XXXVIII National Congress of the Italian Association for the Study on Pancreas (AISP) Bologna, 18th September 2014



# In vivo models as a platform for the discovery of new therapies in exocrine pancreatic neoplasms

## Elisa Giovannetti



VU University Medical Center, Amsterdam, The Netherlands AIRC-Start-Up, Pisa, Italy



# Why we use mice for our cancer models?

- Small size, easy to handle and take care of
- Easily available from commercials and far cheaper than other mammal animals (many mice can be treated at the same time)
- Genetically best characterized of all mammals
- Accommodation of nude mice to human tumor models
- Relatively rapid tumor growth in mice makes them useful in cancer research and drug screening



# **Evolution of mouse cancer models**

#### FIRST GENERATION: GRAFTED TUMOR CELLS

- Xenotransplants or syngeneic transplants
- Subcutaneous or orthotopic transplants

#### SECOND GENERATION: TRANSGENIC MODELS

- Constitutive expression of oncogene
- Conditional expression of oncogene

#### THIRD GENERATION: ADVANCED TRANSGENIC MODELS

 Temporally and spatially controlled expression of oncogenic mutations and combinations thereof

### FOURTH GENERATION

- Spontaneous tumor models mimicking tumor evolution and heterogeneity
- Patient-derived xenografts

### **FIFTH GENERATION**

 Advanced third- and fourth-generation models mimicking metastatic progression (metastasis becoming rate limiting for tumor growth)

### Böck B C et al. Cancer Res 2014;74:4671-4675

# **GEM Mice in pancreatic cancer**

А

Mutant KRAS-driven models.

- (A) Cre/loxP-mediated conditional activation or inactivation of genes can be used for targeting oncogenes and tumour suppressors in the pancreas
- (B) GEMMs develop tumours в that resemble different types of human preneoplastic lesions and PDAC with varying latency depending on the induced genetic alterations



# The advantages of pancreatic GEMMs

 These models maintain an intact immune system and develop tumors histologically similar to human PDACs, including a dense desmoplastic stroma reaction





Olive et al. (Science 2009) showed that PDAC GEMMs

- 1) are poorly perfused and vascularised
- 2) significantly less sensitive to gemcitabine treatment compared to the transplanted tumors (but sensitive to Hh inhibitor)

... but these models failed to predict response to IPI-92 in clinical trials (Rhim et al. 2014)

# ... and their drawbacks

- Not all tissue specific promoters are specific (i.e. since the commonly used transcription factor PDX1 is expressed in the developing foregut (stomach and duodenum) as well as in the epidermis, tumor development may occur in extrapancreatic organs, potentially affecting pancreatic carcinogenesis and responses to therapeutic approaches, as well as the life span of mice)
- Mutant KrasG12D is activated during mice embryogenesis, which probably does not reflect the acquisition of sporadic mutations in adult cells in humans
- Their dependence on a few critical genetic lesions, such as KRAS and P53, might not reflect the genetic diversity that exemplifies human PDAC

# Avatar mice or PDX

Cell lines in vitro	Cell line xenografts	Patient-derived xenografts	Patient with refractory cance
No heterogeneity	Limited intratumoral heterogeneity	Higher Intratumor heterogeneity	High intratumor heterogeneity
Modest diversity of molecular subtypes	Modest diversity of molecular subtypes	Higher number of molecular subtypes	Full range of molecular subtypes
No stroma	Murine stroma; No human stroma	Admixed murine/human stroma	Intact human stroma
Rapid growth (doubling time in days)	Rapid growth (doubling time in days)	Slower growth (doubling time in weeks)	Chronic growth (doubling time in months)
Untreated	Untreated	Mixed untreated and previously treated	Prior treatment in all patients
No linked clinical outcomes	No linked clinical outcomes	Limited clinical outcomes available	Treatment outcomes available
Aixed primary and metastatic sites	Mixed primary and metastatic sites	Mixed primary and metastatic sites	Metastatic sites predominate
No orthotopic studies	Rarely orthotopic studies	Rarely orthotopic implantation	All orthotopic (by definition)
No immune system	Limited immune system	Severely limited immune system	Intact immune system

# *Our models:* new orthotopic bioluminescent models, with primary cell cultures



Avan et al, Cancer Res 2013

## Bioluminescence



Color bar Min:5e+06



# **Other imaging**

<u>MRI</u>



# **Echo-doppler**



## **High-frequency ultrasound**



## **Histological features and metastasis**



## **Genetics of the PDAC3 model**

Chro1	Chro2	Chro3	Chro4	Chro5
Chro6	Chro7	Chro8		Chro10
Chro11	Chro12	Chro13	Chro14	Chro15
Chro16	Chro17	Chro18	Chro19	Chro20

## c-Met in the PDAC-3 model







# BLI measurement of tumor growth and survival



#### **Another model (PDAC2)** Α **Human PDAC-2 PDAC-2 cells Orthotopic PDAC-2** og2ratio og2ratio В chromosome chromosome chromosome chr4 chr1 chr2 chr5 С X R<sup>2</sup> =0.929 PDAC-2 cells chr7 chr6 Human PDAC-2 × × × **Orthotopic PDAC-2** R<sup>2</sup>=0.852 chr14 chr15 chr11 chr12 X X X Human PDAC-2 Ťε. chr16 chr17 chr19 chr20 chr18 R<sup>2</sup> =0.788 **Orthotopic PDAC-2** ΞĒ. chr21 R q22.1 q22.3 q23 p11.31 p11.21 q11.2 = X Chromosome 18 PDAC-2 cells Ш i.

5 MD 10 MD 15 MD 20 MD 25 MD 30 MD 35 MD 40 MD 45 MD 50 MD 55 MD 60 MD 65 MD

20 Mb

-2.0

## **Genomic study**

#### array-CGH data in 44 radically resected pts



Lee, Giovannetti et al., Clin Cancer Res 2012

## Genomic study, part II



## **Role of autophagy**

HSP90AA1 ATG7 ATG9A

DRAM1

AMBRA1 NFKB1

ATG16L2 BAX MAP1LC3A ATG5 CXCR4

• P=0.01

3

2



## Pathways, OS and mts



Giovannetti et al., JNCI 2014

Therapeutics, Targets, and Chemical Biology

### Crizotinib Inhibits Metabolic Inactivation of Gemcitabine in c-Met–driven Pancreatic Carcinoma

Amir Avan<sup>1</sup>, Viola Caretti<sup>3,6</sup>, Niccola Funel<sup>7</sup>, Elena Galvani<sup>1</sup>, Mina Maftouh<sup>1</sup>, Richard J. Honeywell<sup>1</sup>, Tonny Lagerweij<sup>3</sup>, Olaf Van Tellingen<sup>4</sup>, Daniela Campani<sup>7</sup>, Dieter Fuchs<sup>5</sup>, Henk M. Verheul<sup>1</sup>, Gerrit-Jan Schuurhuis<sup>2</sup>, Ugo Boggi<sup>8</sup>, Godefridus J. Peters<sup>1</sup>, Thomas Würdinger<sup>3,9</sup>, and Elisa Giovannetti<sup>1</sup>

JNC JOURNAL OF THE NATIONAL CANCER INSTITUTE

Vol. 106, Issue 1 | djt346 | January 1, 2014

### Role of CYB5A in Pancreatic Cancer Prognosis and Autophagy Modulation

Elisa Giovannetti, Qiuyan Wang, Amir Avan, Niccola Funel, Tonny Lagerweij, Jih-Hsiang Lee, Viola Caretti, Arjan van der Velde, Ugo Boggi, Yisong Wang, Enrico Vasile, Godefridus J. Peters, Thomas Wurdinger, Giuseppe Giaccone

Manuscript received May 11, 2013; revised October 28, 2013; accepted October 29, 2013.

Cancer Research

# Conclusions... "everything is illuminated" (?)

"co-clinical trials" performed in several mouse models in parallel with human patients enrolled in ongoing phase I and II trials should improve the velocity and the outcome of personalized treatments and helps to identify genetic and molecular determinants mediating drug resistance

This approach should enable oncologists to **tailor novel, optimized combinatorial therapies** based on patient stratification

