Generation of *in vivo* tumor models driven by ERBB family of receptor tyrosine kinases and their use in cancer drug discovery

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Abstract

The most commonly used preclinical models of human cancer have been of limited utility in guiding clinical development of anti-cancer agents. Typically, successful inhibition of human cell line xenograft tumors in mice do not accurately predict clinical response in the human tumor type from which the cell line was derived. Further, recent clinical experience with both chemotherapeutic and targeted agents reports significant variation in response even among subpopulations of given human tumor types, as well as significant variations in the genetic contexts of the tumor populations (e.g. EGFR inhibitors in EGFR mutant subsets of NSCLC, or the 30-50% response to Trastuzumab in advanced HER2 amplified breast cancer). In addition, there is a need for relevant *in vivo* models to investigate mechanisms of drug resistance which is often observed upon prolonged treatment with such agents.

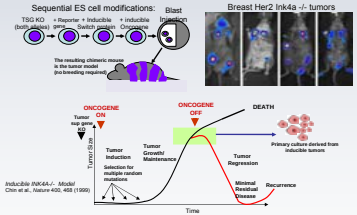
To better understand the relationship between genetic context and drug response, we utilized our inducible cancer breast model to create a panel of genetically-engineered mouse tumors, each driven by a distinct member of the ERBB family of receptor tyrosine kinases (EGFR, HER2, ERBB3, ERBB4). In these models, tumors initially driven by HER2 expression in breast mammary epithelium are re-wired by the addition of another ERBB-family cDNA upon withdrawal of the original HER2 expression. Thus, the newly introduced ERBB family cDNA functionally maintains tumor survival by re-directing downstream effectors of tumor signalling in a process referred to as 'Directed Complementation' (DC). The panel of DC tumors presents a unique pre-clinical opportunity for dissecting subtle differences within complex signalling networks, in part because they were generated on a genetically homogeneous background.

Using this approach, all ERBB family members successfully complemented tumor formation, giving rise to unique models for each ERBB family member, including ERBB4, previously not strongly implicated in cancer. We asked whether these complemented tumors exhibit changes in downstream signalling that would consequently determine their sensitivity to different targeted drugs, by examining their latency and penetrance, mRNA expression, protein phosphorylation, histopathology as well as their respective drug sensitivities. We found that HER2-complemented tumors are indeed sensitive to Trastuzumab treatment, and that EGFR-driven tumors are sensitive to treatment with EGFR inhibitors. Further, wild type EGFR tumors and their derivatives harboring clinically-relevant mutations, EGFRL858R, and EGFR Exon 19 deletion, respond differentially to two ERBB family small molecule inhibitors, Erlotinib and AV-412.

We propose that creation of target-dependent tumors can be powerful tools in guiding pre-clinical development of promising targeted cancer drugs.

Inducible model enables *in vivo* genetic screens based on functional complementation

Complex Models Can Be Generated Rapidly: -Inducible breast and Lung Tumors (Kras, HER2)



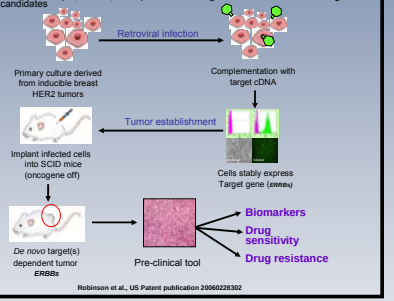
Discovery of Tumor Maintenance Genes: *In vivo* insertional mutagenesis screens in multiple inducible tumor models

Recurrent Egfr hits in Mass Screens

- Comprehensive, large-scale, genetic screens were conducted to identify candidate tumor maintenance genes in multiple inducible tumor models.
- A distinct subset of genes were implicated in all screens.
- The **ERBB pathway** was among the most predominantly hit in the screens, in particular EGFR.

Directed Complementation Technology

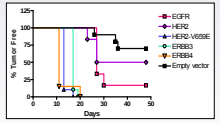
- Creation of tumors driven by chosen target (directed complementation):
  - Allows for study of target biology in an *in vivo* tumor context
  - Allows for correlation between mutational variants and drug response
- Use of tumors driven by EGFR *(wt, L858R, del19)* in drug discovery:
  - Generation of EGFR (wt, L858R, del19) DC tumors
  - Molecular characterization of DC tumors by RT-PCR, IHC.
  - Demonstration of sensitivity to EGFR inhibitors
  - Use of EGFR (wt, L858R, del19) DC tumors to guide selection of clinical drug candidates



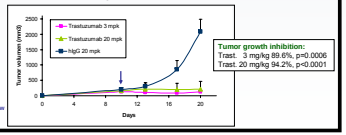
Robinson et al., US Patent publication 20060228302

Generation and Characterization of ERBB RTK Directed Complemented Tumor Models

Adapted Kaplan Meyer Curve - Latency and Penetrance of the ERRBs Models

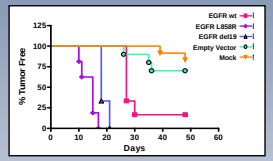


Breast HER2-V659E Directed Complemented tumor is sensitive to trastuzumab

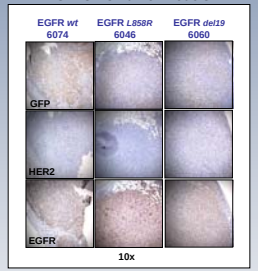


Generation and Characterization of EGFR Directed Complemented Tumor Models EGFR *L858R*, EGFR *del19* and EGFR *wt*

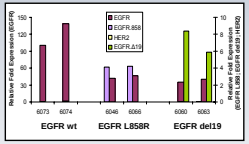
Adapted Kaplan Meyer Curve
Latency and Penetrance of the Models



Immunohistochemistry Analysis
Expression of GFP, HER2 and EGFR in EGFRs DC Tumor Models

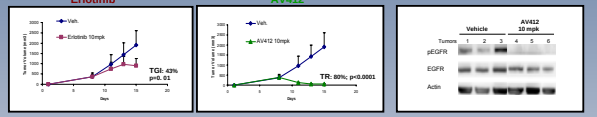


Real Time - PCR Analysis
of HER2 and EGFR (total, L858R, del19)
In EGFRs DC Tumor Models

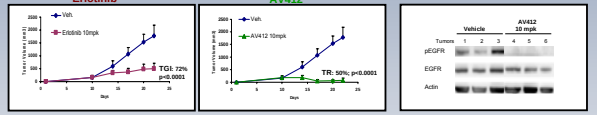


EGFR DC Tumor Models are sensitive to Receptor Tyrosine Kinase inhibitors AV412 and Erlotinib

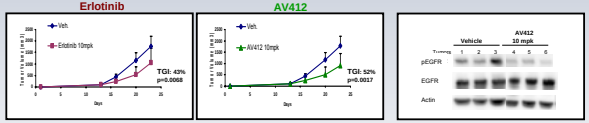
Comparison of Erlotinib and AV412 in EGFR *L858R* DC Tumor (6046_X3); daily, po



Comparison of Erlotinib and AV412 in EGFR *del19* DC Tumor (6060_X3); daily, po



Comparison of Erlotinib and AV412 in EGFR *wt* DC Tumor (6037_X3); daily, po



Summary

- **The ERBB family of receptor tyrosine kinases EGFR, HER2, ERBB3 and ERBB4 were identified as candidate tumor maintenance genes through *in vivo* genetic screens uniquely suited to identifying and validating functional targets in solid tumor models.**
- **Introduction of defined cDNAs into inducible tumor background (Directed Complementation) allows for creation of target-dependent tumor models and enables comparison of different genetic mutations. The *de novo* generated Directed Complemented tumors are no longer dependent on the initiating inducible oncogene, and are instead rewired by the newly introduced target gene, creating a suitable tool to test drug sensitivity *in vivo*. Using this approach, we created various EGFR, HER2, ERBB3 and ERBB4-directed tumor models, validating each one as a tumor maintenance candidate target.**
- **Consistent with clinical reports, the HER2-complemented tumors responded to treatment with Trastuzumab, and EGFR- complemented tumors to EGFR inhibitors Erlotinib and AV412. The various models driven by EGFR mutations afford an experimental system with which to study the relationship between these genetic changes and drug sensitivity towards different targeted inhibitors. For instance, at 10 mpk, the EGFR L858R -complemented tumor responded more dramatically to AV412 than Erlotinib, whereas the EGFR wild type tumors responded similarly to both drugs.**
- **These models also provide an ideal *in vivo* setting to study drug resistance mechanisms.**