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The Niche Microenvironment as a crucial feature in CML LSCs stem cell potential and quiescence maintenance

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Objectives: Chronic Myeloid Leukaemia is a stem cells-driven myeloproliferative triphasic desease which occurs in hematopoietic stem cells due to the reciprocal t(9;22) translocation. This event gives birth to the Philadelphia chromosome that carries the BCR-ABL1 gene which encodes for the constitutively active tyrosine kinase BCR/Abl. Despite the paramount steps forward made throughout the years regarding CML treatment, past and current available drugs are not curative yet. Certainly, the bone marrow stem cell niche microenvironment features such as very low oxygen tension, secreted cytokines (Bone Morphogenetic Proteins (BMPs) above all) and cell adherence to the niche, play a crucial role in the maintenance of Leukaemic Stem Cells' (LSCs) dormancy and persistence, allowing escape from death and a prominent role in therapy-resistant residual desease. Hence, the focus of the work points towards unravelling how very low oxygen-related LSCs metabolism, as well as the interaction with the endosteal niche and BMPs secretion, modulate CML LSCs stem cell potential, alongside dormancy, maintenance.

Methods: To address the role of CML very low oxygen-related metabolism in LSCs persistence, we exploited a hypoxic chamber/manipulator inside which two immortalized CML cell lines (K562 and KCL22) were cultured for a precise time frame. After a primary hypoxic incubation, cells were or transferred into normoxic secondary non-selective cultures to evaluate their stem cell potential maintenance, or lysed to assess BCR/Ablprotein expression and signalling as well as mRNA levels of some Ox-Phos-related metabolic markers. The second part of the project is based on the development of, primarily, an immature CD34+/CD38-,BCR/Abl expressing, immortalized CML cell line (TF1-BA) endowed with the FUCCI system, secondly of a bone marrow-like 3D model allowing us to inquire how

CML LSCs dormancy is fostered, in long-term treatment, both by cells' adherence to the endosteal niche and by the BMP secretion by means of two different stromal cell lines cultured within the 3D system.

Results: Low oxygen tension forced LSCs to dramatically decrease BCR/Ablprotein expression (almost zeroing) during their incubation, with a consequent loss of their oncogene-addiction and thereby to be targetable by Tyrosine Kinase Inhibitors. This phenomenon, mainly dependent on glucose consumption, in turn is strongly regulated by glutamine availability within the niche microenvironment. After a variable lag-phase lasting period, cells rescued from low oxygen cultures were capable to re-gain BCR/Ablprotein expression yielding to the repopulation of the normoxic cultures. The other branch of the project led us to establish that BMP4 promotes the rapid commitment of LSCs in a deep G0 quiescent state under TKI treatment. Moreover, the 3D system has provided consistent data regarding a block of cells into the G1 and a significant decrease in the G2-M cell cycle phases, when subjected to several CML treatments, with a greater extent in Nilotinib-treated cultures.

Conclusions: It is worth to conclude that the loss of LSCs reliance upon BCR/Ablprotein expression and signalling, alongside the fast commitment towards a deep quiescent state, are key events involved in both cells' refractoriness to CML treatment and persistence within the stem cell niche microenvironment.