



***A NATIONAL LABORATORY TO VALIDATE CANCER
TARGETS OF CLINICAL INTEREST***

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Why?

CANCER GENOMES

- Deep sequencing of human cancer genomes has revealed that solid tumors contain dozens of mutations that affect multiple signaling pathways.

Thus, to induce significant and durable anti-tumor responses is going to be necessary to block several signaling pathways

TARGETED THERAPIES

- The development of selective inhibitors of driver oncogenes such as Erlotinib for EGFR⁺ lung cancer, Gleevec for BcrAbl⁺ CML or Vemurafenib for B-Raf⁺ melanoma, has provided significant clinical benefit (albeit limited increased overall survival).

In most cases, these targeted therapies fail primarily due to the appearance of resistance mechanisms caused by secondary mutations in the target or by activation of alternative pathways.

Why?

TUMOR HETEROGENEITY

- It is becoming evident that many advanced tumors are a heterogeneous mix of “cancer clones” that only share a limited number of common (trunk) mutations

Thus, it is essential that we identify key targets that can block those “trunk” pathways common to all cells present in the tumor

What to do?

- These facts are telling us, **in no uncertain terms**, that if we want to make significant inroads in the treatment of solid tumors, we have to treat patients with **drug combinations** that can block, **at once**, as many mutated/altered signaling pathways as possible.
- **Drug combinations** cannot be defined on a random bases. Otherwise the number of combinations will be too large to be even experimentally tested.
- Moreover, they have to be designed keeping in mind that they should not affect, at least significantly, **normal homeostasis**.
- Molecular interrogation of tumors can provide important clues regarding the pathways that need to be inhibited. However, in most cases, such analysis is not sufficient to **predict** what **drug combinations** will be required to induce durable tumor regression.

(For instance, although we have known the Ras/MAPK pathway for over 20 years, there are no selective drugs or drug combinations to effectively treat K-RAS mutant cancers)

What to do?

- On the positive side, deep sequencing of human solid tumors has revealed a series of signaling pathways that appear altered/mutated in multiple tumors, such as:
 - Ras and MAP Kinase pathway
 - PI3Kinase/PTEN/Akt pathway
 - P53/Mdm pathway
 - Rb/Cdk cell cycle pathway
 - Notch pathway
 - Shh/Gli pathway
 - Wnt/Beta-Catenin pathway.....etc., etc.
- Thus, we know to certain extent **where to act**, but we do not know the precise combination of targets needed to block progression/maintenance of each tumor type.

Hence, the goal of this proposal is to establish an experimental platform to address this issue, at least for a limited number of tumor types with unmet medical needs

How to do it?

- Accepting that *in vitro* testing of human tumor cells has a limited value, definition of those combinations of targets whose selective inhibition may have a reasonable chance to work in the clinic, will require the use of preclinical animal models
- The most suitable systems are the Patient Derived Xenograft (PDX) and the Genetically Engineered Mouse (GEM) tumor models

How to do it?

PDX (and CDX) Models

Advantages:

Human origin,

Fairly representative of the actual tumor at least for a few passages

Disadvantages:

Tumor variability (same as the patient population).

Meaningful conclusions that could be extrapolated to the clinic require large cohorts of tumors.

Results depend on the availability of suitable inhibitors

How to do it?

GEM Models

Disadvantages:

Mouse origin.

Multiple initiating events: Tumors develop “too fast”. Thus, unlikely to accumulate as many mutations/alterations as human tumors.

Advantages:

Reproducibility

Systematic analysis of suitable targets/pathways.

Possibility to validate targets by genetic means (do not rely on available drugs)

Possibility to combine genetic with pharmacological validation

Due to logistic reasons (to be discussed by the FNLAC, if deemed appropriate), this proposal will focus exclusively on the use of the GEM models of human cancer

Proposal

To establish a National Laboratory to validate targeted therapies in experimental models of cancer with unmet medical needs.....

.....with the ultimate goal of guiding the design of future clinical trials

Proposal

Objectives

1. To complement ongoing efforts at the FNL's Center for Advanced Preclinical Research on translational research using GEM models
2. To use available GEM models of cancer representing a few relevant tumor types to validate targets of potential therapeutic value
3. To use this information to devise target combinations that can eradicate advanced tumors without significantly affecting normal homeostasis
4. To engage pharma companies to translate these results to a pharmacological scenario using high quality drugs that have entered or will enter clinical trials
5. To use this information to guide the design of future, more effective clinical trials, to ultimately create value for biomedical research at a national level

Proposal

Conceptual Criteria

First of all, this Program does not intend to carry out **basic research**. It will not compete with Government or Academia research labs

Instead, it will collect available information and adapt it to the goals of the Program

As an example, if a research group reports that a target plays a role in the development of a given tumor type, we will add this target to our existing portfolio, ideally in collaboration with the research group

We intend to primarily focus on targets that affect tumor cell proliferation/survival. However, if there are targets of sufficient interest that interfere with the tumor microenvironment, we will also validate them.

Proposal

Experimental Criteria

GEM models should faithfully reproduce the natural history of human cancer

We will focus on GEM models that model human tumors with unmet medical needs and limited 5-year survival

GEM model should be readily available or need minimal modification

GEM models should be amenable to “scaling up” tumor aggressiveness by adding additional driver mutations

Tumors should be detectable by non-invasive techniques

Mice should be treated following standard procedures as similar as possible to those used in clinical trials (co-clinical trials)

Proposal

Experimental Approach

The Experimental Approach will be based on the basic principles of classical bacterial and yeast genetics. That is, we will eliminate those genes encoding potential therapeutic targets to determine to what extent their expression/activity is required for tumor maintenance and tumor progression.

The proposed experimental approach follows three basic steps:

Step 1: Genetic validation of individual targets

Step 2: Genetic validation of combinations of targets

Step 3: Pharmacological validation using selective inhibitors

Proposal: Experimental Approach

Step 1: Genetic validation of individual targets

We will use GEMs in which we can temporally separate tumor development from target ablation. That is, targets will be ablated in tumor-bearing mice **not at the time of tumor development**.

Whenever possible, targets will be genetically **inactivated, not ablated**, to better mimic drug activity (for instance by using conditional knocked-in strains)

Targets will be ablated/inactivated **systemically** to determine potential **toxic effects**

Targets to be validated should be as **druggable** as possible.

Proposal: Experimental Approach

Step 2: Genetic validation of combinations of targets

We will generate compound GEMs in which we could ablate/inactivate as many targets as possible (4-5 targets would be a realistic goal) **at once** in tumor-bearing mice.

These complex compound strains will be generated with the help of the CRISPR editing technology either *in vivo* or (more likely) in ES cells derived from compound mice

We will evaluate the anti-tumor activity of these combinations of targets in tumors of increasing degree of aggressiveness generated by adding sequential driver mutations.

Proposal: Experimental Approach

Step 3: Pharmacological validation using selective inhibitors

We will use selective inhibitors for those targets validated genetically, ideally engaging the pharma industry in order to test their “best in class” compounds.

We will treat the GEM strains used for genetic validation studies with the corresponding inhibitors to compare pharmacological and genetic outcomes with the ultimate goal to evaluate the specificity and potency of each drug candidate as well as the overall anti-tumor effect of the drug combinations.

These studies will also reveal their (putative) undesirable off-target effects

Whenever possible, the drug combinations defined in these studies should be tested in PDX models, so we can compare outcomes in human tumors versus those of GEM models.

Proposal: Experimental Approach

Step 3: Pharmacological validation using selective inhibitors (cont.)

In those cases in which no suitable inhibitors/drugs might be available for all the targets included in the combination, we will combine genetic targeting with drug treatments

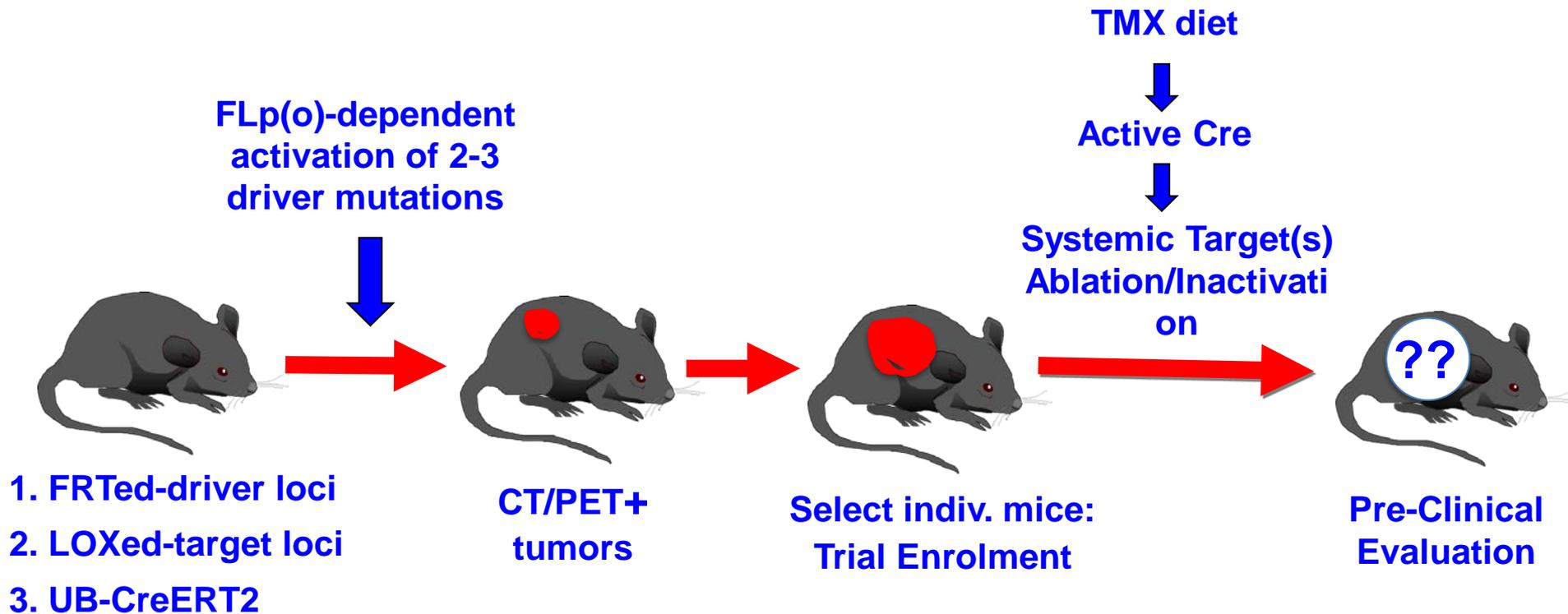
In all the steps of this lengthily process, we will try to maintain the experimental conditions as close as possible to the clinical scenario.

Those drugs/targets that may behave significantly different in mice and human, will not be further pursued (e.g. MEK inhibitors, gamma secretase inh., other....).

Proposal

Experimental Approach: Genetic validation

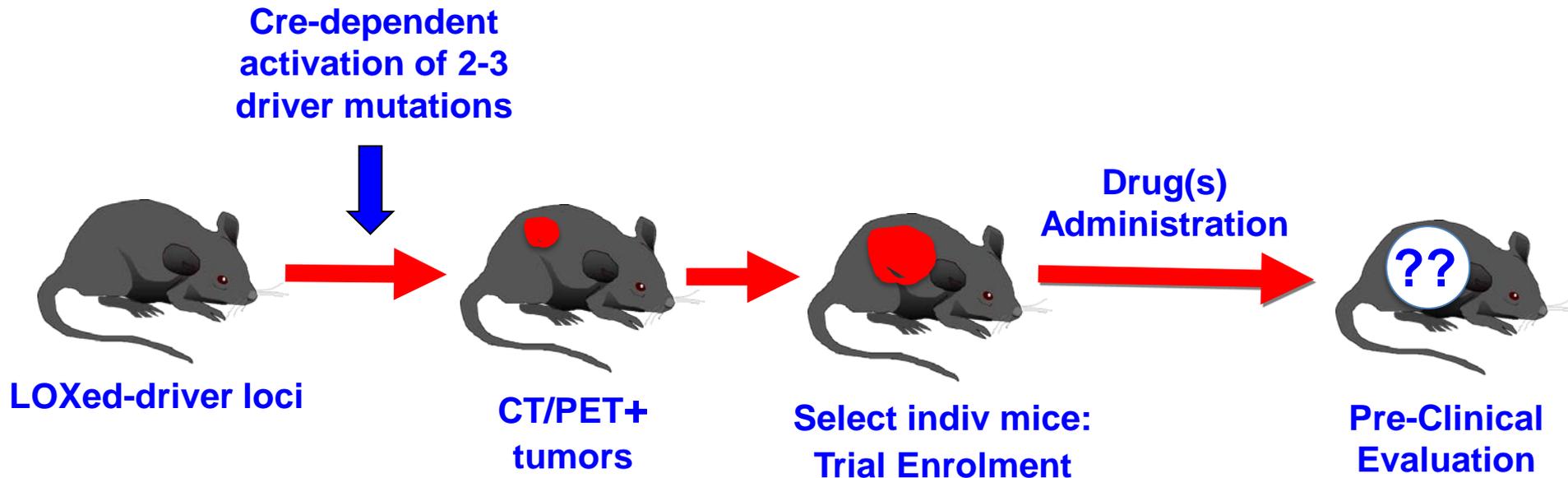
Schematic representation of the basic experimental framework for genetic validation of selected targets



Proposal

Experimental Approach: Pharmacological validation

Schematic representation of the basic experimental framework for pharmacological validation of selected targets



Although we prefer to use Cre-dependent alleles, if necessary we can use the FLpase-dependent alleles

Proposal

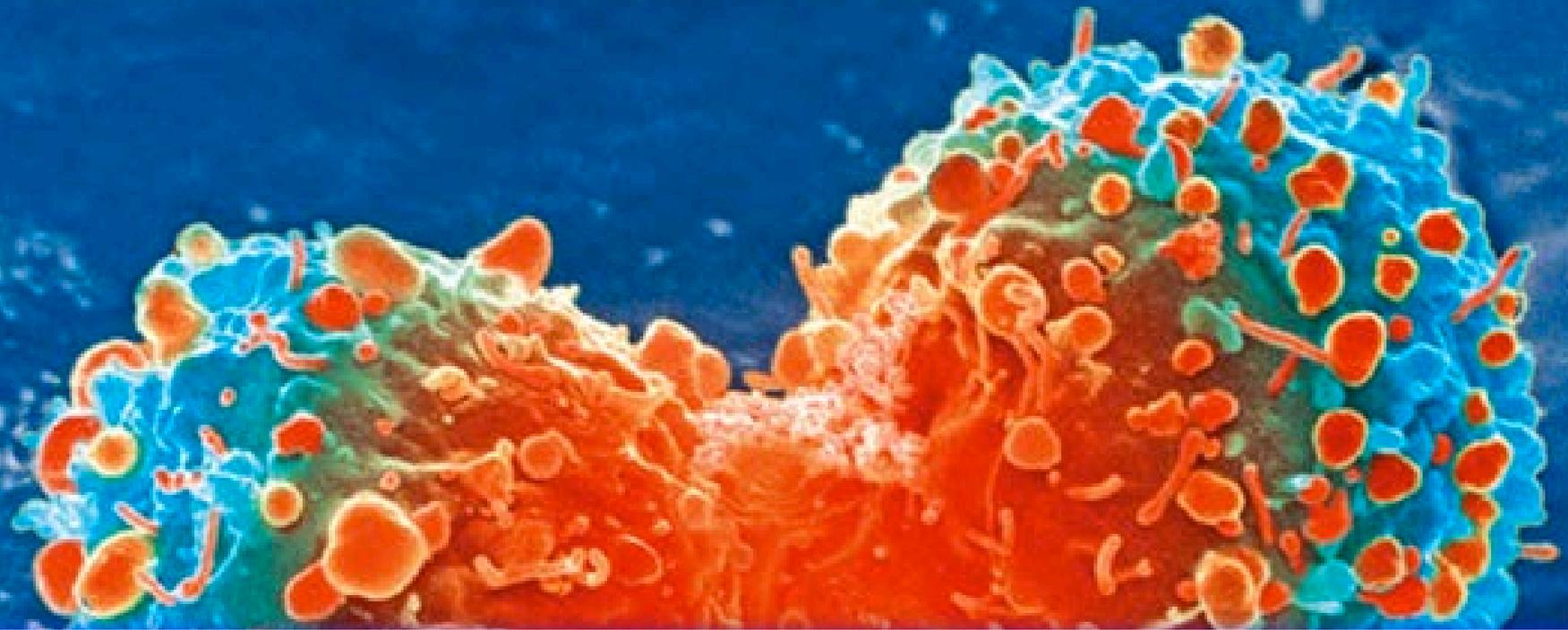
Tumor Types to be studied

To be decided after consultation with experts, based on the criteria mentioned above

An initial suggestion

- Pancreatic ductal adenocarcinoma
- Triple negative breast tumors
- Glioblastoma
- K-RAS mutant lung adenocarcinoma
- Colorectal carcinoma





***SELECTED EXAMPLES OF AVAILABLE INFORMATION OBTAINED
BY EXPERIMENTAL APPROACHES SIMILAR TO THOSE OUTLINED
IN THIS PROPOSAL***



K-Ras driven GEM tumor models

Two of the GEM tumor models proposed to be used in this **Proposal** are those modeling:

- ➔ **K-Ras driven lung adenocarcinoma**
- ➔ **Pancreatic ductal adenocarcinoma** (also driven by K-RAS mutations)

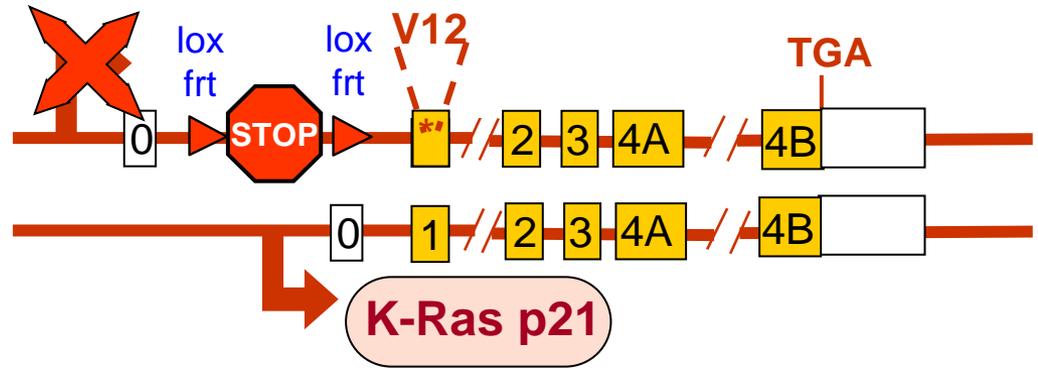
Ras Mutations in Human Tumors (by tissue, COSMOS, 2005)

Tissue/Organ	K-Ras	N-Ras	H-Ras
TOTAL: Samples tested	46,700	18,500	13,900
TOTAL: Percentage	22%	9%	4%
Pancreatic ductal adenocarcinoma	90%	2%	<1%
Intestine/Colon carcinoma	45%	3%	<1%
Lung adenocarcinoma	25%	11%	1%
Endometrium	15%	<1%	1%
All Others	1-8%	1-20%	1-15%

GEM models: K-RAS mutant lung adenocarcinoma

1. K-Ras⁺/LSLG12V

2. K-Ras⁺/FSFG12V



Lung Adenocarcinoma:

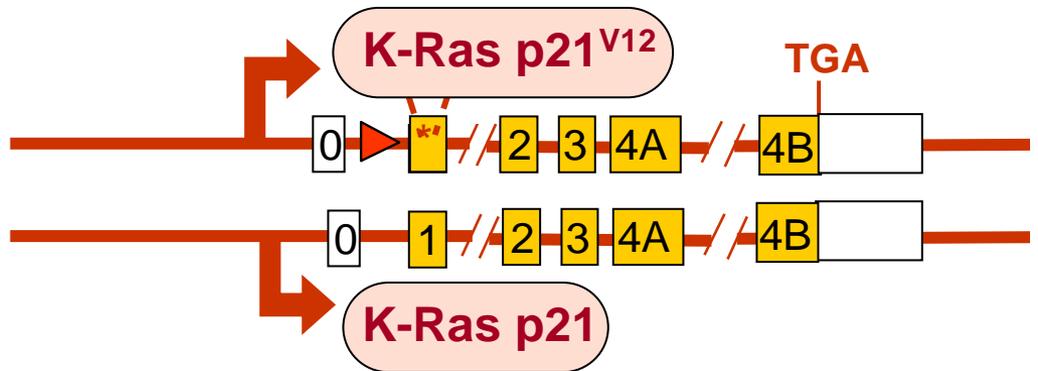
Traqueal Infection with
Adeno-Cre or Adeno-Flp(o)



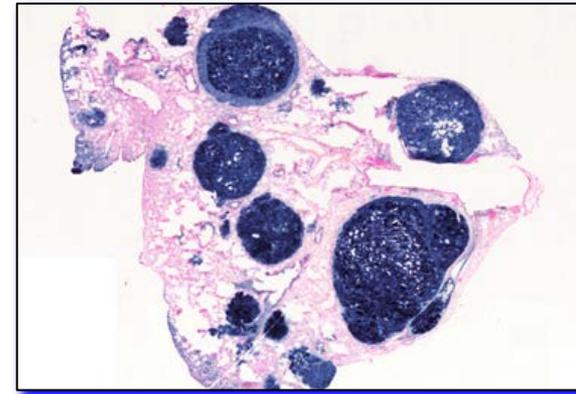
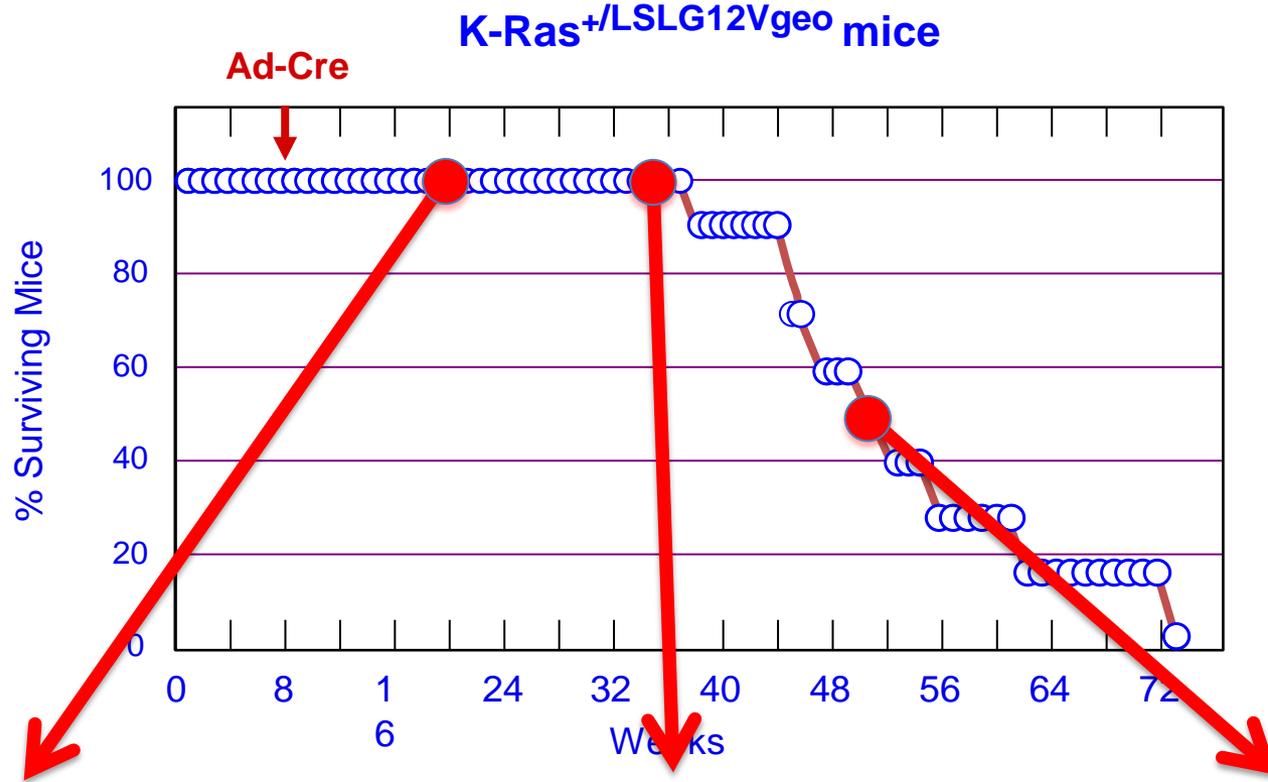
Cre or Flp(o)
recombinase
activity

K-Ras⁺/G12V

expressed in
lung tissue



GEM models: K-RAS mutant lung adenocarcinoma



GEM models: K-RAS mutant lung adenocarcinoma

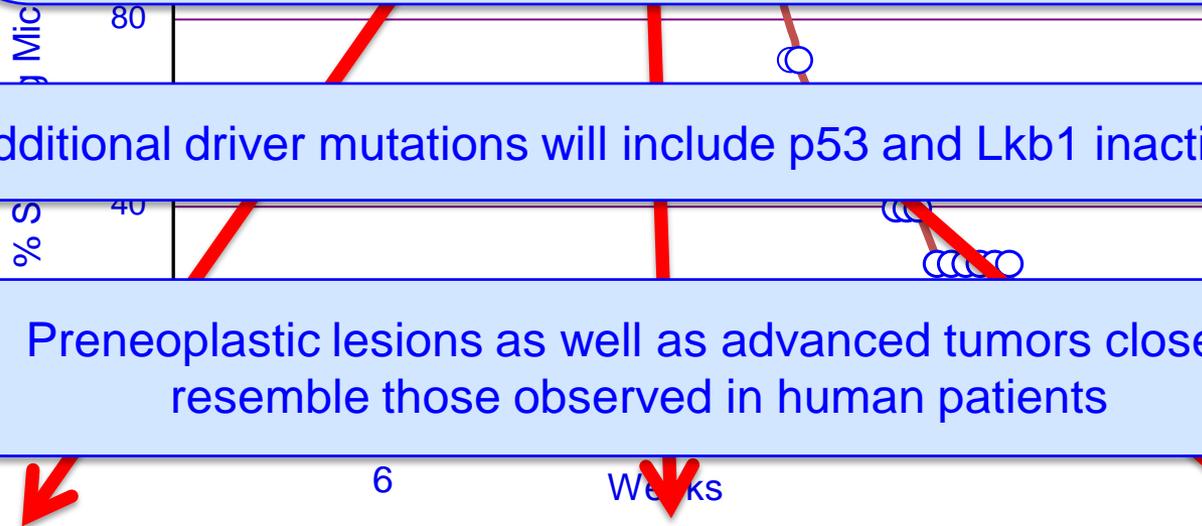
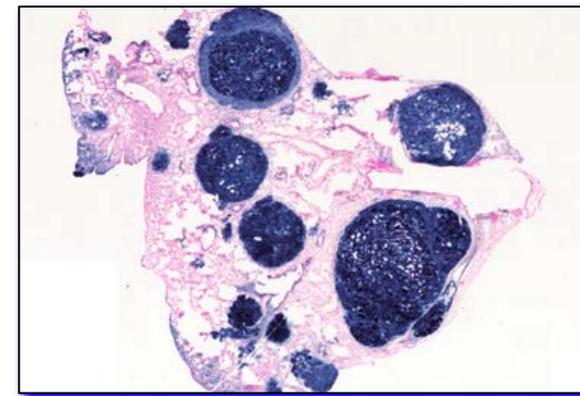
K-Ras^{+/LSLG12V}geo mice

Ad-Cre

Tumor number and latency can be controlled by the titer of infectious adeno-Cre [or adeno-FLp(o)] particles

Additional driver mutations will include p53 and Lkb1 inactivation

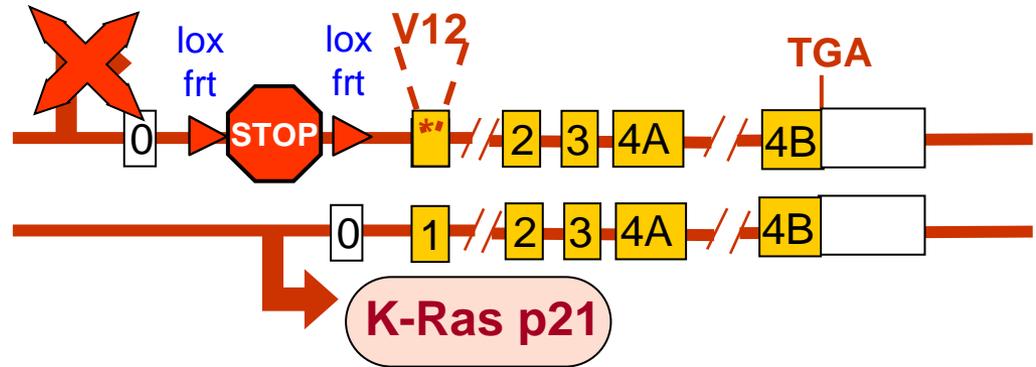
Preneoplastic lesions as well as advanced tumors closely resemble those observed in human patients



GEM models: Pancreatic Ductal Adenocarcinoma

1. **K-Ras⁺/LSLG12V**

2. **K-Ras⁺/FSFG12V**



Pancreatic Ductal Adenocarcinoma

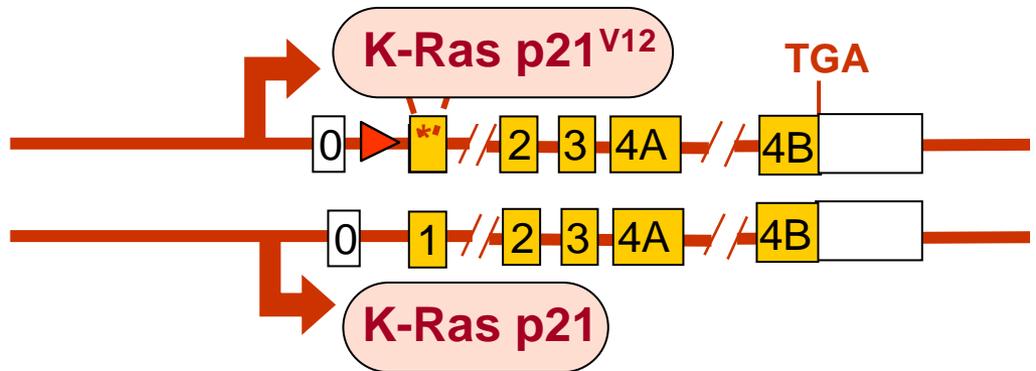
Cross with Tg mice that express Cre or FLp(o) under the control of an inducible Elastase promoter



Cre or Flp(o)
recombinase
activity

K-Ras⁺/G12V

expressed in
pancreatic acinar cells



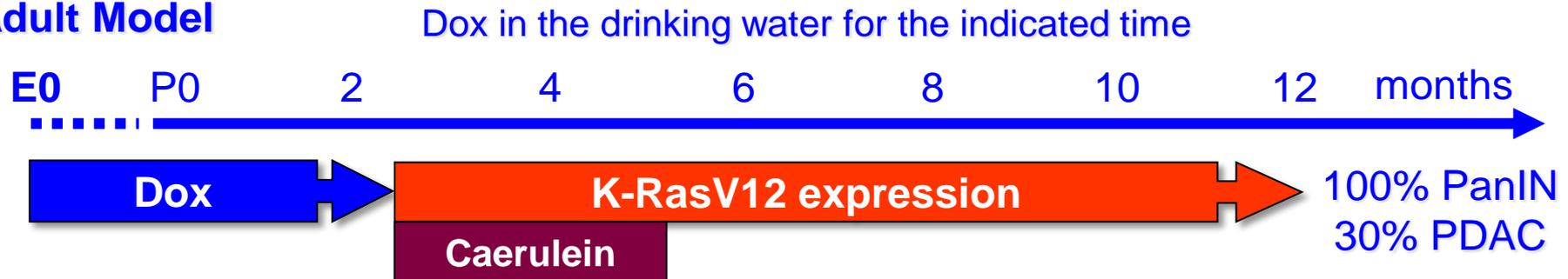
GEM models: Pancreatic Ductal Adenocarcinoma

The resulting strains can be used to generate two different models depending on the time of K-Ras^{G12V} expression

Embryonic Model



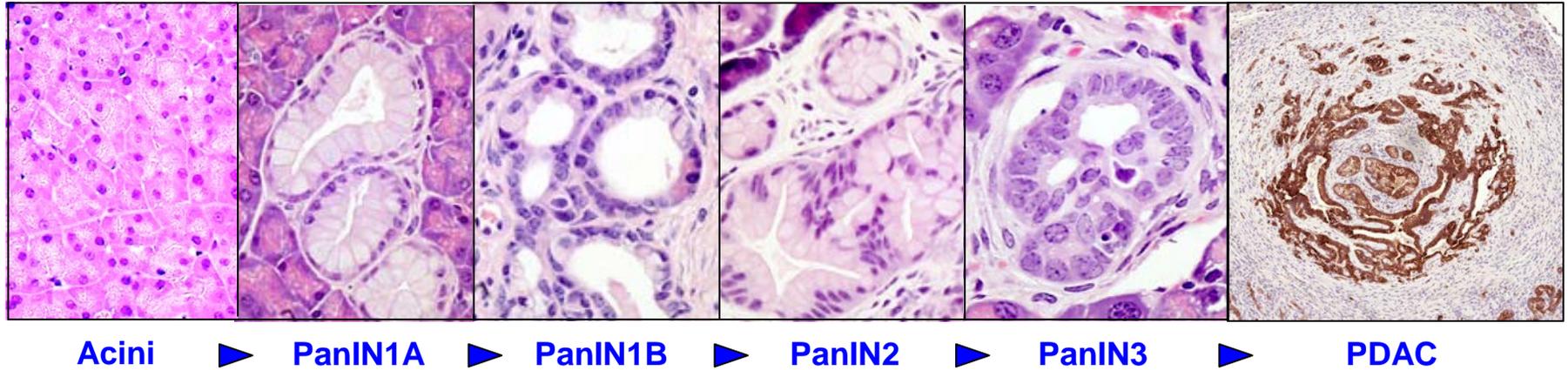
Adult Model



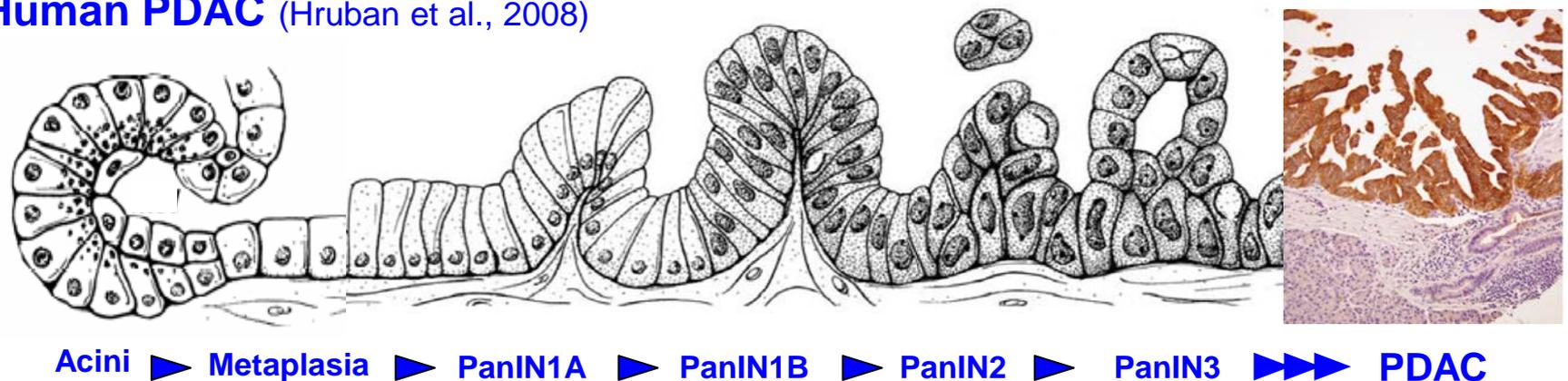
Additional driver mutations including inactivation of p53, p16INK4a or TGFR II/Smad 4, increase PDAC incidence to 100% and decrease tumor latency to 3-4 months

GEM models: Pancreatic Ductal Adenocarcinoma

Both models faithfully reproduce the natural history of PDAC development including the generation of a large desmoplastic component



Human PDAC (Hruban et al., 2008)



Proposal: Experimental Approach

**Selected examples of previous results obtained by the
Experimental Approach outlined in this Proposal**

- #1. Genetic validation of individual targets**
- #2. Pharmacological validation of individual targets**
- #3. Genetic validation of combinations of targets**



c-Raf, but Not B-Raf, Is Essential for Development of *K-Ras* Oncogene-Driven Non-Small Cell Lung Carcinoma

Rafael B. Blasco,^{1,6} Sarah Francoz,^{1,6} David Santamaría,¹ Marta Cañamero,² Pierre Dubus,³ Jean Charron,⁴ Manuela Baccarini,⁵ and Mariano Barbacid^{1,*}

Shortcomings

Ablation of c-Raf prevented tumor development, but we did not evaluate the effect in tumor bearing mice

Since then, we

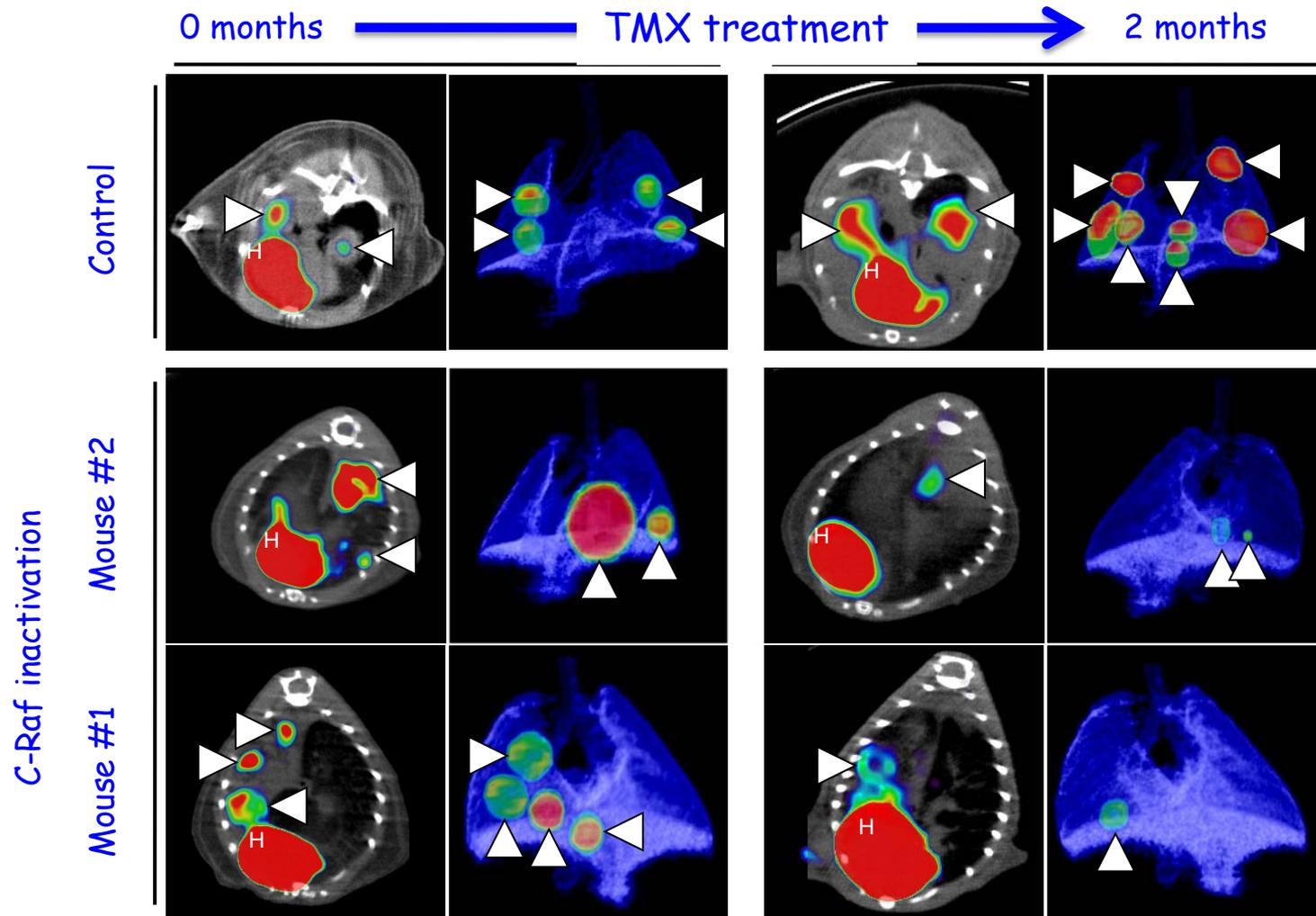
Have validated c-Raf inhibition in tumor bearing mice

Have validated c-Raf inhibition in aggressive p53 *null* tumors

Have evaluated the effect of inhibiting related kinases (mainly B-Raf)

Genetic validation of c-Raf

Expression of a kinase dead isoform of c-Raf (c-Raf^{D468A}) limits progression of K-Ras (only) driven lung tumors



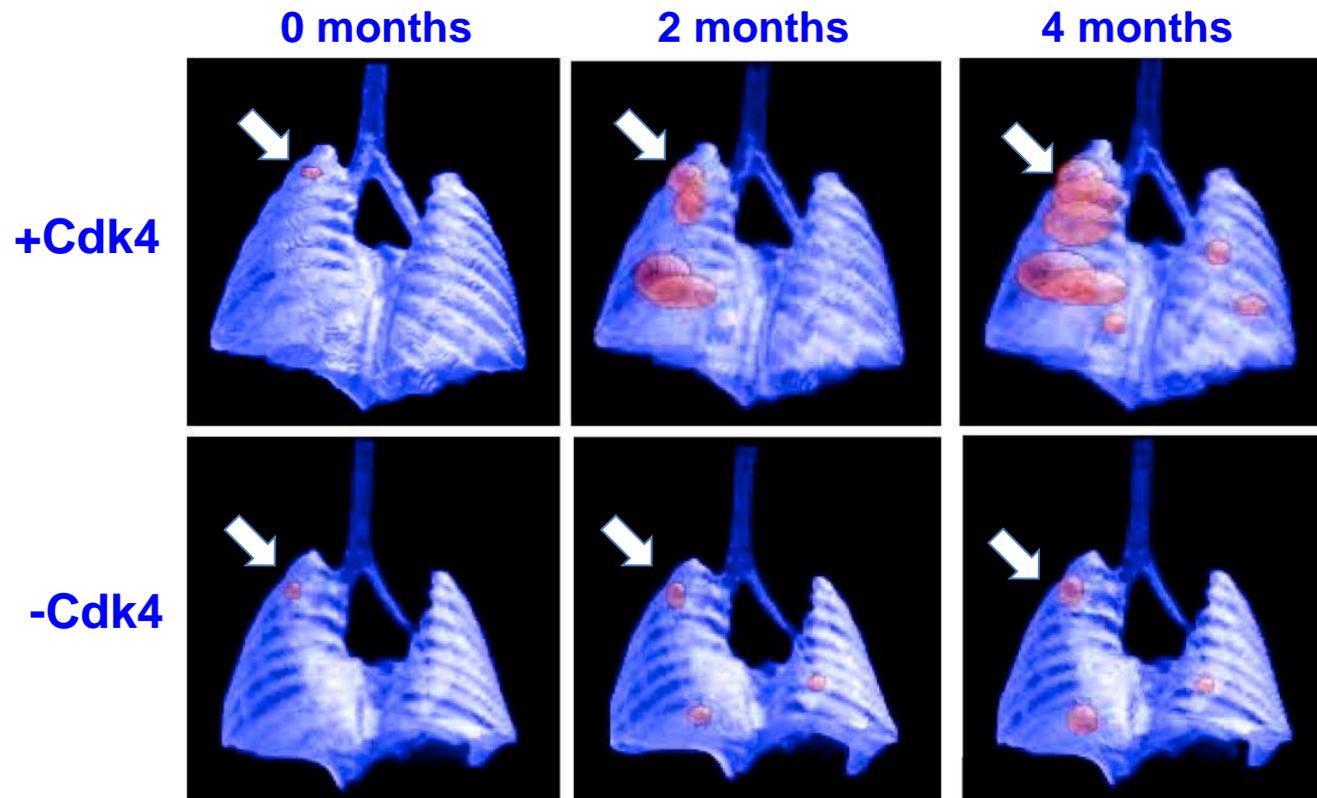
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Genetic validation of Cdk4

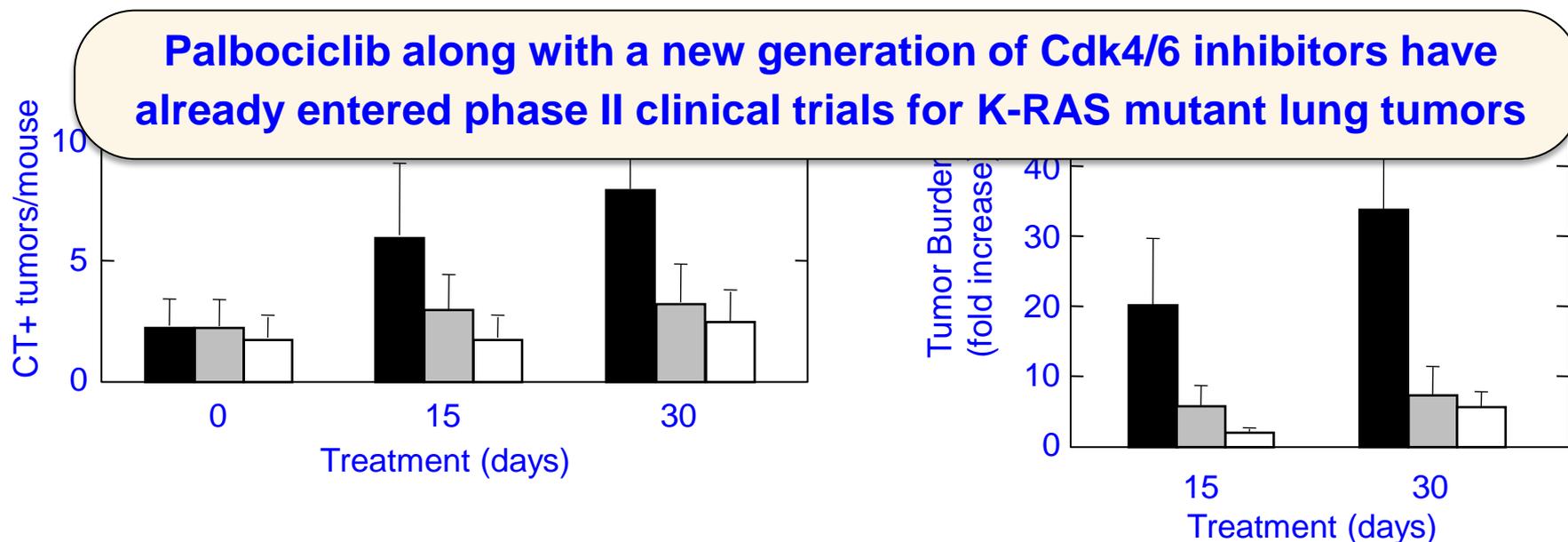
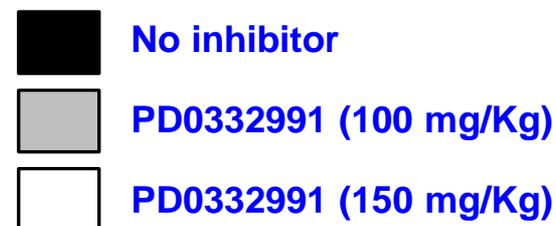
Cdk4 was first genetically validated by eliminating its expression in CT+ tumors



Pharmacological validation of Cdk4

These results led to the pharmacological validation of Palbociclib, a selective Cdk4/6 inhibitor

Palbociclib inhibits progression of K-Ras (ONLY) driven Lung Adenocarcinomas to a similar extent as target ablation



Target Combination: Lung Tumors

Additional targets shown to have potential therapeutic activity against K-Ras mutant lung tumors

<u>Kinases</u>	<u>Other</u>	<u>Non-druggable targets</u>	<u>Metabolic targets</u>
c-Raf	Notch	Myc	Glycine
Cdk4	Bcl-XL	GATA2	Decarboxylase
MEK*¶	NF-kB	Hmga2	Pyruvate
ERK*		Ttf1	Carboxylase
PI3Kinase		RalA/B	
Ddr1		Rac1b	
Ror1			
TKB1			
PLK1			
IKK-beta			
Fak			

* Ablation of Mek1/2 or Erk1/2 is lethal for mice.

¶ Interestingly Mek inhibitors are very effective against K-Ras driven tumors in mice

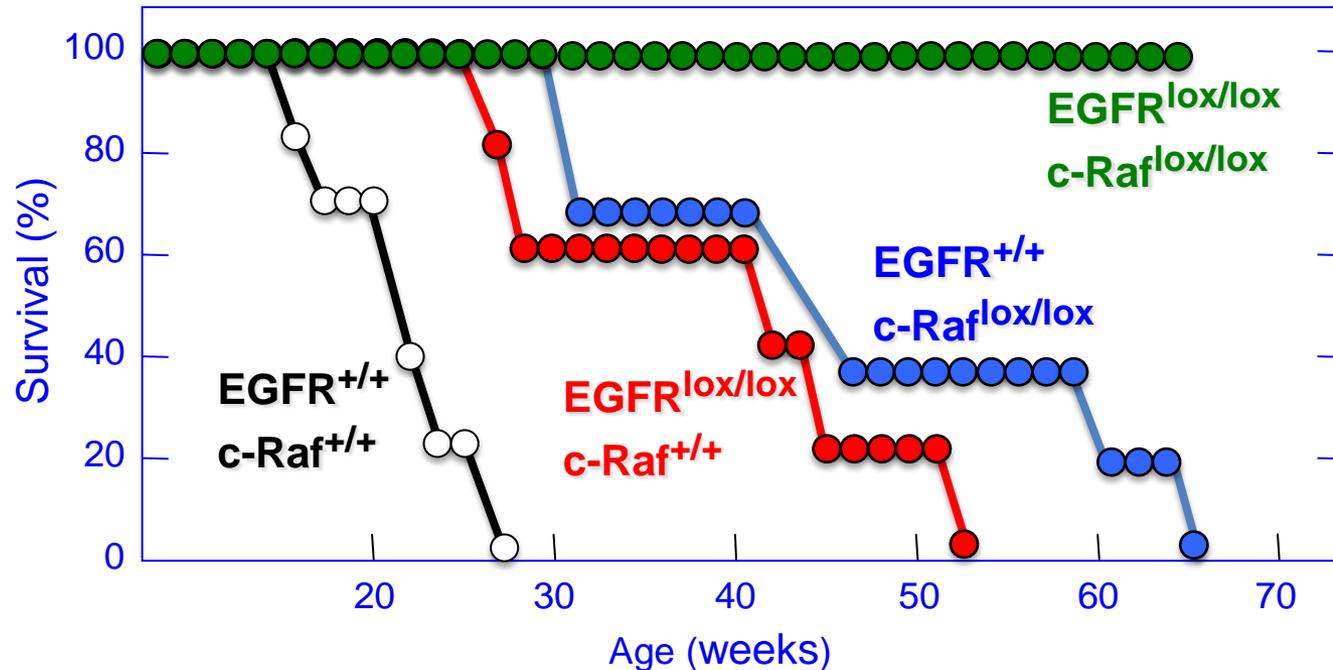
Proposal: Experimental Approach

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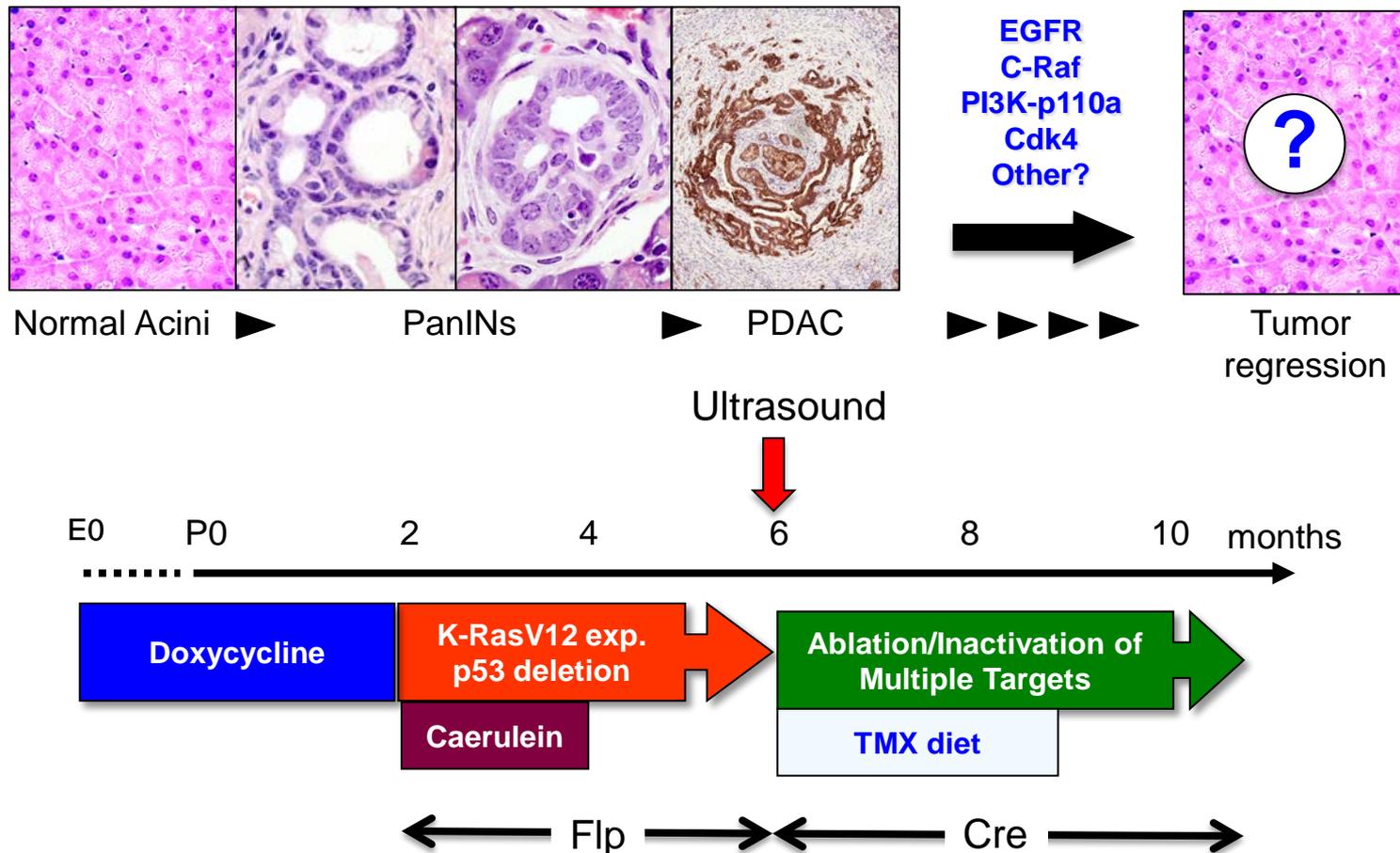
Target Combination: Pancreatic Tumors

- Ablation of either EGF Receptors or c-Raf in a K-Ras (only) driven GEM model of PDAC completely prevents tumor development (including PanIN lesions)
- However, ablation of EGF Receptors or c-Raf only results in tumor delay in a GEM model driven by K-Ras and p53 mutations
- Combined ablation of EGF Receptors and c-Raf **completely prevented** development of PDAC tumors (and PanIN lesions) without causing detectable side effects



Genetic Validation of Multiple Targets

Conditional alleles of PI3K and Cdk4 are being engineered in ES cells of $K-Ras^{+/FSFG12V}; p53^{F/F}; Elaf-tTA; Tet-O-Flp(o); EGFR^{KD/KD}; c-Raf^{KD/KD}; Ub-CreERT2$ mice



Establishing effective drug combinations

- Effective testing of drug combinations in GEM models based on solid genetic data is still in the early stages (most drugs are now being tested in PDX models).
- We are currently testing combinations of Cdk4/6 and c-Raf inhibitors in K-Ras driven/p53 *null* lung tumors as well as in K-Ras driven/p53 *null*/Lkb1 *null* lung tumors in collaboration with industry
- We need to further **engage industry** to provide “**best in class**” inhibitors once we have solid genetic data illustrating the anti-tumor effect of defined target combinations
- Industry should also be engaged in carrying out the necessary pharmacokinetic, pharmacodynamic and toxicology studies (ADME) needed to optimize the results obtained with drug combinations

Proposal

Resources

4 to 6 FTEs per tumor model taking into account the availability of certain core support (animal care takers, imaging support)

Considering the existing infrastructures at the FNLs, I do not think that it will be necessary a significant investment

Yet, I will submit a detailed financial plan if this proposal receives an initial green light

Proposal

To establish a National Laboratory to validate targeted therapies in experimental models of cancer with unmet medical needs.....

.....with the ultimate goal of guiding the design of future clinical trials