
MOBILIZATION FAILURE IN HEMATOPOIETIC STEM CELL TRANSPLANTATION

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G-CSF-based mobilization regimens have a 5%-30% failure rate among healthy donors and patients, but the rate can be up to 60% in high-risk patients such as those exposed to fludarabine. Poor mobilization has significant consequences for the patient with potential loss of the transplant as a treatment option. Repeated attempts at mobilization increase resource use, morbidity, and patient/donor inconvenience. Therefore, there is much interest in the prevention and salvage of mobilization failure with novel strategies.

The role of CD34+ cell dose in haematopoietic stem cell transplantation

Benefits of rapid recovery in HSCT are reduced hospitalization, blood product usage, and infections. Minimum threshold of CD34+ cell dose for autologous transplantation is currently 2×10^6 CD34+ cells/kg body weight (BW) (1). Many centers use a minimum of 3×10^6 CD34+ cells/kg BW for myeloablative and nonmyeloablative allogeneic and matched unrelated transplantations. Furthermore, $> 5 \times 10^6$ CD34+ cells/kg BW is associated with even lower resource use and faster and more complete platelet engraftment and better survival in allogeneic transplantations (2). It is probable that higher cell doses (eg, $\geq 5 \times 10^6$ CD34+ cells/kg BW) may be similarly beneficial in mismatched transplantations. In haplotype mismatched transplantations doses of $\geq 10 \times 10^6$ CD34+ cells/kg BW are often used (3).

Current stem cell mobilization regimens

G-CSF-based mobilization regimens

G-CSF with or without myelosuppressive chemotherapy has been the most commonly used mobilization protocol since the 1990s. When used alone, in both autologous and allogeneic trans-

plantations, G-CSF is given at 10 $\mu\text{g}/\text{kg}$ per day subcutaneously with apheresis beginning on the fifth day until the yield target is reached. There is no evidence that twice daily administration gives a higher yield than once-daily (4).

Combining G-CSF with chemotherapy for mobilization

Combining G-CSF with chemotherapy achieves both mobilization and antitumor activity and has been shown to result in a higher CD34+ cell yield than G-CSF alone (5). Regimens such as cyclophosphamide, doxorubicin, vincristine, and prednisone/dexamethasone, high-dose cytarabine, and cisplatin or cyclophosphamide 1-5 g/m² have been used with G-CSF which is commenced 1-3 days following cyclophosphamide. Apheresis is usually started when leukocyte count reaches $> 2 \times 10^9/\text{L}$. Benefit of higher mobilization yield with higher doses of chemotherapy needs to be balanced against more red cell and platelet transfusions, and, frequently, hospitalization for febrile neutropenia, and it is more justified when significant antitumor effects exist (6). A prospective study that compared 5 versus 10 $\mu\text{g}/\text{kg}$ per day after standard chemotherapy was inconclusive (7). G-CSF 5 $\mu\text{g}/\text{kg}$ per day is used in most units for combined G-CSF/chemotherapy mobilization.

Pegylated G-CSF in mobilization

The pegylated long acting form of G-CSF (pegfilgrastim) showed similar kinetics of mobilization as filgrastim. A study in healthy donors reported suboptimal mobilization with a dose of 6 mg but satisfactory yield with 12 mg (8). Such dose dependence was not seen when pegfilgrastim was used with cyclophosphamide. Pegfilgrastim has the advantages of an earlier start of apheresis, reduction in the number of apheresis procedures and a

reduced number of injections in comparison with unconjugated G-CSF (9) but it is more costly.

Lenograstim compared with Filgrastim in stem cell mobilisation

Lenograstim is a glycosylated form of G-CSF, whereas filgrastim is nonglycosylated. Several prospective studies have shown higher mobilization yield with lenograstim. In contrast to healthy donors CD34+ cell yield appears similar in patients receiving either lenograstim or filgrastim after chemotherapy (10).

Stem cell factor in mobilization

Stem Cell Factor (SCF (Ancestim)) exists as a soluble form as well as a surface molecule on BM stromal cells. It binds to c-kit on HSCs and modulates proliferation and adhesion. As a single agent SCF has limited efficacy in HSC mobilization; however, synergy between SCF and G-CSF exists. SCF at 20 µg/kg subcutaneously is started 4 days before the start of G-CSF and continued with the G-CSF until the end of apheresis. In failed mobilizers SCF plus G-CSF with or without chemotherapy enables adequate mobilization in 50% of patients (11). In prior fludarabine-exposed patients SCF with high-dose G-CSF resulted in a 63% success rate, almost doubling that in historical controls that used G-CSF alone (12).

GM-CSF in stem cell mobilization

GM-CSF is not more effective than G-CSF alone (13) and there is no synergy with G-CSF. Its use is limited by dose-related toxicities. GM-CSF is rarely used in clinical practice.

Plerixafor in stem cell mobilization

Plerixafor is a CXCR4 antagonist. It reduces the binding and chemotaxis of HSCs to the BM stroma. It is used at 240 µg/m² subcutaneously the evening before the scheduled apheresis because it generates peak CD34+ levels 6-9 hours after administration (14). It synergises with G-CSF and chemotherapy. Plerixafor is the latest addition to the list of mobilizing agents and the first driven by understanding how mobilization occurs.

Risk factors for failure of mobilization of stem cells

Poor mobilization may be inherent to a particular genetic combination specific to a person. It can also be acquired as a consequence of aging, prior treatments or disease. The possible defects could be at one or more of three levels:

(1) insufficient number of HSCs because of HSC intrinsic factors

(2) insufficient HSC number because of low number or defective niches

(3) inadequate number or response of effector/supporter cells such as BM macrophages or β-adrenergic nerves.

The following major criteria are proposed by the Italian GITMO working group as the definition of a poor mobilizer: (1) after adequate mobilization (G-CSF 10 µg/kg if used alone or ≥5 µg/kg after chemotherapy) circulating CD34(+) cell peak is <20/µL up to 6 days after mobilization with G-CSF or up to 20 days after chemotherapy and G-CSF or (2) they yielded <2.0 × 10⁶ CD34(+) cells per kg in ≤3 apheresis (15).

Effects of underlying disease on mobilization of stem cells

BM involvement by disease is associated with poor yields (16). There may be reduced HSC numbers partly related to the impairment of healthy niches by malignant cells in the BM or direct competition between HSCs and malignant cells for a limited number of niches.

Effect of prior treatment on mobilization of stem cells

Mobilization failure in autologous donors correlates with the number of previous treatments with chemotherapy. Most cytotoxic treatments and molecules used in targeted therapies can have deleterious effects on HSCs and the niches in which they reside. However, DNA cross-link agents such as melphalan or carmustine, and purine analogs such as fludarabine are associated with a very high risk of mobilization failure. Cytotoxic drugs that do not specifically target cell progressing through the S phase of the cell cycle (eg, anthracyclines, cisplatin, fludarabine, carmustine, melphalan) could potentially kill quiescent HSCs and niche cells, thereby reducing the HSC reserve. Fludarabine itself is toxic both to hematopoietic progenitors and to the niche. Alkylating agents such as chlorambucil given in a continuous manner may also be toxic on HSCs. Highly intensive, dose-dense regimens such as the hyper-CVAD (hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone with methotrexate and cytarabine) regimen are associated with a high risk of mobilization failure beyond the first 2-4 cycles of therapy (17). Lenalidomide is associated with poor mobilization, particularly after > 4 cycles of treatment. Lenalidomide may suppress HSC motility similar to the way

it reduces the motility of marrow endothelial cells in multiple myeloma. The antiangiogenic effect of Lenalidomide could also impair mobilization. Cyclophosphamide and G-CSF based mobilization is recommended for patients previously exposed to Lenalidomide (18). Long-term administration of imatinib has been associated with reduced bone turnover, which could affect HSC niche function (19). Yields were improved if the patients temporarily withheld imatinib for 3 weeks prior to collection (20). Prior radiotherapy to significant amounts of red marrow is associated with mobilization failure, probably because of combined direct HSC toxicity, niche toxicity, and toxicity to the niche-supporting cells (21).

Effect of age on mobilization of stem cells

Poor mobilization is often noted in patients > 60 years of age (21). Firstly, there is an age-related "senescence" of actual HSCs because of progressive telomere shortening. Secondly, there is a reduction in the HSC reserve caused by decreased niche function with depletion of mesenchymal stem cells and osteoprogenitors. Thirdly, aging is frequently associated with a marked decrease in bone formation and osteoblast numbers on endosteal surfaces, so endosteal osteoblastic niches for HSCs are probably reduced (22).

Mobilization failure in patients with no obvious risk factors

Up to 5% of healthy donors and some patients with no obvious risk factors fail to mobilize. Genetic polymorphism analyses in humans have identified polymorphisms associated with mobilizing response to G-CSF in untranslated regulatory regions of genes. Genetic polymorphisms such as GCSFR, adhesion molecules (VCAM-1, CD44), and chemokines (SDF-1), which are all known to regulate HSC trafficking may predict for poor mobilization (23). However, there is currently insufficient evidence to apply such screenings in the clinic to identify poor mobilizers prospectively.

Optimising current mobilization protocols

Donors of male sex, young age, and match at A, B, C, DR, and DP are preferable because of higher HSC yield and more certain engraftment. For patients undergoing chemotherapy, collection of HSCs should be considered early when extensive or intensive chemotherapy such as hyper-CVAD are used and also before extensive use of fludarabine and lenalidomide. Lenalidomide and imatinib should be ceased 3 weeks before mobilization. Clearance of BM disease is also an important factor

for mobilization. For patients undergoing extensive field radiotherapy, pre-treatment harvesting should be considered if autologous transplantation is a potential treatment option .

Optimising apheresis for better mobilization

The peripheral blood CD34+ cell count is the best predictor of apheresis yield (24), and the use of CD34+ cell counts to guide apheresis reduces costs. If the circulating CD34+ cell count is $\geq 20/\mu\text{L}$, 94% of collections performed the following day would be expected to yield $\geq 2.0 \times 10^6$ CD34+ cells/kg (24). In general terms if the CD34+ cell count is $< 5/\mu\text{L}$, the yield will be poor; therefore, apheresis not warranted. Larger volume apheresis (processing ≥ 3 times the blood volume instead of 2 times) results in higher HSC yields and may reduce the number of sessions in some cases.

Improving stem cell collection in mobilization failure

Approaches to failed mobilization after conventional regimens include increased doses of G-CSF or combination with SCF (25). However, the success rate is often still $< 50\%$. Currently, the combinations of G-CSF, SCF, or, more recently, plerixafor with or without chemotherapy are probably the most promising approaches.

Plerixafor in mobilization failure

Plerixafor causes mobilization by disrupting SDF-1/CXCR4 interaction. It synergizes with G-CSF through its different mechanism of action. In clinical practice, plerixafor is usually administered subcutaneously at $240 \mu\text{g}/\text{m}^2$ in the evening 10-11 hours before the planned first day of apheresis. Peripheral blood CD34+ levels should be measured on the morning of apheresis. In healthy donors plerixafor is effective as either a single agent or in combination with G-CSF.

Preemptive plerixafor in predicted poor mobilizers

Rational use of preemptive plerixafor depends on identifying potential poor mobilizers. The adverse effect of BM involvement and prior chemo/radiotherapy have been discussed earlier. However, their specificity as an indicator is low because some of these patients may still mobilize.

Immediate salvage plerixafor in predicted poor mobilizers

Rational use of immediate salvage plerixafor depends on detecting a suboptimal peripheral blood (PB) CD34+ cell level or suboptimal apheresis

yield or both at the expected first day of apheresis. There is as yet no validated data to define cutoffs for the addition of plerixafor; However, one study advises the addition of plerixafor on day 5 of G-CSF if the PB CD34+ level is < 10/ μ L. It has also been shown that a CD34+ cell level of < 5/ μ L on day 4 of G-CSF apheresis indicates the need for salvage plerixafor, because the likelihood of achieving a level > 10/ μ L by the following day is < 40% (26). A first-day apheresis yield of < 0.5×10^6 CD34+ cell/kg also indicates need for salvage.

Remobilization with plerixafor in failed mobilizers

In failed mobilizers, a remobilization regimen with the addition of plerixafor enables reaching the CD34+ cell target in > 70% of patients. One should ensure that there is ≥ 4 weeks of break prior to remobilisation. It is noteworthy that plerixafor-containing regimens have a 30% failure rate among prior failed mobilisers, probably because it could not restore low or defective HSC reserve or niche.

Future agents for stem cell mobilization

CXCR4/SDF-1 inhibition is being further explored with alternative CXCR-4 antagonists such as POL6326 and BTK140 in early phase clinical

trials both show promise as mobilization agents for HSCs. Improved understanding of the HSC niche and HSC reserve has raised possibilities for restoring the niche with the use of agents such as SCF and parathyroid hormone. Triggerring effector cells and pathways more efficiently such as through TNF- α , manipulation of the β -adrenergic circadian pulse (eg, harvesting HSCs in the afternoon, or pretreatment with β_2 agonists), or optimizing macrophage-mediated pathways can be effective. The recent description of S1P and ceramide-1 phosphate as chemoattractants for HSCs also raises possibilities for HSC mobilization. Natalizumab and other α_4 integrin blockers may be useful in plerixafor failures.

Conclusion

Avoidance of HSC and niche damage, the timing of mobilization, and synergism between plerixafor and G-CSF plus chemotherapy are critical in optimising the HSC yield. Plerixafor may be used in up-front, preemptive, immediate salvage and remobilization settings. Novel approaches that explore HSC expansion, β -adrenergic innervation, bone turnover, macrophage function, integrin blockade, and new chemotactic modulators may further improve HSC mobilization in future.

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