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Clinical applications of granulocyte-colony stimulating factor

Lillian Sung [1] **, Yigal Dror** [2]

[1] *Division of Haematology and Oncology, the Departments of Paediatrics, Population Health Research Program at The Research Institute, The Hospital for Sick Children , University of Toronto, 555 University Ave. Toronto, Ontario, Canada, M5G 1X8,* [2] *Division of Haematology and Oncology, the Departments of Paediatrics, Immunity-Infection-Injury-Repair Program at The Research Institute, The Hospital for Sick Children , University of Toronto, 555 University Ave. Toronto, Ontario, Canada, M5G 1X8*

TABLE OF CONTENTS

1. ABSTRACT

Granulocyte colony stimulating factor (G-CSF). is a naturally occurring potent neutrophil growth factor. Recombinant human G-CSF has been developed by pharmaceutical companies, and since the late 1980's, multiple clinical trials have explored its efficacy in a variety of medical conditions. These include various inherited and acquired neutropenia, as well as mobilization of hematopoietic stem cells and progenitors for transplantation. Interestingly, in several type of inherited neutropenia where no randomized controlled studies have ever been conducted, its chronic use is considered critical for survival and deemed a standard of care. Unfortunately, it the settings of cancer treatment-related neutropenia and post hematopoietic stem cell transplantation, controversy still prevails whether universal usage of drug is cost effective despite innumerous randomized clinical trials. This review will focus on the clinical applications of G-CSF in the setting of inherited and acquired bone marrow failure, cancer treatment-related neutropenia and hematopoietic stem cell transplantation.

2. INTRODUCTION

Granulocyte colony stimulating factor (G-CSF). is a hematopoietic colony-stimulating factor (CSF). which stimulates granulocytic differentiation and maturation, and promotes proliferation and survival of neutrophil progenitors. These effects result in maintenance of normal neutrophil counts in healthy individuals, or neutrophilia when exogenous G-CSF is administered (1). G-CSF has varied biological effects which include effects on proliferation, differentiation and function as reviewed by Gaviria and colleagues (2). Formulations of G-CSF include a recombinant non-glycosylated protein (filgrastim)., and a glycosylated form derived in Chinese hamster ovary cells (lenograstim) (3). A new derivative of G-CSF, pegfilgrastim, which is a covalent conjugate of filgrastim and monomethoxypolyethylene glycol is also available (4;5). It has a prolonged half-life, and requires fewer injections to patients.

This review will focus on the clinical applications of G-CSF in the setting of inherited and

acquired bone marrow failure, cancer treatment-related neutropenia and hematopoietic stem cell transplantation.

3. CLINICAL APPLICATIONS IN INHERITED MARROW FAILURE SYNDROMES

3.1. Kostmann syndrome

Kostmann syndrome is an inherited marrow failure syndrome with isolated severe neutropenia from birth. The characteristic bone marrow finding is maturation arrest at the promyelocyte-myelocyte stage. Neutrophil counts are typically $<0.2 \times 10^9$/L, and 90% of the patients present with life-threatening infections during the first 6 months of life (6). More than 80% of the cases with Kostmann syndrome are associated with mutations in one copy of the *ELA2* gene, encoding neutrophil elastase (7). Mutations in *GFI1* (8). and in *WASP* (9). were also identified as a cause of severe congenital neutropenia in rare subsets of patients.

G-CSF has completely changed the natural history of the disease. Before the era of G-CSF, most patients reported to have died in the first year of life (6)., with the leading causes of death were sepsis and pneumonia. After the introduction of G-CSF therapy to patients with Kostmann syndrome in 1989, death rate decreased dramatically. Of the 348 patients with severe congenital neutropenia on the SCNIR, 16% have died at a median age of 12.1 year (range 0.33 to 88.7) (10). The leading cause of death has changed from infections to leukemic transformation. Additional causes include infections in non-responding or non-compliant patients and complications post hematopoietic stem cell transplantation and myelodysplastic syndromes (MDS) (10). Based on literature review, a projected plateau of 75% survival at age 23 years was estimated (11). Life-long treatment with G-CSF is currently the mainstay of management of patients with Kostmann syndrome, and should be initiated as front-line treatment when the diagnosis is established (12). Since the initial report of G-CSF treatment in Kostmann syndrome in 1989 (13). hundreds of patients have been treated**.** The starting dose of G-CSF is 5 μg/kg/day subcutaneously and can be escalated by 5-10 μg/kg/day every 14 days until the desired neutrophil number is achieved (14). Neutrophils > 500/μl generally provide protection from infection, but target counts of about 1000-2500/μl are clearly safer. It is noteworthy that patients, who are successfully treated with G-CSF may continue to suffer from oral infections such as chronic periodontitis. This might be due to persistent deficiency of anti-bacterial peptides such as cathelin-LL-37 in the patients' neutrophils, plasma and saliva (15).

The Severe Congenital Neutropenia International Registry defined complete response to G-CSF therapy by neutrophil counts of 1×10^9/L with doses up to 120 μg/kg/day and absence of life threatening infections; partial response as the occurrence of infections despite an increase in neutrophil counts to 500/ μl with doses of up to 120 μg/kg/day; and no response, as absolute neutrophil counts of less than 500-1000/μl with doses of up to 120 μg/kg/day (16). Ninety percent of the patients with Kostmann syndrome have complete response to G-CSF with doses of <30 μg/kg/day and minimal adverse reactions. The basis for the refractory state to G-CSF is unknown. In one case with Kostmann syndrome and a mutation in the extracellular domain of the G-CSF receptor, the addition of glucocorticoids to G-CSF to the cultured patient's hematopoietic progenitors *in vitro* and to the patient *in vivo*, markedly increased the neutrophil numbers and the patient suffered no further infections (17). The combination increased proliferation of 32D cells carrying the patient mutation and enhanced STAT5 activation.

3.2. Cyclic neutropenia

Cyclic neutropenia is a sporadic or autosomal dominant disorder characterized by a regular, repetitive decrease in peripheral blood neutrophils at 21 $\pm$ 3 days intervals (18; 19). Patients usually present in infancy or childhood, and have a less severe infectious course compared to Kostmann syndrome, however, they may develop life threatening infections such as clostridium species sepsis, fatal acute peritonitis, staphylococcal aureus septicemia and mouth sores during the neutrophil nadir and chronic gingivitis (20;21). Cyclic neutropenia is caused by heterozygous mutations in *ELA2* gene usually at the active site of neutrophil elastase without disrupting the enzymatic substrate cleavage by the active site (7). Patients with recurrent or severe infections, recurrent oral ulcers or chronic gingivitis require treatment. Data from the SCNIR showed that G-CSF induces neutrophil and clinical responses in more than 90% of the patients with cyclic neutropenia (10;14;22). The mean daily G-CSF dose is lower than the mean dose for the congenital neutropenia patients; approximately 2.6 μg/kg/day. As with Kostmann syndrome, oral disease may be partially resistant to G-CSF therapy.

3.3. Glycogen storage disease type Ib

Patients with this autosomal recessive disorder have features of glycogen storage disease type Ia, which includes hepatomegaly and metabolic crises with hypoglycemia and lactic acidosis. In addition, patients have defects in neutrophil respiratory burst, chemotaxis, calcium flux and intermittent neutropenia, which may be severe and cause serious infection and inflammatory bowel disease. The genetic defect resides in the gene encoded for the glucose 6-phosphate translocase enzyme. Bone marrows are hypocellular, and granulocytes are apoptotic with activation and translocation of the Bax proapoptotic protein (23). Most patients require suffer from infections and require therapy (24). G-CSF is effective in almost all patients (24;25). It successfully increases the neutrophil counts, corrects the neutrophil oxygen radical formation, prevents infections, and improves the bowel inflammation (23;24). Of the 29 patients with GSD Ib on the SCNIR, 40% had splenomegaly before starting G-CSF; 81% developed splenomegaly within 1 years after G-CSF, and 100% by the third year (25). Five of the 29 patients had hypersplenism, which either required splenectomy or reduction of the G-CSF dose.

3.4. Fanconi anemia

Fanconi anemia (FA). is characterized by bone marrow failure and a high risk of MDS, leukemia (especially acute myeloblastic leukemia, AML). and solid

tumors (particularly carcinoma of the gastrointestinal tract and skin) (26; 27). FA is a complex disorder composed of at least 12 subgroups (FA A-L, and their associated genes. The inheritance is typically autosomal recessive, except for FA B subgroup, which is X-linked.

A multicenter clinical trial (28). examined the effect of prolonged administration of G-CSF in 12 FA patients with neutropenia. By week 8 of the study, all patients showed an increase in neutrophil counts. Some patients had clinically significant increase also in platelet counts and hemoglobin. In another study, patients without a matched sibling donor received a combination of G-CSF 5 μg/kg daily and erythropoietin 50 units/kg 3 times a week (29). Of 20 patients treated, all but 1 showed improved neutrophil numbers, 20% achieved a sustained rise in platelets, and 33% showed an increase in hemoglobin levels. However, more than one-half of the responders did not sustain the response after 1 year.

Since most patients with FA and neutropenia will ultimately need hematopoietic stem cell transplantation, and since a marked predisposition to cancer is a feature of FA, prolonged use of growth-promoting cytokines for this disorder is a central issue. Development of new clones on G-CSF treatment was reported, and remains a significant concern (28;30).

3.5. G-CSF treatment in other inherited marrow failure syndromes

Several patients with Shwachman-Diamond syndrome and severe neutropenia have been reported to receive G-CSF. The therapy was effective in inducing a clinically beneficial neutrophil response (31;32;33). Of 16 patients with Shwachman-Diamond syndrome and severe neutropenia on the SCNIR, who were treated with G-CSF and were evaluable, 14 had brisk response, which in some patients can be maintained for >11 years (14). Four patients with dyskeratosis congenita were reported who responded to G-CSF therapy with significant increases in absolute neutrophil counts (34;35). A combination of G-CSF with erythropoietin was used in one case with dyskeratosis congenita and severe cytopenia, and improved counts of all cell lineages was observed (36). Therefore, there appears to be potential benefit from G-CSF in selected patients with dyskeratosis congenital and Shwachman-Diamond syndrome while waiting for HSCT or who are not eligible for transplant. Patients with myelokathexis were treated with G-CSF, with increase in the number of neutrophils and clinical improvement during episodes of bacterial infection (37).

4. CLINICAL APPLICATION IN ACQUIRED APLASTIC ANEMIA

Acquired aplastic anemia is characterized by pancytopenia with a hypocellular bone marrow in the absence of an inherited syndrome. Untreated severe aplastic anemia has a high mortality due to either infections or hemorrhagic events. Immunosuppressive therapy and hematopoietic stem cell transplantation have dramatically changed the outlook for children diagnosed with acquire aplastic anemia. In this era of supportive and specific therapies, survival closely depends on the neutrophil count and duration of severe neutropenia.

G-CSF at doses of 400-2000 $\mu g/m^2$/day has been shown to increase neutrophil counts in 75% of children with acquire aplastic anemia, who consequently spent fewer days in hospital (38-40). In addition, several studies have examined the impact of adding G-CSF to immunosuppressive therapy (antithymocyte globulin, cyclosporine and prednisone). on response and long-term survival (41-43).

Gluckman et colleague conducted a randomized, parallel-group, multicentre study to evaluate the efficacy and safety of subcutaneous G-CSF during the first 12 weeks of standard immunosuppressive therapy in 102 patients with de novo severe aplastic anaemia (43). The addition of G-CSF to standard therapy resulted in a higher proportion of patients showing complete neutrophil response (83.0%vs 44.9%; $P < 0.0001$). including in patients with very severe aplastic anaemia (69.2%vs 31.6%; $P = 0.012$). In patients receiving G-CSF, median time to complete neutrophil response was shorter (6.3 vs 16.1 weeks; $P = 0.0001$). and mean duration of first neutrophil response was longer ($P = 0.0248$). than in the control group. However, at a median follow-up of 5 years, there was no difference between the groups in overall survival, hematological response and occurrence of secondary leukemia. Others studies showed similar results (41;42).

5. MYELODYSPLASTIC SYNDROMES

G-CSF has been used in adult patients with MDS who are not candidate for curative option such as HSCT. A randomized, double-blind, placebo-controlled trial involving 87 patients with MDS demonstrated efficacy of Recombinant human erythropoietin in relieving anemia relative to supportive care (44). Of the patients with refractory anemia, 50% responded versus 5.9% to placebo ($P=0.0072$)., of the patients with refractory anemia with ring sideroblasts, 37.5% responded vs. 18.2% ($P=0.6$). and of those with refractory anemia with excess blasts, 16.7% responded vs. 11.1% ($P=1.00$).

Several other one-arm studies suggested that the combination of erythropoietin and granulocyte colony-stimulating factor is more effective treatment for the anemia of MDS than erythropoietin alone with overall erythroid response rates among all MDS groups of around 40%,, (45-49). however, most of them are non-randomized. A recently published randomized study of 50 patients showed a significant effect of a combination of G-CSF and erythropoietin treatment on erythroid response (48). Ten of 24 treated patients responded vs, 0 of 26 in the control group ($P = .01$). However, no effect on quality of life was demonstrated, and the treatment was calculated to be costly (48). Jadersten and colleagues studied long-term outcome of a cohort of 129 MDS patients treated with a combination of erythropoietin and G-CSF, and compared them patients with an untreated cohort from the

Table 1. Quantative systematic reviews of colony stimulating factor in the prophylactic and therapeutic settings

First Author	Setting	Number of Studies	CSF Type	Febrile Neutropenia	Documented Infection	Infection Related Mortality	Reference
Prophylaxis							
Lyman	Adults Solid tumours and malignant lymphomas	8	G-CSF	OR 0.38 95% CI 0.29 to 0.49 P=0.001	OR 0.51 95% CI 0.36 to 0.73 P=0.001	OR of 0.60 95% CI 0.30 to 1.22 P=0.2	6
Bohlius	Adults Malignant lymphoma	11	G-CSF GM-CSF	RR 0.74 95% CI 0.62 to 0.89).	RR 0.74 95% CI 0.64 to 0.85	RR 2.07 95% CI 0.81 to 5.34	7
Berghmans	Adults Small cell lung cancer	12	G-CSF GM-CSF	NA	NA	No difference in survival1	9
Sung I	Children Various tumors	16	G-CSF GM-CSF	rate ratio 0.80 95% CI 0.67 to 0.95 P = .01	rate ratio 0.78 95% CI 0.62 to 0.97 P = .02	rate ratio 1.02 95% CI 0.34 to 3.06 P = 0.97	12
Sasse	Children ALL	6	G-CSF GM-CSF	rate ratio 0.63 95% CI 0.46 to 0.85 P=0.003	rate ratio 0.44 95% CI 0.24 to 0.80 P=0.002	NA	14
Therapeutic							
Berghmans	Adults	11	G-CSF GM-CSF	NA	NA	RR of 0.71 [1] 95% CI 0.44 to 1.15	21
Clark	Adults	13	G-CSF GM-CSF	NA	NA	OR 0.51 [2] 95% CI 0.26 to 1.00 P=0.05	22

[1] Overall mortality or all-cause mortality during febrile neutropenia, [2] Result sensitive to a single trial CSF: colony stimulating factor; G-CSF: granulocyte colony stimulating factor; GM-CSF: granulocyte-macrophage colony stimulating factor; OR: odds ratio; CI: confidence interval; RR: relative risk; NA: not available or not applicable; ALL: acute lymphoblastic leukemia

PSS/IMRAW database (50). The results showed that treatment with G-CSF and erythropoietin induced long-lasting responses and transfusion independency in defined subsets of MDS patients, without influence on overall survival. The same group also reported median response duration for treatment with erythropoietin and G-CSF of around 2 years and that a response to treatment was associated with an improved quality of life (45;46;51).

G-CSF has an anti-apoptotic effect on MDS erythropoiesis by inhibition of mitochondrial release of cytochrome C (52;53). Erythropoietin is also a well-known inhibitor of apoptosis (54). There is a concern when erythropoietin and G-CSF are used clinically for MDS, but no signs of increased blast proliferation after treatment has been seen (46;55;56).

6. CLINICAL APPLICATION IN CANCER PATIENTS

There is a vast number of trials that have examined G-CSF efficacy in adult and pediatric cancer patients. These domains can be conceptualized as those relating to 1). prophylaxis, i.e. prior to the development of febrile neutropenia and invasive infections, and 2). treatment during episodes of febrile neutropenia. The results of quantitative systematic reviews are illustrated in Table 1.

6.1.. G-CSF in the Prophylactic Setting

6.1.1. Adult Trials

Three large randomized controlled trials (RCTs). were particularly influential in subsequent guideline development and recommendations for G-CSF utilization in this setting. One trial conducted in 199 evaluable adults with small cell lung cancer found that G-CSF reduced the risk of febrile neutropenia from 77% in the control arm to 40% in the G-CSF group (57). A second randomized study in 129 adults with small cell lung cancer also demonstrated a reduction in the frequency of febrile neutropenia from 53% to 26% (58). Similar findings were demonstrated in a third study that included 80 patients with non-Hodgkin's lymphoma in which G-CSF reduced the risk of febrile neutropenia from 44% to 23%.

Subsequently, several systematic reviews have examined the question of whether G-CSF is useful for cancer patients in the prophylactic setting. Lyman *et al.* conducted a meta-analysis of 8 randomized trials of prophylactic G-CSF in patients receiving dose-intensive chemotherapy for either solid tumors or malignant lymphomas (59). The use of G-CSF was associated with a reduction in febrile neutropenia, with an odds ratio (OR). of 0.38 (95% confidence interval [CI] 0.29 to 0.49; P=0.001). G-CSF also was associated with a reduction in documented infections, with an OR of 0.51 (95% CI 0.36 to 0.73; P=0.001). However, there was no reduction in infection-related mortality with an OR of 0.60 (95% CI 0.30 to 1.22; P=0.2) (6) (59).

Bohlius *et al.* performed a meta-analysis of prophylactic G-CSF and GM-CSF in patients with malignant lymphoma (60). Eleven randomized trials were included. In this review, CSFs reduced the risk of neutropenia, with a relative risk (RR). of 0.64 (95% CI 0.55 to 0.75). In addition, CSFs reduced the risk of febrile neutropenia (RR 0.74, 95% CI 0.62 to 0.89). and microbiologically documented infection (RR 0.74, 95% CI 0.64 to 0.85). However, CSFs did not affect either overall mortality during chemotherapy (RR 1.21, 95% CI 0.70 to 2.10). or infection-related mortality (RR 2.07, 95% 0.81 to 5.34). Furthermore, CSFs were not associated with a benefit in terms of overall survival at an average observation time of four years (hazard ratio 0.97, 95% CI 0.81 to 1.17) (60). These authors later updated their meta-analysis to include 12 randomized trials; the results of this meta-analysis were qualitatively similar to those of their previous publication (61).

Berghmans and colleagues examined 12 RCTs of prophylactic CSFs in small cell lung cancer (62). CSFs were not associated with improved chemotherapy response

or survival (62). Adams *et al.* reviewed cost-effectiveness models of prophylactic G-CSF use in small cell lung cancer. Of the five reviewed studies, three reported an incremental cost to the use of G-CSF, one reported an incremental cost saving and one reported either an incremental cost or cost saving depending on the specific model used. The frequency of febrile neutropenia in the control arm required to result in cost saving associated with G-CSF use ranged from 35 to 70% (63).

Based on the lack evidence that CSF use affected cure rates and survival, the American Society of Clinical Oncology (ASCO). recommendations in 2000 discouraged the routine use of CSFs for primary prophylaxis of febrile neutropenia. Furthermore, it was felt that the regimens that were myelosuppressive enough to warrant CSF use were too toxic given the lack of survival benefit associated with their use. Instead a recommendation has been made that primary administration of CSFs be reserved for those at particularly high risk of febrile neutropenia for other reasons such as co-morbidity or expected poor bone marrow reserve.

6.1.2. Pediatric Trials

There also have been many RCTs of prophylactic G-CSF use in pediatric cancer. The largest such study included 164 patients with ALL and assessed responses in 73 randomized to G-CSF and 75 randomized to placebo (64). G-CSF did not significantly reduce hospitalization for febrile neutropenia (58% in the G-CSF group compared with 68% in the placebo group (RR 0.85, P=0.23). G-CSF did reduce the median hospital duration (6 days versus 10 days, P=0.01).

The results of 16 RCTs of prophylactic CSFs (both G-CSF and GM-CSF). in pediatric cancer patients were synthesized in a recent meta-analysis (65). In total, the 16 studies included 1,183 children, 592 of whom were randomized to CSF and 591 to control arms. Eleven of the studies evaluated G-CSF while 5 studies evaluated GM-CSF. The mean rate of febrile neutropenia in the control arms was 57% (range 39 to 100%). Using a random effects model, CSFs were associated with a 20% reduction in febrile neutropenia, with a rate ratio of 0.80 (95% CI 0.67 to 0.95; P = .01)., and a decrease in hospitalization length, with a weighted mean difference of –1.9 (95% CI –2.7 to -1.1 days; P < 0.00001). CSF use also was associated with reduction in documented infections (rate ratio 0.78, 95% CI 0.62 to 0.97; P = .02). and reduction in amphotericin B use (rate ratio 0.50, 95% CI 0.28 to 0.87; P = .02). There was no difference in duration of parenteral antibiotic therapy (weighted mean difference –4.29, 95% CI 10.60 to 2.02 days; P = 0.2). or infection-related mortality (rate ratio 1.02, 95% CI 0.34 to 3.06; P = 0.97) (65). Using a stratified analysis, this systematic review suggested that there the effects of G-CSF and GM-CSF were similar. However, a second analysis using the same data found a 90% probability that G-CSF was more effective than GM-CSF in reducing the rate of febrile neutropenia. G-CSF was associated with a 1.6 day greater decrease in duration of hospitalization compared to GM-CSF and there was a 68% probability that G-CSF was better than GM-CSF with respect to this outcome. G-CSF also was associated with a 4.8 day greater decrease in duration of parenteral antibiotic therapy compared to GM-CSF and there was a 98% probability that G-CSF was better than GM-CSF with respect to this outcome (66).

A second pediatric meta-analysis of prophylactic CSFs was conducted in children with acute lymphoblastic leukemia (67). Six studies of 332 children were included. The use of CSFs reduced the risk of febrile neutropenia (rate ratio 0.63, 95% CI 0.46 to 0.85; P=0.003)., duration of hospitalization (weighted mean difference -1.58, 95% CI -3.00 to 0.15; P=0.03). and number of infections (rate ratio 0.44, 95% CI 0.24 to 0.80; P=0.002). However, CSFs did not reduce chemotherapy delays (rate ratio 0.77, 95% CI 0.49 to 1.23; P=0.3) (67).

Cost effectiveness has been examined in six RCTs of prophylactic CSFs in pediatric cancer (64, 68-72). Within individual analyses, significant differences in cost were not found. When the direction of costs were qualitatively described, three studies found that treatment with CSFs was associated with higher costs, (68;70;72). whereas three found that CSFs were associated with lower costs (64;69;71).

6.2.. G-CSF in the Therapeutic Setting

6.2.1. Adult Trials

Little data is available to address the use of CSFs to treat neutropenia in cancer patients in the absence of fever. The largest such study randomized 138 afebrile neutropenic patients to G-CSF or placebo. The only difference in outcomes was a modest 2 day decrease in neutropenia, without an affect on the rate of hospitalization, number of days in the hospital, duration of parenteral antibiotics, or number of culture-positive infections (73).

Much more data are available with respect to therapeutic CSFs for febrile neutropenia. Berghmans and colleagues performed a meta-analysis of therapeutic G-CSF and GM-CSF in febrile neutropenic cancer patients (74). Eleven studies were included; four studied G-CSF, six studied GM-CSF and one studied both. A significant reduction in hospitalization duration was seen, with a HR of 0.63 (95% CI 0.49 to 0.82; P=0.0006). CSFs did not affect mortality during the episode of febrile neutropenia, with a RR of 0.71 (95% CI 0.44 to 1.15). Another meta-analysis of 13 randomized clinical trials using CSFs for febrile neutropenia (75). identified 779 patients who were randomly assigned to receive CSF and 739 to the control arm. There was no significant effect of CSFs on overall mortality, with an OR of 0.68 (95% CI 0.43 to 1.08; P=0.1). Including data from nine trials with 872 patients, a reduction in infection-related mortality was demonstrated, with an OR of 0.51 (95% CI 0.26 to 1.00; P=0.05). However, this finding was sensitive to the results of a single trial, (76). in which the participants with hematological malignancies had an unusually high mortality rate of 15 deaths among 64 patients randomized to antibiotics alone. Thus, there is considerable uncertainty in the effect of CSFs on this outcome,

Based upon the data derived from randomized trials, the 2000 ASCO guidelines suggest that CSFs should not be routinely used either for uncomplicated febrile neutropenia or for those who are neutropenic and afebrile (77).

6.2.2. Pediatric Trials

There have been three RCTs of therapeutic CSFs in children with cancer (78-80). One double-blind RCT randomized 94 subjects to G-CSF and 92 subjects to placebo in children with a wide variety of underlying malignancies with febrile neutropenia (78). This study found that G-CSF decreased the duration of hospitalization, with a median duration of 5 days (range 4 to 8 days). in the G-CSF group and a median duration of 7 days (range 4 to 10 days; P=0.04). in the placebo group. There was also a reduction in the number of days of parenteral antibiotics with a median duration of 5 days (range 3 to 7 days). in the G-CSF group and 6 days (range 4 to 9; P=0.02). in the placebo group. There was no difference in the number receiving amphotericin B and no child died of infection in either group. The second study was also a double-blind RCT that randomized 31 episodes of febrile neutropenia to GM-CSF and 31 episodes to placebo (79). This study also demonstrated that CSF was associated with a reduction in the duration of hospitalization, with a mean duration of 9±2.6 days in the GM-CSF group and 11.7±4.9 days (P<.05). with placebo. There was also a reduction in the duration of antibiotic therapy with a median duration of 7 days in the GM-CSF group and 8.5 days in the placebo group (P<.05). The third study conducted by the Children's Oncology Group included 66 patients and randomized them to G-CSF plus antibiotics or antibiotics alone (80). G-CSF reduced hospitalization by 1 days (median of 4 versus 5 days, P=0.04). but did not affect duration of antibiotics, antifungal therapy or shock.

7. CLINICAL APPLICATION IN STEM CELL TRANSPLANTATION

7.1. Stem Cell Mobilization

In many centers, peripheral blood progenitor cells (PBPCs). have largely replaced bone marrow derived cells for use in transplantation in both the autologous and allogeneic settings (81-83). Multiple studies have shown that G-CSF is effective and safe for PBSC mobilization for transplantation, although the mechanism for mobilization of the hematopoietic stem cells is unclear. G-CSF has become a standard agent for this application (84). It has been shown to be superior to GM-CSF in some studies (85)., but not in others (86;87). At a dose of 10 μg/kg/day, G-CSF can increase the peripheral blood CD34+ cell number by 50 fold. Higher doses or divided doses might be more effective, but are inconvenient or more expensive. In the setting of both autologous and allogeneic HSCT doses of 10–16 μg/kg/day of G-CSF for 5 consecutive days are effective (88-91**).** Leukapheresis is performed starting on day 4 or 5 after G-CSF administration when the CD34+ cells reach a peak levels in peripheral blood (92).

In the setting of autologous PBSCT for malignant diseases, G-CSF can also be used in combination with chemotherapy. In such cases, higher doses of G-CSF (16 μg/kg vs. 8 μg/kg). were associated with a higher CD34+ cell numbers, but not differences in transplant outcomes (93). In heavily pretreated patients, higher doses of G-CSF may be necessary to achieve stem cell mobilization (up to approximately 30 μg/kg/dose) (94).

In comparison to unstimulated bone marrow, the use of cytokine stimulated PBPCs has been associated with more rapid engraftment and shorter length of hospitalization in both the autologous and allogeneic settings. However, higher rates of chronic GVHD occurred when allogeneic PBPC were used, (81;95). including extensive chronic GVHD (44% versus 17%; P=0.004) (96).

7.2. Post Stem Cell Transplantation

The use of CSFs following SCT is controversial. Several large randomized trials have been conducting in both the autologous and allogeneic settings. In a large multi-center study of 315 patients over the age of 2 years with non-myeloid cancer undergoing both autologous and allogeneic bone marrow transplantation, G-CSF was associated with a shorter median time to an ANC of 500/mL (14 versus 20 days; P<.001)., shorter median duration of hospitalization (25 versus 29 days; P=0.02)., lower median number of days of febrile neutropenia (3 versus 5 days; P=0.01)., less infections (10 versus 13; P=0.009)., and shorter duration of parenteral antibiotics (a median duration of 15 versus 19 days, P<.001). There was no difference in the median time to platelet transfusion independence, number of platelet units received, percentage of patients with fever on at least one day, relapse risk or survival at 100 days (97).

In the autologous setting, a double-blind RCT including 192 recipients of autologous PBPC transplantation who were randomized to G-CSF or placebo demonstrated significantly fewer infections in the treatment group (67% vs. 91%, P=0.00005)., lower need for intravenous antibiotics (67.3% vs. 90.4%; P<.001)., shorter median duration of intravenous antibiotics (8 versus 10 days; P=0.04)., and a decrease in the median duration of hospitalization (15 versus 17 days; P<.001) (98). In an additional randomized trial of G-CSF in patients who received PBPCs with or without autologous bone marrow transplantation, G-CSF was associated with a shorter median time to an ANC of at least 500/mL (10.5 versus 16 days; P=0.0001)., shorter median duration of hospitalization (18 versus 24 days; P=0.002)., and shorter duration of non-prophylactic antibiotics (11 versus 15 days; P=0.03). There was no difference in time to platelet engraftment, red blood cell transfusion requirements, number of days of fever or survival (99).

In a pediatric study of 74 children receiving PBPC transplantation, 38 were randomized to G-CSF after transplantation or a control group that did not receive G-CSF. Only 58 children were evaluable because of disease progression, failure to harvest sufficient PBPCs or protocol violations. The median time to an ANC of at least 500/mL was significantly shorter in the G-CSF compared to the placebo group (11 versus 12 days; P=0.03). The median

time to platelet transfusion independence was significantly longer in the G-CSF group compared to placebo (27 versus 13 days; P=0.04). No difference in febrile days was seen (100).

Several RCTs also have been conducted in the allogeneic setting. In one such study, 54 patients with hematological malignancies undergoing related HLA-matched SCT were randomized to G-CSF or placebo. The study was terminated at the first interim analysis. Those randomized to G-CSF has a shorter median time to an ANC of at least 500/mL (11 days versus 15 days; P=0.008). There was no difference in the median time to platelet transfusion independence, platelet transfusion requirements, median time to red blood cell transfusion independence or red cell transfusion requirements. No differences in acute or chronic GVHD or overall survival were seen (101). In a second randomized study of 42 adults receiving HLA-matched PBPC transplantation, those randomized to G-CSF had a shorter time to an ANC of 500/mL (12 versus 15 days; P=0.002). There were no significant differences in days to platelet transfusion independence or number of transfusions required between the two groups. Also, there were no significant differences in the number of days of broad-spectrum antibiotics, proportion of patients experiencing an infection, or acute or chronic GVHD between the groups (102).

Although there has been concern regarding GVHD in those who receive CSFs following allogeneic SCT, a meta-analysis of randomized trials did not demonstrate an association between growth factor use and GVHD, with a RR of 1.08 (95% CI 0.87 to 1.33; P=0.5). for grades 2-4 acute GVHD, 1.22 (95% CI 0.80 to 1.86; P=0.99). for grades 3-4 acute GVHD, and 1.02 (95% CI 0.82 to 1.26; P=0.87). for chronic GVHD (103).

In an economic evaluation of G-CSF prophylaxis following SCT when bone marrow was the progenitor cell source, seven cost-effectiveness studies were examined. Six studies demonstrated cost savings and one study reported cost liability from the administration of G-CSF. In contrast, of seven studies that compared G-CSF prophylaxis following mobilized PBPCs as the source of progenitor cells, cost saving was demonstrated in four and cost liability was demonstrated in three studies. The 2000 ASCO guidelines recommend that CSFs be used in the SCT setting, following infusion of progenitor cells, and in the event of graft failure (77).

8. ADVERSE EFFECTS ASSOCIATED WITH G-CSF

Acute side effects of G-CSF in patients with short or long term use of G-CSF are generally mild and consisted of headache, general musculoskeletal pain, transient bone pain, and rash. None of these required the discontinuation of G-CSF (104).

In patients with chronic use of G-CSF, several serious complications have been reported, although they are rare and the relationship between the usage of the drug and the illness is not always clear. Approximately 50% of the patients on the SCNIR in whom bone mineral density was evaluated had osteopenia; most of these patients were symptomatic. This complication might be related either to the treatment or to the underlying disorder, (105;106). and require periodic evaluation of the bone mineral density. The optimal treatment of Kostmann syndrome patients with symptomatic or advanced osteopenia is still to be determined (10;12).

Patients with neutropenia on chronic use of G-SCF can develop new splenomegaly. This is common in, but is not limited to, patients with glycogen storage disease type Ib. Splenomegaly requiring splenectomy has been reported in patients on G-CSF, but mainly in those who had splenomegaly before commencing G-CSF treatment.

Thrombocytopenia was reported in about 3% of the patients with KN receiving G-CSF. In about one third of the patient the thrombocytopenia developed concurrently with transformation into MDS/AML (10). Vasculitis was reported in 4.1% of the patients on the SCNIR, and glomerulonephritis was reported in 2.1% of the patients who did not have predilection for renal disease.

The main concern and controversy with using G-CSF on a chronic basis is development of malignant myeloid transformation. Of the 200 patients reported in the pre-GCSF era in the literature, 4 developed MDS/AML (2%)., compared to 12.5% crude rate among G-CSF-treated patients on the Severe Congenital Neutropenia International Registry, or an annual transformation rate of 2% (14). Although the data sources are different, this might suggest that a higher percentage of patients develop MDS/AML while on G-CSF. Eighty percent of the patients with severe congenital neutropenia on G-CSF, who develop leukemia harbor G-CSF receptor mutation in their marrow cells. Furthermore, remission of AML after discontinuation of G-CSF was reported in a patient with Kostmann syndrome on G-CSF, who had developed a G-CSF receptor mutation (107). Development of MDS/ AML must be viewed in the context of the underlying primary problem. First, prior to the availability of G-CSF therapy, it was recognized that leukemic transformation occurs occasionally in patients with Kostmann syndrome /congenital neutropenia (108-112). However, in the pre-cytokine era, many Kostmann syndrome / congenital neutropenia patients died in the first years of life from other causes. Of published cases, 42% of patients died at a mean age of 2 years secondary to sepsis and pneumonia (11;14). Thus, the true risk of congenital neutropenia patients developing MDS/AML was not defined. Currently, with G-CSF therapy, most patients do not develop life-threatening infections and survive, which might thereby allow for the natural expression of leukemogenesis in this population. Second, MDS/AML has not seen in patients with cyclic neutropenia or glycogen storage disease Ib on the Severe Congenital Neutropenia International Registry, who had been treated with G-CSF. Third, there is no correlation between the dose and duration of therapy and the occurrence of leukemia. Thus, it is still to be determined whether G-CSF hastens the appearance of leukemia in patients with an underlying genetic

predisposition by giving growth advantage to the malignant clones.

The relationship of this cytokine with MDS/AML in acquired aplastic anemia is controversial (113-115). A prospective randomized study of immunosuppressive therapy with and without G-CSF in children with acquired aplastic anemia reported an overall cumulative incidence of clonal evolution after treatment of about 22% at 8 years at a median of 37 months, and 75% of these patients had morphological dysplasia (113). The risk of clonal evolution was proportional to the duration of G-CSF administration, particularly if it was longer than 180 days. Failure to respond to immunosuppressive therapy at 6 months was another risk factor in multivariate analysis. An earlier report also identified the duration and cumulative dose of G-CSF therapy as a risk factors in the development of MDS/AML in children with acquired aplastic anemia (116).

These observations have not been borne out by other studies (41;117-120). In these reports, there was no difference in the incidence of cytogenetic abnormalities between patients who received immunosuppressive therapy with or without G-CSF. Further studies are, therefore, required to investigate the safety of G-CSF in patients with acquired aplastic anemia.

G-CSF is safe when used for mobilization of hematopoietic stem cells for transplantation. Common side effects include bone pain and reversible elevations of alkaline phosphatase, lactate dehydrogenase, asparate aminotransferase and uric acid. Febrile reactions are less common. Serious complications are rare and include thrombosis, myocardial infarction, adult respiratory distress syndrome, and splenic rupture. A case if AML in a donor of PBSC for transplantation has been reported 14 months after G-CSF administration (121)., but the relationship between G-CSF and leukemia is unlikely (122).

9. PERSPECTIVES

Based on preliminary case series and data from the Severe Congenital Neutropenia International Registry, it seems that in cases of chronic marrow failure with maturation arrest (such as in Kostmann syndrome). or with residual marrow function, G-CSF has prominent clinical response. In these studies no randomized controlled studies have been done. However, the clinical improvement in this patient population was so remarkable, that clinical trials are currently deemed unethical. In cases of inherited and acquired marrow failure with aplasia or MDS, there seems to be improvement of neutrophil response, however, the data is insufficient to draw conclusions about the clinical response in terms of effect on survival.

There have been a tremendous number of randomized trials conducted in adults and children that have examined the potential usefulness of G-CSF in cancer patients. It is clear that G-CSF can reduce the duration of neutropenia in the cancer and SCT settings. There is increasing evidence that G-CSF also can decrease the risk of febrile neutropenia and documented infections, at least in those with a higher baseline risk of these adverse events. While several studies have demonstrated that G-CSF can reduce the duration of hospitalization, this effect is likely to be dependent upon the changing practice of febrile neutropenia management. Current trends in febrile neutropenia include earlier discharge home and outpatient management, both of which would diminish an effect of G-CSF on hospitalization duration.

Another consistent finding is the lack of effect of G-CSF on infection-related mortality or overall mortality. Furthermore, although many cost-effectiveness analyses have been performed that use different methodologies and different assumptions, the striking feature is the lack of consistent directionality in the findings. In other words, many of these analyses have demonstrated a cost saving while others have demonstrated a cost-liability with G-CSF. These findings would suggest that a definitive conclusion on the cost-effectiveness of G-CSF remains unclear.

Based upon the change in ASCO guidelines from 1996 to 2000, (77;123). there appears to be a waning enthusiasm for the use of growth factors. In part, this change is likely to be related to the consistent lack of benefit of growth factors with respect to survival. Even in the context of curable cancers, a beneficial effect of dose intensification has for the most part been disappointing, thus further calling into question any benefit of CSFs. Furthermore, the inconsistent effect on costs also has failed to clarify the role of CSFs. It is likely that there will be a group of patients that will benefit from prophylactic and therapeutic growth factors, although delineation of that group remains uncertain. Future research regarding CSFs and cancer likely will further examine what specific patient population is most likely to benefit from CSFs and clarifying the specific potential benefit in this population.

Although the ASCO guidelines suggest the use of G-CSF for use in SCT, a quantitative systematic review in this area would likely clarify the potential benefit and risks of its use, both for mobilization and administration post stem cell infusion.

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Send correspondence to: Dr. Yigal Dror, Division of Haematology and Oncology, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada, Tel: 416-813-5630, Fax: 416-813-5327, E-mail: yigal.dror@sickkids.ca