Human population based engineered breast tumor model for *in vivo* biomarker discovery

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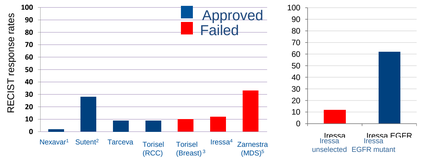
Abstract

Human breast cancer development is a complex pathobiological process driven by genetic changes in normal epithelial cells which lead to uncontrollable growth in a permissive microenvironment. Therefore, it is not surprising that tumors from different patients exhibit variable responses to standard of care therapy with unfortunately only a small percentage of patients benefitting from therapy. It has therefore become a priority in oncology and personalized medicine to match patients to drugs that will result in a favorable treatment outcome. In this report, we describe a population based approach for response prediction featuring naturally occurring variation in tumors derived from genetically defined human-in-mouse models of cancer.

De novo human breast tumors were generated by genetically engineering normal primary human breast epithelial cells with HER2 and SV40 early region (HS) or KRAS and SV40 early region (KS) in an *in vivo* Human-In-Mouse (HIM) tissue transgenic model. The HS and the KS HIM tumors develop as human breast adenocarcinoma that are histologically similar to those observed in patients. Also similar to that observed in human tumors, microarray profiling demonstrated significant inter-tumor variation among the established tumors. Moreover, the KS tumors could be clustered with basal type breast cancers from patients, a poor prognosis human breast cancer subtype. The established HIM tumors exhibited variable responses to treatments with the potent selective triple VEGFR inhibitor, tivozanib. Further characterization of those tumors will help to identify potential biomarkers for tumor response to tivozanib. This population-based approach enables us to identify and validate biomarkers of therapeutic response in an *in vivo* human tumor model.

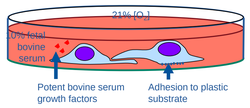
Understanding responsive patient populations can make the difference between approval and failure

•Tumor variation from patient to patient leads to low rates of ORR in most studies
•Iressa failed in an unselected population, but is now approved using EGFR mutation biomarker



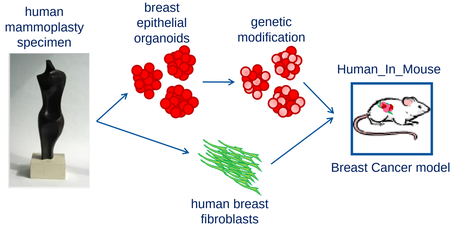
Cell culture creates a very artificial environment in which to assess relevant drivers of tumor maintenance

- Human soluble ligands are missing (e.g. HGF), selects for cells that can survive on cross-reacting bovine growth factors
- Endothelial cells missing
- Stromal cells missing
- Extracellular matrix missing
- Hematopoietic system missing
- Oxygen and nutrients are supplied at supra-physiologic concentrations



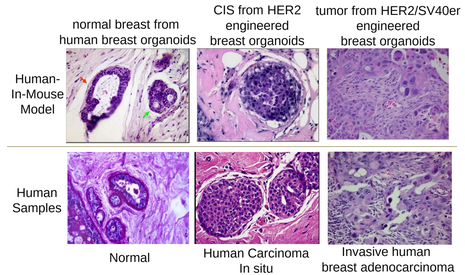
(human tumor derived cell lines are almost always dependent on attachment and serum)

Human-in-Mouse (HIM) tissue transgenic breast tumor model: tissue reconstitution of oncogene-engineered epithelial organoids

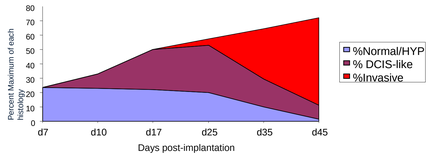


• Tissue transgenic human breast tumors may provide a more accurate model for translational studies with appropriate species and tissue context.

HIM model faithfully recapitulates human breast cancer transformation

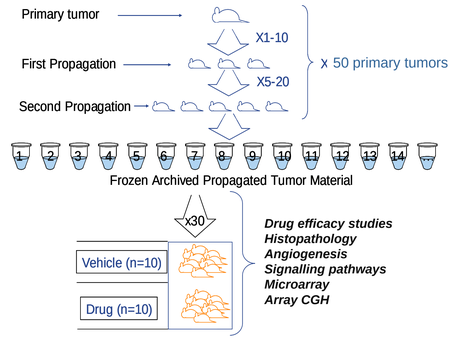


Histological quantitation of tumor progression: tumor development progresses through distinct stages



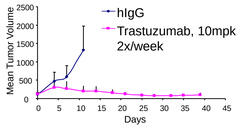
** HER2/SV40er* HIM tissue recombinants were monitored over time.

Propagation and Archiving primary HIM tumors to establish population based breast tumor model

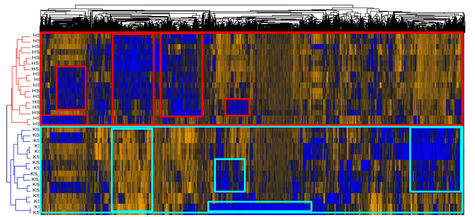


Propagated HER2-driven tumors are sensitive to trastuzumab

• Successful in vivo propagation of HIM tumors surmounted many of the technical challenges of treating primary tumors

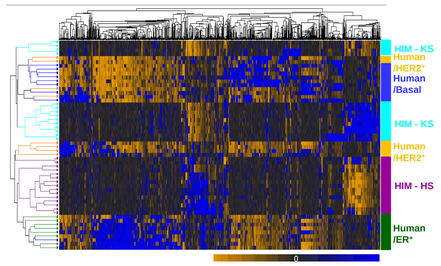


High degree of tumor variation both within and across genotypes



- tumor genotype, *HER2/SV40er (HS)* vs. *KRAS/SV40er (KS)*, is the major variation
- Tumors from the same primary always cluster together

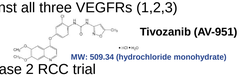
HIM tumors resemble different subtypes of human breast cancer



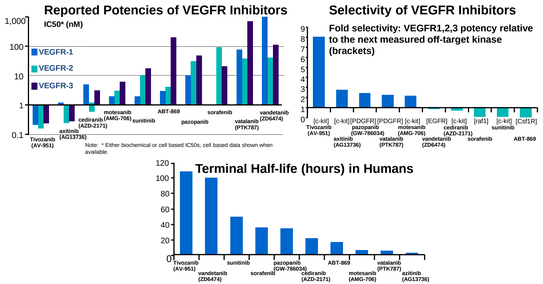
** Hierarchical clustering after removing difference between human and HIM samples*

Tivozanib: potent selective VEGFR TKI

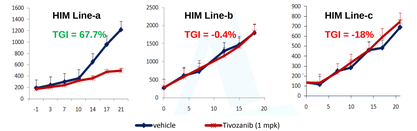
- Extremely potent (~200 pM) against all three VEGFRs (1,2,3)
- Highly selective
- 4.5 day $T_{1/2}$ in human studies
- Robust efficacy in 272 patient Phase 2 RCC trial
  - ORR: 25 - 40% (all RCC independent review--clear cell, nephrectomized investigator review)
  - PFS: 14.8 months in clear cell nephrectomized RCC patients (n=176)
- Safety profile consistent with on mechanism inhibition
  - Most common AEs are Hypertension and Hoarseness



Characteristics of selected VEGFR targeted TKIs

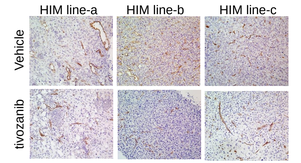


HIM tumor lines exhibited variable responses to treatment with tivozanib



** Three Kras/SV40er HIM tumor lines treated with tivozanib*

Variation in vessel architecture observed across treated tumors



** CD31 IHC of three Kras/SV40er propagated HIM tumors*

Tivozanib is active in virtually all of traditional Xenograft lines tested

Cell Line	Tissue (in vivo, nude mice)	Tumor Growth Inhibition (%)	
		5 mg/kg/day	20 mg/kg/day
A549	Lung carcinoma	68.1***	88.5***
LC6	Lung carcinoma	66.1***	91.5***
Calu6	Lung carcinoma	54.3***	68.8***
HT29	Colon carcinoma	78.6***	87.5***
SW620	Colon carcinoma	52.4***	73.5***
Colo205	Colon carcinoma	74.6***	98.3***
DU145	Prostate carcinoma	>100***	>100***
PC3	Prostate carcinoma	65.5*	57.5*
LNCaP	Prostate carcinoma	99.3***	>100***

***P<0.001, *P<0.05

Conclusions

- Human population based *in vivo* Biomarker Discovery Platform has been established using genetically engineered HIM models.
- HIM tumors exhibited variable response to treatment with tivozanib and will be used to validate tivozanib predictive signatures.
- This population-based approach enables us to identify and validate biomarkers of therapeutic response in an *in vivo* human tumor model.