

## Exploitation of the neural-hematopoietic stem cell niche axis to treat myeloproliferative neoplasms

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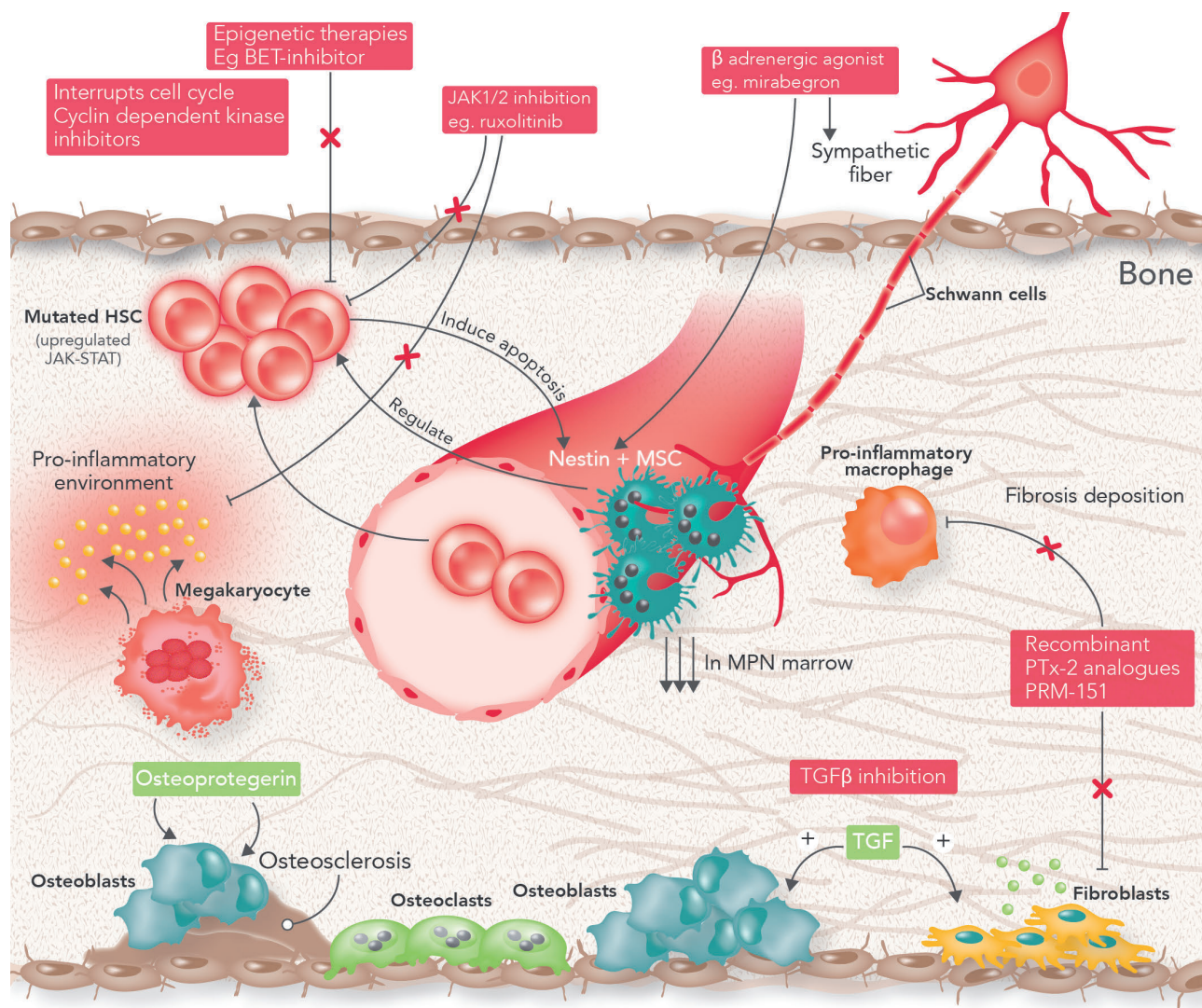
Myeloproliferative neoplasms (MPN) originate from a population of hematopoietic stem cells (HSCs) within the bone marrow (BM) that undergo clonal expansion as a result of factors both intrinsic and extrinsic to the cell. They are characterized by progressive marrow fibrosis, heterogeneous symptomatology, extramedullary hematopoiesis, splenomegaly, a propensity to both hemorrhage and thrombosis, and an inherent risk of transformation to acute myeloid leukemia (AML). Recently, there has been an increasing focus on the role of the BM HSC niche in MPN development and disease maintenance.<sup>1</sup> This niche encompasses complex cellular and signaling networks with multiple interactions and 'cross-talk' between HSC, mesenchymal cells (MSC), perivascular cells identified as chemokine (C-X-C motif) ligand 12 (CXCL12)-abundant reticular (CAR) cells, osteolineage-derived cells and sinusoidal endothelial cells, amongst others<sup>1-3</sup> (Figure 1). Importantly, the acquisition of gain-of-function mutations such as *JAK2-V617F* by MPN HSCs or other cells can result in an alteration of the niche to favor clonal expansion at the expense of background normal HSCs.<sup>4</sup> For example, Zhan *et al.* recently suggested that *JAK2-V617F* mutant endothelial cells in the vascular niche promote clonal expansion of *JAK2-V617F* HSCs at the expense of wild-type progenitors.<sup>5</sup> In chronic myeloid leukemia (CML), one study found *BCR/ABL* transgenic mice create a self-reinforcing leukemic niche that impairs normal hematopoiesis, favors leukemic stem cell function and contributes to development of BM fibrosis by stimulating MSCs to overproduce osteoclastic stem cells.<sup>6</sup> Conversely, there is also mounting evidence that targeted disruption of the HSC niche may result in development of MPN, for example defective Notch activation<sup>7</sup> and ablation of the *retinoic acid receptor gamma*<sup>8</sup> and *retinoblastoma* genes<sup>9</sup> have been evaluated in murine models, suggesting that genetic mutations in the niche itself can also drive the malignant process. Little was known about the role of the MSC population in MPN-pathogenesis and maintenance and data were often conflicting. Avanzini *et al.* described how MPN-BM-derived MSC exhibited decreased proliferative and osteogenic capacity whereas Martinaud *et al.* suggested an enhanced and persistent increase in osteogenic abilities coupled with an altered secretome and transcriptome in primary myelofibrosis (PMF)-derived BM-MSC.<sup>10,11</sup> More recently, Ramos *et al.* demonstrated that BM-MSC derived from *JAK2*-mutated MPN patients favored maintenance of clonal hematopoietic cells.<sup>12</sup> Targeting of this population, in particular increasing the 'beneficial' MSC subgroup, is hence of potential interest.

Nearly a decade ago, Méndez-Ferrer *et al.* originally described how Nestin-positive(+) MSC are essential components of the HSC niche, containing all of the BM colony-forming-unit fibroblastic activity and having the ability to function as so-called 'niche-forming' cells.<sup>13,14</sup> Nestin is a type VI intermediate filament protein and functions as a major component of the cytoskeleton. Méndez-Ferrer *et al.* described that cyclical

HSC trafficking is regulated by noradrenaline release *via* the sympathetic nervous system (SNS), transmitted to niche stromal cells by the beta( $\beta$ )(3)-adrenergic receptor, resulting in reduced nuclear Sp1 transcription factor and downregulation of CXCL12. There is a close association between so-called Nestin<sup>+</sup> MSC and adrenergic nerve fibers of the SNS and this is believed to control HSC maintenance, egress and functional capacity.<sup>15</sup> Of additional interest, Maryanovich *et al.* have recently described how the aging HSC niche is associated with a distinct loss of functional SNS nerve fibers, postulating that denervation-associated remodeling would lead to 'aged' HSC.<sup>16</sup>

Méndez-Ferrer *et al.* extended their original observations and demonstrated that SNS fibers intrinsically involved in supporting Schwann cells and nestin<sup>+</sup> MSC are reduced in the marrow of MPN patients compared to healthy individuals.<sup>14</sup> Schwann cell death is initiated following mutant-HSC IL-1 $\beta$  production, and the resultant denervation ultimately leads to a reduction in so-termed 'beneficial MSC' and facilitates expansion of clonal mutant-HSC within the niche. During disease progression, the supportive microenvironment is further disrupted by mutant-HSC-mediated hypercytokinemia and thus the MSC population and the osteoblast lineage cells continue to modulate. In the same publication, an MPN murine model was used to demonstrate that restoration of sympathetic regulation of Nestin<sup>+</sup> MSC induced *via*  $\beta_3$ -agonist exposure abrogated MPN progression and led to a reduction in disease-associated HSC.<sup>17</sup> The  $\beta_3$ -adrenergic agonist BRL37344 led to reductions in murine BM mutant-HSC-derived progenitors and decreases in neutrophilia, thrombocytosis and marrow fibrosis, associated with a restoration of Nestin<sup>+</sup> MSC. In addition, BRL37344 exposure led to a significant decrease in 'leukemic' stem cells. This pivotal work rapidly led to conceptualization of adrenergic nervous system modulation as a novel therapeutic approach in MPN.

In solid tumor oncogenesis, *in vitro* studies investigating neuro-biological regulation of tumor establishment, aggressiveness and metastases have emerged over the last five years.<sup>18-20</sup> By way of example, in contrast to what has been described above in MPN, Magnon *et al.* studied mice bearing PC-3 prostate tumor xenografts and human prostate tumor specimens, and demonstrated that prostate cancer growth was down-regulated following chemical or surgical sympathectomy or deletion of stromal  $\beta_3$ -adrenergic receptors; tumor samples demonstrated higher densities of adrenergic (surrounding the tumor) and cholinergic (invading the tumor) nerve fibers associated with poorer prognosis, and that the cholinergic parasympathetic nerve fibers were associated with cancer dissemination.<sup>20</sup> Further links between neuro-modulation and prostate cancer have been suggested by a Norwegian epidemiological study suggesting  $\beta$ -blocker use was associated with reduced prostate cancer mortality.<sup>21</sup> Other *in vivo* work has also investigated the importance of SNS modulation in breast, ovarian and melanoma tumorigenesis, amongst others, and how



**Figure 1. Bone marrow hematopoietic stem cell (HSC) niche in the development and disease maintenance of myeloproliferative neoplasms (MPN).** This niche encompasses complex cellular and signaling networks with multiple interactions and ‘cross-talk’ between HSC, mesenchymal cells (MSC), perivascular cells identified as chemokine ligand 12-abundant reticular cells, osteolineage-derived cells and sinusoidal endothelial cells, amongst others.<sup>1,3</sup>

this is intrinsically linked to both adaptive and innate tumor-immune responses (reviewed by Qiao *et al.*<sup>22</sup>).

In this issue of *Haematologica*, Drexler *et al.* report a novel multi-center, phase II trial exploring the refocused use of mirabegron, an oral  $\beta$ -3 adrenergic agonist with a lower receptor affinity than BRL37344 but commonly used for overactive bladder syndrome. This is the first study in MPN to explore the neural-HSC niche.<sup>23</sup> Mirabegron (25 mg titrated to 50 mg once daily) was administered to 39 patients with a diagnosis of MPN fulfilling the World Health Organization (WHO) 2008 diagnostic criteria, and in whom the *JAK2*-V617F mutant allele burden in granulocyte DNA exceeded 20% at study entry. The primary end point was defined as the reduction in the *JAK2*-V617F mutant allele burden of 50% or more after 24 weeks. A sub-project, in which 20 patients participated, assessed whether mirabegron can restore the Nestin<sup>+</sup> MSC population and alter reticulin fibrosis. The primary end point was

not reached in any of the patients, although a 25% reduction in *JAK2*-V617F allele burden at 24 weeks was recorded in one patient. Twenty-four percent of patients with polycythemia vera and 29% of patients with essential thrombocythemia showed hematologic response in accordance with European LeukemiaNet (ELN) and International Working Group Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) criteria. One patient with myelofibrosis became transfusion independent. These clinical findings were considered of minor, possibly insignificant, benefit. Evaluation of BM biopsies showed an increase in Nestin<sup>+</sup> MSC from a median of 1.09 [interquartile range (IQR) 0.38-2.37/mm<sup>2</sup>] to 3.95 (IQR 1.98-8.79/mm<sup>2</sup>) ( $P < 0.0001$ ), and a slight but significant decrease in reticulin fibrosis from a median grade of 1.0 (IQR 0-3) to 0.5 (IQR 0-2) ( $P = 0.01$ ) between the start and end of mirabegron treatment. The decrease in reticulin fibrosis was only observed in patients not previously treated with



hydroxycarbamide. A trend was also observed towards reduction in megakaryocyte cluster formation and decrease in numbers of large megakaryocytes with staghorn-like morphology, both cardinal features of MPN. Possible confounding factors are that the participants had established MPN with a median time between MPN diagnosis and trial registration of 3.6 years (range 1.6-8.6 years) and received a relatively short duration of therapy. It is possible that longer exposure to mirabegron, which was well tolerated, and considering its use earlier in the disease course may have demonstrated different results since potentially the chronicity and degree of increase in nestin<sup>+</sup> MSC may be paramount in ultimately modulating the clinical phenotype. Mirabegron itself, as noted above, is a less potent agent and may have additional effects when compared to BRL37344, which was used in the murine work. Moreover, although significant increases in Nestin<sup>+</sup> MSC were seen, this was limited to those patients not receiving hydroxycarbamide, an agent that most likely affected MSC senescence.

As we begin to explore the complex BM HSC-neurostromal interaction through niche targeting therapies, such as that described in the novel study described above, significant thought needs to be given to the timing of such therapies within the disease course and that logical sequencing / dual agent approaches are considered when targeting of this axis is contemplated. For example, the JAK inhibitor ruxolitinib has been shown to abrogate MSC-growth and an ability to secrete MCP and IL-6 which could potentially be beneficial through downregulation of pro-inflammatory MSC, yet it is unknown what effect prior or concurrent JAK inhibitor therapy would have on  $\beta_3$  adrenergic agonist mediated-increases in Nestin<sup>+</sup> MSC.<sup>24</sup> It is increasingly evident, however, that strategies with the ability to break through the self-perpetuating inflammatory and pro-oncogenic MPN microenvironment are required. Potential combination approaches with drugs acting on this axis to explore include the human pentraxin-2 protein analog PRM-151 (Promedia Pharmaceuticals), which may potentially synergistically reduce fibrosis, or with interferon, which may 'switch on' mutant-HSC-directed immune responses. Both of which are being assessed as single agents and in combination with ruxolitinib (reviewed by Harrison and McLoman<sup>25</sup>). Furthermore, an additional benefit of focusing on the stem cell niche could generate a surrogate marker of disease response that would facilitate more rapid evaluation of niche-targeting therapies in the clinical arena. Surrogate markers of disease response moving beyond the blood count, symptoms and spleen are urgently required in this field. An enhanced understanding of the role of the neural network within the MPN niche and how this can be successfully modulated will accelerate the design of such synergistic therapeutic approaches and help the field to move forward. This work from Méndez-Ferrer and Skoda is also an inspiration in following the academic trail from bench to bedside.<sup>15,14,17,23</sup>

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