

TELOMERES AND TELOMERASE  
GROUP – FUNDACIÓN  
HUMANISMO Y CIENCIA

Maria A. Blasco  
Group Leader

Research Scientists  
Isabel López de Silanes, Rosa M.  
Marión, Paula Martínez



Post-Doctoral Fellows  
Giuseppe Bosso, Buyun Ma, Arpita  
Saha (since May), Sarita Saraswati

Hernández (since Nov.) (TS)\*, Rosa  
M. Serrano

Internship, Univ. of São Paulo, Brazil),  
Ana Carolina Cintra (since Nov.)  
(Erasmus Fellowship, Tras-os-Montes  
e Alto Douro Univ., Portugal),  
(Sep.-Dec.) (Traineeship, Univ.  
Autónoma de Madrid, Spain)

Graduate Students  
José Carlos González, Óscar Laguna,  
Jessica Louzame, Amparo Sánchez,  
Raúl Sánchez

Visiting Students  
Sarah Adetchessi (May -July) (PhD  
Student, Univ. of Paris, France),  
Stavroula Boukoura (Oct.-Dec.) (PhD  
Student, Danish Cancer Society  
Research Center, Denmark), Robson  
Diego Calixto (Aug.-Dec.) (FAPESP

Mariana Deli (June-Dec.) (Erasmus +  
Fellowship, Univ. of Ioannina,  
Greece), Julie Klein (June-Aug.) (PhD  
Student, École de Biologie  
Industrielle, France), Neetij Krishnan  
(until June) (Fulbright Commission  
Fellowship, USA), Cristina Pastor

Technicians  
Ana Guío (until Oct.) (TS)\*, David

OVERVIEW

Immortality is one of the most universal characteristics of cancer cells. We study the mechanisms by which tumour cells are immortal and normal cells are mortal. The enzyme telomerase is present in more than 95% of all types of human cancers and is absent in normal cells in the body. Telomeres are nucleoprotein complexes located at the ends of chromosomes, essential for chromosome protection and genomic stability. Progressive shortening of telomeres associated with organism ageing leads to ageing. When telomeres are altered, adult stem cells have a maimed regenerative capacity.

“Our potential preclinical mouse model *ki-Pot1a<sup>R117C</sup>* for Li-Fraumeni-Like syndrome presenting with high angiosarcoma incidence could be a very useful tool in the therapeutics of these tumours.”

Our research focuses on:

- Generating mouse models to validate telomeres and telomerase as therapeutic targets for cancer and age-related diseases.
- Interplay between telomeres and DNA repair pathways.
- Role and regulation of non-coding telomeric RNAs or TERRA.
- Testing telomerase gene therapy in *telomere syndromes* and age-related diseases.
- Role of telomerase and telomeres in adult stem cell biology and in nuclear reprogramming of differentiated cells to iPS cells.

RESEARCH HIGHLIGHTS

**BRAF<sup>V600E</sup> in adult mouse models elicits early differential responses**

The BRAF gene, which encodes a master kinase of the RAS-pathway, is frequently mutated in human cancers. The most common genetic mutation is a single nucleotide transition that gives rise to a constitutively active BRAF kinase (BRAF<sup>V600E</sup>), which in turn sustains continuous cell proliferation. The study of BRAF<sup>V600E</sup> murine models has so far focused mainly on the role played by BRAF<sup>V600E</sup> in tumour development, so much so that little was known about the early molecular impact of the *in vivo* expression of BRAF<sup>V600E</sup>. We have now provided the first *in vivo* evidence that acute BRAF<sup>V600E</sup> expression elicits instant DNA damage in an organ-specific fashion. The senescence marker p21CIP1, which may be activated by p53 upon genotoxic insults and by oncogene activation via pRb/E2F, promotes cell cycle arrest and senescence by inhibiting CDKs. Despite BRAF<sup>V600E</sup> inducing both DNA damage and p21CIP1 activation *in vitro*, as well as in senescent lung adenomas, we did not find any differences in p21CIP1 levels either in liver or spleen upon BRAF<sup>V600E</sup> expression. BRAF<sup>V600E</sup> in lungs provokes an acute inflammatory state with tissue-specific recruitment of neutrophils to alveolar parenchyma and of macrophages to bronchi/bronchioles, as well as bronchial/bronchiolar epithelium transdifferentiation and development of adenomas.

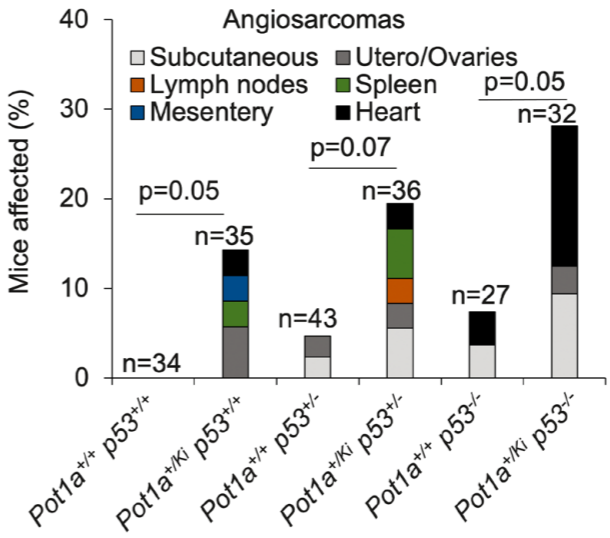
**A mouse model for Li-Fraumeni-Like syndrome with cardiac angiosarcomas associated to POT1 mutations**

Although the telomeric protein POT1 is mutated in many different familial and sporadic cancers, so far there have been no mouse models to understand the pathobiology of these mutations. We have generated a mouse model for the human *POT1<sup>R117C</sup>* mutation found in Li-Fraumeni-Like (LFL) families with cases of cardiac angiosarcoma (CAS) by means of introducing this mutation in the *Pot1a* endogenous locus, *knock-in* for *Pot1a<sup>R117C</sup>*, thus generating *Pot1a<sup>ki</sup>* mice. While

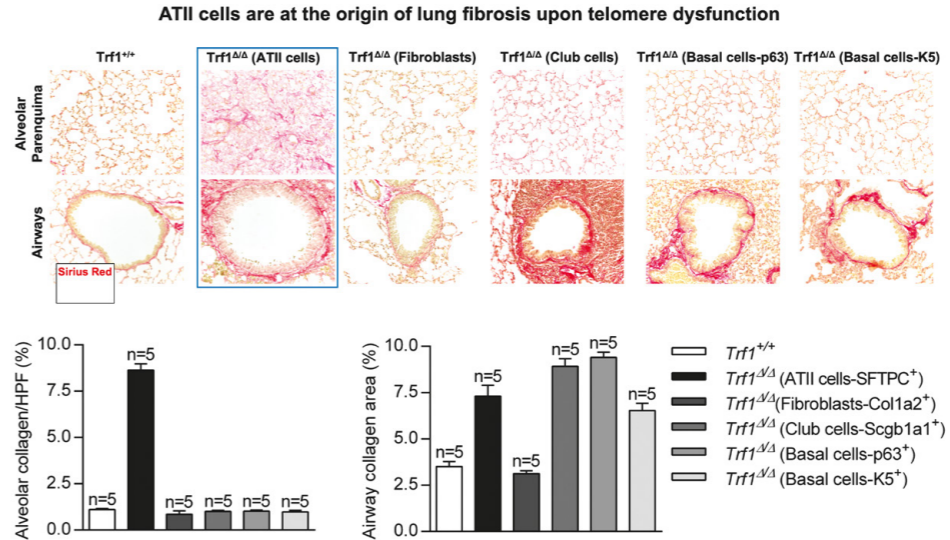
homozygous *Pot1a<sup>ki/ki</sup>* are embryonic lethal, heterozygous *Pot1a<sup>+/-ki</sup>* mice are viable. We also found that both mouse embryonic fibroblasts (MEFs) and tissues from *Pot1a<sup>+/-ki</sup>* mice harbour longer telomeres than wild-type controls. Like human LFL patients, heterozygous *Pot1a<sup>+/-ki</sup>* mice spontaneously develop a high incidence of angiosarcomas (FIGURE 1), including CAS, and this is associated with the presence of abnormally long telomeres in endothelial cells as well as in the tumours. The *Pot1a<sup>+/-R117C</sup>* mouse model therefore constitutes a useful tool to understand human cancers initiated by POT1 mutations.

**Impact of telomere dysfunction in fibroblasts, Club and basal cells on the development of lung fibrosis**

Telomeric protein TRF1 is an essential component of the telomeric protective complex that prevents telomeric DNA damage, chromosome end-to-end fusions and telomere fragility. We previously showed that induction of telomere dysfunction in alveolar type II (ATII) cells is sufficient to induce progressive and lethal pulmonary fibrosis in mice. The pathological consequences of telomere dysfunction in lung fibroblasts, Club and basal cells remained to be investigated. We have now conditionally deleted *Trf1* in the former mouse tissues. We found that while TRF1 deficiency in fibroblasts does not lead to significant lung pathologies, *Trf1* deletion in Club and basal cells from male mice led to lung inflammation and airway remodelling. While dysfunctional telomeres in ATII cells led to alveolar DNA damage, senescence and apoptosis, as well as to interstitial lung fibrosis, their presence in Club and basal cells increased the presence of neutrophils, lymphocytes and macrophages in the lung, as well as airway collagen deposition and fibroblast abundance, features not observed in female mice upon telomere dysfunction. Depletion of TRF1 in fibroblasts, Club and basal cells did not lead to interstitial fibrosis, underscoring ATII cells as the relevant cell type for the origin of interstitial fibrosis (FIGURE 2). ■



**FIGURE 1** Higher incidence of angiosarcomas in *Pot1a<sup>+/-ki</sup>* mice.



**FIGURE 2** Pathological consequences of telomere dysfunction in fibroblasts, Club and basal cells in the lung. Dysfunctional telomeres in ATII cells led to alveolar DNA damage, senescence and apoptosis, and to interstitial lung fibrosis. TRF1 deficiency in Club and basal cells induced telomeric damage and cell cycle arrest, and reduced proliferation. TRF1 deletion in fibroblasts increased telomeric damage, cell cycle arrest, apoptosis, and proliferation. Depletion of TRF1 in fibroblasts, Club and basal cells did not lead to interstitial lung fibrosis.

► **PUBLICATIONS**

- Piñeiro-Hermida S, Martínez P, Bosso G, Flores JM, Saraswati S, Connor J, Lemaire R, Blasco MA (2022). Consequences of telomere dysfunction in fibroblasts, club and basal cells for lung fibrosis development. *Nat Commun* 13, 5656.
- Bosso G, Lanuza-Gracia P, Piñeiro-Hermida S, Yilmaz M, Serrano R, Blasco MA (2022). Early differential responses elic-

ited by BRAF<sup>V600E</sup> in adult mouse models. *Cell Death Dis* 13, 142.

- Martínez P, Sánchez-Vázquez R, Ferrara-Romeo I, Serrano R, Flores JM, Blasco MA (2022). A mouse model for Li-Fraumeni-Like Syndrome with cardiac angiosarcomas associated to POT1 mutations. *PLoS Genet* 18, e1010260.
- Montero-Conde C, Leandro-García LJ, Martínez-Montes ÁM, Martínez P, Moya FJ, Letón R, Gil E, Martínez-Puente N, Guadali-

S, Currás-Freixes M, García-Tobar L, Zafon C, Jordà M, Riesco-Eizaguirre G, González-García P, Monteagudo M, Torres-Pérez R, Mancikova V, Ruiz-Llorente S, Pérez-Martínez M, Pita G, Galofré JC, Gonzalez-Neira A, Cascón A, Rodríguez-Antona C, Megías D, Blasco MA, Caleiras E, Rodríguez-Perales S, Robledo M (2022). Comprehensive molecular analysis of immortalization hallmarks in thyroid cancer reveals new prognostic markers. *Clin Transl Med* 12, e1001.

- Ayora M, Fraguas D, Abregú-Crespo R, Recio S, Blasco MA, Moises A, Derevyanko A, Arango C, Díaz-Caneja CM (2022). Leukocyte telomere length in patients with schizophrenia and related disorders: a meta-analysis of case-control studies. *Mol Psychiatry* 27, 2968-2975.
- Sanz-Ros J, Romero-García N, Mas-Bar-gues C, Monleón D, Gordevicius J, Brooke RT, Dromant M, Díaz A, Derevyanko A, Guío-Carrión A, Román-Domínguez A,

Inglés M, Blasco MA, Horvath S, Viña J, Borrás C (2022). Small extracellular vesicles from young adipose-derived stem cells prevent frailty, improve health span, and decrease epigenetic age in old mice. *Sci Adv* 8, eabq2226.

► **PATENTS**

- Blasco MA, Saraswati S, Martínez P (2022). Telomerase reverse transcriptase therapy for kidney fibrosis and non-human animals thereof. *PCT/EP2022/051505*.
- Blasco MA, Martínez P, Bosch MF, Jiménez V, García M, Casana E. Recombinant TERT-encoding viral genomes and vectors. PCT application (2022). *PCT/EP2022/062990*. *WO2022238557A1*.

► **AWARDS AND RECOGNITION**

- *Fundación Eugenio Rodríguez Pascual* 2022 Award, Madrid, Spain.
- *Averroes de Oro Ciudad de Córdoba* 2022 Award in Scientific Research, Córdoba, Spain.
- Full Member (*Académica de número*) of the Royal Spanish Academy of Pharmacy.
- Doctorate *Honoris Causa*, *Universidad Internacional de Valencia*, Valencia, Spain.

- Honorary Academician of *Academia Malagueña de Ciencias*, Málaga, Spain.
- Member of the Board of Trustees of the International Centre for Ageing Research (ICAR) Foundation, Valencia, Spain.
- President of the Scientific Advisory Board of the International Centre for Ageing Research (ICAR), Valencia, Spain.
- Member of the Advisory Board of the Spanish Foundation of Science and Technology (FECYT), Madrid, Spain.