AHNS 2015
TRANSLATIONAL RESEARCH MEETING

on Transforming Patient Care Through Innovative Research

April 21-22, 2015

Sheraton Boston Hotel, 39 Dalton Street, Boston, MA 02199

AMERICAN HEAD & NECK SOCIETY
1954 ★ 1998 ★ 1958

FINAL PROGRAM

WWW.AHNS.INFO

THE RESEARCH AND EDUCATION FOUNDATION OF THE AMERICAN HEAD AND NECK SOCIETY

Dear Colleagues,

As we all know, head and neck cancer is not one of the more "publicized" cancers and often overlooked by both the general public and research funding sources. However, we believe that this is slowly starting to change. With increased attention on head and neck cancer, the Research and Education Foundation believes this is an opportunity to strengthen and expand our impact. **With your commitment to the Foundation, we can reverse the trend of declining funding for head and neck cancer research!**

Presently the Research and Education Foundation supports two research awards each year. We are proud to fund these grants but there are more to cover. We would like to create more opportunities for young clinicians and researchers to explore unique and innovative treatments which may one day lead to a cure. To do as much, the Foundation needs to increase its asset base.

There are **three targeted ways for you to support** the Foundation which are designed to provide immediate revenue as well as to increase the capital base for the long term with the goal of generating increased annual income perpetually to support more research. The support opportunities include:

1. **Legacy gifts**, such as estate planning, single premium life insurance and charitable lead annuity trusts (CLATs), are meaningful ways to create continuous and sustainable growth for the Foundation in the years and decades to come.

2. Committing to a **five-year pledge** with an annual donation provides the Foundation income in the present and assists us in budgeting several years out. We will be launching a Future in Five campaign with a generous but critical pledge of $5,000/annually. This pledge level entitles one to Centurion Club level membership for life.

3. **General Donations and Centurion Club membership**: Any donation from AHNS members and general public alike provides immediate support and is always appreciated. All gifts of $1,000 or more (including pledge gifts) are considered Centurion Club level donations.

*To make your donation today, please go online to **www.ahnsfoundation.info/donations** or you may complete a donation form available at the AHNS registration desk. Please contact Kelly Honecker at **kelly@ahns.info** with any questions. The future of the Foundation is bright and your support brings great promise to head and neck cancer research. Thank you!*

Sincerely,

Jatin P. Shah, MD
Foundation Chair

Doug Girod, MD
AHNS President

WELCOME

WHO: **The American Head & Neck Society**

WHAT: **2015 Translational Research Meeting on Transforming Patient Care Through Innovative Research**

WHEN: **April 21-22, 2015**

WHERE: **Sheraton Boston Hotel**
39 Dalton Street
Boston, MA 02199

CONTACT: **The American Head and Neck Society (AHNS)**
11300 W. Olympic Blvd., Suite 600
Los Angeles, CA 90064

Via phone: +1-310-437-0559
Via fax: +1-310-437-0585
Via email: registration@ahns.info

www.ahns.info

2015 TRANSLATIONAL RESEARCH MEETING EXHIBITORS

Thank you to our 2015 exhibiting companies!

Genzyme, a Sanofi Company

Novadaq

Synovis Micro Companies Alliance

Veracyte, Inc.

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GENERAL INFORMATION

TRANSLATIONAL RESEARCH MEETING EDUCATIONAL OBJECTIVES

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

- Integrate molecular testing for HPV and other HNSCC subtypes into practice and/or trial design

- Understand that massive sequencing and molecular data exists and use this data to form hypotheses, to design pre-clinical experiments and to translate results into clinical trials

- Be aware of existing and developing technology for minimally invasive surgery and implement currently available tools for care of patients

- Recognize existing best practices based on outcome studies and integrate them into current multi-disciplinary practices at their institutions or practices

- Assess the potential value of immuno-modulatory and combinatorial targeted therapy in HNSCC based on progress made with other tumor types and participate in pre-clinical or clinical research to increase understanding of these therapies for HNSCC

RESEARCH MEETING 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 2015 Translational Research Meeting 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.

ON-SITE REGISTRATION HOURS
Location: Constitution Ballroom Foyer

Monday, April 20	12:00 PM - 6:00 PM
Tuesday, April 21	7:00 AM - 6:00 PM
Wednesday, April 22	7:30 AM - 7:00 PM

EXHIBIT AREA HOURS
Location: Back Bay Ballroom

Tuesday, April 21	12:00 PM - 3:30 PM
	5:00 PM - 6:00 PM
Wednesday, April 22	10:00 AM - 3:00 PM
	6:00 PM - 7:00 PM

POSTER SCHEDULE & VIEWING HOURS
Location: Back Bay Ballroom

Tuesday, April 21

Poster Set-Up Hours	10:00 AM - 11:30 AM
Poster Display Hours	12:00 PM - 3:30 PM
Opening Reception	5:00 PM - 6:00 PM

Wednesday, April 22

Poster Display Hours	10:00 AM - 3:00 PM
Poster Reception	6:00 PM - 7:00 PM
Poster Breakdown Time	7:00 PM - 9:00 PM

SPEAKER READY INSTRUCTIONS

All speakers must have their PPT presentation uploaded 2 hours before their presentation. Please check in your presentation at the AV table in the Constitution Foyer located right outside Constitution Ballroom.

VISIBILITY DONORS

Thank you to our 2015 Visibility Donors!

The following companies have provided generous support for non-CME meeting activities:

Platinum Donor

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ABOUT AHNS

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/member-central** or call +1 310-437-0559, ext. 156.

TRANSLATIONAL RESEARCH MEETING PLANNING COMMITTEE

Wendell G. Yarbrough, MD, MMHC, FACS
Program Chair

M. Boyd Gillespie, MD, MS
Program Co-Chair

Benjamin Judson, MD
Poster Chair

Nishant Agarwal, MD

Joseph A. Califano, MD

Christine G. Gourin, MD

Chris Holsinger, MD

Eduardo Mendez, MD

Gregory L. Randolph, MD

James Rocco, MD, PhD

Eben Rosenthal, MD

John Sunwoo, MD

Adam M. Zanation, MD

PROGRAM LEADERS

PROGRAM CHAIR

Wendell G. Yarbrough, MD, MMHC, FACS

Dr. Yarbrough graduated in 1989 from UNC School of Medicine with honors and distinction after graduating from UNC as a Morehead Scholar. Otolaryngology residency and a surgical oncology fellowship, then a faculty position, K08, then R01 funding followed while at UNC. In 2003, Dr. Yarbrough relocated to Vanderbilt as leader of the Barry Baker Laboratory for H&N Cancer where he was Co-Leader of the Thoracic/H&N Program and helped form the Tennessee Chapter of the Head and Neck Cancer Alliance.

In 2012, Dr. Yarbrough assumed the roles of Chief of Otolaryngology, Director of the Head & Neck Disease Center, and Co-Director of the Molecular Virology Program at Yale. He currently serves as leader of the Research Committee of the AHNS. He also serves on the editorial board of Head and Neck and has extensively published with areas of emphasis including head and neck squamous cell carcinoma and salivary cancers.

Dr. Yarbrough takes great pride in the residents, graduate students, post-doctoral fellows, and junior faculty that he has mentored. His goals continue to focus on advancement of patient care through improvements in prevention, clinical treatment, innovative translational research, and by mentoring clinical and research personnel to be the next generation of leaders in otolaryngology and head and neck cancer.

PROGRAM CO-CHAIR

M. Boyd Gillespie, MD, MSC

M. Boyd Gillespie, MD, MSc, is professor of otolaryngology-head and neck surgery and vice-chairman of clinical outreach at the Medical University of South Carolina. He is internationally recognized investigator and teacher on endoscopic salivary gland surgery. He is actively involved in the design and execution of clinical trials to test new devices in head and neck and sleep surgery. Dr. Gillespie has served on numerous AHNS committees, and is currently co-chair of the 2015 AHNS Translational Research Meeting.

KEYNOTE LECTURERS

Paul Eder, MD

Dr. Joseph Paul Eder's involvement in cancer therapeutics began during his fellowship with the taught him protocol design and management, clinical pharmacology, and translational science. Dr. Eder wrote the first paper on the use of high dose combination chemotherapy with autologous bone marrow transplantation in breast cancer and performed several phase I trials of high dose chemotherapy. From 1990 to 1995 he worked on the biological mechanisms of drug resistance. They provided the first genetic proof in mammalian systems that topoisomerase II was the target of etoposide. Other studies determined that there was co-ordinate expression of the topoisomerase I and II proteins in solid tumors in vitro and in vivo that made it a testable hypothesis in a clinical trial of doxorubicin and topotecan.

Dr. Eder became the principal clinical investigator of the Harvard UO1 Phase I program in 1995, uniting the clinical efforts at the DFCI, BWH, MGH and BIDMC. In 1998, he became the Clinical Director of the Experimental Therapeutics Program for the Dana-Farber/Harvard Cancer Center. As Clinical Director, he assumed overall responsibility for the trials performed at the DF/HCC. In 2004 he assumed the responsibilities as the Clinical Director of the DFCI General Cancer Research Center at the Dana-Farber/ Brigham and Women's Hospital. A NIH U01 grant [UO1CA 62490-13] formed the basic funding mechanism. The program was also a Project on the DF/HCC Cancer Center grant and the Overcoming Barriers to Clinical trials, a NIH award to increase access to early clinical trials for underrepresented populations. These clinical and basic research activities involved particular areas of involvement include high dose chemotherapy, the modulation/reversal of drug resistance, growth factors, vaccines, novel agents, analogue development, signal transduction pathway inhibitors, cell cycle inhibitors, and the therapeutic use of antiangiogenesis agents.

At AstraZeneca PLC 2007-2012, Dr. Eder was the Medical Science Director for AstraZeneca's Boston site, responsible for the medical and clinical aspects of development of agents from chemical lead identification through clinical proof of concept in phase II. This position had global responsibilities with clinical trials in the US, Canada, European Union, Japan and Korea. He was a member of the Strategic Planning & Business Development and was the Global Disease Area Clinical Expert for Hematology.

Dr. Eder joined the Yale Cancer Center in 2012 as Director of the Early Drug Development Program and Assistant Director of Experimental Therapeutics. He am a co-investigator and site PI on an UM1 from Vanderbilt-Ingram Cancer center, Karmanos Cancer Institute. TGen and Yale Cancer center (ViKTrY). He am a member of the senior leadership members of ViKTriY and the site PI responsible for oversight of activities at Yale.

Neil Hayes, MD

D. Neil Hayes is a native of Winston-Salem, NC and graduate of Davidson College with a BS in chemistry. He completed his medical degree at the University of North Carolina, Chapel Hill and then a Masters of Public Health at the Harvard School of Public Health. His residency was at the Boston City Hospital in the Boston University School of Medicine program and then fellowship training in Hematology/Oncology at the Tufts New England School of Medicine where he also completed a Masters degree in Clinical Research. In conjunction with his fellowship he completed post-doctoral training in the lab of Dr. Matthew Meyerson at the Dana Farber Cancer Institute.

Upon completion of clinical training he took a faculty position (currently associate professor) at the University of North Carolina, Chapel Hill School of Medicine where he sees patients with aerodigestive tumors. His efforts include founding and current leadership of the head and neck cancer clinical trials group. He maintains a lab of approximately 10 staff and students dedicated to translational study of cancer genomics. He is co-leader of the Clinical Research Program in the Cancer center and co-director of the Bioinformatics Core. He has numerous grants in his field of study and approximately 100 peer-reviewed publications.

AHNS ACCREDITATION

ACCREDITATION STATEMENT

The American Head & Neck Society (AHNS) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians.

CREDIT DESIGNATION STATEMENT

The AHNS designates this live activity for a maximum of **15.25** *AMA PRA Category 1 Credit(s)*™. Physicians should claim only the 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.

TIME	ACTIVITY	CREDITS AVAILABLE	HOURS ATTENDED
TUESDAY, APRIL 21, 2015			
8:15 AM - 9:00 AM	KEYNOTE LECTURE: "Immunomodulation as Therapy: Opportunities for HNSCC"	0.75	
9:30 AM - 10:30 AM	CONCURRENT SESSION: Salivary Cancer – What's New?	1.5	
	CONCURRENT SESSION: Thyroid Molecular Advances		
11:00 AM - 12:30 PM	CONCURRENT SESSION: Immunomodulation	1.5	
	CONCURRENT SESSION: Epignetics in Cancer		
12:30 PM - 1:30 PM	LUNCH SESSION: NIH, NDCR, NCI & Foundation	1.0	
	LUNCH SESSION: Outcomes Best Practice – How to Get Started		
	LUNCH SESSION: Starting a Biorepository		
1:30 PM - 3:00 PM	CONCURRENT SESSION: Breakthroughs – Paradigm Changes New Thoughts, HNSCC & HPV	1.5	
	CONCURRENT SESSION: Maximizing Function After Treatment of HN Cancer		
3:30 PM - 4:50 PM	Head and Neck Cancer Genetics – Sequencing, Pathways and Targets	1.25	
	Total Credits Available for Tuesday, April 21, 2015:	**7.5**	
WEDNESDAY, APRIL 22, 2015			
8:00 AM - 9:00 AM	*Hynes Convention Center – Ballroom A* AHNS Annual Meeting Scientific Session 4	1.0	
9:00 AM - 9:45 AM	AHNS Annual Meeting Hayes Martin Lecture: Some Things We Know	0.75	
10:30 AM - 11:15 AM	KEYNOTE LECTURE: "Deep Sequencing Profiling and Impact on Therapy"	0.75	
11:15 AM - 12:15 PM	CONCURRENT SESSION: Tumor Imaging	1.0	
	CONCURRENT SESSION: Stem Cells in Cancer		
1:15 PM - 2:30 PM	CONCURRENT SESSION: Advancing Cancer Therapy Best Practices Through Outcomes Research	1.25	
	CONCURRENT SESSION: Surgical Advances		
3:00 PM - 4:30 PM	CONCURRENT SESSION: Personalized Patient Care – How Our Institution Does It	1.5	
	CONCURRENT SESSION: Best Posters		
4:30 PM - 6:00 PM	CONCURRENT SESSION: Late Breaking Hot Topics	1.5	
	CONCURRENT SESSION: Advances Through Recent Cooperative Group and SPORE Trials		
	Total Credits Available for Wednesday, April 22, 2015:	**7.75**	
	TOTAL CREDITS AVAILABLE:	**15.25**	

TO RECEIVE YOUR CME CREDIT:

To receive your CME credit: AHNS has instituted a process for claiming CME credits and printing certificates. All attendees wishing to receive a CME certificate for activities attended at the AHNS 2015 Translational Research Meeting 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 is mailed to you.

COMMERCIAL BIAS REPORTING FORM

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*, 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:
(eg session name, etc)

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

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

COMMERCIAL BIAS ABOUT:
(check all that apply)

❑ 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

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SCIENTIFIC PROGRAM

TUESDAY, APRIL 21, 2015

7:45 AM	**Continental Breakfast**	*Constitution Foyer*
8:00 AM - 8:15 AM	**AHNS Translational Research Opening, Welcome & Overview**	*Constitution Ballroom*
	Douglas A. Girod, MD and Wendell G. Yarbrough, MD, MMHC, FACS	
8:15 AM - 9:00 AM	**KEYNOTE LECTURE: "Immunomodulation as Therapy: Opportunities for HNSCC"**	*Constitution Ballroom*
	INTRODUCTION: Wendell G. Yarbrough, MD, MMHC, FACS	
	KEYNOTE SPEAKER: Paul Eder, MD	
9:00 AM - 10:30 AM	**CONCURRENT SESSIONS**	

Constitution Ballroom

Republic Ballroom

Salivary Cancer – What's New?
CHAIR: Wendell G. Yarbrough, MD, MMHC, FACS

Thyroid Molecular Advances – A Collaborative Head and Neck Surgical, Pathology, and Endocrine Panel
CHAIR: Gregory L. Randolph, MD

Salivary cancers remain difficult to treat with no targeted therapies currently available. Recent advances will be discussed as well as new tools for study of these orphan cancers.

Thyroid nodule evaluation and thyroid cancer management has been increasingly affected by our emerging understanding of the molecular underpinning of thyroid neoplasia. This panel will review our current state-of-the-art understanding of molecular analysis in both thyroid cancer diagnosis and treatment and will range from preoperative assessment to postoperative medical oncologic options.

At the conclusion of this session, participants will be able to:
1. Identify issues and new solutions for identification and testing of new therapies for salivary cancers.
2. Understand pathways that may be targeted in salivary cancers.
3. Understand issues with cell culture of salivary cancers.

At the conclusion of this session, participants will be able to:
1. Better understand the current testing options available for preoperative FNA molecular analysis.
2. Understand better the role of the wrath analysis in determination of extent of nodal surgery.
3. Understand how medical chemotherapeutic options may require knowledge of specific molecular alterations in specific thyroid cancers.

9:00 AM	**Introduction**
	Wendell G. Yarbrough, MD, MMHC, FACS
9:05 AM	**Molecular Targets in Salivary Carcinoma**
	Adel K. El Naggar, MD, PhD
9:17 AM	**Understanding Signaling Pathways in Adenoid Cystic Carcinoma**
	Patrick K. Ha, MD
9:29 AM	**Discussion/Q&A**
9:33 AM	**Gene Expression and Translational Targets in Adenoid Cystic Carcinoma**
	Scott A. Ness, MD
9:45 AM	**S001: Application of Primary Culture of Salivary Gland Benign and Malignant Tumors Under Conditional Reprogramming Conditions for Definition of Tumor-Associated Genetic Changes**
	Priscilla Furth, MD
9:50 AM	**Targeting Neurotrophic Signaling in Adenoid Cystic Carcinoma**
	Sergey Ivanov, PhD
10:02 AM	**Discussion/Q&A**
10:06 AM	**S002: Generation and Characterization of Adenoid Cystic Carcinoma Cultures and Cell Lines**
	Michael Chang, BS
10:12 AM	**Targeting Apoptosis in Adenoid Cystic Carcinoma**
	Jacques E. Nor, DDS, MS
10:24 AM	**Discussion/Q&A**

9:00 AM	**Introduction to the Thyroid Cancer Molecular Landscape**
	Jim Fagin, MD

Diagnosis: Molecular Analysis of the FNA sample

9:25 AM	**FNA Oncogene Panels and the Detection of Malignancy**
	Yuri Nikiforov, MD, PhD
9:45 AM	**Clinical Application of FNA Molecular Analysis**
	Gregory L. Randolph, MD

Treatment: Molecular Analysis and Individualized Treatment

9:55 AM	**Initial Thyroid and Nodal Surgery**
	Robert L. Ferris, MD, PhD
10:05 AM	**Braf Inhibition in Treatment of RAI Non-Avid Thyroid Cancer**
	Lori Wirth, MD
10:20 AM	**Panel Cases and Audience Q&A**

SCIENTIFIC PROGRAM

10:30 AM - 11:00 AM **Coffee Break**   *Constitution Foyer*

11:00 AM - 12:30 PM **CONCURRENT SESSIONS**

Constitution Ballroom

Immunomodulation
CHAIR: John Sunwoo, MD

This session will focus on the latest research on the interaction between the immune system and head and neck cancer. The session will educate the audience on basic concepts of cancer immunotherapy and will highlight novel studies that seek to translate the knowledge gained from this research into novel therapeutic strategies targeting this head and neck cancer.

At the conclusion of this session, participants will be able to:
1. Understand the concepts and mechanisms of immune surveillance and how tumor cells evade this process.
2. Understand the current strategies for modulating the immune response to cancer, including checkpoint blockade and STING agonists.
3. Understand how chemotherapy and radiation therapy interact with the immune response to cancer.

11:00 AM **Introduction**
John Sunwoo, MD

11:05 AM **Clinical Trials to Identify and Reverse Immune Escape in the Tumor Microenvironment**
Robert L. Ferris, MD, PhD

11:20 AM **STING Agonist Immunotherapy for Head and Neck Cancer Patients**
Young Kim, MD, PhD

11:35 AM **S003: Altered Expression of Programmed Death Ligand in the Tumor Microenvironment of Oral Cavity Cancer Following PI3K/mTOR and MPAK Targeted Therapy**
Andria Caruso, MD

11:42 AM **Q&A**

11:47 AM **Manipulation of the B7-H1 – PD-1 Axis for the Treatment of Head and Neck Cancer**
Scott E. Strome, MD

12:02 PM **The Effects of Chemoradiotherapy on Peripheral Anti-Tumor Immunity in HPV+ Head and Neck Cancer Patients**
Andrew G. Sikora, MD, PhD

12:17 PM **S004: PD-1 Blockade Synergizes with Cisplatin Radiation Therapy Aiding in Clearance of HPV+ Oropharyngeal Carcinoma**
William C. Spanos, MD

12:24 PM **Q&A**

Republic Ballroom

Epigenetics in Cancer
CHAIR: Joseph A. Califano, MD, FACS

This session will address the impact of epigenetic alterations in the development of head and neck cancer. Presentations will define the role of epigenetic alterations in carcinogenesis, development of prognostic markers and markers for detection as well as in development of therapeutic approaches to head and neck cancer.

At the conclusion of this session, participants will be able to:
1. Understand the nature of epigenetic alterations in head and neck cancer.
2. Understand the investigational approaches to understanding epigenetic alterations in head and neck cancer prognosis, therapy, and carcinogenesis.

11:00 AM **Introduction**
Joseph A. Califano, MD, FACS

11:05 AM **DNA Methylation as a Classifier of Head and Neck Squamous Cell Carcinoma**
Thomas J. Belbin, PhD

11:30 AM **S005: 5-Azacytidine Selectively Kills HPV-Positive Head and Neck Cancer Cells via Simultaneous Targeting of Multiple Cancer-Related Pathways**
Andrew Sewell, MD

11:40 AM **Discussion/Q&A**

11:45 AM **S006: MGMT Hypermethylation as a Promising Marker for AST1306 Therapy Response**
Lidia M. R. B. Arantes, PhD

11:55 AM **Defining Specific Methylation Alterations in HPV Positive Oropharynx Cancer**
Joseph A. Califano, MD, FACS

12:15 PM **S007: DNA Methylation Regulates TMEM16A Expression Through Alternate Mechanisms at Two Distinct CPG Islands**
Andrey Finegersh, PhD

12:25 PM **Discussion/Q&A**

SCIENTIFIC PROGRAM

12:30 PM - 1:30 PM   **LUNCH SESSIONS** (RSVP Required) or **Lunch On Own**

1) NIH, NDCR, NCI & Foundation   *Constitution Ballroom*
MODERATOR: M. Boyd Gillespie, MD, Msc

The session is designed to update junior investigators on the grants process of the NIH and other foundations.

At the conclusion of this session, participants will be able to:
1. Understand the grants processes of the NCI; NIDCR; and CORE.
2. Discuss differences in the funding priorities of NCI, NIDCR, and CORE.

12:30 PM   **NCI: Process and Priorities in Head and Neck Cancer** – *William C. Timmer*

12:50 PM   **NIDCR: Process and Prioritues in Head and Neck Cancer** – *Marilyn Moore-Hoon*

1:10 PM   **CORE: Opportunities in Head and Neck Cancer research** – *Cherie Ann-Nathan*

1:20 PM   **Q&A** – *Led by M. Boyd Gillispie*

2) Outcomes Best Practice – How to Get Started   *Republic Ballroom*
MODERATOR: Christine G. Gourin, MD

This session will provide an overview of databases currently available for use in outcomes research.

At the conclusion of this session, participants will be able to:
1. Understand how outcomes research is performed.
2. Identify data resources to begin.

12:30 PM   **Outcomes Measurement** – *Andrew G. Shuman, MD*

12:40 PM   **SEER, NCDB, and NSQIP** – *Benjamin Judson, MD*

12:50 PM   **Using Insurance Databases** – *Steve S. Chang, MD*

1:00 PM   **AHRQ Databases** – *Christine G. Gourin, MD*

1:10 PM   **Q&A**

3) Starting a Biorepository   *Independence Ballroom*
MODERATOR: Wendell G. Yarbrough, MD, MMHC, FACS

SPEAKERS:
• David M. Neskey, MD
• Adel K. El-Naggar, MD, PhD
• Benjamin Judson, MD

Biorepositories are critical for translational research, but there are several strategies for contributing or starting a repository. Understanding roadblocks and solutions based on the experience of others will be emphasized.

At the conclusion of this session, participants will be able to:
1. Understand some roadblocks to starting a Biorepository.
2. Understand how to start a Biorepository with different levels of resources available.
3. Understand how they can contribute to and benefit from biorepositories.

SCIENTIFIC PROGRAM

1:30 PM - 3:00 PM   **CONCURRENT SESSIONS**

Constitution Ballroom

Breakthroughs – Paradigm Changes New Thoughts, HNSCC & HPV CO-CHAIRS: James Rocco and Eduardo Mendez, MD

This session will focus on the major breakthroughs that are likely to lead to paradigm shits on how we treat head and neck cancer. Specifically, this session will focus on new insights into and novel therapeutic approaches for two major issues: 1) TP53 biology as a driver of head and neck cancer and 2) intratumor clonal heterogeneity.

At the conclusion of this session, participants will be able to:
1. The attendees will understand the role of TP53 tumor suppressor loss as a biomarker of treatment response.
2. The attendees will evaluate novel therapeutic approaches for p53 mutated head and neck cancers.
3. The attendees will gain insight into the treatment challenges posed by intratumor clonal heterogeneity and how this feature can be used to predict outcomes to treatment.

1:30 PM   **Introduction**
James W. Rocco MD, PhD and Eduardo Mendez MD

1:35 PM   **P53 as a Predictive Biomarker in the Treatment of HNSCC**
Jeffrey N. Myers MD,PhD

1:55 PM   **Increased Mortality in Head and Neck Cancer Patients with High Intra-Tumor Genetic Heterogeneity**
James W. Rocco MD, PhD

2:15 PM   **Functional Genomics Screening of Protein Kinases Identifies WEE1 as a Therapeutic Target in p53-mutated Head and Neck Cancer**
Eduardo Mendez, MD

2:35 PM   **Q&A**

2:40 PM   **S009: Intra-Tumoural Heterogeneity of P53 Expression in Squamous Cell Carcinoma of the Head and Neck**
Sankalap Tandon, FRCS

2:50 PM   **S008: A Regimen Combining the WEE-1 Inhibitor, AZD-1775 with the HDAC Inhibitor, Vorinostat is Highly Active Against Head and Neck Squamous Cell Carcinoma Harboring P53 Mutations**
Natlie Silver, MD, MS

Republic Ballroom

Maximizing Function After Treatment of HN Cancer CHAIR: M. Boyd Gillespie, MD, Msc

Head and neck cancer survivors face numerous disease and treatment-related side-effects. This session will focus on the latest research to improve outcomes and quality of life in head and neck cancer survivors.

At the conclusion of this session, participants will be able to:
1. Describe recent research to improve swallowing after head and neck cancer treatment.
2. Gain insight into ancillary specialists who can improve the function and quality of life in head and neck survivors.
3. Describe common side-effects of head and neck cancer and its treatment and ways to manage these issues.

1:30 PM   **Introduction**
M. Boyd Gillespie, MD, MSc

1:35 PM   **Swallowing Evaluation in the Head and Neck Cancer Patient**
Bonnie Martin-Harris, PhD

1:50 PM   **S010: The Relationship Between Post-Laryngectomy Speech Outcomes and Communicative Participation**
Tayna L. Eadie, PhD

1:56 PM   **Rehabilitation of Late Radiation Toxicity**
Kate Hutcheson, MD

2:11 PM   **Q&A**

2:15 PM   **Treatment of Head and Neck Lymphedema**
Jan S. Lewin, PhD

2:27 PM   **S012: The Role of Adipose Derived Stromal Cells for Reversal of Radiation Fibrosis**
Xiao Zhao, MD

2:33 PM   **Facial Nerve Rehabilitation after Head and Neck Cancer**
Caroline Banks, MD

2:45 PM   **Q&A and Wrap-up**

3:00 PM - 3:30 PM   **Coffee Break**   *Back Bay Ballroom*

SCIENTIFIC PROGRAM

3:30 PM - 4:50 PM **STANDALONE SESSION: Head and Neck Cancer Genetics – Sequencing, Pathways and Targets** *Constitution Ballroom*
CHAIR: Nishant Agarwal, MD, FACS

Comprehensive sequencing has revealed the genomic landscapes of cancer, including head and neck cancers. The mutations identified can generally be classified in core cellular pathways and may help the development of targets to reduce morbidity and mortality from head and neck cancer.

At the conclusion of this session, participants will be able to:
1. Identify the most common genetic alterations in head and neck cancers.
2. Outline the resulting implications for therapy and personalized medicine.
3. Integrate the genomic data to design fundamental, translational, and clinical research.

3:30 PM **Introduction** – *Nishant Agarwal, MD, FACS*

3:35 PM **Clinical Targets from Sequencing Projects** – *Neil Hayes*

3:46 PM **Sequencing Aggressive Cutaneous Squamous Cell Carcinoma: Insights into SCC Biology** – *Curtis Pickering*

3:57 PM **SMAC-Mimetic Birinapant and Radiation Eradicates Human Head and Neck Cancer Xenografts with Genomic Alterations in Cell Death Pathways** – *Carter Van Waes, MD, PhD*

4:08 PM **Immunogenomic Approaches in Head and Neck Cancer** – *Ravi Uppaluri, MD, PhD*

4:19 PM **Clonal Architecture and Evolution in Head and Neck Tumors** – *Luc G.T. Morris, MD, MSc*

4:30 PM **S015: Characterization of Novel Gene Fusions in Human Papillomavirus (HPV) Related Oropharyngeal Squamous Cell Carcinoma** – *Theresa Guo, MD*

4:38 PM **Q&A**

5:00 PM - 6:00 PM **Welcome Reception** *Back Bay Ballroom*
Supported in part by our Visibility Donors: Medrobotics Corporation and IRX Therapeutics, Inc.

SCIENTIFIC PROGRAM

WEDNESDAY, APRIL 22, 2015

10:30 AM - 11:15 AM **KEYNOTE LECTURE: "Deep Sequencing Profiling and Impact on Therapy"** *Constitution Ballroom*
INTRODUCTION: Wendell G. Yarbrough, MD, MMHC, FACS
KEYNOTE SPEAKER: Neil Hayes, MD, MPH

11:15 AM - 12:15 PM **CONCURRENT SESSIONS**

Constitution Ballroom

Tumor Imaging
CHAIR: Eben L. Rosenthal, MD

The use of optical imaging for detection of cancer during surgical procedures both preclinical and clinical studies related to this novel field.

At the conclusion of this session, participants will be able to:
1. Understand the utility of optical imaging to improve subclinical detection of cancer.
2. Plan for future trials using optical imaging to detect cancer.
3. Develop an appreciation for the principles of optical contrast agents and their potential.

11:15 AM Introduction and Background to Optical Imaging
Eben L. Rosenthal, MD

11:22 AM Developments in Intraoperative CT Imaging
Hadi Seikaly, MD, FRCS

11:30 AM S016: Fluorescence Detection of Squamous Cell Carcinoma and Oral Dysplasia with Indocyanine Green-Labeled Chlorotoxin
Eduardo Mendez, MD, MPH

11:37 AM Commentary
by Baran D. Sumer, MD

11:40 AM Discussion

11:45 AM S017: In Vivo Fluorescence Immunohistochemistry: Localization of Fluorescently Labeled Cetuximab in Squamous Cell Carcinomas
Esther de Boer

11:52 AM Commentary
by Mark A.S. Varvares, MD

11:55 AM Discussion

12:00 PM S018: Wide-Field Targeted Fluorescence Guided Multiphoton Microscopy of Oral Epithelial Neoplasia in a Hamster Model of Oral Carcinogenesis
Rahul Pal, MS

12:07 PM Commentary
by D. Gregory Farwell, MD, FACS

12:10 PM Discussion

Republic Ballroom

Stem Cells in Cancer
CO-CHAIRS: Eduardo Mendez, MD and John Sunwoo, MD

This session will focus on the most relevant studies in stem cell biology as they relate to head and neck cancer. The session will highlight novel studies that seek to translate this knowledge into novel therapeutic strategies targeting head and neck cancer stem cells.

At the conclusion of this session, participants will be able to:
1. Identify different phenotypes of cancer stem cell populations in head and neck cancer.
2. Evaluate mechanisms of invasion, metastasis and immunosuppression in head and neck cancer stem cells.
3. Appraise novel therapeutic strategies to target head and neck cancer stem cells.

11:15 AM Cancer Stem Cell Heterogeneity
Quintin Pan

11:25 AM A G0-like Cell State as a Barrier to HNSCC Stem Cell Eradication
Devraj Basu, MD, PhD

11:35 AM S019: CD271 Modulates the Invasive and Metastatic Phenotype of Head and Neck Squamous Cell Carcinoma Tumor-Initiating Cells Through the Upregulation of Slug
Manki Chung, MD

11:40 AM Q&A

11:45 AM Head and Neck Cancer Stem Cells – Two Approaches to Targeted Therapy
Mark E.P. Prince, MD, FRCS

11:55 AM Immunosuppression by Head and Neck Cancer Stem Cells
John Sunwoo, MD

12:05 PM S020: Chemoprevention Efficacy of Curcumin and Metformin Effected Through the Down-Regulation of Cancer Stem Cells
Moni A. Kuriakose, MS, FRCS

12:10 PM Q&A

12:15 PM - 1:15 PM **Lunch on Own**

SCIENTIFIC PROGRAM

1:15 PM - 2:30 PM **CONCURRENT SESSIONS**

Constitution Ballroom

Advancing Cancer Therapy Best Practices Through Outcomes Research
CO-CHAIRS: Benjamin Judson, MD and Christine Gourin, MD

This session will cover the current state of outcomes research in HNCA and clinical applications.

At the conclusion of this session, participants will be able to:
1. Understand how outcomes research can be used to influence clinical practice and policy.
2. Discriminate between guidelines and measures.
3. Implement both guidelines and measures into clinical practice.

1:15 PM **How Outcomes Influence HNCA Policy and Practice – the UK Example**
Nigel J. Beasley, FRCS, MBBS

1:30 PM **Using Outcomes Research to Develop HNCA – Specific NQMC Measures**
Christine G. Gourin, MD

1:45 PM **How the AHNS and AAOHNSF Develop HNCA Guidelines**
Amy C. Hessel, MD

2:00 PM **Using Outcomes Research to Influence HNCA Policy and Practice- the Canadian Example**
Jonathan Irish, MD, FACS

2:15 PM **A Tumor Board Quality Check-List as a Tool for Quality Measurement**
Brian Nussenbaum, MD

Republic Ballroom

Surgical Advances
CHAIR: Adam Zanation, MD

A panel on novel surgical technology and application.

At the conclusion of this session, participants will be able to:
1. Adjust practice to allow for better minimal access surgery.
2. Prioritize methods for controlling bleeding in a small field.

1:15 PM **Current Generation Head and Neck Robotic Platforms and Surgery**
Chris Holsinger, MD

1:26 PM **Next Generation Head and Neck Robotics**
Umamaheswar Duvvuri, MD, PhD

1:37 PM **The Future of Sialoendoscopy for Cancer Care**
Trevor Hackman, MD

1:48 PM **Updates on New Energy Technologies for Hemostasis**
Adam Zanation, MD

1:59 PM **The OR of the Future**
Alex Langerman

2:10 PM **Panel Discussion: Focusing on Health Care Cost and New Technology**
Lead by Adam Zanation, MD

2:30 PM - 3:00 PM **Coffee Break**   *Back Bay Ballroom*

SCIENTIFIC PROGRAM

3:00 PM - 4:30 PM   **CONCURRENT SESSIONS**

Constitution Ballroom

Personalized Patient Care – How Our Institution Does It
CO-CHAIRS: James Rocco, MD, PhD and Joseph A. Califano, MD, FACS

This session will address the impact of personalized therapy in the treatment of head and neck cancer. Presentations will define the role of personalized therapy related to carcinogenesis, development of prognostic markers and markers for of therapeutic approaches to head and neck cancer.

At the conclusion of this session, participants will be able to:
1. Understand and apply personalized therapy approaches to head and neck cancer.
2. Understand the investigational requirements and knowledge gaps in development of personalized therapeutic approaches.

3:00 PM   **Introduction**
           Joseph A. Califano, MD, FACS and James Rocco, MD, PhD

3:00 PM   **S021: Molecular Screening for Cancer Treatment Optimization in Head and Neck Cancer (MOSCATO 01): A Prospective Molecular Triage Trial; Interim Analysis of 78 Patients with Recurrent or Metastatic Head and Neck Cancers**
           Ingrid Breuskin

3:10 PM   **Defining Risk in HPV-Associated Oropharynx Cancer**
           Barbara A. Burtness, MD

3:40 PM   **Q&A**

3:45 PM   **S022: Targeted Sequencing of an Epidemiologically Low Risk Patient Defines Fibroblast Growth Factor Family Aberrations as a Driver of Head and Neck Squamous Cell Carcinoma**
           Brittny N. Tillman, MD

3:55 PM   **Targeting the PI3K/Akt/mTOR/eIF4E Axis in HNSCC**
           Matt Fury

4:25 PM   **Q&A**

Republic Ballroom

Best Posters
CHAIR: Benajmin Judson, MD

This session will highlight the several posters of the meeting. Each presenter will give a short 3 minute talk regarding their presentation and will then be able to answer any questions attendees may have.

P013: Restoration of MIR-124 Reduces EGFR Levels and Potentiates the Anti-Tumor Activity of EGFR/HER2 Tyrosine Kinase Inhibitors
Xiujie Xie, PhD

P015: Treatment Variation for Nasopharyngeal Carcinoma: Impact on Survival
Zachary Schwam, BA

P021: Molecular Subtype Dictates Hypersensitivity To ERBB3 Inhibition in HNSCC
Loren Miche, MD

P022: Surgical Salvage Improves Overall Survival for HPV-Positive and HPV-Negative Recurrent Locoregional and Distant Metastatic Oropharyngeal Cancer
Theresa Guo, MD

P030: Tumour Metabolism in Squamous Cell Carcinoma of the Head and Neck: An In-Vitro Study of the Consequences of TP53 Mutation and Therapeutic Implications
Mark D. Wilkie, MD

P038: A Growth Factor Loaded Modular Hydrogel System Supports Stable Vascular Networks in a Rodent Parotid Gland Resection Model
Robert L. Witt, MD

P051: Modulation of Tumor Growth, Vascularity and Inflammatory Cell Infiltration Following PI3K/mTOR and MAPK Targeted Therapy in a Syngeneic Model of Oral Cavity Cancer
Andria Caruso, MD

SCIENTIFIC PROGRAM

4:30 PM - 6:00 PM   **CONCURRENT SESSIONS**

Constitution Ballroom

Late Breaking Hot Topics
CHAIR: Eduardo Mendez, MD

The Late-Breaking Session will focus on highlighting studies with novel findings worthy of oral presentations either from data finalized after the original abstract deadline or selected by the organizer as late-breaking in the field of translational research. The session will highlight work from four invited faculty (including the organizer's own work) and three abstracts covering late-breaking findings in stem cell biology, immunology, genomics, targeted therapies and robotics.

At the conclusion of this session, participants will be able to:
1. Appraise the most novel late breaking findings in the field of stem cell biology, immunology, genomics, targeted therapies and robotics as they relate to head and neck cancer.
2. Identify future potential treatment options for head and neck cancer patients.
3. Gain understanding of how translational research can be applied to tackle challenging clinical problems in head and neck cancer.

4:30 PM   **Introduction**
Eduardo Méndez, MD

4:35 PM   **Novel Immunosuppressive Mechanisms in the Tumor Microenvironment and Reversal by Therapeutic mAb**
Robert L. Ferris, MD, PhD

4:50 PM   **S023: Cyclic Dinucleotide, A Novel Adjuvant for Squamous Cell Carcinoma**
Shekhar K. Gadkaree, MD

5:00 PM   **Complex Role of PAPR-1 in HPV-associated Head and Neck Cancer**
Natalia Issaeva, PhD

5:15 PM   **Neural Stem Properties of Tumor-Initiating Cells in Adenoid Cystic Carcinoma**
Sergey Ivanov, PhD

5:30 PM   **Translational Functional Genomics Initiative Towards Precision Oncology**
Eduardo Méndez, MD, MS

5:40 PM   **S024: Mutational Landscapes of Oral Tongue Squamous Cell Carcinoma Reveal Recurrent Mutations in Genes of Therapeutic and Prognostic Relevance**
Gopal Iyer, MD, PhD

5:50 PM   **S025: Next-Generation Robotic Head and Neck Surgery: A Novel Single-Arm, Flexible Robotic Surgical System for Transoral Resection of the Tonsillar Fossa and Lateral Oropharyngeal Wall**
Michelle Chen, MD

Republic Ballroom

Advances Through Recent Cooperative Group and SPORE Trials
CHAIR: Chris Holsinger, MD

Over the last decade, substantial progress has been made in head and neck oncology from prospective clinical trials and translational research. Cooperative groups funded by the National Cancer Institute as well the Specialized Programs of Research Excellence (SPORE) have played a central role.

At the conclusion of this session, participants will be able to:
1. Understand the impact of prospective clinical trials research on head and neck oncology.
2. Describe new and innovative approaches to clinical research to improve outcomes in head and neck oncology.
3. Describe how functional outcomes may play a role in future clinical trials and patient care in head and neck oncology.

4:30 PM   **Introduction** – Chris Holsinger, MD

Part I: Cooperative Group Clinical Trials

4:35 PM   **ECOG Cooperative Group Trials in H&N Oncology, Engaging the Whole Multidisciplinary Team, Past Progress and Future Challenges**
Barbara A. Burtness, MD

4:50 PM   **Engaging Surgeons in Cooperative Group Trials: The RTOG Experience**
Erich M. Sturgis, MD MPH

Part II: H&N SPORE Clinical Trials

5:05 PM   **Johns Hopkins School of Medicine: Bi-functional Antibody-Based Strategies To Optimize Treatment of H&N Cancer: EGFR and TGF- in the Tumor Microenvironment**
Atul Bedi, PhD

5:15 PM   **University of Michigan: Targeting Cancer Stem Cells – Cancer Vaccines Revisited**
Mark E.P. Price, MD, FRCS

5:25 PM   **University of Pittsburgh: Neoadjuvant Window Trials: Using Surgery to Reveal Biological Basis of H&N Cancer**
Robert L. Ferris, MD, PhD

5:35 PM   **University of Texas MD Anderson Cancer Center: Assessing Function in H&N SPORE-Funded and Cooperative Group Clinical Trials**
PANEL DISCUSSION: *Jan S. Lewin, PhD; Kate Hutcheson, PhD; University of Texas M.D. Anderson Cancer Center, Houston, Tx, and Heather Starmer, MS-SLP, Stanford University*

6:00 PM - 7:00 PM   **Poster Event Reception**
Social Event supported in part by our Visibility Donors: Medrobotics Corporation and IRX Therapeutics

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D. Gregory Farwell	AO-ASIF Intuitive Surgical	Honoraria RO1 funding with Intuitive Surgical as CoPI	Speaking/Teaching Grant	Faculty
Robert L. Ferris	Bristol Myers-Squibb VentiRX	Research Research	Contracted research PI for research grant	Faculty
Matt Fury	Novartis Novartis Regeneron	Honoraria Research spouse holds employee stock	Advisory Committee investigator spouse holds employee stock	Faculty
M. Boyd Gillespie	Inspire Medtronic Olympus Surgical Specialties	Consulting Fee, Research Support Consulting Fee Consulting Fee, Research Support Consulting Fee, Research Support	Sleep Apnea research Electrosurgical Device Development Sleep Apnea research Sleep Apnea research	Planning Committee, Faculty
Arjun Joshi	Cook Medical	Consulting Fee	Consultant	Program Committee
Stephen Y. Lai	Navidea	Consulting Fee	Medical Affairs Consultant	Program Committee
Bonnie Martin-Harris	Bracco Diagnostics NIH/NIDCD Northern Speech Services Veterans Administration	Unrestricted educational grant Research Royalty Grant	Researcher Researcher Speaking/Teaching Researcher	Faculty
Cherie-Ann Nathan	LSU Health LSU Health Shreveport Novartis	Patient Intellectual Property Rights Royalty Sponsored Grants	Invention Provisional Patent co-PI	Program Committee, Presenter, Faculty
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Baran D. Sumer	OncoNano Medicine Inc	Stocks	Founder	CME Committee, Faculty

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Lori Wirth	Astra Zeneca Eisai	Consulting Fee Consulting Fee	Consultant Advisory Committee	Faculty
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ORAL PAPERS

CONCURRENT SESSION: SALIVARY CANCER – WHAT'S NEW?

S001: APPLICATION OF PRIMARY CULTURE OF SALIVARY GLAND BENIGN AND MALIGNANT TUMORS UNDER CONDITIONAL REPROGRAMMING CONDITIONS FOR DEFINITION OF TUMOR-ASSOCIATED GENETIC CHANGES – <u>Priscilla A Furth, MD</u>[1], Xuefeng Liu, MD[1], Ahmad Alamri, MS[1], Bhaskar V Kallakury, MD[2], Kenneth Newkirk, MD[2], Bruce Davidson, MD[2]; [1]Georgetown University, [2]Georgetown University and MedStar Georgetown University Hospital

INTRODUCTION: There are few existing salivary tumor cell lines and genetic characterization of salivary pathology is limited by the diverse pathology and relative rarity of these tumors. We tested an approach for genetic profiling of tissues obtained from surgical or biopsy specimens. This utilized selective primary culture of epithelial cells using conditionally reprogramming (F medium containing the Rho Kinase (ROCK) inhibitor Y-27632 in the presence of irradiated Swiss 3T3-J2 mouse fibroblasts feeder cells) followed by separation of human cells from murine feeders for transcriptome characterization and exome sequencing.

METHODS: 15 patients with surgical indications for salivary gland resection or biopsy (female n=7; male n=8; age range: 31-80 years) were enrolled after informed consent by our Nontherapeutic Subject Registry Shared Resource. Deidentified samples were bisected and 'mirror image' histology was read to verify pathology of the 'mirrored' half, which was processed for culture. Samples included 1 matched mucoepidermoid carcinoma (ca)/normal; 1 matched metastatic squamous cell ca/normal; 1 matched lymphoma/normal; 4 matched pleomorphic adenoma/normal; 1 primary squamous cell ca; 1 metastatic squamous cell ca; 1 benign pleomorphic adenoma; 1 squamous metaplasia; 2 sialoadenitis; 1 normal specimen from a patient with a history of adenocystic ca that was insufficient material for culture) and 1 fine needle aspirate, n=3 samples from the same lesion, malignant pleomorphic adenoma. Deidentified pathology reports, paraffin-fixed sections of original tumors and frozen tissue (when sample sufficiently large) were also obtained. DNA and RNA were isolated for sequencing following separation of cultured human from murine feeder cells. Three paired normal/tumor samples and the two different aspirate site cultures were subjected to exome sequencing for comparison between normal and tumor samples and reproducibility between biopsy sites. Single nucleotide polymorphisms (SNPs) and insertion or deletion of bases in DNA (indels) were detected by SAMTOOLS (variant database: dbSNP & 1000G) after mapping to UCSChg19. RNAseq was performed on all specimens to assess similarities/differences between cells cultured from normal and tumor tissue, and between different metastatic squamous cell ca and pleomorphic adenoma samples (analysis in progress).

RESULTS TO DATE: Cultures grew from all but the 2 sialoadenitis samples and 1 of 3 needle aspirates (88% success). Viable frozen pellets were prepared from each matched tumor/normal n=7 (mucoepidermoid n=1, benign pleomorphic adenoma n=4, metastatic squamous n=1, lymphoma n=1) and disease only n=5 (malignant pleomorphic adenoma n=1, benign pleomorphic adenoma n=1, primary squamous n=1, metastatic squamous n=1, squamous metaplasia n=1) samples. Cells were regrown from pellets for DNA/RNA sequencing. From exome sequencing data SNPs defined as having clinical non-pathogenic, pathogenic or probable pathogenic significance (http://www.ncbi.nlm.nih.gov/projects/SNP/docs/rs_attributes.html) were analyzed. ~98% of these SNPs were identical between matched normal/tumor samples and two biopsy sites. No defined cancer-causing SNPs were found. At least three SNPs considered in the literature as possibly cancer-related were identified in each paired sample.

CONCLUSIONS: Application of conditional reprogramming culture for collection and genetic characterization was successful for malignant and benign salivary tumors. The technique was also successful with fine needle aspirates. Validation to original tissue is a next step.

S002: GENERATION AND CHARACTERIZATION OF ADENOID CYSTIC CARCINOMA CULTURES AND CELL LINES – Alex Panaccione, BS[1], <u>Michael Chang, BS</u>[2], Bea Carbone, BA[2], Manju Prasad, MD[2], Gary Bellinger, BS[2], Sergey Ivanov, PhD[2], Wendell Yarbrough, MD, MMHC, FACS[2]; [1]Vanderbilt University, [2]Yale University School of Medicine

Salivary gland adenoid cystic carcinoma (ACC) is a slow-growing but often fatal tumor due to its propensity for perineural invasion and distant metastases along nerves to the lungs and brain. Current treatment options are limited to surgery with or without radiation; however, with a lack of targeted therapies-owing to its orphan status and limited molecular insight into its etiology- ACC continues to exhibit high levels of recurrence and metastasis. As recent as 2009, cell lines for ACC were revealed to be contaminated or misidentified leaving the ACC field with a dearth of tools available for improving clinical outcomes of ACC. Here, we utilized a highly efficient ROCK-inhibitor-based cell culture approach to generate several ACC cell lines and short-term cultures from ACC xenografts and primary tumors. Confirmation of cell line identity began with microsatellite analysis of eight short tandem repeats (STRs) to validate the origin of primary cultures as derivatives of parental tumors. As further evidence of derivation, cultures retained expression of at least some markers characteristic for ACC. Finally, a subset of cultures exhibited tumorigenicity when injected into immunodeficient mice. Tumors formed from injected cells recapitulated the morphology and protein expression patterns of the parental tumors. Cell lines and derived xenografts provide an invaluable tool for the study of ACC and open new avenues for research.

CONCURRENT SESSION: IMMUNOMODULATION

S003: ALTERED EXPRESSION OF PROGRAMMED DEATH LIGAND IN THE TUMOR MICROENVIRONMENT OF ORAL CAVITY CANCER FOLLOWING PI3K/MTOR AND MAPK TARGETED THERAPY – Andria Caruso, MD[1], <u>Harrison Cash, BS</u>[2], Sujay Shah, BS[3], Carter Van Waes, MD, PhD[3], Clint Allen, MD[4];[1]Walter Reed National Military Medical Center, [2]Medical Research Scholars Program, National Institutes of Health, [3]National Institutes of Health, [4]Department of Otolaryngology-Head and Neck Surgery, Johns Hopkins School of Medicine

INTRODUCTION: Emerging evidence indicates a critical role for both the phosphoinositide-3-kinase (PI3K)/mammalian target of rapamycin (mTOR) and mitogen associated protein kinase (MAPK) pathways in the pathogenesis and progression of head and neck squamous cell carcinoma (HNSCC). Combining PI3K/mTOR and MAPK pathway targeting therapies results in synergistic tumor growth inhibition and prevents therapeutic resistance observed with inhibition of either pathway alone. Understanding how these targeted therapies alter programmed death ligand (PD-L) expression in the tumor microenvironment is critical for the rational combination of targeted and immune checkpoint antibody therapies. Here, we characterize baseline PD-L expression on different cellular subsets and how this expression is altered following mTOR inhibition with rapamycin and MAPK inhibition with the MEK1/2 inhibitor PD325901 (PD901) in a syngeneic murine model of oral cavity cancer.

METHODS: Ras-mutant mouse oral cancer (MOC) cells demonstrating a co-activated PI3K/mTOR pathway were transplanted into immunocompetent C57BL/6 mice. Mice were treated with systemic rapamycin (1.5 mg/kg QOD), PD901 (1.5 mg/kg QD), combination or control for 21 days following primary tumor growth to 0.1 cm3. At the end of the treatment period, control and treated mice were euthanized, tumor harvested and processed, and PD-L1/2 expression was characterized on CD45-CD31- MOC tumor cells, CD45-CD31+ endothelial cells, and tumor-infiltrating CD45+ hematopoietic cells including Gr1+CD11b+ myeloid derived suppressor cells (MDSCs), CD11b+Ly6ClowF4/80+ macrophages (TAMs) and CD4+Foxp3+ regulatory T-cells (Tregs) using flow cytometric analysis. Results analyzed are from 7 individual tumors for each treatment condition in two biological replicate experiments.

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RESULTS: PD-L1 was expressed on 62.2% MOC tumor cells with an average mean fluorescence intensity (MFI) of 450 at baseline. Tumor cell PD-L1 expression could be stratified by CD44 expression (p<0.001) suggesting co-activation. Further characterization revealed PD-L1 expression on 24.4% of endothelial cells (avg MFI 208), 43.5% of MDSCs (avg MFI 614), 90.7% of TAMs (avg MFI 4167) and 58.6% of Tregs (avg MFI 330). PD-L2 was expressed on less than 5% of all cell subsets analyzed. Following targeted therapy in-vivo, MEK inhibition alone resulted in a significant decrease in PD-L1 expression on MOC tumor cells (p<0.001), MDSCs (p<0.05), TAMs (p<0.01) and Tregs (p<0.001) but a significant increase in tumor endothelial cell PD-L1 expression (p<0.05). Rapamycin alone appears to induce little change in PD-L1, but produces additive effects when combined with MEK inhibition to enhance or suppress PD-L1 expression in different cell subsets (p<0.001 in MOC tumor cells, TAMs, Tregs and endothelial cells, p<0.01 in MDSCs).

CONCLUSIONS: PD-L1 but not PD-L2 was expressed at baseline in varying degrees on all tumor cell subsets analyzed. MEK and mTOR targeted inhibition variably alters PD-L1 expression in-vivo. Interestingly, while targeted therapy reduced PD-L1 expression on most cell subsets analyzed, PD-L1 expression on endothelial cells was enhanced. Knowledge of how different targeted and standard cytotoxic anti-cancer treatments alter checkpoint inhibitor expression and validation of this murine data in human clinical trial samples will be critical for the rational design of combination targeted and immune checkpoint antibody-based immunotherapies.

S004: PD-1 BLOCKADE SYNERGIZES WITH CISPLATIN RADIATION THERAPY AIDING IN CLEARANCE OF HPV+ OROPHARYNGEAL CARCINOMA – Daniel W Vermeer, BS, Steve Powell, MD, William C Spanos, MD, John H Lee, MD; Sanford Research

Squamous cell carcinomas of the head and neck (SCCHN) are the 6th most common malignancy world-wide with approximately 650,000 diagnoses per year. Human papillomavirus (HPV) causes up to 80% of oropharyngeal (tonsil/base of tongue) SCCHN. Despite presenting at an advanced stage, multiple studies show that HPV+ SCCHN are more curable relative to their HPV- counterparts. Clearance of SCCHN during treatment with cisplatin radiation therapy (CRT) is not only related to direct cytotoxicity, but is also dependent on immune mediated clearance. This immune clearance is strongly dependent on an intact CD4+ and CD8+ cellular response to tumor cells harboring these viral oncogenes. Furthermore, the immune system recognizes and clears HPV + SCCHN only after the start of CRT. The immunologic tolerance to these cancers prior to treatment could be explained by immune checkpoints aimed at preventing unregulated immune activity. The programmed death 1 receptor (PD-1) and its ligand, PD-L1, are one such checkpoint axis. Recent data suggests that the PD-1:PD-L1 pathway may play an important role in immune resistance in head and neck cancer. We evaluated PD-1 in combination with CRT in an immune competent HPV+ SCCHN mouse model. HPV + mouse SCCHN cells were injected subcutaneously into immune competent mice and tumors were treated after growing to palpable size. Using the mouse analog to the human PD-1 blocker pembrolizumab, we determined that while single-agent therapy does not have significant activity on primary disease or distant metastasis, PD-1 blockade strongly synergizes with CRT. An overall survival of 58% was evident in mice treated with combination PD-1 and CRT versus 8% in mice treated with isotype control (IgG1) in combination with CRT (p = 0.006). These studies provide the impetus for future work investigating less toxic induction of immune responses in combination with blockade of immune checkpoint pathways.

CONCURRENT SESSION: EPIGENETICS IN CANCER

S005: 5-AZACYTIDINE SELECTIVELY KILLS HPV-POSITIVE HEAD AND NECK CANCER CELLS VIA SIMULTANEOUS TARGETING OF MULTIPLE CANCER-RELATED PATHWAYS – Andrew B Sewell, MD, Asel Biktasova, MD, PhD, Michael Hajek, Cyril Gary, Gary Bellinger, Wendell G Yarbrough, MD, Natalia Issaeva, PhD; Yale University

Although human papilloma virus (HPV)-positive head and neck squamous cell carcinoma (HNSCC) patients have higher cure rates compared to HPV-negative HNSCC, lifelong side effects of currently used treatment are severe; in addition, upon recurrence, treatment options and survival are limited. Therefore, new therapies with better side effect profiles that can also offer therapeutic options for patients with recurrent HPV-associated HNSCC are urgently needed.

Recently, we found that HPV-positive head and neck cancer cell lines and primary cells were more sensitive than HPV-negative cells to the demethylating agent 5-azacytidine. Importantly, even low doses of 5-azacytidine delayed HPV-positive tumor growth and prevented metastatic cell spread in mouse xenograft model. Cancer-related pathways that we found to be altered in HPV-positive, but not in HPV-negative head and neck cancer cells, upon demethylation included: 1) restoration of tumor suppressor p53 levels and associated apoptotic activity; 2) activation of cytotoxic type I interferon response; and, 3) downregulation of matrix metalloproteinases (MMP1 and MMP10) expression. We determined detailed molecular mechanisms and consequences of each modulated pathway.

Demethylation as a therapy for HPV-associated HNSCC is particularly interesting, since simultaneous targeting of multiple molecular pathways that are important for cancer development and progression, may increase efficacy and reduce acquisition of resistance. Based on our data, a small clinical trial with 5-azacytide on patients with HNSC C was initiated at Yale Cancer Center.

S006: MGMT HYPERMETHYLATION AS A PROMISING MARKER FOR AST1306 THERAPY RESPONSE – Lidia M R B Arantes, PhD, Renato J S Oliveira, MsC, Ana Carolina de Carvalho, PhD, Matias E Melendez, PhD, Rui M Reis, PhD, André L Carvalho, MD, PhD; Molecular Oncology Research Center, Barretos Cancer Hospital – Pio XII, Barretos – SP, Brazil

BACKGROUND: Over the last years, diagnosis and management of head and neck cancer (HNSCC) patients have improved through combined efforts in surgery, radiotherapy and chemotherapy, but long-term survival rates have improved only marginally, and the overall 5-year survival rate is around 50%. Late diagnosis and frequent loco-regional recurrences are the major causes for the poor prognosis. Therapies targeting inhibition of multiple points along signal transduction pathways are potential new approaches in the treatment of cancer. Overexpression of epidermal growth factor receptor (*EGFR*), a transmembrane tyrosine kinase growth factor receptor, is found in more than 90% of HNSCC tumors and anti-*EGFR* therapy has shown to be effective against HNSCC. AST1306 is a novel anilino-quinazoline compound, which irreversibly inhibits the enzymatic activities of wild-type *EGFR*. Hypermethylation in the promoter regions of genes is associated with suppression of gene expression and has been considered a potential molecular marker for several tumor types, including HNSCC. O6-methylguanine-DNA methyltransferase (*MGMT*) is a DNA-repair protein that protects glioblastoma tumor cells against alkylating agents including temozolomide (TMZ) by removing alkyl adducts from the O6-position of guanine. Several studies have demonstrated that epigenetic silencing of *MGMT* gene by promoter methylation was of predictive significance for prolonged survival to the combination of TMZ and radiotherapy in glioblastoma patients.

OBJECTIVES: To evaluate the methylation status of *DCC*, *MGMT* and *p16* genes in HNSCC cell lines and associate the molecular findings with the response profile to AST1306.

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MATERIALS & METHODS: Six HNSCC cell lines and A431 (control) were used to test the treatment efficacy of AST1306 by cell viability assays (MTS). Cells were seeded in 96 well plates, exposed to increasing doses of AST1306 (0 - 2.5 µM) for 72 hours. The methylation profile of *DCC*, *MGMT* and *p16* genes was assessed by Pyrosequencing in a PSQ96ID pyrosequencer.

RESULTS: To quantify the response to AST1306, according to the methylation status of *DCC*, *MGMT* and *p16*, areas under the curve (AUCs) were calculated for all cell lines. Interestingly, the *MGMT* hypermethylation status showed the highest level of sensitivity and specificity (AUC=1.000) in discriminating sensitive from resistant HNSCC cell lines to AST1306. The AUC levels for the other genes were 0.500 for *DCC* and 0.542 for *p16*.

CONCLUSION: *MGMT* hypermethylation in HNSCC cell lines predicts response to AST1306, irrespective of HPV status, showing its feasibility as a marker that can potentially be used in a clinical setting.

S007: DNA METHYLATION REGULATES TMEM16A EXPRESSION THROUGH ALTERNATE MECHANISMS AT TWO DISTINCT CPG ISLANDS – <u>Andrey Finegersh, PhD</u>, Scott Kulich, MD, PhD, Ronak Dixit, Umamaheswar Duvvuri, MD, PhD; University of Pittsburgh School of Medicine

RATIONALE: Mechanisms of head and neck squamous cell carcinoma (HNSCC) nodal metastasis are still poorly understood and contribute to tumor recurrence. TMEM16a is a calcium-activated chloride channel whose expression was recently found to act as a switch between tumor growth and metastasis in HNSCC. Given its importance for tumor progression, studying TMEM16a regulation may provide insights into epigenetic markers of HNSCC metastasis. We hypothesized that changes in DNA methylation of key CpG residues near the TMEM16a promoter underlie changes in gene expression in HNSCC.

METHODS: We analyzed all HNSCC samples from TCGA with available 450K DNA methylation array (Illumina) and expression data (n = 475). Using these samples, we studied 25 CpG's across two CpG islands annotated by the UCSC Genome Browser near the TMEM16a transcriptional start site. We calculated the Spearman rank correlation between DNA methylation and TMEM16a expression at each CpG and identified statistically significant relationships using a strict p-value cut-off (p < 0.001). The effect of HPV status on CpG methylation was assessed. We also assessed the effects of TMEM16a expression on patient survival.

RESULTS: TMEM16a expression was significantly correlated with patient survival. Of the 25 CpG's analyzed, 17 were significantly correlated with TMEM16a expression (p < 0.001) (10 negatively correlated; 7 positively correlated). 8/10 negatively correlated CpG's were within the first CpG island and 4/7 positively correlated CpG's were within the second CpG island. For samples with available HPV classification, HPV+ (n = 34) samples had significantly decreased expression of TMEM16a compared to HPV- samples (n = 241) (p < 0.01). There was a significant interaction between HPV status and DNA methylation across the 25 CpGs analyzed (F(25,6625) = 3.465, p < 0.001); Bonferroni post-hoc testing revealed that HPV+ samples had significantly decreased DNA methylation at positively correlated CpG's (cg23876072, cg24891434, cg25818697).

CONCLUSIONS: TMEM16a expression is a clinically significant marker of patient survival, indicating its regulation is important for tumorogenesis. DNA methylation appears to regulate TMEM16a expression by two distinct mechanisms – hypomethylation of CpG's within the first CpG island just before the transcriptional start site increases expression while hypomethylation of CpG's at a second, intergenic CpG island at exon III decreases expression. HPV+ tumors had hypomethylation at the second CpG island, which correlated with decreased TMEM16a expression and suggests a role for HPV infection in epigenetic modulation of TMEM16a. While the specific role of intergenic CpG's on gene expression is currently unknown, they may be an important consideration when treating with modulators of chromatin modifying enzymes. Studies are currently being planned to investigate specific mechanisms of DNA methylation changes at the TMEM16a promoter.

CONCURRENT SESSION: BREAKTHROUGHS – PARADIGM CHANGES NEW THOUGHTS, HNSCC & HPV

S008: A REGIMEN COMBINING THE WEE-1 INHIBITOR, AZD-1775 WITH THE HDAC INHIBITOR, VORINOSTAT IS HIGHLY ACTIVE AGAINST HEAD AND NECK SQUAMOUS CELL CARCINOMA HARBORING P53 MUTATIONS – <u>Natalie Silver, MD, MS</u>[1], Abdullah Osman, PhD[1], Ameeta Patel[1], Jiping Wang[1], Noriaki Tanaka[1], Mitchell Frederick, PhD[1], Faye Johnson, MD, PhD[2], Siqing Fu, MD, PhD[3], Jeffrey Myers, MD, PhD[1]; [1]UT MD Anderson Cancer Center-Department of Head and Neck Surgery, [2]UT MD Anderson Cancer Center-Department of Thoracic/Head and Neck Medical Oncology, [3]UT MD Anderson Cancer Center- Department of Investigational Cancer Therapeutics

INTRODUCTION: The cure rate for patients with advanced head and neck squamous cell carcinoma (HNSCC) remains in the 25-40% range due to resistance to standard therapy primarily consisting of chemoradiation. Since mutation of TP53 in HNSCC occurs in 60-80% of non-HPV associated cases and is in turn associated with resistance to these treatments, novel therapeutic approaches are needed to overcome drug resistance and improve survival outcomes in patients with advanced HNSCC. Wee-1 is a kinase that has been linked to DNA damage induced G2/M arrest, owing to its ability to inactivate cyclin dependent kinase 1 (CDK1) through phosphorylation of the Tyr15 residue. Our laboratory has shown that the Wee-1 kinase inhibitor, AZD-1775, sensitizes HNSCC cells harboring high risk p53 mutations to cytotoxic therapies both in vitro and in vivo. AZD-1775 is currently being evaluated in phase II clinical trials in several solid tumors including HNSCC. Vorinostat (SAHA) is a small molecule inhibitor of histone deacetylase (HDAC), which preferentially induces cancer cell growth arrest and differentiation, in cell culture and xenograft models. HDAC inhibitors increase the hyperacetylation status of histones which strongly affects the structure of the chromosome to increase Wee-1 expression while Cdc25C expression is decreased, resulting in arrest in G2 phase. Treatment with vorinostat shows preferential cytotoxicity for mutant p53 HNSCC cell lines and does not have an effect on wild-type and null p53 tumor cells. This finding supports the rationale to use vorinostat-based regimens for mutant p53-specific anticancer therapy. Therefore, it is of great interest to develop a therapeutic strategy to target histone deacetylase and a cell-cycle checkpoint regulator for achieving maximum synthetic lethality. In this study, we evaluated the efficacy of a regimen combining vorinostat and AZD-1775 or in combination with cisplatin in HNSCC cells with various p53 mutations.

MATERIALS & METHODS: Clonogenic survival assays were performed to examine in vitro sensitivity of several TP53 mutant head and neck cell lines after treatment with vorinostat and AZD-1775 with or without cisplatin. Cell cycle analysis and western blotting was performed to investigate cellular mechanisms.

RESULTS: Vorinostat synergized with AZD-1775 and cisplatin in vitro and reduced cell survival of mutant p53 HNSCC cells. Interestingly, addition of vorinostat had no effect on either AZD-1775 or cisplatin responses in the wild-type p53 HNSCC cells. It appears that the reduction in cell survival with vorinostat treatment is mediated through apoptosis. Treatment of HNSCC cells with vorinostat and AZD-1775 with or without cisplatin increased p21 induction independent of p53 expression. Evaluation of vorinostat and AZD-1775 efficacy with or without cisplatin in an orthotopic mouse model with p53 mutant HNSCC tumors is ongoing.

CONCLUSIONS: The data demonstrate that vorinostat enhances anti-tumor efficacy of cisplatin and when combined with AZD-1775 in preclinical models of mutant p53 HNSCC. This study is important because it demonstrates the potential for a therapeutic strategy aimed at targeting the G2/M checkpoint and histone deacetylation in tumors expressing mutant p53.

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S009: INTRA-TUMOURAL HETEROGENEITY OF P53 EXPRESSION IN SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK – <u>Sankalap Tandon</u>[1], Nazia Munir[1], Jeff Lancaster[1], Nick Roland[1], Shaun R Jackson[1], Tim Helliwell[2], Mark Boyd[3], Terry Jones[4]; [1]University Hospital Aintree, Liverpool, UK, [2]Royal Liverpool University Hospital, Liverpool, UK, [3]Institute of Translational Medicine, University of Liverpool, Liverpool, UK, [4]NWCR Centre, University of Liverpool, Liverpool, UK

INTRODUCTION: Variable responses to standard treatments in squamous cell carcinoma of the head and neck (SCCHN) are potentially a function of intra-tumour heterogeneity. This is relevant when treatment response is inferred from a single, small biopsy of the primary tumour, as there is inevitably a high probability of not detecting treatment resistant clones. A greater understanding of the extent of intra-tumoural heterogeneity of the p53 genotype in SCCHN is particularly pertinent.

AIMS: We aim to assess the inferred p53 status of specimens of SCCHN and to investigate the intra-tumour heterogeneity of p53 expression across whole tumour sections.

METHODS: Whole tissue sections of 50 primary squamous cell carcinomas of the larynx, oropharynx, and hypopharynx were assessed using standard immunohistochemical (IHC) techniques for p53, MDM2 and p21 expression. Eight high-powered fields (HPFs) of X150 magnification from the tumour centre and periphery were each scored for inferred p53 expression using a combinatorial analysis of the 3 markers.

Wild type = raised p53, raised MDM2, raised or low p21
Mutant = raised p53, low MDM2
Functionally inactive p53 = low p53, raised MDM2
Deletion/nonsense = absent p53, absent MDM2, raised or low p21

The degree of heterogeneity of the inferred p53 expression for each HPF across the whole sample was assessed and defined as:
1. As a difference in the pattern of nuclear counts between HPFs within individual tumour samples.
2. As a difference in the expression pattern and classification of individual HPFs as either p53 active (wild type); or p53 inactive (mutant, functionally inactive or deletion) in comparison with the remaining HPFs in the same tumour.

RESULTS: 50% of the specimens were classified as inferred wild type, 24% as inferred mutant, 20% were inferred functionally inactive and 6% were classified as inferred p53 deletion/nonsense mutation. Heterogeneity was seen in several situations. Two cases displayed heterogeneity at the whole slide level with the periphery being classified as a different inferred p53 status than the centre, 7 cases displayed heterogeneity between the four HPFs at the tumour centre, 9 cases displayed heterogeneity between the four HPFs at the tumour periphery whilst 8 cases displayed heterogeneity between the HPFs at both the tumour centre and periphery. A total of 58 HPFs displayed heterogeneity in comparison to the other HPFs assessed for that tumour sample. Heterogenous HPFs account for only 4.8% of all 1200 HPFs assessed over the 50 cases.

CONCLUSIONS: Based on our findings, the heterogeneity of inferred p53 status as determined by IHC expression for p53, MDM2 and p21 in SCCHN is small across whole tumour specimens, although the complexity of tumour development means that some degree of heterogeneity is inevitable. In order to investigate the expression of proteins involved in the p53 pathway in SCCHN, multiple biopsies of the tumour should be taken and assessed, rather than one or two samples alone. Research into the impact of solid tumour heterogeneity on survival is on going.

CONCURRENT SESSION: MAXIMIZING FUNCTION AFTER TREATMENT OF HN CANCER

S010: THE RELATIONSHIP BETWEEN POST-LARYNGECTOMY SPEECH OUTCOMES AND COMMUNICATIVE PARTICIPATION – <u>Tanya L Eadie, PhD, CCCSLP</u>[1], Devon Sawin, MS[1], Steven Cox, MS, CCCSLP[2], Carolyn Baylor, PhD, CCCSLP[1], Kathryn Yorkston, PhD, CCCSLP[1], Philip C Doyle, PhD[2];[1]University of Washington, [2]Western University

BACKGROUND: Total laryngectomy (TL) results in unique challenges to communication. Traditional methods of assessing speech and voice after TL include objective measures (e.g., acoustics), listener-ratings of speech intelligibility and acceptability, as well as patient-reported measures, such as voice-related quality of life scales. Recently, a patient-reported scale that measures communication in everyday contexts, or "communicative participation", was validated in head and neck cancer survivors. Consequently, we examined how this new tool, the Communicative Participation Item Bank (CPIB), relates to traditional post-laryngectomy speech and voice outcomes.

OBJECTIVE: To determine relationships between communicative participation with traditional post-laryngectomy speech and voice outcomes including: a) speech intelligibility; b) speech acceptability; and c) voice handicap.

METHODS: Thirty-six individuals (29 males, 7 females) who had undergone TL (n=11 electrolaryngeal speakers; n=23 tracheoesophageal speakers; n=2 esophageal speakers) completed patient-reported outcomes including the CPIB (Baylor et al., 2013) and the Voice Handicap Index-10 (VHI-10; Rosen et al., 2004). Individuals also recorded 6 sentences (5-15 words) from the Sentence Intelligibility Test (SIT; Yorkston et al., 1996) and a standard reading passage (Rainbow Passage; Fairbanks, 1960). Forty-eight inexperienced listeners each transcribed 6 sentences for 3 speakers using the SIT protocol. Each speaker's intelligibility was based on the average score across 3 listeners. Eighteen listeners also judged speech acceptability for the 36 speakers using 100 mm visual analog scales. Twenty percent of the samples were repeated to determine intra-rater reliability (r = .71); intraclass correlation coefficients were calculated as a measure of interrater reliability (ICC = .96, speech acceptability). To determine the relationships between the CPIB and speech and voice outcomes (VHI-10, speech intelligibility, and speech acceptability), correlational analyses were performed.

RESULTS: Listeners judged tracheoesophageal speakers significantly more intelligible and acceptable than electrolaryngeal speakers (p < .01). However, patient-reported outcomes such as voice handicap and communicative participation were not significantly differentiated by speaker type. In addition, listeners' ratings of speech acceptability were only moderately related to intelligibility (r = .43). Relationships were weak between ratings of speech and CPIB, with speech acceptability tending to be a stronger predictor than intelligibility (see Table 1). The strongest relationship was observed between the two patient-report measures; decreased voice handicap related to increased communicative participation (r = -.756).

TABLE 1: Correlations between ratings of post-laryngectomy speech outcomes with CPIB.

	Patient-Report	Listener-Rated	Listener-Rated
	VHI-10	Intelligibility (%)	Acceptability
CPIB	-0.756	0.055	0.293

CONCLUSIONS: Alaryngeal speakers exhibited a wide range of speech acceptability and intelligibility, although these dimensions were not strongly related. These results suggest that while listeners may be able to understand some alaryngeal speakers, being understood is not sufficient to render the speech "acceptable" to the listener. Results also revealed that listeners' judgments

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of speech are not necessarily predictive of the speakers' own perceptions of communication in everyday contexts. As hypothesized, the patient-reported measures (VHI-10; CPIB) were strongly related. Listener-rated and patient-reported measures are complementary after TL. Therefore, both should be adopted as part of an assessment battery.

S012: THE ROLE OF ADIPOSE DERIVED STROMAL CELLS FOR REVERSAL OF RADIATION FIBROSIS – Xiao Zhao, MD[1], Ju Hee Lee[2], Laurie Ailles, PhD[3], Kenneth Yip, PhD[4], Fei-Fei Liu, MD, FRCPC5; [1]Department of Otolaryngology, University of Toronto, [2]University of Waterloo, [3]Division of Stem Cell and Developmental Biology, Ontario Cancer Institute, [4]University of Toronto, [5]Department of Radiation Oncology, University of Toronto

INTRODUCTION: Up to 70% of patients after radiotherapy develop radiation fibrosis (RF), an irreversible scarring of normal tissue resulting in functional morbidity and increased risk of surgical complications. Adipose-derived stromal cells (ADSCs) have been used effectively for the treatment of complex wounds in animal and clinical trials. We hypothesize that ADSCs may reverse RF by repopulating mesenchymal precursor cells and by providing angiogenic, anti-inflammatory, and matrix remodeling factors.

METHODS: C3H mice were used to create an animal model of RF and for ADSC isolation/expansion. GFP and luciferase labelled ADSCs were used to assess biodistribution after transplantation. To determine the therapeutic effect of ADSC transplantation for RF, we assessed functional changes to leg contracture, oxygen saturation and perfusion and molecular changes to inflammation, vascularization, and matrix remodeling. To uncover the mechanism of ADSC-mediated fibrosis reversal, we performed RNA-seq and reduced representation bisulfite sequencing of RF tissue with and without ADSC transplantation.

RESULTS: A RF model was created by radiating the hind limb of C3H mice. This model showed a dose dependent leg contracture and histological findings of fibrosis. We confirmed the immunophenotype of isolated ADSC and their ability to differentiate into adipogenic, chondrogenic, and osteogenic lineages. ADSC transplantation showed a statistically significant improvement in leg contracture (2-way ANOVA, $p<0.05$). Biodistribution studies confirmed the presence of transplanted ADSCs in the subdermis of RF tissue with persistence for at least 18 days post-transplantation. Transcriptomic and methylation data analysis is underway.

CONCLUSIONS: ADSC transplantation may be an effective treatment for the reversal of radiation fibrosis. As cancer survivorship increases, the prevalence of radiation fibrosis may rise and necessitate an increased focus on effective treatment strategies for this condition.

S013: AN EXPLORATORY EVALUATION OF PERCEIVED BODY IMAGE AND QUALITY OF LIFE IN INDIVIDUALS WITH HEAD AND NECK CANCER – Melissa M Nash, BHSc, MSc[1], Kevin Fung, MD[2], S Danielle MacNeil, MD[2], Grace M Scott, BA[1], John H Yoo, MD[2], Philip C Doyle, PhD[1]; [1]Western University, [2]London Health Sciences Centre

BACKGROUND: One of the most distressing aspects of head and neck cancer (HNC) treatment is the possibility of disfigurement and its potential visibility to others. Such physical changes may directly impact one's perceived body image (BI) with a secondary potential to impact long-term functioning; hence, BI may be seen as a critical influence on quality of life (QOL). Optimized QOL is an identified priority following treatment, among individuals involved in the care of those with HNC. Therefore, identifying factors that may result in a reduction in QOL is essential as doing so has the potential to influence overall outcomes following treatment.

OBJECTIVE: This study sought to identify the potential influence of BI on QOL following treatment of HNC. The study further sought to determine the essential elements to identify body image disturbance among this clinical population.

METHODS: A cross sectional, self-report survey design was utilized. All participants completed four previously validated questionnaires including two related to QOL, the European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) and the site specific module for head and neck cancer (H&N35) and two related to body image, the Body Image Scale and the Body Image Disturbance Questionnaire.

RESULTS: Participants included 80 adults (45 men, 35 women) diagnosed and treated for HNC. Our data suggest that perceived changes in BI are highly variable and individualized secondary to treatment. Data indicate that 30% of both men and women demonstrated some level of concern relative to their perceived BI. This finding revealed concurrent reductions in perceived QOL However, deficits in perceived BI exist independent of clearly identifiable treatment related physical head/neck alteration. Further, the negative influence of reductions in BI directly impact perceived QOL.

CONCLUSIONS: The identification of disturbances in BI can be accomplished with minimal additional consultation or time demand. Thus, potential changes in BI should be monitored in all individuals treated for HNC, regardless of treatment modality. Because alterations in BI may be related in part to other more traditional indices of QOL (e.g., physical, emotional, social and role status concerns), comprehensive and ongoing clinical monitoring is warranted. Because disruption in BI may interfere with one's resumption of previous roles and routines post-treatment, the identification of such concerns and efforts to reduce its impact may serve to facilitate improved rehabilitation outcomes.

STANDALONE SESSION: HTS – DEEP SEQUENCING TARGET AND PATHWAYS

S015: CHARACTERIZATION OF NOVEL GENE FUSIONS IN HUMAN PAPILLOMAVIRUS (HPV) RELATED OROPHARYNGEAL SQUAMOUS CELL CARCINOMA – Theresa Guo, MD[1], Daria Gaykalova, PhD[1], Michael Considine, MS[2], Justin A Bishop, MD[3], Julie Ahn, MS[1], Samantha Gebhart, MS[1], Marla Goldsmith, MS[1], Zubair Khan, MD[1], William H Westra, MD[3], Wayne M Koch, MD[1], Elana Fertig, PhD[2], Joseph A Califano, MD[1]; [1]Department of Otolaryngology, Head and Neck Surgery, Johns Hopkins Medical Institutions, [2]Oncology Biostatistics and Bioinformatics, Sidney Kimmel Comprehensive Cancer, Johns Hopkins Medical, [3]Department of Pathology, Johns Hopkins Medical Institutions

INTRODUCTION: Gene fusions play a significant role in hematologic malignancies and have recently been recognized in solid tumors, such as the MYB-NFIB fusion in adenoid cystic carcinoma. In human papillomavirus (HPV) related head and neck squamous cell carcinoma (HNSCC), few targetable genetic alterations have been identified despite recent sequencing efforts. Thus we sought to use RNA sequencing technologies to discover novel gene fusions in HPV-related HNSCC.

METHODS: Primary tumor samples from 47 patients with HPV-positive oropharyngeal squamous cell carcinoma were collected as well as normal mucosa from 25 uvulopalatopharyngoplasty surgical specimens. HPV tumor status was defined by pathologic HPV in situ hybridization or p16 immunohistochemistry and confirmed with PCR. Tumor RNA was sequenced on Illumina TruSeq platform. MapSplice2 was used for RNA sequence alignment and identification of putative gene fusion candidates.

Gene fusion analysis was performed in R (version 3.1.1). We filtered out putative fusions with less than five spanning reads, fusions detected in normal tissues, and fusions mapping to the same gene. Gene fusions were confirmed using integrative genomics viewer (IGV, Broad Institute). Gene expression was calculated from RNA sequencing read counts and compared in samples containing relevant fusions. Genes associated with identified gene fusions were queried in Molecular Signatures Database to identify associated pathways (MSigDB v4.0, Broad Institute).

RESULTS: Within 47 HPV-related HNSCC tumors, 277 gene fusions were identified involving 349 unique genes. Of these, 220 were intrachromasomal and 57 were interchromasomal (Figure). The average number of fusions per tumor was 9.91 (range 0-34), and 93.6% of tumors harbored at least one fusion. Fusions occurred in 1 to 16 tumors, with a majority (83.7%) of fusions occurring in one tumor. Gene fusions previously reported in other cancers were also identified in our cohort including FGFR3-TACC3 and TFG-GPR128.

Gene expression was analyzed to evaluate whether gene fusions were associated with up-regulation or suppression. The ratio of median gene expression with and without a fusion showed that 49.2% of genes showed less than a 2 fold change in gene expression. Median ratio was 1.28 (fusion samples/non-fusion samples) with 66.4% of genes showing up-regulation and 33.6% of genes with down-regulation of expression. Of note, 12 genes showed significant up-regulation (greater than two standard deviations above the median) in association with a gene fusion, including EGFR, ERBB4 and GPR128. Pathway analysis with MutSigDB showed significant correlation of genes involved in gene fusions with gene sets associated with ERCC3 (DNA helicase involved in nucleotide excision repair) and nasopharyngeal carcinoma.
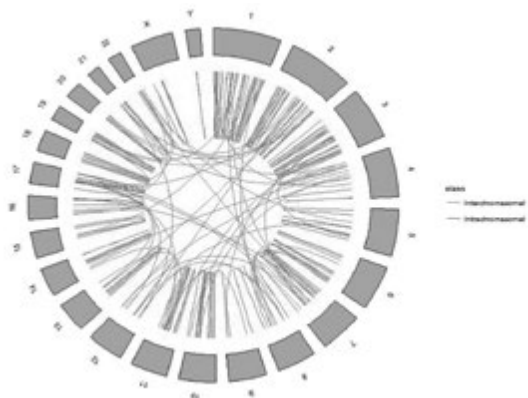
DISCUSSION: This study represents the first description of gene fusions specific for HPV-related HNSCC. Our fusion analysis identified the FGFR3-TACC3 gene fusion, which was also found within HPV-positive HNSCC in the TCGA cohort. Significant changes seen in gene expression suggest functional consequences of these gene fusions. Further studies will allow for elucidation of the potential functional implications of gene fusions in HPV-related disease.

CONCURRENT SESSION: TUMOR IMAGING

S016: FLUORESCENCE DETECTION OF SQUAMOUS CELL CARCINOMA AND ORAL DYSPLASIA WITH INDOCYANINE GREEN-LABELED CHLOROTOXIN – Fred M Baik[2], Chang Xu, PhD[1], Eduardo Mendez, MD, MPH[2]; [2]University of Washington Department of Otolaryngology - Head and Neck Surgery, [1]Fred Hutchinson Cancer Research Center

BACKGROUND: Oncologic surgery is guided by the principles of complete resection and normal tissue preservation. However, the interface between abnormal and normal tissue can be difficult to distinguish. While the bulk of a tumor is easily identifiable, the microscopic edges of tumor are not readily apparent to the naked eye. In addition, tissue surrounding an identifiable tumor is often dysplastic, which does not have a distinct phenotypic appearance.

BLZ-100 is a chlorotoxin-based agent conjugated with indocyanine green (ICG) with tumor targeting characteristics. Here, we test the ability of BLZ-100 to distinguish head and neck squamous cell carcinoma (HNSCC) and oral dysplasia from normal stroma.

METHODS: To assess uptake characteristics of BLZ-100 in HNSCC two cell lines (JHU-019 and PCI-15B) and a low-passage primary culture, FHCRC/UW-SCC1 were orthotopically injected into the tongues of NOD/SCID mice. BLZ-100 (6 nM) was injected via tail vein when tumor was visible at 2-4 weeks. Twenty-four hours following injection, fluorescent images of the oral cavity were obtained using the Xenogen IVIS Spectrum. Fluorescence measurements were determined using the LI-COR Odyssey scanner.

Sensitivity and specificity of BLZ-100 binding was quantified using a digital grid to map fluorescence intensity in tissue sections, and to determine the presence of tumor in each grid box in H&E sections. A receiver operating characteristic (ROC) curve was plotted.

To assess uptake characteristics of BLZ-100 in oral dysplasia, 0.9% 7,12-dimethylbenz(a)anthracene (DMBA) was painted on the right mucosal cheek pouch of Golden Syrian hamsters three times a week to induce dysplasia. BLZ-100 was subcutaneously injected (1mg/kg) at 6, 9 and 12-15 weeks of treatment. Tissue was harvested and fluorescence measurements were made using the Odyssey scanner. Pathologic grading of dysplasia was performed by a veterinary pathologist who was blinded to study data.

RESULTS: Eighteen oral squamous cell carcinoma tumor xenografts (five – JHU-019, five – PCI-15B, eight – FHCRC/UW-SCC1) were analyzed. The fluorescent signal from BLZ-100 corresponded with that from GFP in tongue tumors (Figure 1). The signal-to-background ratio (SBR) of tumors labeled by BLZ-100 was 2.47 +/- 0.66 SD.

Using the digital grid overlay on each tumor-containing tongue, a ROC curve was plotted with an area under the curve of 0.916. A SBR of 2.5 corresponded to a sensitivity and specificity of 92% and 80%, respectively.

A total of 54 cheek pouches were measured for fluorescence intensity (Figure 2). Specimens were histologically classified on a five-point dysplasia scale. The absolute fluorescence counts were significantly different when comparing normal (1379.6 +/- 361 SD) versus mild-to-moderate dysplasia (2121.4 +/- 743.5 SD, p = 0.012), and mild-to-moderate versus severe dysplasia/carcinoma in-situ (CIS) (3297.1 +/- 1092.2 SD, p = 0.013, Student's T-test). The SBRs of mild-to-moderate dysplasia and severe dysplasia/CIS were also significantly different (1.51 +/- 0.34 SD and 2.31 +/- 0.65 SD, p = 0.013).

CONCLUSION: BLZ-100 localizes to HNSCC xenografts with high sensitivity and specificity. BLZ-100 signal increases with the severity of dysplasia, and distinguishes mild-to-moderate from severe dysplasia/CIS. BLZ-100 has the potential to guide the surgeon towards complete oncologic resections.
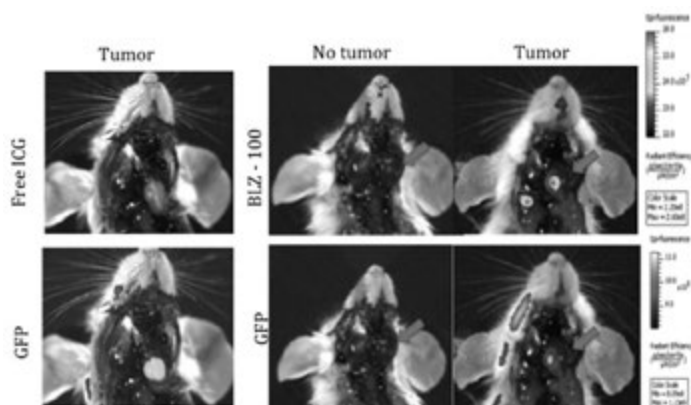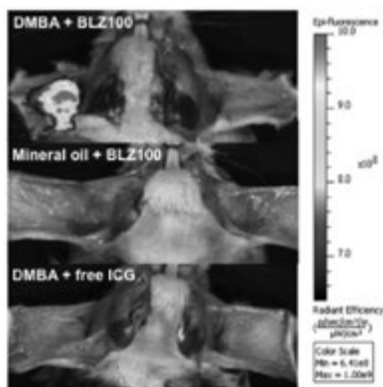

FIGURE 1

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FIGURE 2

S017: IN VIVO FLUORESCENCE IMMUNOHISTOCHEMISTRY: LOCALIZATION OF FLUORESCENTLY LABELED CETUXIMAB IN SQUAMOUS CELL CARCINOMAS – <u>Esther de Boer</u>[1], Jason M. Warram, PhD[1], Matthew D. Tucker, BS[1], Yolanda E. Hartman, BS[1], Lindsay S. Moore[1], Johannes S. de Jong[2], Thomas K. Chung[1], Melissa L. Korb[1], Kurt R. Zinn[1], Gooitzen M. van Dam[3], Margaret S. Brandwein-Gensler[1], Eben L. Rosenthal[1]; [1]University of Alabama at Birmingham, [2]University Medical Center Utrecht, The Netherlands, [3]University Medical Center Groningen

INTRODUCTION: Anti-EGFR (epidermal growth factor receptor) antibody based treatment strategies have been successfully implemented in head and neck squamous cell carcinoma (HNSCC). Unfortunately, predicting a therapeutic response remains a challenge and is far from reliable. Although significant effort has been invested in understanding EGFR-mediated changes in cell signaling with treatment and its efficacy, the delivery and histological localization in (peri-) tumoral compartments of antibody-based therapeutics in human tumors is poorly understood nor made visible. In this first in human study of systemically administered fluorescently labeled cetuximab, we sought to localize antibody delivery to tumor compartments by optical molecular imaging (i.e. denominated as in vivo fluorescence immunohistochemistry) and correlates with biological markers thought to influence antibody delivery to tumors.

MATERIALS & METHODS: Specimens were collected from nine consented patients enrolled in a clinical trial evaluating the safety and tumor-specificity of systemically injected cetuximab-IRDye800 in patients with HNSCC. The distribution of intratumoral anti-EGFR antibody cetuximab-IRDye800 fluorescence as a correlate of antibody distribution in surgical resection specimens was assessed and correlated with histopathological analysis and tumor biological characteristics.

RESULTS: Cetuximab-IRDye800 localized specifically to tumor cells; significantly higher mean fluorescent intensities (MFIs) were measured within the tumor compartment compared with the stromal compartment or normal epithelium (P<0.001). Fluorescence intensity correlated significantly with EGFR density (P<0.001), after excluding keratinizing areas in HNSCC, which demonstrated discordant regions with high EGFR expression and low fluorescence. Consistent with some of the commonly known toxicities related to cetuximab therapy, we found strong fluorescence intensity in sebaceous, submandibular and sublingual glands.

CONCLUSIONS: We were able for the first time in humans to evaluate (peri-) tumoral localization of an anti-EGFR fluorescently labeled antibody by optical molecular imaging. Clearly, in vivo fluorescence immunohistochemistry with fluorescently labeled antibodies correlating morphological characteristics to levels of antibody delivery, may improve treatment paradigms based on understanding intratumoral antibody delivery.

S018: WIDE-FIELD TARGETED FLUORESCENCE GUIDED MULTIPHOTON MICROSCOPY OF ORAL EPITHELIAL NEOPLASIA IN A HAMSTER MODEL OF ORAL CARCINOGENESIS – <u>Rahul Pal, MS</u>, Jinping Yang, MD, Susan McCammon, MD, Gracie Vargas, PhD; UTMB

BACKGROUND & OBJECTIVE: Multiphoton Autofluorescence Microscopy (MPAM) and Second Harmonic Generation Microscopy (SHGM) are a promising imaging combination for revealing indicators of neoplasia and have the ability to represent the microstructural organization of the mucosa in a manner which may be compared to histology. However they provide limited field of view, which is not practical for large area surveillance. On the other hand wide-field fluorescence could provide large area surveillance of areas that may be suspicious for neoplastic features, such as through metabolic indication, but cannot provide subsurface detail. The objective of this study was to investigate the pairing of the microscopic and widefield approaches as a potential combination for identifying high risk precancers and cancer using an animal model for OSCC.

METHODS: A chemically induced Golden Syrian Hamster model of precancer and oral cancer was used for these studies. A fluorescently labelled deoxy-glucose analog, 2-?deoxy-?2-?[(7-?nitro-?2,?1,?3-?benzoxadiazol-?4-?yl)amino]-?D-?glucose (2-NBDG) was used to highlight areas of high glucose uptake hypothesized to correspond to (pre)neoplastic sites due to the Warburg effect as well as additional metabolically active areas. Follow-up MPAM-SHGM was conducted on regions of interests (ROIs) to assess whether microscopy would reveal microscopic features associated with neoplasia to confirm or exclude 2-NBDG based findings. Biopsies were obtained of imaged sites and pathological grading assigned.

RESULTS: 2-NBDG fluorescence was shown to significantly increase with conditions of dysplasia and OSCC compared to normal, however an increase was also observed for the case of inflammation. MPAM-SHGM was useful for revealing cytologic and layer based architectural abnormalities.

CONCLUSIONS: Our results show the feasibility of this combinatorial approach of large area assessment and subsurface microscopic evaluation of neoplastic oral mucosa. This finding will allow us to develop novel diagnostic approaches for oral precancers by involving a combined analysis of tissue metabolism and microstructure.

CONCURRENT SESSION: STEM CELLS IN CANCER

S019: CD271 MODULATES THE INVASIVE AND METASTATIC PHENOTYPE OF HEAD AND NECK SQUAMOUS CELL CARCINOMA TUMOR-INITIATING CELLS THROUGH THE UPREGULATION OF SLUG – <u>Manki Chung, MD</u>[1], Young Ho Jung, MD[2], Joon Kyoo Lee, MD[3], Oihana Murillo-Sauca[4], Ravindra Uppaluri, MD[5], June Ho Shin, PhD[4], John B Sunwoo, MD[4]; [1]Department of Otorhinolaryngology - Head & Neck Surgery, Sungkyunkwan University School of Medicine, [2]Department of Otolaryngology-Head & Neck Surgery, Seoul National University College of Medicine, [3]Department of Otolaryngology-Head and Neck Surgery, Chonnam National University Medical School, [4]Division of Head and Neck Surgery, Department of Otolaryngology; Stanford Cancer Institute, [5]Department of Otolaryngology-Head and Neck Surgery, Washington University School of Medicine

Head and neck squamous cell carcinoma (HNSCC) is comprised of heterogeneous populations of cells. Discrete subpopulations with distinct marker phenotypes and functional characteristics have been defined. Recently, we have identified CD271 (low-affinity nerve growth factor receptor; p75NTR) as a functional marker of a tumor-initiating cell subpopulation in HNSCC[1]. CD271+ cells, which comprise a subset of the CD44+ cells, signals through the MAPK pathway and promote tumor formation. In the current study, we used a murine model of HNSCC (MOC2 cell line derived from DMBA-treated oral cavities of mice) that has been previously characterized and described[2]. As with human HNSCC, the MOC2 cells also have a subpopulation of cells that express CD271.

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When MOC2 cells were transduced to express NGFR by lentivirus (MOC2-NGFR), a more robust metastatic phenotype was observed. Specifically, when MOC2-NGFR cells were implanted orthotopically into the oral cavities of C57BL/6 mouse, there was a significantly higher rate of regional lymph node metastasis in mice implanted with the MOC2-NGFR cells compared to the parental MOC2 cells (91.6%, 11/12 in MOC2-NGFR cells vs. 30.0%, 3/10 in mice implanted with parental MOC2 cells). Furthermore, MOC2-NGFR cells were significantly more invasive in an invasion chamber assay, and this enhanced invasive phenotype could be abrogated by a blocking anti-CD271 antibody.

To understand the molecular basis, mRNA expression profiles of epithelial-to-mesenchymal (EMT) transition-related genes were assessed in the MOC2-NGFR and MOC2 parental cells. We observed a significantly greater expression of *Snai2* mRNA (which codes for the transcription factor Slug) in the CD271-NGFR cells. Notably, the activation of CD271 by recombinant NGF resulted in higher *Snai2* mRNA expression, measured by RT-qPCR, and Slug protein, measured by Western blot analysis. In addition, incubation with NGF enhanced the invasive phenotype in vitro to a significantly greater extent in the MOC2-NGFR cells compared to the parental MOC2, and this effect of NGF was abrogated when Snai2 was knocked down by shRNA, indicating that the invasive phenotype conferred by CD271 activation was dependent on *Snai2* expression.

Finally, we show that these findings are relevant to human HNSCC. Specifically, *Snai2* mRNA expression was observed to be higher in CD271+CD44+ subpopulation compared to the CD271-CD44+ subpopulation, sorted from patient derived xenografts. In addition, when CD271 expression was measured quantitatively by immunohistochemical analysis of human oral SCC tumors (all initially staged clinically as T3-4N0M0), tumors, associated with pathologically discovered nodal metastases, had higher CD271 expression compared to tumors from patients without nodal metastasis.

Thus, our data indicate that activation of the tumor-initiating cell marker CD271 results in upregulation of *Snai2*/Slug, which, in turn, results in a more invasive phenotype and the enhanced capacity for metastasis to regional lymph nodes. These findings point to CD271 as a promising target for therapy.

1. Murillo-Sauca, O., et al. CD271 is a functional and targetable marker of tumor-initiating cells in head and neck squamous cell carcinoma. Oncotarget 5, 6854-6866 (2014).

2. Judd, N.P., et al. ERK1/2 Regulation of CD44 Modulates Oral Cancer Aggressiveness. Cancer Res 72, 365-374 (2012).

S020: CHEMOPREVENTION EFFICACY OF CURCUMIN AND METFORMIN EFFECTED THROUGH THE DOWN-REGULATION OF CANCER STEM CELLS – Gangotri Siddappa, MSc[1], Safeena Kulsum, MTech[1], Ravindra D R, MLT[1], Vinay V Kumar, MDS[2], Babu M, MVSc[2], Benny Antony, PhD[3], Padma L, MD, Pharmacology[2], Amritha Suresh, PhD[1], Moni A Kuriakose, MS, FRCS[4]; [1]DSRG-5, MSCTR, Mazumdar Shaw Medical Centre, Narayana Hrudayalaya, Bangalore 560099, [2]Dr B R Ambedkar Medical College, Bangalore 560054, [3]Arjuna Natural Extracts Ltd., Kerala 683101, [4]Head and Neck Oncology, Mazumdar Shaw Medical Centre, Narayana Hrudayalaya, Bangalore, 560099

Cancer stem cells are known to be involved in oral carcinogenesis, effective chemo-preventive drug may work through targeting these cells and corresponding signaling pathways. The primary objective was to evaluate the role of cancer stem cells in the chemo-preventive efficacy of Curcumin and Metformin. The animal model was established using 4-6 weeks C57Bl/6 mice (N=60); the mice were divided into two arms; i) Control Arm (N=10) administered with plain drinking water and ii) Treatment Arm (N=50). The treatment arm was administered with 4NQO (4-nitroquinoline-oxide) in drinking water (50ppm) for a period of 17 weeks. The mice were then taken off the carcinogen and administered the drugs in drinking water. The mice (N=45) were divided into four arms; i) Arm I (N=15)

with plain water, ii) Arm II (N=15) with Curcumin (64μg/ml), Arm III (N=15) with Metformin (5mg/ml) and iv) Arm IV (N=15) with a combination of both Curcumin and Metformin. The mice were dissected at 17th week (N=5) as well as at the end of the study period and the samples collected for molecular analysis of stem cell related pathways

Histopathology carried out at 17th week, showed dysplastic changes after which the animals were administered with the drugs. The average tumor volume (mm3) was reduced in the combination arm (0.693+0.034) and the individual arms (Curcumin=2.54; Metformin=1.45+0.33) as compared to the 4NQO arm (6.65+2.37). The average number of lesions per mice was also reduced in the combination arm (Avg=0.375+0.17) and the Curcumin arm (Avg=0.25+0.22) as compared to the 4NQO arm (Avg=0.6+0.22). The overall survival of the combination arm was better when compared to individual treatment (p=0.0006). Immunohistochemical studies using NF-κB showed a down-regulated expression in the combination arm as compared to the control arm with a corresponding down-regulation of the stem cell markers, CD44 and Notch1. In vitro treatment of primary cells, generated from the mice tissues, with Curcumin (100μM), Metformin (10μM) and combination treatment confirmed down regulation of CSC markers (CD44, Notch1, Jagged, STAT3) in the treated cells. The effect on the Cancer stem cell is being further confirmed by various functional assays (Migration, colony formation and Colony survival assays) currently been carried out in the lab. The clinical results suggest that the combination arm is more efficient in chemoprevention, while the initial molecular analysis suggest that the stem cell cache might be reduced in parallel. Further studies using the molecular markers for Metformin drug and subsequent functional studies are currently ongoing. Expression profiling studies using molecular markers corresponding to other stem cell related pathways (CD44, Notch1, Jagged1 and STAT3) are also ongoing.

CONCURRENT SESSION: PERSONALIZED PATIENT CARE - HOW OUR INSTITUTION DOES IT

S021: MOLECULAR SCREENING FOR CANCER TREATMENT OPTIMIZATION IN HEAD AND NECK CANCER (MOSCATO 01): A PROSPECTIVE MOLECULAR TRIAGE TRIAL; INTERIM ANALYSIS OF 78 PATIENTS WITH RECURRENT OR METASTATIC HEAD AND NECK CANCERS – Ingrid Breuskin, Caroline Even, Ecaterina Ileana, Christophe Massard, Naima Lezghed, Ludovic Lacroix, Antoine Hollebecque, Rastislav Bahleda, Maud Ngo-Camus, Yohann Loriot, Nathalie Auger, Valerie Koubi-Pick, Thierry De Baere, Philippe Vielh, Vladimir Lazar, Marie-Cécile Le Deley, Catherine Richon, Joël Guigay, François Janot, Jean-Charles Soria, Charles Ferté; Gustave Roussy

BACKGROUND: Patients with recurrent or metastatic head and neck cancers (HNC) have a poor prognosis. After first line chemotherapy, therapeutics are limited. The widespread use of high-throughput molecular techniques has allowed the identification of recurrent and actionable molecular traits across various tumor types. Translating these approaches to bedside may guide the decision-making for cancer patient candidates to early clinical trials.

METHODS: Patients with advanced HNC, referred to our early drug development department, were enrolled in a prospective molecular screening program at Gustave Roussy (France). Surgical or CT-Scan biopsies were performed in metastatic or primary tumor sites to carry out a comprehensive molecular characterization. DNA was extracted from fresh tumor samples and analyzed by comparative genomic hybridization (CGH) (≥ 30% tumor cells required), and by Next Generation Sequencing (NGS) for up to 74 target genes (≥ 10% tumor cells required). A weekly molecular tumor board reviewed all the profiles to identify actionable traits for which the most relevant targeted therapy may be available through early clinical trials or marketed drugs. Treatment efficacy was evaluated by RECIST 1.1 or PERCIST criteria.

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RESULTS: From July 2011 to August 2014, 78 heavily pretreated patients were included in the MOSCATO 01 trial. CGH and NGS profiles were assessed in 64 (82%) and 68 (87%) of biopsied patients, respectively. The median time for delivering results was 20 days. Actionable molecular aberrations were found in 30 patients (38%). Among these patients, 10 patients (33%) were treated with a targeted therapy according to the molecular profile. The most frequent actionable molecular aberrations were observed in the following pathways: FGFs/FGFRs (35%), PI3K/AKT/mTOR (26%), MYC (24%), CDKs/Cyclins (13%), EGFR (9%), HER2 (7%), NOTCH (4%), KIT (2%). Out of the 10 patients treated according to the genomic profile, we observed at first tumor evaluation: 3 partial responses (PR), 3 stable diseases (SD) and 1 clinical progressive disease (PD), while 2 patients were not evaluable. Out of the 20 patients not treated according to the molecular triage whereas actionable traits were detected, we observed: 7 patients with exclusion criteria for clinical trial, 6 patients being dead during the process, 4 patients with feeding tube impairing oral treatment intake, and 3 patients treated by other antineoplastic therapy.

CONCLUSIONS: High throughput genomic analysis is feasible in daily practice and allows the biological-orientation of up to 38% of recurrent or metastatic HNC patients.

S022: TARGETED SEQUENCING OF AN EPIDEMIOLOGICALLY LOW RISK PATIENT DEFINES FIBROBLAST GROWTH FACTOR FAMILY ABERRATIONS AS A DRIVER OF HEAD AND NECK SQUAMOUS CELL CARCINOMA – Brittny N Tillman, MD, Laura M Bou-Maroun, BS, Megan L Yanik, BS, Catherine S Grasso, Michael J Quist, Nallasivam Palanisamy, PhD, Shannon Carskadon, Thomas E Carey, PhD, Carol R Bradford, MD, Scott A Tomlins, MD, Jonathan B McHugh, MD, Matthew E Spector, MD, Chad Brenner, PhD; University of Michigan

BACKGROUND: Despite modifications in treatment over the past decade, overall survival has remained unchanged for advanced head and neck squamous cell carcinoma (HNSCC). One area showing promise to improve these survival rates has been precision medicine protocols, which seek to identify the genetic drivers of the tumor and allow the discovery of druggable targets. In addition, HNSCC has had a unique hurdle in this development due to the mutational complexity of tumors given the high rates of smoking and alcohol associated. Therefore, we postulate that targeted sequencing of epidemiologically low risk (ELR) (low smoking and low alcohol consumption) HNSCC patients could help identify novel drivers or lost suppressors leading to precision medicine protocols and improved survival rates.

METHODS: An ELR-HNSCC patient was selected for targeted sequencing on DNA isolated from a diagnostic formalin-fixed, paraffin-embedded (FFPE) tissue block. We then assessed publically available next generation sequencing cohorts for the presence of alterations in the Fibroblast Growth Factor (FGF) pathway. Data was collected and analyzed from the Oncomine Powertool Database, which contains pan-cancer data from The Cancer Genome Atlas (TCGA).

RESULTS: Targeted next generation sequencing of an ELR HNSCC patient revealed a FGFR1 amplification as a driver of the patient's tumor. Analysis of HNSCC patients from TCGA data demonstrated that FGF family genes were deregulated by mutation, rearrangement or amplification in over 35% of HNSCC cases, with a trend of higher frequency in African American populations. Further analysis if the TCGA data revealed that patients with FGFR alterations are unique from those with other activating mutations and that these patients trend towards mutually exclusive from patients with inactivating NOTCH pathway mutations, activating PIK3CA mutations and EGFR amplifications.

CONCLUSION: Together, this data suggests that FGF signaling is critical for a subset of HNSCC patients independent of other known pathways and provides rationale for leveraging ELR-HNSCC patients to define molecular subsets of high risk HNSCC.

CONCURRENT SESSION: LATE BREAKING HOT TOPICS

S023: CYCLIC DINUCLEOTIDE, A NOVEL ADJUVANT FOR SQUAMOUS CELL CARCINOMA – Shekhar K Gadkaree, Rupashree Sen, Juan Fu, Clint Allen, Young J Kim; Department of Otolaryngology - Head & Neck Surgery, Johns Hopkins University School of Medicine

IMPORTANCE: Cyclic dinucleotides (CDNs), which signal through the STING (Stimulator of Interferon Gene) pathway, are a novel class of adjuvants that have been shown to induce anti-tumor responses in vivo. Preclinical studies will provide the necessary rationale for immunotherapy clinical trials for metastatic/recurrent HNSCC patients.

OBJECTIVE: In this study, we examined the role of CDNs in stimulating human dendritic cells and monocytes. We further examined the anti-tumor immune responses generated by natural and synthetic CDNs on SCCVII squamous cell tumors in a murine model.

DESIGN, SETTING & SUBJECTS: Preclinical animal study and in vitro human sample experiments performed at an academic animal research facility.

INTERVENTIONS: Cultured human dendritic cells (DCs) were stimulated with CDNs to examine cytokine response profiles and activity levels. We further attempted to model the effects of CDNs in a murine system. SCCFVII tumor bearing C3H/HeOUJ mice (n=5 per group) were treated with three intratumoral injections of RR-CDG (synthetic cyclic-di-guanine), CDG (natural cyclic-di-guanine), R848 (TLR 7/8 agonist), or PBS (phosphate buffered saline, control). In addition, a group of RR-CDG treated mice was also treated with anti-CD8 T-cell depletion antibody to evaluate the role of CDNs in stimulating CD8+ T-cells. In vivo tumor growth rate was followed for 23 days, after which spleens were harvested and splenocytes were analyzed for cytokine profile using flow cytometry. In addition to measuring the anti-tumor immune response, the direct toxicity of the CDNs was measured by performing in vitro dose response live/dead cell viability assays.

MAIN OUTCOMES & MEASURES: The primary outcome measure was tumor volume. The secondary outcome measure was comparison of relative expression of Th1 vs. Th2 cytokine profiles from splenocytes in disparate murine treatment groups and from cultured human dendritic cells.

RESULTS: In cultured human DCs, CDN stimulation resulted in higher levels of monocyte activation and increased IFNg and IL-12 levels when compared to PBS controls. In the murine model, RR-CDG and CDG treatment groups showed significantly decreased tumor size when compared to the R848, T-cell deficient RR-CDG, and PBS treatment groups (p<0.05). Splenocytes from the RR-CDG treated mice showed an enhanced Th1 response, as determined by increased expression of IFNγ, when compared to the PBS treatment group. In vitro tumor cell killing assay showed that there was no direct tumor cytotoxicity effect in response to CDNs.

CONCLUSIONS & RELEVANCE: Synthetic and natural CDN treated mice had significantly reduced tumor volumes when compared to R848 (TLR7/8 agonist) and control treated mice. Mice treated with synthetic and natural CDNs also showed a predominant Th1 response when compared to mice treated with R848 (TLR7/8) agonists or controls. A pre-dominant Th1 cytokine response and increased monocyte activation in human cell studies demonstrate the translational nature of the animal studies and strongly support the future implementation of intratumoral injection of CDN into HNSCC patients.

ORAL PAPERS

S024: MUTATIONAL LANDSCAPES OF ORAL TONGUE SQUAMOUS CELL CARCINOMA REVEAL RECURRENT MUTATIONS IN GENES OF THERAPEUTIC AND PROGNOSTIC RELEVANCE – Andre Luiz Vettore, Kalpana Ramnarayanan, Gregory Poore, Choon Kiat Ong, Huang Kie Kyon, Hui Sun Leong, Chong Fui Teen, Kiat-Hong Lim, Weng Khong Lim, Ioana Cutcutache, John R Mcpherson, Yuka Suzuki, Shenli Zhang, Thakshayeni Skanthakumar, Weining Wang, Daniel Shao-Weng Tan, Byoung Chul Cho, Teh Bin Tean, Steve Rozen, Patrick Tan, N Gopalakrishna Iyer; Head and Neck Service, Department of Surgical Oncology, National Cancer Centre

Carcinoma of the oral tongue (OTSCC) is the most common malignancy of the oral cavity, characterized by frequent recurrence and poor survival. The last three decades has witnessed a change in the OTSCC epidemiological profile, with increasing incidence in younger patients, females and never-smokers. Here, we sought to characterize the OTSCC genomic landscape and to determine factors that may delineate the genetic basis of this disease, inform prognosis and identify targets for therapeutic intervention. Seventy-eight Asian OTSCC cases were subjected to whole-exome and targeted-deep sequencing. While the most common mutation was in TP53, the OTSCC genetic landscape differed from previously described cohorts of patients with head and neck tumors: OTSCCs demonstrated frequent mutations in DST, FSIP2 and RNF213, while alterations in CDKN2A and NOTCH1 were significantly less frequent. Despite a lack of previously reportedNOTCH1 mutations, integrated analysis showed enrichments of alterations affecting Notch signaling in OTSCC. Importantly, these Notch pathway alterations were prognostic on multivariate analyses. A high proportion of OTSCCs also presented with alterations in drug targetable and chromatin remodeling genes. Patients harboring mutations in actionable pathways more likely to succumb from recurrent disease compared to those who did not, suggesting that the former should be considered for treatment with targeted compounds in future trials. Our study defines the Asian OTSCC mutational landscape, highlighting the key role of Notch signaling in oral tongue tumorigenesis. We also observed somatic mutations in multiple therapeutically relevant genes, which may represent candidate drug targets in this highly lethal tumor type.

S025: NEXT-GENERATION ROBOTIC HEAD AND NECK SURGERY: A NOVEL SINGLE-ARM, FLEXIBLE ROBOTIC SURGICAL SYSTEM FOR TRANSORAL RESECTION OF THE TONSILLAR FOSSA AND LATERAL OROPHARYNGEAL WALL – F. Christopher Holsinger, MD, FACS, Michelle Chen, MD; Department of Otolaryngology - Head and Neck Surgery, Stanford University

IMPORTANCE: Since FDA approval of the use of first generation robotic surgical system for T1-T2 oropharyngeal cancers, there has been a rapid adoption in the use of transoral robotic surgery (TORS) among community and academic centers. However, the rigidity of the instruments and the limited working space of the oral cavity and pharynx result in significant limitations to the use of these systems in the management of oropharyngeal cancer.

OBJECTIVE: To determine the feasibility of performing transoral resection of the tonsillar fossa and lateral oropharyngeal wall using a novel flexible robotic surgical system.

DESIGN, SETTING, PARTICIPANTS: Preclinical anatomic study using three dentate adult human cadavers.

INTERVENTIONS: Utilization of the Da Vinci Sp, a novel single-arm, flexible robotic surgical system, for transoral resection of the tonsillar fossa and lateral oropharyngeal wall.

MAIN OUTCOMES & MEASURES: Completion of transoral resection of the lateral oropharyngeal wall with mucosal and muscular resection of the tonsillar fossa.

RESULTS: For all three cadavers, the Da Vinci Sp provided sufficient reach and access to the necessary anatomy to successfully complete a transoral lateral oropharyngectomy. Three instruments and the camera were used in each cadaver without collision or movement restriction compared to two instruments and a camera in the traditional robotic surgical systems. The third instrument permitted the surgeon at the console to expose the hidden folds of the oropharynx, which provided improved visualization of the cranial and caudal boundaries of the dissection. While the traditional systems required three separate robotic arms to access the patient, the novel system has one arm from which all four instruments exit, which is ideal for transoral surgery. The ideal placement of the cannula was 10-15cm from the upper lip with the cannula angled from the contralateral side of the oral cavity. The single robotic arm afforded additional workspace in and around the mouth for the bedside assistant. The increased flexibility of the instruments was provided by a second joint, which acted as an elbow and created an additional point of external rotation. This permitted triangulation of the instruments around a small space.

CONCLUSIONS & RELEVANCE: We described the first preclinical evaluation and use of a novel flexible, single-arm robotic surgical system for transoral endoscopic head and neck surgery. The single cannula and flexible multi-jointed instruments was well-suited for transoral surgery and further research is needed in clarifying the potential uses of this system for head and neck surgery in general.

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P001: IN VITRO EFFECTS OF MEBENDAZOLE IN A SPONTANEOUS FELINE MODEL OF HNSCC – <u>Jackie M Wypij</u>, DVM, MS; Univ of Illinois at Urbana-Champaign

INTRODUCTION: Although advancements in therapeutic modalities have improved the quality of life for patients with HNSCC, survival as a whole has not markedly increased. There exists a critical need to overcome the barriers of drug toxicity and inefficacy that hinder new cancer drug development. One potential mechanism is a translational research incorporating 1) drug repositioning of low-toxicity pharmaceuticals with putative anti-cancer activity and 2) use of companion animals with spontaneously arising comparative cancer.

Pet cats represent a novel, relevant, and under-utilized resource in translational oncology research with many advantages over traditional preclinical murine models. Feline oral squamous cell carcinoma (FOSCC) is an excellent model of advanced stage human HNSCC. Malignant SCC is a common feline cancer often diagnosed at a late stage with limited effective treatments and opportunities for translational experimental research including clinical trials of novel drugs. HNSCC is traditionally treated with surgery, radiation therapy, and chemotherapy, with recent studies suggesting a role for incorporation of newer mitotic spindle inhibitors into treatment regimens. Benzimidazole (BZ) anti-parasitics have anti-neoplastic properties in vitro and in murine models.

The goals of this study were to evaluate the in vitro effects of mebendazole in feline squamous cell carcinoma as a model of HNSCC. Specific aims include assessment of cell proliferation, cell-cycle phase distribution, VEGF secretion, and tubulin binding characteristics utilizing commercial assays.

MATERIALS & METHODS: The feline oropharyngeal SCC cell line (SCCF1) was incubated with MBZ at 0-100micromolar. Cell proliferation (Aqueous One MTS Assay, Promega), soluble VEGF (VEGF ELISA, R&D Systems), and tubulin polymerization (Cytoskeleton) were assessed with commercial assays. Combination studies were performed with traditional mitotic spindle poisons with analysis via CompuSyn. Cell cycle was assessed via flow cytometry.

RESULTS: Mebendazole decreases cell proliferation (p<0.05) and reduces VEGF secretion (p<0.05). MBZ decreases the rate and total polymerization of tubulin, alters cell cycle, and is synergistic with mitotic spindle inhibitors.

CONCLUSIONS: Mebendazole demonstrates in vitro anti-neoplastic effects in feline oropharyngeal SCC. Given the known low toxicity, MBZ may be a potential candidate for future clinical trials in pet cats with cancer as a translational model.

P002: CHARACTERIZATION OF HUMAN PAPILLOMAVIRUS ANTIBODIES AND INFLAMMATORY IMMUNE MARKERS IN INDIVIDUALS WITH HEAD AND NECK CANCER – <u>Krystle A Lang Kuhs, PhD, MPH</u>[1], Aimee R Kreimer, PhD[1], Sandra Gibson[2], Sumita Trivedi[2], Michael Pawlita[3], Tim Waterboer[3], Robert L Ferris[2]; [1]National Cancer Institute, [2]University of Pittsburgh Cancer Institute, [3]German Cancer Research Center

BACKGROUND: The increasing incidence of oropharyngeal cancer (OPC) in many developed countries has been attributed to human papillomavirus type 16 (HPV16) infections. Recently, HPV16 E6 seropositivity has been identified as a promising early marker for OPC. HPV16 infection has also been implicated in a small minority of head and neck cancer cases outside of the oropharynx (non-OPC). Given the high tropism of HPV for the oropharynx as compared to other anatomic sites within the head and neck, further characterization of how the immune profiles of OPC and non-OPC cases differ in terms of HPV serology and inflammatory immune marker profiles is warranted.

METHODS: 260 serum samples (253 pre-treatment, 7 post-treatment [drawn within 7 days post treatment]) were collected from incident cases of head and neck cancer (38 OPC, 222 non-OPC cases) seen at the University of Pittsburgh between 2003 and 2006. Using Luminex based technology, all 260 serum samples were tested for antibodies against i) HPV16 proteins (L1, E1, E2, E4, E6, E7) and ii) E6 proteins of non-HPV16 types (HPV6, 11, 18, 33). A subset of 253 cases with pre-treatment serum was additionally tested for levels of 31 inflammatory immune markers.

RESULTS: Overall, 14.2% (n=37) of the 260 the head and neck cancer cases were seropositive for HPV16 E6. By anatomic sub-site, 60.5% (n=23) of the 38 OPC cases were seropositive for HPV16 E6 compared to 6.3% (n=14) of the 222 non-OPC cases (odds ratio [OR] 22.8; 95% confidence interval [CI] 9.8-53.1). A total of 25 cases (15 non-OPC, 10 OPC) had both p16 immunohistochemistry (IHC) and HPV in situ hybridization (ISH) test results. 13.3% (n=2 out of 15) of the non-OPC cases were dual positive for p16 IHC and HPV ISH compared to 60% (n=6 out of 10) of OPC cases; all 6 dual positive OPC cases were seropositive for HPV16 E6. None of the dual positive non-OPC cases had detectable HPV16 E6 antibodies. Compared to non-OPC cases, OPC cases were significantly more likely to be seropositive against all HPV16 proteins tested: L1 (OR 7.1; 95% CI: 3.4-14.8); E1 (OR 13.8; 95% CI: 5.4-35.3); E2 (OR 17.2; 95% CI: 7.0-42.2); E4 (OR 22.6; 95% CI 7.4-68.6); and E7 (OR 11.6; 95% CI: 5.1-26.2) and to be seropositive against E6 proteins from non-HPV16 types: HPV11 (OR 11.1; 95% CI: 2.5-48.5) and HPV33 (OR 14.5; 95% CI: 4.1-51.2). Of the 31 inflammatory markers tested, OPC was associated with lower levels of chemokine C-X-C motif ligand 5 (CXCL5) and chemokine C-C motif ligand 5 (CCL5); adjusted ORs for quartile 1 versus quartile 4 were 0.2 (95% CI: 0.1-0.9; ptrend=0.01) and 0.4 (95% CI: 0.1-1.2; ptrend=0.03), respectively.

CONCLUSIONS: OPC cases appear to be immunologically distinct from other head and neck cancers. In contrast to HPV-driven non-OPC cases, HPV16 E6 seropositivity was highly sensitive (6 of 6, 100%) for HPV-driven OPC, which may reflect the unique immunobiology of the oropharynx and its proximity to the lymphatic system. Follow-up studies are needed to confirm these early findings given our small sample size.

P003: OVEREXPRESSION OF MEMBERS OF THE TUMOR NECROSIS FACTOR RECEPTOR-ASSOCIATED FACTOR (TRAF) FAMILY IN TONGUE SQUAMOUS CELL CARCINOMA IS ASSOCIATED WITH POOR DIFFERENTIATION STATUS AND PROGRESSION TO METASTASIS – <u>Sabrina da Silva</u>[1], Bin Xu[2], Hind Amzil[2], Maisa Ismael Alkailani[2], Krikor Bijian[2], Alex Mlynarek[2], Silvia Regina Rogatto[1], Michael Hier[1], Luiz Paulo Kowalski[3], Moulay A. Alaoui-Jamali[2]; [1]Department of Otolaryngology Head and Neck Surgery, Sir Mortimer B. Davis-Jewish General Hospital, C, [2]Segal Cancer Centre and Lady Davis Institute for Medical Research, Sir Mortimer B. Davis-Jewish Gene, [3]Inter-institutional Grad Program on Bioinformatics, University of São Paulo, Brazil

Molecular mechanisms underlying metastatic oral squamous cell carcinoma (OSCC) are poorly understood. In this study, we utilized array comparative genomic hybridization and genome-wide screening of metastatic and non-metastatic tongue tumors to investigate gene markers potentially contributing to OSCC progression to metastasis. Among multiple gene abnormalities, we identified amplifications of chromosomal regions that encompass members of the tumor necrosis factor (TNF) receptor-associated factor (TRAF) family in metastatic OSCC; these include TRAF2, TRAFD1, TRAF4, TRAF7, genes (9q34.3, 12q24.13, 17q11-q12 and 16p13.3, respectively). Among these markers, TRAF2 was observed in advanced OSCC patients being predictive of poor survival. TRAF2 is a RING finger adaptor protein involved in the regulation of cellular response to cytokines and other stress stimuli, and has been established as an important component in TNF- and Tweak-dependent signaling in particular in relation to inflammatory response. Using the yeast two-hybrid system, we identified an interaction of TRAF2 with proteins involved in focal adhesion (FA) complexes, including the focal adhesion kinase (FAK). In FAK-proficient (TRAF2+/+) and FAK-deficient (TRAF2-/-) and TRAF2-/- reconstituted cells, we demonstrated that TRAF2 interact with the C-terminal portion of FAK, colocalizes with FAK and

other FA markers in cell membrane protrusions. We established the requirement of TRAF2 for FAK-induced cell survival, in particular anoikis. This mechanism was also confirmed in a panel of cancer cell lines, in particular TRAF2 down-regulation by siRNA induced cells to anoikis by stimuli such as the chemotherapeutic drug taxol. Together, these results provide evidences that the up-regulation of TRAF proteins can contribute to OSCC progression to metastasis via regulation of both cell invasion signalling and anoikis.

P004: GENOME-WIDE ANALYSIS OF RAB GTPASES IDENTIFIED THE PROGNOSTIC VALUE OF RAB5, RAB7 AND RAB11 CO-AMPLIFICATION IN METASTATIC TONGUE SQUAMOUS CELL CARCINOMA – Sabrina da Silva[1], Fabio Albuquerque Marchi[2], Bin Xu[3], Faisal Alobaid[1], Alex Mlynarek[1], Silvia Regina Rogatto[2], Michael Hier[1], Luiz Paulo Kowalski[4], Moulay A. Alaoui-Jamali[3]; [1]Department of Otolaryngology Head and Neck Surgery, Sir Mortimer B. Davis-Jewish General Hospital,, [2]NeoGene Laboratory, Department of Urology, Faculty of Medicine, UNESP, and International Research Ce, [3]Segal Cancer Centre and Lady Davis Institute for Medical Research, Sir Mortimer B. Davis-Jewish Gene, [4]Inter-institutional Grad Program on Bioinformatics, University of São Paulo, Brazil

Metastatic oral squamous cell carcinoma (OSCC) is frequently associated with recurrent gene abnormalities at specific chromosomal loci. Here, we utilized array comparative genomic hybridization and genome-wide screening of metastatic and non-metastatic tongue tumors to investigate genes potentially contributing to OSCC progression to metastasis. We identified predominant amplifications of chromosomal regions that encompass the RAB5, RAB7 and RAB11 genes (3p24-p22, 3q21.3 and 8p11–12, respectively) in metastatic OSCC. The expression of these Rab GTPases was confirmed by immunohistochemistry in OSCC tissues from a cohort of patients with a follow-up of 10 years. A significant overexpression of Rab5, Rab7 and Rab11 was observed in advanced OSCC cases and co-overexpression of these Rabs was predictive of poor survival (log-rank test, P=0.006). In preclinical models, mRNA and protein expression levels of these Rab members were elevated and in a panel of invasive OSCC cell lines, their down-regulation prevented focal adhesion disassembly and inhibited cell invasion. Together, the results provide insights into the cooperative role of Rab gene amplifications in OSCC progression and support the potential utility of targeting multiple Rab proteins as a therapeutic approach for advanced OSCC.

P005: FACTORS AFFECTING QUALITY AND COST OF CARE IN IN MAJOR THERAPEUTIC SURGERIES IN HEAD AND NECK CANCER – Paula Wu, BS, Saral Mehra, MD, MBA; Department of Surgery (Otolaryngology), Yale University School of Medicine, New Haven, Connecticut

IMPORTANCE: Understanding national variations in cost and quality of inpatient care for major head and neck surgery is critical to improve the value of surgical care.

OBJECTIVE: To describe national trends and benchmark cost and quality factors in major head and neck surgery for head and neck cancer, and determine factors associated with cost and quality outcomes.

DESIGN: Retrospective National database research.

SETTING: Nationwide Inpatient Sample (NIS) Datafiles

PARTICIPANTS: Patients discharged from NIS-participating institutions between 2003 to 2011, following a major therapeutic surgery for the treatment of head and neck cancer (n=76,468).

MAIN OUTCOME(S) AND MEASURES: Outcome measures included total complications, length of stay (LOS), in-hospital mortality, and total charges. Total charges were used as a proxy for quality, cost, and value of care because it encompasses various metrics not included as distinct outcomes

in the NIS, including but not limited to ICU use, return to OR, and additional diagnostics/treatments. Factors associated with these outcomes included patient demographics (payor, rural/urban location, race, age, gender, and income); patient clinical characteristics (number of chronic conditions, tumor stage, point of admission, and APRDRG Risk Mortality and Severity scores); hospital characteristics (hospital region, membership in a multi-hospital system, teaching hospital, bedsize, percent registered nurses among nurses, number of RN full-time equivalents per 1000 patients, number of licensed practical nurse FTEs per 1000 patients, percent surgeries performed in an outpatient setting, and hospital volume).

RESULTS: There was an average of one complication per discharge (range 0-8), median LOS of 4 days(range 6-336), and mortality rate of 1%. The median total charges were $38,064(range $35-$2,767,402).. On multivariate analysis, increased charges were associated with large metropolitan patient location(p=1.45E-15), non-white race(p<2E-16), Medicaid status(p=1.9E-13), treatment at an urban teaching hospital(p=0.0007), high APRDRG Severity(p=0.0002) or Risk Mortality scores(p<2E-16), age (p=2.24E-13), number of chronic conditions (p<2E-16), hospital volume (P=0.0195), and RN FTEs per 1000 patients (p<2E-16). With the exception of large bedsize and large metropolitan location, these factors also associated with increased LOS (p<=2E-16,<2E-16,<2E-16,0.0012,<2E-16,1.48E-06,0.0162,0.0264, respectively). Patients with higher complication rates were more likely to come from hospitals with large bedsizes (P=0.0011), hospitals outside the northeast, more likely to be Black (p<2E-16) or Hispanic (p=0.0002), with Medicare or Medicaid (both p<2E-16), and in lower income quartiles (p=6.08E-16). Wound complications, infectious complications, and "other" surgical complications resulted in the greatest additional charges and LOS after adjusting for APRDRG Disease Severity and Risk Mortality scores (p<0.05). Cardiovascular complications resulted in no additional LOS and "other" medical complications resulted in no significant relative charge increase (P>0.05). No factor was found to be significantly associated with in-hospital mortality.

CONCLUSION: Charges and quality outcomes in patients undergoing major therapeutic surgery for the treatment of head and neck cancer vary widely nationwide. We identified patient, clinical, and hospital characteristics associated with higher total charges and LOS, both proxies for quality of care. Determining reasons for this variation, sharing best practices, and targeting interventions specific to these factors may help to improve the value of care delivery in this patient population.

P006: PERINEURAL INVASION INCIDENCE IN THE TCGA HEAD AND NECK CANCER COHORT – Christian A Graves, BS[1], Uma Shankavaram, PhD[2], Lucia A Pirisi-Creek, MD[3], James Wells, MD[1]; [1]USC School of Medicine/ Dorn VAMC, [2]National Cancer Institute, [3]USC School of Medcine

BACKGROUND: Perineural invasion (PNI) is an unfavorable prognostic indicator in head and neck cancer (HNC) and the molecular drivers are relatively unknown. We compared clinicopathological, survival, mutational, and gene expression characteristics between PNI+ and PNI- HCN patients in the cancer genome atlas (TCGA) dataset.

METHODS: Gene expression changes were investigated using Bioconductor packages edgeR and DESeq2 . Geneset enrichment was performed by goseq package in R, and network analysis by Ingenuity pathway analysis.

RESULTS: PNI+ patients have significantly worse prognosis than PNI-patients (p<0.05). The most common site of PNI+ lesions was the oral tongue (48% vs. 21%; p<0.0001). PNI- lesions have frequent mutations in AJUBA and EP300. PNI+ lesions had neurogenesis-related gene expression signatures centered around neurogenic drivers while PNI-lesions were characterized by SMAD2 overexpression.

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CONCLUSIONS: Differential mutations and molecular pathways that characterize PNI+ lesions may provide insights for targeted therapies of this aggressive form of HNC.

P007: DEVELOPING BIOMARKERS TO PREDICT RESPONSE TO PARP INHIBITORS IN HEAD AND NECK SQUAMOUS CELL CARCINOMA PATIENTS – Michael Hajek, BA, Asel Biktasova, MD, PhD, Andrew Sewell, MD, Cyril Gary, BA, Wendell G Yarbrough, MD, MMHC, FACS, Natalia Issaeva, PhD; Dept. Surgery, Otolaryngology, Yale School of Medicine

In the past years, several poly-ADP ribose polymerase (PARP) inhibitors have entered clinical trials in patients with different types of cancer, including head and neck squamous cell carcinoma (HNSCC). In breast and ovarian cancer, BRCA1/2 mutations are associated with a great response to the PARP inhibition due to synthetic lethality, most likely explained by the inability to repair specific DNA lesions, as well as overactivation of PARP-1, in the mutant cells. However, molecular markers that can predict sensitivity to PARP inhibitors in HNSCC (and thus help select a specific group of patients for the treatment) have yet to be discovered.

Recently, utilizing reverse phase protein arrays, we found that DNA repair proteins represent the largest differentially expressed group between HPV-positive and negative HNSCCs. Moreover, all diversely expressed total DNA repair proteins, including PARP-1, are up-regulated in HPV-associated tumors. Further studies revealed that PARP is enzymatically overactive in HPV-positive head and neck tumors and cells. In addition, we have shown that both HPV-positive cell lines and primary cells are significantly more sensitive to the inhibition of PARP than HPV-negative cells. PARP-1 is known to be activated by damage to DNA; indeed, utilizing Comet assays, we found elevated levels of DNA damage in HPV-positive, but not in HPV-negative cells. Interestingly, among all HPV-encoded proteins tested, overexpression of major HPV capsid protein L1 in HPV-negative cells resulted in overactivation of PARP, as well as significantly increased sensitivity of cells to both PARP inhibition and carboplatin treatment. We suggest that overactivation of PARP, determined by immunohistochemistry or PARP activity ELISA, and/or expression of HPV L1, analyzed by immunohistochemistry or real time PCR, may predict response to PARP inhibitors in patients with HNSCC.

P008: GENOME-WIDE METHYLATION ANALYSIS OF SALIVARY GLAND ADENOID CYSTIC CARCINOMA BY METHYLATED DNA BINDING DOMAIN SEQUENCING Shizhang Ling, MD, PhD, Sarah J Wheelan, MD, Alexander Favorov, Elana J Fertig, Daria A Gaykalova, Joseph A Califano, MD, Srinivasan Yegsubramanian, Nishant Agrawal, Patrick K Ha, MD; Johns Hopkins University

BACKGROUND: Salivary gland adenoid cystic carcinoma (SGACC) remains a poorly understood malignancy due to the lack of basic understanding of its carcinogenesis. The recent whole-genome sequencing by other groups uncovered relatively consistent results, but identified only a few genes with somatic mutations in SGACC. This suggests that aberrant epigenetic regulation may play a role in the carcinogenesis of SGACC. Aberrant DNA methylation or demethylation of CpG dinucleotides of tumor suppressor genes (TSGs) or oncogenes is an integral part of aberrant epigenetic regulation in carcinogenesis of many types of human cancers. In fact, genome-wide methylation arrays have identified a small number of TSGs and oncogenes that are regulated by methylation in SGACC, but results have been modest due to technical limitations of these arrays.

AIM: In order to identify genes that are regulated by methylation in SGACCs in a genome-wide, unbiased manner, a methylated DNA binding domain (MBD)-based sequencing (MBD-seq) technique was performed with hopes of providing methylation information across the entire genome.

METHODS: MBD-seq was performed in 19 SGACC and 15 normal salivary gland tissues. The raw data obtained was subsequently evaluated by bioinformatic analysis. Using MACS, we delineated peaks of differential methylation among the samples. As there are multiple unmatched tumor and normal samples, we prioritized peaks using a Fisher-based comparison of peak occupancy in predefined genomic regions. A list of genes whose promoters contain differentially methylated regions (DMRs) between SGACC and normal samples was generated. Among these genes, the MACS peaks in each gene on the list were manually checked in each sample to identify the authentic DMRs. The differential methylation status of the gene GPR123 was further validated by quantitative methylation-specific PCR (qMSP) in a separate cohort of formalin fixed primary SGACC samples. Results: The differential methylation in GPR123 was identified using the MBD-seq approach and confirmed by quantitative methylation specific PCR in a larger validation cohort of SGACC and normal FFPE samples (normal vs. SGACC: 456.1±50.95 vs. 105.7±24.14, p<0.001).

CONCLUSION: MBD-seq can be used to successfully identify differentially methylated genes in SGACC in a genome-wide, unbiased manner.

P009: THE IMPACT OF INTRATUMORAL LYMPHATIC VESSEL DENSITY ON SURVIVAL IN LOCALLY ADVANCED LARYNGEAL/HYPOPHARYNGEAL CANCER – Madana Jeevanandam, MD, Jose Jirron, MD, David Cohen, MD, Luz Barona-Lleó, MD, Naweed Raza, MD, Dennis Bojrab II, MD, Andrew Johnson, MD, John Jacobs, MD; Wayne State University

BACKGROUND: Lymphatic vessel density [LVD] has been shown to be an important predictor of survival in head and neck cancers. We report the predictive value of LVD for progression-free (PFS) and overall survival [OS] in locally advanced laryngeal/hypopharyngeal cancer.

METHODS: Fifty-five evaluable, untreated patients, with T3/T4 laryngeal and T4 hypopharyngeal cancer underwent laryngectomy between 1999-2010. Archived paraffin-embedded biopsy specimens were sectioned and stained with hematoxylin-eosin and anti-LYVE-1 antibody, a highly specific marker for lymphatic endothelium. LVDs were determined in tumor vessel "hot spots"using Chalkley point counting. Recursive partitioning analysis identified thresholds for LVDS of peri- [LVDit] and Intra-tumoral [LVDit] for association with PFS and OS.

RESULTS: Patients with mean LVDit of less than 11 vessels/mm2 had 2-year PFS and OS rates of 58% and 65% respectively, compared to 13% and 13% for those with LVDit greater than or equal to 11 vessels/mm2 (p= 0.06 and 0.04, respectively].

CONCLUSIONS: Intratumoral lymphatic vessel density is predictive of PFS and OS in locally advanced laryngeal/hypopharyngeal cancer.

P011: IL-6 PROMOTES STEMNESS AND ANOIKIS RESISTANCE IN CANCER CELLS VIA THE ACTIVATION OF FAK AND NANOG STABILIZATION – Arti Yadav, MS, Bhavna Kumar, MS, Theodoros N Teknos, MD, Pawan Kumar, MS, PhD; Department of Otolaryngology-Head and Neck Surgery, The Ohio State University, Columbus, OH 43210

The process of tumor metastasis is highly complex and involves dissemination of tumor cells from the primary tumor through the vascular system. However, most of the cancer cells, particularly epithelial cancer cells, have very low survival rates in circulation and undergo rapid anoikis. Tumor cells that acquire malignant potential have developed mechanisms to resist anoikis and thereby survive while travelling though the lymphatic or circulatory systems. Recently, we have shown that IL-6 promotes epithelial-mesenchymal transition (EMT) and tumor metastasis. A number of studies have shown that FAK signaling pathways play an important role in inducing anoikis resistance in tumor cells. In this study, we examined if IL-6 promoted tumor metastasis by enhancing stemness and anoikis resistance in cancer cells via the activation of FAK signaling. Our results demonstrate that

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IL-6 significantly enhances anoikis resistance in head and neck cancer cells. IL-6 treatment of cancer cells induced FAK phosphorylation in a time dependent manner and FAK knockdown significantly reversed IL-6-induced anoikis resistance. Recent studies have highlighted the role of FAK in stem cell expansion and maintenance. However, the precise role of FAK in stem cell regulation is not very well explored. Our results show that FAK knockdown significantly decreased IL-6-mediated tumorsphere formation and ALDH expression on cancer cells (cancer stem cell marker). IL-6 promotes stem cell phenotype and anoikis resistance in head and neck cancer cells via FAK activation and nanog protein stabilization.

P012: BRAF MUTATION DRIVES AGGRESSIVENESS OF FOLLICULAR THYROID CARCINOMA IN PRECLINICAL MOUSE MODELS – Andrea Ziegler, BA, Ashley Reeb, BS, Kaveh Karimnejad, MD, Wen Li, MD, Reigh-Yi Lin, PhD; Saint Louis University School of Medicine

BACKGROUND: Follicular thyroid carcinoma (FTC) is the second most common type of thyroid cancer with almost 10,000 cases being diagnosed annually in the United States. BRAF mutations are the most common mutations associated with papillary thyroid carcinoma, but have rarely been associated with FTC. This study explores the role of BRAF mutation in FTC in preclinical mouse models of thyroid carcinoma.

MATERIALS & METHODS: Three human FTC cell lines were studied. FTC-238 and TT2609-CO2 both have a known TP53 mutation, and WRO has a BRAFV600E mutation. qRT-PCR was performed to detect thyroid-specific genes in the three human FTC cell lines. Cell proliferation was performed using Alamar Blue assay. In vivo tumor growth was monitored by orthotopically implanting these cell lines into the thyroids of immunodeficient NOD/ScidIl2rg-/- to initiate primary tumors. These cell lines were also injected into the mouse lateral tail veins in order to monitor for metastatic lung disease. Tumor progression was evaluated in both groups by weekly bioluminescent live imaging as total photon counts per second using IVIS Spectrum. The mice were sacrificed when they reached humane endpoint and tumors were collected for histologic analysis postmortem.

RESULTS: The qRT-PCR analysis showed that FTC-238 and WRO expressed Pax 8, a thyroid transcription factor. One of the cell lines, TT2609-CO2, also expressed thyroid transcription factor 1 (TTF1). In contrast, none of the cell lines expressed thyroid differentiation markers such as thyroglobulin (TG), sodium/iodide symporter (NIS) or the receptor for thyroid stimulating hormone (TSHR), indicating the dedifferentiated state of these FTC cell lines. In vitro cell proliferation studies demonstrated that TT2609-CO2 showed the most aggressive growth, followed by FTC-238, and lastly WRO. This was not the case with the in vivo orthotopic mouse model. The WRO-derived tumors grew faster and were larger than were the tumors arising from FTC-238 and TT2609-CO2 cell lines. Mice implanted with WRO cells survived a total of 14-18 days, while mice implanted with FTC-238 cells survived 27-29 days. It took about 35 days for mice implanted with TT2609-CO2 cells to begin to develop tumors and these mice survived a total of 57-70 days. Post mortem histologic analysis demonstrated the presence of multiple lung metastases in a WRO-implanted mouse just 14 days after orthotopic implantation. The results were similar when we performed a tail vein injection assay for distant metastasis. Mice injected with WRO cell line exhibited more rapid and aggressive growth of bilateral lung metastases than the other two cell lines.

CONCLUSION: The three FTC cell lines display highly varied in vivo growth trends with WRO being the most aggressive cell line in the orthotopic and tail vein models. The increased aggressiveness of WRO compared to the other FTC cell lines is likely due to the BRAF mutation. These findings establish a potential role forBRAFV600E mutation in FTC as a future marker of tumor aggression as well as a therapeutic target for the treatment of similar neoplasms.

P013: RESTORATION OF MIR-124 REDUCES EGFR LEVELS AND POTENTIATES THE ANTI-TUMOR ACTIVITY OF EGFR/HER2 TYROSINE KINASE INHIBITORS – Manchao Zhang, PhD[1], Jharna Datta, PhD[1], Xiujie Xie, PhD[1], James Lang, PhD[1], Theodoros Teknos, MD[1], Anna Mapp, PhD[2], Quintin Pan, PhD[1];[1]The Ohio State University Medical Center, [2]University of Michigan

Over-expression of EGFR is a frequent event in head and neck squamous cell carcinoma (HNSCC). However, EGFR tyrosine kinase inhibitors (TKIs) have shown limited activity in numerous clinical trials for HNSCC. Molecular insight into the lack of therapeutic response with EGFR TKIs in this highly EGFR-positive HNSCC patient population remains to be established. We hypothesized that the therapeutic dose of EGFR TKIs is not sufficient to completely dampen the hyperactive EGFR signaling cascade in HNSCC cells. Therefore, simultaneous targeting of EGFR levels and activity may be an efficient dual-prong approach to maximally suppress the EGFR pathway resulting in an optimal therapeutic response. Epithelial-restricted with serine box (ESX), a member of the ETS transcription factor family, is elevated and associated with EGFR levels in primary HNSCC tumors. ESX was demonstrated to occupy and hyper-activate the EGFR promoter providing a novel molecular mechanism for EGFR dysregulation in HNSCC. miRs are known to modulate target protein levels and may be utilized as a therapeutic to suppress the levels and activity of an "oncogenic" protein. We performed an in silico miR analysis for ESX and found two predicted miR-124 binding sites located in the 3'-UTR of ESX. Delivery miR-124 inhibited the activity of the 3'UTR ESX-luciferase reporter by 50% (p<0.05). Mutations in any of the two miR-124 binding sites in the 3'UTR of ESX completely blocked the inhibitory effect of miR-124, thus, confirming ESX as a direct target of miR-124. Loss of miR-124 was found to a frequent event in primary HNSCC tumors and HNSCC cell lines. miR-124 expression was significantly depleted in the primary tumor compared to matched normal tissue in 100% (12/12) of HNSCC patients; mean miR-124 expression in matched normal tissue is 0.012 and mean miR-124 expression in primary tumor is 0.0011 (p<0.001, n=12). Over-expression of miR-124 decreased ESX and EGFR levels in miR-124low/ESXhigh/EGFRhigh SCC15 HNSCC cells and reduced cell invasion (61% inhibition, p<0.01), migration (40% inhibition, p<0.05), proliferation (64% inhibition, p<0.01) and colony formation (45% inhibition, p<0.01). HNSCC cells with miR-124 restoration were less tumorigenic in vivo than miR-control HNSCC cells (70% inhibition, p<0.01). Importantly, ectopic miR-124 expression in HNSCC cells potentiated (p<0.01) the anti-proliferative efficacy of the EGFR/Her2 TKIs, lapatinib and afatinib. Recapitulation of EGFR (using miR-resistant EGFR) in miR-124 over-expressing HNSCC cells was sufficient to completely block the anti-proliferative effects of lapatinib and afatinib. These results demonstrated that targeting EGFR levels through the miR-124/ESX signaling axis enhances the efficacy of EGFR/Her2 TKIs. In conclusion, our work provides intriguing evidence that novel therapeutic approaches to reduce EGFR protein, in this case, delivery of miR-124, may be used to enhance the response rate of HNSCC patients to current anti-EGFR/Her2 therapies.

P014: TARGETED SEQUENCING IDENTIFIES HER2 AMPLIFICATIONS IN LARYNGEAL SQUAMOUS CELL CARCINOMA – Andrew C Birkeland, MD[1], Megan L Yanik, BS[1], Morgan M Sandelski[1], Brittny N Tillman, MD[1], Laura M Bou-Maroun, BS[1], Thomas E Carey, PhD[1], Carol R Bradford, MD[1], Scott A Tomlins, MD, PhD[2], Jonathan B McHugh, MD[2], Matthew E Spector, MD[1], J Chad Brenner, PhD[1]; [1]University of Michigan, Department of Otolaryngology-Head and Neck Surgery, [2]University of Michigan, Department of Pathology

BACKGROUND: HER2 is a member of the EGFR family of transmembrane receptor tyrosine kinases that are involved in cell proliferation and growth. It has been shown to be amplified in approximately 15-30% of breast cancers and 10-30% of gastric and gastroesophageal cancers. In some breast cancer cases, HER2 has been successfully targeted with monoclonal antibodies (trastuzumab, pertuzumab), with a demonstrated significant improvement in overall survival and disease recurrence. To date, there have been no studies identifying HER2

amplifications in laryngeal cancer specimens. Identification of HER2 positive tumors could lead to new targeted therapy treatment options for head and neck cancers.

METHODS: 42 laryngeal squamous cell carcinoma tumor specimens were identified from patients previously treated at the University of Michigan. Patient demographics, stage, treatments rendered, and outcomes were recorded. Using targeted, amplicon-based sequencing with the Oncomine Cancer Panel, we assessed the copy number and mutation status of several common therapeutic targets. Immunohistochemistry staining was then performed on laryngeal squamous cell carcinoma tissue microarray samples to assess the protein expression of obvious therapeutic targets with genomic amplification.

RESULTS: Of the 42 samples, 4 were positive for HER2 amplification on sequencing. Two of the 4 samples identified with sequencing were scored to be positive for HER2 overexpression on immunohistochemistry. The first patient was a smoker who developed a T2N0M0 SCC of the glottis at age 41, initially treated with radiation. He developed a recurrence and underwent salvage total laryngectomy. The second patient was a smoker who developed a T4N0M0 SCC of the glottis at age 57, initially treated with a right hemilaryngectomy. He developed a recurrence, and was treated with salvage chemotherapy, and salvage laryngectomy and radiation. Both patients are deceased.

CONCLUSIONS: HER2 overexpression is identified in a subset of larynx cancer specimens (5-10%). These tumors are potentially responsive to targeted therapy against HER2, which may be beneficial for organ preservation protocols. Screening for HER2 amplification and applying targeted therapy in HER2 positive patients may provide a useful and successful clinical tool for personalized therapy in head and neck cancer patients, particularly in patients that are refractory to current treatment paradigms.

P015: TREATMENT VARIATION FOR NASOPHARYNGEAL CARCINOMA: IMPACT ON SURVIVAL – Zachary Schwam, BA[1], Julie A Sosa, MD, MA[2], Sanziana Roman, MD[2], Benjamin L Judson, MD[1]; [1]Yale University School of Medicine, [2]Duke University School of Medicine

IMPORTANCE: There is a paucity of data with respect to national treatment patterns for nasopharyngeal carcinoma (NPC), and it is unknown whether treatment variation is associated with overall survival (OS). The National Comprehensive Cancer Network (NCCN) publishes treatment guidelines, but it is not known whether patients are treated within these guidelines, or whether treatment outside of guidelines is associated with OS.

OBJECTIVE: Characterize national treatment patterns, determine treatment relative to NCCN guidelines, analyze whether treatment outside of guidelines is associated with OS, and identify patient and clinical variables associated with receiving care outside of NCCN guidelines for nasopharyngeal carcinoma.

DESIGN, SETTING, AND PARTICIPANTS: Retrospective cohort study of 2718 patients diagnosed with nasopharyngeal carcinoma in the National Cancer Data Base (1998-2011).

INTERVENTION: Treatment of NPC with radiation, chemotherapy, chemoradiation, and/or neck dissection.

MAIN OUTCOMES & MEASURES: Overall survival (OS), proportion of patients not receiving care within NCCN guidelines, and factors associated with treatment outside of NCCN guidelines. Statistical analysis included chi-square, multivariate logistic regression, Kaplan-Meier, and Cox regression.

RESULTS: Patients with NPC had stage I (8%), II (24%), III (31%), IVA/B (32%), and IVC (5%) disease. The proportion of patients receiving treatment outside of NCCN guidelines was 34.3% for stage I, 26.5% for stage II, 20.2% for stage III, 23.0% for stage IVA/B, and 55.9% for stage IVC. Factors associated with treatment outside of NCCN guidelines included: ages 65-79 (OR 1.52, p=.007)

and ≥80 years (OR 3.51, p<.001), being uninsured (OR 1.50, p=.016), Asian race (OR 0.66, p=.001), and having stage III (OR 0.52, p<.001), IVA/B (OR 0.59, p=.002), or IVC disease (OR 2.45, p<.001). Factors associated with decreased OS included receiving care outside of NCCN guidelines (OR 1.30), ages 45-64 (OR 1.78), 65-79 (OR 2.48), and ≥80 years (OR 4.66), having government insurance (OR 1.66), Charlson score of 1 (OR 1.43) (all p<.001), and stage III, IVA/B, and IVC disease (OR 1.46, 2.45, 5.12, respectively, all p≤.002). Asian race was a predictor of increased OS (HR 0.64, p<.001). Receiving care outside of NCCN guidelines was associated with compromised survival for stage II, III, and IVA/B disease in Kaplan-Meier analysis (all log-rank p<.001).

CONCLUSIONS & RELEVANCE: A significant proportion of patients with nasopharyngeal carcinoma have not received care within NCCN guidelines; this is particularly the case for patients with stage I and IVC disease. Receiving care outside of the NCCN practice guidelines was found to be associated with compromised survival for multiple stages of disease. Further study is needed in order to characterize the obstacles to delivering treatment in accordance with practice guidelines.

P016: INCREASING PREVALENCE OF OROPHARYNGEAL CANCER SURVIVORS IN THE UNITED STATES – Mira A Patel, BA, Amanda Blackford, ScM, Carole Fakhry, MD; Johns Hopkins School of Medicine

BACKGROUND: Human papillomavirus (HPV) infection is responsible for a rising incidence of oropharyngeal cancer (OPC) in the United States (U.S.). Patients with HPV-related OPCs are younger than patients with HPV-unrelated OPC and have improved prognosis. This implies that there is an increasing prevalence of survivors with HPV-related OPC. However, the actual disease burden of HPV-related OPC relative to HPV-unrelated OPC in the U.S. is unknown.

METHODS: Data was collected from 18 Surveillance, Epidemiology, and End Results (SEER) program registries (1973-2011) for survival analysis and from 9 SEER registries for all other analyses. Cases of squamous cell histology OPC were classified by anatomic location as HPV-related (n=38,478 in 18 registry [18 regs] analysis, n= 25,404 in 9 registry [9 regs] analysis) and HPV-unrelated (n=43,295 in 18 regs, n= 35,311 in 9 regs). Five-year survival and annual percentage change (APC) in incidence were calculated using the actuarial method and weighted least squares method, respectively. Crude prevalence percentages were calculated at 5-year intervals.

RESULTS: The incidence of OPCs overall has increased from 6.7 per 100,000 in 1973 to 7.2 per 100,000 in 2011. Incidence has increased in males (9.6 to 10.8 per 100,000, APC=.003, p=.97) and decreased in females (4.4 to 3.9, APC= -.67, p<.001). Incidence of HPV-related OPCs increased significantly from 1973 to 2011 (2.13 to 3.73, APC=1.3, p<.001), although this increase was primarily in males (APC=1.83, P<.001) and remained nearly constant in females. Incidence of HPV-unrelated OPCs declined significantly (from 3.9 to 3.1, APC= -.99, p<.001) in both males and females. The 5-year survival rate in OPCs overall rose from 36.7% (95% CI 35.1-38.4) in 1980 to 57.4% (95% CI 56.6-68.3) in 2011. There was a greater increase in 5-year survival rate in HPV-related than HPV-unrelated OPCs (26.5%, CI 23.9-29.1 to 62%, CI 60.9-63.1 vs. 42.0%, CI 39.9-44 to 51.5%, CI 50.3-52.8, respectively). The prevalence of OPC survivors overall rose from 0.06 per 100,000 in the calendar period 1976-1981 to 20 per 100,000 (% Standard Error (SE)=.00001% and 0.0003%, respectively) in the calendar period 2006-2011. The increase in males was 0.07 to 40 per 100,000 (SE=.00002% and 0.0005%, respectively) and in females was 0.05 to 10 per 100,000 (SE.00002% and.0003%, respectively). Prevalence of HPV-related OPC increased from 0.02 to 10 per 100,000 (SE=.00001% and SE=.00022%, respectively), while prevalence of unrelated OPCs rose 0.04 to 10 per 100,000 (SE=.00001% and .0002%, respectively) during this period.

CONCLUSIONS: There is a significant rise in 5-year survivors of OPC in the United States from 1980-2011, which reflects a combination of significantly increasing

incidence of HPV-related OPCs and improving survival. This dramatic increase in prevalence of OPC survivors emphasizes the importance of long-term follow-up and management of the late effects of cancer therapy.

P017: ORAL SQUAMOUS CELL CARCINOMA IN MOROCCAN POPULATION : A RETROSPECTIVE STUDY – Narjiss Akerzoul, RESIDENT, ORAL, SURGERY, DEPARTMENT, Saliha Chbicheb, Professor, of, Oral, Surgery, Wafaa El Wady, Professor, chief, service, of, Oral, surgery; Center of Consultation and Dental Treament of Rabat- Mohammed V University-Rabat-Morocco

INTRODUCTION: Cancers of the oral cavity are an integrate part of cancers of the oropharynx, larynx and pharynx, in the particular disease entity cancers of the upper aero digestive tract (VADS). These oral cancers represent 25-30% of all head and neck cancers.

The aim of the present work is to understand and evaluate the epidemiological characteristics of these cancers in our Moroccan population, especially in the southern region of Morocco.

MATERIALS & METHODS: This is a retrospective study of 100 patients hospitalized between 2010 and 2013 in the service of Oncology of the Ibn Rochd Hospital of Casablanca, and in which we did diagnosed a confirmed cancer of the oral cavity.

RESULTS: Men in our sample represented 66% and women 34%. The patients' ages ranged between 23 and 93 years. Most patients were of low socioeconomic status (61%) and viewed during the second six months of the appearance of the tumor. The most common risk factors were smoking (36%) and poor oral health status (56%). The lip was the most common location in 34% of cases. On histology, squamous cell carcinomas was the most predominant (90%).

DISCUSSION: Epidemiological, clinical and pathological characteristics of cancers of the oral cavity in our population are not different from the literature data.

Our results are comparable to those found in the oncology center of MARAKECH and AGADIR and the National Institute of Oncology of Rabat. They are also close to those found in Tunisia, Myanmar, China and other countries around the world.

However, the lack of awareness and late diagnosis of these lesions appear to be responsible for the dramatic profile of oral cancers.

P018: AN OPTICAL IMAGING THRESHOLD TO DETECT HEAD AND NECK CANCER DURING INTRAOPERATIVE FLUORESCENCE-GUIDED SURGERY – Lindsay S Moore, BS, Esther de Boer, BS, Eben L Rosenthal, MD, Jason M Warram, PhD; University of Alabama at Birmingham

During fluorescence-guided surgery, a cancer-specific optical probe is injected and visualized using a compatible device in the intra-operative setting to provide visual contrast between diseased and normal tissues. However, the current classifications used to define the extent of fluorescence within imaged tissues are often purely qualitative, undefined, and lacking distinct criteria for fluorescence comparison. A quantitative reporting criteria using standardized methods is necessary for widespread approval and advancement of this technique. Here, we introduce a ratiometric value to assess tissue fluorescence in real-time in order to objectively distinguish between normal and cancerous tissue during fluorescence-guided surgery. To accomplish this, imaging was performed on punch biopsy tissues acquired from resected primary tumors of head and neck cancer during a phase 1 dose-escalation trial evaluating the safety and tumor-specificity of cetuximab-IRDye800. During the study, 48 punch biopsies of tumor (n=30) and normal (n=18) tissue were collected from areas of high and low fluorescence intensity in nine resected primary tumors from patients who received the fluorescent study probe. Two fluorescent imaging devices, a wide-field (LUNA) and closed-field (Pearl) device, were used to evaluate the approach and assess the variability of a ratiometric threshold between different imaging devices. Punch

biopsy tissues were subsequently imaged using both imaging devices, and mean fluorescence intensity (MFI) was calculated for each. Additionally, skin and muscle samples were collected and imaged to serve as internal anatomic controls for each patient, thus establishing a patient-matched "background" fluorescence to ensure the ratiometric value would account for inherent variability between patient tissues (Figure 1).
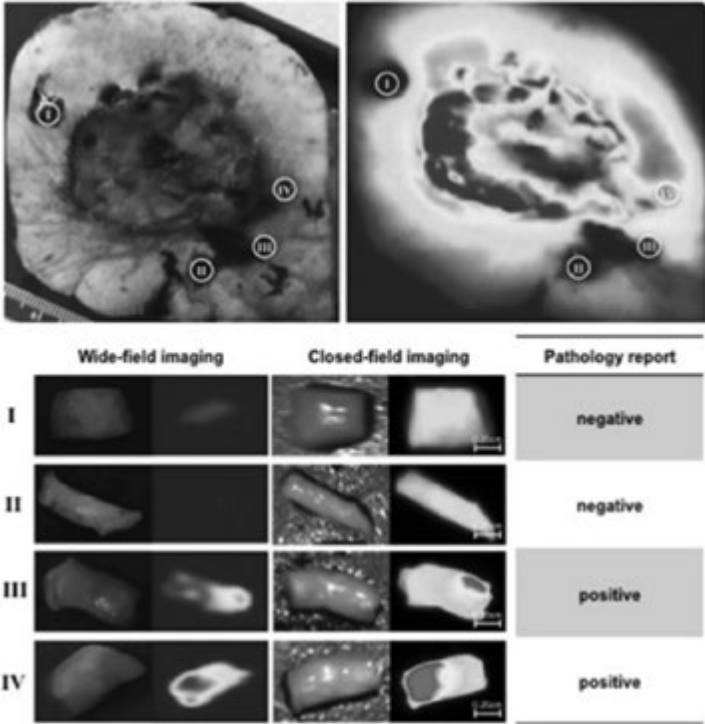

FIGURE 1: Bright-field (left) and fluorescence (right) images of tissue specimen and corresponding biopsy samples.

Specifically, skin and muscle were selected as normalizing tissues in this study given cetuximab's inherent targeting of EGFR in normal skin. For each biopsy specimen, a fluorescence ratio was calculated by dividing the MFI of each specimen by the MFI of patient-matched background tissues (muscle or skin). Ratios were correlated to pathological assessment (gold standard) in order to determine a ratiometric threshold to predict the presence of malignant tissue using fluorescence imaging. For the wide-field intraoperative device, when the muscle-normalized ratiometric value was less than 2.7, the negative predictive value (NPV) was 89%. When skin-normalized values were less than 1.1, the NPV was 100%. When MFI obtained from the closed-field device were analyzed, the muscle-normalized NPV was 100% using a threshold of 3.2 and skin-normalized NPV was 94% using a threshold of 1.5. Table 1 shows the accuracies of disease detection using corresponding thresholds.

Table 1: Accuracy of tumor detection among devices and ratios tested.

Device	Standardizing Tissue	Ratiometric Thresholding Value	Sensitivity	Specificity
Wide-field	Muscle	2.7	95.2%	61.5%
	Skin	1.1	100%	69.2%
Closed-field	Muscle	3.2	100%	72.2%
	Skin	1.5	96.6%	83.3%

To demonstrate the accuracy of the ratiometric value method, receiver operator characteristic (ROC) analysis was performed and demonstrated a significantly

(p<0.01) greater AUC than the chance diagonal with a 95% confidence interval for both skin and muscle normalized ratios using wide- and closed-field imaging devices. This proof of concept study yielded a highly sensitive, semi-quantitative threshold for objective determination of the presence of cancer using fluorescence imaging. Furthermore, statistical assessments (sensitivity, specificity, and ROC analysis) of the ratiometric threshold confirmed the accuracy of this diagnostic tool.

P019: SENSITIZATION OF SALIVARY ADENOID CYSTIC CARCINOMA STEM CELLS TO RADIATION THERAPY – Alex Panaccione, BS[1], Michael T Chang, BS[2], Bea Carbone, BA[2], Manju Prasad, MD[2], Gary Bellinger, BS[2], Wendell Yarbrough, MD, MMHC, FACS[2], Sergey Ivanov, PhD[2]; [1]Vanderbilt University,[2]Yale University School of Medicine

Adenoid cystic carcinoma (ACC), which accounts for nearly one quarter of malignant neoplasms of the salivary gland, is a slow-growing yet unpredictable tumor characterized by insidious local spread, perineural invasion, and distant metastases. Therapeutic options are limited to surgery with or without radiation; however ACC is often resistant to radiation and has a high recurrence rate. ACC currently lacks targeted therapies due to a limited understanding of the molecular basis driving this cancer. Our recent expression analysis of clinical ACC specimens suggested the existence of cells with neural stem properties in this cancer. To validate this hypothesis, we generated cell culture from an aggressive high-grade ACC tumor. These cells showed high proliferation rate, spontaneous spheroidogenesis, and high levels of four markers/genes characteristic for neural stem cells: SOX10, NOTCH1, FABP7, and CD133. CD133-positive cells isolated from cultured ACC cells line co-expressed SOX10, NOTCH1, and FABP7 and showed increased tumorigenicity in nude mice as compared to CD133-negative cells. Since activated NOTCH1 is linked to resistance of cancer stem cells to radiation, we investigated the effects of NOTCH1 signaling inhibition on the sensitivity of CD133-positive cells to irradiation. As we demonstrated by FACS analysis and real-time RT-PCR, the combination of irradiation with DAPT, an inhibitor of g-secretase and NOTCH1, depleted CD133-positive cells but had no obvious effect on NOTCH1-negative CD133-negative cells. Thus, we proved in principle that CD133-positive ACC cells with stem properties could be sensitized to radiation therapy with NOTCH1 inhibitors, opening a new avenue for developing effective combinatorial therapies for this cancer.

P020: POSITIVE CO-RELATION BETWEEN CANCER STEM CELL, RESISTANCE AND EMT PROFILE IN ORAL SQUAMOUS CELL LINE – Safeena Kulsum, MSc, MTech, PhD, Scholar[1], Moni A Kuriakose, FDSRCS, LMSSA, FRCS[2], Amritha Suresh, PhD[1]; [1]DSRG-5, Mazumdar Shaw Centre for Translational Research, Mazumdar Shaw Medical Centre, [2]Head and Neck Oncology, Mazumdar Shaw Medical Centre, Narayana Hrudayalaya

EMT (Epithelial-mesenchymal transition) confers mesenchymal property to epithelial cells and are closely associated with acquisition of aggressive traits by carcinomas cells. Emergence of cancer stem cells is known to be strongly related to the adoption of the features of EMT. Our primary objective was to evaluate any positive correlation between the stem cell and EMT profile in drug resistant oral squamous cell carcinoma cell lines. Single resistant sub-lines (Cal-27 CisR, Cal-27 DoxR, Cal-27 5FuR, HEp-2 CisR, HEp-2 DoxR, HEp-2 5FuR) developed in lab were evaluated for its increased cancer stem cell (CSC) specific gene profile and functional property. CSC enriched resistant sublines were further evaluated for its EMT profile using gene expression EMT specific (QRT), Increased migration profile (Scratch assay) and invasion profile (Gel-invasion assay).

Resistant sublines (Cal-27 CisR, DoxR and 5FuR) showed increased cancers stem cell profile using expression profile (QRT) and various functional assays (migration, colony formation, colony survival, spheroid assays) also validated CSC profile. Currently, we are further evaluating the EMT specific gene profile (E-Cad, N-Cad,

Snail, Slug, Twist, EpCam, Vimentin) of CSC enriched resistant sublines along with other functional assays in an effort to further affirm this hypothesis.

P021: MOLECULAR SUBTYPE DICTATES HYPERSENSITIVITY TO ERBB3 INHIBITION IN HNSCC – Loren Michel, Lamont Jones, Sora Lim, Alex Tinianow, Nathan Redlich, Ravi Uppaluri; Washington University in St. Louis

In head and neck squamous cell cancer (HNSCC), four intrinsic subtypes (or groups) have been identified, and each one possesses a unique biology that will require specific treatment strategies. We recently reported that the mesenchymal subtype of HNSCC is enriched for ErbB3 activation. We also showed that HNSCC xenografts exhibiting ErbB3 activation are hypersensitive to anti-ErbB3 antibodies, which cause a dramatic growth arrest phenotype in vitro and anti-tumor response in vivo. The mechanistic basis for ErbB3 hyperactivation in HNSCC is loss of the neuregulin-1 binding protein Trop2. We have now extended these studies from both a pre-clinical and biomarker perspective. Using patient derived-xenografts (PDX), we find that anti-ErbB3 treatment of an unselected panel of ten of these tumors causes a more robust anti-tumor response than cetuximab, and approximates the efficacy of cisplatin. Gene expression analysis of ErbB3 hyperactivated and ErbB3 treated tumors reveals that ErbB3 is a major regulator of both cell cycle and metabolic genes in HNSCC cell lines and patient derived xenografts. Antibody treatment alone is sufficient to suppress expression of multiple regulators of the Warburg effect, and strikingly, suppresses the Wnt pathway, a known driver of tumor glycolysis. Based on these data, we are moving forward with a biomarker directed clinical trial of ErbB3 inhibition in HNSCC.

P022: SURGICAL SALVAGE IMPROVES OVERALL SURVIVAL FOR HPV-POSITIVE AND HPV-NEGATIVE RECURRENT LOCOREGIONAL AND DISTANT METASTATIC OROPHARYNGEAL CANCER – Theresa Guo, MD[1], Jesse R Qualliotine[1], Patrick K Ha, MD[1], Joseph A Califano, MD[1], Young Kim, MD[1], John R Saunders, MD[2], Ray Blanco, MD[2], Gyspamber D'Souza, PhD[3], Zhe Zhang, MS[4], Christine G Gourin, MD[1], Wayne Koch, MD[1], Jeremy D Richmon, MD[1], Nishant Agrawal, MD[1], David W Eisele, MD[1], Carole Fakhry, MD, MPH[1]; [1]Department of Otolaryngology, Head and Neck Surgery, Johns Hopkins Medical Institutions, [2]Milton J. Dance Head and Neck Center, [3]Johns Hopkins Bloomberg School of Public Health, Johns Hopkins Medical Institutions, [4]Division of Oncology Biostatistics, Johns Hopkins Medical Institutions

BACKGROUND: Human papillomavirus (HPV) is now an established etiologic agent for a growing subset of oropharyngeal squamous cell carcinoma (OPSCC). HPV is a biomarker for improved prognosis in primary OPSCC. Since the identification of this prognostic biomarker, the OPSCC patient population has now matured enough to study the impact of HPV on recurrent and metastatic disease. HPV tumor status and surgical salvage were recently shown to be independently associated with improved prognosis after recurrence, but data on surgical procedures and for patients with distant metastatic disease was limited.

METHODS: A retrospective analysis of patients with recurrent OPSCC from two institutions between 2000-2012 was performed. P16 immunohistochemistry and/or in situ hybridization, as clinically available, were used to determine HPV tumor status. Clinical characteristics, distribution of recurrence site, method of diagnosis, and treatment modalities were compared by HPV tumor status. Time to recurrence and overall survival were examined by Kaplan-Meier and Cox proportional hazards methods.

RESULTS: The study population comprised of 108 patients with 65 locoregional and 43 distant metastatic first recurrences. The majority were HPV-positive (n=80). HPV-positive patients with recurrent OPSCC were more likely to be white (p<0.01), male (p<0.01), and never smokers (p=0.06) but similar age of diagnosis (p=0.95) compared to HPV-negative patients. Site distribution (local, locoregional, distant) of recurrences did not differ by HPV tumor status (p=0.83).

Anatomic site of distant metastatic disease was similar for HPV-positive and HPV–negative patients (p=0.41). The most common site of distant metastatic disease was lung (HPV-positive 69.7% vs. HPV-negative 50.0%). The majority of recurrences occurred within two years after primary diagnosis for both HPV-positive (66.0%, 95%CI 55.4-76.3) and HPV-negative patients (89.3%, 95%CI 74.9-97.3). HPV-positive tumor status was associated with longer time to recurrence (p<0.01). HPV-positive recurrences were primarily diagnosed by imaging (47.4%) whereas clinical examination (46.4%) was the primary method of diagnosis for HPV-negative recurrences (p=0.02).

Of patients receiving salvage treatment (n=94), a majority received surgical salvage (n=60), usually with adjuvant radiation (n=42). Locoregional salvage surgery (n=45) included wide local excision ±neck dissection (n=28, including TORS n=3), total laryngectomy ±total glossectomy (n=7), and neck dissection only (n=10). Twenty-one were extensive enough to require tracheostomy (n=13), gastrostomy tube (n=9), and/or free flap reconstruction (n=14). Surgical salvage for distant metastasis was performed for 16 patients, primarily pulmonary resections (n=9). Other procedures performed were mediastinal lymphadenectomy, craniotomy, dermal excision, hepatectomy and laminectomy.

HPV-positive tumor status (adjusted HR [aHR] 0.29, 95%CI 0.12-0.71, p=0.007), longer time to recurrence (≥1 year; aHR 0.33, 95%CI 0.16-0.70, p=0.004) and surgical salvage (aHR 0.29, 95%CI 0.14-0.63, p=0.002) were independently associated with improved overall survival after recurrence. Surgical salvage was independently associated with improved overall survival compared to non-surgical treatment in both locoregional (aHR 0.25, 95%CI 0.08-0.80, p=0.02) and distant metastatic recurrence (aHR 0.20, 95%CI 0.05-0.76, p=0.02).

CONCLUSIONS: Anatomic sites of recurrent OPSCC do not differ by HPV tumor status. HPV-positive tumor status is associated with longer time to recurrence. Surgical salvage is associated with improved overall survival for both recurrent locoregional and distant metastatic OPSCC, independent of HPV tumor status.

P024: ARTISS® IN PAROTID SURGERY: ARE SUCTION DRAINS STILL REQUIRED? – K Davies, C Heffernan, K Majeed, O Young, J Lang; University Hospital Galway

OBJECTIVE: Superficial parotidectomy traditionally necessitates the insertion of suction drains. Our objective was to assess the safety and efficacy of the fibrin sealant Artiss® in minimizing or eliminating the need for postoperative suction drainage.

METHOD: We carried out a chart review of parotidectomies performed by two consultant head and neck surgeons at a regional referral centre between January 2011 and December 2012 where suction drains were routinely used in all cases. We prospectively compared these results with parotidectomies performed by the same 2 surgeons between January 2013 and July 2014, where Artiss ® was used routinely prior to skin closure with or without suction drainage. Our study comparison included drain output and length of inpatient hospital stay in both sets.

RESULTS: We performed 42 parotidectomies, 17 with neck dissection between January 2011 and December 2012. All patients had suction drains inserted. Mean total drain volume was 20mls and mean length of stay was 5 days. We encountered 2 haematomas and one seroma. We compared these results to 43 parotidectomies, 17 with neck dissection carried out between January 2013 and July 2014. Mean total drain volume, where inserted was 2mls and mean length of stay was 1 day. We encountered one haematoma and one seroma.

CONCLUSION: Artiss® fibrin sealant when applied prior to skin closure, significantly decreases total drain volume. The use of suction drains may be reduced and potentially eliminated, resulting in shorter length of hospitalization, improved patient satisfaction and a decrease in hospital cost.

P025: GENOMIC ANALYSIS AND PERSONALIZED CANCER THERAPY IN MEDULLARY THYROID CANCER – Rajan P Dang, BA, Ross Cagan, PhD, Eric Genden, MD, Michael Donovan, MD, Eric Schadt, PhD, Marshall Posner, MD, Peter Smibert, PhD, Adnan Kahn, BA, Lewis Winder, Nadia Camille, Claire Davis, MSMG, Krzysztof Misiukiewicz, MD; Icahn School of Medicine

BACKGROUND: Standard approaches in cancer research identify new therapies based on observed benefit to average populations, but without emphasis on individual patients whose responses can vary considerably. Further, targeted therapies rarely account for the genomic complexity of patient tumors; the result is poor efficacy and rapid resistance. Ideally, we would identify drugs or drug cocktails that (i) target the details of an individual's tumor and (ii) account for its complexity. Models using the fruit fly Drosophila represent a potential new paradigm in cancer therapy. We have developed fly models that can include up to 10 of a patient's driver mutations; the result is an inexpensive drug screening platform to identify drug cocktails through empirical screening. As validation flies helped identify vandetinib, now approved for treatment of metastatic MTC.

METHODS: Tumor mutations identified by deep DNA and RNA sequencing of individual tumors are screened for tumor drivers, which are then incorporated into the "personal" Drosophila model and tested against a library of FDA approved drugs. Fly mortality is used as a surrogate for toxicity and increased survival to adulthood; improvements in tumor mutation-linked eye and/or wing abnormalities serve to quantify efficacy. This allows rapid and parallel screening of up to 800 drugs and subsequent drug combinations. The most efficacious and least toxic combinations are tested in xenograft models and a multidisciplinary tumor board of experts select the best therapeutic option.

OBJECTIVES: To demonstrate that the personalized drosophila model approach is superior to the current standard, cabozantinib, which performed best in MTC with a 27% response rate (RR) against placebo in a phase III trial but with considerable toxicity. Using the 27% RR as a benchmark, we will apply a sequential Bayesian method for 50 MTC patients enrolled to receive personalized treatment to demonstrate that this approach has greater efficacy, or at minimum, substantially similar efficacy with reduced toxicity.

P026: HEAD AND NECK SURGERY IN THE 21ST CENTURY: TRANSITIONING TO A "WORLD B" CULTURE – Ansley M Roche, MD, Amy Y Chen, MD, Mihir R Patel, J. Trad Wadsworth, Douglas E Mattox, MD, Mark W El-Deiry, MD; Emory University

INTRODUCTION: Delivery of healthcare in the United States faces significant challenges in the current era of cost-containment policies and performance-based payment systems. Complex medical systems, such as high-volume tertiary care centers, must anticipate the impact of policy changes and evolve in order to continue to deliver effective, state-of-the-art, quality healthcare without significantly increasing healthcare costs. The Patient Protection and Affordable Care Act of 2010 introduced payment incentives for improved quality of healthcare delivered, so called "World B," representing a shift from pay-for-service healthcare. Primary care quality metrics, such as tobacco prevention, hypertension screening, and vaccinations, have been clearly described in the literature. Quality assurance of surgical specialties has proven more difficult to characterize, however there is evidence in the literature of the success of a provider-driven pay-for-performance program for acute cardiac surgical patients. This new model is based on a rigorous pre-operative assessment and resulted in decreased length of hospital stay, decrease in hospital costs, and increase in home discharge location. Similar models in the field of otolaryngology in general, and head and neck oncology in particular, are lacking.

METHODS: In early 2014, the Division of Head and Neck Surgery at Emory University created a new model for delivery of integrated care for head and neck cancer patients. Through a financial relationship with Emory University Hospital Midtown, and together with Rehabilitation Medicine, Psychiatry, Social Services,

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Speech Therapy, and patient care coordinators, the Division implemented a pre-operative, perioperative, and post-operative clinical pathway for patients undergoing head and neck surgery. Because of their significant complexity and measurable clinical outcomes, patients undergoing free tissue transfer represent the ideal opportunity to examine the effect of this integrated approach. The perioperative clinical pathway was initiated September 1, 2014 to address costs associated with intensive care management and entails early extubation on post-operative day zero, where possible.

RESULTS: Since implementation of this pathway, 11 patients have undergone free tissue reconstruction. In comparing these patients to preceding free tissue transfer patients at our facility, preliminary data demonstrates that this care pathway has resulted in improved quality outcomes for the division including statistically significant decrease in the number of ventilator days (0.82 days ±2.09 vs 3.09 days ±3.46, p=0.035) as well as statistically significant decrease in the intensive care unit (ICU) length of stay (3.82 days ±1.54 vs 6.63 days ±4.48, p = 0.041). There was a difference, although not statistically significant, in the length of hospital stay (8.84 days ±2.45 vs 12.80 days ±7.52, p=0.08).

CONCLUSIONS: Initiation of a perioperative clinical pathway has resulted in a significant decrease in the number of ventilator days and length of stay in the ICU. Development of new quality-based pathways in the field of otolaryngology provides the foundation on which other surgical specialties can build in the current age of healthcare reform.

P027: METABOLIC COMPARTMENTALIZATION IN ORAL CAVITY SQUAMOUS CELL CARCINOMA – Mehri Mollaee, MD, TingTing Zhan, MS, Madalina Tuluc, MD, Jennifer Johnson, MD, Paolo Cotzia, MD, William Keane, MD, David Cognetti, MD, Adam Luginbuhl, MD, Ubaldo Martinez-Outschoorn, MD, Joseph M Curry, MD; Thomas Jefferson University

IMPORTANCE: Metabolism may differ in varying regions of a tumor, and these compartments may be coupled for energy transfer permitting enhanced growth, invasion, or metastasis. When identified, this metabolic compartmentalization (MC) can have significant prognostic impact. Transporter of mitochondrial membrane 20 (TOMM20) and monocarboxylate transporter 1 (MCT1) are markers of oxidative metabolism. MCT4 is a marker of glycolytic metabolism, and Ki-67 is a marker of cellular proliferation.

OBJECTIVE: To assess the significance of MC in HNSCC in the tumor and peritumoral stroma.

STUDY DESIGN: Retrospective cohort.

METHODS: Clinical data were collected for 86 consecutive patients with oral HNSCC. Tumor resection specimens were IHC stained on tissue microarray for MCT1, MCT4, TOMM20 and Ki-67. IHC staining was assessed and quantified via digital image analysis and staining intensities were compared to clinical risk factors and outcomes.

RESULTS: Low staining of MCT1 was identified in peritumoral stroma. Increased IHC staining for MCT1 was identified in tumor cells typically in the tumor periphery. Increased intensity for MCT1 and TOMM20 was associated with an increased risk of recurrence, OR 4.45 (p=0.001) and OR 7.03 (p=0.02), respectively. Increased IHC staining for Ki-67 in tumor cells was also associated with increased risk of recurrence OR 2.06 (95% CI: 1.33,3.48, p=0.003). Peritumoral stromal cells stained more strongly for MCT4.

CONCLUSIONS: The IHC staining patterns of MCT1, MCT4, and TOMM20 suggest MC occurs between the cancer cells and tumor stroma with both glycolytic and oxidative compartments existing in HNSCC. This coupling may predict recurrence.

P028: A PRELIMINARY STUDY IN THE USE OF DATA MINING TO DETERMINE PATIENT SURVIVAL IN TEMPORAL BONE SQUAMOUS CELL CARCINOMA – At Harris[1], Ma Lones[2], J Wilson[1], N Upile[1], Sc Leong[3], Thj Lesser[3]; [1]University of Liverpool, [2]Heriot-Watt University, [3]Aintree University Hospitals NHS Foundation Trust

BACKGROUND: Temporal bone squamous cell carcinoma (TBSCC) comprises a group of rare, aggressive tumours accounting for approximately 0.2% of head and neck cancers. The scarcity of these tumours means a lack of prospective clinical trials posing a challenge for best treatment options. Evidence for management comes from the clinical experience of the treating clinician and the multidisciplinary team, along with reported small case series. The curative management of advanced stage tumours (T3/T4) comprises radical surgery which usually involves a resection of the temporal bone, a portion of the mandible may be excised, parotidectomy, neck dissection and free tissue transfer reconstruction with post-operative radiotherapy. Outcome for patients undergoing such radical treatment is still in the order of 30%, 5 year survival. Such radical treatment is not always appropriate for each patient; co-morbid factors may prevent such extensive surgery. However, anyone deemed fit enough for the procedure will be offered it as their only alternative even though tumour factors may mean their survival is poor. It would be advantageous to be able to classify patients in to those who would potentially benefit from such treatment and those who would not.

Data mining is used to recognise and extract patterns within datasets allowing the construction of classifiers. This technique employs artificial intelligence, machine learning and statistics to analyse data without a-priori hypothesis. Since this allows the construction of decision trees, we expect data mining could be used to provide an algorithm to determine the survival for patients undergoing surgery for TBSCC.

METHODS: Thirty-five patients over a twenty year period underwent surgery +/- adjuvant radiotherapy with curative intent for TBSCC at Aintree University Hospital. Data collected for these thirty-five patients included patient demographics, tumour stage (83% were stage IV), histological grade, facial nerve and parotid involvement, co-morbidities, type of surgical procedure, whether post-operative radiotherapy was undertaken and patient survival.

Small data sets are challenging for data mining. In order to generate an accurate classifier (a learning algorithm), it is necessary to maximise the proportion of data used for training it. However, this leaves little data to verify that the classifier will generalise to unseen cases. To mitigate this situation, we use ensemble classifiers, which use voting between multiple, diverse classifiers trained on sub-samples of the data. These are known to be more accurate and to generalise better from small training sets than single classifiers. To estimate accuracy on unseen cases, we also used leave-one-out cross-validation, an approach that maximises the data available for training whilst still providing a robust estimate of performance on unseen data.

RESULTS & CONCLUSIONS: Data mining is a novel approach for data analysis within the clinical setting. Our preliminary results suggest that classifiers can predict the likelihood of survival in around 80% of cases. Analysis of the classifiers' decision trees indicated the more extensive the temporal bone resection (petrosectomy compared to lateral temporal bone resection) along with radical parotidectomy was associated with poor 5 year survival. A better functioning facial nerve post-surgery was associated with a greater chance of survival.

P029: ANTITUMOR EFFICACY OF ARUM PALAESTINUM-BASED FORMULATION GZ17 6.02 IN HEAD AND NECK SQUAMOUS CELL CARCINOMA – Vikalp Vishwakarma, Lisa Stehno-Bittel, Sufi Thomas; University of Kansas Medical Center

Head and neck squamous cell carcinoma (HNSCC) have a propensity for aggressive growth and metastasis, with high morbidity and low 5-year survival rates. Despite advances in treatment, the overall survival rate has only modestly improved over the past several years. Herbal tea extract from the root and leaves of Arum palaestinum has been used to prevent and facilitate treatment of cancer in Palestine. The mechanisms and antitumor efficacy of this nutraceutical remain unknown. Here we tested the antitumor efficacy of a fortified racemic mixture of Arum palaestinum extract (GZ17S). In addition, we tested synthetic formulations of the active ingredients; GZ17 6.02 (containing 3 active components) and GZ17 5.0 (containing 17 active components) in preclinical models of HNSCC. Our preliminary data demonstrates that GZ17 S, GZ17 6.02 and GZ17 5.0 have antitumor efficacy in several cancers including HNSCC, lung and ovarian cancers. Further, GZ17 6.02 was the most potent formulation of the three. We hypothesized that GZ17 6.02 inhibits phosphorylation of key molecules that mitigate HNSCC progression and induce apoptotic cell death. Cytotoxicity of GZ17 6.02 was investigated in two HNSCC cell lines, OSC19 and UMSCC1. The ED50 values for GZ17 6.02 was found to be less than 10 µg/ml. A prerequisite for tumor metastasis is the ability of cancer cells to migrate and invade through extracellular matrix. To assess the effect of GZ17 6.02 on invasion and migration, OSC19 cells were seeded in Boyden trans-well chambers with or without matrigel, respectively. The percent invasion/migration in treatment arm relative to the vehicle control (100%) was determined. The GZ17 6.02 significantly reduced invasion of OSC19 cells compared to vehicle control (P<0.001). Further, GZ17 6.02 -induced cytotoxicity was found to be caspase-9-independent in HNSCC cells. Drugs affecting cell proliferation frequently alter cell cycle progression. Flow cytometric analyses of GZ17 6.02 treated HNSCC revealed a significant reduction in cells in the G2-M phase and increased apoptosis compared to vehicle control treated cells. In order to identify the molecular mechanisms of GZ17 6.02 cytotoxicity in HNSCC, we determined the levels of phosphorylated proteins in treated cells. It was observed that the expression of several phospho-proteins including p38, mitogen activated protein kinase (MAPK), mammalian target of Rapamycin (mTOR), heat shock protein 27 (HSP27), src family kinase member Lyn and signal transducer and activator of transcription 5a (STAT5a) were reduced in OSC19 cells treated with GZ17 6.02 compared to the vehicle control treated cells. Further, we tested the in vivo antitumor efficacy of GZ17 6.02 on HNSCC xenografts. GZ17 6.02 was well tolerated in mice. Intratumoral administration of 15 mg/kg/d of GZ17 6.02 significantly reduced the tumor volume compared to the vehicle control treated tumors (P<0.05). In conclusion, by modulating key intracellular tyrosine kinases and inducing apoptosis, the GZ17 6.02 demonstrates therapeutic potential in the treatment of HNSCC.

P030: TUMOUR METABOLISM IN SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK: AN IN-VITRO STUDY OF THE CONSEQUENCES OF TP53 MUTATION AND THERAPEUTIC IMPLICATIONS – Mark D Wilkie, MD, Andrew S Lau, MD, Nikolina Vlatkovic, PhD, Terence M Jones, MD, Mark T Boyd, PhD; Liverpool Cancer Research centre

INTRODUCTION: Survival rates for squamous cell carcinoma of the head and neck (SCCHN) have not improved significantly in recent years. Radiotherapy (RT) remains a cornerstone of treatment, and as such, it is imperative to identify means to augment RT efficacy, for which tumour metabolism is an attractive putative target. Importantly, p53 is a key mediator of metabolism, while TP53 mutations are common in SCCHN and associated with aggressive disease. We sought, therefore, to investigate the metabolic alterations in SCCHN, to elucidate any correlation with TP53 status, and to determine whether targeted anti-metabolic therapy might be employed to potentiate RT effects.

METHODS: Extracellular acidification and oxygen consumptions rates, respective measures of glycolytic flux and mitochondrial respiration, were assayed in real-time for a panel of wild-type (wt) and mutant (mut) TP53 SCCHN cell lines using an XF24 extracellular flux analyser (Seahorse Bioscience, Billerica, USA) during specifically designed stress tests. Sensitivity to RT +/- 25mM 2-deoxyglucose (glycolytic inhibitor) was evaluated using clonogenic assays.

RESULTS: MutTP53 SCCHN cell lines exhibited a distinct metabolic phenotype to that of wtTP53 cells: wtTP53 cells maintained metabolic diversity, displaying robust mitochondrial and glycolytic reserve capacities, while mutTP53 cells displayed glycolytic dependence with markedly reduced mitochondrial and glycolytic reserve, functioning near capacity under basal conditions. This metabolic shift, in turn, correlated with RT response following administration of 2-deoxyglucose, which significantly (p<0.05) potentiated RT effects in mutTP53 but not wtTP53 cells.

CONCLUSIONS: TP53 mutation in SCCHN appears to correlate with a metabolic shift away from mitochondrial respiration towards glycolysis, resulting in sensitivity to the potentiating effects of glycolytic inhibition on RT. Notionally, TP53 status may be applied clinically as a marker of metabolic phenotype in SCCHN, enabling a more tailored therapeutic approach, which will also specifically target the typically aggressive and treatment resistant disease associated with TP53 mutation.

P031: LAMC2 AND LAMININ 332 ACTIONS DIFFERENTIATED USING LAMC2 AND LAMB3 KNOCKDOWNS IN HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC) CELLS – Susan M Fennewald, PhD, Peter Szaniszlo, PhD, Vicente A Resto, MD, PhD; UTMBHealth

Laminin c2 (lamc2) is highly expressed in a variety of cancers and is predictive of outcomes in oral squamous cell carcinoma. Lamc2 is normally present in extracellular basement membranes as part of the laminin 332 trimer which is composed of laminin α3 (lama3), laminin β3 (lamb3) and laminin γ2 (lamc2). In tumors it can often be found in the cytoplasm of cells or at the leading edge of a tumor. Many of the laminin proteins can be found in multiple laminin trimers. Lama3 is present in lam 332, but is also present in lam311 and lam 321. However, lamb3 and lamc2 are never present in any lam trimer in addition to lam332. Many histological studies have used this fact to identify the presence of lam332 using antibodies to lamc2. However, in addition to its participation in laminin 332, lamc2 has been reported to be present as a monomer and to exert effects independent of laminin 332 effects. To distinquish effects of lamc2 from lam332, we have constructed knockdowns of lamb3 in JHU-SCC-012 and JHU-SCC-019 cells using lentivirus delivered shRNA. We have then compared the effects of lamc2 knockdown, which should decrease both the lamc2 monomer and the lam332 trimer, with the effects of lamb3 knockdown, which should only decrease the lam332 trimer, but leave lamc2 monomer affects intact. Both knockdowns have an effect on the growth of JHU-SCC-012 cells in cell culture that can be partially compensated by growing the cells on extracellular matrix. However, the characteristics of the two knockdowns are not identical. We used Next Generation Sequencing to compare and contrast the effect of the laminin knockdowns on transcription in these cells.

P032: DEVELOPING A TONGUE CARCINOMA MODEL COUPLED WITH BIOLUMINESCENT IMAGING – Marisa R Buchakjian, MD, PhD, Michael D Henry, PhD; University of Iowa Hospitals and Clinics

OBJECTIVE: Head and neck squamous cell carcinoma poses a significant clinical challenge, with approximately 37,000 new cases diagnosed annually and a plateaued survival rate despite multiple surgical and medical advances. Mouse models of cancer provide insights into tumor biology that are difficult to extract from patient-based samples and provide valuable insight into disease. We are currently developing a novel viral-mediated inducible model of HNSCC based on the principles of somatic induction and tongue-specific loss of TP53/

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PTEN. Importantly, this model is coupled with Cre recombinase-dependent luciferase expression to allow for longitudinal bioluminescence imaging of gene recombination and tumor formation.

METHODS: C57BL/6 mice with floxed TP53/PTEN and a lox-stop-lox luciferase allele (LSL-luc TP53-/- PTEN-/-) were engineered for the proposed model. Adenovirus or adeno-associated virus (AAV) containing a Cre recombinase vector driven by the CMV promoter was injected into the tongue mucosa to promote site-specific recombination. Mice were followed longitudinally with physical examination and real-time molecular imaging using luciferin substrate to evaluate for allele recombination and tumor development.

RESULTS: A pilot cohort of LSL-luc TP53-/- PTEN-/- mice underwent injection of adenovirus containing CMV Cre-eGFP into the tongue with a range of titers. Mice were imaged 24 hours post-exposure and biweekly thereafter. Luciferase expression was detected in the tongue as early as 24 hours post-injection and appeared to be dependent on viral titer, suggesting efficient viral delivery and recombination. Luciferase signal increased over time as cells with TP53/PTEN loss continued to grow and divide. In addition to expression in the tongue, signal was also observed in regional lymph nodes, alerting us to limitations in viral specificity and prompting us to evaluate AAV serotypes for tissue-specific tropism. In parallel, cohorts of mice were injected with AAV2/1- and 2/6-CMV Cre-eGFP, which demonstrated significantly improved tongue-specific expression as well as more pronounced localized gene recombination. Over time, TP53/PTEN mice injected with both AAV2/1 and 2/6 developed robust tongue tumors. Grossly, these tumors resembled classic human squamous cell carcinomas, however, the samples are currently being analyzed by pathology to determine exact tumor type and invasiveness. Current work aims at fine-tuning vector delivery specificity by engineering a new construct with Cre recombinase driven by the Keratin-5 promoter to restrict recombination to the basal tongue epithelium.

CONCLUSIONS: We are making progress in developing a novel model of tongue SCC based on viral-mediated TP53/PTEN knockout coupled with luciferase expression. Successful development of this model will help us gain a better understanding of HNSCC, and real-time imaging of cancer cells in vivo will allow us to monitor tumor dynamics and metastasis. In the future this model may provide a novel system for monitoring tumor formation, metastatic behavior, and response to treatment.

P033: TO EVALUATE THE EFFECT OF HYPERBARIC OXYGEN THERAPY ON THE HEARING OUTCOME IN PATIENTS WITH SUDDEN SENSORINEURAL HEARING LOSS – Swati Agrawal, MBBS, MS, Nishi Sharma, MBBS, MS, DNB, Neerja Banerjee, MBBS, MD; PGIMER & Dr. Ram Manohar Lohia Hospital, New Delhi, India

AIMS & OBJECTIVES: To ascertain whether the addition of Hyperbaric Oxygen Therapy to the conventional medical treatment improves the hearing outcome in patients with Sudden Sensorineural Hearing loss, to assess the impact of patient-related and audiovestibular parameters on the prognosis of sudden hearing loss and to document any adverse effects of Hyperbaric Oxygen Therapy.

METHODOLOGY: Forty patients with Sudden SNHL, aged 18-60 years, were enrolled in this randomized controlled study. Twenty patients (Group A) received steroids, plasma expander dextran, gingko biloba extract, nicotinic acid, betahistine and antiviral acyclovir. A second group (Group B) of twenty patients received the same basic treatment with the addition of 10 sessions of HBOT. Audiological assessments of all patients were performed with pure tone audiometry on day 5, day 10 and at the end of each month, for next 3 months. The following parameters were noted for each patient: demographics (age and gender); presence of tinnitus; vestibular symptoms; time elapsed between onset of sudden hearing loss and initiation of treatment; severity of hearing loss at presentation. The hearing outcomes were evaluated by four indices: cure rate, marked recovery rate, recovery rate, and hearing gain.

RESULTS: The mean hearing gain was 31.5 + 20.0 dB in Group B, which was significantly higher than that in Group A, 16.8 + 17.5 dB ($p = 0.018$). There was no significant difference in the cure rate and recovery rate between the two groups; however, the marked recovery rate was significantly higher in Group B than in Group A (50% vs. 20%; $p = 0.047$). The patients who were treated within the first seven days of onset of hearing loss showed significantly higher hearing gains and better marked recovery rates. The cure rate was significantly higher in patients without vertigo than in those with vertigo (19% vs. 0%; $p = 0.045$). 20% of the patients suffered from adverse effects of HBOT, the most common of which was found to be otitis media. No other complications were reported.

CONCLUSION: The addition of HBOT to the conventional treatment significantly improves the outcome of sudden deafness, and its use should be encouraged as an adjunctive therapy along with conventional medical treatment in sudden SNHL patients. Also, age, gender, tinnitus and severity of hearing loss were not found to affect the outcome of sudden hearing loss. However, the following were found to be poor prognostic factors: the presence of vertigo and initiation of treatment more than seven days after onset of sudden deafness. HBOT was found to be a relatively benign intervention.

P034: ANTI-TUMOR EFFECT OF THERAPEUTIC INHIBITION OF IL-6R SIGNALING IN SALIVARY MUCOEPIDERMOID CARCINOMA – Daiki Mochizuki[1], April Adams[1], Kristy A Warner[1], Zhaocheng Zhang[1], Alexander Pearson[2], Kiyoshi Misawa[3], Scott A McLean[4], Gregory T Wolf[4], Jacques E Nör[1]; [1]Department of Restorative Sciences, University of Michigan School of Dentistry, Ann Arbor, Michigan, [2]Department of Internal Medicine, University of Michigan School of Medicine, [3]Department of Otolaryngology/Head Neck Surgery, Hamamatsu University School of Medicine, Hamamatsu, [4]Department of Otolaryngology, University of Michigan School of Medicine

Mucoepidermoid carcinoma (MEC) is one of the most frequent salivary gland malignancies. Response to systemic therapy and radiotherapy is modest, and therefore radical surgery remains the most effective treatment strategy. Emerging evidence suggest that Interleukin-6 (IL-6) signaling plays a key role in resistance to therapy in several solid tumors. However, it is unclear if IL-6 signaling is involved in the resistance that MEC exhibits to chemotherapy. Here, we investigated whether inhibition of IL-6 receptor (IL-6R) signaling with tocilizumab (a humanized anti-human IL-6R antibody FDA-approved for treatment of rheumatoid arthritis) sensitizes MEC to cisplatin or paclitaxel therapy. We investigated the effects of tocilizumab on MEC growth *in vitro* and *in vivo*, using the University of Michigan Human Mucoepidermoid Carcinoma (UM-HMC) cell lines and xenograft models of MEC. Primary human MEC and UM-HMC xenografts expressed high levels of IL-6R. Tocilizumab inhibited rhIL-6-induced and endothelial cell conditioned medium-induced STAT3 phosphorylation in UM-HMC-3A and UM-HMC-3B cells. However, tocilizumab had no measurable effects in MEC cell viability (SRB assay), cell cycle (FACS), or apoptosis (propidium iodide staining followed by FACS) *in vitro*. Single agent tocilizumab showed an inhibitory effect on the growth of MEC xenografts that was comparable to cisplatin or paclitaxel alone. Notably, combination of tocilizumab with cisplatin or paclitaxel significantly inhibited the growth of MEC xenografts ($p<0.05$), without measurable systemic toxicities (as determined by stable mouse weight). Combination therapy enhanced the time to failure (defined by a 2-fold increase in tumor volume) when compared with vehicle-treated controls ($p<0.01$). Collectively, these pre-clinical findings demonstrate that combination of tocilizumab potentiates the anti-tumor effect of conventional chemotherapy in mucoepidermoid carcinomas. These results suggest that patients with mucoepidermoid carcinoma might benefit from a therapeutic strategy that combines an inhibitor of IL-6R signaling with a conventional chemotherapeutic agent such as cisplatin or paclitaxel. This work was funded by grants R01-DE21139 and R01-DE23220 from the NIH/NIDCR, and P50-CA97248 (University of Michigan Head Neck SPORE) from the NIH/NCI.

P035: EPIDEMIOLOGY AND SURVIVAL OUTCOMES OF OROPHARYNGEAL SQUAMOUS CELL CARCINOMA IN CANADIAN FIRST NATIONS – Vincent Biron, MD, PhD, FRCSC, Bree Erickson, MD, Han Zhang, MD, Hadi Seikaly, MD, MAL, FRCSC, David Cote, MD, MPH, CCFP, FRCSC; University of Alberta

INTRODUCTION: Oropharygeal squamous cell carcinoma (OPSCC) is an aggressive malignancy that can be caused by tobacco smoking, alcohol and human papillomavirus (HPV). Despite decreasing smoking rates in many populations, the incidence of OPSCC is increasing worldwide due to oncogenic HPV infection. HPV-related OPSCC is known to be associated with favorable survival outcomes. However, there is a paucity of data examining these changing etiological factors and survival outcomes in Canadian First Nations (FN) patients, a population known to have poorer cancer survival and health related quality of life.

METHODS: Demographic, survival, staging and pathologic data from patients diagnosed with OPSCC from 1998-2009 was obtained from the Alberta Cancer Registry. This data was cross-referenced to the Alberta Health and Wellness Registry to identify patients with FN status. Clinicopathological differences were compared between FN and non-FN patients. Overall and disease specific survival was calculated using Kaplan-Meir and Cox regression analyses.

RESULTS: Between 1998 and 2009, 712 patients were diagnosed with OPSCC in Alberta, of which 20 (2.02 %) were First Nations. 345 (48.9%) patients were diagnosed in Northern Alberta, which included 16 (4.6 %) FN patients. Detailed demographic, survival and pathologic data was available for this cohort of patients, which was used for our comparative analysis. Overall, 83.5 % (572) of OPSCC patients in Northern Alberta presented with advanced stage disease, compared to 100% of FN patients with FN status. No significant differences were seen between FN and non-FN patients in terms of demographics at time of diagnosis. FN patients had significantly higher rates of smoking and p16 negative tumors. Despite this, we found no significant differences in 5 year overall and disease specific survival.

CONCLUSIONS: In comparison to non-FN patients, Canadian First Nations patients with OPSCC may more commonly present with a tobacco smoking history and advanced stage p16 negative tumors. However, overall and disease specific survival outcomes are similar between these populations.

P036: ORTHOTOPIC ORAL CANCER MOUSE MODEL FOR ANTI-ORAL CANCER DRUG SCREENING – Manish V Bais, DVM, PhD, Maria Kukuruzinska, PhD, Philip Trackman, PhD; Department of Molecular and Cell Biology, Boston University Henry M. Goldman School of Dental Medici

INTRODUCTION: Oral squamous cell carcinoma (OSCC) represents the majority of head and neck cancers. Distant metastases occur 20-40% of the time, leading to poor survival and failure of therapeutic interventions. The goal of the present study is to establish an orthotopic OSCC mouse models for tumor growth and metastasis, to evaluate the effectiveness of anti-cancer agents, and to identify predictive biomarkers. We hypothesized that orthotopic OSCC mouse models are predictive of anti-oral cancer agent efficacy.

METHODS: We have developed an orthotopic tongue nude mouse model (n=5/conditions) using DsRed protein- expressing metastatic (UMSCC2) non-metastatic (CAL27) cell line, and vehicle injection served as a non-tumor controls. The growth of these orthotopic tumors was monitored by fluorescence-based live imaging and caliper measurements. Western blot and immunohistochemistry were also performed. These models were used to validate biologic and small molecules drugs.

RESULTS: Injection of CAL27 cells resulted in localized primary tongue tumor growth, whereas injection of UMSCC2 cells also resulted in distant metastatic lesions in the submandibular region, lung, kidney, liver and bone by day 24.

Western blot analyses of tongue tumor extracts showed higher expression of LSD1, dimethylated histone, PCNA and cyclin D1. Immohistochemistry analyses showed higher expression of OSCC related proteins including LSD1, MT-MMP1, Ki-67, E-cadherin and Oct 4A in UMSCC2 and CAL27 cells- derived tumors. Validation of these models was demonstrated by inhibition of tumor growth and/ or metastasis with the well-characterized anti-cancer agents as lysyl oxidase pro-peptide and N-glycosylation inhibitor tunicamycin.

CONCLUSION: We have established a mouse model for studying primary tumor growth and metastasis and validated the efficacy of oral cancer targeted therapeutics. These preliminary studies will help to establish patient-derived OSCC mouse models to study the biology and assess therapeutic opportunities with novel anti-cancer agents. Thus, these models are validated as an anti-oral cancer therapeutics screening.

P037: THE CLINICAL UTILITY OF A NEW HUMAN PAPILLOMAVIRUS DETECTING SYSTEM IN METASTATIC CARCINOMA TO LYMPH NODES FROM AN UNKNOWN PRIMARY SITE – Michael T Chang, BS, Bea Carbone, BA, Wendell G Yarbrough, MD, MMHC, FACS; Yale University School of Medicine

Fine-needle aspiration (FNA) is a common diagnostic procedure in head and neck cancer with an unknown primary site and lymph node metastasis. Human papillomavirus (HPV)-associated cancers have recently been demonstrated to predominately arise in the oropharynx. However, in cystic nodes, it is common to lack the epithelial cells necessary to detect HPV by immunohistochemistry. If HPV is undetectable, the next step is often to perform a repeat FNA or a surgical biopsy to investigate potential sites of the primary tumor. Here we discuss a novel, non-invasive HPV detection tool to help guide management of head and neck carcinoma of unknown primary (CUP). For an inconclusive FNA, the Cobas system (Roche, Pleasanton CA) may be appropriate as it detects the presence of the viral HPV capsid gene L1 within the cystic fluid by polymerase chain reaction (PCR). Here we present two cases of patients with CUP whose initial nodal FNA showed no epithelial cells but subsequent L1-detection PCR revealed the tumor to be HPV-positive. In both cases, the primary tumor was located in the oropharynx at the time of surgery. The utilization of this novel diagnostic tool could potentially expedite diagnosis and more effectively guide management of CUP in the head and neck.

P038: A GROWTH FACTOR LOADED MODULAR HYDROGEL SYSTEM SUPPORTS STABLE VASCULAR NETWORKS IN A RODENT PAROTID GLAND RESECTION MODEL – Swati Pradhan-Bhatt, PhD[1], Mary C Farach-Carson, PhD[2], Daniel A Harrington, PhD[2], Xinqiao Jia, PhD[3], Robert L Witt, MD[1]; [1]Helen F. Graham Cancer Center & Research Institute, [2]Rice University, [3]University of Delaware

INTRODUCTION: Radiation therapy (RT) commonly used to treat head and neck cancers decreases salivary secretion leading to xerostomia. Xerostomia affects ~40,000 patients in the United States each year. RT damages secretory acinar cells of the salivary gland leading to a near-complete loss of saliva with difficulty swallowing and speaking, an increase in tooth decay, and other oropharyngeal infections. This project aims to develop a functional implantable salivary gland that can restore the salivary functions of these patients. A successful tissue-engineered implant requires a stable vasculature to survive long-term in vivo. Our current strategy is to use a hydrogel-based system loaded with angiogenic growth factors (GFs) to support the growth of blood vessels into the implant to ensure long-term survival and retention of structures.

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METHODS: Under an IRB approved protocol, human tissues are obtained from patients undergoing head and neck surgery. Tissue specimens are dissociated to obtain human salivary acinar-derived cells (hSACs) that are encapsulated in a hyaluronic acid (HA)-based hydrogel system to form functional spheroids. To ensure long-term survival of these spheroids in vivo, angiogenic GFs were included in the best concentration to create stable vascular networks. In this study, a cell-free hydrogel was seeded with heparin-decorated hydrogel particles (HGPs, 100nm–10μm) loaded with angiogenic GFs such as vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF). A parotid gland resection model was previously developed to simulate acinar cell loss. In this model, ¾ of the left parotid gland is resected and replaced with a hydrogel implant. HA-hydrogels seeded with GF-loaded HGPs were implanted in the resected salivary bed of athymic rats. Implants were removed from rats and assessed for vascular infiltration one-week post-implantation. Implants were analyzed for presence of endothelial cells, which form the blood vessel wall and for pericytes, which envelop and stabilize the vascular tube.

RESULTS: HA-hydrogels containing control/unloaded HGPs were compared to those with 50ng VEGF alone, 50ng VEGF+50ng PDGF, and 100ng VEGF+100ng PDGF. Other than the control implant, all hydrogels showed an increase in local vasculature. Cryosections of all implants showed CD31/PECAM-1 positive blood vessels and α-smooth muscle actin (α-sma) positive pericytes in the tissue surrounding the hydrogels. Robust vasculature was observed in the vicinity of the 100ng VEGF+100ng PDGF implant. Additionally, this implant contained CD31/PECAM-1 and α-sma positive blood vessels within the hydrogel unlike any other implant. The presence of both endothelial cells and pericytes in the hydrogel indicated that the vascular tubes in the implant were likely forming stable blood vessel networks. Hematoxylin and Eosin staining supported these results. Future work involves long-term implantation studies to ensure complete infiltration of the hydrogel over time.

CONCLUSIONS: A hydrogel culture system with GF-loaded HGPs, capable of supporting stable vascular networks in vivo, was established. The hydrogel culture system reported here will aid in the long-term survival and retention of functional salivary units in vivo, which will bring us closer toward the development of an artificial salivary gland that can relieve symptoms of patients suffering from xerostomia.

ACKNOWLEDGMENTS: Supported by NIH/DE RO1-DE022386.

P039: METADHERIN REGULATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION IS DEPENDENT UPON THE PI3K/AKT PATHWAY IN SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK – Gangcai Zhu, Lijun Liu, Yong Liu, Xin Zhang; xiangya hospital

PURPOSE: Our previous study indicated over-expression of Metadherin (MTDH) is an adverse prognostic factor in squamous cell carcinoma of the head and neck (SCCHN) and promotes SCCHN cells proliferation and invasion. However, its mechanism remains unclear. Recent studies have indicated that MTDH is a cancer-metastasis-associated molecule that participates in the process of angiogenesis. Therefore, the study is aim to investigate that whether vascular endothelial growth factor (VEGF), as one of the most potent proangiogenic cytokines, is regulated by MTDH and the role of the PI3K/Akt pathway in this process of regulation and the clinical significance of both MTDH and VEGF in SCCHN.

METHODS: Immunohistochemistry was used to assay the expression of MTDH and VEGF in a cohort of 189 SCCHN patients with intact follow-up information. The expression of MTDH was then up-regulated or inhibited by lentivirus-mediated MTDH cDNA or MTDH shRNA to observe the resulting alterations in VEGF expression and the PI3K/Akt signalling pathway in SCCHN cell lines. In addition, the PI3K/Akt pathway was modulated to observe the resulting changes in the MTDH-mediated expression of VEGF.

RESULTS: The immunohistochemistry data showed that MTDH expression is positively correlated with VEGF expression in SCCHN tissues. Moreover, the overexpression of MTDH in SCCHN Tu686 and 5-8F cells led to increases in the expression of VEGF, and this effect was accompanied by activation of the PI3K/Akt pathway. Conversely, shRNA-mediated knock-down of MTDH led to decreased VEGF expression. In addition, inhibition of the Akt signalling pathway reversed the up-regulation of VEGF resulting from MTDH overexpression. Moreover, the survival analysis revealed that VEGF is an independent prognostic factor; and a combined survival analysis based on both MTDH and VEGF showed synergistic effects in the prognosis evaluation of SCCHN patients.

CONCLUSIONS: The findings of the present study demonstrate that MTDH regulates the expression of VEGF via the PI3K/Akt signalling pathway, indicating the potential role of the MTDH-mediated activation of VEGF signalling pathway in SCCHN angiogenesis and metastasis.

P040: A SYSTEMS APPROACH TO CLINICO-PATHOLOGICAL ANALYSIS IDENTIFIES KEY EVENTS IN IMPROVED CHEMORADIOTHERAPY RESPONSE ASSOCIATED WITH GLUCOSE INDEPENDENT TUMOR METABOLISM AND ALTERED INTRATUMORAL CD8/CD4 RATIO – Rosemarie Krupar, MD[1], Ravi Pathak, PhD[1], Matthias Hautmann, MD[2], Andrew Sikora, MD, PhD[1]; [1]Baylor College of Medicine, [2]Universitaetsklinikum Regensburg

Radiotherapy response of head and neck squamous cell carcinomas (HNSCC) depends on tumor metabolism and antitumoral immune response. While tumor oxygenation improves radiosensitivity, anaerobic tumor metabolism, also known as Warburg effect, causes radioresistance due to increased lactic acid secretion as a consequence of high glycolytic rate. Additionally, tumor cell derived lactic acid is known to inhibit cytotoxic T-cells. However, there have been few studies that have correlated cancer driven metabolic shifts with antitumoral immune responses. This study aimed to investigate the interdependence of tumor metabolism and immune microenvironment in vivo, and analyze its impact on radioresponse. In order to decipher the broader molecular events that drive therapy response, clinico-pathological observations were then integrated with systems wide global network analysis.

74 pretreatment HNSCC biopsies (oropharynx, hypopharynx, larynx, oral cavity, nasopharynx) treated by primary Cisplatin-based chemoradiotherapy were analyzed for infiltration by CD8+ and CD4+ T-cells and expression of GLUT1 as a marker for anaerobic, glucose dependent tumor metabolism. Tumors with high GLUT1 presented a significantly decreased CD8/CD4 ratio and a strong trend towards an increased FOXP3/CD8 ratio indicating an inhibition of antitumoral and an activation of protumoral immune response in anaerobic tumors. While high CD8/CD4 was associated with a trend towards improved therapy response/12 months survival (p=0.075), high CD8/CD4 together with low GLUT1 expression showed a significantly improved therapy response/12 months survival (p=0.046). In order to advance our observations of GLUT1 dependent alteration of antitumoral immune response and identify any additional targets, we constructed a biological network comprising of GLUT1 and immune checkpoint modulators that are frequently expressed on squamous cell carcinomas. The network was selectively perturbed to mimic high GLUT1 expression levels and to determine its effect on the immune checkpoint modulators. Our analysis revealed network-wide alterations of most key immune checkpoint molecules. These included positive regulation of PD-L1, Gal9, CD70, CD40, CD80/CD86, OX-40 and CD137L as well as negative alterations of B7-H3, B7-H4 and PD-L2 in response to increased GLUT1 levels. Analysis of the network identified GLUT1-mediated control of AKT as a modulator of NFKB signaling resulting in changes of the immune checkpoint molecules.

These findings advance our understanding of the mechanisms by which glucose dependent tumor metabolism drives tumor-associated immune suppression and poor radiotherapy response. Furthermore they predict a number of key proteins

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that could be responsible for metabolic-shift associated immune modulation and evaluated as potential targets for immune- and radiation-based combinatory therapy approaches.

P041: INCIDENCE, TREATMENT AND OUTCOMES IN RECURRENT OROPHARYNGEAL CANCER FOLLOWING PRIMARY SURGERY – Bhavna Kumar, Matthew Old, Nicole V Brown, Enver Ozer, Amit Agrawal, Ricardo Carrau, Marino E Leon, Mike J Cipolla, Kasim Durmus, Pawan Kumar, Paul E Wakely Jr, David E Schuller, Theodoros N Teknos; The Ohio State University

PURPOSE: The recent retrospective analysis of patients on the RTOG 0129 and 0522 trials treated with chemotherapy/radiotherapy showed that HPV status was a strong predictor of overall survival after disease progression. In this study we sought to define the predictors of recurrence, optimal therapies, and outcomes in recurrent oropharyngeal cancer after surgery as the primary method of treatment.

METHODS: We conducted a retrospective study of 267 patients diagnosed with OPSCC at the James Cancer Hospital and Solove Research Institute from 2002 to 2012. These patients were all treated with primary surgery followed by appropriate adjuvant radiation and/or chemoradiation. Demographic, pathologic, treatment, and follow-up data were collected including the recurrence rates, patterns of recurrences and overall survival (OS). The human papillomavirus (HPV) and p16 status was determined by CISH and IHC respectively. Odds of recurrence were evaluated via univariate logistic regression models and OS was assessed for those with recurrences via univariate Cox proportional hazards models. Variables found to be significant (0.05 alpha level) in the univariate logistic regression models were included in a multivariable model.

RESULTS: Of the 267 patients with OPSCC, 69 patients (25.8%) developed a recurrence. Thirty seven patients developed locoregional recurrence, 22 had distant spread and 10 had both. Those with distant spread had a greater hazard of death (HR=2.2; p=0.01) as compared to those with locoregional recurrence. A greater proportion of patients treated with open surgical approach for their primary tumor resection had a recurrence compared to those treated with a transoral approach (p<0.0001). A larger proportion of HPV-negative patients (41/103; 39.8%) had a recurrence as compared to HPV-positive patients (25/157; 15.92%); p<0.0001. The patterns of recurrence did not differ significantly between the HPV-positive and negative patients (p=0.78). The time to develop a recurrence also did not differ significantly between the HPV-positive (median : 1 year) and HPV-negative (median : 11 months) groups; p=0.31. Extra capsular spread (ECS), perineural invasion, African American race, >10 pk-year smoking history, open surgical approach, positive margins and higher tumor stage were significantly associated with disease progression in patients after resection of primary tumor/adjuvant therapy. After adjustment for other covariates, surgical approach, ECS, HPV, and mucosal margin status were significantly associated with recurrence in the multivariable model. With a median follow-up of 0.69 years after recurrence (0.07-10.19), the 3 year survival rate of patients was 20.8%. Tumor HPV status was not a predictor of OS after disease recurrence. Receipt of salvage surgery was associated with improved OS (p=0.0004). None of the other variables analyzed (T stage, N stage, AJCC stage, smoking status, margin status, perineural invasion, ECS, gender and race) predicted OS of patients with recurrence.

CONCLUSION: In surgically treated OPSCC patients, the chances of developing a recurrence are greater in patients with HPV-negative tumors as compared to those with HPV-positive tumors. However, once the patient develops a recurrence, tumor HPV status does not significantly influence OS. Despite low survival rates, the ability to perform salvage surgery in the recurrent setting was predictive of improved survival in this patient population.

P042: CURRENT BIOBANKING TECHNIQUES AND THE IMPORTANCE OF VIABLE BIOBANKING IN PERSONALIZED MEDICINE – Bea Carbone, BA, Eriko Kanaya, MD, Gary Bellinger, BS, Wendell G Yarbrough, MD, MMHC, FACS; Yale University School of Medicine

Though non-viable biobanking is a useful technique that has been utilized for years, recently, it has not shown a significant amount of innovation. In more rare cancers, the largest obstacle in progress is often a dearth of samples or cell lines. In head and neck cancers, there is the combined challenge of common recurrence, comparatively low occurrence, and number of therapeutic options. Construction of a non-viable biobank could alleviate some of these issues, but would leave the problem of inability to perform in vitro confirmations of therapies based on next-gen sequencing. We have demonstrated and published on the idea of viable biobanking, wherein cell culture can be produced from tumor or cells disaggregated from tumor can be frozen and later revived. A viable biobank that has the capacity to create personalized in vitro models could be used as a point of reference if a patient recurs years down the line or for immediate testing of possible therapeutic treatment in a nonresponsive tumor. Recently we have experimented with new methods of culturing tumors that have seen great success in growing short-term cultures from patient samples.

P043: FACTORS ASSOCIATED WITH QUALITY AND VALUE IN FIBULA FREE FLAP SURGERY FOR HEAD AND NECK CANCER: A NATIONAL ANALYSIS – Paula Wu, BS, Saral Mehra, MD, MBA; Department of Surgery (Otolaryngology), Yale University School of Medicine, New Haven, Connecticut

IMPORTANCE: Surgical cases involving free tissue transfer are amongst the costliest in the surgical treatment of head and neck cancer, but national-level analysis of quality and value metrics have never been analyzed.

OBJECTIVE: To describe national variation in cost and quality outcomes in free flap surgery for head and neck cancer, and determine factors associated with these outcomes.

DESIGN: Retrospective National database research.

SETTING: Nationwide Inpatient Sample (NIS) Datafiles

PARTICIPANTS: Patient discharged from NIS participating institutions between 2003 to 2011 following fibula free flap surgery as part of the surgical treatment of head and neck cancer.

MAIN OUTCOME(S) & MEASURES: Outcome measures were total complications, length of stay, in-hospital mortality, and total charges. Total charges were used as a proxy for quality, cost, and value of care because it encompasses various metrics that are not included as distinct outcomes in the NIS database including but not limited to use of intensive care unit, return to operating room, and additional diagnostics/treatments.

Factors assessed included patient demographic characteristics (payor, rural/urban location, race, age, gender, and income); patient clinical characteristics (number of chronic conditions, tumor stage, point of admission, and APR-DRG Risk Mortality and Severity scores); and hospital characteristics (hospital region, membership in a multi-hospital system, teaching hospital, bedsize, percent registered nurses among nurses, number of RN full-time equivalents per 1000 patients, number of licensed practical nurse FTEs per 1000 patients, percent surgeries performed in an outpatient setting, and hospital volume).

RESULTS: During the 9-year study period, 678 patients with head and neck cancer had free fibula transfer in this database. The majority of patients were treated at teaching hospitals (99%), hospitals with large bedsizes (80%), and in metropolitan areas (55% large metropolitan areas with population >1 million, 28% small metropolitan areas with populations <1 million). Patients had a median of one complication (range 1-7). Median total charges was $121,368

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(range $12,094-$818,795). Median length of stay was 10 days (range 1-115). The mortality rate was 1%. On multivariate analysis, higher total charges were associated with "other" race (p=0.0138), Medicaid payor (P=0.0275), Western hospital location (P=0.023), higher APRDRG Severity Scores (P=0.0002), and higher RN FTEs per 1000 patients, a common metric for assessing nurse staffing load (P=0.0002). Greater length of stay was associated with Medicaid payor (P<0.000001), and higher APRDRG Severity Score (P=0.0036).

CONCLUSION: There is a wide national variation in charge and quality outcomes in patients undergoing fibula free flap surgery as part of the treatment for head and neck cancer, which can be a proxy for value of care. A number of factors are significantly associated with higher total charges including Medicaid payor, higher APR-DRG Severity score, and Western hospital location. Determining reasons for this variation, sharing best practices, and targeting interventions specific to these factors may help to improve the value of care delivery in this patient population.

P044: CHEMOSENSITIVITY OF HPV-POSITIVE AND HPV-NEGATIVE HNSCC CELL LINES – <u>Chia-Jung Busch, MD</u>[1], Malte Kriegs, PhD[2], Cordula Petersen, MD, PhD[3], Rainald Knecht, MD, PhD[1], Ekkehard Dikomey, PhD[2], Thorsten Rieckmann, PhD[2]; [1]Department of Otorhinolaryngology, Head & Neck Surgery & Oncology University Medical Center Hamburg, [2]Laboratory of Radiobiology & Experimental Radiooncology, [3]Department of Radiotherapy and Radiooncology

BACKGROUND: Patients with HPV-positive HNSCC show remarkably better survival rates than patients with HPV-negative. The favorable survival is described to be independent of treatment but since data from single modality regimes are rare, so far only an enhanced radiosensitivity is truly established. In contrast, an enhanced chemosensitivity and a favorable survival after surgery alone - though often stated - remain speculative. Standard radiochemotherapy for HNSCC is performed with cisplatin, which is associated with high-grade toxicities. As a chemotherapeutic agent cisplatin possesses profound cytotoxicity and, in addition, it is generally referred to as a potent radiosensitizer. Here we compared the cytotoxic and radiosensitizing effects of cisplatin in a panel of HPV-positive and HPV-negative HNSCC cell lines previously described to show pronounced differences in radiosensitivity with HPV-positive strains being far more radiosensitive.

METHODS: Cytotoxicity: Proliferation and colony formation assays were performed with five HPV/p16INK4a-positive and five HPV/p16INK4a-negative HNSCC cell lines. The IC50 dose of cisplatin was defined for each cell line. Radiosensitization: The impact of radiosensitization by cisplatin was assessed by colony formation assay using the individual IC50 dose of cisplatin for each strain.

Results: The HPV-positive cell lines showed a stronger response to cisplatin in the proliferation assay. Regarding colony formation, both panels demonstrated strong heterogeneity in cisplatin-sensitivity but, in contrast to the proliferation assay, HPV-positive strains did not show a more pronounced effect. Combining radiation with cisplatin, we generally observed an additive effect on colony formation but only one out of five HPV-negative and two out of five HPV-positive strains demonstrated radiosensitization due to cisplatin.

CONCLUSIONS: In contrast to the previously described differences in radiosensitivity (Rieckmann2013), we observed no apparent difference in the sensitivity toward cisplatin in this panel of HPV-positive and HPV-negative HNSCC cell lines. Although cisplatin is not generally a radiosensitizer in terms of cellular radiosensitivity, it demonstrated profound cytotoxicity and/or radiosensitization in several HPV-positive HNSCC strains. Therefore it should not just be omitted but rather be replaced by less toxic but equally efficient molecular targeting agents (Busch2013, Güster2014) in order to safely deintensify the therapy for HPV-positive HNSCC.

P045: CANCER STEM CELL NICHE CORRELATES WITH MALIGNANT PROGRESSION OF ORAL POTENTIALLY MALIGNANT LESION – <u>Subin Surendran</u>[1], Gangotri Siddappa[2], Sindhu Govindan[2], Ravindra Ravi[2], Amritha Suresh[3], Austin Price[1], Varun Bhat[2], Christina Mimikos Mimikos[1], Mary Reid[1], Mihai Merzianu[1], Vijay Jayaprakash[1], Wesley Hicks[1], Moni A Kuriakose[3]; [1]Roswell Park cancer Institute, [2]Mazumdar Shaw Medical Centre, Narayana Hrudayalaya, Bangalore, [3]Mazumdar Shaw Medical Center- Roswell Park Collaboration Program, Buffalo, New York

The ability of cancer stem cells (CSCs) to maintain their capacity for self-renewal, pluripotency, and potential for tumor initiation depends on the presence of a supportive perivascular niche. Microvascular density has been found to correlate to the presence of CSC niche in overt carcinoma of the head and neck. We propose that this relationship is maintained in premalignant dysplastic head and neck lesions, and that increasing microvascular density correlates to an increasing degree of dysplasia in oral mucosal lesions. Oral mucosa patients were histologically examined and given a degree of no, mild, moderate, or severe dysplasia (N=93) or as well differentiated (WDSCC), moderately differentiated (MDSCC) and poorly differentiated squamous cell carcinoma (PDSCC) (N=48). These samples were then immunostained with antibodies to CD31, an endothelial marker, and CD44, a cancer stem cell marker in head and neck squamous cell carcinoma. Microvascular density and stem cell density at the sub-mucosal level was then measured at a magnification of 400x. The average density of stem cells and microvessels per high power field (hpf) for patient was then calculated and compared to the densities of normal oral mucosa. Normal mucosa was found to have a microvascular density (MVD) of 12.21/hpf (SD 1.06). Tissues with mild, moderate, and severe dysplasia demonstrated MVDs of 13.3 +/- 1.03/hpf, 21.4 +/-2.45/hpf, and 21.44 +/-3.13/hpf respectively. These tissues showed a higher MVD as compared to the OSCC tissues. An increased average microvascular density correlated with an advancing degree of dysplasia during progression as compared to normal oral mucosa. These findings were statistically significant for mild and severe dysplasia (p<0.05). CD44+ cells were found at a density of 58.57/hpf in normal mucosa, 61.56/hpf in mild dysplasia, 119.44/hpf in moderate dysplasia, and 107.6 in severe dysplasia. The density of CD44+ cells in WDSCC, MDSCC and PDSCC are 183.4, 105 and 160/hpf respectively.Submucosal microvascular density and the presence of stem cells appears to correlate with the degree of dysplasia in oral mucosal lesions. Given this finding, microvascular density along with the CSC cells may represent a marker of malignant progression.

P046: PREDICTION MARKERS FOR RESISTANCE/RESPONSE TO CHEMOTHERAPY IN HEAD AND NECK CANCER - A META ANALYSES APPROACH – Ram B Reddy, MSc, MTECH[1], Vikram Kekatpure, MCh[2], <u>Moni A Kuriakose, MD, FRCS</u>[2], Amritha Suresh, PhD[1]; [1]Head and Neck Oncology, DSRG-5, Mazumdar Shaw Centre for Translational Research, MSMC, [2]Head and Neck Oncology, Mazumdar Shaw Medical Centre, Bangalore,

Chemotherapy resistance/response depends upon the genetic makeup of the patient; a panel of markers that can predict response to treatment will help towards improving treatment management and patient survival. Several high throughput studies have been carried out documenting the molecular basis of resistance, however studies that analyze the data in total and apply it for clinical benefit are very few. In addition, the discordance between the different studies on clinically similar samples makes it difficult to implement this information in the clinics. This study aims to develop a panel of biomarkers of chemotherapy resistance/response to the drugs currently used in HNSCC (Taxol, Platinum and 5-Flurouracil) by a systematic meta-analysis of existing microarray databases. The public databases like GEO (Gene Expression Omnibus), Array Express (EBI) were used to retrieve raw data from Agilent and Affymetrix platforms using specific search criteria. The series data (N=72) were classified into

responders/non-responders (patient samples) and treated/untreated (cell lines samples). The samples were pooled based on the design and analyzed using Gene spring software [v12.6.1, Agilent, USA]. Normalization was by Robust Multichip Averaging (RMA) and statistically significant genes (p-value<0.05, fold value >2) are currently being selected after samples quality control and multiple testing corrections (FDR). The pathways/genes will further be identified after filtering out for the drug-related genes/entities. The top gene entities will then be compared with the publically available mutation database (TCGA http://www.cbioportal.org/public-portal/). The common statistically significant genes specific to the pathways (between the expression and the mutation database) will be validated in retrospective chemotherapy HNSCC patients (N=15 from both responders and non-responders) by QRT PCR. The study is an initial step towards the development of a molecular marker panel that will help in predicting resistance/response towards chemotherapy in HNSCC.

P047: TGF-B1-INDUCED MALIGNANT PHENOTYPES IN SCC AND ADENOCARCINOMA OF HEAD AND NECK ARE ACQUIRED BY DIFFERENT EMT-ASSOCIATED TRANSCRIPTION FACTORS – Kei Ashizawa, Hiroki Ishii, Keisuke Masuyama; University of Yamanashi

Transforming growth factor b1 (TGF-b1) plays multi roles in acquiring and maintaining progressive phenotypes, such as invasion, distant metastasis, and chemoresistance, in head and neck squamous cell carcinoma (SCC). However, little is known whether TGF-b1 is essential for tumor progression in head and neck adenocarcinoma. Moreover, TGF-b1 is also identified as main inducer of epithelial-mesenchymal transition (EMT) by up-regulating several transcription factors, including Snail, Slug, dEF1, and SIP1. Although some reports have shown that EMT promotes invasion and metastasis, it remains unknown which EMT associated transcription factors are essential for tumor progression in head and neck.

To demonstrate that TGF-b1 promotes head and neck cancer progression, we used SCC cell lines (SAS, HSC4, Ca9-22, and Gun1) and adenocarcinoma cell line (HSG) of head and neck.

We observed that TGF-b1 stimulation induced phosphorylation of Smad2/3 and up-regulated PAI-1 and Smad4 mRNA levels in both SCC and adenocarcinoma cells. Interestingly, adenocarcinoma cells were more sensitive to TGF-b1 than SCC cells. In addition, cancer cell motility and viability against docetaxel were enhanced by TGF-b1 stimulation in both SCC and adenocarcinoma cells.

While TGF-b1 stimulation significantly increased Slug expression in SCC cells, Snail expression, by contrast, was increased in adenocarcinoma cells. Next, we suppressed Slug or Snail expression by using siRNA in SCC or adenocarcinoma cells, respectively. Importantly, TGF-b1 effects on malignant phenotypes were canceled.

Our data indicated that TGF-b1-induced malignant phenotypes in SCC and adenocarcinoma of head and neck were acquired by different EMT-associated transcription factors.

P048: ESRP1 SUPPRESSES TUMOR INITIATION BY DOWN-REGULATION OF RAC1B IN HNSCC – Hiroki Ishii, Kei Ashizawa, MD, Keisuke Masuyama, PhD; University of Yamanashi

The epithelial-mesenchymal transition (EMT) is important for acquisition of phenotypes including cancer cell invasion into stroma and distant metastasis. In head and neck squamous cell carcinoma (HNSCC), epithelial splicing regulatory protein 1 (ESRP1) controls EMT process by alternative RNA splicing of EMT-associated genes such as CD44, MENA and FGFR. We have recently reported that ESRP1 knockdown promoted cancer cell motility by up-regulating Rac1b isoform in HNSCC. Rac1b is self-activating variant isoform of Rac1 and associated with enhancing tumor progression. However, little is known whether

Rac1b contributes to acquisition of malignant phenotypes and tumor progression in HNSCC.

In this study, we examined whether ESRP1 knockdown-induced Rac1b could enhance anchorage-independent growth of HNSCC cells by soft agar assay. Interestingly, ESRP1 knockdown in SAS and HSC4 cells significantly increased the number and size of colony. Next, we found that knockdown of Rac1b significantly suppressed the enhanced anchorage-independent cell growth in ESRP1-depleted SAS cells. Moreover, ESRP1 knockdown in SAS cells also up-regulated MMP-3 expression that degrades the structural components of extracellular matrix and permits tumor invasion and metastasis.

To define whether Rac1b expression is associated with malignant characteristics of HNSCC patients, we evaluated the immunoreactivity of Rac1b in 49 oral squamous cell carcinoma (OSCC) patients. Rac1b expression was significantly elevated in grade 2-3 of OSCC compared with grade 1 of OSCC (p<0.01).

These data indicated that ESRP1 suppressed HNSCC initiation in stromal tissues by down-regulation of Rac1b and MMP3.

P049: POINT OF CARE OPTICAL DIAGNOSTIC TECHNOLOGY; USING SALIVA AS A MOLECULAR FINGERPRINT FOR THE EARLY DETECTION OF ORAL AND OROPHARYNGEAL SCC – K Davies, J Connolly, Y Lang, P Owens, P Dockery, M Olivo, I Keogh; National University of Ireland, Galway

OBJECTIVE: As cancer-associated biomarkers precede the clinical manifestations of disease, there is a growing research interest in clinical molecular diagnostics for early cancer detection. Surface enhanced Raman spectroscopy (SERS) is a technique that provides a spectral-based fingerprint of tissues and biofluids such as saliva at the molecular level. The objective of this study was to investigate the potential of saliva as a point of care molecular diagnostic probe for the early detection of oral and oropharyngeal squamous cell carcinoma using SERS.

METHOD: Saliva samples were collected from cohorts of smokers, those with confirmed oral or oropharyngeal squamous cell carcinoma and from healthy, age and sex-matched controls. Saliva was centrifuged to remove oral debris and stored frozen until analysis. Saliva was placed on gold nanoparticle substrates; Raman spectra were then collected using a Witec Raman Spectroscope and analyzed using principle components analysis (PCA).

RESULTS: We have shown that saliva specific Raman spectra are markedly enhanced by the gold nanoparticle substrates. Analyses of the spectral data suggest that there may be some differences in the molecular fingerprint of saliva between those with confirmed oral or oropharyngeal squamous cell carcinoma and from healthy, age and sex-matched controls.

CONCLUSION: Our results demonstrate that by combining the recent advances in biomedical optics and nanotechnology, diagnosis of oral and oropharyngeal malignancy at earlier subclinical stages is achievable. For the patient, this equates to less severe treatment regime with better outcomes and therefore a significant improvement in quality of life.

P050: MDM2 SNP309 STATUS AND OVERALL SURVIVAL IN HPV NEGATIVE SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK – Navdeep S Upile, MBBS, BMedSci, Jon Sheard, Kath Brougham, Nikolina Vlatkovic, PhD, Mark T Boyd, PhD, Terry M Jones, MD, FRCS; University of Liverpool

INTRODUCTION: The prognosis for patients with HPV negative squamous cell carcinomas of the head and neck (SCCHN), has not substantially improved for many decades. Understanding cellular mechanisms that drive oncogenesis in these patients may provide insights which will allow us to improve outcomes. TP53 is the most commonly mutated gene in SCCHN and is negatively regulated by MDM2, an essential gene that is itself a transcriptional target of

p53. MDM2 exhibits a well-characterised T to G polymorphism at nucleotide 309 (SNP309T/G) which lies within the promoter region of MDM2. The G/G haplotype has been shown to increase the intracellular levels of MDM2 mRNA and protein, thereby attenuating the p53 pathway and resulting in enhanced tumorigenesis. We investigated the prevalence of polymorphisms at nucleotide 309 in larynx and hypopharynx carcinoma tissue samples and its effect on 5 year overall survival (OS).

METHODS: Following ethical approval, DNA was extracted from Fresh Frozen Paraffin Embedded blocks of 109 laryngeal and hypopharyngeal tumour samples retrieved intraoperatively (86 and 23 respectively). All patients had been treated within our centre using surgery +/- post operative Radiotherapy or Chemoradiotherapy with curative intent as the primary modality. Long term clinical outcome data was available on all these patients. MDM2 promoter DNA was amplified by Polymerase Chain Reaction (PCR). Amplicons were digested with MspA1i, a restriction endonuclease enzyme that selectively cleaves DNA containing the G allele. Agarose gel electrophoresis was then used to enable determination of the SNP haplotype.

RESULTS: For all 109 cases the 5 year OS was 50.45% (55/109). MDM2 Single Nucleotide Polymorphism (SNP) 309 analysis revealed that 72 cases were T/T, 26 T/G and 11 G/G. The 5 year OS respectively for these groups were 50% (36/72), 53.85 (14/26) and 45.45% (5/11).

DISCUSSION: Studies in other cancers have revealed that MDM2 SNP309 can be a significant factor in disease e.g. Endometrial cancer1. SNP309 is linked with altered levels of MDM2 expression and it seems likely that the association with disease is a direct consequence of MDM2 levels regulating the cellular level of p53. Our data suggest that SNP 309 status does not significantly influence overall survival in laryngeal or hypopharyngeal SCC. However, we recognise there are several limitations with this data. Our case selection is a surgical subset of all laryngeal and hypopharyngeal SCCs which may have introduced a bias into the result. Secondly determination of the haplotype relies on an indirect method. We are proposing to recapitulate this data using direct sequencing methodology and to expand our subsites to HPV –ve oropharynx to robustly determine whether there is any influence of MDM2 SNP 309 on outcome.

1 Hum Cell. 2011 Jun;24(2):57-64. doi: 10.1007/s13577-011-0013-4. Epub 2011 Mar 4

P051: MODULATION OF TUMOR GROWTH, VASCULARITY AND INFLAMMATORY CELL INFILTRATION FOLLOWING PI3K/MTOR AND MAPK TARGETED THERAPY IN A SYNGENEIC MODEL OF ORAL CAVITY CANCER – Harrison Cash, BS[1], Sujay Shah, BS[2], Andria Caruso, MD[3], Carter Van Waes, MD, PhD[2], Clint Allen, MD, PhD[4]; [1]Medical Research Scholars Program, National Institutes of Health, [2]National Institutes of Health, [3]Walter Reed National Military Medical Center, [4]Department of Otolaryngology-Head and Neck Surgery, Johns Hopkins School of Medicine

INTRODUCTION: Emerging evidence indicates that receptor or signal kinase components of the phosphoinositide-3-kinase (PI3K)/mammalian target of rapamycin (mTOR) and mitogen activated protein kinase (MAPK) pathways harbor genetic mutation or copy number alteration in the majority of head and neck squamous cell carcinomas (HNSCCs). Characterization of the HNSCC tumor microenvironment demonstrates infiltration of different immunosuppressive hematopoietic cell subsets leading to dysregulated anti-tumor immunity. The link between these activated signaling pathways and immunosuppressive microenvironment is poorly understood. Here, we characterize direct tumor cell and cellular tumor microenvironment alterations following treatment with the mTOR inhibitor rapamycin and MEK inhibitor PD325901 (PD901) in a syngeneic murine model of oral cavity cancer.

METHODS: Poorly immunogenic, ras and p53-mutant mouse oral cancer 2 (MOC2) cells with coactivated PI3K/mTOR were treated in-vitro with rapamycin, PD901 or combination and evaluated for on-target and pathway inhibition effects with western blot, flow cytometric, RT-PCR and ELISA analysis. MOC cells were then transplanted into fully immunocompetent C57BL/6 mice and tumor bearing mice were followed for primary growth and survival analysis following 21 days of treatment with rapamycin and PD901. A subset of tumors were excised after treatment, processed and evaluated for on-target drug effects as well as alterations in infiltrating hematopoietic cells with flow cytometric, IHC, RT-PCR and ELISA analysis.

RESULTS: In vitro, drug induced inhibition of either pathway alone resulted in enhanced activation of the other as measured by protein phosphorylation in MOC2 cells; this was overcome with combination therapy. MAPK pathway inhibition resulted in cell death, reduced CD44 expression, inhibition of cellular migration and reduced expression of chemotactic cytokines VEGF, MCP-1 and GRO/KC whereas mTOR inhibition alone produced modest effects on these parameters in-vitro. In-vivo, tumor-bearing mice demonstrated significantly reduced MOC2 primary tumor growth with either drug alone and absolute growth inhibition with combination rapamycin and PD901. Tumor progression occurred after therapy was stopped in all mice. Survival was significantly increased with treatment compared to control and limited toxicity was observed. MAPK inhibition significantly suppressed CD44 expression, tumor angiogenesis and expression of chemotactic cytokines. mTOR inhibition produced additive or synergistic effects when combined with MAPK blockade. Combination therapy significantly reduced tumor infiltration but not systemic (splenic) presence of CD11b+Gr1+ myeloid derived suppressor cells, CD11b+Ly6ClowF4/80+ M2 macrophages and CD4+Foxp3+ regulatory T-cells. Combination therapy failed to induce an antigen-specific immune response as measured by H2Kb:p15E-tetramer positive CD8+ T-cell tumor infiltration despite a systemic p15E specific immune response being present.

CONCLUSIONS: In poorly immunogenic, ras and p53-mutant MOC2 tumors, the growth inhibitory effects of mTOR and PD901 inhibition appear to be related to effects on tumor, vascular and infiltrating inflammatory cells tumor cell intrinsic but not related to enhanced antigen specific T-cell immunity. Similar studies are currently underway in a highly immunogenic model where alteration of the immunosuppressive tumor microenvironment following mTOR and MAPK targeted therapy may enhance anti-tumor immunity.

P052: THERAPEUTIC TARGETING OF CYCLIC GMP SIGNALING IN HEAD AND NECK CANCER – Traci R Tuttle, PhD, Michelle L Mierzwa, MD, Keith A Casper, MD, Nira Ben-Jonathan; University of Cincinnati

OBJECTIVES: Common treatments for head and neck squamous cell carcinoma (HNSCC) often result in treatment failure, adverse side effects, and decreased quality of life. These provide a major incentive to develop alternative targeted therapies. Activation of the enzyme soluble guanylate cyclase (sGC) leads to increased intracellular cyclic GMP (cGMP), which is rapidly broken down by phosphodiesterase 5 (PDE5). Activation of the cGMP pathway induces apoptosis and/or suppresses cell proliferation in several cancer cell types, but the role of this pathway in HNSCC has not been investigated. We hypothesized that drugs which increase cGMP levels cause apoptosis in HNSCC under in vitro and in vivo conditions. Our objectives were: 1) to examine the effects of sGC activators or PDE5 inhibitors on viability and apoptosis of several HNSCC cell lines, 2) to determine whether these drugs increase the efficacy of standard chemotherapy, targeted therapy, and/or ionizing radiation, and 3) to determine whether these drugs affect tumor growth in mouse xenograft models.

MATERIALS & METHODS: HNSCC cell lines (CAL 27, UMSCC-1, UMSCC-6 and UMSCC-47) were incubated with increasing doses of sGC activators (YC-1 or BAY 41-2272) or a PDE5 inhibitor (Cialis) alone, or in combination with

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Cisplatin, 5-Fluorouracil, Lapatinib, or ionizing radiation. Following treatment with the drugs and/or radiation, cell viability was analyzed using the MTT assay, and apoptosis was determined by flow cytometry. CAL27 cells (2x106 cells in Matrigel) were inoculated sc in the flank of athymic nude/nude female mice. Tumor growth was monitored with calipers. Once tumor volume reached ~250 mm3, continuous drug delivery was provided via Alzet osmotic mini-pumps implanted sc in the dorsal neck.

RESULTS: Treatment with YC-1, BAY 41-2272 or Cialis dose-dependently decreased the viability of HNSCC cells and induced apoptosis. Co-treatment with low doses of any of the three drugs strongly enhanced the effects of Cisplatin and 5-Fluorouracil, the EGFR inhibitor Lapatinib and ionizing radiation on HNSCC cell viability. Ongoing studies are evaluating the effects of Cialis or BAY 41-2272 on the growth of CAL27 xenografts in nude mice.

CONCLUSIONS: Activators of the cGMP pathway induce apoptosis and enhance the efficacy of both chemotherapy and ionizing radiation in HNSCC cells. Our data suggest that this pathway is targetable in the treatment of HNSCC. PDE5 inhibitors such as Cialis are FDA-approved to treat erectile dysfunction, while an sGC activator, Riociguat, was recently approved for treating pulmonary hypertension. We foresee that these drugs should be considered as novel therapeutic intervention for head and neck cancer. Furthermore, analysis of tumor sGC/PDE5 activity may be predictive of response to these therapies.

P053: ANTIBIOTIC PROPHYLAXIS IN HEAD AND NECK PATIENTS RECEIVING FREE FLAP RECONSTRUCTION – Ryan M Mitchell, MD, PhD[1], Eduardo Mendez, MD, MS[1], Nicole C Schmitt, MD[2], Amit D Bhrany, MD[1], Neal D Futran, DMD, MD[1]; [1]University of Washington, [2]University of Pittsburgh

BACKGROUND: Evidence supports short courses of perioperative antibiotics for patients receiving minor clean-contaminated head and neck procedures. Few studies have addressed antibiotic type and duration for patients receiving free flap reconstruction of head and neck defects. These patients, particularly those with malignant pathology, frequently have a number of characteristics considered possible risk factors for developing postoperative infections and other complications, including the use of tobacco, long procedure duration, previous treatment, and comorbidities such as diabetes mellitus and hypothyroidism.

METHODS: We performed a retrospective cohort study of 427 adults receiving free flap reconstruction of head and neck defects. Data was abstracted from patients' medical records including date of surgery, duration of perioperative antibiotic prophylaxis, type of antibiotic, methicillin-resistant staphylococcus aureus (MRSA) carrier screen test results, sex, free flap donor site and flap components, surgical wound classification, pathology, cervical lymph node stage (N-stage), age-adjusted Charlson comorbidity index (CCI), diagnosis of hypothyroidism, previous resection, previous radiation therapy to the head and neck, previous chemotherapy, alcohol use at the time of the preoperative appointment, tobacco smoking history, and oral tobacco use. Clinical outcomes within 30 days of the reconstruction were determined from patients' charts including infection at the flap site, neck incision, tissue donor site, and distant non-surgical sites; and flap site complications including dehiscence, fistula, or flap compromise.

Patients were compared by logistic regression based on type and duration perioperative antibiotics, as well as other potential risk factors. The primary outcome was any infection within 30 days of surgery.

RESULTS: During the study period, our institutions changed recommended antibiotics for clean-contaminated procedures, and changed policies to recommend short courses of perioperative prophylactic antibiotics. For the patients included in the study, 96 (22.5%) received prophylactic antibiotics for ≤ 24 hrs, while 331 patients received prolonged courses of prophylactic antibiotics. The majority of patients received ampicillin/sulbactam alone for prophylaxis (53.2%), while 36.5% received clindamycin alone, and 10.3% of

patients received an alternative regimen. The use of clindamycin (OR = 2.54, 95% CI: 1.25-5.14), but not duration of antibiotics (OR = 0.63, 95% CI: 0.34-1.19), was associated with an increased risk of postoperative infection.

Subgroup analysis was performed to identify risk factors associated with complications at the flap site including infection, dehiscence, fistula, and flap compromise. By multivariate analysis, use of clindamycin (OR: 2.77, 95% CI: 1.35-5.66) and osteocutaneous flaps (OR: 2.29, 95% CI 1.23-4.26) were associated with increased risk of any flap site complications.

CONCLUSIONS: The choice of antibiotic appears to affect the rate of postoperative infection more than the duration of antibiotics following head and neck free flap reconstruction. At our institutions, we have begun to use ampicillin/sulbactam as the preferred prophylactic antibiotic for major clean-contaminated head and neck procedures when possible.

P054: NAVIGATED OSTEOTOMIES IN ABLATIVE HEAD AND NECK ONCOLOGIC SURGERY: ACCURACY, REPEATABILITY AND FEASIBILITY IN PRE-CLINICAL PHANTOMS AND PATIENTS – Jonathan M Bernstein, MD, FRCS, Michael J Daly, MSc, Harley H Chan, PhD, Jimmy Qiu, MASc, Nidal Muhanna, MD, PhD, Robert Weersink, PhD, David Goldstein, MD, MSc, FRCSC, John R de Almeida, MD, MSc, FRCSC, Ralph W Gilbert, MD, FRCSC, Jonathan C Irish, MD, MSc, FRCSC; University of Toronto

OBJECTIVES: Navigation is used in fields including neurosurgery and orthopedics to improve precision. Intraoperative image guidance may have utility in ablative head and neck surgery [1]. The objectives of this study were to assess the accuracy, reproducibility and clinical feasibility of a prototype surgical navigation system applied to osteogenic tumors of the maxilla and mandible and soft tissue tumors invading bone, comparing navigated and unnavigated osteotomies in pre-clinical phantoms and patients.

MATERIALS & METHODS: Using a 3-dimensional (3D) planning tool, we undertook a prospective study comparing navigated with unnavigated mandibular and maxillary osteotomies using Sawbone and cadaver models in the laboratory (Fig. 1) and validated the technology in patients in the operating room. The in-house research system utilizes real-time optical image tracking (NDI Polaris), 3D visualization with saw plane clipping, and cone-beam computed tomography (CBCT) [2]. Navigated and unnavigated osteotomies for segmental mandibulectomy and maxillectomy were undertaken. Cut planes were analyzed using CBCT and deviation from the cut plans was calculated. The Mann-Whitney U test was used for non-parametric comparisons.

RESULTS: Based on pilot data, the planes of 100 navigated and 100 unnavigated bone cuts in Sawbones models and cadavers will be compared. The navigation of a reciprocating saw blade is accurate to 1-2 mm. Proof-of-principal clinical feasibility of the techniques and processes has been ascertained in three patients in the research-designated operating room at University Health Network.

CONCLUSION: 3D surface rendering in addition to tri-planar views enhances navigation. The present laboratory and clinical study on the accuracy of navigation in ablative head and neck surgery may lead to the translation of this technology into the clinical setting.

REFERENCES: [1] King E, Daly MJ, Chan H, Bachar G, Dixon BJ, Siewerdsen JH, Irish JC. Intraoperative cone-beam CT for head and neck surgery: feasibility of clinical implementation using a prototype mobile C-arm. Head Neck 2013;35:959-67.

[2] Dixon BJ, Daly MJ, Chan H, Vescan A, Witterick IJ, and Irish JC. Augmented real-time navigation with critical structure proximity alerts for endoscopic skull base surgery. Laryngoscope 2014;124:853-59.
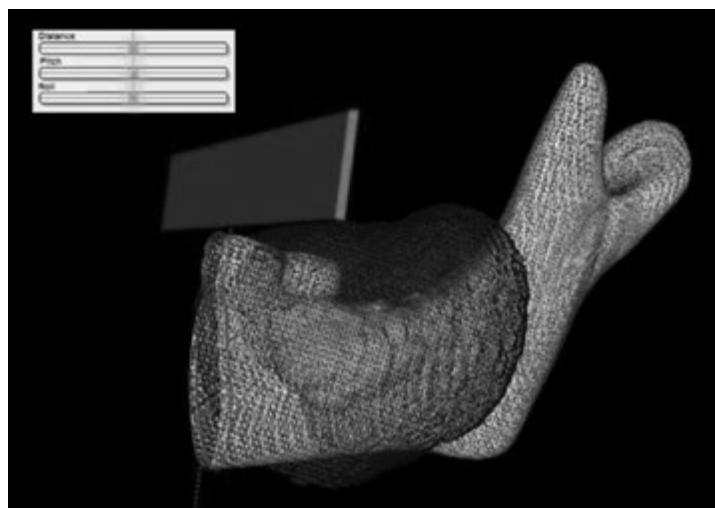
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FIGURE 1: CBCT 3D reconstruction of a Sawbones mandible with virtual tumor (yellow), 1 cm computer-generated margin around the virtual tumor (brown), planned osteotomy plane (red), and navigated saw aligned in position (purple). Three orthogonal views (inset) turn green when the navigated saw blade is precisely aligned with the planned osteotomy plane.

P055: CLEAVED NOTCH1 EXPRESSION PATTERN IN HEAD AND NECK SQUAMOUS CELL CARCINOMA IS ASSOCIATED WITH NOTCH1 MUTATION, HPV STATUS AND HIGH-RISK CLINICAL FEATURES – Eleni M Rettig, MD, Christine H Chung, MD, Justin A Bishop, MD, Jason D Howard, PhD, Rajni Sharma, PhD, Ryan J Li, MD, Christopher Douville, Rachel Karchin, PhD, Evgeny Izumchenko, PhD, David Sidranksy, MD, Wayne Koch, MD, Joseph Califano, MD, Nishant Agrawal, MD, Carole Fakhry, MD; Johns Hopkins

BACKGROUND: The Notch pathway is frequently altered in head and neck squamous cell carcinomas (HNSCCs), however the nature and clinical significance of NOTCH1 dysregulation is poorly understood.

OBJECTIVES: This study was designed to characterize expression of the transcriptionally active NOTCH1 Intracellular Domain (NICD1) in HNSCCs and evaluate its association with NOTCH1 mutation status and clinical parameters.

METHODS: Immunohistochemistry for NICD1 was performed on 79 previously sequenced archival HNSCCs with known NOTCH1 mutation status. Retrospective medical record review was performed to determine clinicopathologic variables of interest. NICD1 expression pattern, NOTCH1 mutation status and clinicopathologic variables were compared. NICD1 expression pattern and NOTCH1 mutation were also evaluated for association with disruptive TP53 mutation.

RESULTS: Three distinct immunohistochemical staining patterns were identified: positive/peripheral (47%), positive/non-peripheral (34%) and negative (19%). NICD1 expression was associated with NOTCH1 mutation status (p<0.001). Most NOTCH1-wild type tumors were peripheral (55%), while mutated NOTCH1 tumors were most commonly negative (47%). Non-peripheral tumors were more likely than peripheral tumors to have extracapsular spread (aOR 16.01, 95%CI=1.92-133.46, p=0.010) and poor differentiation (aOR 5.27, 95%CI=0.90-30.86, p=0.066). Negative staining tumors tended to be poorly differentiated (aOR 24.71, 95%CI=1.53-399.33, p=0.024) and were less likely to be HPV-positive (aOR 0.043, 95%CI=0.001-1.59, p=0.087). NOTCH1 mutation was significantly associated with HPV status, with NOTCH1-wild type tumors more likely to be HPV-positive than NOTCH1-mutated tumors (aOR 19.06, 95%CI=1.31-276.15, p=0.031). TP53 disruptive mutations were not associated with NICD1 expression or NOTCH1 mutation.

CONCLUSION: NICD1 is expressed in three distinct patterns in HNSCC. The immunohistochemical patterns are significantly associated with high-risk clinical features. These findings further support a dual role for NOTCH1 as both tumor suppressor and oncogene in HNSCC. Additional research is necessary to clarify the role of NOTCH1 in HNSCC and understand the clinical and therapeutic implications therein.

P057: SYNERGISTIC CYTOTOXICITY OF REOVIRUS IN COMBINATION WITH HISTONE DEACETYLASE INHIBITORS IN THE TREATMENT OF SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK – Matthew Old, MD, Jun-Ge Yu, MD, Alena Cristina Jamie-Ramirez, PhD, Enrico Caserta, Theodoros Teknos, MD, Flavia Pichiorri, PhD, Balveen Kaur, PhD; The Ohio State University

BACKGROUND & OBJECTIVES: Oncolytic viruses (OVs) are emerging as potentially powerful, targeted anti-cancer agents. A promising OV, Reovirus, is a naturally occurring non-pathogenic, double-stranded RNA virus that was isolated from the human respiratory and gastrointestinal tracts. Moreover, Reovirus type 3 Dearing (Reolysin; Oncolytics Biotech Inc., Calgary, AB, Canada) is currently being tested in phase I-III clinical trials in a variety of tumor types. Currently, even with standard combination treatment regiments, locally advanced head and neck cancer carry a dismal prognosis, where the overall 5-year survival rate for oral cavity and oropharyngeal squamous cell carcinomas (SCC) is still less than 56%. There is a clear and pressing need for novel therapies with activity against these tumors. Histone deacetylase inhibitors (HDACi) comprise a structurally diverse class of compounds that are targeted anticancer agents. The first FDA approved HDACi, vorinostat (suberoylanilide hydroxamic acid-SAHA), is highly effective in the treatment of cutaneous T-cell lymphoma. Moreover, like Reolysin, SAHA, is currently being testing in head and neck cancer clinical trials. Data obtained from our group indicates that treatment with the HDACi (SAHA or AR-42) results in the upregulation of the Reolysin receptor, JAM-1. We hypothesize that treatment of squamous cell carcinoma of the head and neck (SCCHN) with HDACi and reovirus (reolysin) will result in synergistic, anti-tumor effects.

MATERIALS/METHODS: Reolysin was kindly provided by Oncolytics Biotech Inc. (Calgary, AB, Canada). Cell survival experiments were performed with MTT assays alone, and in combination with reovirus and SAHA and AR42. . IC50 values were interpolated from a sigmoidal dose–response curve fit of the log-transformed survival data. The effect of reovirus in combination with HDACi was assessed by the method of Chou and Talalay. CI values were generated using the CalcuSyn software (Biosoft, Cambridge, UK). Western blotting using anti-reovirus antibody was performed to examine the enhancement of HDACi in reovirus replication. JAM-1 surface levels were assessed via flow cytometric analysis. Briefly, tumor cells were treated with Mock, AR-42/SAHA (0.2uM), Reolysin (10 MOI) or the combination. Cells were collected after 48 hours and cell death was assessed via Annexin V and PI staining. JAM-1 levels were assessed using anti-JAM-1-PE staining as compared to a control isotype-PE antibody. Data was analyzed using FloJo software.

RESULTS: Our experiments demonstrated HDACi (SAHA and AR-42) and reolysin treatment alone inhibit SCCHN growth. Combination of HDACi with reolysin exhibit synergistic anti-tumor effects. Western blotting indicated that HDACi increase reovirus replication. JAM-1 is unregulated in SCCHN cells after SAHA or AR-42 treatment. The down regulation of JAM-1 after combinatorial therapy indicates that the receptor is being internalized-most likely with reovirus.

CONCLUSION: This data suggests that the combination of reovirus plus HDACi therapy has significant activity in the treatment of SCCHN. Broader studies are needed to further establish the safety and improved efficacy of Reolysin therapy in combination HDACi prior to translating this novel therapy to phase one clinical trials.

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P058: IDENTIFICATION OF EPIGENETICALLY SILENCED GENES ASSOCIATED WITH THE STATUS OF HPV INFECTION IN HEAD AND NECK SQUAMOUS CELL CARCINOMA – Ana Carolina de Carvalho, PhD, Matias E Melendez, PhD, Lidia Maria R Arantes, PhD, André L Carvalho, PhD; Molecular Oncology Research Center, Barretos Cancer Hospital, Brazil

BACKGROUND: Head and neck squamous cell carcinomas (HNSCC) are characterized by highly incident tumors with high rates of morbidity and mortality resulting from a late diagnostic and the frequent development of recurrences. Tobacco smoking is the major risk factor associated with these tumors; however, high-risk human papilloma virus (HPV) infection has been recently reported as an etiologic factor for a subset of these tumors, mainly in the oropharynx. HPV positive head and neck tumors represent a distinct clinical and epidemiological entity, with differences in clinical presentation, response profiles and treatment outcome when compared to HPV negative tumors. Currently, the therapeutic choice for HNSCC depends on the determination of tumor stage, however, patients with tumors of the same stage and same location may have different outcomes. Therefore, a better characterization of clinical, pathological and molecular factors associated with patient prognosis is of great importance and may help in a more thorough patient management. Changes in DNA methylation are associated with gene silencing. This molecular alteration is involved in malignant transformation and can provide useful markers for early diagnosis, prediction of disease progression and response to therapy in many tumors, including HNSCC.

OBJECTIVE: The aim of this study is to identify the involvement of epigenetic modulation of gene expression through the methylation of specific genes in HNSCC patients and correlate the molecular profiles with HPV status and patient clinical data.

MATERIALS & METHODS: Candidate genes were selected by an in silico search for genes with differential expression and methylation profiles between HPV positive and HPV negative HNSCC from the data available at the TCGA database. The role of methylation in gene expression silencing was evaluated by RT-PCR in HNSCC cell lines (FaDu, JHU-12, JHU-13 and JHU-28) before and after treatment with the demethylating agent 5-aza-2′deoxycytidine (5′-aza-dC).

RESULTS: TCGA expression and methylation data showed differences in the molecular profile of CD40, CDH8, JAK3, KRT8, RUNX2 and STAT5A between HPV positive and HPV negative HNSCC samples (P<0.005, and P<0.00000001, respectively). All genes were downregulated in at least 1 of the cell lines tested. The cell line JHU-28 presented a low or absent expression of all genes evaluated, however, gene expression was restored upon treatment with 1μM of 5′-aza-dC for 3 days, suggesting a role of methylation in the silencing of these genes.

CONCLUSIONS: This study was able to find genes whose expression and methylation profiles were associated with the status of HPV infection in HNSCC samples whose molecular data are available in the TCGA database. Moreover, it was observed that methylation seems to be mediating the transcriptional silencing of the selected genes in HNSCC cell lines. Next, the methylation profile of these genes will be evaluated in HNSCC cell lines and in tumor samples classified according to their HPV status. Finally, the association between the molecular findings, HPV status and the clinical information from the patients will be assessed.

P059: INFLUENCE OF ADJUVANT GLUCOCORTICOID THERAPY ON BODY COMPOSITION IN CANCER PATIENTS UNDERGOING CHEMORADIOTHERAPY – Albert C Chamberlain, MD, Daniel C Jupiter, PhD, E. L Dillon, PhD, Kathleen M Randolph, BS, Christopher Danesi, MS, William J Durham, PhD, Maurice Willis, MD, Randall J Urban, MD, Susan McCammon, MD, Melinda Sheffield-Moore, PhD, Sandra Hatch, MD, Gwyn Richardson, MD; University of Texas Medical Branch

BACKGROUND: Glucocorticoids (GC) are a commonly prescribed class of medication used to decrease inflammation in a broad range of medical conditions including back pain, allergies, rheumatic diseases, gastrointestinal disorders, ophthalmic conditions, dermatological conditions, asthma, chronic obstructive pulmonary disease (COPD), systemic lupus erythematosus, and cancer. In cancer, GC are a mainstay, frontline therapy for patients undergoing chemoradiotherapy (CRT) to reduce CRT symptoms and control systemic inflammation. Paradoxically, while GC therapy is intended to reduce systemic inflammation, severe wasting can occur in skeletal muscle in response to chronic GC therapy. Data from our lab indicates that a single, standard-of-care dose of GC increases in vivo expression of a key skeletal muscle kinase that induces skeletal muscle proteolysis in otherwise healthy men. We also found that this kinase can be counter-regulated by testosterone, thus preventing GC-induced catabolism. Therefore, as part of a recently completed clinical trial examining the efficacy of testosterone as an anabolic/anti-cachectic agent in cancer patients, we additionally examined whether adjuvant GC therapy further influenced body composition in patients with advanced or recurrent cervical or head/neck cancer.

METHODS: A 7-week, prospective, randomized, double-blind, placebo-controlled trial of intramuscular testosterone was administered during 7 weeks of CRT to twenty-one male and female patients with advanced or recurrent cervical or head/neck cancer. Patients received either testosterone enanthate (T, n=9) 100 mg IM injection weekly or placebo (PL, n=12) injection weekly for 7 weeks during CRT. In PL, 9 patients received GC therapy during CRT, while in the T group 8 received GC (dexamethasone). The primary end point examined was change in body composition (leg lean mass, total lean mass, and fat mass) from baseline to week 7.

RESULTS: Total GC, number of days GC was given, and timing of GC did not differ between PL and T. On average, the PL group received more total GC, but this did not reach statistical significance. Using a linear regression model with and without interaction terms, we found no interaction between total GC and total T levels in any measured variable of body composition. Fat mass decreased in both PL and T, with no difference between groups. Notably, without consideration of GC, we found lean body mass markedly increased in the T group while it decreased significantly in PL (+2.3 [± 3.1] v -2.2 [± 3.4] kg; P = 0.005). Interestingly, the anabolic influence of T on lean body mass was more positive during the early phase (weeks 0-3.5 of CRT, p=0.0496) of CRT, without consideration of GC.

CONCLUSION: Adjuvant glucocorticoid therapy given in combination with CRT for symptom management to patients with advanced or recurrent cervical or head/neck cancer does not appear to influence body composition. Conversely, testosterone significantly increased lean body mass in these patients, and appears to have the greatest anabolic influence during the early CRT period. Thus, testosterone and GC therapy appear to each confer a benefit during CRT by providing a means of managing both body composition and CRT symptoms, respectively.

P060: ASSESSMENT OF ANTINEOPLASTIC AGENTS RESPONSE IN AN ESTABLISHED PUTATIVE HEAD AND NECK CANCER STEM CELL LINE – M E Melendez, PhD, K Pepineli, R J da Silva Oliveira, MSc, A L Almeida Vicente, F Cury, M F Zanon, A Tansini, G N Berardinelli, F Escremim, C Scapulatempo, R M Reis, A L Carvalho; Molecular Oncology Research Center - Barretos Cancer Hospital

INTRODUCTION: Current evidence suggests that initiation, growth, and invasion of cancer are driven by a small polulation of cancer stem cells (CSC). Previous studies have identified CD44 cells as cancer stem cells in head and neck squamous cell carcinoma (HNSCC). However, CD44 is widely expressed in most cells in HNSCC tumor samples and several cell lines tested. In this work, we characterize a putative HNSCC cancer stem cell line, HCB289 cell line, which is positive for both CD44 and ALDH markers.

METHODS: A laryngeal primary tumor sample from a 43 years-old at-diagnosis male, with T4N2M0 clinical staging, was submitted to primary cell culture. Authenticity of the HNSCC cells was performed using STR markers. Expression of keratin, vimentin and p16 proteins were analyzed by immunocytochemistry. EGFR copy number was determined by FISH, while mutational status of hotspot regions of EGFR, KRAS, PI3KCA genes and all coding-region of PTEN was assessed by PCR-followed direct sequencing. Genomic instability was evaluated by microsatellite analysis. Cell sorting was performed with FACSAria II, using CD44-PE and Aldefluor reagents. Double negative (DN) and double positive (DP) populations for both markers were sorted out and further amplified in cell culture. Clonogenic potential was analyzed by agar colony formation assay, on DN and DP populations. To evaluate the biologic effect of paclitaxel, cisplatin, cetuximab and AST1306 (a novel irreversible anti-EGFR drug), cell viability and proliferation was measure by MTS.

RESULTS: HCB289 HNSCC cells only expressed keratin and were negative for vimentin or p16 markers. Molecularly, the cells showed absence of microsatellite instability, lack of EGFR copy number alterations, and were wild-type for all cancer genes evaluated. By Flow cytometry, 84% of the HNSCC cells were CD44+, and 2.6% were ALDH+. A putative cancer stem cell subpopulation of CD44+/ALDH+ cells (DP), representing 1.1% of the population, was identified. After cell culture of DP and DN subpopulations, we observed that DP cells showed greater clonogenic potential than the DN population, whereas the DN cells had higher proliferation rate. Cell viability measured by IC50_SD of DP and DN subpopulations for the distinct drugs was: paclitaxel (16.41 ± 2.81 and 9.32 ± 2.22 nM), cisplatin (17 ± 1.84 and 4.35 ± 0.65 μM), cetuximab (247.8 ± 22.6 and >250 μg/mL) and AST1306 (0.811 ± 0.073 and 0.421 ± 0.013 μM).

CONCLUSION: We identified a colony-forming CD44+/ALDH+ subpopulation on a established HNSCC cell line. We showed that this putative head and neck cancer stem cell line exhibited a reduced proliferative rate, and a higher resistance to almost all drugs analyzed. Therefore, the current HCB289 HNSCC cells represent an interesting model for further biological and therapeutically proposes.

P061: SERUM IL-6 LEVELS SHOW A DIRECT CORRECTION WITH CIRCULATING TUMOR CELLS (CTCS) AND POOR PROGNOSIS – Azeem Kaka, MD[1], Arti Yadav, MS[1], Kyoung-Joo Park, BS[2], Peter Amaya, MS[2], Bhavan Kumar, MS[1], James Lang, PhD[1], Jeffrey J Chalmers, PhD[2], Theodoros N Teknos, MD[1], Pawan Kumar, MS, PhD[1]; [1]Department of Otolaryngology-Head and Neck Surgery, The Ohio State University, Columbus, OH 43210, [2]Department of Chemical and Biomedical Engineering, The Ohio State University, Columbus, OH 43210

Distant metastases in head and neck cancer patients almost invariably herald a poor prognosis. Five year survival rates for early stage localized head and neck cancers are over 80% but this drop to 40% where disease has spread to neck nodes, and to below 20% for patients with distant metastatic disease. A key process in this metastatic cascade is the transition of tumor cells from an adherent epithelial phenotype into a highly motile and invasive mesenchymal phenotype (EMT), which plays a critical role in tumor cell dissemination from the primary tumor into circulation. We have recently demonstrated that IL-6 is a potent inducer of EMT-related changes in HNSCC via the JAK-STAT3-Snail signaling pathway. These results therefore suggest a potential role of IL-6 in the release of CTCs from the primary tumors. Recently, we have also shown that low number of circulating tumor cells predict a significantly higher disease-free survival. However, there is no published report showing a correlation between serum IL-6 levels and CTCs. In this study, we examined if serum IL-6 levels directly correlate with CTCs and also examined if high IL-6 levels could predicts poor prognosis in HNSCC patients. The majority of the published studies on the detection of CTCs in the blood of cancer patients use a combination of a negative depletion step (removal of RBCs) and a positive selection step to enrich CTCs using an anti-epithelial antibody bound to a magnetic particle. However, the use of a positive selection

technique for the CTCs introduces a potential, significant bias into the final detection analysis: the CTCs must express the surface marker that is specifically recognized by the antibody. This represents a potential problem because it is likely that tumor cells that have passed through a partial or complete EMT are no longer detectable by epithelial-specific antigens. To circumvent this problem, we have developed an enrichment method that is based only on negative depletion of normal cells. In this study, we used this negative selection method to quantify CTCs in the blood samples of head and neck cancer patients. Our results suggest a direct correlation between serum IL-6 levels and CTCs in same patient (r=0.976, p<0.0001, n=15). HNSCC patients with <10 pg/ml of IL-6 in their serum had 0 detectable CTCs in their blood samples. In contrast, patients with >10 pg/ml of IL-6 showed markedly higher numbers of CTCs. Mean IL-6 level in patients with <1 CTC was 6.5 pg/ml and mean IL-6 level in patients with >1 CTC was 55.1 pg/ml. We are in the process of examining additional blood samples (total n=50) for IL-6 and CTC levels and correlating the IL-6/CTC levels with clinical parameters.

P062: CANCER STEM CELL PATHWAY IN HEAD AND NECK SQUAMOUS CELL CARCINOMA: A POTENTIAL THERAPEUTIC TARGET – Kaveh Karimnejad, MD, Wen Li, MD, Mark A Varvares, MD, Reigh-Yi Lin, PhD; Saint Louis University

OBJECTIVE: Head and neck squamous cell carcinoma (HNSCC) is the sixth most common type of cancer worldwide with an approximate 5-year survival rate of 40-50%. Two thirds of HNSCC present as advanced cancer with lymph node metastases. Thirty-five to 55% of patients with advanced-stage HNSCC remain disease-free 3 years after standard treatment. However, primary site recurrence occurs in 10-30% of patients. Cancer stem cells (CSCs) may be a cause of tumor recurrence and chemoradiation resistance in HNSCC. CD10, CD24, CD44 were previously identified as possible CSC markers in HNSCC while CD10 and ALDH have been associated with therapeutic resistance. This study evaluated the expression of CSC markers in the tumors of HNSCC patients and analyzed the clinicopathological characteristics of the patients to determine the clinical implications of these CSC markers.

METHODS: Archived tumor tissue from 5 HNSCC patients (4 tongue and 1 laryngeal cancer) were analyzed for the expression of CD44, CD24, CD10, and ALDH by immunohistochemistry. A chart review was performed for the five patients to correlate clinical information with laboratory findings.

RESULTS: Cancer staging and tumor grades for the five patients ranged from Stage I-IV and well differentiated to poorly differentiated tumors, respectively. 2/5 tongue cancer patients demonstrated positive staining for CD44 in >75% of tumor cells. One of these patients underwent failed primary treatment with chemoradiation followed by surgical resection with clear margins (7 mm), but ultimately developed a second local recurrence. The other CD44 positive patient underwent surgical resection with clear margins (7 mm) followed by radiation therapy and continues to be disease free three years post-treatment. 1/5 patients demonstrated positive staining for both CD44 in >75% of tumor cells and CD10 in 10% of tumor cells and 50% of surrounding stromal cells. This patient underwent surgical resection with clear margins (9 mm), however within one year she developed primary site recurrence and distant metastases. 1/5 patients demonstrated positive staining for both CD44 and CD24 in the entire tumor area. This patient had clear margins at the time of resection (.5 mm) and has not had a recurrence of his tongue cancer. However, this same patient had previously developed a separate oral cavity cancer close to the primary site which did recur following chemoradiation. The patient with laryngeal cancer demonstrated positive staining for CD44 in > 75% of tumor cells. He developed distant metastases following surgical resection with clear margins (2 mm) and radiation therapy. All 5 patients demonstrated negative staining for ALDH on tumor cells.

CONCLUSIONS: In our pilot study, 3/5 patients had cancer recurrence while the remaining two are only three years post-treatment and still have the potential

for recurrence. Therefore, CD44 may be a marker for CSC's which promote recurrence of HNSCC. Furthermore, combined CD44/CD10 expression appears to correlate with aggressive recurrence while combined CD44/CD24 expression may cause susceptibility to multiple primary HNSCCs. Additional studies in larger cohorts are required to clarify the significance of the expression of CD44, CD10, and CD24 in the pathogenesis of tumor recurrence and resistance to therapy.

P063: UNRAVELING THE CELLULAR HIERARCHY WITHIN HUMAN TONSILLAR CRYPT EPITHELIUM AND OROPHARYNGEAL SQUAMOUS CELL CARCINOMA TUMORS – <u>Vivian Wu, MD, MPH</u>[1], Brette Harding, BS[1], Tennison Yu, MS[1], Robert Bruno, PhD[2]; [1]EVMS, [2]Old Dominion University

OPSCCs most commonly arise from the epithelium lining the tonsillar crypts of the lingual and palatine tonsils. The stratified epithelium at the surface of the tonsils gives way to complex reticulated epithelium in the crypts. The epithelium within these crypts contains a diverse cell population with varying cytokeratin expression patterns and morphology. However, how such a diverse cell population is maintained has not yet been explored. It has been observed that HPV+ HNSCC consistently arise from the tonsillar crypts. In addition, somatic stem and progenitor cells have been identified as the likely targets of tumorigenesis in many tissues, including HPV-induced tumors of the epidermis and cervix. To further evaluate whether tonsillar crypts contain unique multi-potent stem cells that are targets of tumorigenesis, and similar stem cells are maintained in OPSCC tumors of tonsillar origin (including HPV+ OPSCC), we propose the following studies:

SPECIFIC AIM 1: Characterize the cellular populations of tonsillar crypt epithelium and compare to oral cavity epithelium. Human tissue obtained from the IRB approved EVMS Biorepository will be evaluated. We will stain for purported markers of stem cell activity including LGR5/6, CD34, p63, Bmi-1, cytokeratins 15, 19, and hTert using tyramide signal amplification (Life Technologies) when necessary. Cytokeratins 5, 8, 14 and 17 (previously identified in subsets of crypt epithelium) will be evaluated and co-stained with the above markers. Ki67 will be used to identify proliferative regions within the crypt epithelium.

SPECIFIC AIM 2: Characterize the cellular population of oropharyngeal squamous cell carcinoma and compare to normal tonsillar crypt epithelium. Human tumor obtained from EVMS IRB approved Biorepository will be evaluated. HPV+ and HPV- OPSCC cell lines will be quantified for the markers of stem cell activity listed in Aim 1. Similarly, the same markers will be evaluated in HPV+ and HPV-human OPSCC tumors and compared to normal tonsillar epithelium. The cell cycle regulator, p16, which is up-regulated in HPV-induced OPSCC and has previously been identified in a subset of normal tonsillar crypt epithelial cells will also be co-stained with any identified stem cell markers to determine if it is associated with any stem/progenitor cell function. Patient data will be correlated to identify prognostic value of these markers.

SPECIFIC AIM 3: Evaluate clonal expansions of cells in tonsillar crypts and OPSCC tumors by mitochondrial lineage tracing. Acquired deficiencies in the mitochondrial gene for the respiratory chain enzyme cytochrome c oxidase (CCO) can be exploited to identify cellular lineages within human tissues and tumors. Homoplastic mtDNA mutations take many years to be acquired, so deficient lineages are reasonably assumed to arise from a single stem cell. This aim will evaluate if such CCO deficient (CCO-) clones exist within normal tonsillar crypts and OPSCC tumors, which will allow for careful lineage tracing in future experiments to fully understand the cellular hierarchy underlying tonsillar crypt and OPSCC tumor morphology. Elucidating the cellular hierarchy within tonsillar crypts, and identifying whether multi-potent stem cells exist within the tissue is imperative to understanding OPSCC, including those that are HPV-induced.

P064: FACTORS INFLUENCING THE LONGEVITY AND FREQUENCY OF REPLACEMENT OF THE PROVOX VOICE PROSTHESIS – <u>Alper Yenigun</u>[1], Sabri Baki Eren, Assistant, Professor[2], Murat Haluk Ozkul, Associate,

Professor[3], Selahattin Tugrul, Assistant, Professor[2], Aysenur Meric, Associate, Professor[2];[1]Konya Hospital, Otorhinolaryngology Clinic, [2]Bezmialem Vakif University, Department of Otorhinolaryngology, [3]Haseki Education and Resarch Hospital, Otorhinolaryngology Clinic

INTRODUCTION: The goal of the study was to perform an assessment of factors influencing the longevity and replacement frequency of a Provox voice prosthesis following its placement.

PATIENTS & METHOD: The data of 27 patients who received Provox voice prosthesis after total laryngectomy and regularly followed up between the years 1998-2012 were retrospectively examined. All the patients were male, the age of patients ranged between 43 and 78 and the average age was 63. The follow-up period of patients was 60.3 months (6-168 months). The rates and quality of speech of patients, complications, prosthesis replacement frequency and reasons were evaluated.

RESULTS: Fluent and understandable speech was obtained in 85% of 27 patients who received Provox voice prosthesis. The prosthesis replacement periods were minimum 1 month and maximum 36 months with the average period being 17 months. The most frequent complication was fluid leak through the prosthesis. A strong positive correlation of 77.1% was identified between the longevity of prosthesis and operation follow-up duration (r=0.771; p<0.01)

CONCLUSION: Voice prosthesis is a tool that can be delivered in a practical fashion and replaced easily with no serious complications by means of which speech can be achieved at a high rate. In our study, it was concluded that the patient factor was the most important factor influencing the increase in the longevity of Provox voice prosthesis. Postoperative follow up duration is an important predictor of the longevity of Provox voice prosthesis.

P065: CASE PRESENTATION OF A PATIENT WITH HPV-ASSOCIATED OROPHARYNGEAL CARCINOMA DEMONSTRATING A PERSISTENTLY ELEVATED T CELL RESPONSE AGAINST E7 ANTIGEN – <u>Mirabelle B Sajisevi, MD</u>, Robyn Medinas, Kent J Weinhold, PhD, Walter T Lee, MD; Duke University Medical Center

PURPOSE: To report a case of a patient with human papilloma virus associated oropharyngeal squamous cell carcinoma with a sustained elevated CD 8+ effector cell response against E7 antigen.

BACKGROUND: Human Papilloma Virus (HPV) has been linked to the development of oropharyngeal squamous cell carcinoma (OPSCC) in a subset of patients. These patients tend to be younger and often do not have the classic risk factors of smoking and drinking like their HPV negative counterparts. They present with more advanced stage but demonstrate statistically significant improved survival after treatment. Preclinical and clinical studies have demonstrated that an HPV specific adaptive immune response is important in cancer clearance and survival. This therapy induced immune reaction remains not well defined in human OPSCC patients. In this study, we report the unusual finding of a patient with a sustained elevated CD 8+ effector cell response against E7 antigen.

DESCRIPTION: Patients with locally advanced, non-metastatic OPSCC undergoing chemoradiation therapy were approached to give blood for immune studies. Blood samples were acquired at 4 time points: pre-treatment, early treatment, late treatment and post treatment. Peripheral blood mononuclear cells were stimulated with HPV E6, HPV E7, CMV pp65, and CMV IE-1 peptide pools. Intracellular cytokine staining (ICS) and multiparameter flow cytometry were performed to measure HPV-specific and CMV-specific T cell responses. The cytokines analyzed in this assay included interferon gamma (IFN-γ), interleukin 2 (IL-2), tumor necrosis factor alpha (TNF-α).

RESULTS: Six subjects were enrolled in the study. One patient with HPV-associated OPSCC demonstrated a significantly elevated CD8+ response to

HPV E7 antigen. This response was characterized by production of both IFN-γ and TNF-α detected in 10% or more of CD8+ lymphocytes. This HPV-specific response was maintained throughout concurrent chemoradiation and afterward, despite transient therapy-induced lymphopenia. Further characterization of the CD8+ lymphocyte subsets revealed this patient to have a high proportion of effector cells at 81.6% which is significantly elevated (normal control = 38.2%). When stimulated with E7 antigen, these cells were polyfunctional, producing IFN-γ and TNF-α. Similarly, when stimulated with CMV peptides, CD8+ effectors cells produced IFN-γ and TNF-α. The CD8+ effector T cell response of this patient remained persistently elevated across all time points. Given this unusual finding, further blood samples were collected at regular intervals from this patient over a two year time period from 2011 to 2013 and the CD8+ effector T cell response continued to be elevated. This patient remains clinically free of disease 3 years and 6 months post treatment.

CONCLUSION: We report the finding of a patient who is a "super-responder" to E7 antigen with persistently elevated CD8+ effector cells producing IFN-γ and TNF-α. This is an unusual finding given that effector cells are usually eliminated and rather antigen specific memory cells are maintained. The significance of this hyperreactivity is unknown but may provide insight into the immune related control of disease progression. This may also give insight to immune modulating strategies to target HPV related tumor antigens.

P066: SINGLE-PEDICLED FASCIOCUTANEOUS FLAP SURVIVAL IN AGED RAT MODEL OF CHRONIC ALCOHOLISM – Sudeep Roy, MD[1], Edita Aksamitiene[2], Kealan Hobelmann[1], Juliana Rodin[1], Giuseppe Staltari[1], Ryan Heffelfinger, MD[1], Jan Hoek[2], Edmund Pribitkin, MD[1]; [1]Department of Otolaryngology, Thomas Jefferson University, [2]Department of Pathology, Anatomy & Cell Biology, Thomas Jefferson University

OBJECTIVE: Impact of chronic ethanol consumption on signaling events underlying composite pedicled flap survival was examined in isocaloric pair-fed Sprague-Dawley rats that began to consume Lieber-DeCarli '82 liquid 1000 kcal/L diet consisting of 15.1% protein, 35.9% fat and either 49% carbohydrates (control group) or 13.5% carbohydrates and 35.5% ethanol (alcoholic group) as soon as their 2 week adaptation period was over inside animal facility.

METHODS: Six pairs of rats that were fed such diet for at least 12 months underwent surgery in which a 3:8 width to length ratio pedicled fasciocutaneous flap based on the inferior epigastric artery was raised in each rat and rotated into a defect created on the contralateral abdomen. Tissue from the contralateral recipient site was snap frozen to serve as baseline (day 0) for histology (E&H staining) and Western Blotting data comparison with the flap harvested on post-operative day (POD) 9. The endogenous expression of identical cytokines and growth factors, cleaved Caspase-9, ß-Actin, GAPDH and activating phosphorylation of Akt, STAT3, PLC-gamma, eNOS, Src, FAK, p38 MAPK and ERK1/2 were measured in homogenates of proximal, middle and distal segments of the harvested flaps to reflect the pro-angiogenic, inflammatory, anti-apoptotic, stress, motogenic and mitogenic cellular responses.

RESULTS: There was a statistically significant increase in percentage of gross necrosis noted in flaps of alcohol-fed animals compared to controls based on planimetric analysis. This correlated with lower expression levels of activated Akt, STAT3 and p-ERK1/2, and increased Caspase-9 cleavage in the alcohol-fed animals compared to their paired controls in proximal, middle and distal segments.

CONCLUSION: This data confirms previous in vitro reports on ethanol-mediated inhibition of cytokine and growth factor-stimulated JAK/STAT3 activities in various cell types and implies that chronic alcohol consumption renders composite pedicled flap tissue more susceptible to necrosis by impairing multiple signaling pathways whose cross-talk is essential for cell survival and expedient wound healing.

P067: BIOLOGIC EFFICACY OF A NOVEL MICROGRANULAR FORMULATION OF CURCUMIN IN HNSCC PATIENTS – Cherie-Ann Nathan, MD, Brian Latimier, MS, Tara Moore, Neil Nathan, Oleksandr Ekshyyan, Xiaohui Ma, Fleurette Abreo, Vikas Mehta, Timothy Lian; LSU-Health Shreveport

INTRODUCTION: Field cancerization of the head and neck contributes to the development of second primary tumors and local recurrences. Curcumin a nutraceutical has shown potential to slow tumor progression and reduce cancer-related symptoms.To date, most human trials involving curcumin have utilized capsular formulations, which are limited by poor bioavailability due to poor gut absorption and rapid conjugation. In contrast, our study administered curcumin as microgranular beads (MB) contacting the oral mucosa (thus allowing for direct absorption into the bloodstream). Curcumin is known to target multiple pathways but identifying a consistent biomarker that can be validated in chemoprevention trials is critical as the occurrence of cancer as an end point could take years.

MATERIALS & METHODS: After screening, 15 cancer patients took part in the study. All subjects were given a 4g MB dose at time 0, with subsequent blood collections at 15min, 30min, 1hr, 2hr, and 4hr on Day 1. Patients then continued self-administered dosing at 4g (twice daily) with final collection at approx. 3-4wks (Post). Serum samples were then analyzed via multiplex for 13 different analytes: FGF-2, GM-CSF, Interferon (IF) IFNγ, GRO, Interleukin (IL)-13, IL-17, IL-1B, IL-6, IL-8, Inducible Protein (IP)-10, MIP1β, TNFα, and VEGF. All samples were assayed via MilliplexMap Human Cytokine/Chemokine Magnetic Bead multiplex in duplicate. We evaluated various biomarkers of the AKT/MTOR pathway such as cyclin D1, FGF-2, MMP-9, caspase-3 and PARP, all markers of cell cycle progression, cell proliferation, angiogenesis, invasion and apoptosis using IHC.

RESULTS: There was a significant effect of curcumin on the level of FGF-2 at time points 4h (p=0.0078) and a trend towards decreased levels of FGF-2 in serum at 15min (p=0.084), 30min (p=0.083) and 1h (p=0.084) after curcumin administration. There was also a significant effect of curcumin on the level of GM-CSF in serum at time points between 15min and 2h (p<0.05) after curcumin administration. We also detected a significant effect of curcumin on serum level of IL-17 (1h; p=0.0342). Curcumin treatment also caused a substantial numerical decrease in the levels of the following serum biomarkers: IFNγ, IL-13, TNFα and VEGF at various time points and a robust numerical increase in the level of MIP1β. However, because of data variability there was no statistically significant difference between the time points for the aforementioned serum biomarkers. FGF-2 was significantly decreased in post-treatment biopsy samples using IHC in 7 out of 11 patients with evaluable matched tumor samples (p = 0.0261)

CONCLUSION: Interestingly the results of IHC and Multiplex Assay analyses of FGF-2 corroborate and strongly indicate that FGF-2 is potentially an important biomarker for future clinical trials of curcumin. Although many other markers appear promising and trend towards significance, FGF-2 was consistent. It also indicates a potential effect of curcumin in suppressing angiogenesis in head and neck premalignant lesions. In future studies we plan to increase the number of study participants, thus lessening the likelihood of variation between sample.

P068: INTEGRINS BETA-1 AND BETA-4 PLAY COMPLEMENTARY ROLES IN HEAD AND NECK SQUAMOUS CELL CARCINOMA PROGRESSION – Peter Szaniszlo, MD, PhD, Susan M Fennewald, PhD, Ruwen Cui, BS, Suimin Qiu, MD, PhD, Tammara L Watts, MD, PhD, Vicente A Resto, MD, PhD; UTMB Health

BACKGROUND & OBJECTIVE: Targeted molecular therapies have gained great interest in recent years in cancer research. Inhibiting key oncogenic signaling pathways has been shown to be efficient in various malignant diseases, however, toxic effects if these therapies and recurrence of therapy-resistant cancer after initial success often hamper recovery. Using several of these therapies simultaneously could prevent cancer recurrence, and result in reduced toxicity as well as increased efficacy; therefore, it is essential to find new targets for

combined molecular therapies. Earlier studies suggest that interfering with integrin-ligand interactions and/or with intracellular integrin signaling pathways have the potential to inhibit tumor growth and several anti-integrin antibodies and FAK-inhibitors have entered clinical trials for cancer treatment in the last few years. Integrins beta-1 and beta-4 (ITGB1 and ITGB4) have been implicated in the progression of different tumors including head and neck cancer, but detailed understanding of the mechanisms by which they support cancer growth is still missing. In this study we have investigated the role of ITGB1 and ITGB4 in head and neck squamous cell carcinoma (HNSCC) progression.

METHODS: We used ITGB1- and ITGB4-knockdown HNSCC cell lines to study the effects of impaired beta-integrin functions on cancer cells in vitro, as well as on their tumorigenic potential in vivo using an orthotopic, xenograft mouse model of HNSCC. ITGB1 and ITGB4 expression levels were determined by flow cytometry and immunoblotting. Tumor cell proliferation was assessed by growth assays in tissue culture and by measuring tumor size after short-term xenograft experiments. Cell motility was monitored and analyzed using a Nikon BioStation IM-Q compact cell incubator and monitoring system and by wound healing assays. For integrin-related pathway analysis we assesed total and activated protein levels in vitro by immunoblotting and ex vivo by immunohistochemistry. Overall tumorigenic potential of ITGB1- and ITGB4-knockdown (ITGB1-KD and ITGB4-KD) HNSCC cell lines was evaluated by animal model survival assays.

RESULTS: Both ITGB1 and ITGB4 are highly expressed in HNSCC cells, but their relative expression levels significantly differ in the parent cell lines used in this study. In the different assays the relative effect of integrin-knockdown correlated well with the original relative ITGB1/ITGB4 expression levels. While the knockdown of either beta-integrin decreased cell proliferation rates, they had different effects on cell motility: ITGB1-KD cells showed lower, ITGB4-KD cells showed higher rates than controls. In vivo tumor size decreased after ITGB1-KD in one of our cell lines while did not affect the other. ITGB4-KD did not alter tumor size significantly in short-term animal model experiments. Nevertheless, the knockdown of either beta-integrin, in either HNSCC cell line increased the lifespan in animal model survival assays. Pathway analysis revealed alterations in both common and beta-integrin subunit-specific signaling pathways in integrin-KD cell lines.

CONCLUSIONS: Our results suggest that ITGB1 and ITGB4 are not only both important for HNSCC cell proliferation, survival, and migration, but they play complementary roles in cancer progression. Co-targeting ITGB1- and ITGB4-related intracellular signaling pathways could be an important part of combined targeted molecular therapies for head and neck cancer.

P069: A MODULAR POLYMER PLATFORM THAT DELIVERS RECOMBINANT CYTOKINES AND CISPLATIN ALLOWS FOR DE-ESCALATION OF RADIATION THERAPY IN AN ANIMAL MODEL – Jon Mallen-St. Clair, MD, PhD, Yuan Lin, PhD, Julianna Pesce, Arnold Suwarnasarn, BS, Sherven Sharma, PhD, Ben Wu, DDS, PhD, Maie A St. John, MD, PhD; Department of Head and Neck Surgery, University of California, Los Angeles

OBJECTIVES: To evaluate the therapeutic efficacy of a novel modular polymer platform in the treatment of HNSCC. 50% of HNSCC patients fail primary management, and salvage of recurrent disease patient is of paramount importance. We had previously shown the antitumor efficiency of this novel polymer in delivering chemokines (CCl21) and cisplatin in the animal model. Herein we and evaluate the efficacy of this polymer in combination with radiation therapy (RT) in an effort to see if this combination allows for a de-escalation of RT.

STUDY DESIGN: in vivo study.

SETTING: Academic research laboratory.

SUBJECTS AND METHODS: C3H/HeJ mice were randomized to receive implantation of (1) no polymer; (2) plain polymer; (3) CCl21-polymer; (4) cisplatinum polymer; and (5) combination CCl21 and cisplatinum secreting polymer; +/- RT at three different doses. 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. Cisplatinum-polymer, CCL21-polymer and the combination CCl21-cisplatinum polymer secreting polymer effectively reduced SCCVII/SF tumors in the C3H/HeJ mice by over 16-fold (P < 0.01) as compared to control and plain polymer groups. Additionally, treatment with Cisplatinum-polymer, CCL21-polymer and the combination CCl21-cisplatinum polymer allowed for a 4-fold reduction in the dose of RT required.

CONCLUSION: Our promising results indicate that this polymer may represent a new therapeutic modality for patients with HNSCC. Our data provides a strong rationale for further evaluation of this polymer in de-intensification of radiation therapy, thus reducing toxicity. 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.

P070: CHANGES IN HEAD AND NECK SQUAMOUS CELL CANCER OF UNKNOWN PRIMARY ORIGIN IN THE ERA OF HUMAN PAPILLOMAVIRUS – Kevin Motz, MD, Jesse Qualliotine, BS, Jeremy Richmon, MD, Justin Bishop, MD, David Eisele, MD, Carole Fakhry, MD, MPH; Johns Hopkins Hospital

BACKGROUND: Head and neck squamous cell carcinoma (HNSCC) of unknown primary (UP) is a rare entity which accounts for less than 3% of head and cancers. Unknown primary evaluations have historically been targeted to the nasopharynx, hypopharynx, larynx and oropharynx. In the era of human papillomavirus (HPV), however, HPV-positivity is considered a biomarker for the oropharynx. Although unknown primaries of HNSCC are strongly associated with HPV-positive tumor status, the prevalence of HPV in UP HNSCC is unknown. With the rising incidence of HPV-related oropharyngeal cancer, it is presumed that UPs are similarly rising. Whether the incidence of UPs has increased, and whether HPV tumor status and transoral robotic surgery (TORS) have increased detection rate is unknown.

OBJECTIVE: 1) To determine the change in frequency of HNSCCs of UP evaluated over time. 2) To estimate the prevalence of HPV-positive HNSCC of UP. 3) To evaluate the detection rates of primaries in HNSCC UP over time. 4) To determine the rate of synchronous primaries in patients diagnosed with UP lesions.

METHODS: A single institution retrospective review of patients diagnosed with HNSCC of UP and evaluated at the Johns Hopkins Hospital (JHH) from 2005-2014. HNSCC of UP was defined as metastatic cervical squamous cell carcinoma without clinical evidence of a primary lesion as determined by a head and neck surgical oncologist. Clinical records, operative notes, and pathology reports were reviewed and abstracted. Descriptive statistics and tests of comparison (chi-square and ANOVA) were performed. P-value <0.05 was considered statistically significant.

RESULTS: The frequency of HNSCC of UP significantly increased from calendar periods 2005-08 to 2012-2014 (p<0.01). For the 76 UP cases with available HPV tumor status, 69 (90.7%) were HPV-positive. HNSCCs of UP that were HPV-positive were significantly more likely to be male (63 of 69) as compared with HPV-negative cases (p<0.01). HPV-positive HNSCC of UP patients were significantly younger than HPV-negative HNSCC UP patients (56.1 vs. 67.7 years; P<.01). There was a non-significant increase in the detection rate of primary lesions from calendar periods 2005-08 to 2012-2014 (46.1% vs 64.9%). Among patients with an identified primary site who underwent bilateral palatine tonsillectomy or lingual tonsillectomy, 10.3% (3/29) had synchronous primary lesions. All of these synchronous lesions were HPV-positive. Since TORS was integrated into the UP paradigm at JHH in 2011, a non-significant increase in the detection of primaries was observed (52.3% vs 65.9%, p>0.05). The average number of procedures (direct laryngoscopy with biopsy, palatine tonsillectomy, and TORS) per individual before and after 2011 was similar (1.85 vs 1.88, p>0.05).

CONCLUSION: The frequency of HNSCC UP has increased significantly in recent calendar periods. The overwhelming majority of HNSCC of UP are HPV-positive. As would be expected, patients with HPV-positive HNSCC UP tend to be male and younger. A non-significant increase in detection of primaries was observed in recent calendar periods that included the addition of TORS to the paradigm.

P071: DEVELOPMENT OF A RAT MODEL FOR PERSISTENT VOCAL FOLD PARALYSIS DUE TO RECURRENT LARYNGEAL NERVE CRUSH – Scott S Harris, MD, Punam Thakkar, MD, Satish Balasubramanian, BS, Ramiz Shuminov, BS, Mark Stewart, MD, PhD, Ko Nakase, MD, Joshua B Silverman, MD, PhD, Krishnamurthi Sundaram, MD, Richard Kollmar, PhD; SUNY Downstate Medical Center, Brooklyn, NY

OBJECTIVE: Unilateral vocal-fold paralysis is a major complication from thyroid surgery and commonly results from trauma to the recurrent laryngeal nerve (RLN). The loss of tone in the intrinsic muscles of the larynx due to RLN injury can eliminate voice, produce breathing difficulties, and significantly increase risk for aspiration. For crush injuries to the RLN, the only non-invasive treatment currently available is "watchful waiting" for spontaneous regeneration of axons and reinnervation of laryngeal muscles, which is inefficient and can take many months. As a first step towards developing an animal model for testing drug treatments to promote RLN regeneration, we investigated which crush conditions result in persistent rather than transient vocal-fold paralysis in the rat.

METHODS: The right RLN was crushed in 36 eight-week-old male Sprague-Dawley rats. The first experimental variable was the degree of exposure of the RLN—it was either skeletonized completely at the crush site or left attached to the inferior thyroid artery as a 'neurovascular bundle'. The second experimental variable was the severity of the crush—it was varied by using a range of surgical tools with closing forces between 0.15 and 28 N. Sham surgery and RLN transection were conducted as negative and positive controls, respectively. Vocal fold motion was recorded by using quantitative video laryngoscopy immediately before, during, and at 1, 2, 4, 8, and 16 weeks after injury.

RESULTS: All but the mildest RLN crushes as well as transection resulted in immediate paralysis of the right vocal fold. In most animals, vocal fold motion recovered spontaneously within two weeks after injury, even if the RLN appeared flattened and translucent at the time of injury. Persistent paralysis up to 16 weeks was only observed with crush forces greater than 16 N or after transection. Persistent paralysis was also more likely if the skeletonized RLN was crushed rather than the neurovascular bundle. In some cases, however, the vocal fold paralysis resolved itself within two weeks even for skeletonized RLNs crushed with the greatest force.

CONCLUSION: The RLN of the rat is remarkably impervious to crush injury, despite or possibly because of its small diameter (less than 0.5 mm). Additional experiments to refine our crush protocol are underway with the goal to produce persistent paralysis in a reproducible fashion. We are also conducting histological experiments to correlate the degree of vocal fold paralysis with the degree of tissue damage as defined by Sunderland in his classification of nerve injuries.

P072: METHODS LIQUID-BASED CYTOLOGY IN THE DIAGNOSIS OF HEAD AND NECK TUMORS – Elena Slavnova, PhD; P.A.Hertsen Cancer Research Institute

In recent years, liquid-based cytology methods are being actively implemented in clinical oncology.

The purpose of this study is to compare the diagnostic accuracy, especially the morphological changes of quality immunocytochemical studies using thin preparations obtained by using E-prep Processor and Cytospin centrifuge with the routine preparation of cytological preparations.

MATERIALS & METHODS: Material for cytological study was obtained from 60 patients with various malignant tumors of the head and neck: salivary gland – 21, thyroid-9, lymph nodes – 30. In the two methods of liquid formulations were evaluated: background, cellularity, morphology features. Always cyto-histological comparison. In 18 cases performed immunocytochemical study.

RESULTS: Conducting cyto-histological comparisons showed that the effectiveness of cytological method was 90.8%, the accuracy of 97.7%, 2.3% discrepancy amounted unsuccessfully taken material 6.9%. Application of the method of liquid-based cytology in conjunction with routine cytology improves the efficiency of cytological diagnosis by reducing the amount of material taken unsuccessfully. Ensured the safety of cytological material, even single abnormal cells get into the test material. Using E-prep- drugs can reduce the background, allowing you to increase the sensitivity of cytological method. Liquid formulation technology avoids contamination of their detritus, red blood cells, inflammation of the elements, which greatly facilitates their browsing. What is more pronounced when using E-Prep, than Cytospin preparations. Preparation of monolayer reduces the number of false-negative results and facilitates viewing cytological smears. Formulations prepared using the E-Prep, differed more uniform distribution of cells on glass. However, the partial fragmentation of cells, epithelial-stromal violation ratios, scattered arrangement of cells, lysing slight wrinkling and more rounded or elongated shape, resize cells in most cases do not allow the liquid on one smears without routine cytological preparations to establish a cytologic diagnosis. Smears prepared using Cytospin cell changes are less pronounced due to the application of the nutrient medium, which allows to preserve cells. However, the cells change shape, becoming more rounded, resize cells often become larger, and even more rarely decreased wrinkling that without sharing routine cytological smears hampered the formulation of diagnosis. Thus, to establish the cytological diagnosis by liquid preparations in both cases it is necessary to conduct a study in conjunction with the traditional Pap smear. Obtained by liquid preparations technologies can be used for immunocytochemical studies. Reduces consumption of expensive reagents, which is especially important in immunocytochemical, genetic studies.

CONCLUSION: The method of liquid-based cytology allows high-quality standard, monolayer cytological preparations is more effective in comparison with sensitive routine method. It can be used successfully in the diagnosis of tumors of the thyroid, salivary gland, soft tissue tumors, metastasis to lymph nodes in combination with a routine method, since currently there is no sufficient experience in viewing such preparations. Cytological preparations made by liquid cytology can be successfully used for the immunohistochemical study, morphometry, computer image processing. It should be noted that the method of liquid-based cytology compared with the routine has its morphological features that must be considered.

P073: PRIMARY COMBINED SMALL AND SQUAMOUS CELL CARCINOMA OF THE HYPOPHARYNX: A CASE REPORT – Kiyoshi Misawa, MD, PhD[1], Daiki Michizuki, MD[1], Atsushi Imai, MD[1], Takeharu Kanazawa, MD, PhD[2], Hiroyuki Mineta, MD, PhD[1]; [1]Hamamatsu University School of Medicine, [2]Jichi Medical University

BACKGROUND: We report a very rare case of combined small cell carcinoma (SmCC) of the hypopharynx.

CASE: A 74-year-old man presented with a 3-month history of throat pain. We performed a total laryngectomy and neck dissections on both sides and diagnosed the malignancy as combined SmCC. One month after surgery, we administered concomitant chemoradiotherapy with cisplatin and etoposide.

RESULTS: Immunohistochemically, the SmCC element was positive for CD56 and Ki-67 (50.2% labeled), and the squamous cell carcinoma (SqCC) element was positive for CK(34bE12) and Ki-67 (47.5% labeled). Furthermore, the SmCC element was positive for KIT and platelet-derived growth factor-α (PDGFRα), while the SqCC element was positive for epidermal growth factor receptor (EGFR) and PDGFRα. By genetic analysis, a silent mutation in PDGFRα was recognized.

CONCLUSIONS: Expression of KIT, PDGFRα, and EGFR in this case provided evidence that combined SmCC may be a candidate for molecular targeted therapy, although further investigations are necessary.

P074: THE DISCOVERY OF NOVEL GSN ALTERNATIVE SPLICING IN HEAD AND NECK SQUAMOUS CELL CARCINOMA – Daria A Gaykalova, PhD[1], Elana J Fertig, PhD[1], Theresa Guo, MD[1], Ilse Tiscareno[1], Veronika Zizkova, MS[2], Michael Considine, MS[1], Justin A Bishop, MD[1], Julie Ahn, BS[1], Samantha Gebhart, BS[3], Marla Goldsmith, BS[1], Chi Zhang[4], Wayne M Koch, MD[1], William H Westra, MD[1], Zubair Khan, MD[1], Michael Ochs, PhD[5], Joseph A Califano, MD[1]; [1]Johns Hopkins University, [2]Palacky University, [3]Greater Baltimore Medical Center, [4]University of Virginia, [5]The College of New Jersey

In order to define the biology of HNSCC, several high throughput analyses have been performed. Yet these have only detected a limited number of genetic alterations, which incompletely describe the HNSCC specific pathway alterations. The heterogeneous nature of these alterations has postponed the discovery of reliable HNSCC biomarkers and therapeutic targets for this disease.

We have performed alternative splice events (ASEs) analysis to enhance our understanding of HNSCC biology and to provide opportunities for the development of novel biomarkers and targeted therapy. Indeed, ASEs are significant components of potential oncogenic pathways alterations and play a critical role in malignant cell transformation in a variety of solid and liquid tumors. To define ASEs specific to HNSCC we designed a novel pipeline: we employed RNA-Seq, MapSplice and outlier analysis to detect, align and prioritize tumor-specific ASEs. Evaluating the top scoring candidates, we have found four highly promising ASE candidates, including GSN, Gelsolin, an actin-binding protein, a key regulator of actin filament assembly and disassembly.

Scientific literature proposes that GSN demonstrates tumor-suppressor properties by reducing cell proliferation in vivo and in vitro via suppression of protein kinase C (PKC is part of the PI3K pathway, found altered in HNSCC). The alternative splicing that we discovered occurred between 14th and 15th exons of its longest transcript variant, with insertion of 110 bp from the 14th intron. Such insertion has stop codon in frame, and splice variant gives truncated (562 aa) protein with only 4 Gelsolin domains (instead of full-length 731 aa protein with 6 Gelsolin domains). Overall we found 40% tumor samples to harbor GSN-ASE. QRT-PCR confirmed that while total expression of GSN is decreased in HNSCC samples, GSN is expressed in the alternative truncated form specifically in HNSCC. The scientific literature supports our data and demonstrates that GSN is downregulated in breast, lung and colon cancers, and that epigenetic alteration plays a role inGSN expression regulation. The role of GSN alternative splicing in different tumor types, including HNSCC, is unknown.

We conclude that HNSCC specific ASE expression can be characterized using high throughput genomic techniques combined with appropriate ASE defining algorithms.

P075: SERUM MIRNAS AND THEIR ROLE AS BIOMARKERS FOR ORAL SQUAMOUS CELL CARCINOMA – Cathie Garnis, PhD; University of British Columbia

BACKGROUND: Individuals with oral cancer have a poor survival rate and a high rate of disease recurrence due mainly to the late stage of diagnosis. Novel tools (biomarkers and targeted therapies) to address this issue are required in order to increase survival rates. An ideal molecular biomarker for cancer detection or management will be evaluable from patient samples collected in a non- or minimally-invasive manner. Biomarkers detectable in biological fluids from at-risk patients (e.g. blood) can represent such ideal candidates. However – although conventional strategies for blood-based biomarkers show promise – the development of clinically-validated detection markers remains an unmet challenge for many cancer types. MicroRNAs (miRNAs) are highly conserved, small, endogenous RNAs that, once processed into a mature form, interact with

the 3' UTR of target mRNAs, driving degradation or translational repression of that mRNA. Previous work has shown the importance of miRNAs in various biological processes, however this work has largely focused on miRNA functions within cells. More recently, it has been demonstrated that miRNAs can be detected in blood as freely circulating nucleic acids (despite the presence of abundant ribonucleases). Further, tumor cells have been shown to release miRNAs into circulation and profiling of plasma and serum has demonstrated altered miRNA levels associated with cancer and other disease states.

OBJECTIVE: To determine the ability of serum miRNAs to act as a biomarker for oral squamous cell carcinoma.

METHODS: Serum was collected from patients with oral squamous cell carcinoma (OSCC) and oral carcinoma in situ (n=48) as well as demographically matched non-cancer controls (n=51). RNA extracted from the serum samples was profiled using miRCURY LNA Universal RT miRNA PCR panels to assess the expression of 742 miRNAs. miRNAs known to be affected by haemolysis in blood samples were excluded from analysis. A model to distinguish between OSCC/CIS and non cancer individuals was created using logistic regression on miRNAs included in the model by LASSO analysis, a method that preferentially creates statistical models with fewer miRNAs.

RESULTS: Performing statistical analysis after randomly dividing our sample set into training and test sets determined that our model was able to achieve a higher than 80% accuracy in differentiating between cancer and control samples by including 18 miRNAs in our model/biomarker.

CONCLUSIONS: We have identified a circulating miRNA signature with utility as an oral cancer biomarker. The analytical approaches described here will have utility for developing similar circulating miRNA biomarkers for other cancer types, as well as recurrent disease.

P076: INTEGRATIVE ANALYSIS OF DNA METHYLATION AND GENE EXPRESSION PATTERNS THROUGHOUT THE PROGRESSION OF ORAL SQUAMOUS CELL CARCINOMA – Cathie Garnis, PhD; University of British Columbia

Oral squamous cell carcinoma (OSCC) has a dismal 5 year survival rate of ~50%. This is in part due to the inability to differentiate a lesion at risk for progression at the earliest stages of this disease. Analyzing DNA methylation and gene expression patterns in the different stages of this disease, we aim to gain a better understanding of the genes involved in OSCC progression and identify those that may be used as biomarkers or therapeutic targets.

Multiple biopsies were obtained from each of ten OSCC patients representing hyperplasia, dysplasia and carcinoma in situ (CIS)/squamous cell carcinoma (SCC). Tissues were microdissected and both DNA and RNA were obtained. Extent of DNA methylation was assessed using the Illumina HumanMethylation27K microarray, and gene expression levels were profiled using the Agilent Human Gene Expression 4x44k microarray. Data from both platforms were integrated and recurrently deregulated genes identified.

Numerous genes exhibiting concurrent aberrant DNA promoter methylation and gene expression patterns were identified. We observe recurrent hypermethylation of the promoter region and decreased gene expression of 106 genes in oral dysplasias and 134 genes in the CIS/SCC biopsies. 15 genes were recurrently hypomethylated in the dysplasias and 99 in the OSCC/CIS samples. The role of the top candidate genes in oral tumourigenesis has been evaluated in cell model systems.

This is the first report integrating profiles of DNA methylation and gene expression data in various histological stages of OSCC. Given the frequency of methylation and expression correlating with one another in these candidate genes, we give evidence that DNA methylation may be a potential critical mechanism in OSCC progression.

POSTER LISTING

P077: EFFICACY AND TOXICITY OF CARBOXY-TERMINAL HSP90 INHIBITORS IN AN ORTHOTOPIC MURINE MODEL OF HEAD AND NECK SQUAMOUS CELL CARCINOMA – Michael W Sim, MD[1], Avinaash Kalidindi[2], Chitra Subramanian, PhD[2], Huiping Zhang[3], Brian Blagg, PhD[3], Mark S Cohen, MD[1]; [1]University of Michigan Health System, [2]University of Michigan, [3]University of Kansas

BACKGROUND: Locally advanced head and neck squamous cell carcinoma (HNSCC) is associated with poor prognosis and remains a treatment challenge. Platinum-based chemotherapy with radiation had been the standard of care for decades but lacks treatment-durability and carries a significant toxicity profile. Inhibition of the molecular chaperone HSP90 has gained interest as an anticancer approach since this inhibition leads to simultaneous inhibition of numerous kinases responsible for cancer cell growth, migration and metastatic spread. Early clinical trials with HSP90 inhibitors (17-AAG, geldanamycin) that bind the N-terminus of the protein have not progressed due to toxicity concerns, however we have developed a novel group of compounds that inhibit the carboxy terminus of HSP90 (CT-HSP90i) and have been shown in HNSCC models in vitro and in vivo to be safer and more effective than their N-terminal counterparts. From a robust SAR analysis of over 300 analogues, we identified two novel CT-HSP90i with low nanomolar IC50 levels against HNSCC for translational evaluation in vivo.

OBJECTIVES: To evaluate the efficacy and toxicity of novel CT-HSP90 inhibitors KU711 and KU757 compared to cisplatin and 17-AAG in an orthotopic HNSCC in vivo model to determine their translatability for future clinical trials in this disease.

METHODS: Using our previously published orthotopic oral cavity in vivo model we inoculated 45 athymic nude mice with 1x106 MDA1986 cancer cells into the buccal mucosa. Once tumor volumes reached 100 mm3, treatment was rendered in a randomized, controlled manner, with 9 mice per group receiving either control (saline), KU711 (5 mg/kg/day i.p.), KU757 (5 mg/kg/day i.p.), cisplatin (3mg/kg/week i.v.) or 17-AAG (50mg/kg/qod). Subjects were treated for a single 3 week course and then monitored for tumor size, body weight, body score condition, and survival.

RESULTS: 100% of control and 17-AAG treated mice died by post-treatment day 35 compared to 0% of KU711, KU757, and cisplatin treated mice (p<0.001). Tumor response by modified RECIST criteria demonstrated the best response with KU757 (85% average tumor size reduction) followed by KU711 (70% reduction) which were both significant (p<0.005) vs. the other arms which all showed continued tumor progression. None of the KU711 or KU757 treated mice showed clinical toxicity by weight loss or decreased body score compared to 100% of animals in each of the cisplatin and 17-AAG group (p<0.001). Survival was improved by 3 weeks in the CT-HSP90i treated mice compared to cisplatin (p<0.01).

CONCLUSIONS: CT-HSP90 inhibition was superior to cisplatin and N-terminal inhibitors both in terms of efficacy and tolerability in an orthotopic HNSCC in vivo model. These promising results support further translation of CT-HSP90 inhibition for future clinical evaluation.

P078: FOLLICULAR THYROID CARCINOMA CYTOTOXICITY OF FDA APPROVED ANTINEOPLASTIC THERAPIES – Andrea Ziegler, BA, Ashley Reeb, BS, Reigh-Yi Lin, PhD; Saint Louis University School of Medicine

BACKGROUND: Follicular thyroid carcinoma (FTC) is the second most common type of thyroid cancer with almost 10,000 cases being diagnosed annually in the United States. Traditional antineoplastic agents, such as Cisplatin and Doxorubicin, have been proposed as palliative treatment in patients with advanced disease. Cisplatin works by cross linking DNA while Doxorubicin intercalates DNA leading to a decrease in cell proliferation. Recently, tyrosine kinase inhibitors, such as Imatinib and Sorafenib, have been proposed as adjuvant treatment for well differentiated thyroid cancers that are resistant to radioactive iodine. Imatinib targets the abl, c-kit, and platelet derived growth factor receptor (PDGFR)

domains. Sorafenib is specific for vascular endothelial growth factor receptor, PDGFR, and BRAF and CRAF kinases. Both tyrosine kinase inhibitors ultimately inhibit cell signaling and proliferation. This study was designed to determine the in vitro cytotoxicity of Cisplatin, Doxorubicin, Imatinib, and Sorafenib in three follicular thyroid carcinoma cell lines. The three cell lines utilized in this study were FTC-238, TT2609-CO2, and WRO. It is well defined that FTC-238 and TT2609-CO2 have a TP53 mutation, and WRO has a BRAFV600E mutation.

MATERIALS & METHODS: Cell viability was assessed using Alamar Blue assay. After preculture, cells were treated with medium containing different doses of Cisplatin, Imatinib, Sorafenib, and Doxorubicin for 72 hours. Alamar Blue dye was added directly to the culture media and absorbance was read at 540 nm with an ELISA plate reader.

RESULTS: Data of cell viability was analyzed to determine the IC50 of each drug for each individual cell line. Cisplatin showed the lowest IC50 in FTC-238 at 11.3 ± 0.6 µM compared to 55.9 ± 2.3 µM in TT2609-CO2 and 43.3 ± 8.3 µM in WRO. Doxorubicin displayed the lowest IC50 in TT2609-CO2 at 1.1 ± 0.2 nM compared to 9.8 ± 0.1 nM in FTC-238 and 23.5 ± 4.5 nM in WRO. There were no significant differences in the IC50 of Imatinib in the three cell lines. Sorafenib had the lowest IC50 in WRO at 1.6 ± 0.2 µM compared to 4.2 ± 0.8 µM in FTC-238 and 3.9 ± 0.7 µM in TT2609-CO2. (Table 1)

CONCLUSION: This study demonstrates that the cytotoxicity of the drugs of interest varies significantly amongst these follicular thyroid carcinoma cell lines. Cisplatin was the most cytotoxic to FTC-238, Doxorubicin had the most cytotoxicity to TT2609-CO2, and Sorafenib was most cytotoxic to WRO with no significant differences in the IC50 of Imatinib. The results show that traditional chemotherapeutic agents have the potential to be an effective treatment in FTC. It also suggests that personalized antineoplastic therapy for specific gene mutations, such as BRAFV600E, may provide the most favorable outcomes for patients.

TABLE 1

Cell Line	Cisplatin (µM)	Doxorubicin (nM)	Imatinib (µM)	Sorafenib (µM)
FTC-238	11.3 ±0.6	9.8 ± 0.1	51.7 ± 2.6	4.2 ± 0.8
TT2609-CO2	55.9 ± 2.3	1.1 ± 0.2	40.8 ± 4.9	3.9 ± 0.7
WRO	43.3 ± 8.3	23.5 ± 4.5	48.3 ± 6.3	1.6 ± 0.2

P079: HUMAN PAPILLOMA VIRUS INFECTION SENSITIZES HEAD AND NECK SQUAMOUS CELL CARCINOMAS TO ONCOLYTIC VIRUS INDUCED CYTOTOXICITY: A POTENTIAL NOVEL THERAPEUTIC STRATEGY – Stephanie Johnson-Obaseki, MD, Jim Dimitroulakos, PhD, Michael Odell, Patrick J Villeneuve, MD, Sekhon Harmanjatinder, Tabassom Baghai; University of Ottawa

BACKGROUND: Human papillomavirus (HPV) is now the leading cause of oropharyngeal squamous cell carcinoma (OPSCC). It appears that the patient profile for HPV- related (HPV+) OPSCC is distinct from non-HPV related OPSCC in that patients present at a younger age and with small primary tumors but advanced regional disease. The mainstay of treatment is chemoradiotherapy, which is extremely toxic and has the potential for significant long-term morbidity including the potential for secondary cancers. As such, there is an urgent need to uncover innovative therapeutic strategies for this distinct disease entity. Oncolytic viruses (OV) are known to specifically target tumour cells that have defects in interferon (IFN) signaling. HPV-expressed proteins (E6, E7) are known to inhibit IFN signaling. As such, the goal of this study was to evaluate the sensitivity of HPV-related OPSCC to certain oncolytic viruses, such as vesicular stomatitis virus (VSV).

METHODS: This was a pre-clinical study to assess the potential of VSV to infect and replicate in both tumor-derived cell lines and ex-vivo surgically excised tumour tissue. Using Alamar Blue viability assay and standard viral tittering, we

assessed effects on cell viability and OV replication. We evaluated the sensitivity of 4 cervical carcinoma (CC) (HPV+) and 4 HNSCC (HPV-) derived cell lines to VSV oncolysis. To address the susceptibility of HPV+ and HPV- HNSCC tumour cells, we evaluated a number of ex-vivo surgical specimens from 15 HNSCC and 20 early stage non-small cell lung cancer NSCLC patients. HPV status was determined by employing a nested PCR method and sequencing that can identify all known serotypes of HPV.

RESULTS: The cervical cancer cell lines were consistently very sensitive to VSV cytotoxicity. In addition, exogenous expression of both HPV proteins E6 and E7 in a resistant HNSCC (HPV-) derived cell line, rendered it sensitive to infection, replication and oncolysis with VSV treatment. Importantly, when HPV+ OPSCC was compared with HPV- OPSCC and NSCLC tissues, only the HPV+ OPSCC tissues displayed a robust infection and viral replication.

CONCLUSIONS: Our results demonstrate that HPV-related tumors are more susceptible to oncolytic viruses than non-HPV related tumors. With further study, this could represent a novel therapeutic approach in HPV+ OPSCC patients.

P080: SALIVARY CANCER VIABLE BIOREPOSITORY CONSORTIUM – Gary Bellinger; Yale University

Salivary gland carcinomas are a destructive/devastating group of head & neck cancers comprising ~5% of these tumors in the United States. Salivary cancer is treated exclusively by surgical excision with or without radiation as the mainstay of treatment. Due to low incidences, histological diversities, lack of molecular characterization, and a scarcity of cell lines/animal models have contributed to the lack of effective therapies. Making matters worse, claimed salivary carcinoma cell lines used in the past have been proven not to be of salivary origin.

Without availability and validity of cell lines or xenografts, pre-clinical studies are not possible. Today, we have an ongoing project to grow in vitro and model salivary cancer. To accelerate this project we've enlisted institutions capable of sending viable salivary cancer tissue at the time of biopsy or resection. Our ultimate goal is to accumulate adequate sample numbers for identification and testing of new therapies. Immediate goals are to create short-term cultures, cell lines and xenograft models as tools to advance care of patients with salivary cancer. Xenografts and cell lines created through this project will be validated for patient of origin using microsatellite testing and for tumor specificity by mutational analyses or development of histologically similar tumor in xenograft models. As part of the initial effort, we have enlisted six (6) institutions and have successfully created short-term cultures of six (6) salivary cancers (1- carcinoma ex-pleomorphic adenoma, 2 - salivary ductal carcinoma, 3,4 - squamous cell carcinoma, 5 – acinic cell carcinoma, 6 – Mucoepidermoid carcinoma) received after overnight shipping. We have the infrastructure for collection of salivary cancers from resections and have developed expertise in the processing of these tumors for short term culture and murine xenograft modeling. To continue this great work, we are actively seeking additional collaboration from other institutes to join us in this great effort.

P081: THE ROLE OF LARINGECTOMY IN SELECTED CASES OF LARYNX INVASION IN ADVANCED DIFFERENTIATED THYROID CARCINOMA. REVIEW OF 16 CASES IN TWO CENTERS – Andres Ignacio Chala Galindo, MD[1], Alvaro Sanabria, MD[2]; [1]Surgeon; Associate professor; Chief, Head and Neck Service, Medicine Faculty. University of Caldas, [2]Head and neck surgeon. Associate professor Medicine Faculty. University of Antioquia

INTRODUCTION: The laryngectomy is almost never used in differentiated thyroid carcinoma since even in presence of locally advanced or metastatic disease it has been associated with high survival rates so a radical resection is not easy to support. Locally advanced disease specially in case of larynx or cricoid invasion is a challenge to the surgeon who must decide between a shave resection, cricoid and tracheal resection, hemi-laryngectomy or a total laryngectomy almost

always trying to solve an obstructive or bleeding complication. With less radical procedures even adding postoperative treatments the relapse is frequent and the persistent growing of an intraluminal mass, followed by vocal fold paralysis, airway obstruction, and death by asphyxiation or bleeding usually happens so a more radical resection should be considered.

OBJECTIVE: Review the laryngectomies done in the advanced obstructive differentiated thyroid carcinoma in two head and neck referral Institutions focusing in the larynx or cricoid invasion, the related complications, morbidity, mortality and outcome.

MATERIALS & METHODS: We reported sixteen patients with locally invasive well differentiated thyroid cancer between 2002 and 2015; female 10, male 6, mean age was 63 ±8.8 years. The main related symptoms were dysphonia, dysphagia, respiratory obstruction and bleeding from tumor. The patient's images and endoscopies realized preoperatively showed larynx invasion in all cases. The invasion was also confirmed with the surgical findings. Besides total thyroidectomy and neck dissection, four patients underwent total pharyngolaryngectomy, eleven total laryngectomy and one hemi-laryngectomy. Reconstruction was made with regional flaps in ten patients (7 pectoral/Bakamjian flaps and 3 gastric pull-through procedures) and with a jejunum free flap in one patient; one patient needed a carotid artery reconstruction with a saphenous vein graft. The final pathology report confirmed gross larynx invasion. Five tumors were classic papillary carcinoma, the rest were aggressive histological varieties (insular, tall cell, sclerosing). The tumor mean size was 4.3 ±1.6 cm; All tumors had linfovascular invasion and twelve had positive lymph nodes. Concomitant esophageal/hypopharynx invasion was present in 7 cases and invasion of carotid vessels in 2 cases. Three patients had positive microscopic margin. There were two postoperative deaths (one due to carotid blowout and other for carotid graft thrombosis with brain infarction). There were two anastomotic leaks treated conservatively. The mean overall survival was 31 ± 33 months (median 27.6 months, range 0-120)

CONCLUSION: Even there is no proved benefit on survival, even with the addition of postoperative radio iodine or radiotherapy, laryngectomy provides an alternative surgical procedure to control selected cases of larynx invasion in advanced thyroid carcinoma especially when a less radical procedure was feasible to relieve obstructive or bleeding symptoms. The decision is always hard to take and should be concerned with the patient according to his expectations.

P082: SMALL BOWEL OBSTRUCTION A RESULT OF METASTATIC HEAD AND NECK SQUAMOUS CELL CARCINOMA – Laura Garcia-Rodriguez, MD, Matthew Smith, MD; Henry Ford Hospital

OBJECTIVES: To describe a case of metastatic squamous cell carcinoma of the larynx to the bowel.

STUDY DESIGN: Case report.

METHODS: We describe a clinical case of a patient with recurrent T1bN0Mx Stage I squamous cell carcinoma of the glottis with metastasis five years after initial diagnosis and treatment, presenting itself as a small bowel obstruction. Review of current literature of distant metastasis was performed.

RESULTS: The patient underwent emergent small bowel resection to relieve the obstruction, with intraoperative findings revealing a palpable mass. Histopathologic findings of the mass demonstrated metastatic squamous cell carcinoma, p63 positive.

CONCLUSIONS: Squamous cell carcinoma from the head and neck has a low incidence of distant metastasis especially in those with locoregional control after initial treatment. When present it most commonly metastasizes to the lung followed by the liver and bone. Metastasis to the bowel is an even rarer entity.

POSTER LISTING

P083: ACQUIRED RESISTANCE TO PI3K INHIBITION IN PIK3CA-MUTANT HNSCC CELLS – Kara S Davis, MD, Nayel I Khan, BS, Carolyn Kemp, BS, Kevin Steehler, Sucheta Kulkarni, PhD, Jennifer R Grandis, MD, Umamaheswar Duvvuri, MD, PhD; University of Pittsburgh Medical Center, Eye and Ear Institute, Department of Otolaryngology--Head and Neck Surgery

BACKGROUND: Head and neck squamous cell carcinoma (HNSCC) is characterized by genetic heterogeneity, resulting in variable genetic driver events and complex signaling pathways. This heterogeneity creates challenges to developing effective therapies which are clinically efficacious among HNSCC patients. Recent data, including from our own institution and others, suggests that the Phosphoinositol-3-Kinase (PI3K) pathway is the most commonly altered oncogene in HNSCC; ~38% of all tumors in our cohort have alterations in this pathway, nearly half of which are in the PIK3CA gene. Mutations in the PI3K pathway, especially inPIK3CA, are known to be oncogenic in HNSCC, although the exact mechanisms are poorly understood. Epidermal growth factor receptor (EGFR) is a family of four related receptor tyrosine kinases: EGFR (ERBB1), HER2, HER3, and HER4. This family is dysregulated in numerous cancers including HNSCC, with mutations in EGFR often serving as "driver" events to activate key growth factor signaling pathways such as the PI3K/AKT pathway. Expression of EFGR family member proteins has been shown to be upregulated in response to acute PI3K pathway inhibition, with subsequent increase in HER3 activity and reactivation of the PI3K pathway. However, it is unknown if this phenomenon is present in cell lines with acquired resistance to PI3K inhibition. Therefore, we sought to create a consistently inhibited PIK3CA-mutant cell line as a model of acquired resistance to PI3K inhibition, and to elucidate the mechanisms contributing to acquired resistance in a pre-clinical model.

METHODS: To create a resistant HNSCC cell line, Cal33 cells (PIK3CA mutant, H1047R) were cultured in continuous exposure of increasing doses of BYL719 (selective PI3Kα inhibitor) and DMSO control to reach the IC50 of BYL719 in creating a PI3Kα1 inhibitor-resistant cell line, Cal33-BR, over 3 months' time. Whole cell lysates were prepared and separated in SDS PAGE gel followed by immunoblot analyses with corresponding antibodies. For determination of IC50 concentrations, cells were treated with vehicle control, and increasing doses of inhibitors of interest. At 48 hours, cell viability was assessed by luminescence via CellTiterGlo assay.

RESULTS: The Cal33-BR phenotype was confirmed to be resistant to BYL719 (IC50: 6.63uM) versus DMSO control (IC50: 2.44uM). Cal33-BR cells were also less sensitive to the EGFR inhibitors cetuximab and erlotinib. Furthermore, Cal33-BR cells had decreased pAKT/AKT profiles versus sensitive cells; this phenomenon was negated when Cal33-BR cells were allowed to grow without BYL719 for 3 weeks. Cal33-BR cells had an increased pERK/ERK profile versus control. As a measure of metabolic activity, Cal33-BR cells had significantly less total ATP when quantified by cell viability via luminescence versus control.

CONCLUSION: This study suggests a model for examining acquired resistance to PI3KCA inhibitors. Cal33 cells (PIK3CA mutant, H1047R) engineered to develop acquired resistance to BYL719 were less sensitive to inhibitors of the EGFR-family proteins, which is consistent with the decreased pAKT/AKT profile in these cells versus control. The observed increase in pERK/ERK protein expression and decreased metabolic activity suggest alternative mechanisms of cell resistance, such as cell senescence, in this model and is a platform for further investigation.

P084: COMPLIANCE WITH QUALITY ASSURANCE MEASURES FOR IMPROVING OROPHARYNGEAL CANCER PATIENT CARE – Omar Gayar, Dina Golbin, Tamer Ghanem, Francis Hall, Farzan Siddiqui, Steven Chang; Henry Ford Hospital

PURPOSE: Head and neck cancer therapy practices vary widely between institutions. There are currently no well accepted methods to evaluate the quality of care provided. We evaluated the quality of care provided to patients with oropharyngeal squamous cell cancers (OPSCC) at our institution based on compliance with American Head and Neck Society and National Comprehensive Cancer Network (NCCN) guidelines.

METHODS: An IRB approved retrospective chart review of 84 patients treated for T1-4bN0-3 OPSCC from 2010-2013 was performed. Quality measures included smoking and alcohol history, fiberoptic/mirror and physical exams, radiographic work up, proper staging, multidisciplinary tumor board (MTB) discussion, speech-swallow evaluation (SLP), and HPV testing. For surgical patients, tumor size, grade, margin status, lymphovascular invasion (LVI), perineural invasion (PNI), neck dissection, extracapsular extention (ECE), severe surgical complications, appropriate referral for adjuvant therapy, appropriate adjuvant treatment, and adjuvant therapy within 11 weeks were checked.

RESULTS: Of 84 patients, 66 completed treatment at our institution and were deemed eligible for review. 48 (74%) patients had SLP evaluation. 43 (65%) were tested for HPV. All patients were presented at a MTB. 36 (54.5%) had surgery inclusive of a neck dissection: of which all patients had tumor size, margin status, ECE, LVI and PNI documented. Of 24 pathologically node positive patients, all were checked for ECE. 5 (13.8%) had ECE, 11 (30.5%) had PNI, 12 (33.3%) had LVI. Immediate severe surgical complications were also reviewed, inclusive of hemorrhage, emergent tracheostomy and CVA. 5 patients (13.8%) had a severe complication associated with surgery. 21 of the 36 surgical patients needed adjuvant therapy of which, appropriate referral was made in 20 (95%). The time between surgery and adjuvant RT was kept under 11 weeks in 11 (55%) patients. Of all 66 patients, 55 (83%) received RT, and appropriate RT dose was given in 54 (98%) of them. 49 patients received Ch and appropriate Ch was given in 48 (98%) of them. Appropriate total treatment for all 66 patients was given in 64 (97%) with appropriate clinical follow up at our institution in 65 (99%) patients. 36 (55%) patients had surveillance imaging within 12 weeks of treatment completion.

CONCLUSION: Compliance with delivery and documentation of certain quality of care measures for OPSCC appear to be acceptable. Through this analysis we have identified areas for improvement. To our knowledge, this is the first attempt to establish quality guidelines for OPSCC treatment. Standardization of quality metrics and adherence to them is imperative to allow for meaningful cross-institutional comparison of local control and survival data.

P085: DOSIMETRIC ANALYSIS OF 10 FRACTION STEREOTACTIC RADIATION THERAPY FOR EARLY-STAGE GLOTTIC LARYNX CANCER – Baran D Sumer, MD, Stephen G Chun, MD, Chuxiong Ding, PhD, Lucien A Nedzi, MD, John S Yordy, MD, PhD, Joseph C Hodges, MD, MBA, Weihua Mao, PhD, Susan Cooley, RN, David L Schwartz, MD, Robert D Timmerman, MD; Harold C. Simmons Comprehensive Cancer Center, University of Texas at Southwestern Medical Center

BACKGROUND: Laryngeal cancer is the most common non-cutaneous head and neck malignancy in the USA, for which radiotherapy is a standard treatment for early-stage tumors. However, conventional radiotherapy using opposed lateral beams delivered over 6-7 weeks is inconvenient, costly, and unnecessarily irradiates large volumes of normal tissue including the thyroid gland, carotid arteries, uninvolved vocal cord, and the laryngeal skeleton. We hypothesized that stereotactic body radiotherapy (SBRT) of only the involved hemilarynx has potential to deliver therapeutic doses to tumor while providing substantial sparing of adjacent normal tissue through geometric avoidance. We present dosimetric data of patients treated for early-stage larynx cancer on a single-institutional phase I clinical trial with SBRT in 10 fractions.

METHODS: Institutional review board approval was obtained for this Phase I clinical trial. Patients with carcinoma in situ, or cT1a-T2bN0M0 carcinoma of the glottic larynx cancer were eligible. Nine patients were treated with SBRT to

a dose of 45 Gy in 10 fractions to the involved hemilarynx. Radiation dosimetric parameters of each patient treated were analyzed with respect to dose distribution and using dose volume histograms. Protocol specified normal tissue contours included the arytenoids, cord, carotid arteries, thyroid, and skin.

RESULTS: The average GTV was 1.24 cm3, CTV 2.7 cm3, and PTV 6.6 cm3 (STD 3.85, range 1.22-14.45 cm3). Dose was prescribed to the 85-88% isodose line, which provided 95-97% PTV coverage and conformity indices of 1.11-1.33. The average dose to the ipsilateral arytenoid was 49 Gy (STD 2.3, range 46.7-51.8), and contralateral arytenoid was 28.6 Gy (STD 7.9, range 22-42.2). The average maximum dose to the ipsilateral carotid artery was 20.3 Gy (STD 10, range 10.6-34.9), and average maximum dose to the contralateral carotid artery was 13.1 Gy (STD 6.9, range 6.35-22.2). The mean dose to the thyroid was 4.35 Gy (STD 2.7, range 1.1-7.8). SBRT plans delivered a 4.9 Gy lower contralateral arytenoid dose, 4.3 Gy lower ipsilateral carotid artery maximum dose, and similar mean thyroid doses, in comparison to IMRT plans developed for the same patients.

DISCUSSION: We have analyzed the dosimetric parameters of 9 patients treated with a 10 fraction SBRT protocol for early-stage larynx cancer. SBRT was able to achieve reductions in high doses of radiation delivered to normal structures such as the carotid arteries and contralateral arytenoid compared to IMRT plans. These dosimetric parameters will be used to guide future prospective protocols using SBRT for larynx cancer.

P087: THE EFFECT OF PATIENT-DRIVEN ALCOHOL ABSTINENCE ON POSTOPERATIVE OUTCOME IN ALCOHOL MISUSERS WITH CANCER OF THE HEAD AND NECK – Azeem Kaka, MD; Otolaryngology - Head and Neck Surgery, The Ohio State University Wexner Medical Center

Recent studies have shown that patients who drink at least 60 grams (g) of alcohol (4-5 standard US drinks) per day have a three-fold increase in postoperative complications. Alcohol misusers have been shown to have longer hospital stays, increased need for second operations, and a higher risk of withdrawal as compared to nonusers. In general, the duration of abstinence to counteract the negative effect of alcohol ranges from one to eight weeks, depending on organ system. Alcohol abstinence for just two weeks has been shown to effectively recover platelet function and reduce bleeding times. The risk of withdrawal is known to diminish after one week of abstinence. Given this compelling data, along with the high prevalence of alcoholism in the head and neck population, the Division of Head and Neck at our institution has implemented an official clinical protocol in late 2013 for alcohol misusers. It is a rigorous and safe protocol developed in conjunction with the Department of Internal Medicine, nursing and social work. The protocol asks patients who are misusers of alcohol to sign an alcohol abstinence contract. The contract asks patients to agree to be abstinent of alcohol prior to surgery, at a minimum of 7 days. They are informed about possible signs/symptoms of withdrawal and are given a script for a benzodiazepine (Lorazepam) to treat withdrawal issues. This is preferentially done with family present such that accountability is increased. Several safety nets are present ranging from admission to detox facilities to preoperative inpatient admission for appropriate medical care of the withdrawal if they are too high risk to undergo abstinence at home.

After receiving IRB approval, we performed an early analysis of our outcomes of the abstinence protocol. We compared patients who had undergone a cessation contract (n=15) to those who were abusers of alcohol prior to the inception of the protocol (n=71). Previous abusers of alcohol were found by searching our surgical logs for patients who consumed greater than 4 US drinks/day (>60 g alcohol/day). In both cohorts, no statistically significant difference was found between initial staging, surgery performed and preoperative morbid conditions. Our contracted group had an average of 14.8 days of abstinence prior to surgery. Abstinence was achieved at home for 11 of our patients, in a detox facility for 2 patients and with pre-operative admission for

2 patients. Comparisons between non-contracted and contracted groups for continuous measures were made via two-sample t-tests or Mann-Whitney U tests, depending on normality of the distributions. Categorical measures were compared between non-contracted and contracted groups by Fisher's Exact tests. In univariate comparisons between the groups, rate of alcohol withdrawal was statistically significant. A higher proportion of those in the non-contracted group had alcohol withdrawal compared to those that were contracted (39.4% vs 0%; p=0.0018). Further, hospital stay was significantly longer in those that had alcohol withdrawal compared to those who were contracted (13 days vs 9 days; p=0.0464). These early results are encouraging and promote the necessity of personal responsibility in the head and neck patient.

P088: PROGNOSTIC IMPLICATIONS OF TP53 MUTATION FREQUENCY IN ORAL SQUAMOUS CELL CARCINOMA DETERMINED BY NEXT-GENERATION SEQUENCING – Luiz Paulo Kowalski[1], Ana Flavia Costa[1,2], Frederico Gleber-Netto[2], Natalie Kelner[1], Curtis Pickering[3], Mitchell Frederick[3], Abdullah Osman[3], Diana Noronha Nunes[2], Jeffrey Myers[3], Emmanuel Dias-Neto[2]; [1]Department of Head and Neck and Otorhinolaryngology Surgery, AC Camargo Cancer Center, Sao Paulo, Brazil, [2]Laboratory of Medical Genomics, AC Camargo Cancer Center, Sao Paulo, SP, Brazil, [3]Department of Head and Neck Surgery, The University of Texas MD Anderson Cancer Center, Houston, Texas

INTRODUCTION: There are no currently available biomarkers to guide therapeutic decisions in head and neck squamous cell carcinomas (HNSCC). Since TP53 mutation is the most frequent molecular driver, reported in about 60% of HNSCC, we investigated whether the frequency of these mutations, revealed by Next-Generation-Sequencing (NGS) would have possible prognostic implications. TP53-mutations, particularly those that occur in the DNA binding-domain have been associated with a worse outcome, including in patients with HNSCC. However, the effect of the number of simultaneous mutations/tumor as well as the frequency of these mutations in the tumor has not been addressed yet.

PURPOSE: The present study aimed to investigate the prognostic impact of TP53 mutations in HNSCC, including their number, location and frequency in HNSCC.

METHODS: Mutations were investigated in DNA derived from 65 primary oral HNSCC tumor samples. None of the patients were treated by radio or chemotherapy prior to surgery. TP53 gene was amplified using a TP53 Ampliseq panel, sequenced in an Ion PGM and evaluated using IGV and the Ion Reporter. Particular attention was given to aspects that could be revealed by the large coverage given by NGS: the number of reads carrying mutations as well as the number of distinct TP53 mutations for each patient.

RESULTS: After generating 8.5 million reads, with an average coverage of above 1000X/patient, we observed TP53 mutations in 63% of cases. Most were missense mutations (68.5%), followed by nonsense (20%), frameshift deletions (8.5%) and frameshift insertions (3%). Worse outcome, i.e. patients that died due to this cancer, was most frequent for individuals carrying tumors with nonsense or frameshift-mutations, especially when these were located in the DNA binding-domain. Death from disease was also more frequent for patients carrying >1 mutation, particularly in association with frameshift deletions and for those with a mutant allele fraction >50%, regardless of the mutation type or location.

CONCLUSIONS: The previously described poor prognosis for patients with tumors bearing nonsense TP53 mutations was confirmed but we have also identified that having more two or more distinct TP53 mutations per specimen and/or with an allele fraction of TP53 mutation >50% also is associated with worse outcomes. This finding reinforces the driver effect of TP53 genes and the impact of multiple TP53 mutations and a high allele fraction of mutation, as tumors with a mutation frequency >50% had worse prognosis.

POSTER LISTING

P089: TRANSORAL ROBOTIC SURGERY (TORS) PARAPHARYNGEAL APPROACH FOR SUBMANDIBULAR GLAND EXCISION IN A CADAVER MODEL – Christopher H Rassekh, MD, FACS, Francis Marchal, Bert W O'Malley, Jr., Gregory S Weinstein; Department of Otorhinolaryngology-Head & Neck Surgery, University of Pennsylvania

BACKGROUND: Removal of the submandibular gland is the standard for management of neoplasms as well as intractable chronic inflammatory conditions. Transoral excision of the SMG has been described but has not gained popularity because of questions about the safety of this operation for both neoplastic and inflammatory diseases. In particular, concerns about postoperative hemorrhage, wound infection and most importantly, lingual nerve injury still exist. Transoral Robotic Surgery (TORS) has emerged as a major advance in the management of a variety of lesions in the pharynx and parapharyngeal space.

DESIGN AND OBJECTIVES: We investigated Transoral Robotic Surgery (TORS) Parapharyngeal Approach combined with the traditional anterior transoral approach in cadaver specimens which were obtained after approval from the institution's committee on use of anatomic gifts. All 10 submandibular glands were removed in 5 specimens.

ANATOMIC FINDINGS: The parapharyngeal approach facilitated identification of the proximal lingual nerve and the posterior and deep aspects of the SMG. The anterior approach provided access to the duct and the sublingual gland and the distal branches of the lingual nerve. A progressive decrease in the length of lingual nerve exposure and the degree of retraction occurred with each subsequent dissection. Ultimately, it was discovered that early transection of the submandibular duct just posterior to the sublingual gland and early transection of the submandibular ganglion allowed access to the mylohyoid with minimal retraction of the lingual nerve. The duct provided a handle for upward retraction of the gland for the dissection. The fundamental principles of TORS proved to be advantageous in removal of the submandibular gland. The magnification, wristed instrumentation with tremor filtration and separation of the assistant from the surgeon were beneficial. In particular, the assistant was able to provide varying degrees of retraction and had tactile feedback while the console surgeon was able to visualize the interface between the gland and the critical anatomic landmarks. Communication between the console surgeon and the assistant was important and improved during over the course of the dissections as well. Water-tight closure of the wound was always deemed feasible with the incisions used.

CONCLUSIONS: This series of cadaver experiments demonstrates the feasibility of the TORS Parapharyngeal Approach for resection of the entire SMG . TORS appears to provide several advantages over non-robotic transoral approaches. This project results in a standardized sequential stepwise surgical approach for TORS SMG excision. In addition, the approach helped minimize trauma to the lingual nerve. Another potential advantage of the TORS approach is that if TORS-assisted or combined approach posterior sialolithotomy fails, the operation can be converted intraoperatively to a TORS SMG excision allowing a single-stage management of the patient without the risk of orocutaneous fistula.

P090: ASSESSMENT OF ERK PHOSPHORYLATION PROFILES USING NANOIMMUNOASSAY: DIFFERENTIAL REGULATION OF ERK1 AND ERK2 IN HEAD AND NECK SQUAMOUS CELL CARCINOMA – Matthew A Hubbard, MD, Ashraf Khalil, MD, PhD, Rolando Mendez, BS, Mark Jameson, MD, PhD; University of Virginia Department of Otolaryngology - Head and Neck Surgery

BACKGROUND: Recent studies have identified differential roles of ERK1 and ERK2 in cellular behavior. ERK2 activity promotes cell proliferation and epithelial-to-mesenchymal transition, while ERK1 action can be antagonistic to these effects. Measuring ERK1 vs. ERK2 activation independently may have clinical utility but is challenging using standard Western blot techniques.

OBJECTIVE: To demonstrate the differential activation of ERK1 and ERK2 in head and neck squamous carcinoma (HNSCC) cells using nanoimmunoassay (NIA).

DESIGN: ERK phosphorylation profiles were assessed using NIA, which evaluates nanoscale protein samples using capillary isoelectric focusing followed by immunoassay detection similar to a Western blot. This allows for quantification of all six phosphoisoforms of ERK1/2 in a single sample using a single antibody.

RESULTS: HNSCC cell lines were stimulated with growth factors in vitro and their ERK phosphorylation profile was evaluated using NIA. Increased relative ERK2 phosphorylation in response to treatment correlated with proliferative response to stimulation. An orthotopic mouse xenograft model was used to assess in vivoERK phosphorylation profiles. Mice were treated with either the insulin-like growth factor-1 receptor (IGF1R) antagonist BMS-754807 or vehicle; mice treated with BMS-754807 were noted to have reduced IGF1R, AKT and ERK phosphorylation by IHC, as well as reduced size of the primary tumor and metastatic nodes. On NIA analysis, no ERK2 phosphorylation was detected in normal tongue tissue. In the primary tumor and metastatic nodes, 40% and 15% of ERK2 was in the diphosphorylated form, respectively. Treatment with BMS-754807 resulted in elimination of the phosphorylated forms of ERK2, with a profile similar to normal tongue tissue. In contradistinction, ERK1 was noted to be 95% monophosphorylated in normal tongue tissue but 65% unphosphorylated in the primary tumor and metastatic nodes. On treatment with BMS-754807, both the primary tumor and metastatic node profiles demonstrated 95% monophosphorylated ERK1.

CONCLUSION: The ERK phosphorylation profile may be a useful marker of malignancy. Additionally, ERK2 phosphorylation in particular may predict response to targeted therapy. These studies are consistent with the growing body of literature suggesting differential roles of ERK1 and ERK2 in cell signaling and highlight the utility of NIA in assessment of ERK phosphorylation status.

P091: MAMMARY ANALOGUE SECRETORY CARCINOMA: A NEW DIAGNOSIS – Lauren Bohm, Amy Anne Lassig; Department of Otolaryngology - Head and Neck Surgery, University of Minnesota

BACKGROUND: Mammary analogue secretory carcinoma is a salivary gland neoplasm first described in 2010 of which many head and neck surgeons are unfamiliar.

METHODS: We present a case of a 43 year old healthy male who developed a mammary analogue secretory carcinoma of the minor salivary glands in the lower lip. Excisional biopsy was completed with histological characterization, followed by imaging, tumor conference evaluation, and re-excision. He was not treated with adjuvant therapy.

RESULTS: The patient is currently doing well without evidence of recurrence and continues to undergo clinical surveillance.

CONCLUSIONS: Mammary analogue secretory carcinoma is a newly described salivary gland malignancy. As such, most clinicians are not familiar with the diagnosis or characteristics. We present this case to share information about this tumor including histopathological correlation. The diagnostic features and prognosis of the lesion will be extensively reviewed.

POSTER LISTING

P092: LOSS OF LZAP INACTIVATES P53 IN HEAD AND NECK CANCER AND REGULATES SENSITIVITY OF CELLS TO DNA DAMAGE IN THE P53-DEPENDENT MANNER – James J Wamsley, Natalia Issaeva, Xinyuan Lu, Gary Bellinger, Mi Zou, Brandee Brown, Asel Biktasova, Wendell Yarbrough, MD; Yale UniversityWe reported that LZAP has tumor suppressor activity, is lost in a portion of head and neck squamous cell carcinoma (HNSCC), and inhibits NF-κB. Current anticancer therapies, including those used to treat HNSCC, are associated with severe side effects limiting dose and efficacy. Because these side effects are, at least in part, dependent on p53-mediated apoptosis, transient downregulation or suppression of p53 in healthy tissues has been explored as a therapeutic strategy to protect normal cells during cancer treatment. Wild-type p53 is nearly always inactivated in human cancers through various mechanisms. These include binding to viral proteins, alterations in genes whose products serve as positive or negative regulators of p53, or directly by mutations within the TP53 gene. TP53 mutations occur within approximately 50% of human tumors, particularly in HNSCC, and are associated with rapid tumor progression and resistance to anticancer therapy through both abrogation of the tumor suppressor capabilities of p53 and novel "gain-of-function" oncogenic properties.

Here, we show that LZAP downregulation diminishes both mutant and wild-type p53 protein levels. Importantly, downregulation of LZAP protects wild-type p53 cells from radiation while sensitizing cells expressing no or mutant p53. These data suggest that inhibition of LZAP activity toward p53 for patients with tumors harboring p53 mutations may simultaneously sensitize the tumor and protect normal surrounding tissues from damage due to radiation and chemotherapy.

p53 induces apoptosis through both transcription-dependent and transcription-independent mechanisms, the latter mediated by translocation of p53 to the mitochondria. Using small-molecule inhibitors that block 1) the transactivation function of p53 or 2) the translocation of p53 to the mitochondria, we demonstrated that loss of LZAP inhibits the transactivation potential of p53. Consistent with this finding, zeocin-induced upregulation of p53 target genes involved in apoptosis (Bad, Bax, PIDD, APAF1), as well as others (MDM2 and Wip1), was attenuated following LZAP knockdown.

Interestingly, we showed that LZAP depletion downregulates p53 at multiple levels. Knockdown of LZAP resulted in a small (20-30%) reduction in TP53 mRNA levels. Since this effect alone cannot explain the entire decrease in p53 protein level often observed (sometimes up to 70%), we speculated that loss of LZAP may destabilize p53 at the protein level. Indeed, treatment with MG132 fully restored the decrease in p53 levels that accompany LZAP knockdown.

Non-small cell lung cancers share a number of molecular signatures with HNSCC, including frequent mutations of the TP53 gene. Interestingly, we found that in both adenocarcinomas and squamous cell carcinomas of the human lung, LZAP and p53 protein levels display a statistically significant positive correlation, as measured by immunohistochemical staining of tissue microarrays (TMAs). Similar analysis is underway with newly obtained HNSCC TMAs. Since high p53 protein levels often indicate a mutation within the TP53 gene, these results suggest that loss of LZAP may represent a novel mechanism of p53 inactivation. Sequence analysis of a subset of these tumors is underway to determine whether tumors with low LZAP levels demonstrate reduced pressure to mutate TP53.

NOTES

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