

## Abstract

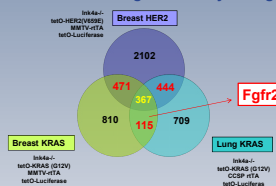
Rapidly emerging data implicate Fibroblast Growth Factor Receptor-2 (FGFR-2) in the genesis of a variety of human cancers, including breast and lung malignancies. However, few FGFR-2-driven tumor models exist and they are not amenable to biomarker discovery and therapeutic response prediction. Using our inducible breast and lung cancer models we followed two approaches to create Fgfr-2/FGFR-2 driven tumors. First, we turned off KRAS expression in established lung cancers and conducted *in vivo* genetic screens using Moloney retroviral insertional mutagenesis. A large fraction of tumors carried retroviral insertions targeting the endogenous *Fgfr-2* locus, and integration hotspot mapped to the promoter and region encoding the carboxyl terminal end of the Fgfr-2 kinase domain. The retroviral insertions are analogous to *FGFR-2* mutations in human cancer cell lines that lead to protein truncation and activation. Second, we turned off HER2 expression in engineered breast tumors and assessed the oncogenic potential of introduced *FGFR-2* alleles. A human *FGFR2* (IIb) allele carrying the cancer-relevant carboxyl terminal truncation efficiently drove tumor growth and was more oncogenic than wild type *FGFR-2* (IIb) (8-12 vs. 12-15 days tumor latency, respectively;  $p < 0.001$ ). Wild type *FGFR2* (IIb), in turn, was a stronger tumor promoter than wild type *FGFR-2* (IIc) (12-15 vs. 18-25 days tumor latency, respectively;  $p < 0.05$ ), pointing to isoform-specific differences in oncogenicity. For each *FGFR-2* allele we generated numerous independent tumors and, surprisingly, observed substantial molecular heterogeneity within each cohort that might underlie variation in response to pathway inhibitors. In line with this notion, we are using our Fgfr-2/FGFR-2-driven cancer models to probe two critical translational priorities: 1) to determine the *in vivo* activity of different pathway inhibitors on tumors driven by the various endogenous and exogenous Fgfr-2/FGFR2 alleles, and 2) to exploit the molecular tumor variation within each tumor cohort in discovering biomarkers that might predict sensitivity or resistance to pathway antagonists.

## Insertional Mutagenesis Genetic Screens in AVEO Models

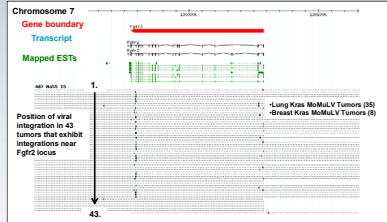
*In vivo* Moloney insertional mutagenesis screens in multiple inducible tumor models



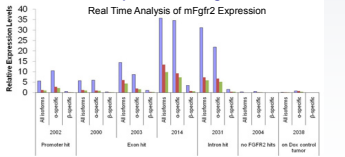
### Nomination of Candidate Target Genes by Triangulation



### Recurrent Fgfr2 hits in Mass Screens



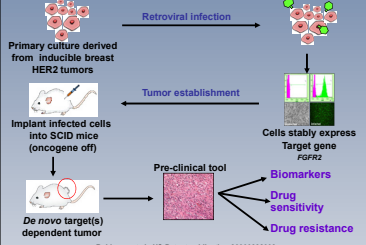
Moloney insertion in the *Fgfr2* locus correlate with overexpression of the gene



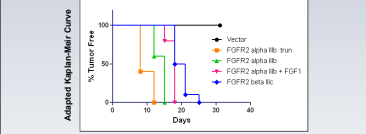
\*Moloney driven tumors with insertions in the *Fgfr2* locus overexpress mFgfr2 compared to either Moloney-driven tumors with no insertion in that locus or with ON Doxy control tumors

## Generation of FGFR2 Directed Complemented Tumor Models

- Directed Complementation Technology
  - Creation of tumors driven by chosen target (directed complementation);
  - Allows for study of target biology in an *in vivo* context
  - Allows for correlation between mutational variants and drug response

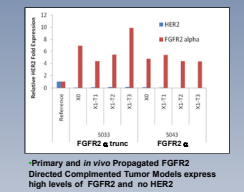


### Latency and Penetrance of the Directed Complemented Tumor Models

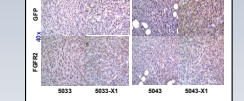


## *In vitro* and *in vivo* Validation of Directed Complemented Tumor Models: FGFR2 DC Tumors are Sensitive to FGFR Tyrosine Kinase inhibitor PD173074

### FGFR2 $\alpha$ Directed Complemented (DC) Tumor Models Retain Expression Upon *in vivo* Propagation

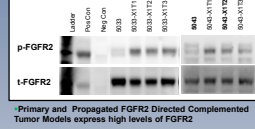


\*Primary and *In vivo* Propagated FGFR2 Directed Complemented Tumor Models express high levels of FGFR2 and no HER2



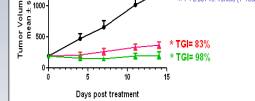
\*Primary and *in vivo* Propagated FGFR2 Directed Complemented Tumor Models express high levels of FGFR2

### *In Vivo* Propagation FGFR2 $\alpha$ Directed Complemented Tumor Models



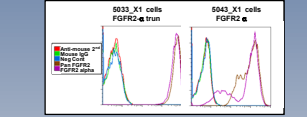
\*Primary and Propagated FGFR2 Directed Complemented Tumor Models express high levels of FGFR2

### Tyrosine Kinase Inhibitor PD173074 Induced Tumor Growth Inhibition in FGFR2 $\alpha$ truncated DC Tumor Model



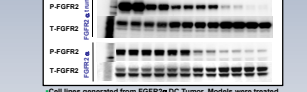
\*FGFR2 $\alpha$  truncated Directed Complemented Tumor cells were established by injecting disrupted tumor cells with Matrigel into 6 weeks old female NCR Nude mice subcutaneously. When tumors reached approximately 150 mm<sup>3</sup>, 30 tumor-bearing mice were randomized into three groups. Group 1 received vehicle, group 2 received PD173074 at 10 mg/kg, group 3 received PD173074 at 20 mg/kg ip, daily.

### *In Vitro* Validation of FGFR2 $\alpha$ and FGFR2 $\alpha$ truncated DC Tumor Models

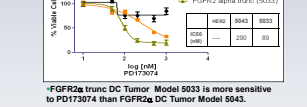


\*Cell lines generated from FGFR2 $\alpha$  and FGFR2 $\alpha$  trunc DC Tumor Models express FGFR2 in the cell membrane

### PD173074 inhibits p-FGFR2 and cell Viability in FGFR2 $\alpha$ DC Tumor Models



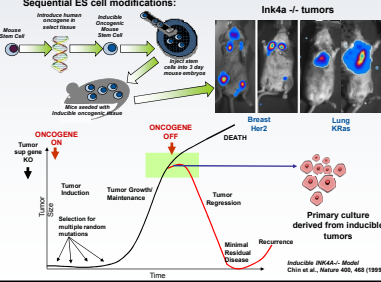
\*Cell lines generated from FGFR2 $\alpha$  DC Tumor Models were treated with the indicated concentration of PD173074 or vehicle for 24 hrs.



\*FGFR2 $\alpha$  trunc DC Tumor Model 5033 is more sensitive to PD173074 than FGFR2 $\alpha$  DC Tumor Model 5043.

## Inducible Tumor Models

Complex Models Can Be Generated Rapidly Inducible Breast and Lung Tumors (Kras, HER2)



## Summary

- \*Comprehensive, large-scale, genetic screens were conducted to identify candidate tumor maintenance genes in multiple inducible tumor models (AVEO's inducible Breast HER2, Breast Kras, and Lung Kras Tumor Models). A large fraction of tumors carried retroviral insertions targeting the endogenous *Fgfr-2* locus, in two integration hotspots clustered either near the promoter or in the exon encoding the carboxyl end of the Fgfr-2 kinase domain. The retroviral insertions are analogous to *FGFR2* mutations in human cancer cell lines that lead to protein truncation and activation (Breast Cancer Res 2000, 311-322). Moloney-driven Tumors with insertions in the *Fgfr2* locus have elevated mFgfr2 ( $\alpha$  isoform) mRNA expression relative to expression in either Moloney-driven tumors without insertions in the same locus or in control tumors expressing the original initiating oncogene (ON Doxy tumors).
- \*Introduction of defined cDNAs into inducible tumor background (Directed Complementation or DC) allows for creation of target-dependent tumor models and enables comparison of different genetic variants or mutations. Using this approach, we created FGFR2-directed complemented tumor models, validating each one as a tumor maintenance candidate target.
- \*FGFR2 $\alpha$  DC Tumor Models could be successfully propagated *in vivo* and retained expression of the introduced transgene. One such model, expressing the truncated form of FGFR2 $\alpha$  IIb, was tested with PD173074, a small-molecule RTK inhibitor known to inhibit FGFR (The EMBO Journal 1998, 5896-5904). Treatment with the compound inhibited tumor growth *in vivo*.
- \*Similarly, cell lines established from FGFR2 $\alpha$  DC Tumor Models were also sensitive to PD173074 treatment, as measured by cell proliferation in culture.
- \*Together both *in vivo* and *in vitro* models provide ideal preclinical settings for the development of targeted anti-FGFR2 therapies, as well as a tool for dissecting their accompanying molecular correlates of response.