Concise Review: Next-Generation Cell Therapies to Prevent Infections in Neutropenic Patients

MARION E. G. BRUNCK, LARS K. NIELSEN

Key Words. Neutrophils • Chemotherapy • Cell therapy • Neutropenia • Hematopoiesis

ABSTRACT

High-dose chemotherapy is accompanied by an obligate period of neutropenia. Resulting bacterial and fungal infections are the leading cause of morbidity and mortality in neutropenic patients despite prophylactic antimicrobials and hematopoietic growth factor supplements. Replacing neutrophils in the patient through transfusion of donor cells is a logical solution to prevent fulminant infections. In the past, this strategy has been hampered by poor yield, inability to store collected cells, and possible donor morbidity caused by granulocyte colony-stimulating factor injections and apheresis. Today, neutrophil-like cells can be manufactured in the laboratory at the clinical scale from hematopoietic stem and progenitor cells enriched from umbilical cord blood. This article reviews the rationale for focusing research efforts toward ex vivo neutrophil production and explores clinical settings for future trials. Stem Cells Translational Medicine 2014;3:541–548

PREVENTING INFECTIONS IS VITAL FOR NEUTROPENIC PATIENTS

Neutropenia is a disorder characterized by an abnormally low number of circulating blood neutrophils. Patients are considered neutropenic when their absolute neutrophil count (ANC) falls below 1,500 cells per microliter of peripheral blood. Subclassifications are reported based on the depth of neutropenia, which range from mild (1,000–1,500 cells per microliter) to moderate (500–1,000 per microliter) to severe (<500 cells per microliter) [1]. In their landmark study, Bodey et al. observed that the rate of infections increases with the depth of neutropenia, and the severity of infections correlates with duration of neutropenia [2]. About one third of severely neutropenic patients develop life-threatening infections within a week. Intensive chemotherapy schedules cause obligatory neutropenic periods and are associated with a higher risk of infections [3]. Therefore, patients treated for leukemias and myeloproliferative disorders are particularly at risk. Moreover, neutropenia frequently dictates dose reduction or treatment interruptions, thereby worsening outcomes, especially in pediatric and elderly patients [4–6].

Established infections threaten the lives of neutropenic patients and considerably burden the health care system. Early diagnosis of infection is vital but difficult because these patients cannot mount potent and specific inflammatory responses [7,8]. Improvement of diagnostic tools has ameliorated patient prognosis, but 10% of admitted febrile neutropenic patients still die of infections [9]. Therefore, robust prophylactic strategies are crucial to prevent the onset of infections and related complications. Antibacterials such as fluoroquinolones were initially successful at preventing gram-negative bacterial infections. However, their systematic use progressively led to the emergence of resistant strains and frequent shifts between gram-negative and gram-positive pathogens [10]. In addition, the use of antibacterial agents eliminates niche competition for yeast and molds, which may enhance the risk for fungal infections [11]. Prophylactic antifungals reduce infection-related mortality, but the associated toxicity, emergence of resistant strains, and possible drug reactions render this option a solution for small patient cohorts only. Autopsy studies in multiple institutions reveal that the rate of fungal infections is increasing among patients treated for leukemias, with up to 78% positive cases. Within this group, a dramatic 30% of deaths can be attributed to fungal infections in spite of antifungal prophylactic use [12,13]. Moreover, Viscoli et al. suggested a correlation between patients receiving antifungal prophylaxis and a higher risk of bacteremia [14]. The available literature demonstrates that the success of antibiotics in prophylactic, empiric, or pre-emptive strategies depends on an educated guess of the most probable pathogen for a particular neutropenic patient. This practice can cause toxicity and resistance, with associated negative impact for the patient and the broader community.

Although antibiotics reduce morbidity by preventing the spread of infecting pathogens, the
prevailing determinant for the onset of infection remains blood ANC. Neutrophils being short-lived cells, the maintenance of a healthy blood ANC relies on constant neutrophil production from hematopoietic stem and progenitor cells (HSPCs). Chemotherapy-induced niche exhaustion is the underlying cause for neutropenia. A strategy to dampen the myelosuppressive effect of chemotherapy is to maintain HSPCs in a quiescent state transiently during the anticancer treatment. The crucial role of HSPC binding to E-selectins on the epithelium is exemplified by a significant decrease in proliferation and self-renewal of HSPCs in E-selectin knockout mice [15]. Injections of the E-selectin antagonist GMO-1017 during chemotherapy or irradiation protect the HSPC pool from exhaustion, which significantly accelerates the recovery of ANC in wild-type mice [15]. Similarly, Lucas et al. demonstrated that protecting adrenergic nerves during chemotherapy by administration of 4-methylcatechol or glial-derived neurotrophic factor maintained the HSPC niche, which significantly improved hematopoietic recovery [16]. Although these results are promising, the current procedure is to facilitate neutrophil recovery from the remaining HSPC pool to restore patient immunity. Granulocyte colony-stimulating factor (G-CSF) promotes neutrophil production and release from the bone marrow. In patients in whom myeloablation is incomplete, support with G-CSF achieves significant reduction in neutropenic days and related complications [17, 18]. In contrast, for patients receiving high-dose chemotherapy that damages the HSPC niche, such as for treating acute myeloid leukemia (AML), restoring protective blood ANC takes too long to significantly affect infection-related morbidity and mortality despite G-CSF support [19, 20]. Furthermore, although G-CSF may shorten the neutropenic window, complete abrogation cannot be achieved as neutrophil production takes several days. A constant pursuit for alternatives or complementing factors to G-CSF has been motivated by these pitfalls. Among these agents, recent efforts have proposed prostaglandin E2 (PGE2). Administration of PGE2 agonists, such as dimethyl PGE2, increases the stem cell pool in zebrafish and mice [21]. A recent study showed that injections of dimethyl PGE2 to irradiated mice enhanced hematopoietic recovery and survival [22]. These results propose that, similar to G-CSF, administration of PGE2 agonists to neutropenic patients may boost healthy ANC recovery. However, the effect of PGE2 is biphasic as continued exposure eventually inhibits expansion of the progenitor pool [23]. Therefore, clinical trials are expected in the future to confirm the role of early PGE2 injections in neutropenic patients and to contrast with results currently obtained with G-CSF. Meanwhile, despite some benefits provided by combinations of G-CSF and antimicrobial prophylactics, neutropenic infections remain the leading cause of chemotherapy-associated death.

In contrast to G-CSF support, neutrophil transfusions can potentially restore protective ANC immediately. Similar to red blood cell and platelet transfusions used to treat anemia and thrombocytopenia, respectively, neutrophils can be collected from healthy donors for transfusion into neutropenic patients. Neutrophils are harvested using density sedimentation during apheresis. Although neutrophils usually make up >70% of collected cells, the product also contains the other granulocytes, eosinophils and basophils. Hence, neutrophil transfusions have been commonly named granulocyte transfusions (GTx) [24]. The first GTx trials were conducted in the early 1960s, but the low cell yields possible at the time barely impacted host ANC upon transfusion. Transfusions of granulocytes harvested from chronic myelogenous leukemia patients did provide proof of concept as recipients showing blood ANC increment demonstrated rapid clinical improvement post-transfusion [25]. G-CSF administration to healthy donors has enabled collection of clinically meaningful neutrophil numbers and several small trials have suggested some degree of efficacy. Additional large-scale trials of therapeutic GTx have been proposed in light of the current knowledge of neutrophil biology and transfusion [26].

Even if efficacy is demonstrated in large-scale trials, significant logistic and donor safety issues remain. Neutrophils have a short half-life; therefore, GTx is usually performed daily or every second day during the neutropenic period. Neutrophils do not store well and must be infused within 24 hours of collection. Finally, whereas G-CSF is usually well tolerated in healthy donors, it can cause bone pain, flu-like symptoms, and spleen enlargement that in rare cases leads to spleen rupture [27, 28]. There are also long-term safety concerns for G-CSF-mobilized donors [29]. Finally, steroids such as prednisone and dexamethasone, frequently used in combination with G-CSF to mobilize donor neutrophils, may increase the risk of developing subcapsular cataracts later in life [30, 31]. Accordingly, there is a clear need for alternative cell products.

### Stem Cell-Derived Therapies to Treat Neutropenia: Rationale and Options

In order to mitigate neutropenia, possible strategies are to regenerate the hematopoietic niche or to immediately replace functional cells in the blood (Fig. 1). Autologous transplantation of mobilized peripheral blood (mPB) HSPCs coupled with cytokine support promotes engraftment and blood cell recovery [32]. Clinically significant delays to protective blood ANC recovery are common and have prompted efforts to expand HSPCs ex vivo prior to transfusion [33]. The development of the Amgen-defined serum-free medium containing a key cocktail of the three cytokines stem cell factor (SCF), thrombopoietin (TPO), and G-CSF eased large-scale ex vivo expansion of HSPCs, with bias toward differentiation into a neutrophil phenotype [34]. Transfusion of ex vivo-expanded HSPCs (eHSPCs) to chemotherapy-treated patients caused significantly fewer neutropenic days, febrile episodes, and shorter hospital stays [35, 36]. Furthermore, the reduction in time to engraftment corresponded to the transfused dose, reinforcing the need for better ex vivo expansion protocols [36, 37]. Subsequent studies have assessed a wide range of parameters for increasing eHSPC yields, including different culture vessels, seeding densities, feeding schedules, oxygen tensions, addition of small molecules to culture medium, and genetic manipulations of developmental factors, all of which have been extensively discussed in recent reviews of the literature [38–40].

A fundamentally distinct approach promotes HSPC expansion by preventing intercellular communication and ensuing repressive feedback. Czaszar et al. [41] and Kirouac et al. [42] have modeled and validated the hypothesis that secreted factor-mediated intercellular communication regulates the fate of HSPCs. Preventing autologous feedback through a dilution strategy caused an 11-fold expansion of umbilical cord blood (UCB)-enriched HSPCs without impacting self-renewal or differentiation potential [41]. A remaining concern is the concomitant mobilization of tumor cells during autologous HSPC collection that has caused relapse in transfused patients [43, 44]. Therefore, this strategy may be restricted to nonhematopoietic cancer treatments only, although some studies suggest that purging the graft from malignant cells using chemotherapy is...
When autologous transplantations are not advised, patients may turn to allogeneic options. Allogeneic mPB HSPC transfusions are an alternative if human leukocyte antigen (HLA)-matched donors are available. Successful neutrophil engraftment relies on further expansion of eHSPCs in vivo; therefore, the product cannot be irradiated before transfusion (Fig. 1). Hence, a significant concern with allogeneic transfusions is the risk of developing acute and chronic graft-versus-host-disease (GVHD) due to engrafting lymphoid cells. Cellerant Therapeutics, Inc. (San Carlos, CA, www.cellerant.com) has demonstrated that the protocol for producing the off-the-shelf cell therapy CLT-008 overcomes this problem [46]. mPB HSPC are expanded in a myeloid-driving, defined medium consisting of X-VIVO 15 (Lonza, Basel, Switzerland, www.lonza.com) supplemented with SCF, Fms-related tyrosine kinase 3 ligand, interleukin-3, and TPO. Elimination of lymphoid cells is achieved passively, alongside expansion of myeloid progenitors (Fig. 1). Over 8 days of culture, this process averages a 40-fold expansion. The CLT-008 product is cryopreserved and it is suggested that HLA matching is not required, facilitating access to patients. At the time of writing, Cellerant Therapeutics, Inc. is recruiting patients to investigate the safety and efficacy of CLT-008 to abrogate neutropenia in chemotherapy-treated patients [47]. Despite clear improvements over the current therapies, a major issue with the CLT-008 strategy remains the source of stem cells. Cellerant Therapeutics, Inc. uses mobilized healthy donors to source a sufficient number of HSPCs. Furthermore, the average fold expansion of the myeloid compartment is similar to that obtained by Paquette et al. a decade ago [36, 37]; therefore, several mobilized blood donations may be required per patient. UCB has been used as a source of HSPCs for transplantations for the past 25 years and is a promising alternative to using mPB [48]. Historically, UCB HSPCs have been transfused in patients for whom autologous transplantations are not advised and matched donors are unavailable. Advantages of UCB as a source of HSPCs include the ease of procurement and decreased incidence of GVHD; however, delays in engraftment remain an issue. Ex vivo expansion protocols have been developed to transfuse a larger number of cells, and downstream transplantations in vivo have been performed successfully [49, 50]. In specific settings, UCB eHSPCs have yielded better expansion than mPB eHSPCs [51].

The delay to engraftment of transfused eHSPCs can be clinically significant. A natural extension to the eHSPC protocol consists of expanding and differentiating a fully mature neutrophil product from HSPCs. Our group has developed a protocol to differentiate UCB HSPCs toward a neutrophil-like phenotype [52]. Over 15 days of culture, an average 5,800-fold expansion of UCB HSPCs is reached. Accounting for the mean CD34+ HSPC yield (10^6 cells per day) [53], this expansion is estimated sufficient for a single prophylactic dose of donor neutrophils (10^9 cells per day) [54]. However, during the culture process, the entire HSPC pool inevitably enters differentiation, so that the ex vivo-manufactured neutrophils (eNeut) yield is currently finite. These eNeut can be produced under good manufacturing practice conditions at the clinical scale. The mature product is composed in its majority of postmitotic neutrophils that exhibit bactericidal functions in vitro. eNeut are poorly immunogenic as assessed by the granulocyte immunofluorescence test (GIFT) and the granulocyte agglutination test (GAT), endorsing the product for allogeneic use. eNeut transfusions should cause immediate increments in ANC, as observed post-GTx with donor neutrophils [54]. Interestingly, the culture process used in this work merges a dilution feeding strategy to the key culture medium protocol to enable the highest yield of eNeut to date from expanded UCB-enriched HSPCs. Again, a major challenge is to produce large quantities of cells. Current studies are attempting clever combinations of culture protocols to further enhance eNeut yields [55].

Approval of eNeut by regulatory bodies will be crucial for implementation in the clinic. The U.S. Food and Drug Administration has approved a Phase I clinical trial using eNeut as a prophylactic for severe neutropenia following chemotherapy. This approval is a significant milestone in the development of allogeneic neutrophil therapies. The use of allogeneic neutrophils has the potential to improve patient outcomes by reducing the risk of infection and complications associated with neutropenia.
Administration (FDA) is evolving its regulatory processes on regenerative medicine products, with a handful of ex vivo-generated cellular therapies recently approved for the market. These therapies include autologous cultured cells like chondrocytes (Carticel) and allogeneic solutions like Gintuit, a product containing keratinocytes and fibroblasts cultured in bovine collagen. FDA approval for biologic licensing has doubled in the last 2 years, which suggests the imminent infiltration of cellular therapies in the clinic [56]. Nonetheless, emerging therapies must be fully supported by clinical data before licensure, especially in the booming field of ex vivo-cultured hematopoietic cells. Although the clinical potential of eNeut is promising, controlled randomized trials remain to be implemented (Fig. 2).

A major determinant holding preliminary human trials is the high cost of manufacture for cell therapies (Fig. 2). For instance, a unit of platelet pool, which is considered a blood product by the FDA, costs $450 Euros to the receiving hospital [57]. By comparison, Provenge (sipuleucel-T), an autologous dendritic cell therapy for prostate cancer, is sold by its manufacturer for U.S. $93,000 per patient [58]. The preparation of a platelet unit involves donor apheresis, leukocyte depletion, resuspension in a platelet additive solution, storage, and irradiation prior to infusion. This process evokes the culture protocol for eNeut, although the differentiation part of the protocol may suggest that eNeut be considered under the “cell therapy” scheme. Although the regulatory format framing eNeut production will dramatically impact product cost, other factors may contribute to lowering manufacture expenses, such as automation and implementation of closed systems, discussed in detail elsewhere [38]. A direct comparison of the cost of eNeut treatment to the mean patient cost of neutropenia-associated hospitalization, estimated at U.S. $19,100 per episode of febrile neutropenia [9], may not be sufficient as cost-benefit analysis should also consider patients’ reduced morbidity and increased survival.

LESSONS LEARNED FROM GTX AND THEIR RELEVANCE FOR ENEUT PRODUCTION AND CLINICAL USE

Lesson 1: Keeping the Transfusion Recipient Safe

In transfusion medicine, part of the “non-self” nature of the transfused product is dealt with through lymphoreduction and irradiation to prevent GVHD. A remaining source of concern is alloimmunization, which occurs when antibodies in the transfusion recipient target their cognate antigen present on the recipient cells. Consequently, the infused product survives poorly in the recipient who becomes refractory to future transfusions [59, 60]. Alloimmunization against human leukocyte antigens usually causes transplant rejection, and if the involved antibodies target human neutrophil antigens (HNAs), the transfused neutrophils may become activated, pool in the lungs, and exhibit limited chemotaxis to infection sites [61, 62]. In addition to limiting transfusion efficacy, alloimmunization can initiate a cascade of events leading to life-threatening complications such as transfusion-related acute lung injury (TRALI) [62]. Lastly, transfusion of incompatible neutrophils causes delayed HSPC engraftment [63]. These issues prompt HLA and HNA typing prior to granulocyte collections, further burdening the health care system and restricting potential donor availability [64]. On the other hand, mature eNeut cultures have been tested by GIFT and GAT techniques using a positive serum pool. Mild or complete absence of reaction confirmed that eNeut are not immunogenic and therefore may present a lower risk of transfusion-associated complications compared with donor neutrophils [52]. Also, eNeut are generated in a chemically defined medium, devoid of animal products, potential pathogens, or blood contaminants responsible for adverse reactions. These precautions further suggest that eNeut may be a safe donor granulocyte alternative for transfusions (Fig. 2), which may
not require frequent, time-consuming, and expensive immuno-
typing and serotyping.

Despite these measures, eNeut remain allogeneic in nature. γ
irradiation of blood products is an obligatory practice to prevent
engraftment and GVHD. γ-irradiated myeloid progenitor cells dis-
play dose-dependent viability over 6 days, which suggests arrest
of expansion of mitotic cells [65]. Although further studies are
necessary to assess the effects of the 25-Gy irradiation dose re-
quired for blood product transfections, these data demonstrate
that irradiation does not prevent final maturation of progenitor
cells. This feature is attractive if one considers that current neu-
rophil differentiation protocols do not use synchronized cells.
It will be interesting to see whether irradiated eNeut transfusions
confer longer lasting protection than donor GTx through post-
transfusion late-phase maturation in vivo. In addition, if non-
synchronized HSPCs are used to initiate eNeut cultures, the
mature cells will appear in waves. As a result, the product pheno-
type can be characterized as soon as the first mature eNeut are
available. This feature is important to avoid delays in patient
transfusions given that typing assays can be time consuming [66].

Lesson 2: Timing of GTx Is Key to Benefit Recipients

To date, no randomized controlled trial has clearly demonstrated
the benefit of GTx. However, most clinical studies set to assess
GTx have been performed in the context of febrile or septic neu-
 tropenic patients for whom the pathogen replication has not
been contained by innate immunity. Arguably, choosing the set-
ting of an established infection, it is inappropriate to expect im-
provement using GTx as there is a large discrepancy between
the dose of neutrophils needed to abrogate the infection and
the dose of neutrophils actually transfused. In a healthy individ-
ual, 10^{11} neutrophils are released every day from the bone mar-
row to maintain homeostasis in tissues and steady blood ANC, and
the bone marrow reserve contains approximately 6 times more
neutrophils [67]. Following a microbial insult, neutrophils are mo-
obilized from the bone marrow reserve, which creates a dramatic
increment in blood ANC [68]. Apheresis of G-CSF-mobilized neu-
trophils typically yields only 4–8 × 10^{10} neutrophils, leaving
donors in a state of transient neutropenia [54, 69]. It is unreason-
able to expect that a lower-than-homeostatic neutrophil dose
should be able to treat an established infection. Therefore, the
apparent lack of benefit of GTx may be attributable to trials in
inadequate settings rather than fundamental misconceptions of
GTx antimicrobial potential.

A more favorable situation is prophylactic GTx in at-risk pop-
ulations, such as AML patients who receive aggressive myeloabla-
tive chemotherapy. The proposed objective of prophylactic GTx
would be to maintain peripheral immunological surveynase. This
suggestion is supported by successful prevention of infection re-
lapse in patients receiving GTx as secondary prophylaxis [70]. In
addition, a landmark meta-analysis of GTx prophylaxis trials indi-
cates that a transfusion dose of as few as 10^{10} neutrophils is su-
ficient to improve prognosis [53].

Lesson 3: Patient Outcome Depends on the Phenotype
of Transfused Granulocytes

The immunological phenotype of donor neutrophils is likely to
change during harvest and ex vivo procedures, which may have
a dramatic impact on transfusion efficacy. Cell purification pro-
cesses can inadvertently damage or activate neutrophils. For
instance, neutrophil enrichment using filtration leukapheresis
leads to neutrophil phenotype modification including activation.
Furthermore, enriched concentrates may contain apoptotic neu-
trophils and other damage-associated molecular patterns, which
cause activation of neutrophils. Activated neutrophils exhibit an
altered phenotype of apoptosis, decreased deformity potential,
and spontaneous degranulation, all of which promote tissue dam-
age in the transfused host and may participate in the initiation of
TRALI [43]. Determining factors for activation are diverse and in-
clude the type of anticoagulant used, temperature and length of
storage period, and contamination by other blood products. Mo-
bilization agents also have an impact on the phenotype of col-
lected neutrophils: collection using G-CSF plus dexamethasone
increased CD11b and CD18 surface receptors, which suggests cell
activation. [71]. G-CSF-mobilized neutrophils are larger than ho-
meostatic blood neutrophils, have a different surface molecule
phenotype, and some reports suggest reduced efficacy in vivo.

Similar to G-CSF-elicted neutrophils, eNeut display a larger
surface area compared with unstimulated donor neutrophils.
However, eNeut are maintained under consistent conditions
throughout their production, which may limit prospects for acti-
vation. Furthermore, mature eNeut can be harvested quickly and
directly prior to transfusion, while the lengthy apheresis process
and associated temperature variations may contribute to donor
neutrophil activation. Current expansion strategies used for
eNeut production grant the equivalent of at least one protective
dose from a single UCB donation, eliminating the need for inva-
sive procedures on donors. Defined durations of cultures to a ma-
ture product promise definite availability of protective neutrophil
doses at a particular time without relying on donors. Because
standard protocols lead to a uniform eNeut phenotype, transfus-
ping patients using eNeut standardizes the transfused product
by eliminating the naturally occurring variability within donor
neutrophil phenotype and subpopulations. Therefore, from a re-
search perspective, transfusing eNeut instead of donor neutro-
phils may emphasize correlations between patient conditions
and GTx success without being confounded by inconsistencies
in donor neutrophil characteristics and numbers. Furthermore,
using a single homogenous product devoid of contaminants, such as
antibodies and serum proteins, reduces donor exposure and possi-
bly the rate of complications (Fig. 2).

CONCLUSION

Despite obvious advantages of eNeut over donor neutrophils,
critical milestones must be met before clinical implementation
can be suggested. Quality control guidelines, automation of bio-
reactors, and costs consideration are examples of current optimi-
ization concerns, discussed recently elsewhere [38]. The ultimate
optimization strategy for eNeut production would be conditional
immortalization of UCB HSPCs. In addition to solving the yield
issue, this outcome would standardize the manufactured product
into a well-characterized, off-the-shelf solution for neutropenic
patients. Although neutrophil-like cell lines are currently avail-
able, they originally derive from human leukemic samples, which
may hamper safe clinical use. Lin et al. have shown that HL-60-
differentiated neutrophil-like cells, named ATAK, improve overall
outcome in a chemotherapy-treated neutropenic mouse model
challenged with Candida albicans and Aspergillus fumigatus
[72]. Their effort to engineer a “suicide trap” within these cells
points toward the significant safety concern of transplanting
immortalized cells into patients. Conditional immortalization of primary cells through targeted genetic manipulation may therefore be preferred. Wang et al. have demonstrated unlimited production of neutrophils through conditional regulation of Hoxb8 in HSPCs from mouse bone marrow [73]. Further studies are warranted in human cells, however, as similar manipulations of HSPCs may lead to different outcomes in different species [74]. Regardless of the used strategy, the risk of tumorigenesis post-transplant must be carefully examined before implementing a human trial. We feel that assessments of phenotypic stability and long-term survival of transplanted cells must also be performed for non-immortalized but in vitro-manipulated eHSPCs.

It is relevant to consider the extreme condition of common candidates for GTx. Chemotherapies damage the gut mucosa dramatically, facilitating dissemination of microbes. Therefore, transfused neutrophils must be highly mobile, respond to the finest chemotactic signals, and must be able to kill microbes efficiently. Hence, it may be appropriate to investigate prospects of producing more competent eNeut by culture manipulation. Li et al. showed that SF1670 enhances neutrophil function and efficacy in vivo [75]. Similarly, addition of the retinoid agonist Li et al. showed that SF1670 enhances neutrophil function and efficacy in vivo [75]. Similarly, addition of the retinoid agonist Am80 to eNeut cultures gives rise to neutrophils with enhanced bactericidal abilities compared with G-CSF-derived neutrophils [76]. We recommend extreme caution in the exploration of this field, however, as there is a fine balance between functional superiority and promotion of inflammation that might culminate in patient tissue damage.

Successful studies of GTx highlight specific patient subpopulations and treatment context. Exploration of this niche is critical in the emerging era of personalized medicine to confirm and reveal new degrees of responses. Using eNeut instead of donor-mobilized neutrophils to facilitate future GTx studies is motivated by their practical and physiological advantages including relatively weak immunogenicity. Using a more generic and readily available eNeut product may eliminate issues associated with donor safety and accessible dose of neutrophils. Finally, research in the field of eNeut production and transfusion should attract maximal allocation of resources as the rate of cancers and associated neutropenic disorders is increasing dramatically with general population aging. Therefore, we foresee an increase in the neutropenic population requiring neutrophil support.

ACKNOWLEDGMENTS
We gratefully acknowledge the financial support of StemCells Australia and the University of Queensland.

AUTHOR CONTRIBUTIONS
M.E.G.B.: conception and design, manuscript writing; L.K.N.: conception and design, manuscript writing, financial support.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST
L.K.N. is an inventor of a patent involving eNeut (US patent 7173427).

REFERENCES

1 Schwartzberg LS. Neutropenia: Etiology and pathogenesis. Clin Cornerstone 2006;8(Suppl 5):S5–S11.
2 Bodey GP, Buckley M, Sathe YS et al. Quantitative relationships between circulating leukocytes and infection in patients with acute leukemia. Ann Intern Med 1966;64:328–340.
3 Sung L, Buxton A, Gamis A et al. Life-threatening and fatal infections in children with acute myeloid leukemia: A report from the Children’s Oncology Group. J Pediatr Hematol Oncol 2012;34:e30–e35.
4 Kimby E, Nygren P, Glimelius B. A systematic overview of chemotherapy effects in acute myeloid leukaemia. Acta Oncol 2001;40:231–252.
5 Hämäläinen S, Kuitinen T, Matinlauri I et al. Neutropenic fever and severe sepsis in adult acute myeloid leukaemia (AML) patients receiving intensive chemotherapy: Causes and consequences. Leuk Lymphoma 2008;49:495–501.
6 Cassileth PA, Lynch E, Hines JD et al. Varying intensity of postremission therapy in acute myeloid leukemia. Blood 1992;79:1924–1930.
7 Aisner J, Schimpff S, Wiernik P. Treatment of invasive aspergillosis: Relation of early diagnosis and treatment to response. Ann Intern Med 1977;86:539–543.
8 EORTC International Antimicrobial Therapy Cooperative Group. Empiric antimicrobial therapy in febrile granulocytopenic patients. Am J Med 1989;86:668–672.
9 Kuderer NM, Dale DC, Crawford J et al. Mortality, morbidity, and cost associated with febrile neutropenia in adult cancer patients. Cancer 2006;106:2258–2266.
10 Viscoli C, Varnier O, Machetti M. Infections in patients with febrile neutropenia: Epidemiology, microbiology, and risk stratification. Clin Infect Dis 2005;40(Suppl 4):S240–S245.
11 Lin MY, Carmeli Y, Zumsteg J et al. Prior antimicrobial therapy and risk for hospital-acquired Candida glabrata and Candida krusei fungemia: A case-control study. Antimicrob Agents Chemother 2005;49:4555–4560.
12 Chamilos G, Luna M, Lewis RE et al. Invasive fungal infections in patients with hematologic malignancies in a tertiary care cancer center: An autopsy study over a 15-year period (1989–2003). Haematologica 2006;91:986–989.
13 Paffenbach B, Donhuijzen K, Pahinke J et al. Systemic fungal infections in hematologic neoplasms. An autopsy study of 1,053 patients [in German]. Med Klin (Munich) 1994;89:299–304.
14 Viscoli C, Paesmans M, Sanz M et al. Association between antifungal prophylaxis and rate of documented bacteremia in febrile neutropenic cancer patients. Clin Infect Dis 2001;32:1532–1537.
15 Winkler IG, Barbier V, Nowlan B et al. Vascular nicheα-intern e-selectin regulates hematopoietic stem cell dormancy, self renewal and chemoresistance. Nat Med 2012;18:1651–1657.
16 Lucas D, Scheiermann C, Chow A et al. Chemotherapy-induced bone marrow nerve injury impairs hematopoietic regeneration. Nat Med 2013;19:695–703.
17 Freyer G, Jovenin N, Yazbek G et al. Granocyte-colony stimulating factor (G-CSF) has significant efficacy as secondary prophylaxis of chemotherapy-induced neutropenia in patients with solid tumors: Results of a prospective study. Anticancer Res 2013;33:301–307.
18 Ladenstein R, Valteau-Couanet D, Brock P et al. Randomized trial of prophylactic granulocyte colony-stimulating factor during rapid COI PIC induction in pediatric patients with high-risk neuroblastoma: The European HR-NBL1/SIOPEN study. J Clin Oncol 2010;28:3516–3524.
19 Heuser M, Zapf A, Morgan M et al. Myeloid growth factors in acute myeloid leukemia: Systematic review of randomized controlled trials. Ann Hematol 2011;90:273–281.
20 Godwin JE, Kopecky KJ, Head DR et al. A double-blind placebo-controlled trial of granulocyte colony-stimulating factor in elderly patients with previously untreated acute myeloid leukemia: A Southwest Oncology Group study (9031). Blood 1998;91:3607–3615.
21 North TE, Goessling W, Walkley CR et al. Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. Nature 2007;447:1007–1011.
22 Hoggatt J, Singh P, Stilger KN et al. Recovery from hematopoietic injury by modulating prostaglandin E(2)signaling post-irradiation. Blood Cells Mol Dis 2013;50:147–153.
23 Hoggatt J, Pelus LM. Eicosanoid regulation of hematopoiesis and hematopoietic stem and progenitor trafficking. Leukemia 2010;24:1993–2002.
24 Thoraus K, Schulz M, Bialleck H et al. Granulocyte collections: Comparison...
stimulating factor early in culture improves ex vivo expansion of neutrophils. Cytotherapy 2011; 366–377.
56 Osborne R. Fresh from the biotech pipeline—2012. Nat Biotechnol 2013; 31: 100–103.
57 Hajjema R, van der Wal J, van Dijk N. Blood platelet production: Optimization by dy-
amic programming and simulation. Comput Oper Res 2007; 34:760–779.
58 Longo DL. New therapies for castration-
resistant prostate cancer. N Engl J Med 2010; 363: 479–481.
59 Brown CJ, Navarrete CV. Clinical rele-
ance of the HLA system in blood transfusion. Vox Sang 2011; 101:93–105.
60 McCullough J, Clay M, Hurd D et al. Ef-
fect of leukocyte antibodies and HLA matching on the intravascular recovery, survival, and tis-
sue localization of 111-indium granulocytes. Blood 1986; 67:522–528.
61 Dutcher JP, Schiffer CA, Johnston GS et al. Allomimunization prevents the migration of transfused indium-111-labeled granulocytes to sites of infection. Blood 1983; 62:354–360.
62 Fung YY, Silliman CC. The role of neutro-
phils in the pathogenesis of transfusion-related acute lung injury. Transfus Med Rev 2009; 23:
266–283.
63 Adkins DR, Goodnough LT, Shenoy S et al. Effect of leukocyte compatibility on neu-
rophil increment after transfusion of granulo-
cyte colony-stimulating factor–mobilized prophy-
ac忪を含む人工粒細胞。Tissue localization of 111-indium granulocytes. Blood 1986; 67:522–528.
61 Dutcher JP, Schiffer CA, Johnston GS et al. Allomimunization prevents the migration of transfused indium-111-labeled granulocytes to sites of infection. Blood 1983; 62:354–360.
62 Fung YY, Silliman CC. The role of neutro-
phils in the pathogenesis of transfusion-related acute lung injury. Transfus Med Rev 2009; 23:
266–283.
63 Adkins DR, Goodnough LT, Shenoy S et al. Effect of leukocyte compatibility on neu-
rophil increment after transfusion of granulo-
cyte colony-stimulating factor–mobilized prophy-
acсход, Leukocyte Transplant 2001; 11:1464–1468.
31 Clayton JR, Vitale S, Kim J et al. Preval-
ce of posterior subcapsular cataracts in vol-
unteer cytapheresis donors. Transfusion 2011; 51:921–928.
32 To LB, Roberts MM, Haylock DN et al. Comparison of haematological recovery times and supportive care requirements of autolo-
gous recovery phase peripheral blood stem cell transplants, autologous bone marrow transplants and alogeneic bone marrow transplants. Bone Marrow Transplant 1992; 9:277–284.
33 Haylock DN, To LB, Dowse TL et al. Ex vivo expansion and maturation of peripheral blood CD34+ cells into the myeloid lineage. Blood 1999; 94:105–141.
34 Paquette RL, Gonzales E, Yoshimura R et al. Ex vivo expansion and differentiation of unselected peripheral blood progenitor cells in serum-free media. J Hematother 1998; 7: 481–491.
35 Reiflers J, Cailliot C, Dazev E et al. Ab-
rogation of post-myeloablative chemotherapy by expanded autologous CD34-positive cells. Lancet 1999; 354:1092–
1093.
36 Paquette RL, Dergham ST, Karpf E et al. Ex vivo expanded unselected peripheral blood: Progenitor cells reduce posttransplantation neutropenia, thrombocytopenia, and anemia in patients with breast cancer. Blood 2000;96:
2385–2390.
37 Paquette RL, Dergham ST, Karpf E et al. Culture conditions affect the ability of ex vivo expanded peripheral blood progenitor cells to accelerate hematopoietic recovery. Exp Hematol 2002; 30:374–380.
38 Csaszar E, Cohen S, Zandstra PW. Blood stem cell products: Toward sustainable bench-
marks for clinical translation. Bioessays 2013; 35:201–210.
39 Dahlberg A, Delaney C, Bernstein ID. Ex-
vivo expansion of human hematopoietic stem and progenitor cells. Blood 2011;117:
6083–6090.
40 Walasek MA, van Os R, de Haan G. Hema-
topoietic stem cell expansion: Challenges and opportunities. Ann NY Acad Sci 2012;1266:
138–150.
41 Csaszar E, Kirouac DC, Yu M et al. Rapid expansion of human hematopoietic stem-
cells by automated control of inhibitory feed-
back signaling. Cell Stem Cell 2012; 10:
218–229.
42 Kirouac DC, Zandstra PW. Understanding cellular networks to improve hematopoietic stem cell expansion cultures. Curr Opin Biotech-
nol 2006; 17:538–547.
43 Brugger W, Bross KJ, Glatt M et al. Mo-
bilization of tumor cells and hematopoietic progenitor cells into peripheral blood of patients with solid tumors. Blood 1994; 83:
636–640.
44 Gorin NC, Labopin M, Reiflers J et al. Higher incidence of relapse in patients with acute myelogenic leukemia infused with higher doses of CD34+ cells from leukapheresis products autografted during the first remission. Blood 2010;116:3157–3162.
45 Yang H, Robinson SN, Nieto Y et al. Ex vivo graft purification and expansion of autologous blood progenitor cell products from patients with multiple myeloma. Cancer Res 2011; 71:
5040–5049.
46 Mandalam R, Karsunky H, Tressler R. De-
velopment of CLT-008 (novel universal myeloid progenitors) for treatment of hematopoietic syndrome caused due to exposure to radiation. NATO Science and Technology Organization 2012; RTO Human Factors and Medicine Panel (HFM) Symposium, Ljubljana, Slovenia.
47 Cellerer Therapeutics, Inc. Safety Study of Human Myeloid Progenitor Cells (CLT-008) After Chemotherapy for Leukemia. Available at http://clinicaltrials.gov/show/NCT01279543 (2011). ClinicalTrials.gov ID: NCT01297543.
48 Ballen KK, Gluckman E, Broxmeyer HE. Umbilical cord blood transplantation: The first 25 years and beyond. Blood 2013;122:491–498.
49 McNiece I, Kubagdo N, Kerec P et al. In-
creased expansion and differentiation of cord blood products using a two-step expansion cul-
ture. Exp Hematol 2000; 28:1181–1186.
50 Shpall EJ, Quinones R, Giller R et al. Transplantation of ex vivo expanded cord blood. Biol Blood Marrow Transplant 2002;8:
368–376.
51 Tanavde VM, Malehorn MT, Lumkul R et al. Human stem-progenitor cells from neo-
nal cord blood have greater hematopoietic ex-
ward to sites of infection. Am J Physiol 1998; 275:
L261.
57 Brown CJ, Navarrete CV. The kinetics of granulopoiesis in normal man. Blood 1964;24:780–803.
58 Sato Y, Van Eeden SF, English D et al. Pul-
monary sequestration of polymorphonuclear leukocytes released from bone marrow in bac-
ericemic infection. Am J Physiol 1998; 275:
L255–L261.
59 Anderlini P, Przepiorka D, Seong D et al. Transient neutropenia in normal donors after G-CSF mobilization and stem cell apheresis. Br J Haematol 1996;94:155–158.
60 Mousset S, Herrmann S, Klein SA et al. Prophylactic and interventional granulocyte transfusions in patients with haematological malignancies and life-threatening infections during neutropenia. Ann Hematol 2005; 84:
734–741.
61 Dale DC, Liles WC, Llewellyn C et al. Neu-
trophil transfusions: Kinetics and functions of neutrophils mobilized with granulocyte-colony-stimulating factor and dexamethasone. Transfusion 1998;38:713–721.
Lin L, Ibrahim AS, Baquir B et al. Safety and efficacy of activated transfected killer cells for neutropenic fungal infections. J Infect Dis 2010;201:1708–1717.

Wang GG, Calvo KR, Pasillas MP et al. Quantitative production of macrophages or neutrophils ex vivo using conditional Hoxb8. Nat Methods 2006;3:287–293.

Zhang XB, Schwartz JL, Humphries RK et al. Effects of HOXB4 overexpression on ex vivo expansion and immortalization of hematopoietic cells from different species. STEM CELLS 2007;25:2074–2081.

Li Y, Prasad A, Jia Y et al. Pretreatment with phosphatase and tensin homolog deleted on chromosome 10 (PTEN) inhibitor SF1670 augments the efficacy of granulocyte transfusion in a clinically relevant mouse model. Blood 2011;117:6702–6713.

Ding W, Shimada H, Li L et al. Retinoid agonist Am80-enhanced neutrophil bactericidal activity arising from granulopoiesis in vitro and in a neutropenic mouse model. Blood 2013;121:996–1007.