Molecular and cellular features of CTLA-4 blockade for relapsed myeloid malignancies after transplantation

Livius Penter,1,4 Yi Zhang,5,6 Alexandra Savell,1 Teddy Huang,1,7 Nicoletta Cieri,1,3 Emily M. Thrash,8 Seunghee Kim-Schulze,9 Aashna Jhaveri,5 Jingxin Fu,6 Srinika Ranasinghe,8 Shuqiang Li,1,2,7 Wandi Zhang,1 Emma S. Hathaway,8 Matthew Nazzaro,8 Haesook T. Kim,5 Helen Chen,10 Magdalena Thurin,10 Scott J. Rodig,11 Mariano Severgnini,6 Carrie Cibulskis,2 Stacey Gabriel,2 Kenneth J. Livak,1,7 Corey Cutler,1,3,12 Joseph H. Antin,1,3,12 Sarah Nikiforow,1,3,12 John Koreth,1,3,12 Vincent T. Ho,1,3,12 Philippe Armand,1,3,12 Jerome Ritz,1,3,12 Howard Streicher,10 Donna Neuberg,5 F. Stephen Hodi,1,8 Sacha Gnjatic,9 Robert J. Soiffer,1,3,12 X. Shirley Liu,5,6 Matthew S. Davids,1,3,12 Pavan Bachireddy,1,2,3,12 and Catherine J. Wu1,2,3,12*

1Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA; 2Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge, MA; 3Harvard Medical School, Boston, MA; 4Department of Hematology, Oncology, and Tumorimmunology, Campus Virchow Klinikum, Berlin, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany; 5Department of Data Science, Dana-Farber Cancer Institute, Boston, MA; 6Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA; 7Translational Immunogenomics Laboratory and 8Center for Immuno-Oncology, Dana-Farber Cancer Institute, Boston, MA; 9Human Immune Monitoring Center at the Icahn School of Medicine at Mount Sinai, New York, NY; 10Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, MD; and 11Department of Pathology and 12Department of Medicine, Brigham and Women’s Hospital, Boston, MA

**KEY POINTS**

- Increased T-cell infiltration, activation, and peripheral chemokine expression mark ipilimumab response to relapsed leukemia after HSCT.
- Similar to the nontransplant setting, ipilimumab alters peripheral memory T-cell populations and leads to global T-cell activation.

Relapsed myeloid disease after allogeneic stem cell transplantation (HSCT) remains largely incurable. We previously demonstrated the potent activity of immune checkpoint blockade in this clinical setting with ipilimumab or nivolumab. To define the molecular and cellular pathways by which CTLA-4 blockade with ipilimumab can reinvigorate an effective graft-versus-leukemia (GVL) response, we integrated transcriptomic analysis of leukemic biopsies with immunophenotypic profiling of matched peripheral blood samples collected from patients treated with ipilimumab following HSCT on the Experimental Therapeutics Clinical Trials Network 9204 trial. Response to ipilimumab was associated with transcriptomic evidence of increased local CD8 T-cell infiltration and activation. Systemically, ipilimumab decreased naïve and increased memory T-cell populations and increased expression of markers of T-cell activation and costimulation such as PD-1, HLA-DR, and ICOS, irrespective of response. However, responding patients were characterized by higher turnover of T-cell receptor sequences in peripheral blood and showed increased expression of proinflammatory chemokines in plasma that was further amplified by ipilimumab.

Altogether, these data highlight the compositional T-cell shifts and inflammatory pathways induced by ipilimumab both locally and systemically that associate with successful GVL outcomes. This trial was registered at www.clinicaltrials.gov as #NCT01822509.

Introduction

Relapsed acute myeloid leukemia (AML) following allogeneic hematopoietic stem cell transplantation (HSCT) is associated with poor prognosis, and therapeutic options remain limited.1 Immune escape mechanisms contribute to relapse post-HSCT2 and suggest a reinvigorated graft-versus-leukemia (GVL) effect could improve outcomes.3 Indeed, the Experimental Therapeutics Clinical Trials Network 9204 trial demonstrated that immune checkpoint blockade (ICB) can induce regression of relapsed AML after HSCT through CD8 T-cell recruitment to leukemic sites.3,5 Through unbiased molecular profiling of the leukemic microenvironment and peripheral blood immunophenotyping of samples from study subjects on this trial, we sought to elucidate the molecular and cellular features of immunologic responses to ICB. These fresh insights may inform new strategies to control relapsed myeloid malignancies after HSCT and broaden our understanding of leukemia-specific immune responses.5,7

Study design

Additional information is provided in the supplemental Appendix, available on the Blood Web site.

Bulk RNA sequencing

RNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissue scrolls and sequenced as previously described (supplemental Figure 1A-D).4
Flow cytometry and mass cytometry

Flow cytometry data were acquired using antibody panels (supplemental Tables 1 and 2) on a BD Fortessa flow cytometer. Cytometry by time-of-flight (CyTOF) data were acquired using a 35-antibody panel (supplemental Table 3) on a Fluidigm Helios Mass Cytometer.8 Data analysis was performed using CATALYST9 and manual gating (FlowJo 10.7.1).

Bulk T-cell receptor sequencing

RNA was extracted from T cells enriched with CD3 MicroBeads and MACS columns (Miltenyi) using the RNeasy Midi Kit (Qiagen). Complementarity-determining region 3 (CDR3) sequences were obtained using rhTCRseq.10

Plasma analyte analysis

Protein concentration in plasma samples was determined using the Proximity Extension Assay (Olink Bioscience, Sweden).11 Normalized protein expression was calculated from cycle threshold values.12

Results and discussion

Transcriptomic evidence of T-cell activation in long-term responders to ipilimumab

We focused on patients enrolled on Experimental Therapeutics Clinical Trials Network 9204 with relapsed myeloid disease, which constituted the majority of subjects (38/71 [54%]; Figure 1A). To
define transcriptomic characteristics underlying successful GVL responses with ipilimumab, we performed bulk RNA-sequencing (RNA-seq) on 33 high-quality disease-site biopsies from 13 patients (3 complete responders [CR; response > 12 months], 3 transient responders [TR; response < 12 months], and 7 nonresponders [NR]) before post-ipi and after post-ipi (ipilimumab treatment [supplemental Tables 4-7]). Disease sites included bone marrow, skin, and extramedullary manifestations. In addition, we generated RNA-seq data from 9 biopsies from sites of graft-versus-host disease (GVHD) or ICB-associated toxicity (supplemental Figure 1E).

Differential gene expression analysis (DGEA) between all pre- and post-ipi CR samples demonstrated enrichment of T cell–specific genes post-ipi but revealed no consistent change in NR samples post-ipi (Figure 1B; supplemental Figure 1F). DGEA on 4 site-matched paired pre-post samples from 3 CR patients revealed a signature of 47 up- and 3 downregulated genes.
Hypothesizing increased TCR diversity post-ipi, we performed CyTOF on 10 patients using CyTOF. Even though these analyses were all performed on NR, we observed that pre-ipi and post-ipi samples clustered separately (Figure 2B). Consistent with T-cell activation, we detected increased expression of PD-1 on both CD4⁺ and CD8⁺, and HLA-DR and ICOS on CD8⁺ T cells post-ipi (all \( P < .05 \); Figure 2C). The proliferation marker Ki-67 was higher on CD8⁺ T cells post-ipi (\( P < .05 \)), but no changes in absolute lymphocyte counts were detected (supplemental Figure 2D). CD69 was upregulated on regulatory T cells post-ipi (false discovery rate \( < .05 \)), which may reflect a compensatory mechanism to ipilimumab-induced T-cell activation (supplemental Figure 2E-F). Altogether, ipilimumab thus alters differentiation and activation states of circulating T cells independent of clinical response.

Hypothesizing increased TCR diversity post-ipi, we performed TCR sequencing on longitudinally collected peripheral blood samples of 9 AML/myelodysplastic syndrome patients (3 CR, 1 TR, 5 NR). However, TCR repertoires remained relatively stable without consistent changes in TCR diversity (supplemental Figure 2G). Of 57,201 total CDR3/β sequences, only 776 dynamic CDR3s (0.13%) changed in abundance after 1 cycle of ipilimumab (adjusted \( P \) value \( < .01 \); Figure 2D). Increased T-cell infiltration of disease biopsies post-mpi suggested ipilimumab may mobilize T cells from systemic sites into the leukemic microenvironment. Indeed, we observed an increase in CDR3 sequences shared between tissue sites and blood post-mpi regardless of clinical outcome (Figure 2E; supplemental Figure 2H). We also observed differences in the systemic effects of ipilimumab between CR/TRs and NRs. Dynamic CDR3s were more frequent in CR/TR (613 vs 163 of 776, \( P < .01 \); Figure 2F). Moreover, ipilimumab induced higher plasma expression of pro-inflammatory factors modulating a broad range of cell types in CR/TR (n = 4) compared with NR (n = 8; adjusted \( P \) value \( < .05 \), log fold change > 1) (Figure 2G-H; supplemental Figure 3; supplemental Table 9). Thus, peripheral TCR repertoires largely remain stable, and increased T-cell infiltration at tissue sites occurs independent of outcome. In addition, responders show more dynamic changes in a subset of the peripheral TCR repertoire and greater systemic expression of chemokines associated with leukocyte activation and trafficking.

In summary, T-cell reinvigoration accompanies clinical response to ipilimumab. Similar observations following response to donor lymphocyte infusion suggest common mechanistic pathways for effective GVL reinstatement. Moreover, the convergence both locally and peripherally of gene and chemokine response signatures that invoke GVL and GVHD processes speak to the involvement of CD28-CTLA-4 signaling to each pathway. Future studies that compare tissue sites of GVL and GVHD will be critical to divorce these processes to improve HSCT outcomes. Because of the limitations imposed by small cohort size and tissue heterogeneity, longitudinal high-resolution studies on larger cohorts are urgently needed to deconvolute the heterogeneous cellular populations driving clinical outcomes to ICB.

Acknowledgments

The authors thank the Cancer Moonshot CIMAC-CIDC Network for their tremendous support of the work and particularly appreciate the support and feedback from Holden Maeccker, Ignacio Wistuba, Ethan Cerami, James Lindsay, Radim Moravec, and many others. The authors are grateful for support from Carol Reynolds and the members of the DFCI Flow Cytometry Core, the Longwood Medical Area CyTOF Core, DFCI Center for Immuno-Oncology, Doreen Hersey and the members of the Ted and Eileen Pasquarella Tissue Bank in Hematologic Malignancies for provision of samples; the patients who generously consented for the research use of these samples; the research coordinators, research nurses, advanced practice providers, and site staff for their support of the trial; Irene Ghobrial and Lee Greenberger and Michael Yaffe for support of the trial through the LLS BCIP; Edwin P. Hyman, David Avigan, and Yi-Bin Chen for clinical data; the Center for Advanced Molecular Diagnostics (CAMD) at the Brigham and Women’s Hospital for isolation of FFPE samples; Sam Pollock and Candace Patterson for excellent project management support; and members of the Wu laboratory for their valuable feedback.

This work was supported by National Institutes of Health, National Cancer Institute grant P01CA229902 (C.J.W.) and grants R01CA183559, R01CA183560, and SUM1CA186709 (principal investigator: Geoffrey Shapiro), National Cancer Institute Cancer Therapy Evaluation Program, Bristol-Myers Squibb, and LLS Therapy Accelerator Program. L.P. is supported by a research fellowship from the German Research Foundation (DFG, PE 3127/1-1). S.L. is supported by the National Institutes of Health, National Cancer Institute Research Specialist Award (R50CA251956-01). P.B. is supported by National Institutes of Health, National Cancer Institute grant K08CA248458 and the Amy Streler Manasevit Research Program, which is funded through Be The Match Foundation. P.B. is a Scholar of the American Society of Hematology. M.S.D. and P.A. are both Scholars in Clinical Research from the Leukemia & Lymphoma Society. S. Gnjatic is supported by National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases grant...
U01DK124165 and National Cancer Institute grant P01CA190174. N.C. is supported by an AACR-Incyte Immuno-oncology Research Fellowship (20-04-46-CEIR). The Human Immunology Monitoring Center at ISMMS received support from the Cancer Center Support Grant CA196521. Scientific and financial support for the CIMAC-CIDC Network is provided through National Institutes of Health, National Cancer Institute Cooperative Agreements U24CA224219 (to the Icahn School of Medicine at Mount Sinai CIMAC), U24CA24331 (to the Dana-Farber Cancer Institute CIMAC), and U24CA24316 (to the CICD at Dana-Farber Cancer Institute). Additional support is made possible through the National Institutes of Health, National Cancer Institute CTMS contract HHSN26120160002C. Scientific and financial support for the PACT project are made possible through funding support to the FNHI by: AbbVie Inc., Amgen Inc., Boehringer-Ingelheim Pharma Gmbh & Co. KG, Bristol-Myers Squibb, Celgene Corporation, Genentech Inc, Gilead, GlaxoSmithKline plc, Janssen Pharmaceutical Companies of Johnson & Johnson, Novartis Institutes for Biomedical Research, Pfizer Inc, and Sanofi. The CIMAC-CIDC website is found at https://cimac-network.org/.

Authorship

Conflict-of-interest disclosure: C.J.W. holds equity in BioNTech, Inc, and receives research support from Pharmacyclics. D.N. holds stock in Madrigal Pharmaceuticals and received research funding from Pharmacyclics. E.M.T. performs research in the field of immunology, and receives grants and research funding from both the National Institutes of Health and the Department of Defense. E.M.T. received grant support from the National Institutes of Health (NIH) for the project described in the manuscript. E.M.T. is a member of the editorial board of Blood.

Funding

The current affiliation for P.B. is Department of Hematopoietic Biology and Malignancy, University of Texas MD Anderson Cancer Center, Houston, TX. P.B., 0000-0002-8698-4957. 

Conflict-of-interest disclosure: S. K.-S. and S. Gnjatic provided proximity extension assay data; M.S.D., P.B., and C.J.W. supervised the study; and P.B. and C.J.W. interpreted results and wrote the manuscript. Conflict-of-interest disclosure: C.J.W. holds equity in BioNTech, Inc, and receives research support from Pharmacyclics. D.N. holds stock in Madrigal Pharmaceuticals and received research funding from Pharmacyclics. E.M.T. performs research in the field of immunology, and receives grants and research funding from both the National Institutes of Health and the Department of Defense. E.M.T. received grant support from the National Institutes of Health (NIH) for the project described in the manuscript. E.M.T. is a member of the editorial board of Blood.

Funding

The current affiliation for P.B. is Department of Hematopoietic Biology and Malignancy, University of Texas MD Anderson Cancer Center, Houston, TX. P.B., 0000-0002-8698-4957.

Acknowledgment

Correspondence: Pavan Bachireddy, The University of Texas, MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, e-mail: pbachireddy@mdanderson.org.
REFERENCES

1. Rautenberg C, G erming U, Haas R, Kobb G, Schroeder T. Relapse of acute myeloid leukemia after allogeneic stem cell transplantation: prevention, detection, and treatment. Int J Mol Sci. 2019;20(1):E228.

2. Zeiser R, Vago L. Mechanisms of immune escape after allogeneic hematopoietic cell transplantation. Blood. 2019;133(12):1290-1297.

3. Penter L, Wu CJ. Personal tumor antigens in blood malignancies: genomics-directed identification and targeting. J Clin Invest. 2020;130(4):1595-1607.

4. Davids MS, Kim HT, Bachireddy P, et al. Leukemia and Lymphoma Society Blood Cancer Research Partnership. Ipilimumab for patients with relapse after allogeneic transplantation. N Engl J Med. 2016;375(2):143-153.

5. Davids MS, Kim HT, Costello C, et al. A multicenter phase 1 study of nivolumab for relapsed hematologic malignancies after allogeneic transplantation. Blood. 2020;135(24):2182-2191.

6. Boddu P, Kantarjian H, Garcia-Manero G, Allison J, Sharma P, Daven N. The emerging role of immune checkpoint based approaches in AML and MDS. Leuk Lymphoma. 2018;59(4):790-802.

7. Stahl M, Goldberg AD. Immune checkpoint inhibitors in acute myeloid leukemia: novel combinations and therapeutic targets. Curr Oncol Rep. 2019;21(4):37.

8. Thrash EM, Kleinsteinber K, Hathaway ES, et al. High-throughput mass cytometry staining for immunophenotyping clinical samples. STAR Protoc. 2020;1(2):100055.

9. Crowell HL, Zanotti VRT, Chevrier S, Robinson MD. CATALYST: Cytometry Data Analysis tools. Available at https://rdr.io/bioc/CATALYST/. Accessed 22 March 2021.

10. Li S, Sun J, Allese R, et al. RNase H-dependent PCR-enabled T-cell receptor sequencing for highly specific and efficient targeted sequencing of T-cell receptor mRNA for single-cell and repertoire analysis. Nat Protoc. 2019;14(8):2571-2594.

11. Assarsson E, Lundberg M, Holmquist G, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. PLoS ONE. 2014;9(4):e95192.

12. RStudio Team. RStudio: Integrated Development Environment for R. Available at https://rstudio.com/products rstudio/. Accessed 22 March 2020.

13. Benci JL, Xu B, Qiu Y, et al. Tumor interferon signaling regulates a multigenic resistance program to immune checkpoint blockade. Cell. 2016;167(4):1540-1554.e12.

14. Jacquelot N, Yamazaki T, Roberti MP, et al. Sustained type I interferon signaling as a mechanism of resistance to PD-1 blockade. Cell Res. 2019;29(10):846-861.

15. Weber JS, Hamid O, Chasalow SD, et al. Ipilimumab increases activated T cells and enhances humoral immunity in patients with advanced melanoma. J Immunother. 2012;35(1):89-97.

16. Felix J, Lambert J, Roelens M, et al. Ipilimumab reshapes T cell memory subsets in melanoma patients with clinical response. Oncolimmunology. 2016;5(7):1136045.

17. Robert J, Tsao J, Wang X, et al. CTLA4 blockade broadens the peripheral T-cell receptor repertoire. Clin Cancer Res. 2014;20(9):2424-2432.

18. Cossarizza A, Chang H-D, Radbruch A, et al. Guidelines for the use of flow cytometry and cell sorting in immunological studies (second edition). Eur J Immunol. 2019;49(10):1457-1973.

19. Hughes CE, Nibbs RJ. A guide to chemokines and their receptors. FEBS J. 2018;285(16):2944-2971.

20. Bachireddy P, Hainz U, Rooney M, et al. Reversal of in situ T-cell exhaustion during effective human antileukemia responses to donor lymphocyte infusion. Blood. 2014;123(9):1412-1421.

21. Penter L, Dietze K, Ritter J, et al. Localization-associated immune phenotypes of clonally expanded tumor-infiltrating T cells and distribution of their target antigens in rectal cancer. OncoImmunology. 2019;8(6):e1586409.

22. Gohil SH, Iorgulescu JB, Braun DA, Keskin DB, Livak KJ. Applying high-dimensional single-cell technologies to the analysis of cancer immunotherapy. Nat Rev Clin Oncol. 2021;18(4):244-256.