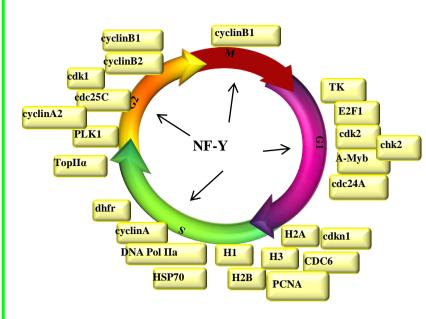
Mice and fishes illuminate diseases. A new era of preclinical oncology models.

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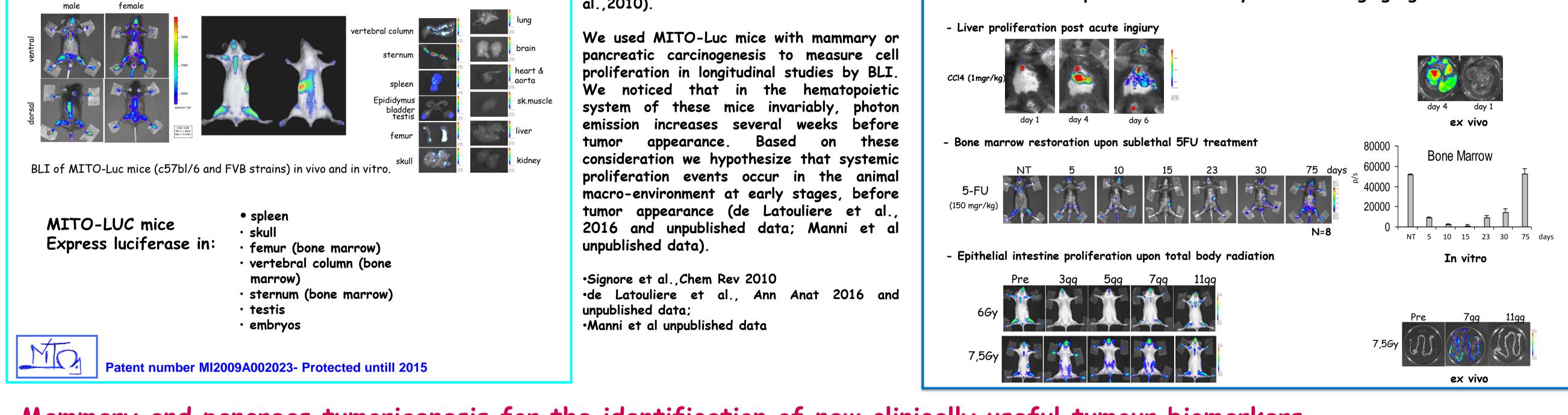
Isabella Manni¹, Luisa De Latouliere¹, Federica Pisati², Aymone Gurtner¹, Gianluca Deflorian², Giulia Piaggio¹ ¹SAFU Unit, IRCCS Regina Elena National Cancer Institute, Rome, Italy; ²IFOM - Zebrafish Unit, Milan, Italy

NF-Y exerts its activity in proliferating cells



-Farina et al., Oncogene 1999 -Bolognese et al., Oncogene 1999 -Manni et al., J Biol Chem 2001 -Gurtner et al., EMBO Rep 2001 -Imbriano et al., J Biol Chem 2001 -Sciortino et al., Embo rep 2001 -Gurtner et al., Mol Biol Cell 2003 -Di Agostino et al., Cancer Cell 2006 -Gurtner et al., PLoS ONE 2008 -Gurtner et al., Cancer res 2010 -De Santis et al., Bioinformatics 2014 -Cicchillitti et al., Oncotarget 2016 -Gurtner et al., BBA 2017

The luciferase gene is expressed in every district contain proliferating cells.

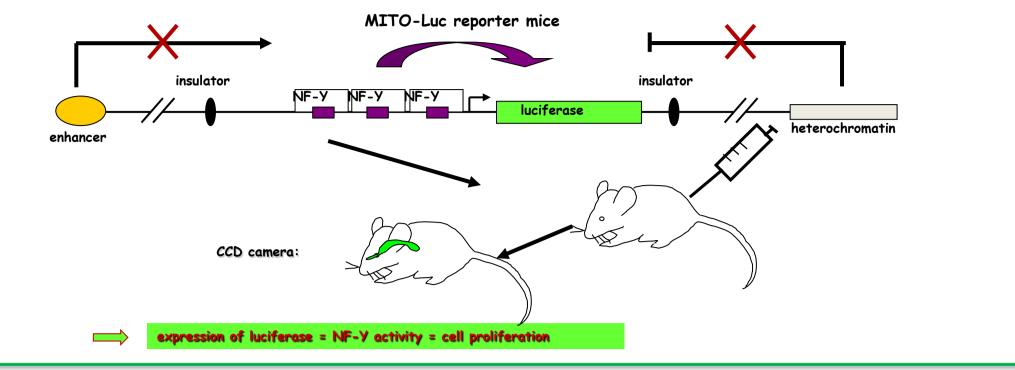


NF-Y is a highly conserved transcription factor that plays a fundamental role in cellular proliferation and it exerts its activity only in proliferating cells

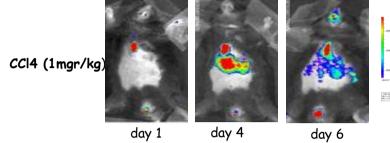
Bioluminescence imaging (BLI) strategies offer the advantage of noninvasive in vivo assessment of the molecular and cellular events that are often targets of therapy. This reduces the number of animals required for a given study and improves the data set, as the temporal data allow for each animal to serve as its own internal control. One update goal is to develop animal models useful for drug screening that allows measurement of the proliferative status of all cells, hence of the activity of the compound under investigation, on cell proliferation in all tissues, both normal and cancerous in longitudinal studies (Signore et al.,2010).

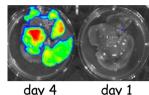
Generation of the reporter mouse

We cloned a promoter fragment of cyclin B2, containing three oxes, in front of a luciferase gene using as a backbone a construct containing insulator sequences that are useful to limit position transcriptional effects coming from the surrounding chromatin.

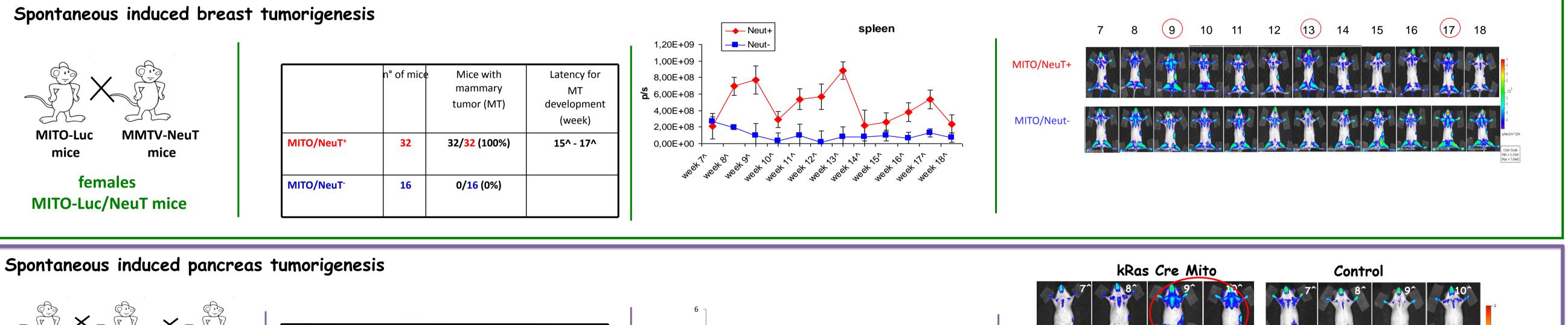


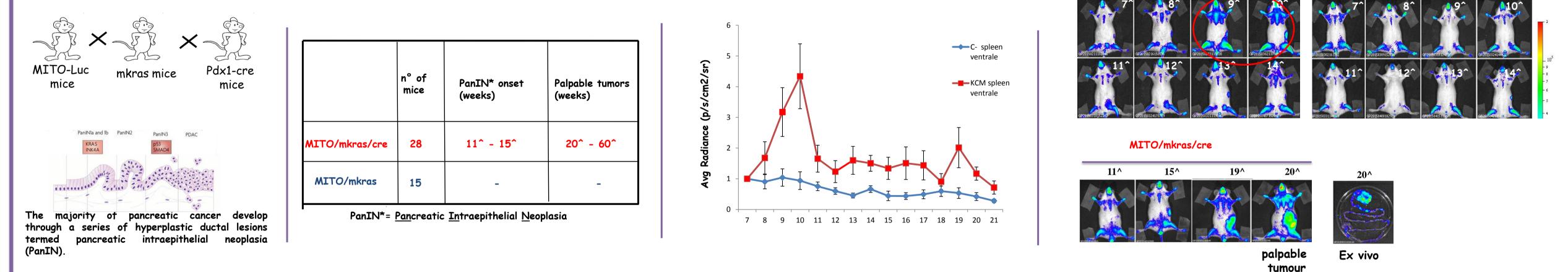






Mammary and pancreas tumorigenesis for the identification of new clinically useful tumour biomarkers.

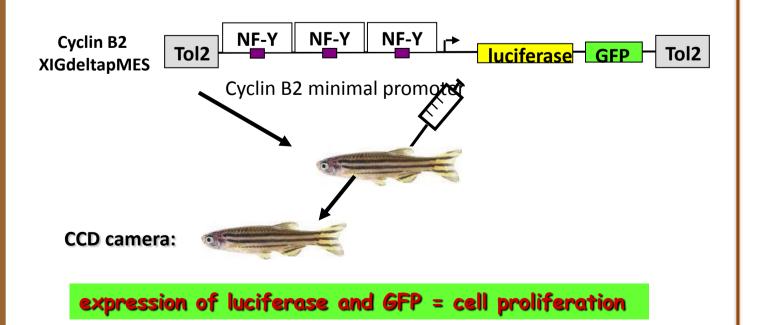




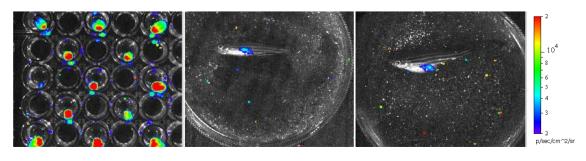
Mito-Luc Zebrafish model to visualize proliferation events in whole animals

MITO-LUC transgenic zebrafish

We are generating a Zebrafish model which allows us to visualize through bio luminescence imaging (BLI) any proliferation events in the context of the entire alive animal during development and adult life.



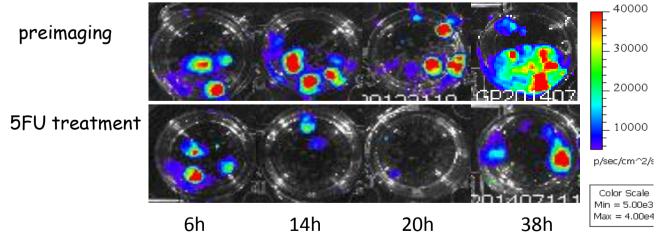
Luciferase expression analysis



Bioluminescence signal in living embryos, juveniles and adult zebrat y BLI. The luciferase expression had been confirmed by Luc-assay "in vitro" analysis on embryos, juveniles and adult zebrafish.

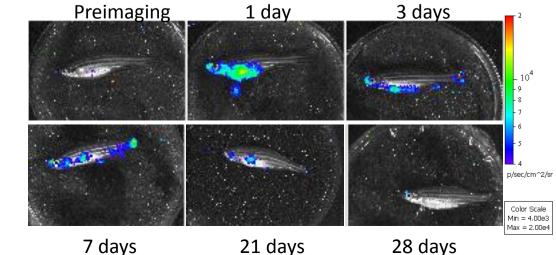
5FU treatment

Effect of 5FU, well-known drugs on proliferation rate, of zebrafish embryos both by luciferase assay and by BLI. 10mM of 5FU has reduced the proliferation level in zebrafish embryos.



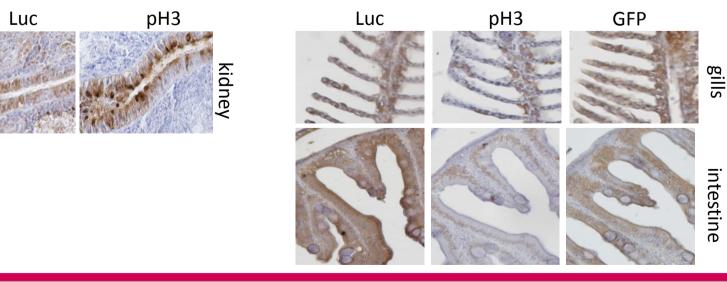
Fin clip treatment

Expression of the luciferase reporter gene in alive adult zebrafish after fin clip treatment. During early days after fin clip there is a systemic proliferation signal in the whole animal. Than, the signal is focused on the tail during regeneration. Later the fish comes to the initial condition, as before the fin clip.



Immunofluorescence and histological analysis

Expression of luciferase and GFP proteins in proliferative tissues by immunofluorescence and histological analysis.



Conclusions

-We have developed reporter animal models, Mito-Luc mice and zebrafishes, in which it is possible to measure physiological and/or pathological proliferation in any tissue of the body.

-In mice we have identified systemic proliferative waves during early neoplastic transformation in breast and pancreatic cancer.

Ongoing

-We are collecting peripheral blood, bone marrow and spleen to investigate circulating cell and/or cytochine/chemochine profiles in the identified preneoplastic steps.

-We plan to use Mito-Luc zebrafish model to study the host response in terms of systemic cell proliferation after injection of human cells into young fishes



