The adjuvant therapeutic technology we developed, quadrapeutics, addresses several critical needs for treatment of aggressive, drug-resistant and often unresectable cancers in head and neck. Quadrapeutics ensures clean surgical margins, prevents tumor local recurrence, improves patients' survival rates, and enhances their quality of life. The method selectively detects and destroys tumors in vitally important anatomic locations after standard therapies fail or risk high functional and cosmetic damage. Quadrapeutics selectively enhances the efficacy of chemoradiation therapy in cancer cells in patients who have failed standard treatments and cannot tolerate high therapeutic doses. Specifically, the method (1) intra-operatively detects and eliminates microscopic residual disease after incomplete tumor resection and (2) delivers adjuvant therapy post-operatively to treat local recurrence. This is achieved through a cancer cell-specific on-demand intracellular enhancement of the reduced doses of clinically-approved drugs and radiation. Quadrapeutics employs four clinically validated components (Fig. 1): encapsulated drugs, gold colloids, near-infrared laser pulses and radiation, each being applied in a low and safe dose.

Quadrapeutics is administered in three stages (Fig. 1). At stage I, gold colloids and encapsulated drug are systemically administered prior to the surgery. Cancer aggressiveness regulates the clustering of drug nanocarriers and gold nanoparticles in cancer cells but not in normal cells (Fig. 1). At stage II, laser pulses are intra-operatively applied to surgical margins via endoscope (Fig. 1). Gold cluster in cancer cell, upon exposure to a laser pulse of low energy, generates a plasmonic nanobubble, an intracellular transient vapor nanobubble (Fig. 1). Plasmonic nanobubble mechanically disrupts drug nanocarrier, ejects the drug into cytoplasm and mechanically damages the host cell. At stage III, gold clusters amplify the radiation locally in cancer cells (Fig. 1). Intracellular synergy of the mechanical impact of plasmonic nanobubble, ejected drug and amplified radiation improves the efficacy of standard chemoradiation in resistant and aggressive head and neck squamous cell carcinoma (HNSCC) by more than 100-fold in vitro and in vivo 17-fold for primary tumors, 7-fold for microscopic residual disease and more than 20-fold for the local recurrent disease. Such radical therapeutic enhancement has been achieved with the very low therapeutic doses of drugs (Doxil or Lipoplatin), 2-3%, and radiation, 6%, of their clinical doses. Cancer cell-specific mechanism of such therapeutic enhancement coupled with the low therapeutic doses efficiently spares normal cells. At the same time, acoustic emission by plasmonic nanobubbles provides the high sensitive intra-operative detection of microscopic residual disease in seconds (Fig. 1) and thus allows efficient surgical guidance.

Quadrapeutics converts established macro-therapies into an on-demand intracellular modality with the high therapeutic efficacy and selectivity. Diagnosis and treatment are united into a single theranostic procedure, whose speed, sensitivity and efficacy enable the real-time intra-operative detection and treatment of microscopic residual disease, reduce efficient therapeutic doses of drugs and radiation to 2-6 % of their standard doses and minimize non-specific toxicity. Mechanical and physical, rather than chemical, nature of therapeutic effects makes this technology very universal. Finally, the use of already clinically-validated components ensures rapid translation of quadrapeutics to clinic.
I. Systemic neoadjuvant treatment

Gold colloids & encapsulated drug

II. Intra-operative diagnostics + treatment

Optical guide

Acoustic monitoring

Pulsed laser

Laser

III. Post-operative iMRT

Amplification of radiation
Reactivation of p53 in HPV-positive SCC by small molecule Minnelide

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Importance: The incidence of human papilloma virus (HPV)-positive oropharyngeal squamous cell carcinoma continues to rise. Even though the prognosis is favorable, patients experience severe toxicity with the current treatment modality. No agents that reactivate p53 are available to treat head and neck carcinoma, and the development of such an agent might augment our ability to de-escalate treatment to avoid highly toxic side effects of current treatments.

Objective: We report on a novel chemotherapy agent that reactivates wild-type p53 in HPV-positive head and neck carcinoma. The agent under study, triptolide, and its water soluble pro-drug "Minnelide," were used.

Design and Methods: In the in vitro phase of the study, two HPV-positive head and neck carcinoma cell lines (UM SCC 47 and 93-VU-147), one HPV-negative cell line (UM SCC 11A) and a normal human epidermal keratinocyte cell line (HEKa) was used. Cells were incubated with different concentrations of triptolide for different time points. Activation of p53 was evaluated by western blot, immunofluorescence, and reporter gene assay. In the in vivo phase of the study, Minnelide was injected intra-peritoneal at 0.42mg/Kg to treat two different HPV-positive animal models, one with a subcutaneous injection of UM SCC 47 cell line and the second with a patient-derived tumor xenograft model. Control animals were treated with saline solution. In vivo reactivation of p53 was evaluated by western blot and quantitative PCR.

Results: Incubation of HPV-positive head and neck carcinoma cell lines with triptolide showed a significant decrease in cell viability and activation of caspase 3 activity in a time and dose dependent manner. We demonstrated significant increases in total and phosphorylated p53 on western blot and immunofluorescence assays. Reporter gene assay demonstrated significant increased of p53 reporter activity when compared with the control. Furthermore, our in vivo data confirmed increased p53 phosphorylation and levels of TP53 mRNA. Minnelide also induced apoptosis as evaluated by TUNEL and significantly decreased tumor volume. Finally, Minnelide effectively decreased tumor volume when compared to control in a HPV patient-derived tumor xenograft.

Conclusions and Relevance: Our findings strongly support the notion that Minnelide reactivates p53 both in vitro and in vivo in HPV positive oropharyngeal squamous cell carcinoma, and offers a promising and unique targeted treatment.
LOCALIZATION OF LIPOSOMAL MTHPC FORMULATIONS WITHIN NORMAL EPITHELIUM, DYSPLASTIC TISSUE, AND CARCINOMA OF ORAL EPITHELIUM IN THE 4NQO-CARCINOGENESIS RAT MODEL

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Background and objective. Foslip® and Fospeg® are liposomal formulations of the photosensitizer meta-tetra(hydroxyphenyl)chlorin (Foscan®), which is used for Photodynamic Therapy (PDT) of head and neck malignancies. Literature suggests that liposomal mTHPC formulations have better properties and increased tumor uptake compared to Foscan. To investigate this, we used the 4-nitroquinoline-1-oxide (4NQO) induced carcinogen model to compare the localization of the different mTHPC formulations within normal, precancerous and cancerous tissue. In contrast to xenograft models, the 4NQO model closely mimics the carcinogenesis of human oral dysplasia.

Materials and Methods. 54 rats drank water with the carcinogen 4NQO. When oral examination revealed tumor, the rats received 0.15 mg/kg mTHPC (Foscan, Foslip or Fospeg). At 2, 4, 8, 24, 48 or 96 hours after injection the rats were sacrificed. Oral tissue was sectioned for hematoxylin and eosin (HE) slides and for confocal microscopy. The HE slides were scored on the severity of dysplasia by the Epithelial Atypia Index (EAI). The slides for confocal microscopy were measured for mTHPC fluorescence intensity using a confocal fluorescence microscopy. The calibrated fluorescence intensity per formulation or time point in a region of interest was correlated to EAI. A total of 280 regions of interest were measured.

Results. Fospeg showed higher mTHPC fluorescence in normal and tumor tissue compared to both Foscan and Foslip. Significant differences in fluorescence between tumor and normal tissue were found for all formulations. However, at 4, 8 and 24 hours only Fospeg showed a significant higher fluorescence in tumor. The Pearson’s correlation between EAI and mTHPC fluorescence proved weak for all formulations.

Conclusion. In our induced carcinogenesis model, Fospeg exhibited a tendency for higher fluorescence in normal and tumor tissue compared to Foslip and Foscan. In contrast to Foscan and Foslip, Fospeg showed significantly higher fluorescence in tumor vs normal tissue at earlier time points, suggesting a possible clinical benefit compared to Foscan. Increasing grade of dysplasia was not strongly related to increasing mTHPC fluorescence. This suggests that increased mTHPC fluorescence in tumor tissue versus normal tissue is a result of increased uptake of liposomal mTHPC due the structural tissue changes. Preclinical studies on PDT efficacy of Fospeg at early time points should be assessed.
APPLICATION OF INFRARED-BASED MOLECULAR IMAGING IN A MOUSE MODEL WITH HEAD AND NECK CANCER

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Introduction:

The presence or absence of tumor cells remaining in the surrounding area (the surgical margins) following tumor removal is essential in achieving a successful surgical outcome for head and neck squamous cell carcinoma (HNSCC). Mouse models with HNSCC are invaluable tools used to advance our understanding of the mechanisms of regional and distant metastatic spread of HNSCC, and various subcutaneous xenograft mouse models of HNSCC have been developed in recent years. Molecular imaging (MI) is a promising new technology that may improve current HNSCC mouse models. While the MI of HNSCC mouse model with visible fluorescent proteins, such as tdTomato (excitation/emission: 554/581 nm) has been reported to be effective due to its non-invasiveness and convenience as a means of detecting the tumor margins, it is limited by its inability to penetrate deep tissues, therefore representing a challenge for accurately identifying tumor free margin or distant metastasis. Recently, a near-infrared fluorescent protein (iRFP), with an excitation/emission maxima at 690/713 nm, was developed to overcome this challenge. These wavelengths exist within the near-infrared window where mammalian tissues have the lowest absorbance and less light scattering than in the shorter wavelength spectrum. Therefore, we hypothesized that an iRFP-based mouse model with human HNSCC would result in better original and distance tumor detections, as compared to existing visible-fluorescent model, such as tdTomato. The present study investigates the difference between iRFP and tdTomato proteins in a mouse model with human HNSCC.

Materials and methods:

Both HNSCC-iRFP and HNSCC-tdTomato stable cell lines were established via retroviral transfection of an original human HNSCC cell line, JHU012, with pLNCX2-iRFP and pBabe-Blast-tdTomato plasmid, respectively. The stable cells expressing either iRFP or tdTomato were selected and obtained with antibiotics and cell sorting. HNSCC-iRFP and HNSCC-tdTomato cells were injected subcutaneously in the flank of athymic nude mice to develop fluorescent tumor xenografts. The differences between iRFP and tdTomato tumor in these mice were evaluated using external caliper measurements, and a MI system. Furthermore, \textit{in vivo} and \textit{ex vivo} experiments were conducted to assess the permeability of iRFP and tdTomato tumor signals, as well as the signal-to-background ratio.

Results:

The fluorescent signal from both iRFP and tdTomato xenografted tumors was consistently detected throughout the study. The iRFP signal per unit tumor volume significantly increased and exceeded the tdTomato one by three-fold in the second week after HNSCC cells implantation. The signal permeability experiments showed that iRFP signal was significantly greater than the tdTomato one both \textit{in vivo} and \textit{ex vivo}. Furthermore, the tumor margin of the iRFP mouse model was visualized much more clearly than that of the tdTomato one throughout this study.
Conclusion:

The results of our study support our hypothesis that near-infrared-fluorescence-based MI is superior to visible-fluorescence imaging due to the improved signal-to-noise ratio, and the ability of penetrating deep tissues. Because near-infrared imaging is better suited for detecting deep signals, we believe that iRFP would be very useful not only to study original local HNSCC tumor but also for distant metastases which may occur in underlying tissue.
S332 COOVEREXPRESSION OF ERBB1/4 RECEPTORS PREDICTS POOR OUTCOMES IN PN+ SQUAMOUS CELL CARCINOMA WITH CAPSULAR RUPTURA

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Overexpression of members of the ErbB receptor family is common in oral squamous cell carcinomas (OSCC); however, their prognostic value for aggressive OSCC has been debated. Extranodal spread to cervical lymph nodes is the most significant prognostic indicator in OSCC. In the present study, we investigated the clinical significance of single versus paired overexpression of members of the ErbB receptor family in 82 OSCC patients with lymph nodes metastasis (pN+), with or without capsular rupture (CR) followed by at least 10 years. Immunohistochemistry analysis revealed a common overexpression of ErbB1 (P=0.021), ErbB2 (P=0.001), ErbB4 (P=0.048), as well as MMP-2 (P=0.043) in OSCC cases with CR+. Increased expression of ErbB1 was associated with MMP-2 in tumors with advanced clinical stages, including poorly differentiated (grade III) tumors (P<0.050). Vascular embolization was associated with MMP-2 (P=0.021) and MMP-13 (P=0.010) overexpression. Survival analysis revealed a lower survival probability in tumors overexpressing ErbB1 (P=0.038), ErbB4 (P=0.043), and MMP-12 (P=0.050). As well a strong association was observed in cases with high risk of recurrence and strong immunostaining for ErbB1 (P=0.017), ErbB4 (P=0.008), MMP-1 (P=0.003), MMP-2 (P=0.016), MMP-10 (P=0.041), and MMP-13 (P=0.005). Stratified multivariate survival analysis revealed a strong prognostic interdependence of ErbB1 and ErbB4 cooverexpression in predicting the worst overall and disease-free survivals (P=0.0013 and P=0.0004, respectively). Taken together, these results support a cooperation of ErbB1, ErbB4, and members of the MMP family in predicting OSCC invasion and poor clinical outcomes.
S333 CHROMOSOMAL ABERRATIONS AND PROGNOSIS IN PATIENTS WITH ADJUVANT CHEMORADIOThERAPY FOR RESECTED HEAD AND NECK CANCER

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Objectives: The purpose of this study was to identify alterations in genetic markers related to treatment failure in patients head and neck squamous cell carcinoma (HNSCC) treated with the radical surgery plus postoperative chemoradiotherapy.

Methods: Genome wide copy number alterations were analyzed in 18 HNSCC with (n=9) or without (n=9) recurrence using 180K array-comparative genomic hybridization.

Results: A total of recurrently altered regions (RARs) were identified in the 18 HNSCC cases. Two RARs on chromosomes 7p11.2 and 18p11.32-11.31 were found to be significantly more common in HNSCC with recurrence (P<0.05). Gain of 7p11.2 (where the GBAS gene is located) and 18p11.32-11.31 (where the TYMS gene is located) were the most frequent (5 out of 9 patients with recurrence, while 0 of the 9 without recurrence), and most significantly associated with treatment failure (P=0.029 and P=0.029, respectively). Gain of 7p11.2 and 18p11.32-11.31 were associated with poor disease specific survival (P=0.032 and P=0.032, respectively).

Conclusion: Our findings show copy number gain of GBAS and TYMS is associated with recurrence and prognosis in patients with HNSCC. These profiles may be valuable as predictive markers of treatment failure.
ANALYSIS OF PHYSICAL STATUS OF HPV-16 AND TP53 CODON 72 POLYMORPHISM IN JAPANESE PATIENTS WITH OROPHARYNGEAL SQUAMOUS CELL CARCINOMA

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Objective:

The purpose of this study was to assess the value of the physical status of human papillomavirus (HPV) DNA and the TP53 codon 72 polymorphism in Japanese patients with oropharyngeal squamous cell carcinoma (OPSCC) and to clarify the clinical features.

Methods:

This study included 152 patients with OPSCC who underwent curative-intent therapy from a single institution. To screen for HPV-related OPSCC, tumor samples were immunohistochemically stained for p16. The physical status of HPV-16 was examined using real-time polymerase chain reaction targeting the E6 and E2 open reading frames (ORFs). The TP53 codon 72 polymorphism was screened for by direct sequencing of genomic DNA.

Results:

Sixty patients (39%) were p16 positive and further confirmed for HPV DNA using ISH. Using the E2/E6 ratio as a surrogate marker for integration, we determined the physical status of HPV-16 in 46 tumors among the 60 patients with HPV-related OPSCC. Twenty-eight patients (61%) had integrated forms, and 18 (39%) had mixed or episomal forms. Of 45 patients, 42 carried the Arg/Arg or Arg/Pro genotypes, and only three patients carried the Pro/Pro genotype. Clinicopathological analysis of 46 patients revealed that patients with the integrated form (age: 42-81 years, median age: 62.5 years) were younger than those with the mixed/episomal form (age: 44-81 years, median age: 68 years) (p < 0.05). No differences were observed between patients with the integrated and mixed/episomal form in terms of prognosis, TNM classification, and smoking/alcohol consumption.

Conclusion:

Although the physical status of HPV-16 in patients with HPV-related OPSCC was heterogeneous, no substantial impact on prognosis was observed. It was suggested that HPV-16 may synergize with the Arg/Arg or Arg/Pro genotypes.