Immune landscapes predict chemotherapy resistance and immunotherapy response in acute myeloid leukemia

Jayakumar Vadakekolathu1, Mark D. Minden2, Tressa Hood3, Sarah E. Church3, Stephen Reeder1, Heidi Altmann4, Amy H. Sullivan3, Elena J. Vibo3, Tasleema Patel5, Narmin Ibrahimova2, Sarah E. Warren3, Andrea Arruda2, Yan Liang3, Thomas H. Smith3, Gemma A. Foulds1, Michael E. Bailey3, James Gowen-MacDonald3, John Muth6, Marc Schmitz7,8,9, Alessandra Cesano3, A. Graham Pockley1,10, Peter J. M. Valk11, Bob Löwenberg11, Martin Bornhäuser1,9, Sarah K. Tasián3, Michael P. Retting1,10, Jan K. Davidson-Moncada6, John F. DiPersio12, Sergio Rutella1,10*.

Acute myeloid leukemia (AML) is a molecularly and clinically heterogeneous hematological malignancy. Although immunotherapy may be an attractive modality to exploit in patients with AML, the ability to predict the groups of patients and the types of cancer that will respond to immune targeting remains limited. This study dissected the complexity of the immune architecture of AML at high resolution and assessed its influence on therapeutic response. Using 442 primary bone marrow samples from three independent cohorts of children and adults with AML, we defined immune-infiltrated and immune-depleted disease classes and revealed critical differences in immune gene expression across age groups and molecular disease subtypes. Interferon (IFN)-γ–related mRNA profiles were predictive for both chemotherapy resistance and response of primary refractory/relapsed AML to flotetuzumab immunotherapy. Our compendium of microenvironmental gene and protein profiles provides insights into the immune-biology of AML and could inform the delivery of personalized immunotherapies to IFN-γ–dominant AML subtypes.

INTRODUCTION

Acute myeloid leukemia (AML) is a malignant disorder characterized by the accumulation of myeloblasts in the bone marrow (BM) and blood (1). The discovery of the genomic landscape of AML, including the identification of targetable mutations (2), has propelled the development of novel antileukemic agents and is enabling disease classification and patient stratification into risk groups (3). Despite success in many areas, AML is cured in only 35 to 40% of patients <60 years of age and in 5 to 15% of patients >60 years of age. While chemotherapy resistance is common, most patients die of disease relapse. Investigation of new molecularly targeted and immunomodulatory agents therefore remains a high priority for both children and adults (4).

Tumor phenotypes are dictated not only by the neoplastic cell component but also by the immunologic milieu within the tumor microenvironment (TME), which is equipped to subvert host immune responses and hamper effector T cell function (5). In silico approaches have been instrumental for the identification of immunogenomic features with therapeutic and prognostic implications. In solid tumors, six immune subtypes have been described: wound healing, interferon (IFN)-γ–dominant, inflammatory, lymphocyte-depleted, immunologically quiet, and transforming growth factor (TGF)–β–dominant (6). These are characterized by differences in macrophage or lymphocyte signatures, T helper type 1 (Th1)–to–Th2 cell ratio, extent of intratumoral heterogeneity and neoadjuvant load, aneuploidy, cell proliferation, expression of immunomodulatory genes, and patient survival (6).

Although immunotherapy may be an attractive modality to exploit in patients with AML (7), the ability to predict the groups of patients and the forms of leukemia that will respond to immune targeting remains limited (8–11). Clinical studies in patients with solid tumors have shown that responses to anti–programmed cell death 1 (PD-1)/programmed death ligand 1 (PD-L1)–targeted immunotherapy occur most often in individuals with immune-inflamed lesions that are characterized by preexisting CD8+ T cell responses, release of proinflammatory and effector cytokines (12–14), and an augmented T cell receptor (TCR) clonal diversity pretreatment (15, 16). The T cell inflamed gene expression profile (GEP) is a measure of IFN-γ–responsive genes that are related to adaptive immune resistance mechanisms of immune escape such as indoleamine 2,3-dioxygenase-1 (IDO1) and PD-L1 (17) and is predictive of clinical benefit with pembrolizumab immunotherapy (18, 19). Although IFN-γ plays a critical role in eliciting antitumor T cell activity and enabling tumor rejection, prolonged IFN-γ signaling under conditions of persistent antigen exposure has been shown to activate a PD-L1–independent, STAT1-driven

1John van Geest Cancer Research Centre, Nottingham Trent University, Nottingham NG11 8NS, UK. 2Division of Medical Oncology and Hematology, Princess Margaret Cancer Centre, Toronto, ON MS2 C1, Canada. 3NanoString Technologies Inc., Seattle, WA 98109, USA. 4Department of Medicine, Universitätssklinikum Carl Gustav Carus, 01307 Dresden, Germany. 5Department of Pediatrics, Division of Oncology and Centre for Childhood Cancer Research, Children’s Hospital of Philadelphia and University of Pennsylvania School of Medicine, PA 19104, USA. 6MacroGenics Inc., Rockville, MD 20850, USA. 7Institute of Immunology, Faculty of Medicine Carl Gustav Carus, Technische Universität Dresden, 01307 Dresden, Germany. 8National Center for Tumor Diseases (NCT), Partner Site Dresden, 01307 Dresden, Germany. 9German Cancer Consortium (DKTK), Partner Site Dresden, and German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany. 10Centre for Health, Ageing and Understanding Disease (CHAUD), Nottingham Trent University, Nottingham NG11 8NS, UK. 11Department of Hematology, Erasmus University Medical Centre, 3000CA Rotterdam, Netherlands. 12Division of Oncology, Department of Internal Medicine, Washington University in St. Louis, St. Louis, MO 63110, USA.
*Corresponding author. Email: sergio.rutella@ntu.ac.uk

multigenic program, which confers resistance to radiotherapy and anticytotoxic T lymphocyte antigen 4 (CTLA-4) immunotherapy in mouse models of melanoma (20).

Here, we used targeted immune GEP (IGEP) and spatially resolved multiplexed digital spatial profiling (DSP) for the high-dimensional analysis of the immunological contexture of a broad collection of BM samples from patients with AML and for the identification of molecular determinants of immunotherapeutic benefit. We reveal unifying immune features and critical differences that define classes and subclasses of TMEs and deliver predictions of chemotherapy resistance, survival, and immunotherapy response that are beyond the current capabilities of single molecular markers.

RESULTS
Targeted IGEP identifies immune subtypes of AML
We first analyzed unfractored, archival BM samples from treatment-naive patients with nonpromyelocytic AML [Princess Margaret Cancer Centre (PMCC) discovery series; n = 290 cases; Table 1] (21). We derived immune scores from mRNA expression, similar to those of previous publications, and devised an RNA-based, quantitative metric of immune infiltration (22, 23). As shown in fig. S1 (A and B), patients with adverse cytogenetic features exhibited a shorter relapse-free survival (RFS) and overall survival (OS) compared with patients with intermediate and favorable cytogenetic risk, thus confirming the overall performance of well-established European Leukemia-Net (ELN) categories (24). A Pearson correlation matrix of immune gene sets allowed us to identify coexpression patterns of predefined immune cell types and immune biological activities. Immune signature modules in pretreatment BM samples reflected the coexpression of genes associated with (i) type I and type II IFN biology, (ii) adaptive immune responses, and (iii) myeloid cell abundance (macrophages, neutrophils, and dendritic cells); Fig. 1A). We then computed the sum of the individual scores in each signature module in Fig. 1A and generated three immune scores (IFN-dominant, adaptive, and myeloid), which individually separated AML cases according to high and low expression values (fig. S1C). Specifically, the IFN-dominant gene module was calculated as the sum of IFN-γ signaling, IFN downstream, immunoproteasome, myeloid inflammation, inflammatory chemokine, IL-10, MAGEs, PD-L1, and PD-L2 scores. When considered in aggregate, IFN-dominant, adaptive, and myeloid scores dichotomized BM samples into two immune subtypes, here termed immune-infiltrated and immune-depleted (Fig. 1B and fig. S1, D and E) (25), which expressed comparable amounts of leukemia-associated antigens CD34, CD123 (IL3RA), and CD117 (KIT). This observation suggests that targeted IGEP of bulk BM specimens largely captured elements of the immunological TME rather than features of the tumor cell compartment (fig. S1F).

As shown in Fig. 1C, AML cases with immune-infiltrated profiles had higher expression of IFN-stimulated genes and T cell recruiting factors (STAT1, CXCL10, and IFRF1), T cell markers and cytotoxic effectors (CD8A, CD8B, GZMB, and PRF1), counter-regulatory immune checkpoints and immunotherapy drug targets (IDO1, CTLA4, PD-L1, and BTLA), and molecules involved in antigen processing and presentation (TAP1, TAP2, HLA-A, HLA-B, and HLA-C). Conceivably, high T cell infiltration and expression of major histocompatibility complex and PD-L1 in the immune-infiltrated AML subtype reflected a preexisting IFN-γ-driven adaptive immune response. This type of response has previously been associated not only with suppressed antitumor immune reactivity (13, 26) but also with immunotherapy responses in patients with solid tumors (9, 18, 27) and AML (11). STAT1, a central component of the IFN-γ signaling pathway and predictor of response to immune checkpoint blockade (28), was more strongly correlated with the presence of T cell inhibitory receptors TNFRSF14 (a ligand for the immunoglobulin superfamily members BTLA and CD160), PD-L1, HAVCR2 (Tim-3), and LAG-3, and with IFN-stimulated genes MX1, IFIT1, and IRF1 in the immune-infiltrated relative to the immune-depleted subtype, consistent with their coordinated regulation in an inflamed TME (Fig. 1D).
genomic events (Fig. 2D), significantly correlated with TP53 mutation status, an established adverse prognosticator in AML (P value from mutation enrichment analysis = 0.0285). SIG3 were enriched for Gene Ontology (GO) biological processes related to T cell lineage commitment, positive T cell selection, and T cell homeostasis (Fig. 2E). De-regulation in SIG3 genes, which included PD-L1, FoxP3, GZMB, PTEN, and BCL2, were predominantly observed in patients with immune-infiltrated mRNA profiles (Fig. 2F) and correlated with higher number of mutations (P = 0.021) and with adverse ELN cytogenetic features ($\chi^2$ = 25.03; P < 0.001), but not with other disease characteristics at presentation, including white blood cell (WBC) count, and percentage of AML blasts in pretreatment blood and BM samples. Last, patients with abnormalities in SIG3 genes experienced poor clinical outcomes, as shown by the significantly lower RFS and OS rates (Fig. 2G). Although the mutually exclusive pattern of SIG3 genomic abnormalities would suggest that the altered genes are linked in a common biological process or pathway (30), GZMB and FoxP3 were the only SIG3 genes that individually predicted shorter OS (fig. S6). Collectively, highly multiplexed in situ detection of IO proteins highlights critical differences in T cell–infiltrated versus T cell–depleted AML subtypes and identifies protein signatures with prognostic potential in T cell–infiltrated pretreatment samples.

### Table 1. AML cohorts selected for targeted immune gene expression profiling.

<table>
<thead>
<tr>
<th>Patient series</th>
<th>PMCC</th>
<th>CHOP</th>
<th>SAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>290</td>
<td>34</td>
<td>46</td>
</tr>
<tr>
<td>Age (0–39), n</td>
<td>76</td>
<td>34</td>
<td>13</td>
</tr>
<tr>
<td>Age (40–59), n</td>
<td>126</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>Age (≥60), n</td>
<td>88</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Median age (years, range)</td>
<td>52 (18–81)</td>
<td>10 (0.1–20)</td>
<td>53.5 (23–75)</td>
</tr>
<tr>
<td>WBC count at presentation</td>
<td>$19.15 \times 10^3/\mu l$ (0.7–399)</td>
<td>N.A.</td>
<td>$56.45 \times 10^3/\mu l$ (0.84–320.2)</td>
</tr>
<tr>
<td>Cytogenetic risk group, n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELN favorable</td>
<td>35</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>ELN intermediate</td>
<td>155</td>
<td>20</td>
<td>27</td>
</tr>
<tr>
<td>ELN adverse</td>
<td>59</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>N.A.</td>
<td>41</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Disease status at time of BM sampling, n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td>290</td>
<td>34</td>
<td>46</td>
</tr>
<tr>
<td>Complete remission</td>
<td>0</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>Relapse</td>
<td>0</td>
<td>11</td>
<td>24</td>
</tr>
<tr>
<td>No. of BM samples analyzed</td>
<td>290</td>
<td>61</td>
<td>91</td>
</tr>
<tr>
<td>No. of BM samples analyzed using the GeoMx DSP platform</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>De novo/secondary/therapy-related</td>
<td>244/46/0</td>
<td>36/4/0</td>
<td>39/5/2</td>
</tr>
<tr>
<td>Response to induction chemotherapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>210</td>
<td>26 (M1*)</td>
<td>33</td>
</tr>
<tr>
<td>No</td>
<td>80</td>
<td>3 (M2*)</td>
<td>4</td>
</tr>
<tr>
<td>Primary induction failure</td>
<td>39</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>N.A.</td>
<td>–</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Relapse</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>132</td>
<td>18</td>
<td>31</td>
</tr>
<tr>
<td>No</td>
<td>118</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>N.A.</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Median follow-up time (months)</td>
<td>101.23</td>
<td>70.2</td>
<td>55.54</td>
</tr>
<tr>
<td>Median RFS (months)</td>
<td>19.1</td>
<td>25.6</td>
<td>13.64</td>
</tr>
<tr>
<td>Median OS (months)</td>
<td>21.37</td>
<td>66.8</td>
<td>50.58</td>
</tr>
</tbody>
</table>

*M1, M2, and M3 BM remission status was defined as <5%, 5 to 24%, and >25% AML blasts after induction chemotherapy, respectively (73).*
dendrogram_cut

**Fig. 1.** Immune gene sets stratify bone marrow samples from patients with newly diagnosed AML. (A) Unsupervised hierarchical clustering (Euclidean distance, complete linkage) of the correlation matrix of immune and biological activity signatures identifies coexpression patterns (gray boxes) of immune gene sets (correlation value color-coded per the legend, where blue denotes a Pearson correlation coefficient of -1.0 and red indicates a Pearson correlation coefficient of 1.0) in the bone marrow (BM) microenvironment of patients with AML (PMCC cohort; n = 290), namely, IFN-dominant, adaptive, and myeloid gene modules. Immune cell type (23) and signature scores (22) were calculated from mRNA expression as predefined linear combinations (weighted averages) of biologically relevant gene sets. Morpheus, an online tool developed at the Broad Institute (MA, USA), was used for data analysis and visualization. DCs, dendritic cells. (B) IFN-dominant, adaptive, and myeloid scores in aggregate stratify patients with newly diagnosed AML into two distinct clusters, which are referred to in this study as immune-infiltrated and immune-depleted (25). ClustVis, an online tool for clustering of multivariate data, was used for data analysis and visualization (71). (C) Violin plots summarizing the expression of IFN-stimulated genes (ISGs), T cell and cytotoxicity markers, negative immune checkpoints, genes implicated in antigen processing and presentation, and immunotherapy targets in AML cases with an immune-infiltrated and immune-depleted tumor microenvironment. Data were compared using the Mann-Whitney U test for unpaired data (two-sided). *P < 0.05; **P < 0.0001. (D) Spearman correlation coefficients between STAT1, ISGs [IRF1, MX1, IFIT1, TNFRSF14, PD-L1 (CD274)], surrogate markers for cytotoxic T cells (CD8A, GZMA), and negative immune checkpoints [LAG3, HAVCR2 (Tim-3)] under conditions of high and low immune infiltration. NS, not significant.

**Interactions between immune subgroups, common cytogenetic alterations, and clinical factors**

We next correlated immune signature scores with clinical and demographic factors, including WBC count and blast cell count at diagnosis, ELN cytogenetic category (available in 249 cases from the PMCC discovery series), and patient age. Leukemia burden was significantly lower in the immune-infiltrated AML subtype (median WBC count at diagnosis P < 0.0001; median percentage of BM blasts P < 0.0001; and median number of AML blasts per microliter of blood P < 0.0001; fig. S7A to C). Immune signature scores were correlated with the ELN cytogenetic risk category when considered individually (fig. S7D). Last, patients with high immune infiltration tended to be of a more advanced age at diagnosis compared with patients with low immune infiltration (P < 0.0001; fig. S7E).

**Immune subtypes improve survival prediction**

The activation of immune pathways has context-dependent prognostic impact that differs between tumor types (6). We next assessed the ability of the immune subtype to refine the accuracy of outcome prediction separately for each ELN cytogenetic risk category. Among patients with favorable risk, RFS and OS times were significantly longer in individuals with an immune-infiltrated TME (Fig. 3A). In contrast, clinical outcomes in ELN adverse risk cases were worse in individuals with an immune-infiltrated TME (Fig. 3A). CD8 exhaustion...
scores and PD1 scores were significantly higher in patients with ELN adverse risk compared with ELN favorable risk (Fig. 3B), suggesting that leukemia progression may be sustained by tumor cell-extrinsic (immune) mechanisms in patients with more aggressive disease. We also observed that the ELN classifier assisted outcome prediction only in the immune-infiltrated subtype (Fig. 3C), allowing the identification of patient subgroups with excellent survival estimates (87.5% RFS and 77.8% OS) or with very unsatisfactory
and AUROC = 0.5 would denote no predictive ability. 

Cytogenetically defined categories stratify survival in patients with immune-infiltrated AML.

P < 0.001. 

Expression of PD1 and markers of CD8+ T cell exhaustion across cytogenetically defined patient categories. Data were compared using the Kruskal-Wallis test for unpaired determinations. FAV, favorable ELN risk. INT, intermediate ELN risk. ADV, adverse ELN risk.

The inclusion of immune gene signatures also improved the predictive ability of molecular risk (blue curve) and immune subtype (red curve) for OS. 

Outcomes [10.4% RFS (log-rank $\chi^2 = 15.07; P < 0.0001)$ and 7.2% OS (log-rank $\chi^2 = 25.75; P < 0.0001)$]. Unexpectedly, our immunological classifier was unable to stratify survival in patients with intermediate ELN risk. ELN intermediate patients with NPM1 mutation and FLT3-ITD-ITD information (available in 100 cases) were then subclassified into molecular low-risk (NPM1 mutations without FLT3-ITD-ITD), molecular intermediate-risk (NPM1 wild type without FLT3-ITD-ITD or with low FLT3-ITD-ITD allelic ratio), and molecular high-risk cases (NPM1 wild type with high FLT3-ITD-ITD allelic ratio) (31). As shown in Fig. 3D, the molecular classifier separated survival both in patients with immune-infiltrated AML and in those with an immune-depleted TME. The inclusion of immune gene signatures also improved the ability of NPM1 and FLT3-ITD mutation status to predict survival in multivariate logistic regression models relative to molecular risk alone [area under receiver operating characteristic (AUROC) = 0.938 versus 0.765; Fig. 3E]. Specifically, the IFN downstream score (Wald $\chi^2 = 4.1; P = 0.043$), CD8 score (Wald $\chi^2 = 4.2; P = 0.04$), exhausted CDB8 T cell score (Wald $\chi^2 = 4.4; P = 0.037$), IDO1 score (Wald $\chi^2 = 4.01; P = 0.045$), and CTLA4 score (Wald $\chi^2 = 5.2; P = 0.022$) significantly contributed to the model. 

By performing Cox proportional hazards regression, we also discovered a set of 21 differentially expressed (DE) immune genes [false discovery rate (FDR) < 0.05] between the favorable and adverse-risk ELN category (Fig. 4A and table S1). This gene set was highly expressed in immune-infiltrated AMLs (Fig. 4B) and separated ELN intermediate patients into subgroups with low and high gene expression.

values, with the former being closely similar to ELN favorable-risk patients while the latter resembled ELN adverse-risk patients. The 21-gene classifier exhibited enrichment of GO and pathways related to T cell activation, TCR downstream signaling, and regulation of cytokine production (Fig. 4C). RFS and OS estimates were significant differences for intermediate-risk patients with high versus low expression of the 21 DE genes (Fig. 4D). This finding was validated in silico using transcriptomic data from two independent AML expression cohorts (TCGA (Fig. 4E) and HOVON (Fig. 4F)).

Last, we used informative censoring to evaluate the impact of the immune subtype on outcome while excluding potential clinical benefit from graft-versus-leukemia (GVL) effects after allogeneic hematopoietic stem cell transplantation (HSCT). As shown in Fig. 4A and compared to all patients, survival differences remained evident when patients were censored on the date of allogeneic HSCT, suggesting that immune gene profiles largely predicted outcome after conventional chemotherapy. A competing risks regression analysis in which allogeneic HSCT was treated as a potential confounder (32, 33) confirmed that the IFN-dominant gene module, but not the adaptive and myeloid gene modules, predicted for shorter OS (Table S2). We also assessed the potential impact of the immune subtype on posttransplantation outcomes. Causes of death were not different in patients with immune-infiltrated and immune-depleted AML (Table S3). In contrast to patients with immune-depleted AML, individuals with immune-infiltrated AML benefited from a more profound GVL effect after allogeneic HSCT, as suggested by a significantly longer median OS time (31.9 months) compared with patients receiving chemotherapy only (18.9 months, log-rank P = 0.018). Patients with immune-infiltrated AML and adverse ELN features derived the greatest benefit from allogeneic HSCT (28%
Immune landscapes stratify patients with AML in independent validation sets and differ across age groups and disease stages

AML is a disease with age-dependent biological specificities (34, 35). Furthermore, pediatric AML is inherently of low immunogenicity and is therefore less likely to respond to single-agent checkpoint inhibition (36). To characterize the immunological landscape of AML across age groups and longitudinally in patients who initially achieve complete remission (CR) and then experience disease recurrence, we profiled BM samples from a pediatric [Children’s Hospital of Philadelphia (CHOP) series, n = 34 cases, 61 BM specimens in total] and an adult AML cohort (SAL series, n = 46 cases, 91 BM specimens in total). In line with findings in the PMCC discovery series, we identified IFN-dominant, adaptive, and myeloid mRNA profiles (fig. S9, A to D), which individually separated AML cases according to high and low expression values and, when considered in aggregate, dichotomized AML cases into an immune-infiltrated and immune-depleted subtype (fig. S9, E and F). As summarized in Fig. 5A, comparison between children and adults with AML revealed a set of DE immune genes involved with cytokine and chemokine signaling, as indicated by GO (fig. S10A and table S4) and protein interaction network analysis (fig. S10B). Specifically, genes encoding proinflammatory and proangiogenic cytokines and chemokines, including IL8, CCL3L1, CCL3, and CXCL2, were more expressed in adult AML relative to childhood AML (Fig. 5A). The tumor inflammation signature (TIS) score and the IFN-γ score were significantly higher in elderly patients (>60 years of age) relative to younger adults (<60 years of age) and to children aged less than 18 years (fig. S10C).

When comparing matched BM samples from a subset of adult patients in the SAL series (n = 22) at the time of diagnosis and achievement of CR after induction chemotherapy (Fig. 5B and fig. S11), we identified a set of DE genes that were enriched for GO biological processes related to immune responses, apoptosis, drug resistance, transcriptional mis-regulation, and cell surface receptor/chemokine-mediated signaling (table S5). As shown in Fig. 5B, FLT3, CD99, and milk-fat globule EGF-8 (MGFEB8), which have previously been implicated in cancer stem cell self-renewal (37), were lower in AML cases with CR, serving as a data reliability check. In agreement with recently published observations (38), CTLA4 expression was higher in CR relative to disease onset, with concomitant down-regulation of CD244 coinhibitory molecule, suggesting the occurrence of T cell activation after treatment (fig. S10D). Furthermore, immune genes significantly associated with relapsed AML (SAL series) largely captured CD8+ T cell infiltration, elements of T cell biology, including TCR downstream signaling (CD8A, CD8D, CD3ζ [CD247]), leukocyte differentiation, and immune regulation (Fig. 5C and table S6). The increased expression of surrogate markers of terminal T cell differentiation, senescence, and exhaustion [TBX21 (T-bet) (39, 40), TIGIT, KLRD1 (38), and KLRF1] in relapsed AML suggests that BM-infiltrating cytotoxic T cells may fail to restrain leukemia growth (fig. S10E). The DE genes between patient subgroups with newly diagnosed, CR, and relapsed AML in the SAL cohort, and between childhood and adult cases, were largely nonoverlapping, as shown in Fig. 5D.

Upon activation with a polyclonal stimulus, intracellular cytokine staining of BM suspensions showed higher concomitant production of IFN-γ and tumor necrosis factor–α (TNF-α) by microenvironmental CD4+ and CD8+ T lymphocytes in the immune-infiltrated compared with the immune-depleted subgroup (P = 0.0273; fig. S12, A to D). Polyfunctional IFN-γ-TNF-α+ T cells were significantly reduced in remission BM samples compared with diagnostic and relapse BMs (P = 0.0257) and were particularly low in patients with documented minimal residual disease (MRD) negativity (fig. S12E).

IFN-related gene sets improve the prediction of therapy resistance

We then asked whether the IFN-dominant gene module may assist the prediction of therapeutic resistance, which we empirically defined as failure to achieve CR in patients who survived at least 28 days (primary refractory AML) or as early relapse (<3 months) after achieving CR, as previously published by others (41). When patients in the PMCC cohort were dichotomized based on higher or lower median IFN scores, a higher percentage of patients with primary refractory disease was observed in the IFN-score high AML cases (65.4% versus 34.6%; P = 0.0022, Fisher’s exact test), suggesting that transcriptional programs orchestrated by microenvironmental IFN-γ might render AML blasts resistant to chemotherapeutic agents (20, 42). In contrast, the frequency of primary refractory cases was not different when comparing patients with higher or lower than median adaptive module scores (29.6% versus 25.4%; P = not significant (NS)) and myeloid module scores (26.7% versus 28.1%; NS). In multivariate logistic regression analysis, the IFN-related module scores improved the ability of the ELN category to predict therapeutic resistance but not patient survival (Fig. 6, A and B, and table S7). Specifically, the myeloid inflammation score (P = 0.003), IFN-γ signaling score (P = 0.014), and IFN downstream score (P = 0.034) significantly contributed to the model (table S7). Gene sets defining gene modules 2 and 3 in Fig. 1, reflective of adaptive immune responses, and BM infiltration with cells of the myeloid lineage, respectively, were not associated with either therapeutic resistance or patient survival.

We next tested the predictive and prognostic power of immune scores in silico using a broad collection of public transcriptomic data. We initially devised binary logistic regression models using RNA sequencing data from 196 patients on the Beat AML Master Trial with clinical response information (43). When considering disease type (primary versus secondary), WBC count, and patient age at diagnosis, the inclusion of genes capturing IFN-γ-related biology significantly improved the predictive ability of the ELN risk category (AUROC = 0.921 versus 0.709 with ELN cytogenetic risk alone; model χ² = 106.4 versus 29.6; increased specificity = 4%; increased sensitivity = 17%; decreased false-positive rate = 39%; decreased false-negative rate = 18%; Fig. 6C).

Confirming our findings in the PMCC and Beat AML cohorts, IFN-dominant, adaptive, and myeloid mRNA profiles, when used in aggregate, stratified patients in the HOVON database [618 non-promyelocytic AML cases (44)] into subgroups with high and low immune infiltration (Fig. 6, D and E). Individuals with immune-infiltrated AML had lower leukemia burden (median percentage of BM blasts = 56% versus 71% in patients with immune-depleted AML; P < 0.0001) and tended to have more advanced age at diagnosis (median = 51 years, range 15 to 74, versus 46 years, range 15 to 77; P = 0.0067). A higher percentage of patients with IFN-dominant AML failed to achieve CR in response to induction chemotherapy when compared to non–IFN-dominant AML cases (27.2% versus 3% survival rate after chemotherapy with or without allogeneic HSCT, respectively; fig. S8, B and C).
In contrast, the occurrence of primary induction failure (PIF) was not different when patients were dichotomized based on higher or lower than median adaptive module scores (21.4% versus 21.0% PIF rate; $P = \text{NS}$) or myeloid module scores (21.7% versus 20.7% PIF rate; $P = \text{NS}$). Gene set enrichment analysis (GSEA) with all transcripts in the HOVON dataset provided as input and ranked by the log$_2$ fold change between nonresponders and responders confirmed the overexpression of curated hallmark gene sets linked to IFN-$\gamma$ responses and inflammatory responses in chemotherapy-refractory patients (Fig. 6F). When tested in a multinominal logistic regression model incorporating patient age, leukemia burden, and ELN cytogenetic risk (available in 618 HOVON cases) (45), immune gene sets defining the IFN-dominant module significantly and independently predicted whether patients responded to induction chemotherapy and whether they experienced disease relapse (table S8). In contrast, immune gene signatures were unable to assist the prediction of nonleukemic deaths (table S8).

Mutations in tumor suppressor genes and transcription factors are enriched in immune-infiltrated AML cases

It has recently been shown that genetic drivers of solid malignancies dictate neutrophil and T cell recruitment, thus affecting the immune milieu of the tumor and assisting patient stratification (46). We asked whether clonal driver mutations may correlate with the immune subtypes that we identified here. We therefore retrieved TCGA AML RNA sequencing data from cbioPortal (http://cbioportal.org/) and computed immune cell type–specific and biological activity scores (22). IFN–related gene sets, including the TIS score, were higher in TCGA-AML cases with $TP53$ and $RUNX1$ mutations relative to molecular lesions that confer favorable or intermediate risk (fig. S13A). In contrast, most TCGA-AML cases with $NPM1$ mutations with or without $FLT3$-ITD (intermediate-risk and favorable-risk cases, respectively) were classified as immune-depleted. Clonal hematopoiesis of indeterminate potential (CHIP) is a hematological malignancy precursor condition defined by somatic mutations in
leukemia-associated driver genes, including \( \text{DNMT3A} \), \( \text{TET2} \), and \( \text{ASXL1} \), and associated with increased risk for inflammatory diseases of aging \((47)\). The TIS score, but not the IFN-\( \gamma \) score, was significantly higher in TCGA AML cases with CHIP-defining mutations compared with patient subgroups with other molecular lesions (fig. S13B). When extending our in silico analysis to the Beat AML cohort (281 cases in total), 16 of 17 (94\%) \( \text{TP53} \)-mutated AMLs expressed higher amounts of genes implicated in downstream IFN signaling and higher \( \text{CD8} \) transcripts and markers of cytotoxicity compared with \( \text{TP53} \) wild-type cases (fig. S14, A and B).

IFN-\( \gamma \)-related gene expression and protein profiles correlate with antileukemia responses after flotetuzumab immunotherapy

Last, we hypothesized that higher expression of IFN-\( \gamma \)-related genes in immune-infiltrated AML cases, while underpinning chemotherapy resistance, might identify patients with AML who derive benefit from immunotherapy with flotetuzumab \((48)\), a \( \text{CD3} \times \text{CD123} \) dual affinity re-targeting molecule. BM samples collected before flotetuzumab treatment from 30 adult patients with chemotherapy-refractory or relapsed AML enrolled in the CP-MGD006-01 clinical trial (NCT#02152956) were profiled using the PanCancer IO360 gene expression assay. Patients’ characteristics are summarized in table S9 and flotetuzumab antileukemic activity was defined as either \( \text{CR} \), \( \text{CR} \) with partial hematologic recovery (\( \text{CRh} \)), \( \text{CR} \) with incomplete hematologic recovery (\( \text{CRI} \)), and partial response or overall benefit (>30\% reduction in BM and/or blood blasts). BM samples from 92\% of patients with evidence of flotetuzumab antileukemic activity (11 of 12) had an immune-infiltrated TME relative to nonresponders (Fig. 7A). The IFN-dominant module score was significantly higher in patients with chemotherapy-refractory AML compared with relapsed AML at time of flotetuzumab treatment, and in individuals with evidence of antileukemic activity compared to nonresponders (Fig. 7B). The TIS score was a strong predictor of antileukemic responses to flotetuzumab, with an AUROC value of 0.847 (Fig. 7C).

On-treatment BM samples (available in 19 patients at the end of cycle 1) displayed increased antigen presentation and immune activation relative to baseline samples, as reflected by higher TIS scores.
(6.47 ± 0.22 versus 5.93 ± 0.15; \(P = 0.0006\)), antigen processing machinery scores (5.67 ± 0.16 versus 5.31 ± 0.12; \(P = 0.002\)), IFN-\(\gamma\) signaling scores (3.58 ± 0.27 versus 2.81 ± 0.24; \(P = 0.0004\)), and PD-L1 expression (3.43 ± 0.28 versus 2.73 ± 0.21; \(P = 0.0062\); Fig. 7D).

GeoMx DSP of BM FFPE biopsies from a subgroup of 11 patients identified protein profiles at baseline that distinguish responders from nonresponders (Fig. 7, E and F and fig. S15). The IFN-\(\gamma\)–inducible molecule stimulator of interferon response (STING) was up-regulated after cycle 1 of flotetuzumab in two patients who achieved CR (Fig. 7G). In these individuals, T cell activation markers (CD27, CD45RO, and CD44), Bcl-2, immune checkpoints [ICOS, PD-L2, CTLA4, and 4-1BB (CD137)], and CD4 were highly expressed in ROIs with T cell clustering around CD123+ AML blasts (Fig. 7H), supporting a local immune-modulatory effect of flotetuzumab. Overall, these data suggest a clinical benefit for patients with AML with an immune-infiltrated TME and validate the translational relevance of our findings.

**DISCUSSION**

Using large cohorts of subjects, the current study reveals underlying transcriptomic features that stratify the TME of AML into immune subtypes and may assist therapeutic predictions by defining patients who will potentially derive the greatest benefit from immunotherapies. We identified two subtypes of differentially immune-infiltrated tumors, an observation that was validated in independent childhood and adult AML series, reinforcing the notion that unique molecular features can distinguish AML across age groups (35). The IFN-related gene sets identified in our study improved the prediction of therapeutic resistance after conventional “3 + 7” cytarabine and anthracycline chemotherapy beyond that provided by the ELN cytogenetic risk category [area under receiver operating characteristic (AUROC) = 0.815 in PMCC cases (discovery series) and 0.870 in Beat AML cases (in silico validation series)] (49). In recent Southwestern Oncology Group (SWOG) and MD Anderson Cancer Center clinical
trials, pretreatment covariates such as cytogenetic risk and age only yielded AUROC of 0.65 and 0.59 for therapeutic resistance, respectively (41). Models that were established in the present study by incorporating IFN-related mRNA profiles also outperform a recently developed 29-gene and cytogenetic risk predictor of chemotherapy resistance (AUROC = 0.76) (50). Our observation that CD8 exhaustion scores and PD1 scores are significantly higher in patients with ELN adverse risk compared with ELN favorable risk is congruent with a previous report showing unique transcriptional programs associated with ELN cytogenetic risk groups in CD8⁺ T cells from patients with AML (51). An IFN-related DNA damage resistance signature (IRDS) correlates with resistance to adjuvant chemotherapy and with recurrence after radiotherapy in patients with breast cancer (42), suggesting that tumor cells overexpressing IRDS genes, including STAT1, ISG15, and IFIT1, as a result of chronic activation of the IFN signaling pathway suppressed, as indicated by elevated expression of IFN-inducible type I immune response genes. The higher expression of CD8 and GZMB that we observed in ROIs with higher PTEN suggests that PTEN may represent an unidentified molecular driver of T cell infiltration in AML. In addition, higher expression of GZMB correlated with worse clinical outcomes and individually separated patients with AML into subgroups with different survival probabilities. In this respect, signatures of dysfunctional T cells, including high expression of GZMB and other transcripts associated with effector CD8⁺ T cell differentiation such as IFNG, may be increased in patients with AML at diagnosis and persist with higher frequency only in chemotherapy nonresponders (38). Last, IFN-γ-related gene expression programs in the AML TME, including the TIS score, correlated with response to flotetuzumab immunotherapy in 30 heavily pretreated patients with relapsed/refractory AML on clinical trial CP-MGD006-01.

FLOTETUMAB THERAPY

Flotetuzumab treatment was associated with increased expression of antigen processing machinery components, IFN-γ signaling molecules such as STING, negative immune checkpoints, and lymphocyte activation markers, including heightened PD-L1 mRNA and protein, in BM ROIs with CD3 hotspots. This finding provides a biological rationale for designing clinical studies with sequential flotetuzumab and immune checkpoint blockade in patients with AML in remission with MRD.

The use of bulk BM aspirates is a potential limitation of our analysis. Future studies should use single-cell RNA sequencing of purified CD8⁺ T cells to further dissect individual variation in response to T cell engagers (62). We also acknowledge that the detailed phenotypes, antigen specificities, and intratumoral TCR repertoires of T cells in patients with immune-infiltrated AML remain to be established (63).

In conclusion, our work unveils the heterogeneity of the immune landscape of AML and provides a novel precision medicine-based conceptual framework for AML targeting immunotherapy to subgroups of patients with IFN-γ–dominant AML, who may be refractory to conventional cytotoxic chemotherapy but responsive to T cell engagers. The immunological stratification of pretreatment BM samples may therefore enable rapid risk prediction and selection of frontline therapeutic modalities (11), in conjunction with cytogenetic and mutational information.

MATERIALS AND METHODS

Study design

The aim of this study was the high-dimensional analysis of the immunological contexture of BM samples from patients with AML and the identification of immune correlates of therapeutic benefit. For the discovery cohorts (442 archival BM samples from 370 patients with newly diagnosed AML; Table 1), immune gene expression scores were correlated with disease characteristics and with outcomes, including chemotherapy resistance, RFS, and OS. The ability of immune gene sets defining the IFN-dominant module to predict whether patients responded to induction chemotherapy was validated using in silico data sources (data S1). For the immunotherapy
clinical trial \( (n = 30 \text{ patients with relapsed/refractory AML}) \), BM samples were collected at baseline and after one or two cycles of flotetuzumab immunotherapy. Immune gene expression scores were correlated with disease status at time of study entry (chemotherapy-refractory versus relapsed AML) and with antileukemic activity from flotetuzumab. No data, including outlier values, were excluded. Sample sizes used for each experiment are detailed in the figure legends.

**Patients’ demographics (discovery cohorts)**
Patient and disease characteristics are detailed in Table 1. Primary patient specimens (nonpromyelocytic AML) and associated clinical data were obtained on research protocols approved by the Institutional Review Boards of the CHOP, USA and PMCC, Canada and by the Ethics Committee of TU Dresden and SAL, Germany.

**Patients’ demographics (immunotherapy cohort)**
The CP-MGD006-01 clinical trial (NCT#02152956) is a multicenter, open-label, phase 1/2 dose escalation and dose expansion study. Thirty patients with primary refractory \( (n = 23) \) and relapsed AML \( (n = 7) \) treated with flotetuzumab at the recommended phase 2 dose \( (500 \text{ ng/kg per day}) \) were included in the current analysis. Patients received a lead-in dose of flotetuzumab for week 1, followed by 500 ng/kg per day during weeks 2 to 4 of cycle 1, and a 4-day on/3-day off schedule for cycle 2 and beyond. Eligible patients were 18 years of age or older, with relapsed or refractory AML (according to WHO criteria) unlikely to benefit from cytotoxic chemotherapy defined as (i) refractory to ≥2 induction attempts (primary induction failure), (ii) first relapse with an initial CR duration <6 months (early relapse), (iii) relapse in patients that achieve a CR lasting ≥6 months after prior therapy (late relapse), or (iv) prior failure of hypomethylating agents. All participants were required to have an Eastern Cooperative Oncology Group performance status of ≤2, a peripheral blast count of <20,000/mm² at the time of first treatment, and adequate organ function. Patients with a prior history of ageneic HSCT, active untreated autoimmune disorders, or active central nervous system leukemia were excluded. The trial was approved by the Institutional Review Boards of participating centers and was conducted according to the current International Conference on Harmonization (ICH) Guideline for Good Clinical Practice (ICH E6) and all applicable local and national regulations and ethical principles in accordance with the Helsinki Declaration. All participants provided written informed consent before enrollment.

BM aspirates were collected at baseline \( (n = 30) \) and after cycle 1 of flotetuzumab \( (n = 19) \) to evaluate the temporal immunological effects associated with therapeutic response. Disease status was assessed by modified International Working Group (IWG) criteria. Antileukemic response was defined as either CR, CRi, CRh, PR, or “other benefit” (>30% decrease in BM blasts). Nonresponders were individuals with either treatment failure, stable disease, or progressive disease. Patient and disease characteristics are detailed in table S8.

**RNA isolation and processing**
Messenger RNA was isolated and processed as previously described \( (45) \). For the PMCC, SAL, and CHOP patient cohorts, 100 to 150 ng per sample of RNA extracted from 442 bulk BM aspirates from patients with AML treated with curative intent was analyzed on the NanoString nCounter FLEX analysis system using the PanCancer Immune (PCI) profiling panel (for research use only and not for use in diagnostic procedures), which measures mRNA expression of 770 genes representing 14 immune cell types, common checkpoint inhibitors, cancer testis antigens, and genes covering both the innate and adaptive immune response without the need for amplification \( (25, 64, 65) \). BM samples from patients receiving flotetuzumab immunotherapy were analyzed using the PanCancer IO 360 panel (for research use only and not for use in diagnostic procedures).

**nCounter data quality control, data normalization, and signature calculation**
The reporter probe counts, i.e., the number of times the color-coded barcode for that gene is detected, were tabulated in a comma separated value format for data analysis with the nSolver software package (version 4.0.62) and nSolver Advanced Analysis module (version 2.0.115, NanoString Technologies). The captured transcript counts were normalized to the geometric mean of the housekeeping reference genes included in the assay and the code set’s internal positive controls. The relative abundance of immune cell types and IO biological signatures were computed as previously published \( (22, 23) \). For samples run on the PCI profiling panel, we also calculated an approximation of the TIS using 16 of the 18 functional genes and 5 of the 10 housekeeper genes that are present in the PanCancer IO360 mRNA panel \( (18) \).

**GO and GSEA**
Metascape.org was used to enrich genes for GO biological processes and pathways. GSEA was performed using the GSEA software v.3.0 (Broad Institute, Cambridge, USA) \( (66) \). The hallmark IFN-γ response \( (M5911) \) and inflammatory response gene sets \( (M5913) \) were downloaded from the Molecular Signature Database \( (22, 23) \).

**GeoMx DSP**
Ten FFPE BM biopsies from patients with newly diagnosed AML (SAL series) and 19 FFPE BM biopsies from patients receiving flotetuzumab immunotherapy \( (n = 11 \text{ at baseline and } n = 8 \text{ after cycle 1}) \) were profiled using the prototype or commercial GeoMx DSP platform, respectively \( (fig. S15) \). Samples were stained using three fluorescent visualization markers, CD3 (T cell), CD123 (myeloid blast), and SYTO 83 or 13 (nuclei), and ultraviolet (UV)–cleavable oligolabeled antibodies \( (panels \text{ are shown in table S10}) \). Stained slides were loaded on the DSP instrument and digitally scanned. Fluorescent scans were used to select 24 geometric ROIs for molecular profiling \( (27, 67) \). The DSP instrument then UV-illuminated selected ROIs to release conjugated oligos and the microcapillary fluidics system collected released oligos, which were counted on the nCounter system. Data were normalized to technical controls and area.

**Intracellular cytokine staining**
Cells were aliquoted into \( 12 \times 75 \text{ mm tubes (0.5 } \times 10^6 \text{ cells per tube}) \) in 500 μl of RPMI 1640 (Lonza) + 10% fetal bovine serum (FBS) \( (v/v) \). Two tubes were set up per sample, the first as an unstimulated control and the second stimulated with phorbol 12-myristate 13-acetate \( (50 \text{ ng/ml}) \) and ionomycin \( (1 \mu g/ml) \) \( (both \text{ from Sigma-Aldrich}) \). Samples also received brefeldin A \( (10 \mu g/ml) \) (BioLegend), then were vortexed gently, and incubated at \( 37°C \) for 5 hours. After incubation, cells were washed in phosphate-buffered saline (PBS), then incubated in 100 μl of PBS containing 5 μl of Human FcR Blocking Reagent \( (Miltenyi Biotec) \), fluorescently labeled mAbs for surface markers of interest \( (CD3, CD4, CD8; \text{BioLegend}) \), and fixable LIVE/DEAD viability stain \( (Molecular \text{ Probes}) \) for 30 min at \( 4°C \) protected from

light. Unbound antibody was washed off using PBS and then cells were fixed by incubating in 200 μl of 1× True-Nuclear Fix Buffer (True-Nuclear Transcription Factor Buffer Set, BioLegend) for 20 min. Cells were washed in 1× Perm Buffer (True-Nuclear Transcription Factor Buffer Set, BioLegend), then resuspended in 100 μl of 1× Perm Buffer and fluorescently labeled mAbs for intracellular cytokines of interest [interleukin-2 (IL-2), IL-4, IL-10, IL-17, IFN-γ, and TNF-α; antibody clones are provided in table S11], and incubated for 20 min at room temperature protected from light. Unbound antibody was washed off using 1× Perm Buffer, cells were resuspended in 400 μl of PBS and immediately analyzed on a 3-laser, 10-color Gallios flow cytometer (Beckman Coulter).

**Data sources for in silico analyses**

The first data series (E-MTAB-3444), hereafter referred to as the HOVON series (44), was retrieved from Array Express and encompassed three independent cohorts of adults (≥60 years) with de novo AML (last accessed on 4 March 2019). BM and blood samples were collected at diagnosis and were analyzed on the Affymetrix Human Genome U133 Plus 2.0 Microarray (44, 68). Patients were treated with curative intent according to the Dutch-Belgian Hematology-Oncology Cooperative Group and the Swiss Group for Clinical Cancer Research (HOVON/SAKK) AML-04, -04A, -29, -32, -42, -42A, -43, or -92 protocols (available at www.hovon.nl). Clinical annotations were provided by the authors. The second data series, hereafter referred to as TCGA series, consisted of RNA sequencing data (Illumina HiSeq2000) from 162 adult patients with AML complete cytogenetic, immunophenotypic, and clinical annotation who were enrolled on Cancer and Leukemia Group B treatment protocols 8525, 8923, 9621, 9720, 10201, and 19808 (69). RNA and clinical data were retrieved from the TCGA data portal (https://tcga-data.nci.nih.gov/tcga/tcgaDownload.jsp). The third data series (Beat AML) was retrieved using the VIZOME user interface (www.vizome.org/aml) and consisted of RNA sequencing data from primary specimens from 281 patients with AML with detailed clinical annotations, including diagnostic information, treatments, responses, and outcomes treated on the Beat AML Master Trial (43).

**Statistical analyses**

Descriptive statistics included calculation of mean, median, SD, and proportions to summarize study outcomes. Comparisons were performed with the Mann-Whitney U test for paired or unpaired data (two-sided), as appropriate, or with the analysis of variance (ANOVA) with correction for multiple comparisons. IBM SPSS Statistics (version 24) and GraphPad Prism (version 8) were used for statistical analyses. A two-sided P < 0.05 was considered to reflect statistically significant differences. The log-rank (Mantel-Cox) test was used to compare survival distributions.

Therapeutic resistance was defined as failure to achieve CR despite not experiencing early treatment-related mortality (within 28 days of chemotherapy initiation; primary refractory cases) or as early relapse (<3 months) after achieving CR (41). OS was computed from the date of diagnosis to the date of death. RFS was measured from the date of first CR to the date of relapse or death. Subjects lost to follow-up were censored at their date of last known contact.

Binary logistic regression and multinomial logistic regression were used to ascertain the relative contribution of immune subtypes and other pretreatment covariates selected a priori based on known clinical relevance (ELN risk group, FLT3-ITD status, NPM1 mutational status, patient age at diagnosis, and primary versus secondary AML) toward the predicted likelihood of response to induction chemotherapy, AML relapse, and patient death (45). Competing risks regression analyses by the method of Fine and Gray were performed using STATA/IC (version 16.0) (32). Allogeneic HSCT, a potential confounder, was treated as an event whose occurrence precluded the occurrence of the primary clinical end point (death) (32).

**SUPPLEMENTARY MATERIALS**

stm.sciencemag.org/cgi/content/full/12/546/eaaz0463/DC1

Fig. S1. Immune gene signatures and survival in the AML discovery series.
Fig. S2. GeoMx DSP and RIO selection in a representative pretreatment BM trephine biopsy (SAL series) with high T cell infiltration.
Fig. S3. GeoMx DSP and RIO selection in a representative pretreatment BM trephine biopsy (SAL series) with low T cell infiltration.
Fig. S4. Highly multiplexed protein profiling in the SAL patient cohort.
Fig. S5. Correlation between CD3 infiltration and expression of IO-related proteins as revealed by GeoMx DSP of BM trephine biopsies (SAL series).
Fig. S6. Expression of SIG3 genes in TCGA-AML cases and in healthy tissues.
Fig. S7. Association between immune gene signatures and patients’ characteristics in the AML discovery series.
Fig. S8. Clinical outcomes of patients with immune-infiltrated and immune-depleted AML treated with allogeneic HSCT (PMCC cohort).
Fig. S9. Immune gene signatures in the CHOP and SAL cohorts.
Fig. S10. GO and pathway analyses.
Fig. S11. Immune scores in patients with AML (SAL cohort) at time of diagnosis and achievement of CR (paired samples).
Fig. S12. Intracellular cytokine staining of BM samples from patients with immune-infiltrated and immune-depleted AML.
Fig. S13. Immune subtypes associate with cancer driver gene mutations in TCGA-AML and Beat AML trial specimens.
Fig. S14. Genes implicated in IFN downstream signaling in Beat AML trial specimens.

**REFERENCES AND NOTES**


S. Jacobiell; EBMT Statistical Committee, Suggestion on the use of statistical methodologies in studies of the European Group for Blood and Marrow Transplantation. Bone Marrow Transplant. 48 (Suppl 1), S1–537 (2013).


Capturing the P. A. M. D. N. L. C. T. EMT-related genes linked to and M. expression profiling and therapy is a remission and complexity of hematopoietic stem cells drives a highly invasive AML expressing EMT-related genes linked to poor outcome. Cancer Cell 30, 43–58 (2016).


Funding: This work was supported by grants from the Qatar National Research Fund (NPRP 2297–3–494 to S. Rutella); the Roger Counter Foundation, United Kingdom (to A.G.P. and S. Rutella); the John and Lucille van Geest Foundation (to A.G.P. and S. Rutella); the James Killington Challenge for Leukemia (to A.G.P.); the NCI K08 CA184418 and 1 U01 CA223486 and the Andrew McDonough B+ Foundation (to S.K.T.); and R35CA210084 (to J.F.D.). We also acknowledge support from the National Cancer Institute of the National Institutes of Health under Award Number R50CA211466 (M.P.R.) and St Baldrick’s Foundation—Stand Up To Cancer (SU2C) Pediatric Cancer Dream Team Translational Research Grant (SU2C-AACR-DT-27-17 to S.K.T.). Stand Up To Cancer is a division of the Entertainment Industry Foundation Research grants are administered by the American Association for Cancer Research, the scientific partner of SU2C. The Study Alliance of Leukemia (www.sal-aml.org) is gratefully acknowledged for providing primary patient material and clinical data.


Submitted 7 August 2019
Resubmitted 20 January 2020
Accepted 21 April 2020
Published 3 June 2020
10.1126/scitranslatmed.aaz0463

Immune landscapes predict chemotherapy resistance and immunotherapy response in acute myeloid leukemia


Sci Transl Med 12, eaaz0463.
DOI: 10.1126/scitranslmed.aaz0463

Informing immunotherapy decisions

Predicting which patients with acute myeloid leukemia (AML) are likely to respond to immunotherapy is currently difficult. To better understand the immune heterogeneous landscape in AML, Vadakekolathu et al. studied gene expression in bone marrow biopsies and clinical outcomes of multiple AML cohorts. They observed variable T cell infiltration and interferon-driven signatures that could be associated with outcomes such as chemotherapy resistance. Matched samples from patients before or after flotetuzumab indicated that an IFN-γ signature could identify patients likely to respond to this therapy. These data lead to a better understanding of the immune environment of AML and could help inform immunotherapy treatment decisions in the future.