

# Switching Oncogene Dependence in Lung Tumors *in vivo*

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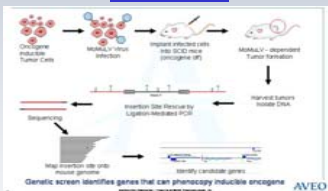
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## ABSTRACT

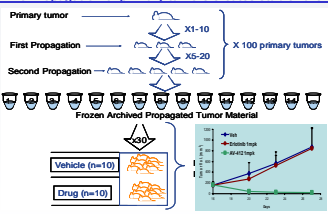
Lung cancer is one of the most prevalent and deadly diseases in the world, with more than 213,000 new cases diagnosed each year in the US and a five year survival rate of 15%. Activating mutations in a number of key signaling genes including KRAS, EGFR, and HER2 have been identified. These mutations are mutually exclusive, indicating that they are playing a redundant role during tumorigenesis. We have used the ES cell chimera based technology to generate inducible mouse models driven by these three oncogenes (LK, LE, LH). When we compared five key signaling pathways activated in these tumor models, we found that HER2 and EGFR driven tumors utilize the same pathways whereas KRAS driven tumors signal through a distinct set of pathways. These data suggest that the function of KRAS and HER2/EGFR are different. We set out to test this hypothesis by replacing HER2 in Her2 driven inducible lung tumors with KRAS or EGFR. To accomplish that, we infected orthotopically propagated LH tumors with lentiviral vectors harboring KRAS or EGFR, and implanted the infected tumor cells into recipient mice that are kept off doxycycline to turn off the expression of HER2. In this setting, tumors will grow only when the gene expressed from the viral vector can functionally complement the loss of HER2. Surprisingly, both KRAS and EGFR are able to replace HER2 as the driving oncogene in the resultant tumors. The switching of oncogenes effectively changed their response to targeted therapeutics. This system also provides a tool for rapid generation of tumors driven by new mutations after they are discovered.

## Applications of Inducible Tumor Models

### Virus Insertional mutagenesis screen in solid tumors identifies novel cancer targets



### In vivo Propagation of primary tumors creates stable "archive"



## Features of Chimeric Inducible Tumor Models

**Expression of Luciferase allows early tumor detection and monitoring**

**Cassette approach combines speed and flexibility**

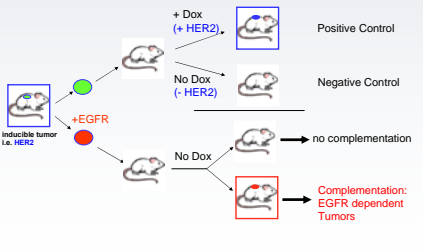
- 1) Knock out tumor suppressor gene
- 2) Add inducible gene for promoter (e.g. LoxP)
- 3) Add oncogene (e.g. KRAS, EGFR, HER2)
- 4) Add inducible oncogene (T and driven)

**Oncogene dependence, release and minimal residual disease**

**Established Models**

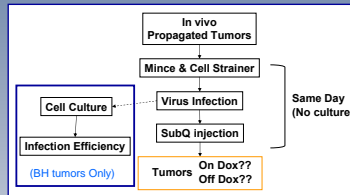
Model	Tissue	Oncogene
BH	Breast	Her2 <sup>WT</sup>
LK	Lung	KRAS <sup>G12S</sup>
LH	Lung	Her2 <sup>WT</sup>
LER	Lung	EGFR <sup>L858R</sup>
LEM	Lung	EGFR <sup>L858R/T790M</sup>
CB	Colon	$\beta$ -Catenin <sup>WT</sup>
CP	Ovary	p110 <sup>WT</sup>

## Directed Complementation

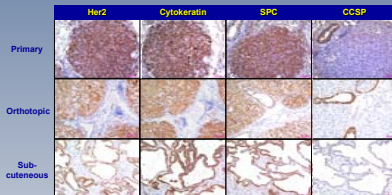


## RESULTS

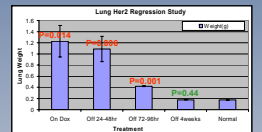
### Directed Complementation in Lung Tumors



### In vivo Propagation of primary lung Her2 tumors



### Doxycycline dependence of lung Her2 tumors



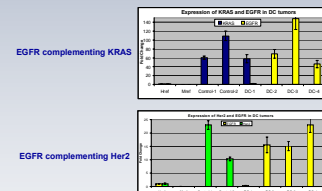
### The Complementation Matrix

Primary Tumor	KRAS	Her2	EGFR
LK	✓	✓	✓
LH	✓	✓	✓

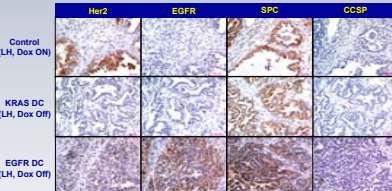
### Future Experiments: Drug response in DC tumors

Primary Tumor	KRAS	Her2	EGFR
LK	Trastuzumab	Trastuzumab	Erlotinib
LH	Trastuzumab	Trastuzumab	Trastuzumab

### Expression Analysis of DC tumors by qRT-PCR



### Immunohistochemical Analysis of DC tumors



## SUMMARY

1. Lung tumors with tumor suppressor knock out and inducible oncogene over expression can be propagated in vivo without losing tissue specificity.
2. The inducible feature of our lung models makes it possible to switch off the tumor driving oncogene and assess the ability of novel targets to complement for it.
3. qRT-PCR and immunohistochemical analysis demonstrated that most of the DC tumors express the new oncogene.
4. Surprisingly, KRAS and the RTKs (Her2 and EGFR) can complement one another. We are investigating whether the signaling pathways are also rewired in these DC tumors.