ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

PERSONALISED MEDICINE SYMPOSIUM

ESMO SYMPOSIUM ON SIGNALLING PATHWAYS IN CANCER

Targeting the MAPK pathway: From RAS to MEK

13 – 14 March 2015, Barcelona, Spain

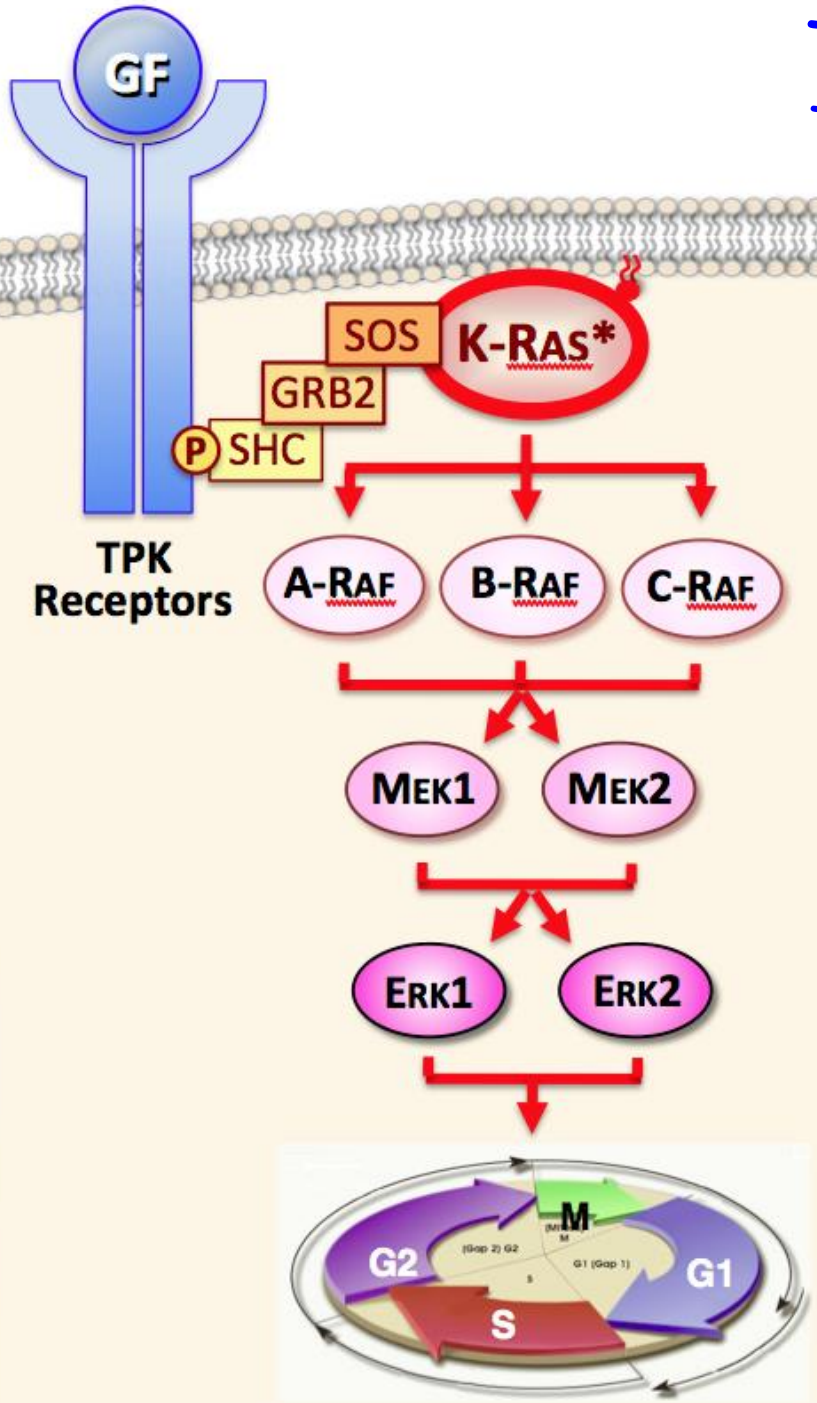
cnio Centro Nacional de Investigaciones Oncológicas

TARGETING THE MAPK PATHWAY IN K-RAS DRIVEN LUNG TUMORS: UNVEILING ITS COMPLEXITIES WITH GENETICALLY ENGINEERED MOUSE MODELS

MARIANO BARBACID
CENTRO NACIONAL DE INVESTIGACIONES ONCOLÓGICAS (CNIO)

Targeting K-Ras driven lung tumors:

The Ras signaling cascade

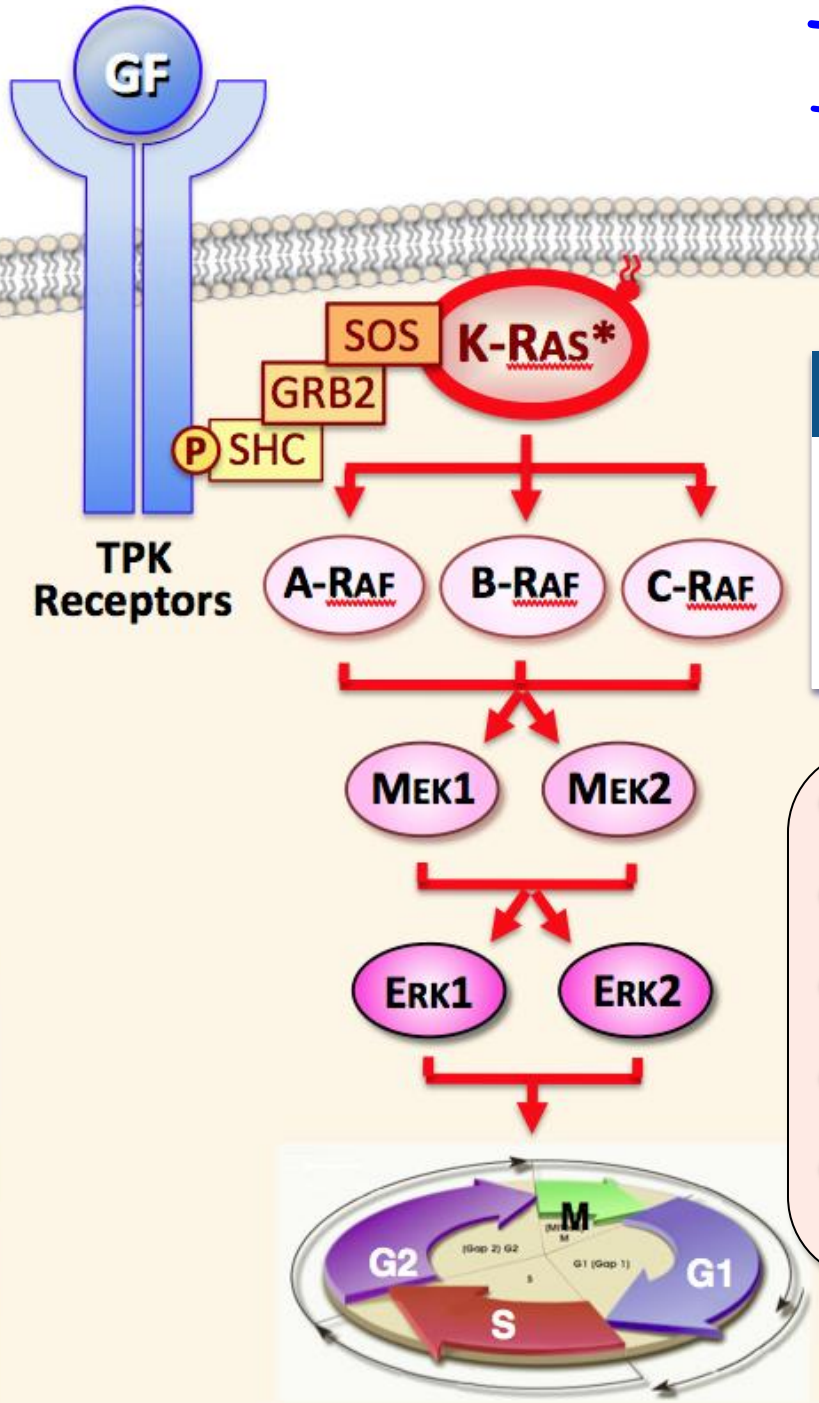


We have known this pathway for over two decades.....

....but we still do not know how to target it

Targeting K-Ras driven lung tumors:

The Ras signaling cascade



Cell PRESS

Cancer Cell

Article

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

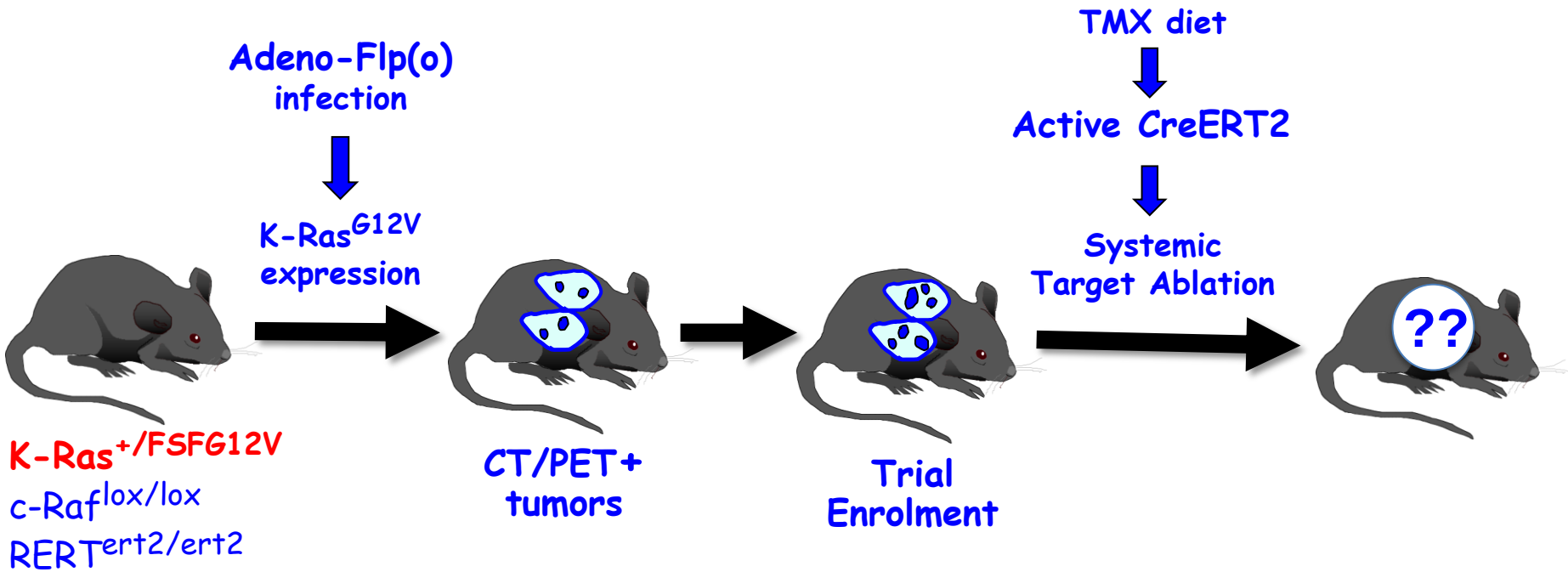
Rafael B. Blasco,[1,6] Sarah Francoz,[1,6] David Santamaría,[1] Marta Cañamero,[2] Pierre Dubus,[3] Jean Charron,[4] Manuela Baccarini,[5] and Mariano Barbacid[1,*]

- Mek and Erk kinases are fully compensatory
- Ablation of Mek and Erk kinases is highly toxic
- Raf kinases do NOT compensate each other
- ONLY c-Raf is essential for tumor development
- Ablation of c-Raf has limited but acceptable toxicity

Genetic validation of c-Raf

Next step was to validate the therapeutic effect of ablating c-Raf in existing CT+ tumors

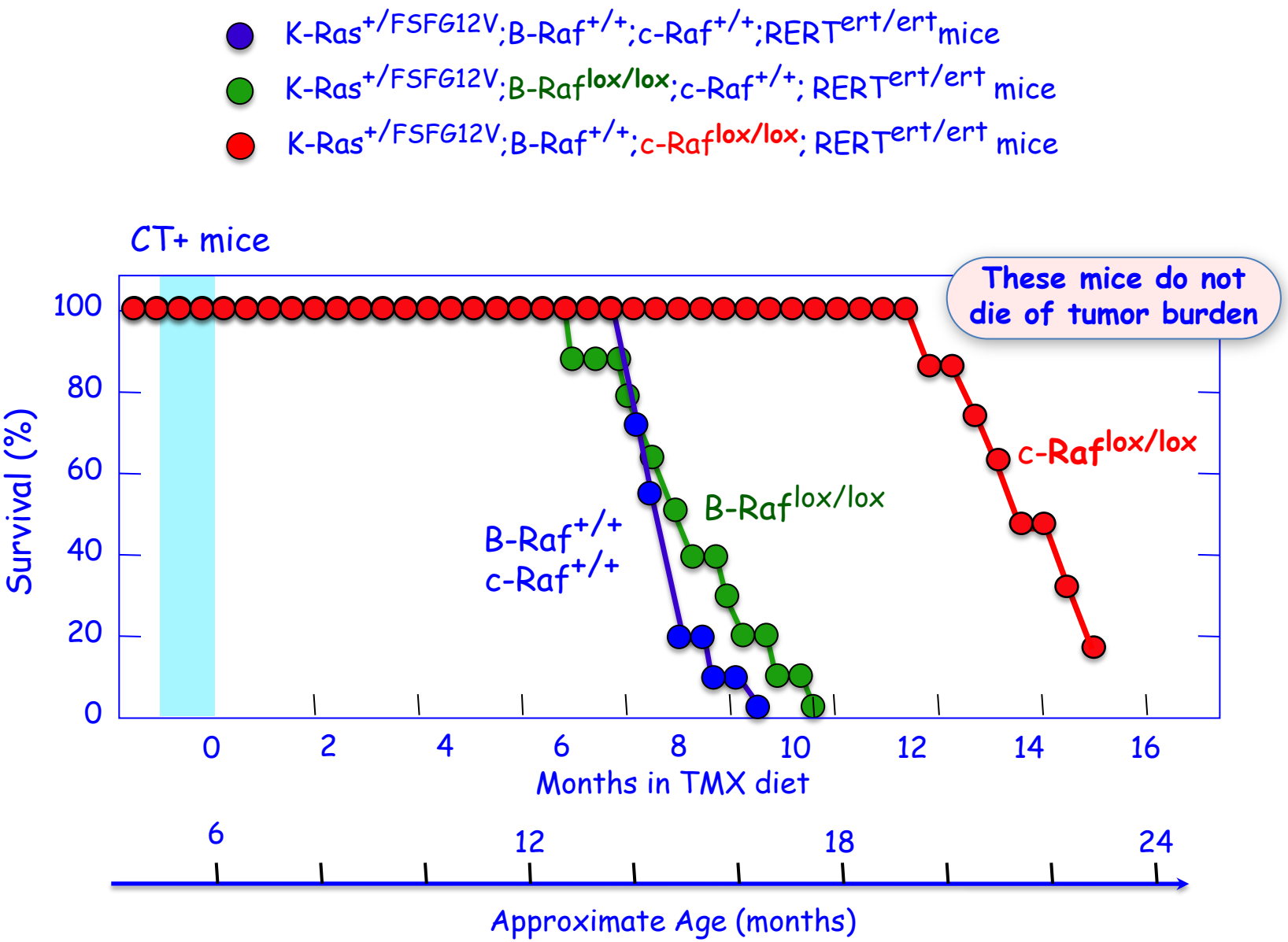
Therapeutic Model: K-Ras$^{+/FSFG12V}$;c-Raf$^{lox/lox}$;RERT$^{ert2/ert2}$ mice



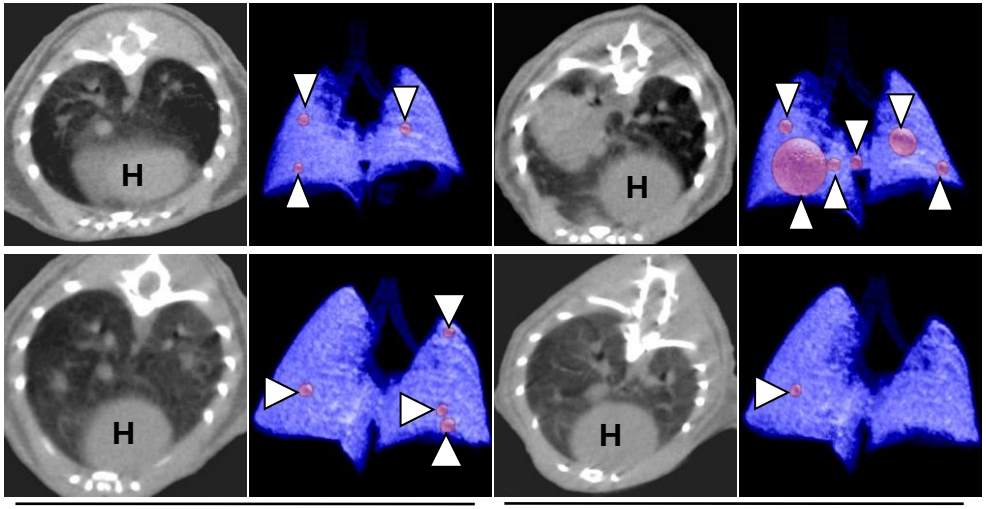
Tumor development: **Flp(o)**-dependent

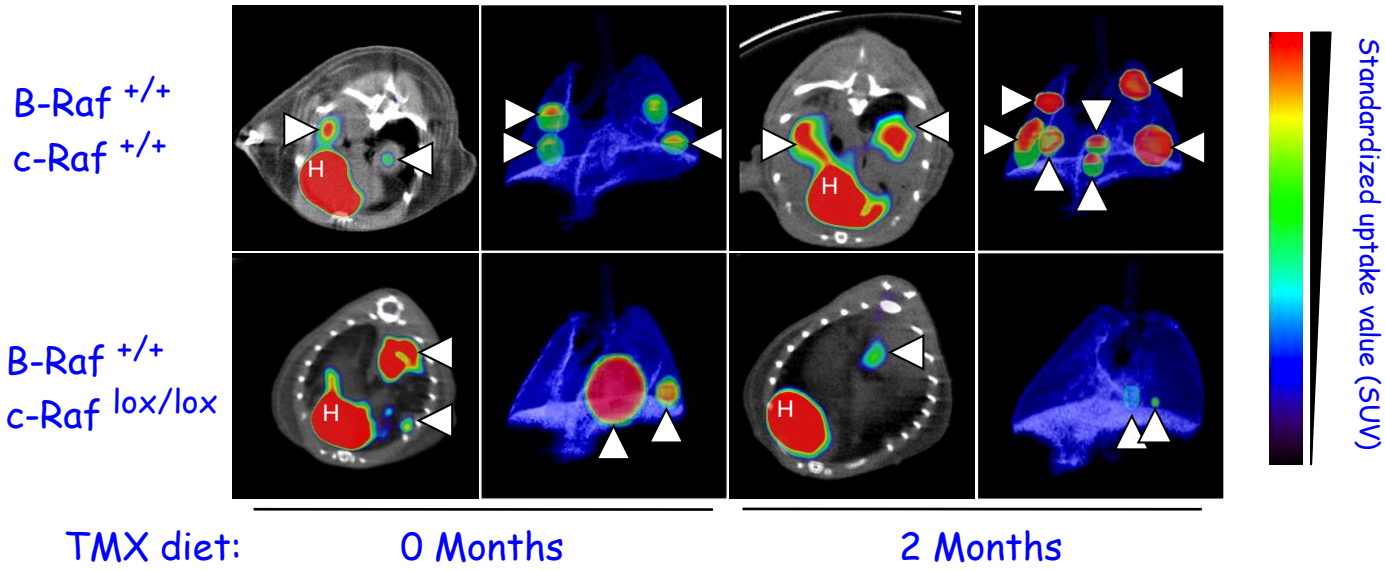
Target Ablation: **Cre**-dependent

Genetic validation of c-Raf



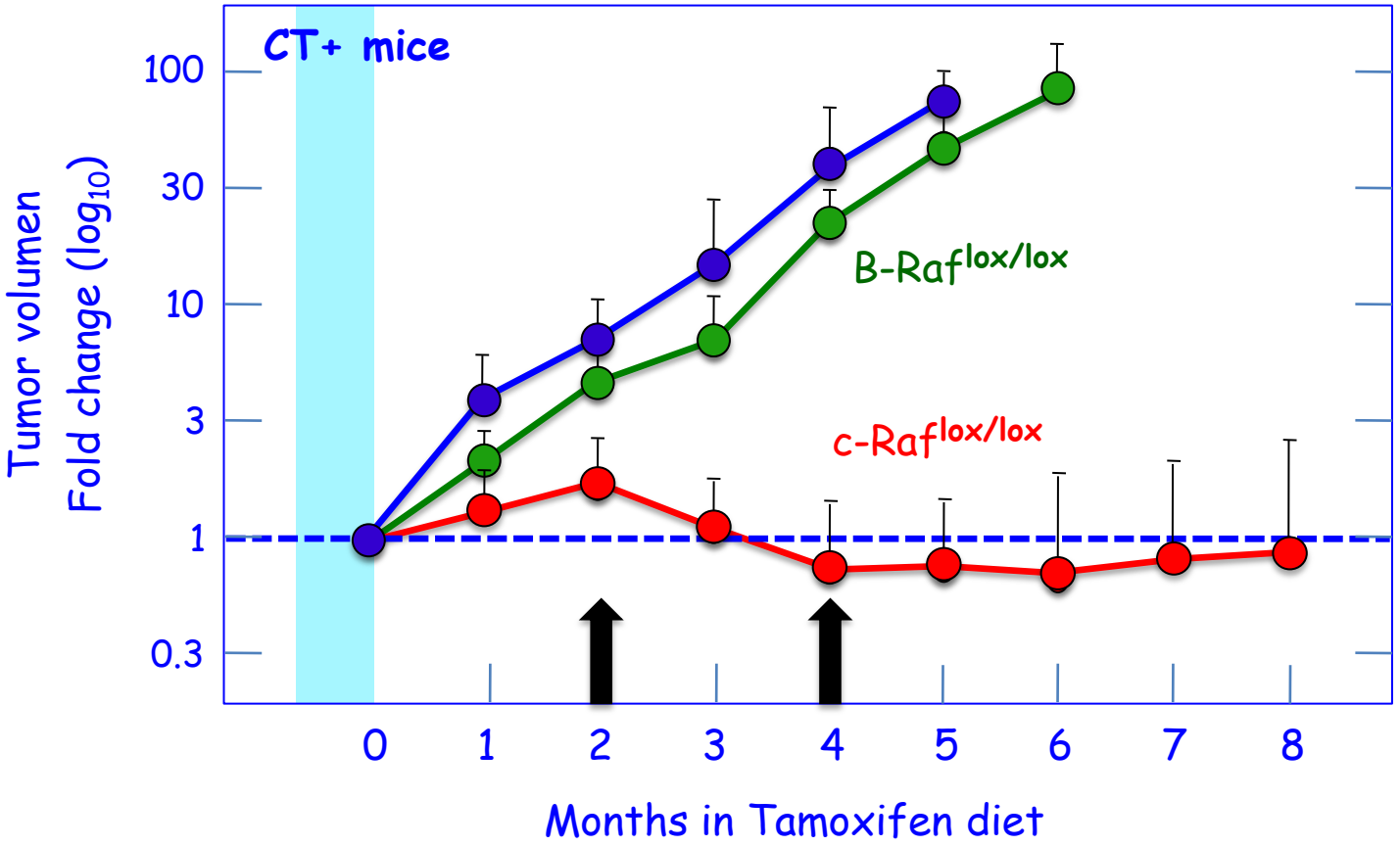
Genetic validation of c-Raf





Genetic validation of c-Raf

- K-Ras$^{+/FSFG12V}$;B-Raf$^{+/+}$;c-Raf$^{+/+}$;RERT$^{ert/ert}$ mice
- K-Ras$^{+/FSFG12V}$;B-Raf$^{lox/lox}$;c-Raf$^{+/+}$; RERT$^{ert/ert}$ mice
- K-Ras$^{+/FSFG12V}$;B-Raf$^{+/+}$;c-Raf$^{lox/lox}$; RERT$^{ert/ert}$ mice



Genetic validation of c-Raf

Tumor development at **2 months** in TMX diet

	c-Raf$^{+/+}$ (n=25)		c-Raf$^{lox/lox}$ (n=22)	
	Tumors	Tumor Vol.	Tumors	Tumor Vol.
Δ Tumor development	46	Δ3.7±0.5 fold	41	Δ1.2±0.1 fold
De novo CT+ tumors	49	3.0±1.4 mm3	15	0.5±0.1 mm3
Partial regression (>20%)	0		7 (17%)	
Complete regression (CT-)	0		7 (17%)	

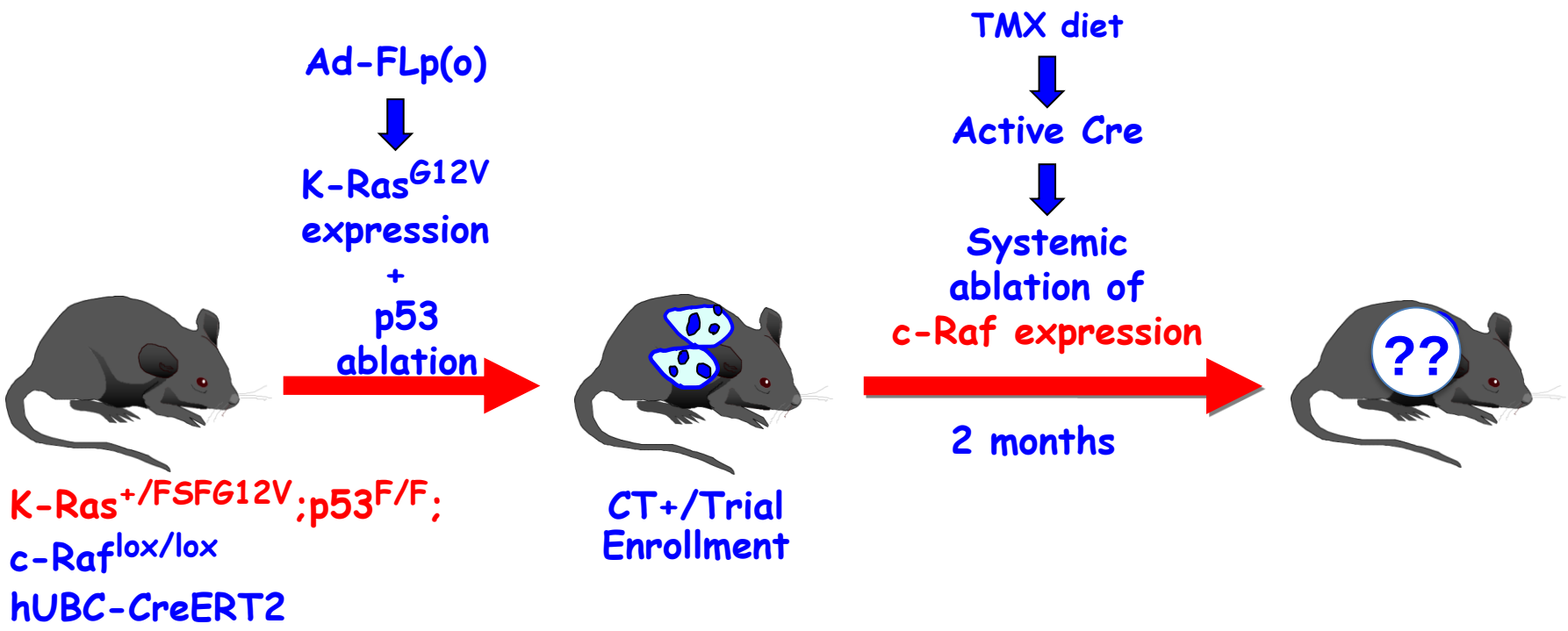
Tumor development at **4 months** in TMX diet

	c-Raf$^{+/+}$ (n=19)		c-Raf$^{lox/lox}$ (n=22)	
	Tumors	Tumor Vol.	Tumors	Tumor Vol.
Δ Tumor development	36	Δ15.3±6.3 fold	42	Δ1.0±0.2 fold
De novo CT+ tumors	67	9.0±3.5 mm3	9	0.4±0.1 mm3
Partial regression (>20%)	0		12 (29%)	
Complete regression (CT-)	0		14 (33%)	

Genetic validation of c-Raf: p53*null* tumors

Next we have validated the therapeutic effect of ablating c-Raf in more aggressive, K-Ras^{G12V} driven, p53*null* tumors

Therapeutic Model: K-Ras$^{+/FSFG12V}$;p53$^{F/F}$;c-Raf$^{lox/lox}$;hUBC-CreERT2$^{+/Tg}$



K-Ras^{G12V} & p53 *null* tumors: **Flp(o)**-dependent

Target Inactivation: **Cre**-dependent

c-Raf Ablation vs. c-Raf inactivation: p53*null* tumors

K-*Ras*$^{+/FSFG12V}$;*p53*$^{F/F}$;*hUBCCreERT2*^{T};**c-*Raf*$^{lox/lox}$** CT+ mice (2 months in TMX diet)

	c-Raf$^{+/+}$ (n=13)		c-Raf$^{lox/lox}$ (n=14)	
	Tumors	Tumor Vol.	Tumors	Tumor Vol.
Δ Tumor development	36	Δ5.8±0.9 fold	43	Δ0.8±0.1 fold
De novo CT+ tumors	37	3.7±0.7 mm3	3	0.5±0.2 mm3
Partial regression (>20%)	0		22 (51%)	
Complete regression (CT-)	0		8 (18%)	

Ablation of c-Raf expression in advanced K-Ras driven, p53 *null* lung adenocarcinomas induces a significant therapeutic effect:

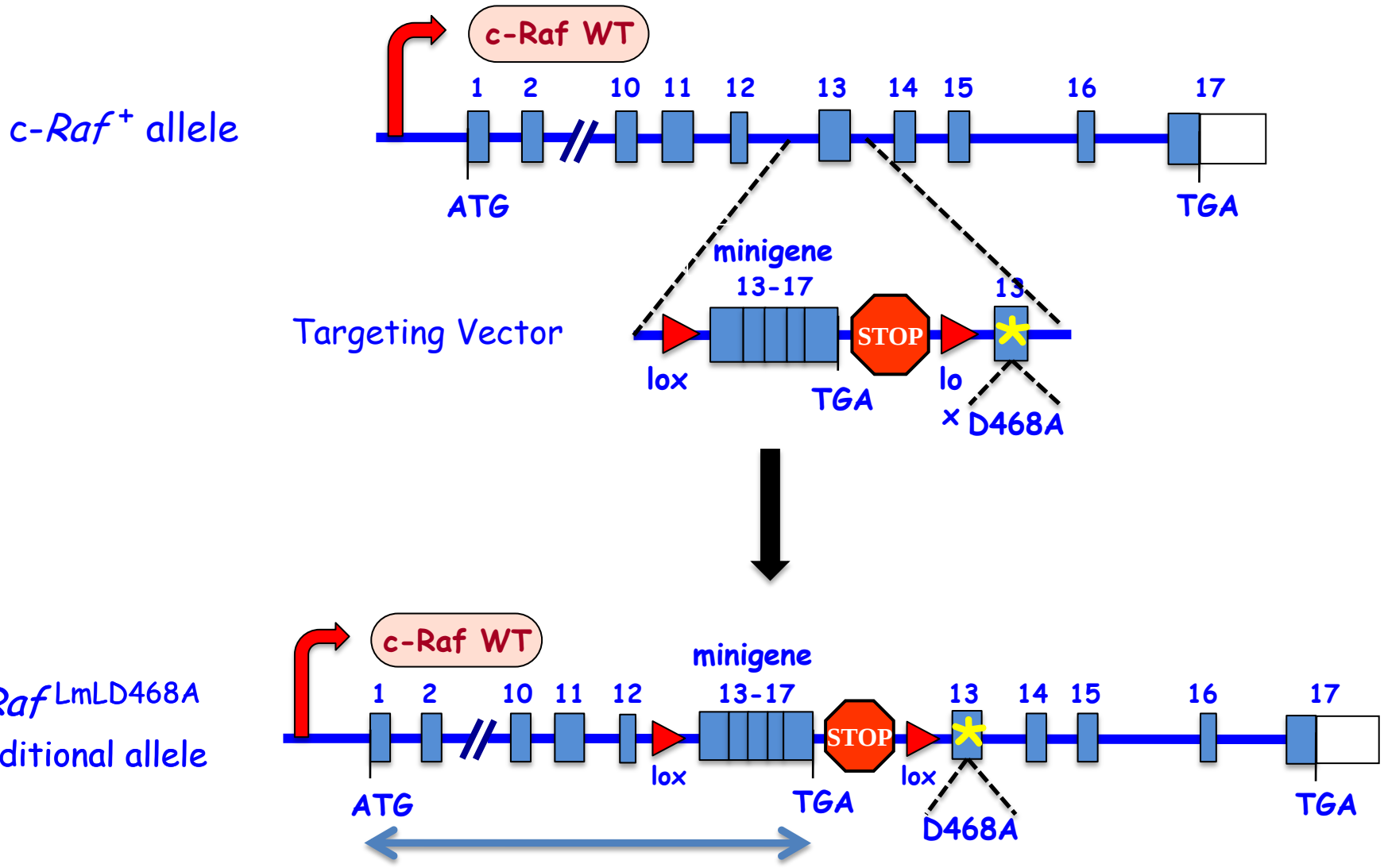
- Prevents progression of most tumors
- Induced partial or complete regression of about half of the tumors

c-Raf Ablation vs. c-Raf inactivation

However, it is important to realize that these models are based on **target ablation**, not **target inactivation**, which is the way we will have to block their activity in the clinic, at least for the time being since, thus far, there are no therapeutic strategies based on inhibition of c-Raf expression or on inhibitors that block c-Raf dimerization

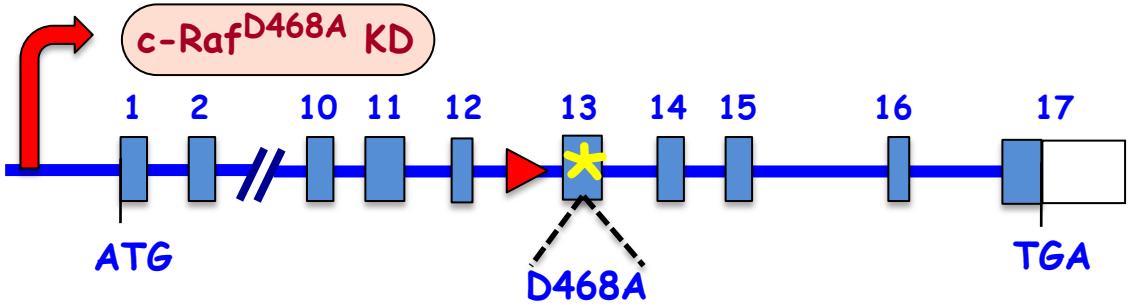
Hence, we have decided to modify our targeting strategy to validate c-Raf by expressing, in a conditional Cre-mediated fashion, **c-Raf kinase dead isoforms** instead of ablating **c-Raf protein expression**

c-Raf Ablation vs. c-Raf inactivation

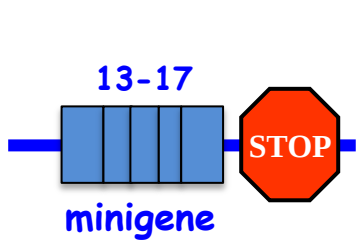


c-Raf Ablation vs. c-Raf inactivation

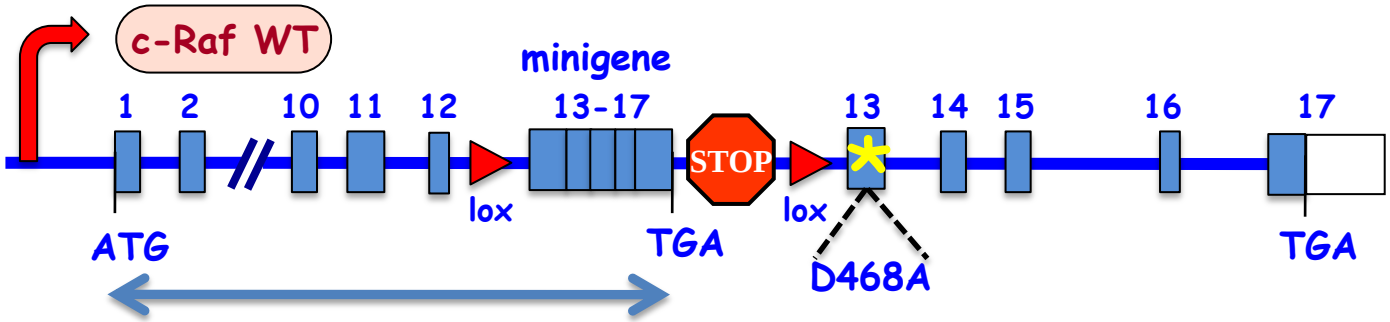
c-Raf^{D468A}
kinase dead allele



Cre
recombinase



c-Raf^{LmLD468A}
conditional allele



c-Raf Ablation vs. c-Raf inactivation

Ablation of c-Raf protein in the germ line results in embryonic lethality

ELSEVIER

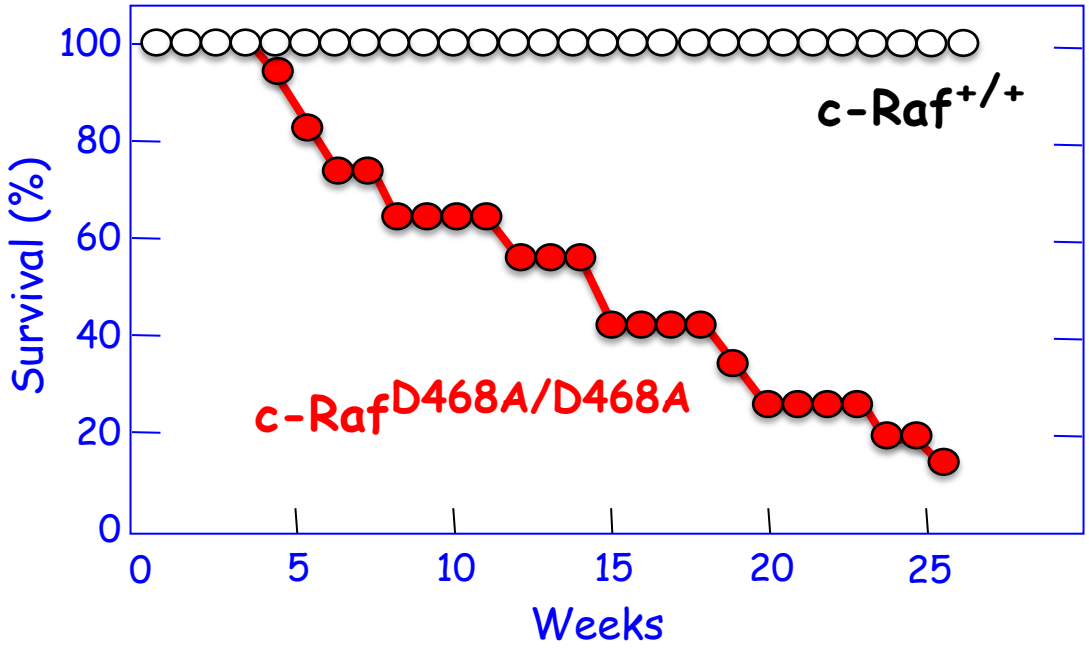
Mechanisms of Development 76 (1998) 141–149

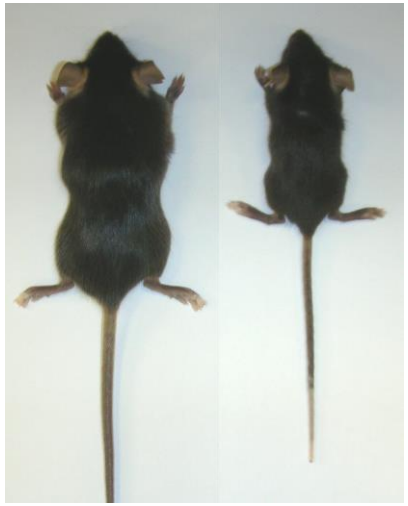
Craf-1 protein kinase is essential for mouse development

Leszek Wojnowski[a], Louis F. Stancato[b], Anne M. Zimmer[a], Heidi Hahn[a], Thomas W. Beck[c], Andrew C. Larner[b], Ulf R. Rapp[d], Andreas Zimmer[a,*]

Embryonic lethality and fetal liver apoptosis in mice lacking the c-*raf-1* gene

Mario Mikula, Martin Schreiber[1], Zvenislava Husak, Lucia Kucerova, Jochen Rüth, Rotraud Wieser[2], Kurt Zatloukal[3], Hartmut Beug[4], Erwin F.Wagner[4] and Manuela Baccarini[5]





WT D468A mutant

The c-Raf protein has important kinase-independent biological activity

c-Raf Ablation vs. c-Raf inactivation

K-Ras$^{+/FSFG12V}$
c-Raf$^{LmLKD/LmLKD}$
hUBC-CreERT2

Ad-FLp(o)

K-Ras^{G12V} expression

CT+/Trial Enrollment

TMX diet

Active Cre

Systemic expression of a c-Raf KD isoform

2 months

Tumor development: **Flp(o)**-dependent

Target Inactivation: **Cre**-dependent

c-Raf Ablation vs. c-Raf inactivation

K-*Ras*$^{+/FSFG12V}$;*p53*$^{+/+}$;*hUBCCreERT2*^{T};**c-*Raf*$^{LMLD468A/LMLD468A}$** CT+ mice (2 months in TMX diet)

	c-Raf$^{+/+}$ (n=12)		c-Raf^{LMLD468A} (n=21)	
	Tumors	Tumor Vol.	Tumors	Tumor Vol.
Δ Tumor development	34	Δ2.8±0.5 fold	42	Δ1.5±0.1 fold
De novo CT+ tumors	7	2.4±0.6 mm3	7	1.3±0.7 mm3
Partial regression (>20%)	0		5 (12%)	
Complete regression (CT-)	0		1 (2%)	

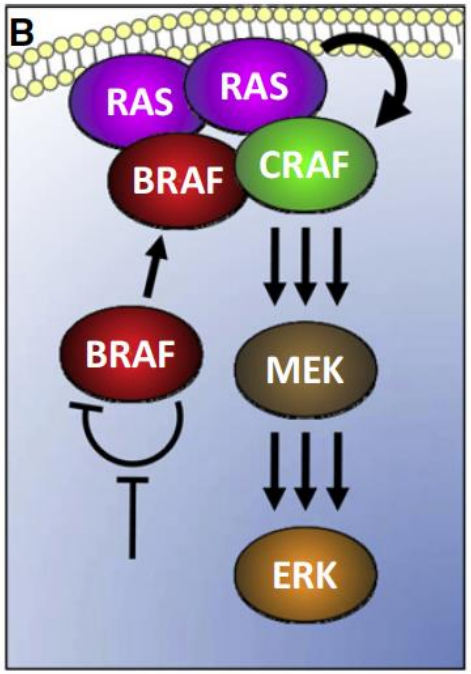
K-*Ras*$^{+/FSFG12V}$;*p53*$^{+/+}$;*RERT*$^{ert/ert}$;**c-*Raf*$^{lox/lox}$** CT+ mice (2 months in TMX diet)

	c-Raf$^{+/+}$ (n=25)		c-Raf$^{lox/lox}$ (n=22)	
	Tumors	Tumor Vol.	Tumors	Tumor Vol.
Δ Tumor development	46	Δ3.7±0.5 fold	41	Δ1.2±0.1 fold
De novo CT+ tumors	49	3.0±1.4 mm3	15	0.5±0.1 mm3
Partial regression (>20%)	0		7 (17%)	
Complete regression (CT-)	0		7 (17%)	

Raf inhibiton and the "paradoxical" effect

Kinase-Dead BRAF and Oncogenic RAS Cooperate to Drive Tumor Progression through CRAF

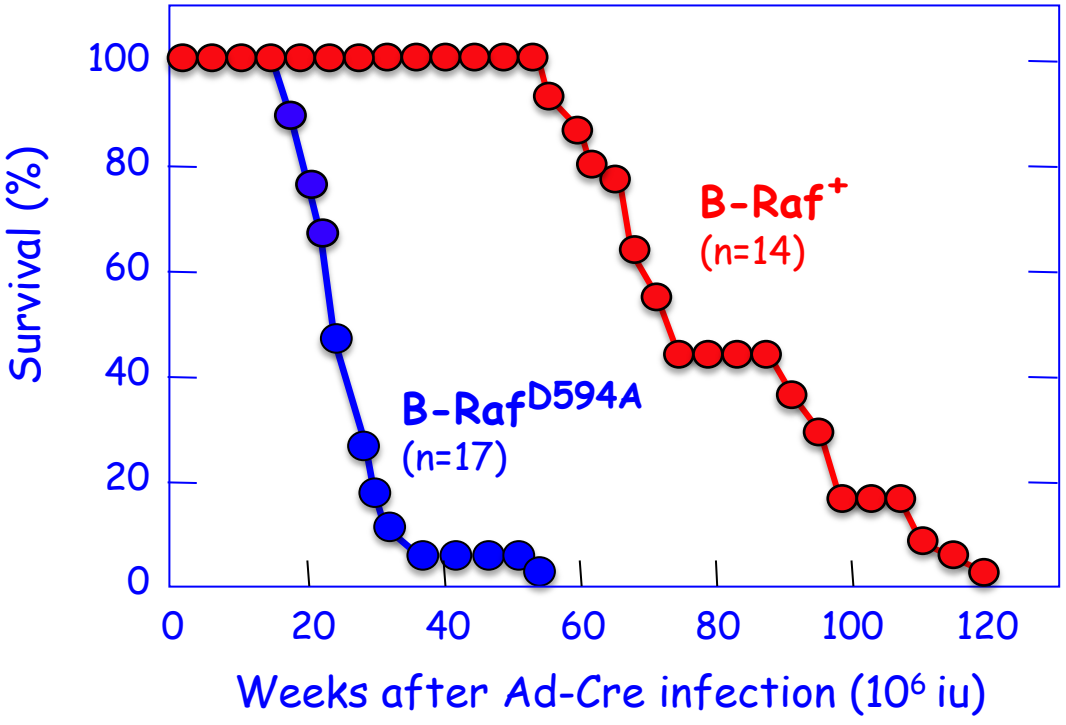
Sonja J. Heidorn,[1,5] Carla Milagre,[1,5] Steven Whittaker,[1] Arnaud Nourry,[2] Ion Niculescu-Duvas,[2] Nathalie Dhomen,[1] Jahan Hussain,[3] Jorge S. Reis-Filho,[4] Caroline J. Springer,[2] Catrin Pritchard,[3] and Richard Marais[1,*]



Accelerated tumor growth

The "paradoxical" effect also takes place in K-Ras driven lung tumors

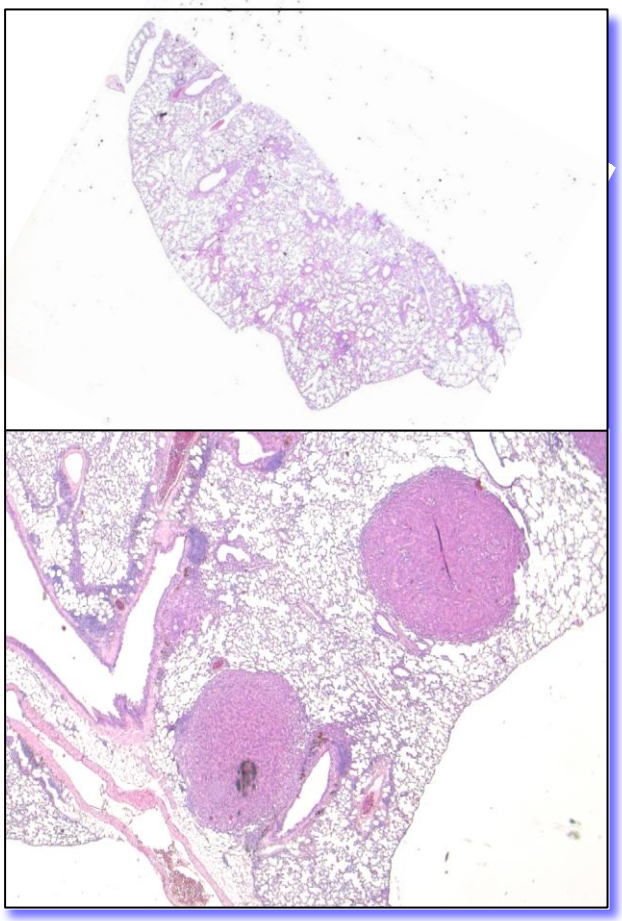
K-Ras$^{+/LSLG12Vgeo}$;B-Raf$^{+/LmLD594A}$ mice



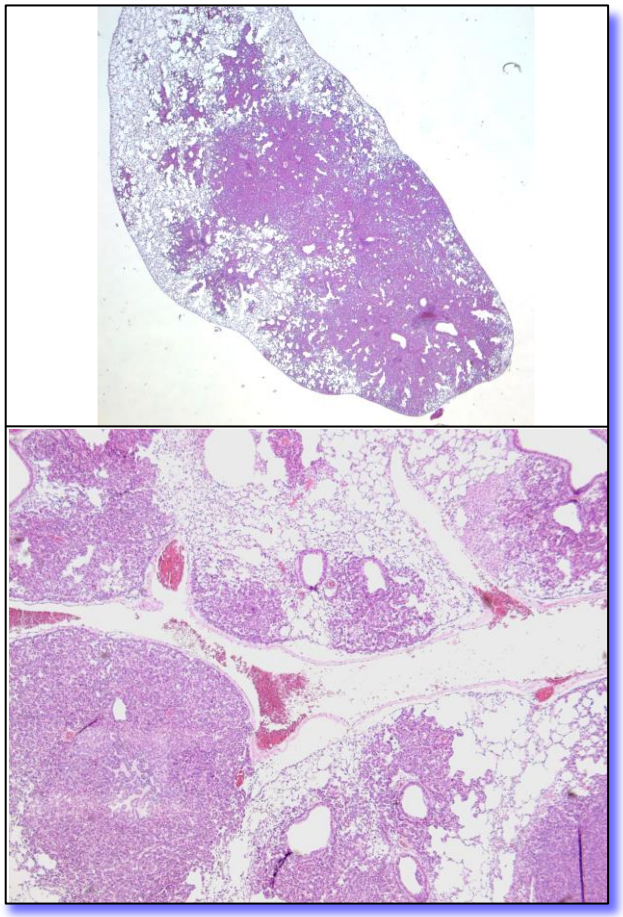
In collaboration with R. Marais

Raf inhibiton and the "paradoxical" effect

K-Ras$^{+/G12V}$ lungs



K-Ras$^{+/G12V}$;B-Raf$^{+/D594A}$ lungs



B-Raf^{D594A} expressing lungs display increased numbers of lesions that result in the death of the animal due to respiratory problems

In collaboration with R. Marais

Raf inhibiton and the "paradoxical" effect

Is the "paradoxical" effect mediated by c-Raf?

Kinase-Dead BRAF and Oncogenic RAS Cooperate to Drive Tumor Progression through CRAF

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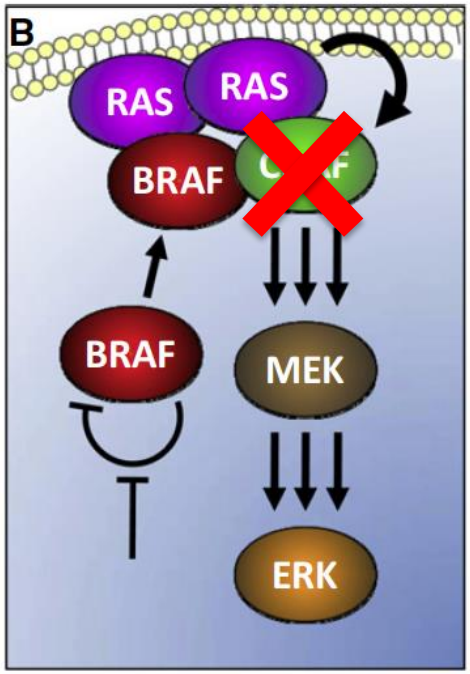
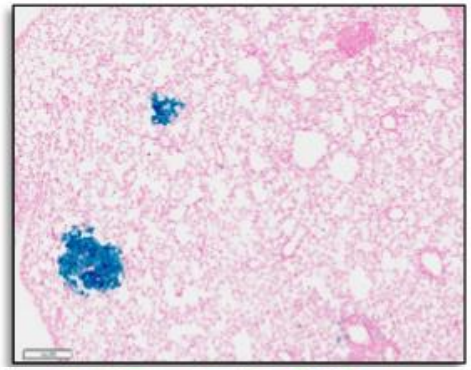
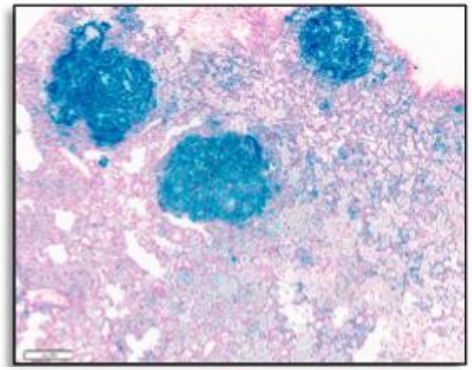


Accelerated tumor growth

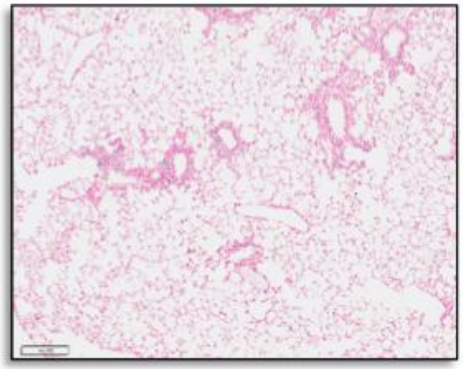
K-Ras$^{+/G12V}$



K-Ras$^{+/G12V}$;
B-Raf$^{+/D594A}$



K-Ras$^{+/G12V}$;
B-Raf$^{+/D594A}$;
c-Raf$^{D468A/D468A}$



Raf inhibiton and the "paradoxical" effect

Kinase-Dead BRAF and Oncogenic RAS Cooperate to Drive Tumor Progression through CRAF

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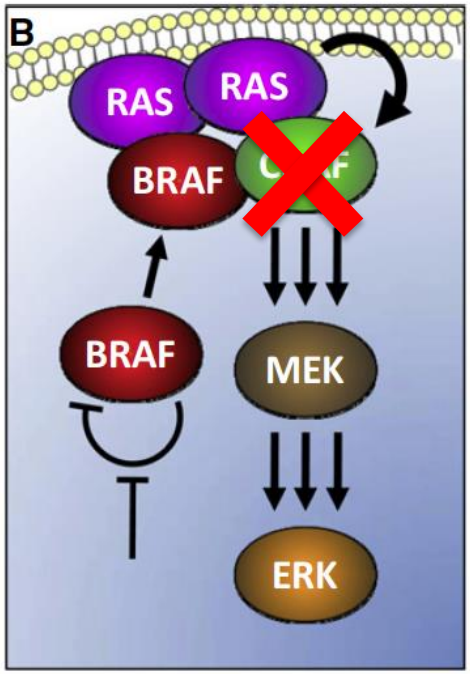
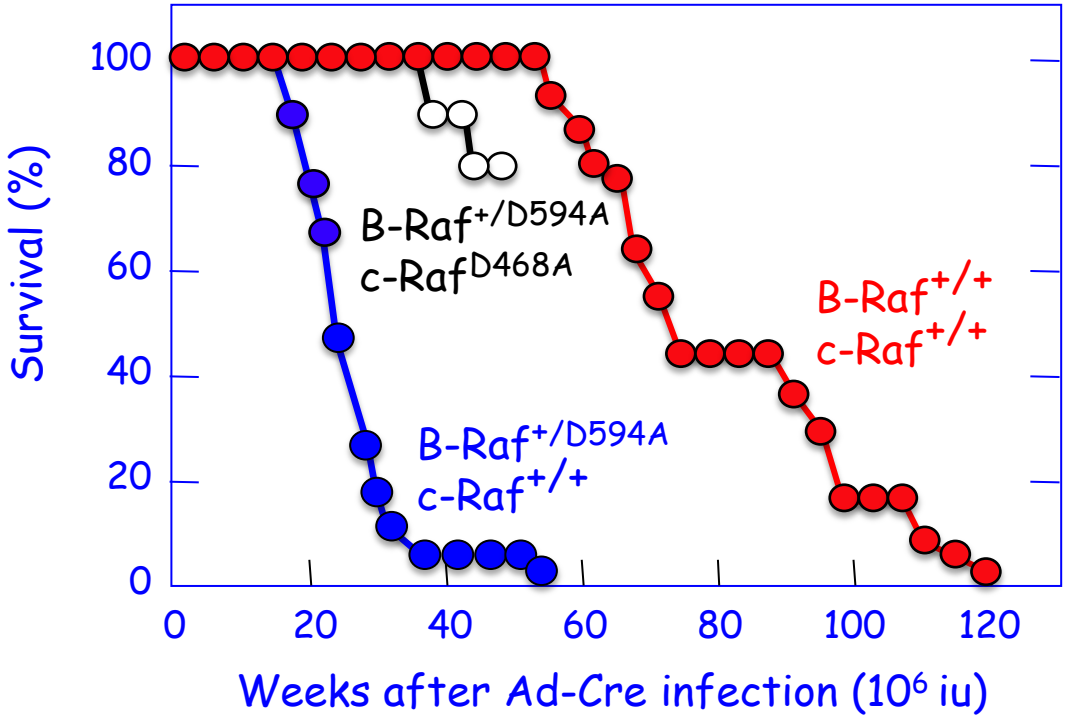
Is the "paradoxical" effect mediated by c-Raf?





Accelerated tumor growth

In collaboration with R. Marais

Raf inhibiton and the "paradoxical" effect

Kinase-Dead BRAF and Oncogenic RAS Cooperate to Drive Tumor Progression through CRAF

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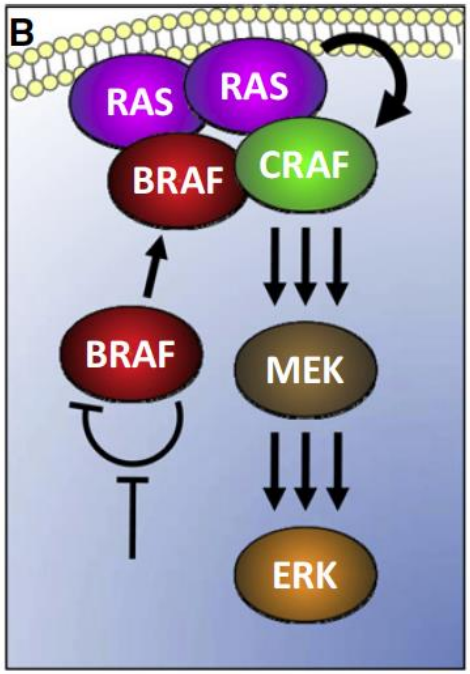
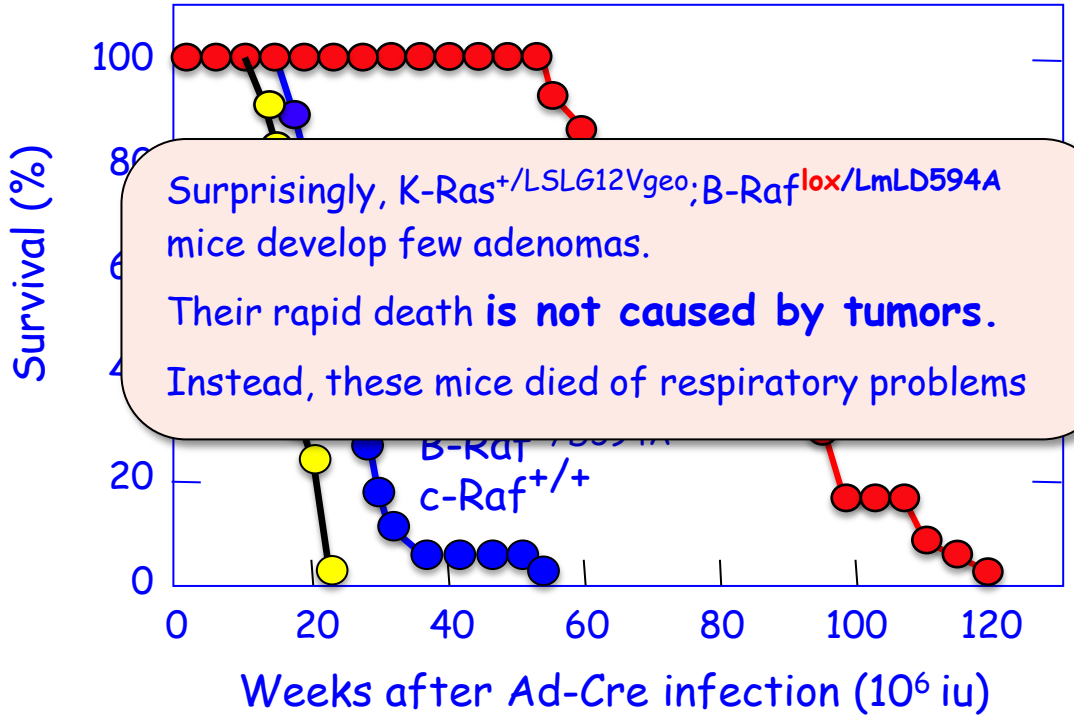


Accelerated tumor growth

What is the role, if any, of the wild type B-Raf kinase in the "paradoxical effect"?

K-Ras$^{+/LSLG12Vgeo}$;B-Raf$^{lox/LmLD594A}$ mice



Surprisingly, K-Ras$^{+/LSLG12Vgeo}$;B-Raf$^{lox/LmLD594A}$ mice develop few adenomas.

Their rapid death **is not caused by tumors.**

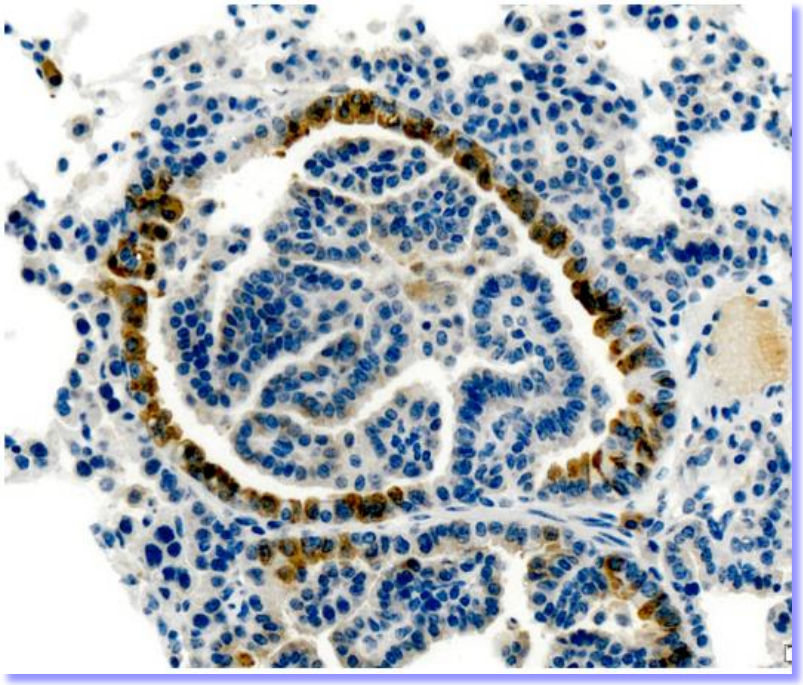
Instead, these mice died of respiratory problems

In collaboration with R. Marais

Raf inhibiton and the "paradoxical" effect

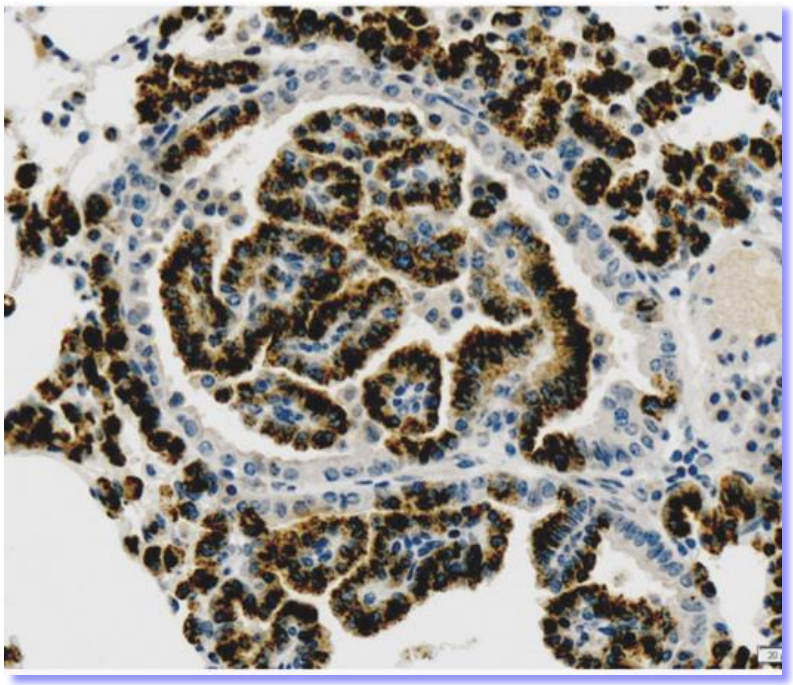
Loss of wild type B-Raf protein in the presence of kinase dead B-Raf^{D594A}, induces a high number of intrabronchiolar lesions which seems to arise from a transdiferentiation process from CC10 positive to SPC positive.

K-Ras$^{+/G12V}$



Anti-CC10

K-Ras$^{+/G12V}$;B-Raf$^{-/D594A}$



Anti SPC

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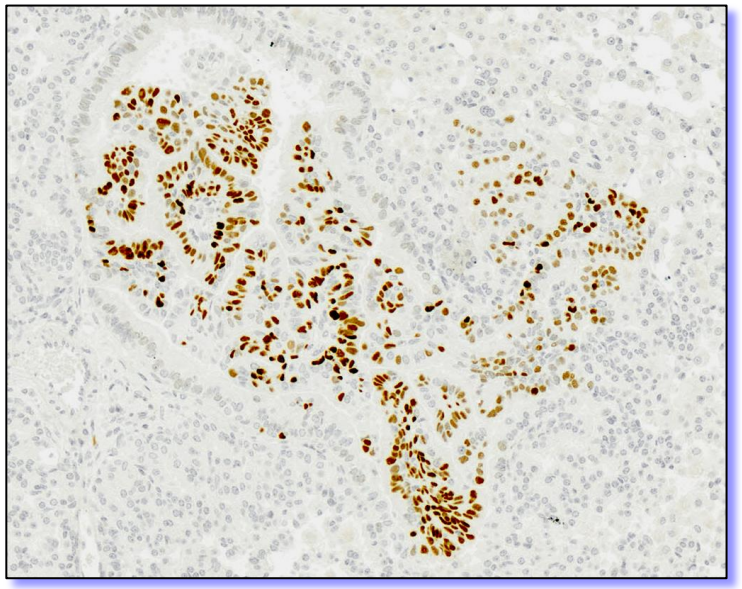
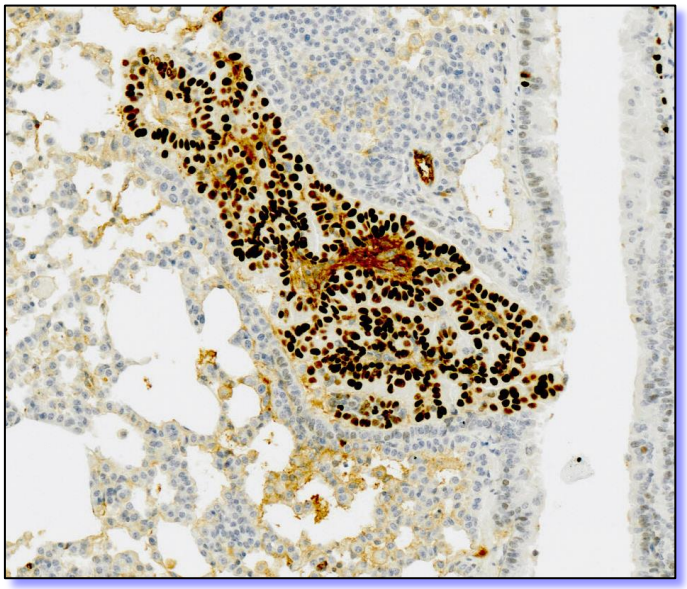
Raf inhibiton and the "paradoxical" effect

These intrabronchiolar hyperplasias express some markers of squemous cell carcinomas such as p63

$K\text{-}Ras^{+/G12V};B\text{-}Raf^{-/D594A}$



p63 IHC



p63 IHC

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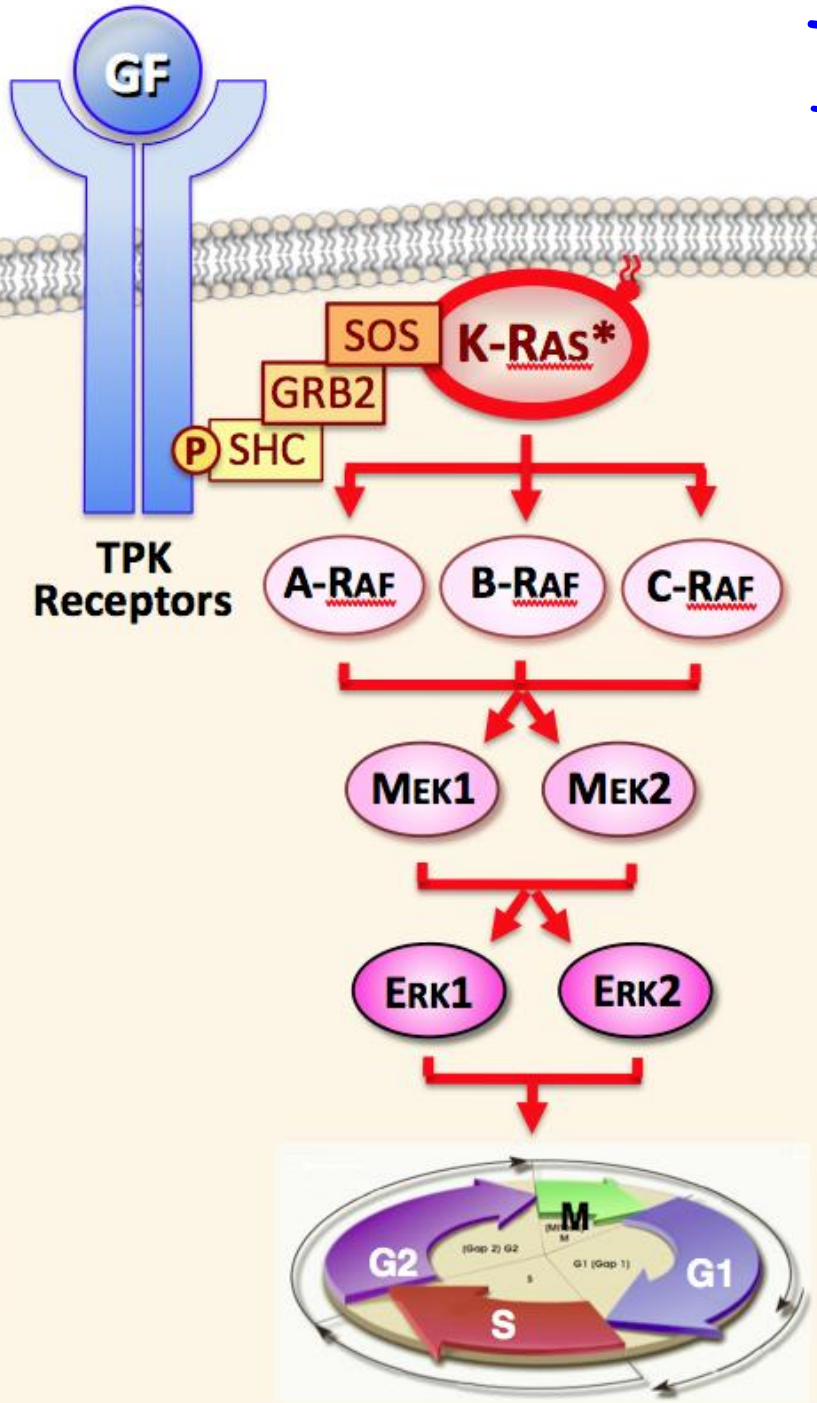
Targeting K-Ras driven lung tumors:

The Ras signaling cascade



We have known this pathway for over two decades.....

....but we still do not know how to target it

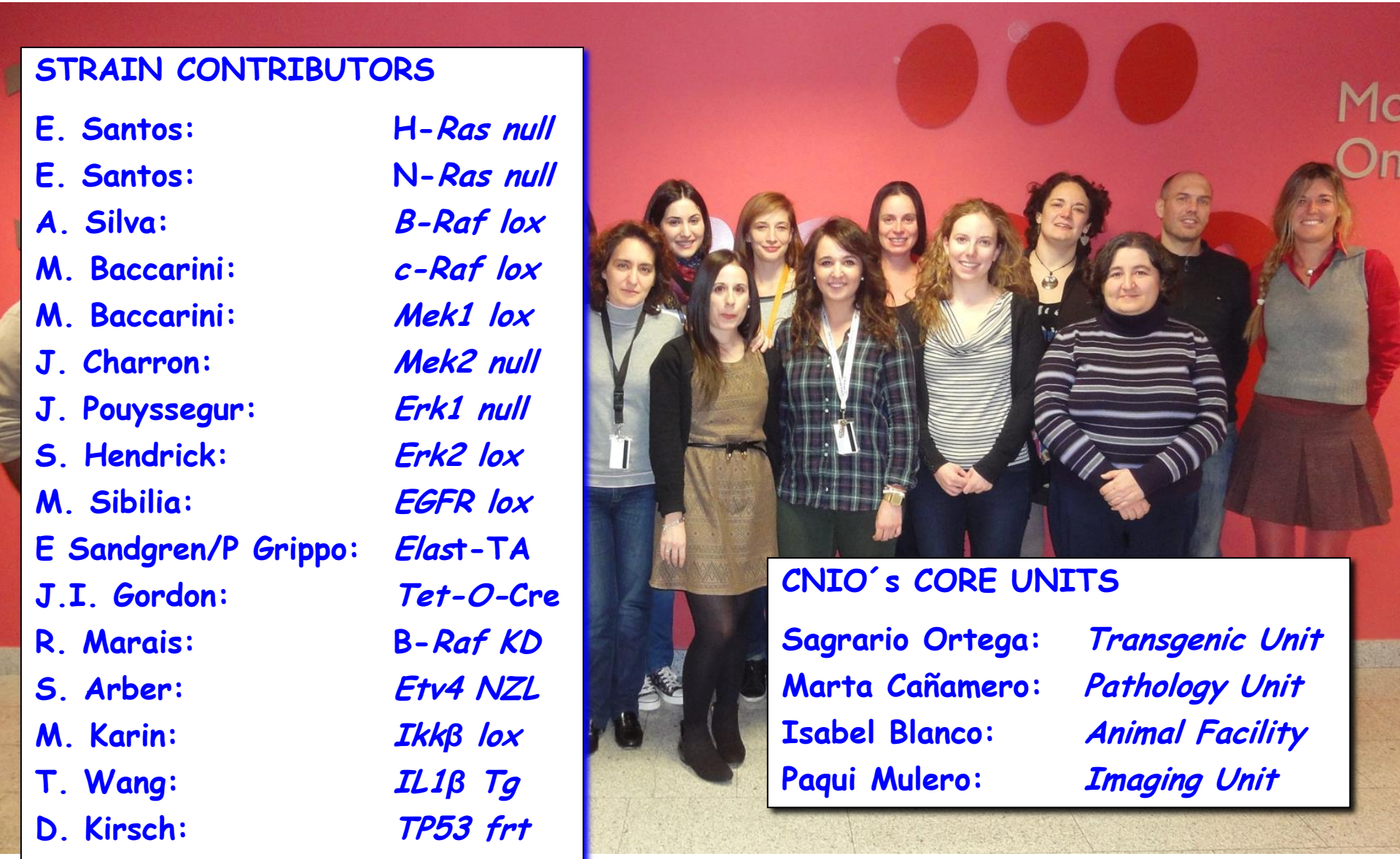
The Barbacid Lab

Lung group

The Barbacid Lab

STRAIN CONTRIBUTORS

E. Santos:	H-*Ras null*
E. Santos:	N-*Ras null*
A. Silva:	*B-Raf lox*
M. Baccarini:	*c-Raf lox*
M. Baccarini:	*Mek1 lox*
J. Charron:	*Mek2 null*
J. Pouyssegur:	*Erk1 null*
S. Hendrick:	*Erk2 lox*
M. Sibilia:	*EGFR lox*
E Sandgren/P Grippo:	*Elast-TA*
J.I. Gordon:	*Tet-O-Cre*
R. Marais:	B-*Raf KD*
S. Arber:	*Etv4 NZL*
M. Karin:	*Ikkβ lox*
T. Wang:	*IL1β Tg*
D. Kirsch:	*TP53 frt*



CNIO´s CORE UNITS

Sagrario Ortega:	*Transgenic Unit*
Marta Cañamero:	*Pathology Unit*
Isabel Blanco:	*Animal Facility*
Paqui Mulero:	*Imaging Unit*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