OMIP-080: 29-Color flow cytometry panel for comprehensive evaluation of NK and T cells reconstitution after hematopoietic stem cells transplantation

Sarka Vanikova | Abhishek Koladiya | Jan Musil

Abstract

This 29-color panel was developed and optimized for the monitoring of NK cell and T cell reconstitution in peripheral blood of patients after HSCT. We considered major post-HSCT complications during the design, such as relapses, viral infections, and GvHD and identification of lymphocyte populations relevant to their resolution. The panel includes markers for all major NK cell and T cell subsets and analysis of their development and qualitative properties. In the NK cell compartment, we focus mainly on CD57+ NKG2C+ cells and the expression of activating (NKG2D, DNAM-1) and inhibitory receptors (NKG2A, TIGIT). Another priority is the characterization of T cell reconstitution; therefore, we included detection of CD4+ RTEs based on CD45RA, CD62L, CD95, and CD31 as a marker of thymus function. Besides that, we also analyze the emergence and properties of major T cell populations with a particular interest in CD8, Th1, ThCTL, and Treg subsets. Overall, the panel allows for comprehensive analysis of the reconstituting immune system and identification of potential markers of immune cell dysfunction.

1 | BACKGROUND

Hematopoietic stem cell transplantation (HSCT) remains the only curative treatment available to patients with acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) [1, 2]. Furthermore, it is indicated as a treatment for other malignant and non-malignant diseases [3–5]. Although HSCT represents the most effective immunotherapy to date, it is associated with severe post-transplant complications that are the result of improper immune system function. These include relapse of the original disease, graft-versus-host-disease (GvHD) and infectious complications, such as reactivation of latent human cytomegalovirus (HCMV) infection [6]. Therefore, monitoring markers of immune system dysfunction is highly sought as it opens up the possibility for early therapeutic intervention. For example, the transfer of virus-specific T cells is actively investigated in clinical trials for the treatment of viral complications [7].

Flow cytometry represents the method of choice when evaluating the status of the post-transplant immune system as it can identify quantitative and qualitative differences between the regenerating and healthy immune system (Table 1).

Therefore, we have developed a 29-color panel to monitor the reconstitution of NK (natural killer) cell and T cell subpopulations as they are the major drivers of anti-leukemia and anti-pathogen responses as well as gatekeepers of tissue tolerance.

NK cells represent the first lymphocyte population to recover after HSCT and play an essential role in controlling viral infections and mediating the graft-versus-leukemia (GvL) effect [8–10].
However, their function, that is, cytotoxicity and cytokine production, is heavily impaired, and it can take up to 6 months until full functionality is restored [11, 12]. To be able to comprehensively monitor the reconstitution of the NK cell compartment, we have included several types of maturation, functional and qualitative markers (Table 2).

Maturation of the NK cell compartment is monitored based on the identification of the four major subsets. These include CD56hiCD16− cells representing producers of cytokines such as interferon-gamma (IFNγ) and tumor necrosis factor alpha (TNFα) [13], CD56loCD16+ highly differentiated cytotoxic cells, CD56hiCD16− cells representing a transitional phenotype sharing functional characteristics with CD56hiCD16− and CD56loCD16+ such as cytotoxicity and cytokine production, and CD56loCD16− which is a heterogeneous population composed of activated NK cells that downregulate CD16 and potential precursors of the CD56loCD16+ subset [14, 15].

HSCT does not only affect the distribution of NK cell subsets but has also been shown to affect their functional properties, such as the production of perforin and IFNγ [16]. Therefore, we have included

| TABLE 1 | Summary table for application of OMIP-080 |
| Purpose | T cells and NK cells |
| Species | Human |
| Cell types | PBMC |
| Cross-reference | OMIP-004,007,013,015,017,024,030,036,037,039,042,050,058,060 |

| TABLE 2 | Reagents used for OMIP-080 |
| Antigen | Fluorochrome | Clone | Purpose |
|---|---|---|---|
| 1 CD62L | BUV395 | DREG-56 | T cell differentiation |
| 2 Viability | Live-Dead Blue | — | Viability |
| 3 CD69 | BUV496 | FN50 | NK and T cell activation marker |
| 4 CCR6 (CD196) | BUV563 | 11A9 | Chemokine receptor; Th subset identification |
| 5 CD27 | BUV615 | M-T271 | T cell differentiation |
| 6 PD1 (CD279) | BUV661 | EH12.1 | T cell inhibitory receptor |
| 7 CD25 | BUV737 | 2A3 | T cell activation; Treg identification |
| 8 CD8 | BUV805 | RPA-T8 | CD8 T cell and NKT-like cell lineage marker |
| 9 NKG2A (CD159a) | BV421 | 131411 | NK and NKT-like cell inhibitory receptor |
| 10 CD45RA | PB | HI100 | T cell differentiation |
| 11 Tim3 | BV480 | 7D3 | T cell inhibitory receptor |
| 12 CD4 | BV570 | RPA-T4 | CD4 T cell lineage marker |
| 13 CD57 | BV605 | QA17A04 | T cell and NK cell differentiation |
| 14 CD95 | BV650 | DX2 | T cell activation and differentiation |
| 15 γδ TCR | BV711 | 11F2 | γδ T cells |
| 16 DNAM-1 (CD226) | BV750 | DX11 | T cell and NK cell activating receptor |
| 17 CD31 | BV786 | WM59 | Adhesion molecule; identification of RTE |
| 18 CCR10 | BB515 | 1B5 | Chemokine receptor; Th subset identification |
| 19 CCR4 (CD194) | BB700 | 1G1 | Chemokine receptor; Th subset identification |
| 20 TIGIT | BB750 | 741182 | T cell and NK cell inhibitory receptor |
| 21 NKG2D (CD314) | BB790 | 1D11 | NK cell activating receptor |
| 22 NKG2C (CD159c) | PE | FAB138P | NK cell activating receptor |
| 23 CD56 | PE-CF594 | NCAM16.2 | NK cell and NKT-like cell lineage marker |
| 24 CXCXR3 (CD183) | PE-Cy5 | 1G6/CXCXR3 | Chemokine receptor; Th subset identification |
| 25 FoxP3 | PE-Cy5.5 | PCH101 | Master transcription factor for Tregs |
| 26 CD39 | PE-Cy7 | A1 | Treg activation marker |
| 27 CD3 | AF647 | UCHT1 | T cell and NKT-like cell lineage marker |
| 28 CD16 | AF700 | 3G8 | NK cell differentiation |
| 29 Perforin | APC Fire750 | B-D48 | Cytolytic function |

Abbreviations: AF, Alexa Fluor; APC, Allophycocyanin; BB, Brilliant Blue; BUV, Brilliant Ultraviolet; BV, Brilliant Violet; Cy, cyanine; PB, Pacific blue; PE, R-phyceroerythin.
perforin and Tim3 as a proposed regulator of IFNγ production in NK cells [12]. Furthermore, regarding relapse of the original disease expression of activating receptors NKG2D and DNAM-1 and inhibitory receptors, NKG2A and TIGIT are monitored due to their role in leukemia immune escape [17–19].

T cells represent the orchestrators of adaptive immunity and are indispensable for long-lasting protective immune responses. In comparison to NK cells, their reconstitution is more complex as it is highly influenced by the type of graft, that is, umbilical cord blood, bone marrow, mobilized blood stem cells [20]. Two pathways of reconstitution exist, namely homeostatic expansion and de novo differentiation via the thymus [11].

In this panel, we define two main subsets of T cells based on the type of T cell receptor (TCR), namely γδ T cell receptor (TCR), namely γδ T cells represent the orchestrators of adaptive immunity and are indispensable for long-lasting protective immune responses. In comparison to NK cells, their reconstitution is more complex as it is highly influenced by the type of graft, that is, umbilical cord blood, bone marrow, mobilized blood stem cells [20]. Two pathways of reconstitution exist, namely homeostatic expansion and de novo differentiation via the thymus [11].

In this panel, we define two main subsets of T cells based on the type of T cell receptor (TCR), namely γδ TCR+ cells and γδ TCR−, which consider to be γδ T cells. We further subtype γδ T cells based on CD56 expression into CD56+ T cells and classical T cells (Figure 1).

γδ T cells represent a bridge between innate and adaptive responses, they recognize infected or malignant cells either through the expression of activating receptors which they share with NK cells like NKG2D, NKG2C and DNAM-1 or by recognition through their TCR, that recognizes phosphoantigens including intermediates from the isoprenoid biosynthesis pathway [21, 22]. In our panel, we subtype γδ T cells based on the expression of CD57 and NKG2A into CD57 + NKG2A− terminally differentiated highly cytotoxic cells, CD57−NKG2A− cytotoxic cells and cytokine-producing CD57-NKG2A+ cells. Furthermore, we are able to evaluate the expression of costimulatory molecules such as NKG2D, which mediates TCR unrestricted effector functions, and CD27 which has been proposed to co-stimulate and thereby boost recognition of target cells via TCR [23].

NKT-like cells represent another subset of T cells that share similarities with NK cells. In our panel, we define them as CD3 + γδ TCR- CD56+ cells. It has to be noted that this population is highly heterogeneous, and besides, NKT-like cells contain CD1d-restricted type I (iNKT) and type II NKT cells; however, these represent a minority [24]. Currently, it has been proposed that NKT-like cells arise from classical T-cells that start to express NK cell markers after prolonged antigen stimulation [25]. Their physiological role is currently under investigation. As there is no clear agreement about the nomenclature of these cells, we further refer to them as CD56+ T cells. In our panel, we identify three subsets of CD56+ T cells: CD4+, CD8−, and CD4− CD8− (DN) cells. We further subdivide the CD8 subset into NKG2C+ and NKG2C− cells as these might represent cells with specific roles for HCMV. These cells are dominated by the CD45RA+ CD62L− effector phenotype, are highly cytotoxic and lack expression of the NKG2A receptor. CD4+: CD56− T cells are also highly differentiated with an effector memory phenotype. These cells contain a substantial population of cytotoxic cells, as evidenced by the expression of perforin and CD57.

Classical T cells are defined in our panel as CD3 + CD56− γδ TCR−, however it has to be noted that the population of CD3 + CD56− γδ TCR− is actually heterogeneous and includes true classical αβ T cells and mucosal-associated invariant T (MAIT) cells characterized by the expression of an invariant αβ TCR [26]. αβ T cells are the centerpiece of adaptive immune responses. However, they are heavily affected by HSCT. The most prominent change, when compared to healthy, is the inverse ratio of CD4:CD8 T cells which is caused by faster regeneration of CD8+NKT-like cells; however, this population is highly heterogeneous, and besides, NKT-like cells contain CD1d-restricted type I (iNKT) and type II NKT cells; however, these represent a minority [24]. Currently, it has been proposed that NKT-like cells arise from classical T-cells that start to express NK cell markers after prolonged antigen stimulation [25]. Their physiological role is currently under investigation. As there is no clear agreement about the nomenclature of these cells, we further refer to them as CD56+ T cells. In our panel, we identify three subsets of CD56+ T cells: CD4+, CD8−, and CD4− CD8− (DN) cells. We further subdivide the CD8 subset into NKG2C+ and NKG2C− cells as these might represent cells with specific roles for HCMV. These cells are dominated by the CD45RA+ CD62L− effector phenotype, are highly cytotoxic and lack expression of the NKG2A receptor. CD4+: CD56− T cells are also highly differentiated with an effector memory phenotype. These cells contain a substantial population of cytotoxic cells, as evidenced by the expression of perforin and CD57.

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As early post-transplant responses heavily rely on CD8+ T cells, we have included several types of markers for assessing the functional status of the CD8+ T cell compartment. First of all, we monitor the distribution of different memory subsets such as CD45RA+ CD62L+ CD95+ memory stem cells (Tscm), CD45RA− CD62L+ central memory cells (Tcm), CD45RA− CD62L− effector memory cells (Tem), and finally CD45RA+ CD62L− effector cells (Teff). Besides that, we have also included activation markers such as the early activation marker CD69, CD25, and perforin as a marker for effector function.

Furthermore, we included markers of T cell exhaustion and senescence, that is, PD1, TIGIT, Tim3, and CD57, as these are important when considering AML relapse [30–32].

CD4+ T cells are the main regulators of innate and adaptive immune responses. We have designed our panel to separate the main T helper (Th) subsets, that is, CD25+ FoxP3 regulatory T cells (Tregs), perforin+ CD27− Th cytotoxic lymphocytes (ThCTL), CCR10-CCR4-CCR6+ Th1, CCR10+ CCR4+ CCR6+ Th22, CCR10− CCR4+ CCR6+ Th17, CCR10− CCR4+ CCR6− Th2, CCR10+ CCR4+ CCR6− Th granulocyte-macrophage colony-stimulating factor (ThGM-CSF), and finally CCR10− CCR4− CCR6− Th9 cells.

Monitoring of Tregs in this panel is a priority as they have been shown to adversely affect anti-leukemic responses and can influence the occurrence of GvHD [33–35]. We monitor Th-like Treg phenotypes, as it has been proposed that Tregs mirror the effector cells they suppress [36]. Additionally, we included markers of highly suppressive Tregs, i.e., CD39, PD1, Tim3 and TIGIT [37, 38].

Another priority of our panel is the identification of exhaustion markers on Th1 and ThCTL cells, as these represent important mediators of antiviral and anti-leukemic responses [39, 40].

Other non-Treg subsets like Th17 and Th22 can play an important role in immunity against bacterial pathogens causing post-HSCT complications such as Pseudomonas aeruginosa, Streptococcus pneumoniae or Pneumocystis jiroveci [41–43]. Finally, we utilized the EmbedSOM algorithm to identify important cell subsets from live single cells (Figure 1H). [20].
FIGURE 1 Manual gating strategy and algorithmic analysis. (A) Before the identification of individual subsets, debris, doublets and dead cells were removed. (B) Cell lineages were identified based on the expression of the following phenotypes: CD56+ (NK cells), CD56−CD3− γδ TCR+ (γδ T cells), CD3+γδ TCR−CD56+ T cells, CD3+γδ TCR−CD56−CD4+ (T helper cells) and CD3+γδ TCR−CD56−CD8+ (T cytotoxic cells). (C) CD4+ T cells were delineated into CD25+FoxP3+ Tregs and non-Treg cells. Activation of Tregs was evaluated based on the expression of CD39. Furthermore, expression of PD1, TIGIT, and Tim3 identifying highly suppressive Tregs was evaluated. Cells in the non-Treg gate were divided into individual T helper subsets based on the combination of chemokine receptors. These cells were first gated for perforin+CD27−CTL cells and perforin− cells. The perforin− cells were gated for CCR10+ CCR4+ CCR6+ Th22 cells, CCR10+ CCR4+CCR6− ThGM-CSF, CCR10−CCR4−CCR6− Th2 cells, CCR10−CCR4+CCR6+ Th17 cells, CCR10−CCR4+CCR6−CCR3− Th9 cells and CCR10−CCR4−CCR6−CCR3− CXCR3+ Th1 cells. Thymic function was evaluated on CD4+ gated cell via identification of CD45RA+CD62L−CD95−CD31+ cells. (D) Gated CD8+ cells were subtyped into individual memory subsets based on CD45RA and CD62L expression of inhibitory receptors, activation markers and perforin was evaluated. Inhibiting receptors are only weakly expressed on T cells from healthy donors, and therefore we show a comparison between healthy and patients after HSCT. (E) Gating of γδ T cells based on CD57+ NKG2A+ cytokine-producing cells, CD57+ NKG2A+ terminally differentiated cytotoxic cells and CD57− NKG2A− with intermediate phenotype. Expression of perforin and TIGIT on these cells is depicted using a histogram. (F) NK cells were subtyped based on the expression of CD56 and CD16, and a comparison of a healthy donor and post-HSCT patient is shown. CD56hiCD16− cells represent less differentiated cytokine-producing cells characterized by low expression perforin and high expression of CD45RA+CD62L+CD95−CD31+ cells. (G) CD56+ T cells can be divided into three main lineages based on CD4 and CD8. The CD4+ cells are enriched with cytotoxic perforin expressing cells mostly terminally differentiated, as evident by expression of CD57. The CD8+ cell can be split into two subsets based on the expression of NKG2C. These subsets show distinct differentiation status, as evident by the expression patterns of CD45RA and CD62L. (H) Identification of individual cell types was performed on gated live single cells using the Embedding Guided by Self-Organizing Map (EmbedSOM) algorithm [20]. Expression pattern of markers on individual cell subsets is shown in Figure S8. FSC, forward-scatter; SSC, side-scatter [Color figure can be viewed at wileyonlinelibrary.com]
Additional details regarding cell phenotypes and markers can be found in the online material.

2 | SIMILARITIES TO PUBLISHED OMIPs

Currently, there is no OMIP tailored explicitly to the need of monitoring a regenerating immune system post-HSCT. OMIP-080 represents a unique combination allowing for comprehensive monitoring of NK cell, NKT-like cell and both γδ and αβ T cell reconstitution using qualitative and functional markers. It identifies NK cell subsets similarly to OMIP 007, 039, 070 and evaluates the expression of activating and inhibitory receptors. However, OMIP-080 also includes DNAM-1, which plays roles in the regulation of relapses. Furthermore, it evaluates functional properties of NK cells such as perforin production.

T cell reconstitution is also a priority of the panel and therefore it maps the emergence of RTE defined similarly to OMIP 013 and monitors the differentiation status Th subsets similarly to OMIP 017 and 030. In contrast to OMIP 030, it defines memory subsets using CD62L and identifies cytotoxic CD4 T cells as a unique subset. Furthermore, our panel allows for deeper analysis of Treg function through evaluation of CD39 expression similarly to OMIP 004 and 015, and identification of highly suppressive populations based on expression of PD1, TIGIT, Tim3. Similarly, to OMIP 037 and 050 our panel detects markers of exhaustion, senescence and activation, but also allows to map these markers to individual Th subsets.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

Sarka Vanikova: Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); validation (equal); visualization (equal); writing – review and editing (equal). Abhishek Koladiya: Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); validation (equal); visualization (equal); writing – review and editing (equal). Jan Musil: Conceptualization (equal); formal analysis (equal); funding acquisition (lead); methodology (equal); project administration (equal); resources (equal); supervision (lead); visualization (equal); writing – original draft (equal); writing – review and editing (equal).

PEER REVIEW

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REFERENCES

1. Wall SA, Devine S, Vasu S. The who, how and why: allogeneic transplant for acute myeloid leukemia in patients older than 60 years. Blood Rev. 2017;31:362–9.
2. Bartenstein M, Deeg HJ. Hematopoietic stem cell transplantation for MDS. Hematol Oncol Clin North Am. 2010;24:407–22.
3. Naïk S, Martinez CA, Omer B, Sasa G, Yassine K, Allen CE, et al. Allo- geneic hematopoietic stem cell transplant for relapsed and refractory non-Hodgkin lymphoma in pediatric patients. Blood Adv. 2019;3: 2689–95.
4. Palumbo A, Cavallo F, Gay F, di Raimondo F, Ben Yehuda D, Petrucci MT, et al. Autologous transplantation and maintenance therapy in multiple myeloma. New Engl J Med. 2014;371:895–905.
5. Pai S-Y, Logan BR, Griffith LM, Buckley RH, Parrott RE, Dvorak CC, et al. Transplantation outcomes for severe combined immunodeficiency, 2000–2009. New Engl J Med. 2014;371:434–46.
6. Gratwohl A, Brand R, Frassoni F, Rocha V, Niederwieser D, Reusser P, et al. Cause of death after allogeneic haematopoietic stem cell transplantation (HSCT) in early leukaemias: an EBMT analysis of lethal infectious complications and changes over calendar time. Bone Marrow Transplant. 2005;36:757–69.
7. Kaeuferle T, Krauss R, Blaeschke F, Willier S, Feuchtinger T. Strategies of adoptive T-cell transfer to treat refractory viral infections post allogeneic stem cell transplantation. J Hematol Oncol. 2019;12:13.
8. Cichocki F, Verneiris MR, Cooley S, Bachanova V, Brunstein CG, Blazar BR, et al. The past, present, and future of NK cells in hematopoietic cell transplantation and adoptive transfer. Curr Top Microbiol Immunol. 2016;395:225–43.
9. Shaffer BC, Hsu KC. How important is NK alloreactivity and KIR in allogeneic transplantation? Best Pract Res Clin Haematol. 2016;29: 351–8.
10. Storek J, Geddes M, Khan F, Huard B, Helg C, Chalandon Y, et al. Reconstitution of the immune system after hematopoietic stem cell transplantation in humans. Semin Immunopathol. 2008;30:425–37.
11. Ullah MA, Hill GR, Tey SK. Functional reconstitution of natural killer cells in allogeneic hematopoietic stem cell transplantation. Front Immunol. 2016;7:144.
12. Cooper MA, Fehniger TA, Turner SC, Chen KS, Ghaheri BA, Ghayur T, et al. Human natural killer cells: a unique innate immunoregulatory role for the CD56bright subset. Blood, 2001;97:3146–51.
13. Grzywacz B, Kataria N, Verneiris MR. CD56dimCD16+ NK cells downregulate CD16 following target cell induced activation of matrix metalloproteinases [4]. Leukemia, 2007;21:356–9.
14. Amand M, Iserentant G, Poli A, Sleiman M, Fievez V, Sanchez IP, et al. Human CD56dimCD16dimCells as an individualized natural killer cell subset. Front Immunol. 2017;8:699.
15. Simonetta F, Pradier A, Bosshard C, Masouridi-Levrat S, Chalandon Y, Roosnek E. NK cell functional impairment after allogeneic hematopoietic stem cell transplantation is associated with reduced levels of T-bet and Eomesoderm. J Immunol. 2015;195:4712–20.
16. Hilpert J, Grosse-Hovest L, Grünebach F, Buechele C, Nuebling T, et al. Comprehensive analysis of NKGD2 ligand expression and release in leukemia: implications for NKG2D-mediated NK cell responses. J Immunol. 2012;189:1360–71.
17. Carlsten M, Baumann BC, Simonsson M, Jädersten M, Forsblom AM, Hammarstedt C, et al. Reduced DNAM-1 expression on bone marrow NK cells associated with impaired killing of CD34+ blasts in myelodysplastic syndrome. Leukemia. 2010;24:1607–16.
18. Stringaris K, Sekine T, Khoder A, Alsuliman A, Razzaghi B, Sargeant R, et al. Leukemia-induced phenotypic and functional defects in natural
killer cells predict failure to achieve remission in acute myeloid leukemia. Haematologica. 2014;99:836–47.

19. Bosch M, Khan FM, Storek J. Immune reconstitution after hematopoietic cell transplantation. Curr Opin Hematol. 2012;19:324–55.

20. Kratochvíl M, Koladiya A, Vondrášek J. Generalized Embed SOM on quadtree-structured self-organizing maps. F1000Res. 2020;8:2120.

21. Simões AE, di Lorenzo B, Silva-Santos B. Molecular determinants of target cell recognition by human γδ T cells. Front Immunol. 2018;9:929.

22. Pauza CD, Liou ML, Lahusen T, Xiao L, Lapidus RG, Cairo C, et al. Gamma delta T cell therapy for cancer: it is good to be local. Front Immunol. 2018;9:1305.

23. Lawand M, Déchanet-Merville J, Dieu-Nosjean MC. Key features of gamma-delta T-cell subsets in human diseases and their immunotherapeutic implications. Front Immunol. 2017;8:761.

24. Seggewiss R, Einsele H. Immune reconstitution after allogeneic transplantation and expanding options for immunomodulation: an update. Blood. 2010;115:3861–8.

25. Ogonok J, Juric MK, Ghimire S, Varanasi PR, Holler E, Greinix H, et al. Immune reconstitution after allogeneic hematopoietic stem cell transplantation. Front Immunol. 2016;7:616.

26. Godfrey DI, Koay HF, McCluskey J, Gherardin NA. The biology and functional importance of MAIT cells. Nat Immunol. 2019;20:1110–28.

27. Seggewiss R, Einsele H. Immune reconstitution after allogeneic transplantation and expanding options for immunomodulation: an update. Blood. 2010;115:3861–8.

28. Ogonek J, Juric MK, Ghimire S, Varanasi PR, Holler E, Greinix H, et al. Immune reconstitution after allogeneic hematopoietic stem cell transplantation. Front Immunol. 2016;7:507.

29. Ravkov E, Slev P, Heikal N. Thymic output: assessment of CD4+ T-cells to aging, and viral and tumor antigens. Front Immunol. 2016;7:616.

30. Schnorfeil FM, Lichtenegger FS, Emmerig K, Schlueter M, Neitz JS, Draenert R, et al. T cells are functionally not impaired in AML: increased PD-1 expression is only seen at time of relapse and correlates with a shift towards the memory T cell compartment. J Hematol Oncol. 2015;8:93.

31. Kong Y, Zhu L, Schell TD, Zhang J, Claxton DF, Ehmann WC, et al. PD-1hiTIM-3+ T cells associate with and predict leukemia relapse in AML patients after allogeneic stem cell transplantation. Blood Cancer J. 2015;5:e330.

32. Kong Y, Zhu L, Schell TD, Zhang J, Claxton DF, Ehmann WC, et al. T-cell immunoglobulin and ITIM domain (TIGIT) associates with CD8+ T-cell exhaustion and poor clinical outcome in AML patients. J Hematol Oncol. 2016;22:3057–66.

33. Shenghui Z, Yixiang H, Jianbo W, Kang Y, Laixi B, Yan Z, et al. Elevated frequencies of CD4+ CD25+ CD127lo regulatory T cells is associated to poor prognosis in patients with acute myeloid leukemia. Int J Cancer. 2011;129:1373–81.

34. Bansal AK, Sharawat SK, Gupta R, Vishnubhatla S, Dhawan D, Bakhshi S. Regulatory T cells in pediatric AML are associated with disease load and their serial assessment suggests role in leukemogenesis. Am J Blood Res. 2020;10:90–6.

35. Elias S, Rudensky AY. Therapeutic use of regulatory T cells for graft-versus-host disease. Br J Haematol. 2019;187:25–38.

36. Duhuen T, Duhuen R, Lanzavecchia A, Sallusto F, Campbell DJ. Functionally distinct subsets of human FOXP3+ Treg cells that phenotypically mirror effector Th cells. Blood. 2012;119:4430–40.

37. Gu J, Ni X, Pan X, Lu H, Lu Y, Zhao J, et al. Human CD39hi regulatory T cells present stronger stability and function under inflammatory conditions. Cell Mol Immunol. 2017;14:521–8.

38. Anderson AC, Joller N, Kuchroo VK. Lag-3, Tim-3, and TIGIT: co-inhibitory receptors with specialised functions in immune regulation. Immunity. 2016;44:989–1004.

39. Lim EY, Jackson SE, Wills MR. The CD4+ T-cell response to human cytomegalovirus in healthy and Immunocompromised people. Front Cell Infect Microbiol. 2020;10:202.

40. van Balen P, van Bergen CAM, van Luxemburg-Heij S, Klerk W, van Egmond EHM, Veld SAJ, et al. CD4 donor lymphocyte infusion can cause conversion of chimerism withoutGVHD by inducing immune responses targeting minor histocompatibility antigens in HLA class II. Front Immunol. 2018;9:3016.

41. Wu W, Huang J, Duan B, Traficante DC, Hong H, Risech M, et al. Th17-stimulating protein vaccines confer protection against Pseudomonas aeruginosa pneumonia. Am J Respir Crit Care Med. 2012;186:420–7.

42. Marqués JM, Rial A, Muñoz N, Pellay FX, van Maelle L, Léger H, et al. Protection against Streptococcus pneumoniae serotype 1 acute infection shows a signature of Th17- and IFN-γ-mediated immunity. Immunobiology. 2012;217:420–9.

43. Hoving JC, Kolls JK. New advances in understanding the host immune response to pneumococcus. Curr Opin Microbiol. 2017;40:65–71.

SUPPORTING INFORMATION
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