment determine the metastatic potential of the cancer cell. Methods: To examine whether HNSCC cells secrete tumorassociated cytokines, we collected serum-free conditioned medium from human HNSCC cell lines over a time-course of 72 hours and assayed via multiplex assay. The screen included a panel of stage- and anatomic sitespecific primary, recurrent and metastatic HNSCC cell lines established by our group (UMSCC 10A, 10B, 11A, 11B, 12, 14A, 22A, 22B, 47). The levels of the cytokines assessed were compared to corresponding levels in normal oral keratinocytes (NOK). Results: Conditioned medium from metastatic and recurrent tumor cell lines showed significantly higher amounts of interleukin 6 (IL-6), IL-6 receptor, tumor growth factor (TGF)-beta and vascular endothelial growth factor (VEGF) than conditioned medium from non-metastatic cells or NOK. The highest IL-6 was secreted by HNSCC originated in the oral cavity with known poor clinical outcome (UMSCC14A). No significant levels of interferon (IFN)gamma or IL-2, 3, 4 and 7 were measured. Finally, tumor necrosis factor (TNF)-alpha was secreted by the least aggressive of HNSCCs and NOK. Conclusion: Tumor progression and metastasis positively correlate with the presence of immune cells that can enhance metastatogenesis through the tumoral cytokines. Furthermore, certain carcinomas recruit VEGF receptor1-positive haematopoietic progenitors to pre-metastatic niche. TGF-beta can promote tumor cell proliferation through an autocrine effect. The IL-6 high expression in patients' serum correlates with poor clinical outcome in various human cancers. The cytokine release profile of HNSCC cells includes high levels of IL-6 and IL-6R, TGF-beta, VEGF, and therefore provides valuable insight into their metastatic potential. This also suggests that a combination test of these four soluble factors may provide a stronger predictive value for the HNSCC tumor progression and clinical outcome.

#### **P154:** SLEX EXPRESSION IN ORAL, HEAD AND NECK

**CARCINOMAS** - <u>Michael J Czerwinski</u>, Maria Athanassiou, PhD, Randall Knibbs, PhD, Ron Craig, PhD, John Henry Owen, BS, Cezar Lapadatescu, MD, Omar Shkeir, Jonathan R McHugh, MD, FACS, Carol E Bradford, MD, FACS, Thomas E Carey, PhD, Mark E Prince, MD, FACS, Llyod Stoolman, MD, FACS, Silvana Papagerakis, MD, PhD; Otolaryngology Head Neck Surgery; Pathology; University of Michigan Ann Arbor

Objective: Among the clinical challenges of head and neck squamous cell carcinomas (HNSCC), the morbidity and mortality of advanced oral cancer (OC) remains paramount. The molecular mechanisms controlling OC progression and metastasis are thus key to the improvement of clinical outcomes. Metastatic dissemination begins with adhesion of circulating tumor cells to the E-selectin-expressing endothelium with high levels of the main E-selectin ligand, sLeX, having been implicated in various carcinomas. Our study represents a novel investigation of the expression and potential roles of sLeX in HNSCC. Methods: A panel of stage- and anatomic site-specific primary, reccurent and metastatic HNSCC cell lines along with primary HNSCC were assayed. Flow cytometry was used to quantify cell-surface sLeX expression. Secreted sLeX expression was measured by Western-blot in serum-free conditioned medium. Relative mRNA levels of fucosyl-transferase VII (FucVII), the main enzyme involved in sLeX neosynthesis in OC, were assessed by quantitative real time PCR (qRT-PCR). HNSCC-derived sLeX-positive cells were injected in the flank of immunodeficient mice to evaluate their tumorigenic potential. Preparations of the cultured cells, as well as of human and mouse tumors were immunohistochemically stained for sLeX. Results: sLeX was predominantly expressed in HNSCC originating in the oral cavity, and was highest in cells from a recurrent (HNSCC14B) and a metastatic (UMSCC103) tumor with known poor clinical outcomes. The expression of secreted sLeX was also high in the HNSCC supernatants of OC. Flank injections of sLeX-positive tumor cells developed SCC in immunodeficient mice, while the mice injected with sLeX-negative cells formed no tumors. Staining of HNSCC and primary and murine tumor specimens showed cytoplasmic and membranous sLeX. Conclusions: Among various HNSCC, both cell-bound and soluble sLeX was predominantly expressed in tumor cells of OC and associated with recurrent and metastatic tumors. The mechanism of sLeX involvement in OC progression is under investigation.

#### **P155:** CD44 VARIANTS EXPRESSION IN THE HEAD AND NECK SQUAMOUS CELL CARCINOMAS - <u>Silvana Papagerakis</u>, <u>MD, PhD</u>, Maria Athanassiou, PhD, Vasu Divi, MD, FACS, Vijay Jarodiya, John Henry Owen, BS, Carol R Bradford, MD, FACS, Thomas E Carey, PhD, Mark E Prince, MD, FACS; Otolaryngology Head Neck Surgery, University of

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Objectives: Cancer stem cells are defined as the subpopulation of tumor cells that have distinctive gene expression profiles, exhibit self renewal, and are highly tumorigenic in immunodeficient mice giving rise to the heterogenous cellular phenotypes of the original tumor. We were the first to isolate head and neck squamous cell carcinomas (HNSCC) stem cells using the cell surface marker CD44 and flow cytometry (Prince et al, 2008). CD44 is a trans-membrane glycoprotein with a multitude of key functions that regulate cancer cell proliferation and metastasis. CD44 variety of functions is due to tissue-specific patterns of glycosylation of the extracellular portion, and to the multiple protein isoforms (CD44 variants) generated by alternative splicing. The expression pattern of CD44 variants was investigated in HNSCC. Methods: To characterize CD44 variants differential expression in HNSCC, ten HNSCC cell lines generated by our group from the most common HNSCC locations and representative of various clinical outcomes along with six primary HNSCC were assayed by quantitative real-time PCR comparatively with normal oral keratinocytes (NOK). Results: Among the CD44 variants tested in our collection of HNSCC primary tumors and cell lines comparatively to NOK, v4 and v6 were exclusively abundant in HNSCC while the isoform v1,2 was expressed in normal oral keratinocytes. Conclusion: HNSCC differentially expressed CD44 variants and that preferential expression might contributes to the tumor behavior. The mechanism of CD44 variants involvement in HNSCC progression is under investigation.

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### **FUTURE MEETINGS**

AHNS 2011 Annual Meeting during the Combined Otolaryngology Society Meetings (COSM) April 27-28, 2011 Sheraton Chicago Hotel & Towers • Chicago, IL

**8**<sup>th</sup> **International Conference on Head & Neck Cancer** July 21-25, 2012 Metro Toronto Convention Center + Toronto, ON, Canada AHNS 2013 Annual Meeting during the Combined Otolaryngology Society Meetings (COSM) April 10-11, 2011 JW Marriott Grand Lakes + Orlando, FL

5<sup>th</sup> World Congress of the International Federation of Head & Neck Oncologic Societies (IFHNOS) July 26-30, 2014 New York Marriott Marquis + New York, NY