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REVIEW

Filgrastim support in allogeneic HSCT for myeloid malignancies: a review of the role of G-CSF and the implications for current practice

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The cytokine G-CSF stimulates myeloid progenitors and is routinely used to accelerate neutrophil recovery in the treatment of hematological malignancy and blood or marrow transplantation. Despite significant reductions in the frequency and duration of febrile neutropenia episodes, infections and the length of hospitalization, filgrastim has never been conclusively proven to produce a survival benefit in allogeneic HSCT and is considered a supportive measure. In this review, we analyze the conflicting evidence and appraise the utility of G-CSF in allogeneic HSCT. G-CSF administration after allogeneic HSCT needs to take into consideration the impact on immune reconstitution, risk of leukemic progression in patients with chromosome 7 abnormalities and the absence of proven benefit in patients receiving marrow or peripheral blood progenitors as the stem cell source.
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Introduction

G-CSF is the major cytokine produced by cells of the monocyte/macrophage lineage that enhances neutrophil production by inducing myeloid progenitor proliferation and differentiation.[1] It induces functional activation of terminally differentiated neutrophils by enhancing phagocytic activity, priming the respiratory burst and antibody-dependent killing. G-CSF plays an essential role in steady-state neutrophil production and in 'emergency' granulopoiesis during infections; G-CSF and G-CSF receptor (G-CSF-R) knockout mice are severely neutropenic.[2,3] Filgrastim (Neupogen, Amgen Inc., Thousand Oaks, CA, USA) is a recombinant methionyl human G-CSF, a 175 amino acid protein with a molecular weight of 18 800 Da.[4] It is FDA approved for patients with non-myeloid malignancies receiving chemotherapy associated with a significant neutropenia, following induction or consolidation chemotherapy treatment of adults with AML, for patients undergoing myeloablative chemotherapy followed by marrow transplantation for non-myeloid malignancies for mobilization of hematopoietic progenitor cells into the peripheral blood for collection by leukapheresis and for patients with severe chronic neutropenia.[4–6] The majority of controlled studies have shown that overall survivals for patients with leukemia or BMT treated with G-CSF have not significantly differed from control and that regulatory approval has resulted in the widespread use of G-CSF.[7–9]

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Filgrastim in myeloid malignancies

The risk of leukemogenesis has been a concern with the use of filgrastim because it can induce the release of immature myeloid precursors into the circulation. Myeloid leukemic cells may express G-CSF and/or GM-CSF receptors. These CSFs induce primary blast cell proliferation *in vivo* and *in vitro* from cells taken from patients with AML or myelodysplastic syndromes (MDS).[10,11] Early in the development of G-CSF, myeloid malignancies were considered to be a contraindication to their use. With extensive clinical use, G-CSF and GM-CSF have been found to be safe and without an increase in mortality in patients undergoing induction therapy for AML.[7–9] The administration of G-CSF to patients with early myelodysplastic syndrome has not been shown to increase the risk of leukemic transformation.[12]

Lessons from aplastic anemia and severe chronic neutropenia

Karyotypic abnormalities involving chromosome 7 produce the most adverse outcomes in patients with AML and MDS.[13,14] AML patients with chromosome 7 abnormalities are usually considered candidates for early allogeneic transplantation. Data from patients with severe aplastic anemia or severe chronic neutropenia (SCN) who receive long-term G-CSF suggest that caution needs to be exercised in patients with chromosome 7 abnormalities.

Clonal evolution, especially chromosome 7 abnormalities, are a frequent late occurrence in patients with severe aplastic anemia (SAA) who have hematological responses to immunosuppressive therapy.[15] FISH is more sensitive

than traditional metaphase karyotyping and may identify a greater proportion of patients with subclinical disease who have chromosome 7 abnormalities at the time of diagnosis.[16,17] It is unclear whether clonal evolution is an escape mechanism from the underlying bone marrow failure state or is induced by therapy. G-CSF administration can drive proliferation of the chromosome 7 clone, and in some cases this appears to be reversible.[18–20] Some retrospective studies have reported the duration of G-CSF exposure as a potential factor in clonal evolution.[21,22] However, the duration of G-CSF could have been a surrogate for more refractory SAA. A different retrospective study of 112 Japanese children with SAA treated with immunosuppressive medication with or without G-CSF found that long-term G-CSF (3.7 years) did not induce clonal evolution.[23] In the largest analysis conducted by the EBMT, Socie *et al*.[24] described 840 patients who received a first-line immunosuppressive therapy with (43%) or without (57%) G-CSF. The incidences of MDS/AML in patients who did or did not receive G-CSF were 10.9 and 5.8%, respectively, with a significantly higher hazard (1.9) of MDS/AML associated with the use of G-CSF. Relapse of aplastic anemia was not associated with a worse outcome in patients who did not receive G-CSF as first therapy, whereas relapse was associated with a significantly worse outcome in those patients who received G-CSF. Ultimately, the question whether G-CSF administration is truly causal is expected to be answered by the ongoing randomized EU trial comparing immunosuppressive therapy with or without G-CSF in patients with SAA.

The G-CSF receptor (CSF3R gene) is capable of transducing proliferative and maturational signals. Patients with SCN may transform into AML related to the development of a G-CSF receptor truncation mutation that is capable of inducing proliferation while blocking differentiation. Functionally similar to the truncated CSF3R mutations observed in the clonal evolution in patients with SCN, the 'Class IV' splice variant isoform of the G-CSF receptor (CSF3R gene) is 'differentiation defective.' Normal mature neutrophils predominantly express the class I isoform capable of inducing myeloid maturation, whereas the class IV isoform is aberrantly increased in leukemic blasts.[25,26]

Sloand *et al*.[27] have examined the relationship between the administration of filgrastim and the development of monosomy 7 to determine whether this chromosomal abnormality develops *de novo* or by the favored expansion of a pre-existing clone. Bone marrow mononuclear cell culture in the presence of 400 ng/ml G-CSF showed significant expansion in the proportion of monosomy 7 cells only when the marrow was derived from individuals with a pre-existing clone but not from karyotypic normal individuals with aplastic anemia, myelodysplastic syndrome, or healthy individuals. In CD34 cells from monosomy 7 patients, no mutation was found in the CSF3R gene. However, G-CSF receptor expression was increased, and increased expression of the G-CSF-R class IV mRNA isoform was found. G-CSF-R signal transduction through the Jak/Stat system was abnormal in monosomy 7 CD34 cells, with increased phosphorylated signal transducer and activation of transcription protein, STAT1-P and increased STAT5-P relative to STAT3-P. These results suggest that pharmacologic doses of G-CSF increase the proportion of pre-existing monosomy 7 cells and that monosomy 7 is a specific karyotypic abnormality with susceptibility to proliferation induction by G-CSF because of the presence of the class IV G-CSF-R isoform.

Filgrastim in healthy donors

Filgrastim is routinely used to mobilize hematopoietic progenitor cells into the circulation from normal adult and selected pediatric donors and is considered to have an acceptable short-term safety profile. The majority of studies show transient bone pain as the only major side effect.[28–30] In a retrospective analysis of 341 normal donors, the main adverse events related to filgrastim were bone pain (84%), headache (54%), fatigue (31%) and nausea (13%). Adverse events prompted discontinuation in two donors (0.5%).[28] Normal donor adverse events include reports of splenic rupture, anaphylactoid reaction, gouty arthritis, stroke, vasculitis, angina, rash, capillary leak syndrome and keratitis. There are no definite contraindications for G-CSF administration to normal donors; potential contraindications include the presence of inflammatory, autoimmune or rheumatologic disorders, as well as atherosclerotic or cerebrovascular disease.[31]

Immunomodulatory activity of G-CSF

Although G-CSF is primarily a myeloid growth factor, emerging data suggest a previously under-recognized role for the action of G-CSF on the immune system. G-CSF is a significant immune regulator inducing T-cell tolerance through actions mediated through cytokines, T cells and dendritic cells, as reviewed by Rutella *et al*.[32] As post transplant immune reconstitution is critically dependent on the post thymic T cells present in the allograft, peripheral blood progenitor cell collections could be susceptible to lasting effects from G-CSF mobilization. G-CSF-mobilized stem cell grafts contain $\sim$1-log greater T cells than do conventional bone marrow harvests, but the incidence and severity of acute GVHD is similar, whereas chronic GVHD is increased.[33–36] Thus, G-CSF-mobilized stem cells or the use of G-CSF post transplant may affect immune reconstitution.

G-CSF induces a switch in T-cell cytokine production from a Th1 profile (IL-1b, IL-12, IFN-g, IL-18, TNF-a) to a Th2 (IL-1Ra, soluble TNFRs) response.[37] Earlier work has suggested that a Th2 phenotype may downregulate acute GVHD. Thus, G-CSF use theoretically would diminish the effect of T-cell activation. However, Th2 polarization of the donor lymphocytes can be durable, increasing late infectious complications, particularly in haploidentical transplantation.[38] Functionally, G-CSF-mobilized allografts exhibit inhibition of mitogen-induced T-cell-proliferative responses.[39,40]

Retrospective studies in allogeneic transplantation

The role of G-CSF after allogeneic transplantation has been debated extensively and is summarized in Table 1. One large retrospective review from the EBMT (Ringden), two from the CIBMTR (Khoury and Eapen) and two

Table 1 Clinical studies of filgrastim support in allogeneic HSCT

Study–author	*Transplant population*	*Stem cell source*	N	*Comparison*	*Primary conclusion*
CIBMTR–Khoury *et al.*[43]	AML, CML	BM = 2110 PBSC = 609	2719	Patients who received G-CSF in first 7 days post HSCT versus others	G-CSF shortened time to ANC recovery; no change in D30 or D100 TRM. No changes in GVHD, LFS or OS
EBMT–Ringden *et al.*[41]	AML	BM = 1789 PBSC = 434	2223	Patients who received G-CSF in first 14 days post HSCT versus others	G-CSF worsened acute and chronic GVHD, TRM, OS and DFS in BM but not in PBSC transplants
CIBMTR–Eapen *et al.*[42]	Pediatric and adolescent	BM = 630 PBSC = 143	773	Children who received G or GM-CSF in first 7 days post HSCT versus others	G-CSF worsened TRM, treatment failure and OS
Meta-analysis–Ho *et al.*[45]	9 prospective randomized trials, 8 retrospective cohort comparisons, 1 case-controlled study	BM = 1056 PBSC = 142	1198	Patients who received G or GM-CSF post HSCT versus others	No difference in TRM, GVHD, or 100 day survival
Meta-analysis–Dekker *et al.*[44]	34 randomized controlled trials	BM and PBSC		Patients who received G- or GM-CSF post auto or allo HSCT prior to neutrophil engraftment versus others	Growth factors reduced documented infections but did not impact acute GVHD or TRM

Abbreviations: DFS = disease free survival; LFS = leukaemia free survival.

meta-analyses (Dekker and Ho) have summarized the available data, and consequently the discussion of individual trials is outside the field of this review.

Ringden *et al.*[41] analyzed data from 1789 patients with acute leukemia receiving BMT, and 434 patients receiving PBSCs from HLA-identical siblings reported to the EBMT on the basis of the administration of G-CSF in the first 14 days after stem cell infusion. G-CSF hastened neutrophil but delayed platelet engraftment. In the BMT patients who received G-CSF, increases were noted in grades II to IV acute graft-versus-host disease (GVHD) (relative risk (RR), 1.33), chronic GVHD (RR, 1.29) and transplantation-related mortality (RR, 1.73). These resulted in decreased overall survival (RR, 0.59) and leukemia-free survival (RR, 0.64). No such effects of G-CSF were seen in patients receiving PBSC.

Eapen *et al.*[42] compared outcomes in children and adolescents receiving HLA-identical sibling allogeneic peripheral blood versus marrow transplants for acute leukemia and reported to the CIBMTR. TRM and survival were worsened in patients receiving PBSC allografts. This study also found that growth factor administration post transplant reduced survival after both PBSC and BMT.

Khoury *et al.*[43] performed a large retrospective analysis of 2719 patients who reported to the CIBMTR with AML or CML to undergo myeloablative conditioning followed by stem cells from an HLA-identical sibling (either PBSC or BM) or an unrelated donor (BM only). Patients received cyclosporine plus methotrexate-based GVHD prophylaxis. Patients who received G-CSF within 7 days of stem cell infusion were compared with patients who did not receive G-CSF before day +7. A 7-day cutoff was selected to reduce a selection bias by ensuring that patients had not engrafted before starting G-CSF. Selection bias was also minimized by restricting enrollment to centers that routinely give G-CSF to >80% of patients. Subjects who received G-CSF before day +7 had a shorter time to ANC recovery ranging from 3–5 days depending on the graft source. Despite faster neutrophil recovery, this did not translate into a difference in TRM at day 30 or d100. The incidences of acute or chronic GVHD, overall survival and leukemia-free survival were not different.

The contradiction between the CIBMTR (Khoury) and the EBMT (Ringden) studies may be related to the selection of the cutoff day: G-CSF administration before 7 days (Khoury) captures patients for whom the intent is to aid count recovery, whereas a cutoff date of 14 days (Ringden) also captures patients receiving G-CSF for delayed recovery.

Neither the CIBMTR nor the EBMT retrospective studies were randomized and could therefore be subjected to unconscious biases. Dekker *et al.*[44] performed a meta-analysis of studies that randomized patients to receiving a growth factor (either G- or GM-CSF) versus placebo/no therapy, and the growth factor was given after HSCT but prior to neutrophil recovery. Thirty-four studies were identified. Growth factors were associated with a small reduction in the risk of documented infections but did not affect the incidence of grades 2–4 acute GVHD or infection or TRM. The Ho meta-analysis identified 18 studies in allogeneic HSCT and found no alteration in risk for GVHD in patients receiving growth factors.[45]

Umbilical cord blood (UCB) transplants are associated with prolonged time to engraftment and infectious morbidity and mortality related to the low frequency of hematopoietic progenitors. In a study of 113 pediatric UCB transplants compared with marrow controls, 41% of cord blood recipients received growth factors compared with 21% of marrow recipients and the median time to absolute neutrophil count $\geqslant 500/\mu l$ was 26 days in UCB transplant versus 18 days for marrow. The likelihood of recovery of the neutrophil count was significantly lower in the first month after cord blood transplantation (relative risk, 0.40), and in multivariable analysis it was accelerated by post transplant growth factor use. Infectious mortality in the first 100 days was 23% for UCB recipients versus

17% for marrow.[46] In 68 adult recipients of UCB transplants who received post transplant filgrastim, the median time to absolute neutrophil count $\geqslant 500/\mu l$ was 27 days. Infectious mortality in the first 100 days was 25%.[47] In another study, 102 patients (median age 7.4 years) received UCB transplants between 1994 and 2001. Patients enrolled after 1997 were given G-CSF ($n = 80$) and had an incidence of neutrophil engraftment by day 42 of 0.90 at a median of 21 days as compared with 0.80 at a median of 31 days in those not receiving G-CSF. Fifteen patients died of opportunistic infection.[48]

Discussion

Filgrastim is a clinically useful therapy for diverse indications in hematology and oncology.

Apart from the significant benefit in reducing neutropenic complications,[49] the use of myeloid growth factors are accompanied by health-economic benefits.[50,51]

There are clinical situations where G-CSF administration may pose a risk for allogeneic stem cell transplant patients. G-CSF does not appear to be directly leukemogenic, but it can potentiate signaling through the G-CSF-R. When G-CSF-R is mutated as in SCN or monosomy 7, G-CSF could result in clonal expansion of the abnormal cell population. Thus, there is a theoretical risk that G-CSF administration could stimulate the underlying disease during allogeneic stem cell transplant. This is especially of concern in monosomy 7 AML where the CD34 monosomy 7 cells proliferate better than do normal CD34 cells. Chromosome 7 abnormalities denote an extremely high risk in AML, in data predating the use of G-CSF.[52,53] The risk of expansion of a pre-existing monosomy 7 clone in patients with AML/MDS by the administration of filgrastim is unacceptably high and could predispose to further mutagenic events. Monitoring clone size with FISH is recommended in the context of myeloid growth factor administration to patients with a pre-existing chromosome 7 abnormality. Also unproven is whether GM-CSF is a safe alternative for patients with monosomy 7. In our limited experience, GM-CSF has not produced significant clonal expansions in patients with monosomy 7, but this issue is best examined prospectively. It is highly unlikely that there will be a willingness to conduct prospective clinical trials to evaluate for a deleterious effect of G-CSF in patients with a chromosome 7 abnormality, and a retrospective review of large data sets will be necessary to provide confirmation.

Given the critical importance of G-CSF-R in granulopoiesis, the widespread distribution of G-CSF-R and emerging data of its importance in other malignancies, it is reasonable to expect that there will be further unraveling of the G-CSF axis in the future.

Several studies have evaluated clinical outcomes for patients administered G-CSF after allogeneic BMT. The benefit of Filgrastim in accelerating count recovery may be marginal in the era of peripheral blood progenitor cell transplantation. Filgrastim has important immunomodulatory effects that may affect outcomes after allogeneic transplantation.[54–57] The Th2 polarization effects have not been described with plerixafor, the CXCR4 antagonist, or with sargramostim (GM-CSF) in clinical trials. It is recognized that routine post transplant G-CSF is controversial, with conflicting reports based on retrospective data analyzed from large cohorts of patients.[41,43,44]

To these concerns we now add the risk of progression for patients with G-CSF-R aberrations, including those associated with monosomy 7.

Conclusions

(1) G-CSF mobilization of donor progenitor cells exerts a durable effect on immune reconstitution post allogeneic transplantation by skewing the cytokine profile and induction of regulatory T cells and tolerogenic dendritic cells. The immunological and clinical consequences of G-CSF administration in conjunction with newer stem cell-mobilizing agents need to be carefully evaluated.
(2) Filgrastim may enhance leukemic transformation through actions mediated by the G-CSF receptor. G-CSF receptor mutations predispose to expansions of clonal populations by exogenous G-CSF. G-CSF is best avoided in all clinical situations with chromosome 7 abnormalities.
(3) Although there is conflicting evidence whether the administration of G-CSF post allogeneic transplant worsens survival, there is no apparent benefit. In the final risk–benefit analysis, the immediate benefits of G-CSF related to count recovery, which were substantial in the case of cord blood grafts, may be insignificant for a peripheral blood progenitor graft. Improvements in the supportive care of neutropenic patients diminish the enthusiasm for the routine administration of G-CSF post allogeneic transplant to all but those at highest risk of severe prolonged neutropenia.

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