Editorial comment: variables affecting the presence of mesenchymal stromal cells in the peripheral blood and their relationship with apheresis product

Guido Moll,[1,2] Norman Drzeniek,[1,2] Julian Kamhieh-Milz,[3] Sven Geissler[1,4] and Petra Reinke[1,5,6]

[1]BIH Center for Regenerative Therapies (BCRT), [2]Berlin-Brandenburg School for Regenerative Therapies (BSRT), [3]Department of Transfusion Medicine, [4]Julius Wolff Institute (JWI), [5]Department of Nephrology and Internal Intensive Care Medicine and [6]Berlin Center for Advanced Therapies (BECAT), all Charité Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health (BIH), Berlin, Germany

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In this issue of the *British Journal of Haematology*, Jain *et al.* identified circulating mesenchymal stromal cells (MSCs) in peripheral blood (PB) and apheresis product (AP) collected in the context of haematopoietic stem cell (HSC) mobilization for HSC transplantation (HSCT). As pointed out by the authors themselves and others (Fox *et al.*, 2007; Jones & McGonagle, 2008; Moll *et al.*, 2019), the presence of circulating MSCs in PB is debatable and their identification may be hampered, among others, by: (i) their low frequency in peripheral blood, (ii) their unclear phenotypic characterization, and (iii) the large biological variations related to donor pathology, disease status, and corresponding treatment regimens.

Within the light of these challenges, it is still not clear today if MSCs naturally extravasate and circulate within blood, or if the presence of MSCs in the circulation is a mere artefact resulting from the blood sampling procedure (e.g. vessel puncture), major micro- or macrovascular injury (e.g. severe fractures or surgery), or non-physiological mobilization strategies (e.g. mobilization drug toxicity) (Marquez-Curtis *et al.*, 2011). Either point could likely lead to an artificial presence of perivascular MSCs in the blood stream, resulting from the breakdown of vascular barrier integrity (Alm *et al.*, 2010; Hoogduijn *et al.*, 2014; Moll *et al.*, 2019).

Jain *et al.* investigated if MSCs can be mobilized into the blood stream in response to drug regimens already approved/applied for HSC collection in HSCT. Two reviews give an elaborate outline on the strategies used for progenitor cell mobilization (Marquez-Curtis *et al.*, 2011; To *et al.*, 2011). One prime example comprises the targetting of cytokine/chemokine and growth factor signalling with granulocyte colony-stimulating factor (G-CSF) and the CXCR4-antagonist plerixafor (AMD3100) for the modulation of the stromal-derived factor-1a (SDF-1a)/CXCR4-signalling axis that controls the retention of HSCs in the bone marrow (BM) (Fig 1).

Intriguingly, Larsen *et al.* noted in a baboon model that MSC mobilization and colony-forming unit fibroblast (CFU-F) in PB in response to G-CSF did only occur when adding stem cell factor (To *et al.*, 2011). Rankin *et al.* found in mice, that MSCs/CFU-F were not found in PB post-mobilization with G-CSF, but when adding vascular endothelial growth factor and CXCR4-antagonist (Pitchford *et al.*, 2009).

Nonetheless, first reports exist that documented small quantities of MSCs/CFU-F in PB of G-CSF-mobilized patients (Wexler *et al.*, 2003; Kassis *et al.*, 2006). This is in line with reports implicating the SDF-1a/CXCR4-axis in BM retention of various progenitor cell linages, but also in chemoattraction of CXCR4-expressing BM progenitors to sites of tissue damage and ischaemia through hypoxia-inducible factor-1 (HIF-1)-mediated induction of SDF-1a (Petit *et al.*, 2002; Ceradini *et al.*, 2004; Kucia *et al.*, 2005; Otsuru *et al.*, 2008; Yin *et al.*, 2010).

Jain *et al.* here report for the first time the detailed kinetics of: (i) circulating MSCs in PB of HSCT patients before HSC mobilization, (ii) in AP obtained at day 5 post start of mobilization with G-CSF (Grafeel, 10 μg/kg/day for 4 days), and (iii) in PB and AP at day 6 upon mobilization with G-CSF (10 μg/kg on day 6) and plerixafor (0·24 mg/kg given 12 hours prior), obtained in a second round of apheresis for those with inadequate $CD34^+$ counts in the first apheresis (<2 × 10^6/kg).

To identify $CD34^+$ HSCs and $CD34^{dim}$ MSCs, and to calculate their frequency in blood relative to white blood cells (WBCs), Jain *et al.* employed a dual platform strategy based on multiparameter flow cytometry and total leukocyte

Correspondence: Guido Moll, BIH Center for Regenerative Therapies (BCRT), Charité Universitätsmedizin Berlin, Campus Virchow Klinikum, Augustenburger Platze 1, D-13353 Berlin, Germany.
E-mail: guido.moll@charite.de

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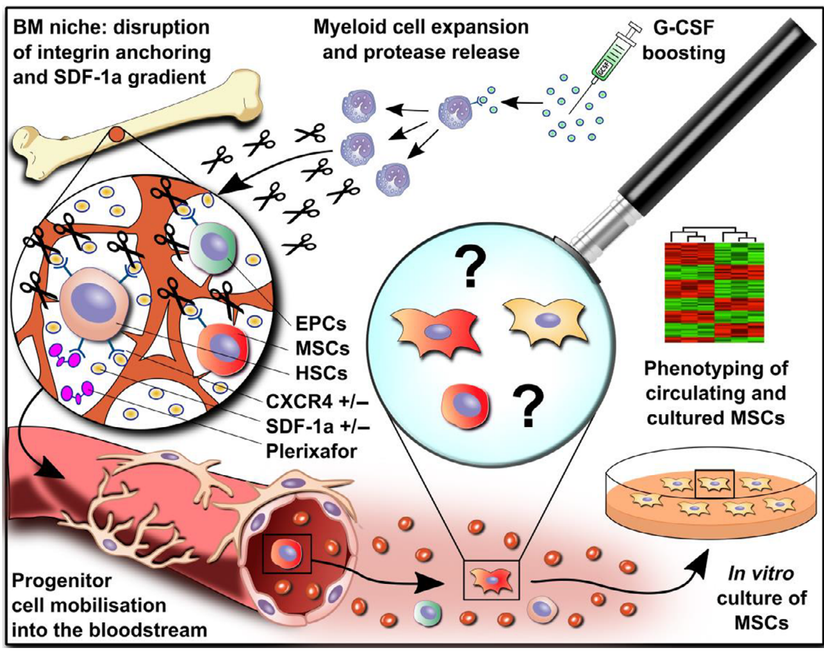

Fig 1. Different types of progenitor/stem cells (e.g. endothelial, haematopoietic, or mesenchymal lineages, EPCs, HSCs and MSCs, respectively) are lodged in extravascular bone marrow (BM) niches, lining the stromal tissue network and the BM microvascular sinusoids. The HSCs (and EPCs/MSCs?) are kept in the BM niche due to the chemokine gradient of locally produced stromal-derived factor 1a (SDF-1a/CXCL12) detected by their expression of the corresponding receptor CXCR4. It is already well established that the HSCs can be mobilized into the bloodstream with granulocyte colony-stimulating factor (G-CSF) and CXCR4-antagonist plerixafor. The injection of G-CSF leads to a strong expansion of myeloid cells within the BM and concomitant release of proteases, which can disrupt the SDF-1a gradient and local integrin tethering of progenitor cells and thus release them into the blood stream (Marquez-Curtis et al., 2011). The MSCs are typically found in a perivascular location surrounding the microvasculature, but on occasion small amounts of circulating MSCs can also be found in blood. Jain *et al.* studied whether apart from HSCs, MSCs can also be mobilized into blood and apheresis product by the typical HSC mobilization regimen. A crucial aspect is the phenotyping of circulating and *in vitro* cultured MSCs. [Colour figure can be viewed at wileyonlinelibrary.com]

counts. The MSCs were identified as $CD34^{dim}/CD73^{+}/CD90^{+}/CD105^{+}$ cells, with dual positivity for either $CD73^{+}/CD105^{+}$ or $CD73^{+}/CD90^{+}$ or $CD90^{+}/CD105^{+}$. The flow cytometry gating strategy and controls are shown in the primary article and the MSC frequency was calculated as follows: MSC (%) = (MSC Number × 100)/Viable WBCs.

The flow cytometry was performed according to Hass *et al.* (2011) in line with the current guidelines for the use of flow cytometry and cell sorting. In addition, another method to enrich MSCs from blood is elutriation based on their physical properties (e.g. size/density) in a liquid centrifuge (Zvaifler *et al.*, 2000). Furthermore, adhesion to fibrin micro-beads may also be used for MSC enrichment before further phenotyping with flow cytometry and other methods (Kassis *et al.*, 2006).

Considering both patient background and sampling time points, the levels of MSCs detected by Jain *et al.* in PB and AP relative to viable WBCs were very similar in all three groups: healthy controls (0·0013–0·0033% MSCs in >50% of the $n = 8$ patients tested positive), multiple myeloma group (0·0011–0·0041% MSCs in >35% of the n = 20 patients), and lymphoma group (0·0016–0·0077% MSCs in >60% of the $n = 5$ patients). The typical MSC-like fibroblastic phenotype of the cells was confirmed upon plating of the sorted cells *in vitro* onto standard cell and tissue culture plastic.

Although the authors found small amounts of circulating MSCs in all three groups of donors, their relative number, expressed as percentages of MSCs relative to viable WBCs, did not appear to be increased in PB post G-CSF treatment. Here, the mathematical expression chosen by Jain *et al.* may carry a risk of misinterpretation, since WBC numbers in PB have been shown to be increased in response to G-CSF (De Felice *et al.*, 2016; Melve *et al.*, 2018). This may suggest that the absolute numbers of circulating MSCs may have actually increased in response to G-CSF.

Noteworthy, HSCs, endothelial progenitor cells (EPCs) and MSCs are not the only stem cell niches mobilized upon G-CSF treatment (Melve *et al.*, 2018). Immune memory cells, like memory B cells, T central memory and T stem cell memory cells are also affected. Thus, a closer look at subsets of bone marrow-resident cells may yield great potential for more targeted mobilisation strategies as a new research directive.

In analogy to identification of MSCs within tissues (Mendez-Ferrer *et al.*, 2010; Consentius *et al.*, 2018), a crucial

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aspect for flow cytometry-based identification of circulating endothelial, haematopoietic, or mesenchymal progenitor lineages is their clear phenotypic distinction with appropriate marker panels (Pitchford *et al.*, 2009). Of importance is their distinction from circulating HSCs (Mendez-Ferrer *et al.*, 2008), circulating EPCs and also circulating mature endothelial cells (CEC) (Asahara *et al.*, 1997; Lanuti *et al.*, 2018; Farinacci, *et al.*, 2019). In particular, the latter two are phenotypically closely related to MSCs, also adhere to culture plastic, and may therefore potentially easily be mistaken for MSCs.

A typical marker for identification of EPCs is the endothelial marker CD31, commonly employed to distinguish MSCs from endothelial cells (ECs) (Dominici *et al.*, 2006), which was not included in the current panel of Jain *et al.* However, a major contamination with EPCs is unlikely, due to the exclusion of $CD34^+$ cells (Farinacci *et al.*, 2019). In line with the confirmation of the MSC identity as plastic-adherent fibroblastic cells, another concern is the potential phenotypic transformation of circulating progenitor cells once plated on tissue culture plastic, akin to the processes of endothelial or epithelial to mesenchymal transition (EndMT/EMT) (Krenning *et al.*, 2010).

In conclusion, the studies of circulating MSCs in PB of humans are more than two decades old (Levesque *et al.*, 2007). Most investigators agree that their frequency in blood is very low in healthy individuals, but that the amounts of circulating MSCs may increase under challenging conditions, thus supporting the notion that MSCs can be transiently found 'circulating' in blood. Jain *et al.* here provide further evidence that MSCs can be found in PB and AP of patients treated with a typical G-CSF-based HSC mobilization regimen. Further phenotyping of circulating MSCs and studies on the mechanism of MSC mobilization should be conducted.

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Conflicts of interest

All the authors declare that they have no conflicts of interests.

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