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ISCT Committee Paper

Strategic infection prevention after genetically modified hematopoietic stem cell therapies: recommendations from the International Society for Cell & Gene Therapy Stem Cell Engineering Committee

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ABSTRACT

There is lack of guidance for immune monitoring and infection prevention after administration of *ex vivo* genetically modified hematopoietic stem cell therapies (GMHSCT). We reviewed current infection prevention practices as reported by providers experienced with GMHSCTs across North America and Europe, and assessed potential immunologic compromise associated with the therapeutic process of GMHSCTs described to date. Based on these assessments, and with consensus from members of the International Society for Cell & Gene Therapy (ISCT) Stem Cell Engineering Committee, we propose risk-adapted recommendations for immune monitoring, infection surveillance and prophylaxis, and revaccination after receipt of GMHSCTs. Disease-specific and GMHSCT-specific considerations should guide decision making for each therapy.

Introduction

Genetically modified cellular therapies (GMCTs) [1] using *ex vivo* autologous hematopoietic stem cells (HSCs) are being investigated as potential treatments for various inherited hematological disorders, including inborn errors of immunity (IEIs), hemoglobinopathies, inborn errors of metabolism (IEMs), and inherited bone marrow failure syndromes [2–11]. As of December 2023, the US Food and Drug Administration (FDA), European Medicines Agency (EMA), and UK Medicines and Healthcare products Regulatory Agency (MHRA) have granted new drug approvals for five genetically modified hematopoietic stem cell therapies (GMHSCTs) treating transfusion-dependent beta-thalassemia (TDT), sickle cell disease (SCD), cerebral adrenoleukodystrophy (c-ALD), and metachromatic leukodystrophy (MLD), with several other products of this type expected to receive approval in the near future [12–18].

In clinical practice, GMHSCT is essentially a hematopoietic stem cell transplant (HSCT) comprising of a conditioning phase, stem cell

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graft infusion, and a hematopoietic recovery phase. Multiple factors contribute to the pace and order of immune reconstitution after HSCT including the pre-HSCT immune status of the recipient, the specific myeloablative and immune ablative therapy administered during the conditioning, the composition of the stem cell graft infused, and post infusion barriers to immune reconstitution such as immune-suppressive agents administered after graft infusion. This complex process has been extensively described for standard autologous and allogeneic HSCT, but is only partially extrapolatable to immune reconstitution after GMHSCTs. Although immune compromise attributable to GMHSCTs is believed to be distinct from that associated with standard myeloablative HSCT procedures, immune function and reconstitution after the administration of such products has yet to be described in the literature.

Even as many GMHSCTs are gaining regulatory approval, clinicians lack guidance on immune monitoring and infection prevention in both the clinical trial and postapproval settings. The unique immunologic landscape associated with the rapidly emerging field of GMCT, including GMHSCT, requires the development of best practices and guidelines for infection prevention. To date, relevant accreditation or regulatory bodies have not issued such guidance due to limited published literature regarding the retention and reconstitution of immunity. Prescriber labels do not comment on infection prevention strategies, but often disclaim that the safety of vaccines after GMHSCT has not been established [30–35]. Therefore, we reviewed current infection prevention practices as reported by clinicians experienced with GMHSCTs and assessed the anticipated immunologic compromise based on knowledge gleaned from experiences with autologous and allogeneic HSCT, as well as administration of GMHSCTs to date. In accordance with the consensus of members of the International Society for Cell & Gene Therapy (ISCT) Stem Cell Engineering Committee, we propose preliminary general and risk-based recommendations for the strategic prevention of infections after receipt of GMHSCTs.

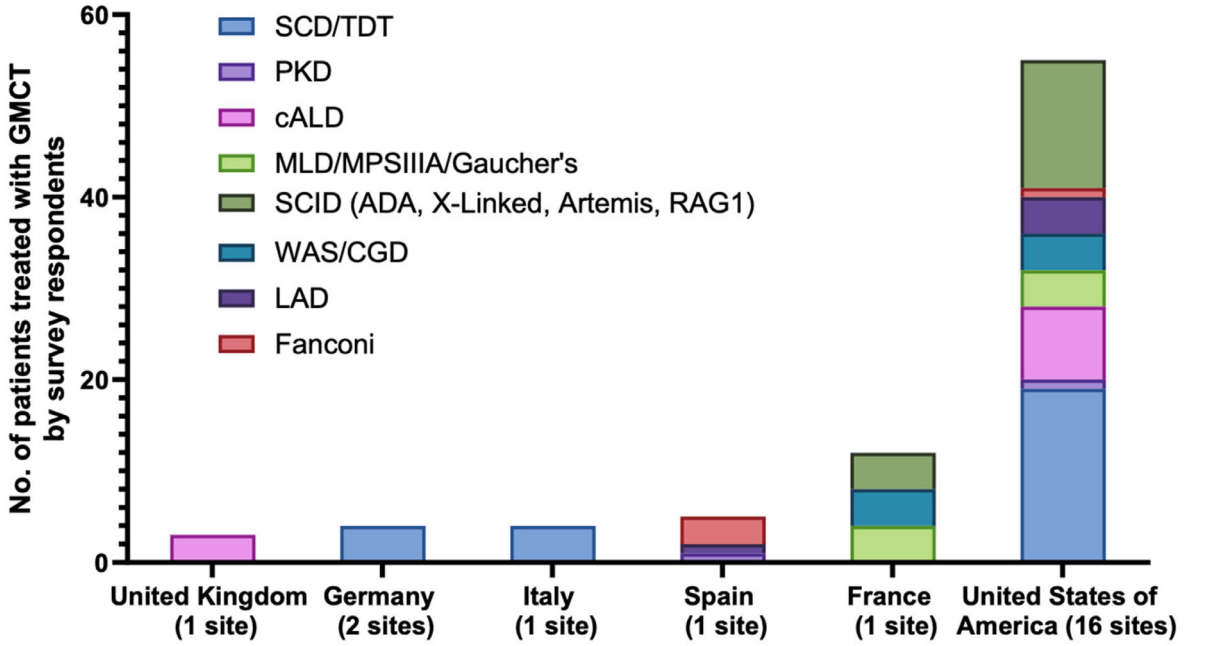
Methods

A literature review related to immune reconstitution after allogeneic HSCT, autologous HSCT, and GMHSCT, and in relation to the administration of various chemotherapeutic agents, was performed. Additionally, a survey was conducted of cellular therapy providers experienced in administering GMHSCTs regarding their current practices related to immune monitoring and infection surveillance and prevention. Individuals with experience administering GMHSCTs were identified by searching for the term “gene therapy” on ClinicalTrials.gov. The survey form was created with Microsoft Forms and distributed to representatives at centers in North America, Europe, Australia, and China via e-mail after approval by the St. Jude Institutional Review Board. Separate surveys were created for experience with GMHSCTs for IEIs and for non-IEIs because of the specific considerations associated with each. Responses were collected and stored within Microsoft Forms.

Results

Survey results: clinician-reported experience

Ninety-eight representatives (cellular therapy providers) from 11 countries, representing 29 US centers and centers in Canada, France, Belgium, Poland, the United Kingdom, Italy, Spain, Germany, China, and Australia were invited to participate. The IEI-directed survey was completed by representatives of 21 institutions, with seven reporting experience with GMHSCTs at six institutions. The non-IEI–directed survey was completed by 26 respondents, with 19 reporting their experience and representing 17 institutions. Two additional non-IEI survey responses were excluded from the compiled results because they were repeat surveys completed as a result of differing practices with Fanconi anemia with nonchemotherapy conditioning (Figure 1).



GMCT: gene modified cellular therapy; SCD: sickle cell disease; TDT: transfusion dependent beta-thalassemia; PKD: pyruvate kinase deficiency; cALD: cerebral adrenal leukodystrophy; MLD: metachromatic leukodystrophy; MPSIIIA: mucopolysaccharidosis type IIIA; SCID: severe combined immune deficiency; ADA: adenosine deaminase; WAS: Wiskott-Aldrich syndrome; CGD: chronic granulomatous disease; LAD: leukocyte adhesion disorder

Fig. 1. Number of patients treated with GMHSCT for various indications by survey respondents.

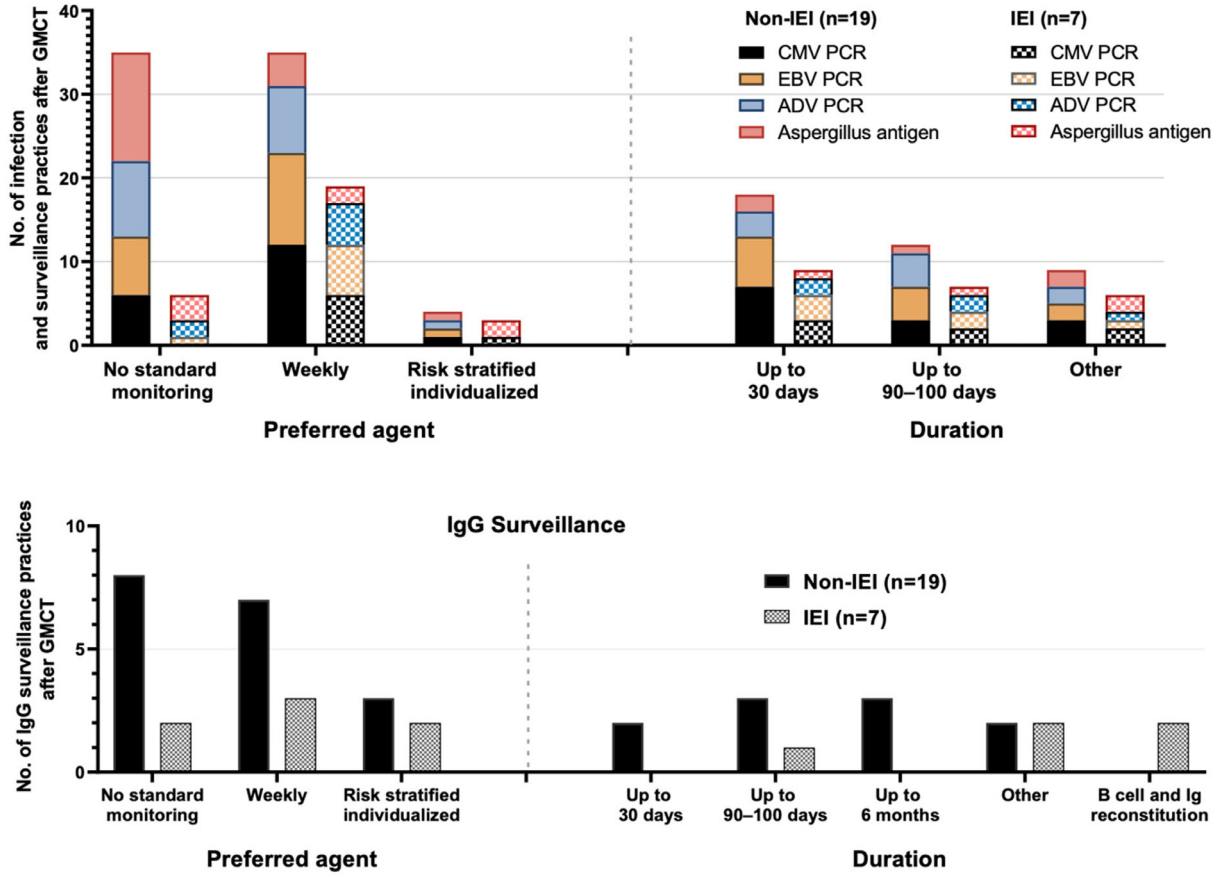
Infection surveillance

Reported infection surveillance practices were somewhat similar across institutions when GMHSCTs were used to treat IEIs, but they varied widely after therapy for non-IEIs. All seven respondents who reported experience with GMHSCTs for IEI indications reported performing standard viral surveillance for cytomegalovirus (CMV) with polymerase chain reaction (PCR) monitoring weekly, and most reported regular monitoring for Epstein–Barr virus (EBV) (n = 6) and adenovirus (ADV) (n = 5). Four respondents reported performing regular Aspergillus antigen surveillance either weekly (n = 2) or based on individual risk (n = 2). Five respondents reported measuring IgG levels every 1–2 weeks (n = 3) or monthly (n = 2). Of 19 respondents to the survey regarding non-IEI therapies, some reported performing standard monitoring for CMV (n = 13), EBV (n = 12), and ADV (n = 9) every 1–2 weeks or based on individualized risk assessment (n = 1). Aspergillus antigen surveillance was not a commonly reported practice (n = 5), and IgG levels were measured by 10 respondents every 1–2 weeks (n = 7) or monthly (n = 3). The durations of surveillance practices were generally variable for both groups; reported durations included until engraftment, 30 days, 60 days, 90–100 days, 1 year, and until institution-specific immune reconstitution standards were met after GMHSCT (Figure 2).

Infection prophylaxis

Similarly, reported prophylaxis strategies for recipients of GMHSCTs were more uniform for individuals treated for IEIs but widely variable for those treated for non-IEI indications. All seven IEI respondents reported using standard Pneumocystis jirovecii (PJP) and fungal prophylaxis, specifically favoring trimethoprim/sulfamethoxazole (TMP/SMX) for PJP (n = 5) and fluconazole for yeast targeted prophylaxis (n = 5). Four respondents reported using CMV prophylaxis with high-dose acyclovir (n = 1) or based on an individualized strategy (n = 3). Prophylaxis with (val)acyclovir was common for herpes simplex virus (HSV) (n = 5) and less so for varicella-zoster virus (VZV) (n = 3). IgG replacement was a commonly reported practice (n = 5) and was scheduled regardless of IgG serum levels (n = 2) or given based on the IgG serum levels (n = 3). Three respondents reported using antibacterial prophylaxis with levofloxacin (n = 1), TMP/SMX (n = 1), or piperacillin/tazobactam (n = 1). Three respondents reported using prophylaxis for encapsulated organisms as a universal practice, based on individual risk, or based on age and/or disease, respectively.

For the 19 non-IEI responders, the standard use of prophylaxis for PJP (n = 18), fungus (n = 15), and HSV (n = 17) was common, with TMP/SMX being used for PJP (n = 12), fluconazole for yeast (n = 9), and (val) acyclovir for HSV (n = 15) and/or VZV (n = 9). The use of prophylaxis for CMV was infrequently reported (n = 4). IgG replacement based on an IgG target of >400mg/dL was practiced by nine respondents. The use of antibiotic prophylaxis was reported by nine respondents, who used levofloxacin (n = 3), ciprofloxacin (n = 4), penicillin (n = 1), or piperacillin–tazobactam (n = 1), primarily during the neutropenic period (n = 5) but sometimes beyond (n = 4). More than half of the respondents (n = 11) reported using prophylaxis for encapsulated bacteria for a minimum of 1 year (n = 3), until there was evidence of normal splenic function (n = 3), or until there was evidence of immunity or revaccination (n = 5). Ten (of 17) respondents treating hemoglobinopathies and one treating IEMs reported using encapsulated organism prophylaxis around GMHSCT. The durations of all



IEI: inborn error of immunity; CMV: cytomegalovirus; EBV: Epstein-Barr virus; ADV: adenovirus; PCR: polymerase chain reaction

Fig. 2. Reported infection and IgG surveillance practices after GMHSCT for IEI and non-IEI indications.

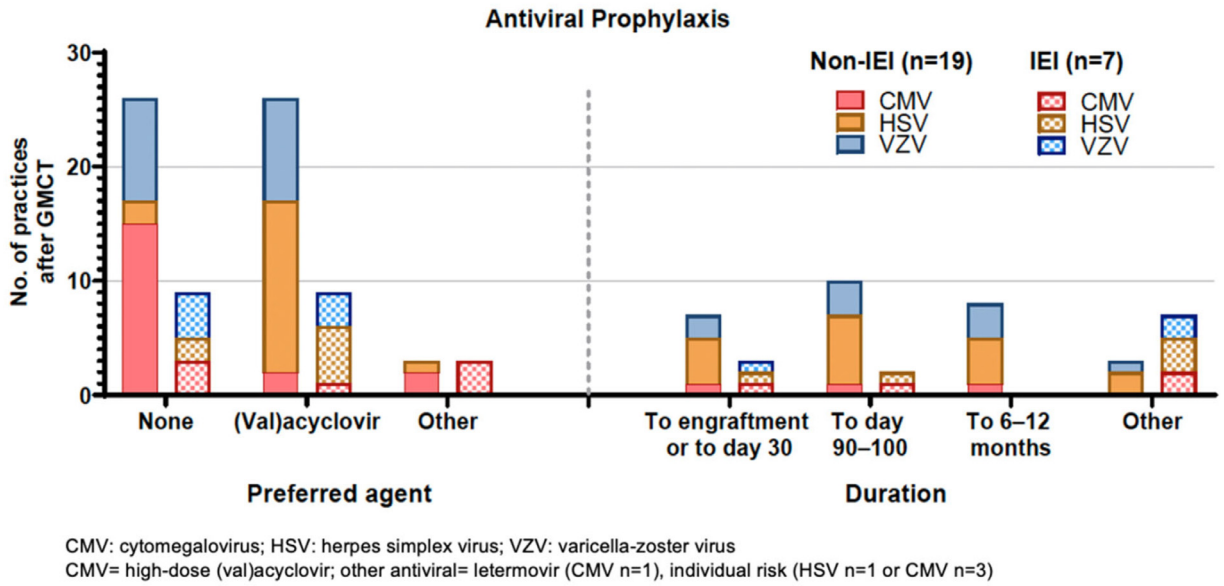


Fig. 3. Reported antiviral prophylaxis practices after GMHSCT for IEI and non-IEI indications.

prophylaxis practices were variable for both groups; they included until engraftment, 30 days, 60 days, 90–100 days, 4 months, 6 months, 1 year, and until institution-specific immune reconstitution standards were met after GMHSCT (Figures 3 and 4).

Vaccination practices

Most respondents acknowledged the need for revaccination after GMHSCT, regardless of the treatment indication. For IEI indications, five respondents reported following a revaccination protocol irrespective of pre-existing immunity (n = 3) or based on serology (n = 2). For non-IEI indications, 16 respondents reported following a revaccination protocol irrespective of pre-existing immunity (n = 9) or based on serology (n = 7). Most respondents reported revaccinating for influenza (n = 5 for IEI indications; n =15 for non-IEI indications) and COVID-19 (n = 5 for IEI indications; n = 11 for non-IEI indications) after GMHSCT. Across both groups, the timing of revaccination was inconsistent, ranging from 3 months to 1 year after GMHSCT. For IEI indications, the timing of revaccination was sometimes based on specific immune reconstitution standards (Figure 5).

Review of risks to immunity

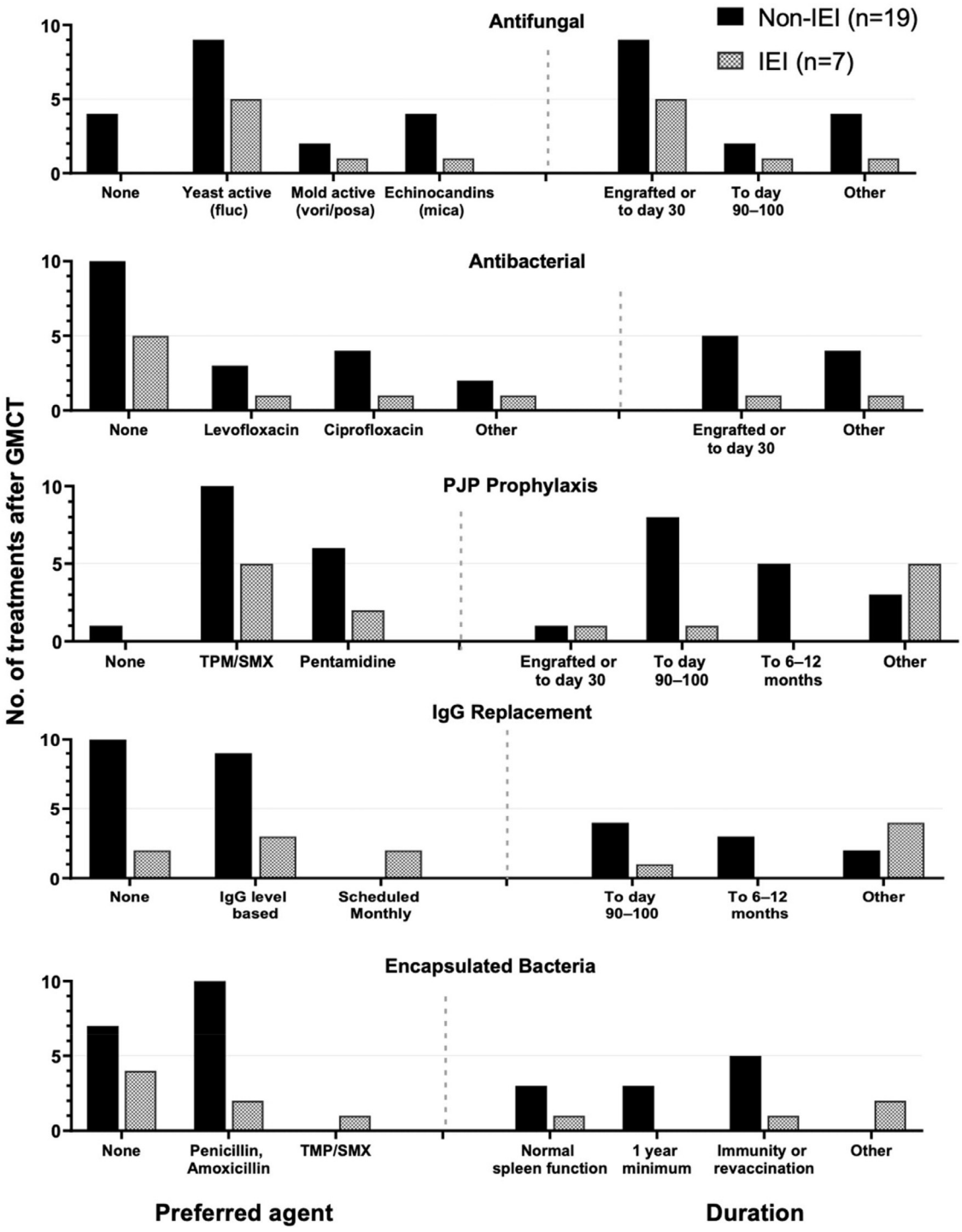
Disease-specific considerations

Individuals receiving autologous or allogeneic HSCT for malignant diseases are frequently immune compromised before HSCT, having previously received multiple courses of multiagent chemotherapy prior to undergoing a consolidative HSCT. In contrast, many patients with non-IEIs treated with GMHSCT are expected to have robust immune function before therapy [22–24], although they may have specific immune compromise related to organ impairment associated with the primary disease. As an example, functional or anatomical asplenia is commonly seen in individuals with SCD secondary to auto-infarction, or in patients with TDT after they undergo surgical splenectomy. Individuals with asplenia experience an increased risk of severe infection by encapsulated organisms, which is mitigated by prophylactic penicillin and comprehensive pneumococcal and meningococcal vaccination courses [36]. Splenic function may improve in young children with SCD after GMHSCT, just as it does after allogeneic HSCT [37], although adolescents, adults, and those with surgical asplenia are not expected to experience splenic recovery. A second example involves individuals with IEMs, particularly lysosomal storage disorders (LSDs), who are cross-reactive immune material (CRIM) negative and have a hypothesized risk of developing a high antibody titer or a T cell–mediated immune response to the newly produced enzyme after successful GMHSCT. Strategies used to mitigate these risks may result in associated immune impairments such as prolonged B-cell aplasia caused by rituximab or T-cell impairment from cyclophosphamide or fludarabine [38,39].

For individuals with IEIs, susceptibility to infection during GMHSCTs is most affected by the underlying associated immune deficiency, and immune reconstitution is dependent on the success of the genetically modified HSCs and their progeny in reversing the disease phenotype. A description of every immune deficiency disorder and immune reconstitution is beyond the scope of this review; however, consideration of the pretreatment infection history of a patient and the general underlying defect is critical. Individuals with lymphocyte deficiencies may experience broad and gradual lymphocyte immune reconstitution after successful GMHSCT, such as has been described for severe combined immune deficiency (SCID; ADA, ARTEMIS-deficient, or RAG1) [4,40–42]. Wiskott–Aldrich syndrome (WAS) [10], and leukocyte-adhesion disorder (LAD) [43]. However, these individuals are at high risk for developing severe viral and fungal infections related to profound compromise of their T, B, and/or NK lymphocytes before receipt of therapy and until immunity is restored by *de novo* immune cell production from GMHSCTs. Similarly, pretherapy susceptibility in those patients with impaired neutrophil function, such as chronic granulomatous disease (CGD), may affect post-treatment management, despite prompt neutrophil engraftment after genetically modified HSC infusion. Individuals with IEIs who have disseminated infections (e.g., invasive aspergillosis, CMV infection, *Mycobacterium bovis* BCGitis, or toxoplasmosis) before transplant may be at risk for immune reconstitution inflammatory syndrome (IRIS) after GMHSCT and, therefore, require immune modulation [44–46].

Conditioning-specific considerations

Conditioning approaches prior to GMHSCT vary according to the disease being treated. Success of the GMHSCT often requires a receptive and available bone marrow niche (either innately empty or myeloablated with chemotherapy), although immune ablation may be needed based on individualized risk. In contrast to the multiagent conditioning regimens that are used before autologous and allogeneic



PJP: *Pneumocystis jirovecii*; TPM/SMX: trimethoprim-sulfamethoxazole; fluc: fluconazole, vori: voriconazole; posa: posaconazole; mica: micafungin
Other antifungal: liposomal amphotericin 2 mg/kg/week (n=1) and individual risk (n=3)
Other anti-bacterial: penicillin (n=1), piperacillin/tazobactam (n=1), TMP/SMX (n=1); individual risk (n=1)

Fig. 4. Reported antimicrobial prophylaxis practices (nonviral) after GMHSCT for IEI and non-IEI indications.

HSCT, preparatory regimens for GMHSCTs generally use a single myeloablative agent (such as busulfan) with or without additional lymphodepleting agents (e.g., cyclophosphamide, fludarabine, or rituximab) [2–5,19,20].

Busulfan is a primarily myelotoxic agent that is commonly used before administering genetically modified HSCs as monotherapy or in combination with lymphocyte targeting agents. Busulfan is administered intravenously, using targeted pharmacokinetics [47,48]. For diseases characterized by normal or hypercellular bone marrow, such as hemoglobinopathies, IEMs, and some IEIs, high busulfan AUC targets of 80–100 mg*h/L are required for sufficient marrow ablation [49]. For some therapies, including those for SCID, low-dose busulfan

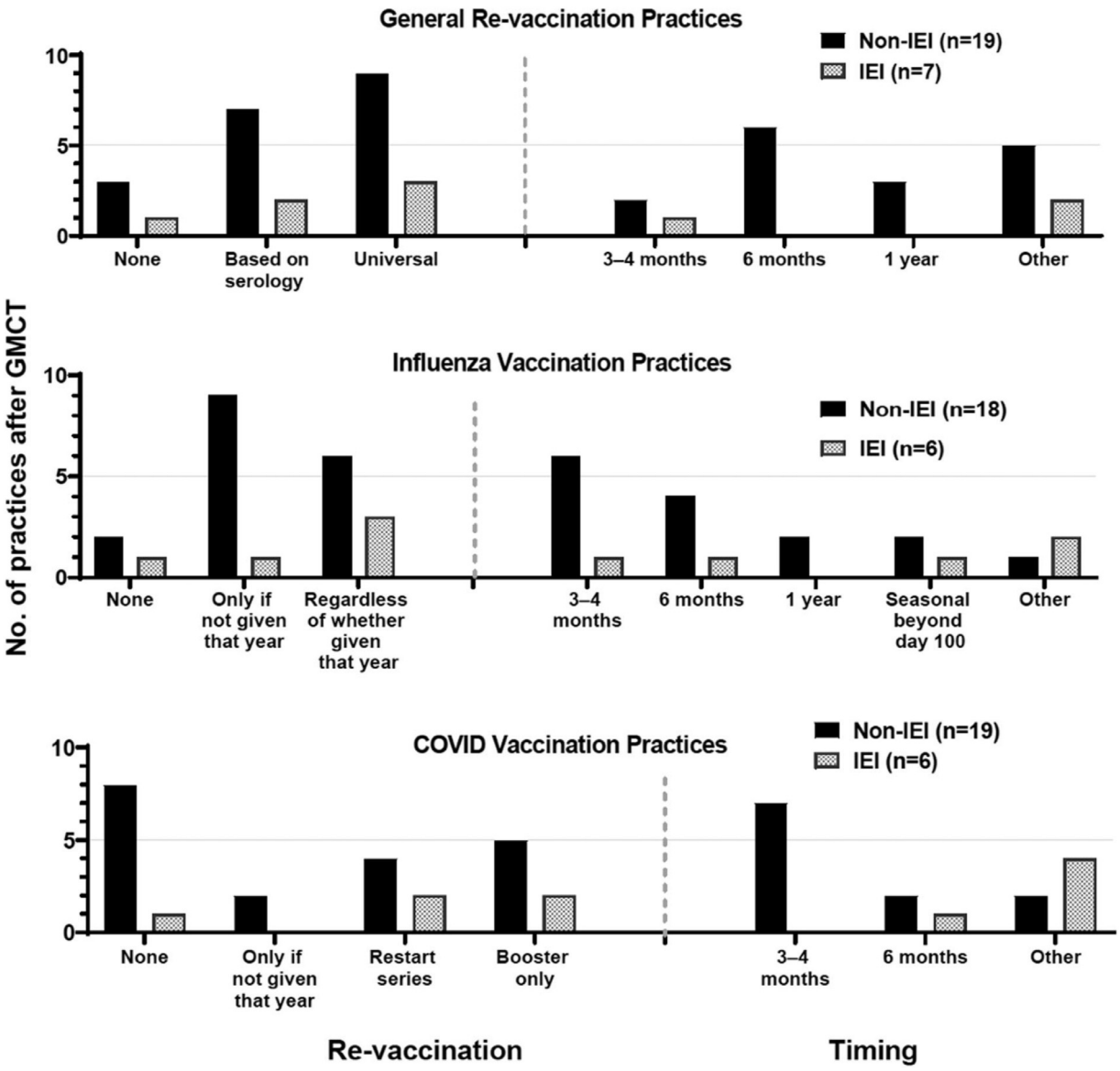


Fig. 5. Reported revaccination strategies after GMHSCT for IEI and non-IEI indications.

with an AUC target of ~20 mg*h/L may be sufficient for adequate engraftment [42]. This myeloablation causes transient neutropenia and may adversely affect lymphocyte populations but the lymphodepletion may not be as extensive as in standard allogeneic HSCT. Preclinical studies have shown that busulfan suppresses myeloid proliferation while preserving lymphocyte formation, and although busulfan may affect T- and B-cell production and function minimally in the long term, it does diminish NK-cell function in a dose-dependent fashion [50,51]. In clinical trials of GMHSCTs, busulfan monotherapy has not led to significant prolongation of lymphopenia or morbidity related to infections [2,3,5,19,20]. Because mature T- and B-cell populations are not expected to be significantly altered after busulfan therapy, the risk of infection is highest during the transient neutropenic period that also coincides with chemotherapy-induced mucosal barrier injury, especially with higher targeted doses. An absolute neutrophil count nadir of $<500/\mu L$ typically occurs 10–12 days after single-agent busulfan exposure, and neutrophil engraftment occurs 12–35 days after product infusion [2,3,19,42].

Melphalan is an alkylating agent with myeloablative properties that has been preliminarily used in a single clinical trial of GMHSCT for SCD [52]. High dose melphalan monotherapy is commonly used for autologous HSCT for multiple myeloma. It has a generally minimal toxicity profile that includes marrow aplasia, gastrointestinal toxicities, and sepsis risk [53]. The neutrophil count nadir is expected to occur earlier after exposure to melphalan than after exposure to busulfan, and in the autologous HSCT setting it lasts 5–10 days [54]. In addition to its myeloablative properties, melphalan is presumed to have immune effects similar to those of cyclophosphamide, including lymphodepletion properties (targeting regulatory T cells), and it been associated with infections that are controlled by T cell–mediated immunity, such as PJP, HSV, and CMV infections [55,56].

High doses of cyclophosphamide and/or fludarabine are often used in combination with busulfan in allogeneic HSCT because of their lympholytic effects [57]. In the allogeneic HSCT setting, immune ablation is necessary to prevent graft rejection caused by immune-competent recipient cells. In patients with IEMs such as cALD, high protein or enzyme expression and CNS penetration without acceleration of neuroinflammation is crucial to achieve success and, as has been the experience with allogeneic HSCT, cyclophosphamide or fludarabine have been used as preparation for GMHSCT. For LSDs,

cyclophosphamide, fludarabine, and/or rituximab have been used in GMHSCT (as in allogeneic HSCT) to prevent an immune response against the "neo" antigen/enzyme [58]. The target cells depleted by such agents and the duration of immune ablation are expected to be limited after GMHSCT. This is in contrast to allogeneic HSCT, for which pre-HSCT serotherapy combined with post-HSCT immunosuppressive therapies cause broad, profound, and prolonged iatrogenic immune deficiency. Nonchemotherapy conditioning has been used successfully in clinical trials of GMHSCT for Fanconi anemia. It has proved effective because of the baseline hypocellularity of the marrow niche related to the underlying disease process and the relative survival advantage and subsequent preferential expansion of even a small number of genetically corrected HSCs [21]. In such cases, the infection risk is associated with the primary disease and prior infections; hence, it may not be further exacerbated by the conditioning process of GMHSCT. Potential future incorporation of nongenotoxic conditioning agents using stem cell targeting antibodies to reduce chemotherapy toxicity may preserve lymphocyte-mediated immunity while promoting the engraftment of genetically modified HSCs [59]; however, newer agents and approaches will require review for potential novel effects on the bone marrow milieu and associated changes in immune function [60].

Gene modified hematopoietic stem cell product considerations

The manufacturing of GMHSCT products begins with selection of CD34+ cells which are manipulated *ex vivo* with genomic editing (e.g., zinc finger nucleases [ZFN], transcription activator-like effector nucleases [TALENs], clustered regular interspaced short palindromic repeats [CRISPR]-CRISPR associated [Cas]) and/or viral vector transduction, and processing which may include electroporation, cell culture, expansion, and cryopreservation. Although basic research has demonstrated that culture conditions and other manufacturing procedures can affect biological properties of HSPCs, it has not been systematically studied yet, of how such procedures affect the long-term immunity after HSC transplantation. Since GMHSCT products consist of CD34+ selected cells, they do not confer adoptively transferred immunity and immune-reconstitution requires maturation from naïve bone marrow cells to functional mature B-cells, NK cells, and thymic educated effector memory T-cells. In a clinical trial for SCID with lentiviral vector transduced GMHSCTs, naïve T-cells (indicating bone marrow derivation) have been detected and numbers sustained after therapy in most subjects. Further, reconstitution of T, NK, and to a lesser degree B-cell function has been described [42]. While most clinical trial reports focus on safety and efficacy of disease amelioration, we stress the necessity for comprehensive analyses and reporting on the contribution of differing manufacturing processes to changes in bone marrow function affecting short- and long-term immunity.

General postinfusion considerations

Generally, neutrophil recovery occurs over a period of 10–25 days after GMHSCT infusion, with higher CD34+ cell doses being associated with faster recovery [61]. Delayed lymphocyte reconstitution occurs after graft infusion in a patient undergoing autologous or allogeneic HSCT with CD34+ selected HSCs in comparison to that observed with unmanipulated T-cell replete grafts, taking as long as 3–6 months in young children and 1–2 years in adults [62,63,29]. However, in the case of GMHSCT, extensive lymphodepletion is not needed and immune reconstitution may not depend solely on the reconstitution of progenitor-derived populations. Therefore, restoration of normal immune function is expected to be accelerated after successful GMHSCT, especially in individuals who have normal lymphocyte immunity before receiving the therapy especially when no lymphocyte-targeted agents are administered.

Graft versus host disease (GVHD) causes prolonged and profound immune dysregulation after allogeneic HSCT including diminished thymic function and subsequent negative impact on T-cell education. Risk for, and presence of GVHD often dictates the duration of infection monitoring and prevention strategies, prophylaxis against opportunistic infections, and revaccination plans after therapy [64]. Individuals receiving autologous GMHSCTs are unlikely to be at risk for immune-mediated graft rejection or GVHD and, hence, do not require standard post-therapy immune modulation [25–28].

Recommendations

After reviewing the reported clinician practice and assessment of anticipated immune compromise, and in accordance with the consensus of members of the ISCT Stem Cell Engineering Committee, we propose the following standard and risk-based recommendations for immune monitoring, infection surveillance and prophylaxis, and revaccination after receipt of GMHSCTs (summarized in Table 1 and 2). Notably, disease-specific and product-specific considerations should guide decision making regarding infection prevention strategies necessary for each GMHSCT indication. Recommended laboratory assessments should be completed pursuant with College of American Pathologists/Clinical Laboratories Improvement Amendments (CAP/CLIA) regulations, as available. Flow cytometry should be performed with adherence to recognized standards as described by the International Society for Advancement of Cytometry (ISAC).

Baseline recommendations for immune monitoring

We recommend longitudinal quantification of major lymphocyte subsets to assess numerical reconstitution of immune cells and evaluation of immune function via lymphocyte proliferation response to nonspecific mitogens, immunoglobulin quantification, and serologic response to common inactivated vaccines.

Table 1
Recommendations for immune monitoring and vaccinations.

	Intervention/evaluation	Special circumstances	Schedule
Standard	1. Basic lymphocyte saubsets[a]	1. B-cell phenotyping	Longitudinal surveillance; at preconditioning, day +30, 3 months, 6 months, and 1 year
	2. Lymphocyte proliferation	2. T-cell maturation[b]	
	3. Immunoglobulins	3. NK cell maturation[d]	
		4. TRECs and TCR repertoire (if applicable)	
		5. ELISpot	
Vaccine-preventable diseases	Tetanus and pneumococcal serology	Serology for other vaccine-preventable diseases	3 to 6 months after GMHSCT and with established minimum immunity[c]

[a] Basic lymphocyte subsets: CD3, CD4, CD8 (T cells), CD19 (B cells), and CD16/CD56 (NK cells).
[b] T-cell maturation: CD25, $\alpha\beta$TCR, $\gamma\delta$TCR, and CD45RA/RO.
[c] Minimum immunity: CD4 count >200 cells/μL and IgG >400 mg/dL without IVIG supplementation in the prior 3 months.
[d] NK cell maturation: CD56dim/CD56bright.

Table 2
Recommendations for infection surveillance and prophylaxis after GMHSCT.

Surveillance practices	Frequency	Duration
CMV/EBV in blood by PCR		
Standard	Weekly	From conditioning through neutrophil engraftment
High-risk conditions and with impaired T-cell immunity	Weekly	From conditioning until establishment of minimum protective T-cell immunity[a]
Other viral surveillance	As needed	As needed
Aspergillus antigen in blood	Not recommended	Not recommended
IgG surveillance		
Standard	Not recommended	Not recommended
High-risk conditions with impaired B-cell immunity	Every 2–4 weeks	From conditioning until >3 months without need for IVIG replacement
Prophylaxis strategies	**Frequency**	**Duration**
Antibacterial	Not recommended	Not recommended
Asplenia (functional or surgical)	Penicillin VK	From conditioning until evidence of regained splenic function and/or with evidence of pneumococcus and meningitis protection
Antifungal		
Standard	Fluconazole/micafungin	From conditioning until neutrophil engraftment
High-risk conditions with impaired neutrophil function	Voriconazole/posaconazole	From conditioning until neutrophil engraftment and based on individual risk assessment
CMV		
Standard	Not recommended	Not recommended
High-risk conditions with impaired T-cell immunity or history of CMV infection	<12 years old: (val)ganciclovir, foscarnet	From conditioning until establishment of minimum protective T-cell immunity[a]
	>12 years old: letermovir	
HSV/VZV		
Standard	Not recommended	Not recommended
High-risk conditions with impaired T-cell immunity, HSV/VZV serology positive	(val)acyclovir	From conditioning until establishment of minimum protective T-cell immunity[a]
PJP prophylaxis	trimethoprim/sulfamethoxazole	From conditioning through 3 months and with minimum protective T-cell immunity[a]
IgG replacement		
Standard	Not recommended	Not recommended
High-risk conditions with impaired B-cell immunity	IgG < 400g/dL	From conditioning until >3 months without need for IVIG replacement
Vaccinations	**Indication**	**Timing**
Individual series or booster	Lack of protective immunity (Table 3) Special circumstance[c]	3 to 6 months
Comprehensive (re)vaccination	Previously unimmunized Waning serology B cell–targeted therapy	3 to 6 months and with minimum immunity[b]
SARS-CoV-2 and influenza	All	Seasonal, yearly after 3 months

[a] Minimum protective T-cell immunity: absolute CD4 count > 200 cells/μL.
[b] Minimum immunity: CD4 count > 200 cells/μL and IgG > 400 mg/dL without IVIG supplementation in the prior 3 months.
[c] Asplenia: give special consideration to monitoring, series, and/or boosters for *Streptococcus pneumoniae* and *Neisseria meningitidis* A/B.

a. Lymphocyte subsets should be quantified via multiflow cytometry to include CD3, CD4, CD8 (T cells), CD19 (B cells), and CD16/CD56 (NK cells) and immunoglobulin levels (IgG, IgM, and IgA) quantified by immunoturbidimetry.
b. Lymphocyte function should be measured via proliferation response to nonspecific mitogens (like phytohemagglutinin (PHA)) or specific antigens (candida and tetanus) producing a memory response.

Longitudinal evaluation of lymphocytes and immunoglobulins before and after GMHSCT, including at day +30, is preferred. If abnormal, repeat (at minimum) at 3 months, 6 months, and 1 year.

c. Pneumococcus and tetanus serologic evaluation for those patients with a history of vaccinations is suggested to evaluate protection status. A full evaluation of vaccine response is preferred although serology may not be widely available.

Longitudinal evaluation is preferred before therapy, at 3 months and 1 year, and then at yearly visits after GMHSCT.

Extended recommendations with academic interest

We recommend longitudinal evaluation of immune cell production and maturation.

a. B-cells phenotyping to identify maturation and class switching.
b. T cell phenotyping looking for evidence of maturation from naïve to effector/memory can help discriminate homeostatic proliferation and thymic output (CD25, $\alpha\beta$TCR, $\gamma\delta$TCR, and CD45RA/RO) [65] and is of particular interest in understanding immune reconstitution for those patients who have innate or acquired defects in lymphocyte immunity.
c. NK cell phenotyping (CD56dim/CD56bright) to identify maturation status may be of particular interest in understanding protection from viral reactivations.
d. Quantification of T-cell receptor excision circles (TRECs) and TCR repertoire are of specific interest for patients with certain IEIs with disease-associated severe lymphocyte deficiencies as indicators of endogenous T cell production and thymic maturation.
e. Post GMHSCT, the interferon-gamma response should be assessed via enzyme-linked immunosorbent spot (ELISpot) to CMV (and

other as available) antigens at day +30, 3 months, and 6 months to determine time to virus specific immunity [66].
f. For other vaccine-preventable diseases (beyond pneumococcus and tetanus), obtaining longitudinal serologies, when available, could be helpful to understand protection status.

Recommendations for infection prophylaxis and monitoring

Bacterial:

a. Standard prevention of bacterial sepsis while the patient is neutropenic, including precautions related to indwelling devices and mucosal barrier injury, are warranted. There is no evidence to support antibacterial prophylaxis during the neutropenic period, as severe neutropenia is not expected to be prolonged [67]. In individuals with splenic dysfunction, disease-specific recommendations should be followed. Close monitoring, timely workup, and prompt initiation of empiric antimicrobial therapy for fever in the setting of neutropenia are warranted.

Fungal:

a. Routine yeast prophylaxis may be considered during the neutropenic period, particularly for patients who are expected to present with significant mucositis associated with the conditioning chemotherapy [68]. Fluconazole is the preferred first-line agent, although micafungin may be considered if drug interactions preclude the use of azoles. Standard Aspergillus antigen monitoring is not recommended, given the anticipated short duration of neutropenia and its lack of efficacy in detecting infection in patients receiving antifungal prophylaxis [69].
b. For those patients with a history of previous mold infection, innate neutrophil compromise, or an expectation to have neutropenia for >30 days, mold-active prophylaxis is warranted, with voriconazole or posaconazole as preferred first-line agents [68].

Viral:

HSV and VZV

a. Routine antiviral prophylaxis for HSV and VZV is probably not warranted.
b. (Val)acyclovir should be considered for high-risk patients, such as those who are seropositive, have a history of serious infection, or have innate or acquired lymphocyte function defects, including lymphocyte-targeted conditioning [64].

HSV/VZV prophylaxis should start with conditioning, if not previously initiated, and should continue until minimum protective T-lymphocyte immunity (commonly defined as an absolute CD4+ count of >200 cells/μL) is established [70].

CMV, EBV, other herpesviridae, and adenovirus

c. Until additional data are generated regarding viral protection in the early post-GMHSCT period, viral monitoring via polymerase chain reaction (PCR) for CMV and EBV is a reasonable, albeit conservative, approach [64].

Monitoring should start at the time of conditioning and should continue weekly until neutrophil engraftment.

d. In high-risk individuals, such as those who are seropositive, have a history of serious end organ infection, or have innate or acquired lymphocyte defects, consider CMV prophylaxis with letermovir if they are older than 12 years [71]. (Val)ganciclovir or foscarnet may be considered for CMV prophylaxis in younger individuals, but the risk of CMV reactivation should be balanced against the risk of myelosuppression and renal dysfunction.

Monitoring or prophylaxis should start at the time of conditioning, if not already initiated, and should be continued until minimum protective T-lymphocyte reconstitution is established with an absolute CD4+ count of >200 cells/μL [70].

e. Other regular viral PCR monitoring (e.g., for human herpesvirus 6 [HHV-6] or adenovirus) is not warranted, but testing based on clinical suspicion should be employed.

Pneumocystis jirovecii

a. Prophylaxis with trimethoprim/sulfamethoxazole (TMP/SMX) is warranted for 3 consecutive months and/or until minimum protective T-cell immunity is established with an absolute CD4+ count of >200 cells/μL [72].

Immunoglobulin replacement:

a. Routine IgG monitoring and replacement is not warranted [73].
b. For individuals with innate or acquired defects in B-lymphocyte immunity, IgG monitoring every 2 weeks and immunoglobulin replacement for IgG < 400 mg/dL should start with conditioning, if not already initiated, and should continue until >3 months have passed without need for replacement [72].

Splenic dysfunction:

a. Prophylaxis for encapsulated organisms with oral penicillin (penicillin VK) is warranted for those patients with functional or surgical asplenia.

Prophylaxis should start with conditioning, if not already initiated, and should continue until there is evidence of regained splenic function via splenic perfusion scanning or blood tests showing an absence of Howell–Jolly bodies (<665/10^6 RBC) or pitted RBCs (<4.5%) [74]. If splenic function cannot be assessed or is not expected to recover (as in patients with surgical asplenia), continue prophylaxis until serological evidence of pneumococcal and meningococcal immunity is established and/or until completion of the pneumococcus and meningitis revaccination series or booster [75].

Recommendations for vaccination practices

a. For individuals who were not previously immunized or who have received B cell–depleting therapies, we recommend initiating a standard immunization schedule based on the experience from allogeneic HSCT, with the best response anticipated to be at least 3 months after GMHSCT and when minimum immunity, including an absolute CD4+ count of >200 cells/μL and IgG > 400 mg/dL without IVIG supplementation in the prior 3 months, is achieved [76,77].
b. For individuals who were previously immunized and are at low risk for loss of protective response, the ideal approach is to obtain serologies for vaccine-preventable diseases (pneumococcus, diphtheria, pertussis, tetanus, VZV, measles, mumps, and rubella) and to consider reimmunizing if titers are negative or show a lack of protection based on conventional standards (Table 3). Note, protection is defined differently for each antigen, and immunoglobulin titer values consistent with protection are not available for all vaccines [78,79]. A person may attain protective titers without reaching the 4-fold increase necessary for seroconversion [80].
c. For individuals who lack protective immunity, have evidence of gradual waning of the serologic response, or are at high risk for

Table 3
Antigen testing and titers defining protective immunity.

Antigen	Test	Protection
Diphtheria and tetanus	IgG antibodies	> 0.1 IU/mL
Haemophilus influenza B	IgG antibodies	> 1.0 μg/mL
Hepatitis B	Surface antibody	> 10.00 IU/L
Poliovirus (type 1 and 3)	IgG antibodies	> 1:10
N. meningitidis A, C, W-135 and Y, IgG	IgG antibodies	> 2.1 μg/mL
S. pneumoniae	IgG antibodies	≥ 0.2 μg/mL for invasive pneumococcal disease (IPD) and ≥1.3 μg/mL for non IPD
Varicella	IgG antibodies	Positive IgG
Measles	IgG antibodies	Positive IgG for measles
Rubella	IgG antibodies	Positive IgG for rubella
Mumps	IgG antibodies	Positive IgG for mumps

Protection values per manufacturer product insert.
The following tests are not recommended because no immune correlation of protection has been established: HPV, meningococcal B, hepatitis A, B. pertussis.
Serology levels are as reported by ARUP Laboratories, Salt Lake City, UT 84108.

the aforementioned infections (e.g., as a result of receiving B cell–targeted therapies), comprehensive reimmunization based on previously published schedules is recommended [77,81].

Serology may be obtained as early as 3 months after, but no later than 6 months after GMHSCT and at 1 year, and should be repeated at yearly visits after GMHSCT. Serology should be repeated at 1–3 months post completion of the vaccine series, if applicable.

d. For those patients with asplenia (functional or surgical), special consideration should be given to reimmunization with a complete series or booster for encapsulated organisms such as Streptococcus pneumoniae and Neisseria meningitidis A/B [77].
e. All individuals should be vaccinated for influenza and COVID-19 yearly [77,82].

Yearly influenza and SARS-CoV-2 vaccinations should start no later than 3 months after therapy or as specified in current local guidelines.

Conclusions

Immune impairment after the administration of *ex vivo* genetically modified HSC products is disease and therapy specific. As various GMHSCTs are approved by healthcare regulatory authorities and become available commercially, standardized and evidence-based approaches to infection prevention will be necessary. For those patients with intact immunity before GMHSCT, severe and prolonged lymphocyte-mediated immune deficiency related to chemotherapy is not expected. In these circumstances, the risk of infection is expected to be highest during the period of neutropenia. In contrast, patients with IEIs or significant lymphocytotoxic drug exposure require a more conservative approach to infection monitoring and prophylaxis that is tailored to the individual. Understanding the effect of GMHSCT manufacturing on bone marrow function and subsequent immunity is of utmost importance. Until a more concrete understanding of immune function after administration of each GMHSCT product is available, we have proposed recommendations for infection prevention based on expert experience and incorporating currently understood innate and anticipated risks extrapolated from experiences with autologous and allogeneic HSCT. Although beyond the scope of this manuscript, some of these considerations regarding infection prevention and revaccination would hold true for other genetically modified cellular therapies (e.g., chimeric antigen receptor (CAR) therapies) that include pretherapy conditioning and manipulation of immune cells.

To further develop these recommendations, clinical research protocols should include longitudinal immune monitoring of lymphocyte quantification, maturation and function, immunoglobulin production, and vaccine-related seroprotective immunity to understand the defects in protective immunity that are specific to each therapy and indication. Given the evolving experience with GMHSCTs, we recognize that these recommendations for infection monitoring and prophylaxis are preliminary and disclaim that they were designed with flexibility and caution to emphasize prudent protection of patients from infections. We envision these recommendations to simulate subsequent research, and we stress the necessity for collaborative study of prevalence and incidence of opportunistic infections with GMHSCTs to evaluate the sufficiency of these recommendations.

Author Contributions

Conception and design of the study: TDJ, GM and AS. Acquisition, analysis and interpretation of data: TDJ, GM, ZC and AS. Drafting or revising the manuscript: All authors. All authors have approved the final article.

Ethical Statement

The survey of providers from centers administering genetically modified cellular therapies to patients was determined to not meet the definition of research as defined in the Common Rule at 45 CFR 46.102(l) by the St. Jude Children's Research Hospital IRB.

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Declaration of Competing interest

Tami D. John has participated as an advisory board member and received consulting fees from bluebird bio and Vertex Pharmaceuticals. She is a medical monitor for BMT CTN 3001 GRASP study for which she receives compensation. Dr. John is the Stanford site

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