**Personalized Cancer Care for Head and Neck Squamous Cell Carcinoma**

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**ABSTRACT**

**Objectives:** Tumor resistance to single-agent chemotherapy is ubiquitous, and recurrence after initial therapy remains a therapeutic challenge. The objectives of our study are:

1. Characterize primary tumor cells from a recurrent head and neck squamous cell carcinoma (HNSCCa) patient for functional therapeutic targets.
2. Determine a personalized drug combination accomplished by mathematical modeling.

**Methods:** A patient with Stage IVa HNSCCa of the floor of mouth refused surgery and received cytotoxic chemoradiation. The disease progressed, so he underwent radical excision in 2012. The specimen was converted to a primary-cell culture. Cells were screened for functional therapeutic targets with a unique high-throughput 60-drug panel of molecularly-targeted agents. A personalized drug combination was derived utilizing a probabilistic Boolean algorithm which cross-correlates drug-screen results with known drug targets.

**Results:** The kinase ALT, if inhibited simultaneously with BTK, CLK2 or WEE1, should result in tumor cell inhibition. PRKCD, if inhibited simultaneously with ALT, BTK, CLK2 or WEE1, should also result in inhibition. Based up this data, Crizotinib (an ALK inhibitor) plus MK-1775 (a WEE1) inhibitor would yield cell death. In order to create 2 distinct points of failure, all 3 drugs could be combined.

**Conclusion:** Molecularly-targeted drug screening and mathematical modeling provides a feasible mechanism for choosing combinations of targeted therapies that may abrogate tumor cell resistance. This treatment paradigm is a potential method for choosing therapy when a patient has developed resistance to the standard-of-care.

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**INTRODUCTION**

Introduction: Over the last decade, personalized cancer care has undergone significant advancement from bioinformatic sciences like genomics and utilization of molecularly targeted therapy like the epithelial growth factor receptor (EGFR) inhibitor, cetuximab. TMra-TM攫 tumor cell resistance, however, remains a significant problem which stems from a multitude of redundant signaling pathways which are not uniformly dependent on a single mechanism. Our study attempts to utilize drug screening of small molecule inhibitors in combination with mathematical modeling to predict vulnerable molecular targets in an individualized manner.

The patient example for the current study is a 63 year old male with T4aN2M0 (Stage IVa) squamous cell carcinoma of the floor of mouth who initially refused surgery and underwent chemoradiation with TPF (docetaxel, cisplatin and 5FU). The patient’s disease progressed during chemotherapy. According to the NCCN guidelines, this patient with recurrent, resectable Stage IVa oral cavity SCCa could be offered surgery with post-op radiation or chemoradiation. Our next step is to inhibit the PTIM “circuit” in two locations in “series.” Breaking the circuit in multiple should further abrogate resistance. These investigations are ongoing. We are also creating patient derived xenografts in which we can test the PTIM algorithm in vivo.

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**METHODS AND MATERIALS**

Please see our workflow diagram in Figure 1. The patient described above underwent surgical resection and tissue was taken from the OR. A primary cell culture was established. After 11 days, 1.7 million cells available for drug screening on against 60 individual small molecule inhibitors. After 72 hours, cell viability was assessed. Data from the drug screen was then analyzed by a probabilistic Boolean algorithm to generate a Personalized Target Inhibition Map (PTIM).

Our model takes advantage of the fact that small molecule inhibitors have overlapping sets of targets. The algorithm cross correlates the dissociation constants (Kd) for each known target with the effective concentration (IC50) of each drug found in the screen. By knowing which drugs worked at which concentrations, in addition to which targets were inhibited at each concentration, the model can deduce which targets are important for inhibiting that tumor cell. For additional details on the PTIM model, please see [4] and [5].

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**RESULTS**

The PTIM for our patient can be seen in Figure 2. One target combinations which may provide synergistic inhibition is WEE1 and ALK. In the drug screen, he ALK inhibitor crizotinib reduced cell growth by 95% and the WEE1 inhibitor, pelitinib reduced cell growth by 79%. MK-1775 is a WEE1 inhibitor not in our drug screen but was chosen for additional studies, because the inhibition of WEE1 is the only necessary component.

Figure 3 reveals drug inhibition curves for crizotinib and MK-1775 delivered alone and in combination. The IC50 for crizotinib was 1.22 μM (95% confidence interval 0.86-1.71 μM) and the IC50 for MK-1775 was 1.09 μM (0.86-1.78 μM). With crizotinib held at its IC50 of 20nM, MK-1775 was varied across 10 concentrations in quadruplicate. The drug combination produced an IC50 of 0.51 μM (0.30-0.86 μM). The R-squared values for crizotinib, MK-1775 and the combination curves are 0.80, 0.63 and 0.6 μM, respectively. In the lower half of Figure 3, the combination index is seen to be less than 1 at lower concentrations. This result supports the concept that PTIMs can successfully predict drug targets that when inhibited together can be more effective than the sum of either one alone.

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**DISCUSSION**

Combination drug therapy is one mechanism used to abrogate tumor cell resistance, but current combinations for HNSCca have shortcomings. Small molecule inhibitors like cetuximab are a new method of targeting tumor cell vulnerability without widespread toxicity. Our methodology attempts to customize therapy with a personalized drug combination of targeted, small molecule inhibitors.

We are able to show that the PTIM algorithm can successfully predict drug synergy at low concentrations. The model is informed by drug screen results and each drug’s Kd for a given target. There is no information about biochemical pathways. We, therefore, have an outcomes derived model that focuses on what actually worked on the tumor cells. This process provides a distinct advantage over bioinformatic sciences that provide copious data which may not be clinically relevant.

In the above example, we were able to derive results 14 days after surgery. This timeline is adequate to inform adjuvant therapy that would typically begin 4-6 weeks after surgery.

Our next step is to inhibit the PTIM “circuit” in two locations in “series.” Breaking the circuit in multiple should further abrogate resistance. These investigations are ongoing. We are also creating patient derived xenografts in which we can test the PTIM algorithm in vivo.

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**CONCLUSIONS**

Tumor cell resistance to therapy remains a significant problem that is being addressed by personalized cancer therapy. We utilize primary cell culture, drug screening and mathematical modeling to predict vulnerable targets for individual patient’s tumors. In the above example, we show that our model is able to predict drug synergy. Utilizing targeted drug combinations of small molecule inhibitors will hopefully help abrogate tumor cell resistance while minimizing patient toxicity.

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**REFERENCES**