In Vivo Target Validation *Section*

Pilar Pizcueta

Section Head

Pilar Pizcueta was born in Zaragoza, Spain, and graduated in Biology at the *Universitat Autònoma de Barcelona*. She completed her thesis at the *Hospital Clínic* of Barcelona where she studied the role of vasodilators as mediators of the pathophysiology of portal hypertension and cirrhosis. She obtained her PhD in Pharmacy from the *Universitat de Barcelona* in 1992.

Supported by a Fulbright Postdoctoral Fellowship she first worked at Harvard Medical School and later at Duke's University, Durham (USA), investigating the functional role of adhesion molecules in inflammation using *in vivo* models.

Upon returning to Spain in 1995, she became an independent researcher at the *Fundació Clínic-IDIBAPS* in Barcelona where her work examined the contribution of adhesion molecules to inflammatory disturbances in the liver (e.g. cirrhosis and hepatitis) using knock-out mice and function-blocking monoclonal antibodies.

In 2001 she moved to the pharmaceutical industry as Head of the *In Vivo* Target Validation Unit in the Drug Discovery Division of Almirall. During this time she generated and characterised experimental models of diseases. In addition, she worked on target identification and validation of therapeutically valuable new molecules involved in inflammatory and autoimmune diseases. After 3 years she became programme leader in Drug Discovery, supervising projects related to autoimmune diseases. During this period two drug candidates were forwarded to Development and one of them has now entered clinical trials.

She joined the CNIO as Head of *In Vivo* Target Validation in January 2009.

Summary

Conventional preclinical tumour models may not reliably predict clinical outcome. New mouse models must therefore be characterised and developed to improve the prediction of *in vivo* testing for the evaluation of experimental therapies.

Our Group focuses on validating targets of therapeutic interest in genetically engineered mouse tumour models that resemble the clinical settings common in humans, comparing genetic ablation and pharmacological intervention approaches. In addition, comparative mouse trials using drug candidates for selective targets either alone or in combination will be carried out to better mimic current cancer treatments.

Main Objectives

- Validate molecular targets of therapeutic interest in genetically engineered mouse (GEM) tumour models
- Pharmacologically validate new oncogenic mouse models
- Elucidate the relevant signalling pathways in tumour development for therapeutic applications
- Characterise novel drug candidates generated by the Experimental Therapeutics Programme in these GEM tumour models

Technicians: Enara Aguirre and Elena Gómez-Casero.

Highlights

During this year our research has focused on the pharmacological inhibition efficacy of our Programme´s PI3kinase compounds, on a lung tumour mouse model driven by a K-*Ras*^{G12V} oncogenic mutation that resembles human non-small-cell lung cancer (NSCLC). This immunocompetent animal model has been incorporated into the screening cascade for the Lead Optimisation phase to evaluate the efficacy/toxicity of the drug candidates. Drug efficacy is defined by the reduction in number and size of the tumours analysed by computed tomography (CT) as well as the avidity of these tumours for ^{18}fluorodeoxyglucose (FDG) analysed by positron emission tomography (PET), similar to the clinical assessment of the course of NSCLC.

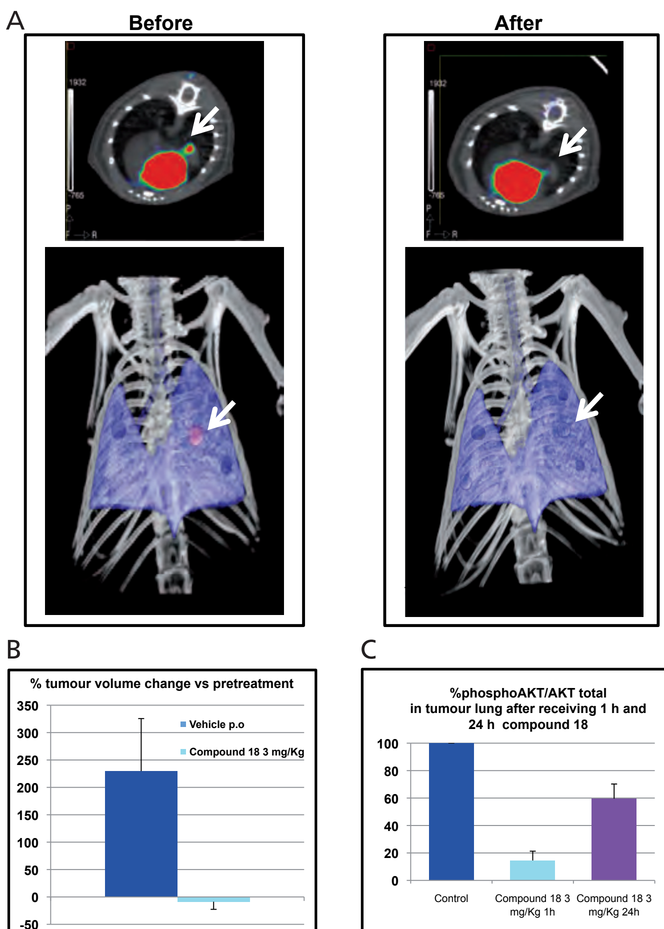

Figure: (A) Representative Computed Tomography (CT) – Positron Emission Tomography (PET) scans of K-*Ras*$^{+/LSLG12Vgeo}$;RERT$^{ert/ert}$ conditional mice before and after treatment. The K-*Ras*^{G12V} mutation was induced by tamoxifen and 3 months later when lung tumours developed, mice were treated with compound 18 (3 mg/kg per day) for 3 weeks. White arrows on the scans indicate one tumour. Dark blue and red colours show tumour and FDG avidity, respectively. (B) Tumour volumes in 4 mice from each treatment group after 3 weeks of daily treatment shown as percentage change relative to pretreatment tumour volumes. Values are means ± s.d. (C) Western Blot analysis of the effect of compound 18 on pAkt in K-*Ras*$^{+/LSLG12Vgeo}$;RERT$^{ert/ert}$ conditional mice, 1 and 24 hours after single compound administration (3 mg/Kg p.o.). Results are expressed as percentage of pAkt vs. total Akt.

Five proprietary compounds with different ADME and selectivity profiles were tested and compared with other PI3K reference compounds currently in clinical trials. Results showed that our compounds are active and well tolerated either as single agents or in combination with other novel and conventional therapies. FDG-PET imaging is the major pharmacodynamic (PD) marker in the clinic used for testing the efficacy of PI3K inhibitors in cancer patients. Likewise, our compounds showed a substantial reduction in FDG-PET uptake as well as marked decrease in tumour volume and size judged by CT (Figure, panels A and B). Other efficacy/toxicity read outs also included pharmacokinetic (PK) and pharmacodynamic (PD) studies (Figure, panel C) as well as biochemical and haematological analysis. These results have allowed us to characterise and select the best candidates.

We also characterised a second GEM mouse model this year, a K-*Ras*^{G12V} oncogene driven murine model carrying an activating *PIK3CA*^{H1047R} mutation. The presence of two mutations increased the number of tumours as well as the inhibitory effect of selective PI3K compounds, compared with a mouse carrying only the K-*Ras* oncogenic mutation.

Our next project will involve setting up other GEM tumour models that recapitulate other cancer pathologies (breast and colon cancer, melanoma) with different oncogenic mutations (*B-RAF, PIK3CA, K-RAS*) and deficient in tumour suppressors.

Publication

Puyol M, Martín A, Dubus P, Mulero F, Pizcueta P, Khan G, Guerra C, Santamaría D, Barbacid M (2010). A Synthetic Lethal Interaction between K-*Ras* Oncogenes and Cdk4 Unveils a Therapeutic Strategy for Non-small Cell Lung Carcinoma. *Cancer Cell* 18, 63-73.