**Publishable summary report**

Over the past four years, Simón Méndez-Ferrer’s group has studied the regulation of hematopoietic stem cells niche in the bone marrow by the microenvironment and long-distance cues, such as the autonomic nervous system and particular endocrine signals (estrogens). They have tackled this project from two different angles, developmental biology and pathophysiology. This has allowed them to understand the basis for this connection, the potential relevance in leukemias and the changes observed in a particular set of leukemias (myeloproliferative neoplasms). These findings might offer new potential therapeutic avenues, some of which are under way to be tested in patients in a multicenter phase-II clinical trial.

The main results obtained are summarized below.

**1. Neural crest origin for hematopoietic stem cell niche-forming mesenchymal stem cells in the delevoping bone marrow** (Isern J et al. **eLIFE** 2014). Commented by Bernitz JM and Moore KA (eLife 2014 Nov 11;3. doi: 10.7554/eLife.05041).

**Abstract**: Mesenchymal stem cells (MSCs) and osteolineage cells contribute to the hematopoietic stem cell (HSC) niche in the bone marrow of long bones. However, their developmental relationships remain unclear. In this study, we demonstrate that different MSC populations in the developing marrow of long bones have distinct functions. Proliferative mesoderm-derived nestin− MSCs participate in fetal skeletogenesis and lose MSC activity soon after birth. In contrast, quiescent neural crest-derived nestin+ cells preserve MSC activity, but do not generate fetal chondrocytes. Instead, they differentiate into HSC niche-forming MSCs, helping to establish the HSC niche by secreting Cxcl12. Perineural migration of these cells to the bone marrow requires the ErbB3 receptor. The neonatal Nestin-GFP+ Pdgfr− cell population also contains Schwann cell precursors, but does not comprise mature Schwann cells. Thus, in the developing bone marrow HSC niche-forming MSCs share a common origin with sympathetic peripheral neurons and glial cells, and ontogenically distinct MSCs have non-overlapping functions in endochondrogenesis and HSC niche formation.

**Digest**: Developing long bones contain distinct mesenchymal stem-cell populations derived from mesoderm and neural crest, which have specialized functions in skeleton formation and the establishment of the hematopoietic stem-cell niche, respectively.

During the earliest phases of development, the embryo is formed by groups of stem cells that can develop into all the different types of tissue in the body—from bones to brain tissue. Later in life, small stockpiles of adult stem cells are found in various tissues and provide a reservoir of new cells available for replacing old or damaged cells. The most important source of blood stem cells is the bone marrow, which produces and stores cells that are capable of developing into blood and immune system cells. These processes are assisted by different bone marrow cells called stromal cells, which create a specialized local environment or “niche”.

But are the stromal stem cells that form the skeleton the same ones that form this niche during development? Or do the various types of stromal stem cells develop from distinct groups of cells in the embryo? Furthermore it is unclear which cells guide blood stem cells towards the forming bones.

Other types of cells, including some of the cells of the nervous system, can communicate with the stem cells in the adult marrow and influence their behavior. This led scientists to wonder whether the stem cells in the bone marrow niche and the cells that communicate with them developed from the same type of embryonic stem cell.

Isern et al. tracked down the developmental origins of different types of bone marrow stromal stem cells by examining the bone marrow from the long bones (for example, the bones in the leg) of unborn and infant mice. It turns out that not all stromal stem cells in the developing bone marrow are alike. In fact, one pool of stromal stem cells forms the skeleton and loses stem cell activity in the process. In contrast, a different population of stromal stem cells develops from the same group of embryonic cells that gives rise to the cells of the nervous system. The stromal stem cells in this second group function as a niche to recruit and store the incoming blood stem cells, and retain their stem cell activity throughout life.

The findings of Isern et al. help to explain why the nervous system is able to communicate with stem cells in the adult marrow, and provide a model for understanding how stem cell niches in organs that contain nerve tissue are established.

**2. Neuroglial damage caused by mutated haematopoietic stem cells is essential for the manifestation of myeloproliferative neoplasms and represents a novel therapeutic target** (Arranz L et al. **Nature** 2014).

**Abstract**: Myeloproliferative neoplasms (MPNs) are diseases that are caused by mutations in the HSC compartment. Most MPN patients have a common acquired mutation of the *Janus* kinase 2 (*JAK2*) gene in HSCs that renders this kinase constitutively active, leading to uncontrolled cellular expansion. The bone marrow (BM) microenvironment might contribute to the clinical outcomes of this common event. We previously showed that BM nestin+ mesenchymal stem cells (MSCs) innervated by sympathetic nerve fibres regulate normal HSCs. Here we demonstrate that abrogation of this regulatory circuit is essential for MPN pathogenesis. Sympathetic nerve fibres, supporting Schwann cells and nestin+ MSCs were consistently reduced in the BM of MPN patients and mice expressing the human *JAK*2-V617F mutation in HSCs. Unexpectedly, MSC reduction is not due to differentiation contributing to the abnormal stromal expansion. It is caused instead by BM Schwann cell death triggered by interleukin-1 produced by mutant haematopoietic progenitors, sympathetic neural damage and ensuing MSC apoptosis. In turn, MSC loss worsens the disease. *In vivo* depletion of nestin+ cells or their production of CXCL12 expanded mutant haematopoietic progenitors and accelerated MPN progression. In contrast, administration of neuroprotective or sympathomimetic drugs prevented mutant HSC expansion. Treatment with 3-adrenergic agonists that restored the sympathetic regulation of nestin+ MSCs prevented the loss of these cells and blocked MPN progression by indirectly reducing the number of leukaemic stem cells. Our results demonstrate that damage to the HSC niche, induced by genetically mutated HSCs, critically contributes to disease manifestation in MPN and identify niche-forming MSCs and their neural regulation as promising therapeutic targets in MPN.

**Digest**: Myeloproliferative neoplasms (MPN) are clonal stem cell disorders characterized by aberrant proliferation of the erythroid, megakaryocytic and myeloid lineages. They are associated with decreased survival, thromboembolic complications, hemorrhage and an inherent tendency towards leukemic transformation. The majority of MPN patients carry a somatic JAK2-V617F mutation. Very recently, somatic mutations in the calreticulin gene were discovered in patients negative for JAK2-V617F. Quantitative tests are now available to follow the mutant allele burden of each of these mutant alleles. No curative treatment exists for MPN, possibly with the exception of HSC transplantation. Currently available drugs reduce the symptoms of the disease, but with the exception of pegylated interferon alpha and HSC transplantation, have little effect on the size of the neoplastic clone. Research by Dr. Simon Mendez-Ferrer has shown that sympathetic nerve fibers regulate HSCs by interacting with bone marrow MSCs. New findings by his laboratory show that these cells are reduced in marrow from patients with MPN and in mice expressing the human JAK2-V617F mutation. This effect was found to be due to MSC apoptosis in models of MPN. These studies furthermore showed that early MPN events could be prevented by compensating for sympathetic neuropathy by the treatment with a 3-sympathicomimetic drug. Mice with JAK2-V617F driven MPN treated with a 3-sympathicomimetic agonist showed correction of thrombocytosis, neutrophilia, marrow fibrosis, and efficiently reduced mutant hematopoietic progenitor numbers in BM and peripheral blood. Thus, correcting the damage of the MPN clone on the stem cell niche led to a dramatic improvement of the MPN and represents a promising novel therapeutic target in MPN. Based on these findings, in collaboration with the group of Dr. Radek Skoda (Basel University Hospital) they have obtained funding to perform a multicenter phase II study in Switzerland using 3-sympathicomimetic drugs in MPN patients. The study has already recruited all the patients and is currently ongoing (https://clinicaltrials.gov/ct2/show/NCT02311569).

Currently treatment for MPN are not very effective, or extremely costly. Ruxolitinib, recently developed for myelofibrosis is only available for a minority of patients worldwide, the same is true for HSC transplantation. Beta-sympathicomimetic drugs are widely used to treat asthma and hyperactive bladder and are relatively cheap. If proven effective this would make treatment of MPN much easier. Dr. Mendez-Ferrer's approach is novel and also fundamentally different from the current strategies, since the primary target of therapy is the correction of the HSC niche damage, rather than the malignant clone itself. Although it is likely that combined therapies, targeting both the genetically altered cells directly as well as the environment are needed, the approach described here needs to be tested to further develop the field.

**3. Regulation of hematopoietic progenitor self-renewal, proliferation and survival might explain gender differences in hematological neoplasias and has anti-leukemic potential** (Sánchez-Aguilera A et al. **Cell Stem Cell** 2014).

**Abstract**: Estrogens are potential regulators of the HSC niche and have effects on mature hematopoietic cells; however, whether estrogen signaling directly regulates normal and malignant HSC remains unclear. We demonstrate differential expression and specific roles of estrogen receptors (ER) in hematopoietic progenitors. ER activation in short-term HSC and multipotent progenitors induced apoptosis. In contrast, the selective ER modulator (SERM) tamoxifen induced proliferation of quiescent long-term HSC, altered their self-renewal signature and compromised hematopoietic reconstitution following myelotoxic stress. Treatment with tamoxifen alone abolished hematopoietic progenitor expansion induced by *JAK2V617F* by restoring normal levels of apoptosis, blocked *JAK2V617F*-induced myeloproliferative neoplasm *in vivo*, and sensitized *MLL-AF9+* leukemias to chemotherapy. Tamoxifen showed selective effects on mutant cells compared to normal ones, and had only a minor impact on steady-state hematopoiesis in disease-free animals. These results uncover specific regulation of hematopoietic progenitors by estrogens and potential anti-leukemic properties of SERM.

**Digest**: Estrogens, a major class of female sex hormone, can regulate the activity of the hematopoietic stem cells in the bone marrow and in this way influence the development of some types of leukemia. This discovery has a potential application in the treatment of certain blood disorders for which there is currently no cure. The study’s authors have demonstrated in mice that tamoxifen, a drug already approved and widely used for the treatment of breast cancer, blocks the symptoms and the progression of a specific group of blood disorders known as myeloproliferative neoplasms.

Scientists have known for some time that men have a higher risk than women of developing leukemia. Although estrogens were known to regulate some types of blood cells, very little was known about their influence on blood stem cells, including those that cause myeloproliferative neoplasms. From this starting point, the researchers discovered an important practical application. The authors demonstrated that tamoxifen has specific effects on HSCs and their immediate descendants, known as multipotent progenitors. The researchers found that tamoxifen had very distinct effects depending on whether the mice were healthy or sick. When administered to healthy animals, tamoxifen induced cell death of the multipotent progenitors, whereas the stem cells accelerated their division and partially lost their functionality. But when tamoxifen was administered to sick animals, symptoms disappeared and disease progression was blocked. In short, an effective therapy. And there’s another advantage. Surprisingly, these effects cause hardly any discernable alteration in the rest of the blood cells, which are maintained at normal levels even after prolonged treatment with the drug, showing no appreciable toxicity. Unlike the situation in breast cancer, where tamoxifen blocks the action of estrogens, in blood cells the team found that the drug acts by imitating the function of the hormone.

Myeloproliferative neoplasms cause an accumulation of abnormal blood cells and the degeneration of the bone marrow, and in mice both these processes are blocked by treatment with tamoxifen. The treatment is able to eliminate the abnormal stem cells, the root cause of the disease, something that current therapies, including JAK2 inhibitors, don’t manage. This is explained by restoration in mutated HSCs of normal apoptosis levels, the quality control that allows the physiological elimination of cells that do not work properly. The fact that tamoxifen is already approved for clinical use and has an appropriate safety profile has facilitated gettting funding to initiate a Phase-II clinical trial in patients with myeloproliferative neoplasms in the UK.