blood advances

TO THE EDITOR:

CD34$^+$-selected stem cell boost can safely improve cytopenias following CAR T-cell therapy

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Adoptive immunotherapy with chimeric antigen receptor (CAR) T-cell therapy has improved outcomes in patients with relapse/refractory B-acute lymphoblastic leukemia (r/r B-ALL), including those who relapse after stem cell transplant (SCT), with an event-free survival of 50% at 1 year.[1-4] Prolonged cytopenias are an underrecognized toxicity of CAR T-cell therapy. For example, in the ELIANA study, 41% and 53% of patients demonstrated grade 3 to 4 thrombocytopenia and neutropenia, respectively, persisting beyond day 30. Impaired hematological recovery after CAR T-cell therapy can lead to susceptibility to opportunistic infections, bleeding, and/or transfusion dependence, causing significant morbidity and mortality.[5] Pre–CAR T-cell therapy lymphodepletion causes cytopenia that generally recovers in most patients within a month after CAR T-cell therapy. However, a proportion of patients develop persistent cytopenia, particularly neutropenia, associated with bone marrow (BM) hypoplasia that can persist for several months after CAR T-cell therpay.[6] We hypothesized that for those patients who have undergone allogeneic SCT before CAR T-cell therapy and develop persistent cytopenia after CAR-T cell therapy, an unconditioned CD34$^+$-selected stem-cell boost (SCB) from the SCT donor could be used to improve the cytopenia, similar to its use in those who have poor graft function after SCT.[7] We hereby report the outcomes of patients receiving SCB for persistent cytopenia after CAR T-cell therapy. A cohort of 101 pediatric and young adults from 2 centers in the United Kingdom with r/r B-ALL were treated with CAR T-cell therapy from May 2016 through December 2021. Of those patients, 2 were not evaluable for assessment of cytopenias after day +28 of CAR infusion (1 died on post-infusion day 4, and 1 had a morphological relapse on day 28). Of the 99 evaluable patients, 52 had undergone SCT before CAR therapy and 23 (44.2%) of them developed grade 3 to 4 cytopenia. Of the 47 patients who did not undergo an SCT, 23 (48.9%) developed grade 3 to 4 cytopenia. The difference in the rate of cytopenia between the 2 groups was not significant ($P = .5999$).

The cytopenia improved spontaneously with time in most patients. Of the 23 patients who had cytopenia and had undergone prior SCT, 7 (30.4%) were treated with a CD34$^+$-selected SCB derived from mobilized peripheral blood from their SCT donor. The indications for SCB were red cell and/or platelet transfusion and/or granulocyte-colony stimulating factor (G-CSF) dependence as a result of grade ≥3 CTCAE anemia, thrombocytopenia, and/or neutropenia (bicytopenia, n = 1; pancytopenia, n = 6), with or without infections beyond 1 month after CAR T-cell therapy. In all 7 patients, viral and drug-induced etiologies for myelosuppression were excluded, and all demonstrated hypoplastic BM for age with measurable residual disease negativity.

The median duration between CAR T-cell therapy and CD34$^+$-selected SCB was 2.6 months (range, 1.9-16.5 months) and median CD34$^+$-cell dose infused was 6.75×10^6/kg (range, 2.5×10^6 to 11.2×10^6/kg). Demographic details, disease status and cytopenias pre-SCB, toxicity and outcomes after SCB were collected retrospectively for this study. Patient, disease status, CAR T-cell constructs, SCB composition, and response characteristics are summarized in Table 1. All patients were heavily

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Original data are available by e-mail request to the corresponding author (khushnuma.mullanfiroze@gosh.nhs.uk).

Table 1. Patient characteristics who received CD34$^+$-selected SCB

Patient characteristics, n = 7		
Median age, y		
At initial diagnosis of B-ALL	6	Range, 1-23
At SCT	14	Range, 7-24
At CAR-T therapy	15	Range, 10-27
Baseline characteristics pre CAR T-cell therapy		
Previous SCT	7	–
Relapses, ≥2	6	–
CAR T-cell therapy characteristics, n = 7		
CARPALL study (#NCT02443831)	3	Autologous CD19 directed
	1	Autologous CD19/CD22 directed
AMELIA study (#NCT03289455)	1	Autologous CD19/CD22 directed
CARD study (#NCT02893189)	1	Allogenic CD19 directed
Tisagenlecleucel (licensed product)	1	Autologous CD19 directed
Post–CAR T-cell therapy, n = 7		
CRS	4	Grade 1, n = 1; Grade 2, n = 2; Grade 3, n = 1
ICANS	3	Grade 3, n = 1; Grade 4, n = 2
Median time to CD34$^+$ SCB infusion after CAR-T therapy, mo	2.6	Range, 1.9-16.5
Median Age at CD34 SCB, y	16	Range, 11-27
Pre-CD34$^+$–selected SCB, n = 7		
Cytopenias, n		
Pancytopenia	6	–
Bicytopenia	1	–
MRD negativity, n	7	MRD method: molecular n=2, flow n=3, both n=2
Chimerism (data available on 5/7 patients), n*		
Full donor	4	
Mixed	1	
Infections		
Invasive fungal infection	3	–
Viral infection(s)	1	–
Bacterial infection(s)	1	–
CD34$^+$-selected SCB characteristics, n = 7		
Median dose, per kg		
CD34$^+$ cells	6.75×10^6	Range, $2.5\text{-}11.2 \times 10^6$
CD3$^+$ T cells	0.19×10^4	Range, $0.07\text{-}1.22 \times 10^4$
Toxicities after CD34 stem-cell infusion, n = 7		
CRS†	1	–
ICANS	0	–
GVHD (acute or chronic)	0	–
Response to CD34 SCB‡		
Median time to recovery, d		
Neutrophils $>1 \times 10^9$/L without GCSF	42	Range, 11-192
Blood transfusion independent	33	Range 4-106
Platelet transfusion independent	33	Range 7-73
Status at last follow-up§		
Deceased		
Relapse	2	
Infection related	2	
Further therapy related	1	
Alive‖	2	

N = 7 patients.
ICANS, immune effector cell–associated neurotoxicity syndrome; MRD minimal residual disease.
*Of the 4/7 pts who responded to SCB, data were unavailable in 1 patient, 2 had full donor chimerism, 1 had mixed chimerism (WB 82%, CD3 95%, CD15 31%) pre-SCB.
†Treated with methylprednisolone and siltuximab.
‡Two patients died, 1 at day 24 and 1 at day 38. One patient had transient recovery of bicytopenia followed by ongoing cytopenias until death. Response data shown depicts hematological recovery for the remaining 4 evaluable patients..
§After CD34 stem-cell boost (median, 9 mo; range, 24 d–2.5 y).
‖Both patients in CR and with complete hematological recovery at last follow-up.

pretreated, including SCT, and 6 of 7 received CAR T-cell therapy in second or later relapses. Four patients received CAR-T cells in the CARPALL study (registered on http://clinicaltrials.gov as #NCT02443831). In the AMELIA study, 1 received CAR T cells and 1 received the licensed product tisagenlecleucel (#NCT03289455), and 1 received allogenic CD19 directed CAR T cells on the CARD study (#NCT02893189). SCB was well tolerated with no patient developing infusional reactions or acute or chronic graft versus host disease (aGVHD or cGVHD) at a median follow-up of 9 months (range 24 days-2.5 years). One patient developed grade 2 cytokine release syndrome (CRS) at post-SCB day 10. Two of 7 patients were not evaluable for response to SCB since 1 died of a gastrointestinal hemorrhage caused by disseminated mucormycosis on day 24, and 1 relapsed at day 38 after SCB. Of the 5 evaluable patients, 1 had transient recovery of the bicytopenia followed by recurrence of cytopenias until demise, while 4 patients recovered neutrophils $>1 \times 10^9$/L without G-CSF, were blood and platelet transfusion independent by a median of day 42 (range, 11-192 days), day 33 (range, 4-106 days), and day 33 (range, 7-73 days), respectively. At a median follow-up of 9 months after SCB, 3 of 7 patients had a B-ALL relapse (2 eventually died after relapse, and 1 died of subsequent therapy-related complications), 2 of 7 died of infectious complications related to prolonged cytopenia and 2 of 7 are alive, in CR and with complete hematological recovery at last follow-up.

Published data suggest that prolonged cytopenia after CAR T-cell therapy is associated with recent SCT (≤1 year before CAR T-cell therapy), baseline cytopenia, CAR construct, and grade ≥3 CRS

and/or immune effector cell–associated neurotoxicity syndrome, the latter implying that early inflammation plays a role.[8,9] In this cohort of 7 patients, the median interval between SCT and CAR T-cell therapy was 16 months (range, 10-53 months) and only 1 patient received SCT within 12 months before the therapy. Baseline hematological characteristics were available for 5 of 7 patients and 3 of 5 had pancytopenia before CAR T-cell therapy. CRS grade ≥3 was noted in only 1 of the 7 patients and 3 had grade ≥3 immune effector cell–associated neurotoxicity syndrome.

Rejeski et al[6] have recently proposed a CAR-HEMATOTOX model to predict post–CAR T-cell hematological toxicity in adult patients with r/r large-cell lymphoma. In this model, BM biopsy preceding lymphodepletion as a marker of baseline BM reserve is postulated as a predictor of prolonged cytopenia in heavily pretreated patients. In our study, all 7 patients were heavily pretreated, and BM aspirate demonstrated hypocellularity in 5 of 7 and effacement with blasts in 2 of 7. BM trephine was performed in 1 of 7 and demonstrated severe hypocellularity.

In the post-SCT setting, the reported incidence of aGVHD and cGVHD after SCB infusion is low because of depletion of the GVHD causing $CD3^+$ T cells associated with $CD34^+$ selection.[10-14] In our analysis of 7 patients, where the median number of infused $CD3^+$ T cells was 0.19×10^4/kg (range, 0.07×10^4 to 1.22×10^4/kg body weight), no aGVHD or cGVHD was observed. Of note, 1 patient developed a second episode of grade 2 CRS 10 days after SCB, despite B-cell aplasia and measurable residual disease negativity before the SCB. This phenomenon may be explained by the fact that $CD19^+$ progenitor cells are present in the SCB,[14] which may lead to activation of circulating CAR-T cells, thus precipitating CRS. This effect has not been reported after autologous stem cell infusion to treat delayed count recovery after CAR T-cell therapy in adult patients with lymphoma or myeloma.[15-17]

Prolonged neutropenia and lymphopenia predispose patients to significant infections after CAR T-cell therapy.[18] In this analysis, 4 of 7 patients had significant infections before SCB: probable invasive fungal infections (IFIs) in 3 of 7 and multiple viral and bacterial infections in 1 of 7. Of the 3 patients with IFIs, all received systemic antifungals and G-CSF and 1 patient also received granulocyte infusions before SCB. Of these 3 patients with IFI, 1 of 3 recovered neutrophils at day 42 and IFI eventually resolved, 1 of 3 died on day 24 from complications of disseminated gastrointestinal mucormycosis, and 1 of 3 relapsed at day 38 after SCB so that the impact of SCB on IFI could not be assessed in the latter 2 patients. One of the 7 patients suffered resistant HSV-1 orbital infection, protracted BK hemorrhagic cystitis and eventual fatal bacterial septicemia. Neutropenia in this patient improved transiently after the SCB and then recurred until the patient died.

Unfortunately, circulating CAR T-cell cell levels were not available for 3 of the 4 responding patients. However, we speculate that SCB is most likely to be effective once the number of CAR T cells have contracted and the inflammatory milieu in the BM has settled.

With the limitations of a retrospective study and the small number of patients analyzed, our data suggest that an unconditioned $CD34^+$-SCB may ameliorate prolonged cytopenia after CAR T-cell therapy. We recommend $CD34^+$ SCB in patients who have a severely hypocellular BM and demonstrate transfusion dependence and/or severe neutropenia persisting at 3 months after CD19 CAR T-cell therapy or at earlier time points in the context of severe neutropenic infection (eg, IFI). Based on data from SCB after allogeneic SCT, we recommend a minimal $CD34^+$ cell dose of 3×10^6/kg. It is not possible to recommend an upper limit of $CD19^+$ progenitors that can safely be infused. We demonstrated that this approach is safe with low risk of GVHD. However, we highlight that CRS can occur because of the presence of normal $CD19^+$ progenitors within the $CD34^+$-selected product, and clinicians should be vigilant for such events, particularly in patients with high levels of circulating CAR T cells.

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