

Abstract

A major source of variation among human tumors lies in their different genetic alterations which impact different signaling transduction pathways that in turn govern their sensitivity to drugs. For cancer drug discovery, there is a need to rapidly and precisely model these genetic differences. To address this need, we have taken advantage of our chimeric, inducible mouse cancer models to develop broad panels of tumors with defined genetic contexts by directed complementation (DC). In the inducible models, the driving oncogene can be conditionally turned off, resulting in regression of the primary tumor, unless a gene capable of functionally complementing the original oncogene is introduced. Thus, introduction of the desired gene generates a novel tumor model whose proliferation and survival is dependent on the newly introduced gene.

We have generated over 20 different tumor models using components in major oncogenic pathways. Additional, parallel DC models were rapidly generated using mutations of the same gene relevant to human cancers – providing an experimental platform to examine the functional relationship between genotype and phenotype in an *in vivo*, yet genetically defined context. Examples include either wild type or oncogenic mutants of human HER2, KRAS, EGFR, ERBB3, PLK3 and many others. Molecular characterization of these tumors document the alteration in signaling at the level of mRNA, protein, phosphorylation status, histopathology, reflecting their dependence on the newly introduced gene. More important, the broad panel of tumor models can then be used to refine our understanding of how each signaling change affects their sensitivity to drugs.

Here we describe the generation of HGF-driven DC tumor models, their molecular characterization, and how such target-labeled tumors can serve to guide pre-clinical development of an anti-cancer agent AV-299, a potent HGF neutralizing antibody. Starting with an inducible, murine breast tumor driven by HER2, we generated a panel of HGF-driven DC tumors whose proliferation and survival is no longer dependent on HER2, but has instead been re-wired to be uniquely driven by HGF. We present evidence that this genetic alteration is accompanied by a change in drug response: the HGF-driven DC tumors are now sensitive to a neutralizing antibody against HGF. Finally, these tumor models can be exploited to guide selection of clinical candidates *in vivo*, and to model drug resistance.

Insertional Mutagenesis Genetic Screens

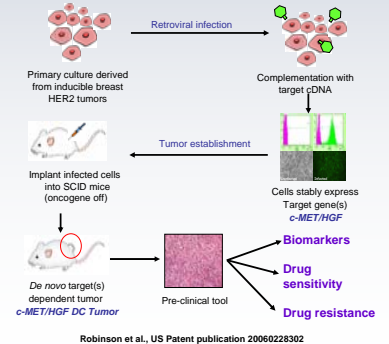
Comprehensive, large-scale, genetic screens were conducted to identify candidate tumor maintenance genes using AVEO's Breast Her2, Breast KRAS and Lung KRAS inducible tumor models. These screens identified well-known, as well as many unexpected cancer targets not previously known to be related to tumor maintenance. A distinct subset of genes, representing 10% of the total, were implicated in all three screens. The MET-HGF pathway was among the most predominantly hit in the screens. A more detailed overview of the screens will be presented by **Isabel Chiu** at the Minisymposium - Integrated Genomic Analysis of Human Cancer on Wednesday, Apr 18, 10:40 AM -10:55 AM at Room 304 AC (#5686).

Recurrent c-MET hits in Mass Screens



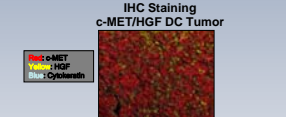
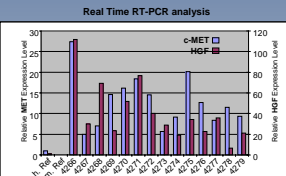
Directed Complementation Technology

- Creation of tumors driven by chosen target (directed complementation):
 - Allows for study of target biology in an *in vivo* tumor context.
 - Allows for correlation between mutational variants and drug response.
- Use of tumors driven by c-MET/HGF in drug discovery:
 - Generation of c-MET/HGF DC tumors
 - Molecular characterization of DC tumors by RT-PCR, IHC.
 - Demonstration of sensitivity to a neutralizing anti-HGF antibody
 - Use of c-MET/HGF DC tumors to guide selection of clinical drug candidates

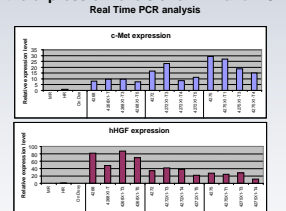


Anti-HGF inhibition of propagated directed complemented c-MET/HGF tumors

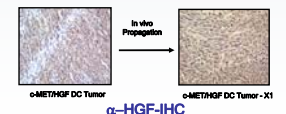
Directed complementation: Generation of breast c-MET/HGF tumors



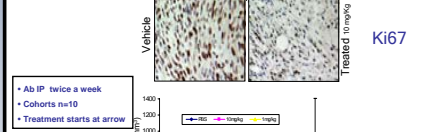
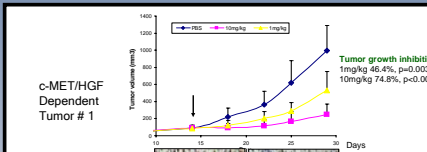
Propagated DC Tumors maintained the expression levels of c-MET and HGF



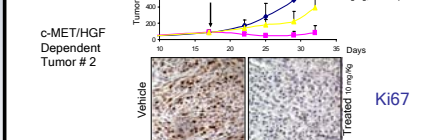
HGF protein levels are maintained through *in vivo* propagation



c-MET/HGF complemented tumors are sensitive to anti-HGF antibody inhibition *in vivo*, exhibiting a significant decrease in cellular proliferation via IHC (Ki67)



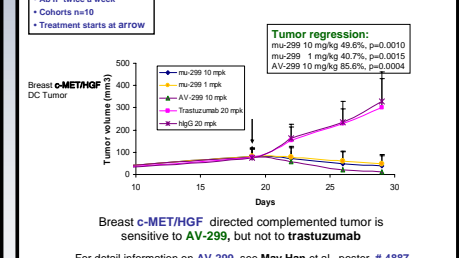
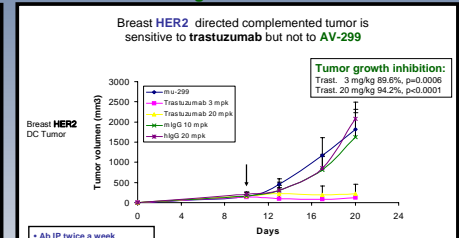
Directed complementation can create unique target dependent tumor models



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Rewiring of drug sensitivity in Breast c-MET/HGF directed complemented tumors. AV-299 induces tumor regression of c-MET/HGF driven tumor



Breast c-MET/HGF directed complemented tumor is sensitive to AV-299, but not to trastuzumab

For detail information on AV-299, see [May Han et al.](#), poster # 4887

Summary

- AVEO has generated an *in vivo* genetic screen uniquely suited to identifying and validating functional targets in solid tumor models.
- Introduction of defined cDNAs into inducible tumor background allows for creation of target-dependent survival tumor models and enables comparison of different genetic mutations (for further information, please see [Jie Lin et al.](#), poster #4008, showing AV-412 drug response in AVEO's lung tumor models).
- The *de novo* generated Directed Complemented tumors are no longer dependent on the original inducible oncogene, but they are rewired by the newly introduced target gene(s), creating a suitable tool to test drug sensitivity *in vivo*.
- AVEO's lead antibody program has generated antibodies that can inhibit the growth of Breast c-MET/HGF Directed Complemented tumors. These c-MET/HGF DC tumors no longer respond to trastuzumab.
- The c-MET/HGF directed complemented tumors are being utilized to identify biomarkers for tumors that are sensitive to a potent neutralizing anti-HGF antibody, AV-299. These biomarkers will facilitate the identification of patient populations likely to be responsive to AV-299 treatment.
- This model also provides an ideal *in vivo* setting to study drug resistance mechanisms.