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ONCOGENIC SIGNALING AND IMMUNE EVASION IN HEMATOLOGIC MALIGNANCIES

Escape from T-cell–targeting immunotherapies in acute myeloid leukemia

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Single-cell and spatial multimodal technologies have propelled discoveries of the solid tumor microenvironment (TME) molecular features and their correlation with clinical response and resistance to immunotherapy. Computational tools are incessantly being developed to characterize tumor-infiltrating immune cells and to model tumor immune escape. These advances have led to substantial research into T-cell hypofunctional states in the TME and their reinvigoration with T-cell–targeting approaches, including checkpoint inhibitors (CPIs). Until recently, we lacked a high-dimensional picture of the acute myeloid leukemia (AML) TME, including compositional and functional differences in immune cells between disease onset and postchemotherapy or posttransplantation relapse, and the dynamic interplay between immune cells and AML blasts at various maturation stages. AML subgroups with heightened interferon gamma (IFN-γ) signaling were shown to derive clinical benefit from CD123×CD3–bispecific dual-affinity retargeting molecules and CPIs, while being less likely to respond to standard-of-care cytotoxic chemotherapy. In this review, we first highlight recent progress into deciphering immune effector states in AML (including T-cell exhaustion and senescence), oncogenic signaling mechanisms that could reduce the susceptibility of AML cells to T-cell–mediated killing, and the dichotomous roles of type I and II IFN in antitumor immunity. In the second part, we discuss how this knowledge could be translated into opportunities to manipulate the AML TME with the aim to overcome resistance to CPIs and other T-cell immunotherapies, building on recent success stories in the solid tumor field, and we provide an outlook for the future.

Introduction

Acute myeloid leukemia (AML) is a model of highly efficient metastatic spread and is characterized by tremendous molecular, immunological, and clinical heterogeneity.[1] The treatment landscape of AML has changed dramatically in recent years.[2] However, most patients eventually relapse and have disappointing outcomes, with a 5-year overall survival (OS) rate of 10%.[3] Checkpoint inhibitors (CPIs) restrain T-cell suppressive signals delivered through cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) and promote antitumor immune responses. Distinct cellular mechanisms underlie CTLA-4 and PD-1 checkpoint blockade, with the former affecting $CD4^+$ T-cell clonal expansion and trafficking, and the latter largely affecting the exhausted $CD8^+$ T-cell compartment.[4,5] Patients with certain solid tumor types, including glioblastoma and pancreatic cancer, demonstrate de novo resistance to CPIs, and a substantial proportion of patients with CPI-sensitive solid tumors eventually develop adaptive resistance.[4] Although CPIs offer long-term clinical benefit in a large proportion of patients with solid tumors, results in AML, which has historically been considered an immunologically "cold" tumor,[6,7] have been less impressive.

Integrative immunogenomic approaches can refine the accuracy of outcome prediction by supporting a more granular stratification of AML within existing European LeukemiaNet (ELN) risk categories.[8,9] In particular, immune gene expression profiling of primary bone marrow (BM) samples uncovered novel immune-infiltrated and interferon gamma (IFN-γ)-dominant AML subtypes associated with poor outcome after standard-of-care chemotherapy and with response to immunotherapy with flotetuzumab, a bispecific T-cell engager targeting CD123.[9,10] The IFN-dominant gene module in pretreatment BM specimens from patients with immune-infiltrated AML reflected the abundance of IFN-γ signaling; immunoproteasome; myeloid inflammation; inflammatory chemokine; *IL-10*, *PD-L1*, and *PD-L2* gene expression scores; as well as higher expression of molecules involved in antigen processing and presentation. BM-resident $CD8^+$ T cells from patients with immune-infiltrated AML were polyfunctional, as suggested by their coexpression of intracellular IFN-γ and tumor necrosis factor α (TNF-α).[9]

A large-scale study of genotype-immunophenotype correlations across lymphoid and myeloid malignancies identified associations between immune checkpoint ligands and disease subgroups, including expression of *PD-L1*, *ARG1*, *CD86*, and

VSIR (VISTA), encoding an inhibitory T-cell checkpoint of the B7 family, in monocytic AML as well as high infiltration of natural killer (NK) and $CD8^+$ T cells in AML with myelodysplastic syndrome–like features.[8,11] This observation suggests that the differentiation stage of the blasts may enable AML-specific immune evasion mechanisms. Furthermore, an inflammation-associated gene expression metric has been correlated with reduction in T-cell clonal expansion, increase in $CD8^+GZMK^+$ and regulatory T (Treg) cells, and worse clinical outcomes both in children and adults with AML.[12]

This review will summarize recent discoveries on T-cell functional states in AML and their impact on response to CPIs, bispecific T-cell engagers, and adoptive cell therapies. We discuss how resistance to immunological treatments could be overcome, and provide an outlook for the future.

Advances in defining T-cell–dysfunctional states

Both senescent and exhausted T cells accumulate in the tumor microenvironment (TME), in which they exhibit impaired antitumor functions. Senescent T cells acquire a unique transcriptional profile, downregulate costimulatory molecules (CD27 and CD28), and highly express CD57 and killer cell lectin-like receptor subfamily member 1 (KLRG1).[13] Although senescent T cells are in a state of cell cycle arrest, do not proliferate in response to T-cell receptor (TCR) triggering, and manifest defective killing abilities, they produce high amounts of proinflammatory and suppressive cytokines and remain metabolically active.[14] Senescent-like $CD27^-CD28^-CD8^+$ T cells expressing NK markers expand in older individuals and lose the signaling activity of the TCR.[15] T-cell exhaustion in tumors is a proxy for tumor antigen-driven T-cell activation and is believed to be a dynamic and gradual state analogous to that elicited by continuous antigen exposure during viral infections.[16] Remarkable phenotypic diversity is observed within intratumoral T cells, which can be broadly compartmentalized into memory T cells and exhausted T cells,[17] and reside along a continuum of progressively declining T-cell function that spans progenitor exhausted T cells (T_{PEX}),[18] $GZMK^+$ predysfunctional or "transitional" T cells (T_{EX}), and early-to-late dysfunctional T cells (Table 1).[24] During chronic lymphocytic choriomeningitis virus (LCMV) infection in mice, T_{PEX} self-renew and exhibit multipotent repopulation capacity, which is governed by sustained expression of *MYB*.[26] In sharp contrast to their $CD62L^-$ T_{PEX} and $CX3CR1^+$ or $CX3CR1^-$ T_{EX} descendants, a subpopulation of $CD62L^+$ T_{PEX} has been reported to proliferate vigorously and to acquire enhanced potential for effector cell generation in response to CPIs.[26] Studies in melanoma-bearing mice identified subsets of stem-like or T_{PEX} and terminally exhausted $CD8^+$ T cells, which differ in gene expression, transcription factor activity, cytokine production, cytolytic function, and epigenetic landscape.[27] Importantly, a larger fraction of $CD8^+$ T_{PEX} with a $TCF1^+PD\text{-}1^+$ phenotype in pretreatment biopsies from patients with advanced melanoma correlated with significantly longer progression-free survival and OS after nivolumab or ipilimumab.[16]

By applying longitudinal single-cell RNA sequencing (scRNA-seq) and single-cell assay for transposase-accessible chromatin sequencing, novel T_{EX} subsets have been revealed in LCMV-infected mice, including a distinct T-cell population coexpressing NK markers and high levels of *Tox*, *Bcl2*, and *Lag3*, that is dependent on the transcription factor *Zeb2* and retains superior functional and proliferative capacity compared with other intermediate T_{EX} states.[21,22] Treatment of LCMV-infected mice with PD-1 blocking agents substantially altered the frequency of T_{EX} and induced their differentiation to functionally intermediate T_{EX} states.[21] Interestingly, addition of interleukin-2 (IL-2) to PD-1 immunotherapy improved responses and expanded stem-like $PD\text{-}1^+TCF1^+CD8^+$ T cells resembling highly functional effector $CD8^+$ T cells.[28] These findings suggest that T_{PEX} are not fate-locked into the exhaustion program and warrant the evaluation of synergistic cytokine effects in future clinical trials of AML and other cancers.

A novel cellular stress state (T_{STR}) characterized by unique expression of stress-related heat shock genes, including *HSP1A1* and *HSPA1B*, has been uncovered in a recent study integrating single-cell and spatial transcriptomes from 308 048 high-quality T cells across 16 cancer types.[23] Notably, T_{STR} predicted inferior clinical outcomes in 6 independent solid tumor immunotherapy cohorts and were enriched in mutation-associated neoantigen-specific $CD8^+$ T cells in tumors from patients with no major pathological response.[23] It would be of great interest to explore the prognostic and/or predictive relevance of an analogous T_{STR} functional state for AML.

Cancer cell–intrinsic signaling and immune dysfunction

Oncogenic drivers

In addition to T-cell–intrinsic mechanisms, the immune milieu of solid tumors can be shaped by cancer-cell–intrinsic features, including genetic aberrations in oncogenes, tumor suppressor genes, or DNA damage repair genes, which affect CPI sensitivity.[29,30] *TP53* is the most commonly altered tumor suppressor gene in cancer. In epithelial tumors, tumor-infiltrating lymphocytes can recognize autologous TP53 neoantigens, pointing to the immunogenicity of *TP53* hotspot mutations and providing a biological rationale for *TP53*-specific immunotherapies.[31] In this respect, bispecific antibodies targeting a common *TP53* neoantigen (arginine-to-histidine substitution at codon 175; R175H) bound to HLA-A*02:01 on the cell surface can activate T cells and promote tumor cell lysis both in vitro and in vivo.[32]

TP53 abnormalities occur in 8% to 10% of de novo AML and portend a high risk of primary induction failure and relapse and a dismal prognosis.[33] AML cells carrying *TP53* missense mutations express gene signatures of *TP53* inactivation, suggesting dominant-negative activity without evidence of neomorphic gain-of-function capacity.[34] AML cases with *TP53* mutations from The Cancer Genome Atlas (TCGA) cohort express higher levels of cytolytic molecules in contrast to the common driver mutations *FLT3* and *NPM1*, which preferentially occur in AML samples with low cytolytic activity (Table 2).[8] Both de novo and secondary *TP53*-mutated AML harbor an inherently immunosuppressive TME, with expanded Treg cells and myeloid-derived suppressor cells, decreased NK cells, enhanced IFN-γ signaling, and increased PD-L1 in hematopoietic stem cells (HSCs).[35,36] Importantly, mouse double minute 2 inhibition in

Table 1. T-cell states in cancer

T-cell compartment	Subpopulation	Phenotype/ signature genes	Function	Other remarks
Memory T cells (reduced overall clonal expansion)	Stem cell memory T cells[19]	TCF1 IL7R CD62L CCR7	Display increased proliferative capacity and mediate superior antitumor responses compared with known memory populations	Do not exhibit tumor-specific localization Retain the ability to regenerate a vast progeny of effector cells
	Central memory T cells[20]	TCF1 IL7R CCR7	Express lymph node homing receptors and lack immediate effector function, but efficiently stimulate DCs and differentiate into $CCR7^-$ effector cells upon secondary stimulation	
	Effector memory T cells[20]	TCF1 IL7R CD62L	Express receptors for migration to inflamed tissues and display rapid production of IFN-γ and immediate effector function	
Exhausted T cells (highly clonotypically expanded)[17]	T_{PEX}	PD1 CTLA4 LAG3 TOX CD62L TCF1	Are early dysfunctional T cells A subset of predysfunctional (or "transitional") T cells is defined by high expression of *GZMK* Lack effector functions	Can self-renew and differentiate and are a reservoir of T_{EX} cells Are often found in lymph nodes and TLS Responsible for immunotherapy efficacy
	T_{EX}	PD1 CTLA4 LAG3 TIM3 CD39 TOX CXCL13 BCL2L11	Have impaired cytotoxic function (gradually lose effector functions)	Even if terminally differentiated, T_{EX} can proliferate in an antigen-dependent fashion
	Tissue-resident memory–like T_{EX}	CD103 HOBIT (*ZNF683*)	Represent a continuum in the spectrum of TIL phenotypes Have high cytotoxic potential Express high levels of inhibitory molecules	Likely reflect a variation of T_{EX} differentiation in the TME
	NK-cell receptor–positive T_{EX}	CD8 KLRG1 CD57 *Bcl2* *LAG3*	Are dysfunctional (ie, have reduced cytotoxicity against autologous AML blasts)	Are more abundant in R/R AML but can also be detected in mice with LCMV infection, in which they may be dependent on the transcription factor *Zeb2*[21,22]
Stress response state (T_{STR})[23]	Both $CD8^+$ and $CD4^+$ T cells	Stress-related heat shock genes (*HSPA1A*, *HSPA1B*); NF-κB signaling molecules	Are highly correlated with IFN-response $CD4^+$ and $CD8^+$ T-cell subsets	Are detectable in situ in the TME across various cancer types (especially those with aggressive phenotypes) Have a potential role in immunotherapy resistance

Intratumoral T cells are characterized by a remarkable phenotypic and functional diversity. This gradient of T-cell states has been described mostly for $CD8^+$ TILs residing along a continuum of dysfunction.[17,24] The current status of $CD4^+$ T cells in cancer has been reviewed elsewhere.[25] HOBIT, homologue of BLIMP1 in T cell; TCF1, T-cell–specific transcription factor 1; T_{EX} cells, terminally exhausted T cells; TILs, tumor-infiltrating lymphocytes; TIM3, T-cell immunoglobulin mucin receptor; TLS, tertiary lymphoid structures; TOX, thymocyte selection–associated high mobility group box protein.

patient–derived AML cells counteracts immune evasion by enhancing TNF–related apoptosis-inducing ligand receptor 1 and 2 and major histocompatibility complex class II (MHC-II) expression in a *TP53*-dependent manner, thereby restoring AML susceptibility to allogeneic T-cell–mediated cytotoxicity.[40] An interesting finding that could be relevant in the context of AML therapies that target phagocytosis such as monoclonal antibodies blocking CD47, a "don't eat me" signal overexpressed on AML blasts and leukemia stem cells (LSCs),[41] is that *TP53* loss in lymphoma cells modulates macrophage phagocytic capacity by enhancing the biogenesis of PD-L1$^+$ extracellular vesicles from tumor B cells.[42] Notably, a phase 1b trial of azacitidine and magrolimab in frontline treatment of *TP53*-mutant AML has shown durable responses and encouraging OS, although immune correlates of success have not yet been reported.[43]

However, a complete evaluation of safety and efficacy of azacitidine and venetoclax, with or without magrolimab, in patients with untreated AML (ENHANCE-3; ClinicalTrials.gov ID: NCT05079230) has shown a CR rate of 39.7% in the magrolimab-containing arm and 42.9% in the control arm, leading to study termination.[116] These negative results underscore the existing challenges in enhancing outcomes for patients unfit for

Table 2. Driver mutations and the immunological TME

Study	Driver mutation	Disease	Immune correlates
Vadakekolathu et al[9]	*RUNX1*, *TP53*	AML	Higher TIS, IFN signaling, and cytotoxicity scores relative to patients with favorable or intermediate-risk AML
Vadakekolathu et al[35]	*TP53*	AML	High expression of *IFNG*, *FOXP3*, immune checkpoints, markers of immune senescence, and phosphatidylinositol 3-kinase-Akt and NF-κB signaling intermediates
Sallman et al[36]	*TP53*	AML	Reduced numbers of BM-infiltrating $OX40^+$ cytotoxic T cells and helper T cells Decreased $ICOS^+$ and $4\text{-}1BB^+$ NK cells Expansion of myeloid-derived suppressor cells and Treg cells Increase of PD-L1 expression in HSCs
Dufva et al[8]	*TP53* *NPM1* *RUNX1*	AML AML AML	High cytolytic score (especially in MDS-like cases) and high expression of PD-L1 Increased expression of *VISTA* and *ULBP1* (NKG2D ligand) High expression of B-cell–associated markers (*BTN2A2*, *SLAMF7*, and *LY9*) in addition to HLA II
Abbas et al[37]	chr7/7q loss	AML	Higher Treg and $CD8^+$ T-cell infiltration, downregulation of IFN-γ pathway genes, and worse survival compared with AML cases with intact chr7/7q
Yeaton et al[38]	*TET2*	AML	Emergence of inflammatory monocyte-like cells during progression to myeloid transformation
Notarangelo et al[39]	*IDH1/IDH2*	Human *IDH*-mutant cancers	Acute but reversible inhibition of $CD8^+$ T-cell proliferation, cytotoxicity, and IFN-γ signaling by oncometabolite D-2HG

D-2HG, D-2-hydroxyglutarate; ICOS, inducible T-cell costimulator; MDS, myelodysplastic syndrome; TIS, tumor inflammation signature.

intensive induction chemotherapy and emphasize the necessity for immunotherapy agents with improved safety and efficacy profiles.

The epigenetic regulator ten-eleven translocation 2 (*TET2*) is mutated in 10% to 30% of AML cases. In mice carrying a patient–derived *TET2* missense mutation, progression to myeloid transformation has been correlated with the emergence of inflammatory monocyte-like cells,[38] whose transcriptional signature independently predicted shorter OS in a broad AML validation cohort. Exploring whether a proinflammatory TME affects response of *TET2*-mutated AML to CPIs could have promising translational potential.

Isocitrate dehydrogenase (*IDH1*) mutations, which are identified in 6% to 10% of patients with AML and impart a poor prognosis,[44] constrain T-cell accumulation and expression of IFN-γ–inducible chemokines in patients with lower-grade glioma.[45] Mechanistically, the oncometabolite D-2-hydroxyglutarate, which is produced by cancers with gain-of-function mutations in both *IDH1* and *IDH2*, alters glycolysis in $CD8^+$ T cells and reversibly inhibits their proliferation, antitumor killing capacity, and expression of IFN-γ gene programs.[39] These findings highlight a potential role for specific inhibitors of mutant *IDH* in improving immunotherapy efficacy through the removal of immunosuppressive D-2-hydroxyglutarate.

Inferred chr7/7q loss correlates with higher Treg and $CD8^+$ T-cell infiltration, downregulation of IFN-γ pathway genes, and worse survival compared with AML cases with intact chr7/7q.[37] Finally, an immunogenomic analysis of TCGA cohorts and 8 clinical trials of anti–PD-1/PD-L1 therapy in >1000 patients with solid tumors has uncovered that homozygous deletion of 9p21.3 (9p21 loss), a genomic defect occurring in ~13% of all cancers and eliminating *CDKN2A/B* tumor suppressors, confers "cold" tumor-immune phenotypes, with reduced abundance of tumor-infiltrating lymphocytes, diminished immune-cell trafficking/activation, decreased rate of PD-L1 positivity, activation of immunosuppressive signaling, and primary resistance to CPIs.[46] A "response score" incorporating 9p21 loss, PD-L1 expression, and tumor mutational burden in pretreatment tumors was shown to outperform PD-L1, tumor mutational burden, and their combination in identifying patients who are likely to achieve sustained response after CPI treatment. Furthermore, 9p21.3 deletions encompassing a cluster of 16 type I IFN genes have been associated with dendritic cell (DC) and $CD8^+$ T-cell dysfunction, derepression of solid tumor metastasis, and immunotherapy resistance.[47] Notably, homozygous deletions of type I IFN genes are very rare in AML (<1% of patients who are queried), as indicated by our interrogation of 1661 cases from the TCGA,[48] Beat-AML,[49] and TARGET-AML[50] cohorts accessed through the cBioPortal for Cancer Genomics (30 April 2023). Determining whether AML with *KMT2A-MLLT3* fusion caused by t(9;11) is immunogenetically distinct could be an interesting avenue for future research.

Type I/II IFN signaling

IFNs are pleiotropic cytokines implicated in cancer immunosurveillance and immunotherapy response, although also exerting protumorigenic effects in a context-dependent manner.[51] Melanoma tumors harboring genomic defects in type II (IFN-γ) pathway genes and amplification of IFN-γ pathway inhibitors *SOCS1* and *PIAS4* may be resistant to anti–CTLA-4 and anti–PD-1 immunotherapy.[52,53] The relationship

between IFN-γ signaling and CPI responses may indeed be more complex than currently appreciated, as suggested by a meta-analysis of 29 studies encompassing 2154 patients with solid tumors from 7 different tissues.[54] Tumor-intrinsic mutations of core IFN-γ signaling genes, including *JAK1*, *JAK2*, *IFNGR1*, *IFNGR2*, and *STAT1*, correlated with a greater likelihood of responding to CPIs. IFN-γ signaling gene alterations had opposing effects in vitro, with tumor screens identifying positive selection for alterations in IFN-γ pathway genes in the setting of host immune pressure. Inflammatory and IFN-γ gene signatures, suggestive of preexisting anti-AML immune responses, are associated with poor outcome across all ELN2017 stratifications.[9] Although IFN-γ–related RNA profiles are good predictors of response to CPIs in solid tumors as well as in AML,[7,9,55] prolonged IFN-γ signaling in tumor cells has been shown to promote resistance to genotoxic damage through STAT1-dependent molecular circuits.[56] Whether pretreatment IFN-γ signaling gene alterations in tumor cells preclude or favor responses of AML to CPIs remains to be conclusively established.

Genes typically associated with type I IFN signaling are largely detected in cancer cells and their tonic expression is maintained by *OAS1*.[57,58] Intact type I IFN signaling in cancer cells mediates resistance to $CD8^+$ T-cell cytotoxicity after genotoxic damage with or without anti–PD-L1 immunotherapy.[59] Responsiveness to type I IFN in peripheral immune cells, that is, higher levels of basal IFN-I–stimulated proteins, is epigenetically predetermined and correlates with solid tumor progression after anti-PD1 immunotherapy.[60] Conversely, T cells from "low responders" to type I IFN are enriched in pathways for immune effector functions, RNA metabolism and oxidative phosphorylation, and demonstrate a reduced exhaustion signature.

Interrogation of TCGA solid tumors has indicated that immune dysfunction is associated with high IFN resistance signatures in cancer cells relative to a nonoverlapping set of IFN-stimulated genes that are preferentially expressed by immune cells, such as T cells, NK cells, and macrophages.[58] Intriguingly, the establishment of acquired CPI resistance correlated with epigenetic features of IFN-associated "inflammatory memory," that is, with prolonged chromatin accessibility after signal termination, in Res 499 melanoma tumors.[58] Abolishing type I IFN signaling through knock out of IFN α and β receptor subunit 1 (*Ifnar1*) in cancer cells increased immune-cell expression of IFN-stimulated genes in myeloid cells, DCs, NK cells, and $CD8^+$ T cells and restored the antitumor response.[58] Orthogonal single-cell transcriptome analyses predicted strong interactions between $CD8^+$ T cells and proinflammatory type 3 DCs,[61] and

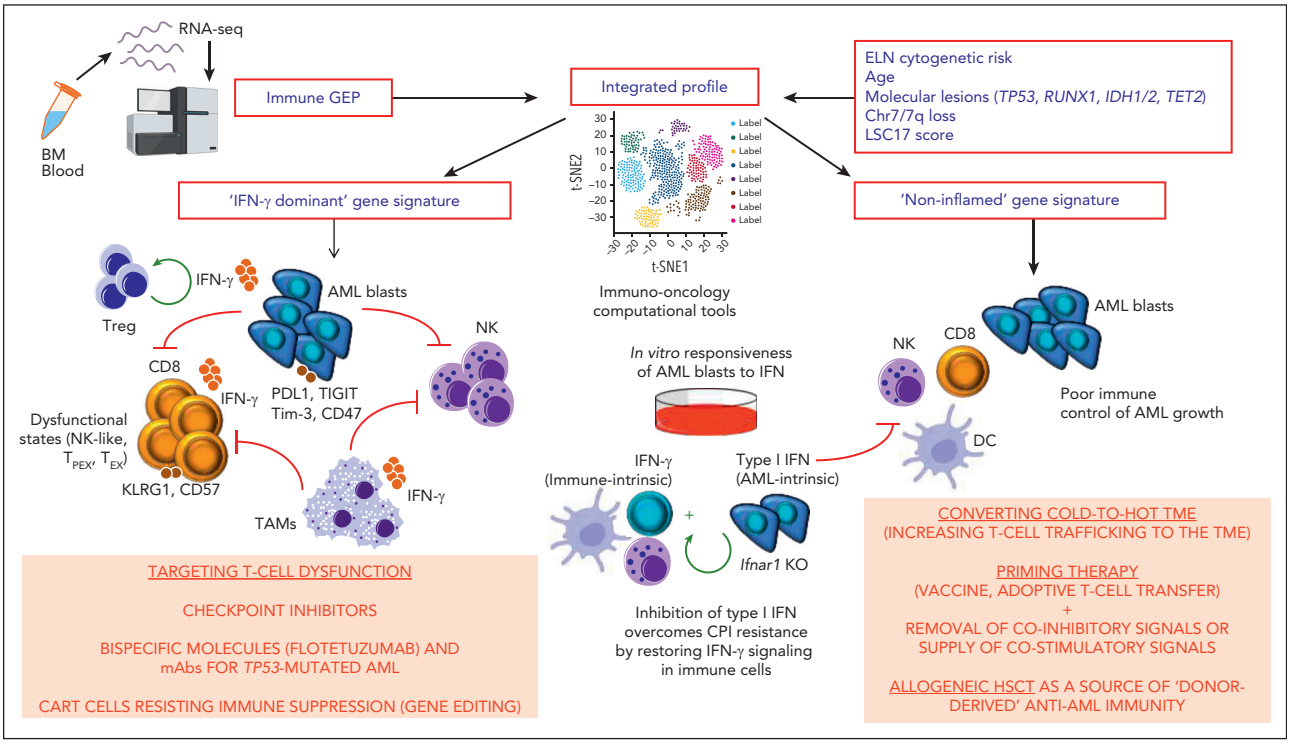

Figure 1. AML immune interplay and potential avenues for clinical translation. Current understanding of T-cell functional states and their effect on AML response to chemotherapy, bispecific molecules, and CPIs. Immune gene expression profiling (bulk and/or scRNA-seq) should be integrated with clinically validated prognosticators, including ELN risk category, LSC17 score, and molecular lesions (*TP53, RUNX1, IDH1/2,* and *TET2* mutational status), to accurately stratify patients with AML into subgroups with substantially different survival probabilities. Patients with an IFN-γ–dominant, immune-enriched TME could be allocated immunotherapies that target AML-induced T-cell dysfunctional states, including T-cell engagers and CPIs. *TP53*-mutated AML have been shown to respond to a CD123-targeting bispecific molecule. Conversely, patients with a "cold," immune-depleted profile could benefit from increasing T-cell trafficking to the TME and/or from priming therapies such as vaccines, adoptive T-cell transfer, or allogeneic HSCT. Interventions that balance type I (tumor-cell intrinsic) and type II (immune-cell intrinsic) IFN signaling could be instrumental to overcoming resistance to CPIs and other T-cell–based immunotherapies. In this respect, IFN-I hyporesponsiveness in tumor cells before anti-PD1 treatment has been correlated with long-term survival, as discussed in the main text. Furthermore, abrogating cancer cell IFN-I signaling increases IFN-II signaling in immune cells, thereby expanding T cells toward effector-like functional states. Red arrows denote inhibition; green arrows denote stimulation. GEP, gene expression profiling; LSC17, 17-gene leukemia stem cell; mAbs, monoclonal antibodies; TAM, tumor-associated macrophage.

highlighted an enhanced transition of $CD8^+$ T cells to an effector-like state. This elegant body of work suggests that inhibition of type I IFN signaling in cancer cells could antagonize CPI resistance through remodeling of inflammatory epigenetic states and feedback modulation of immune cells. Further discovery work on whether inducibility of type I IFN genes correlates with immune function may offer potential therapeutic relevance by allowing the identification of molecularly defined AML subgroups unlikely to benefit from CPIs (Figure 1). A systematic evaluation of inherent AML blast responsiveness to type I IFN might also reveal the molecular basis for AML cell susceptibility and/or resistance to immune attack.[62] Future prospective studies in AML should ascertain whether loss of IFN-γ signaling pathway genes, including *JAK1* and *JAK2*,[53] affects immunotherapy responses by limiting the release of T-cell–attracting chemokines and/or the upregulation of antigen processing machinery genes. Despite the complexity and rapidly evolving scenario of the cancer genome–immunophenotype relationships, these examples illustrate that select cancer-cell–intrinsic features and downstream immunoregulatory pathways, including primary and acquired insensitivity to IFNs, affect immunotherapy response. This knowledge should inform the development of novel immune interventions tailored to individual patients.

Impact of T-cell exhaustion and senescence on therapy response

Chemotherapy

How T-cell derangement affects AML response to standard-of-care chemotherapy, molecularly targeted therapies, and immunotherapies is incompletely understood. Patients with prolonged first complete remission (CR) (lasting >5 years) have a TME at presentation that is relatively less immunosuppressive and T cells that express lower levels of activation- and exhaustion-associated genes.[63] A landmark scRNA-seq study highlighted quantitative defects in T/NK cells, an increase in Treg cells, and predominance of T/NK cells after chemotherapy in a relatively small number of patients with AML.[64] Another study using high-dimensional flow cytometry has shown that the transcriptional profile of $CD8^+$ T cells diverges between chemotherapy responders and nonresponders, with the former patient group showing upregulation of costimulatory molecules, downregulation of apoptotic and inhibitory pathways, and overall reversal of $CD8^+$ gene expression signatures to a healthy-like pattern.[65] A silenced gene expression profile in $CD8^+$ T cells may correlate with longer OS in patients with ELN favorable-risk AML, an observation that establishes previously unappreciated links between clinically validated cytogenetic abnormalities and the AML-immune TME.[66] We have shown that NK-like $CD8^+$ T cells with transcriptional features of immune effector dysfunction (IED) are more abundant in patients with *TP53* and *RUNX1* mutations and predict significantly shorter relapse-free survival and OS.[55] These $CD8^+CD57^+KLRG1^+$ senescent-like T cells were impaired in their ability to lyse autologous AML blasts when activated with an anti–CD33/CD3 bispecific T-cell engaging antibody construct. T_{PEX} with a $GZMK^+IL7R^+CD8^+$ phenotype clonally expand in complete responders to induction chemotherapy compared with in nonresponders, and correlate with prolonged OS.[67] T cells with NK-like (*KLRG1*$^+$), effector memory (EM1), and cytotoxic T-lymphocyte (CTL11) transcriptional profiles may be more abundant in relapsed/refractory (R/R) AML compared with in newly diagnosed AML.[68] The analysis of gene regulatory networks associated with the aforementioned T-cell clusters revealed high activity of *NFYB* and *STAT1* regulons, consistent with enhanced longevity and type I IFN production. The latter finding was also confirmed by in silico prediction of cytokine signaling activity.[68] In agreement with previous reports,[9] higher CD8 gene scores correlated with worse survival in treatment-naive TCGA AML cases.[68]

Finally, a high inflammation risk score has been associated with adverse-risk molecular features, expanded Treg cells and $GZMK^+CD8^+$ T cells, reduced event-free survival and OS, and a myeloid-like phenotype,[12] suggesting that inflammatory programs may be driven by AML blasts.[69]

T-cell immunotherapies

Although conserved TME subtypes have been shown to correlate with immunotherapy efficacy in multiple solid tumor types,[70] little is known concerning biomarkers of immunotherapy success and/or failure in AML (Table 3). A clinical trial of azacitidine and nivolumab reported an overall response rate of 33% vs 20% in historical controls treated on hypomethylating agent–based clinical trials, with pretherapy BM $CD3^+$ and $CD8^+$ T-cell frequencies being significantly associated with improved overall response rate.[72] Responses were largely measured in patients who had received only 1 or 2 lines of prior salvage therapy, suggesting that T-cell dysfunction associated with AML progression and/or extensive treatment with chemotherapy negatively affects response to CPIs. In nonresponders, Th17-like T cells coexpressing RORγt and inducible T-cell costimulator were expanded on treatment.

In the recent CP-MGD006-01 clinical trial, 47% (7 of 15) of patients with R/R AML and *TP53* abnormalities derived benefit from flotetuzumab immunotherapy and had a significantly higher tumor inflammation signature, *FOXP3*, and *CD8* gene expression scores at baseline compared with nonresponders,[35] likely reflecting an IFN-γ–driven TME.[7,78] In line with this observation, loss of *TP53* correlates with enhanced expression of immune checkpoints and effector T-cell–associated genes, including IFN-γ and CXCL9, in lung adenocarcinoma and with improved responses to CPI treatment.[79] In contrast, $TP53^{R172H}$ gain-of-function mutations in pancreatic tumors drive the recruitment of immunosuppressive $CD11b^+Ly6G^+$ neutrophils, which promote resistance to combination CD40 immunotherapy and chemotherapy.[80] Further studies should dissect the functional state of BM-infiltrating neutrophils in *TP53*-mutated AML, both in patients who are treatment naive and after immunotherapy, also in light of recent evidence supporting a key role for $Sell^{high}$ neutrophils in mediating response of lung cancer to T-cell–targeting approaches.[81] Intriguingly, anti-OX40 antibodies might elicit neutrophils with a distinct antitumorigenic, activated phenotype in mice with melanoma.[82]

CTLA-4 inhibition with ipilimumab in relapse of AML after hematopoietic stem cell transplantation (HSCT) and other hematological malignancies induces responses in approximately one-third of patients, with an acceptable safety profile.[71,76] Correlative analyses revealed upregulation of *CD8A*

Table 3. Immune correlatives of immunotherapy success and failure in AML

Study	Treatment	Disease	Number of patients, N	Immune correlates	Trial ID
Davids et al[71]	Ipilimumab	Post-HSCT relapse	28	Infiltration of cytotoxic $CD8^+$ T cells, decreased activation of Treg cells, and expansion of effector T cells in responders	NCT01822509
Daver et al[72]	Azacitidine + nivolumab	R/R AML	70	Pretherapy BM $CD3^+$ and $CD8^+$ T cells associate with improved ORR Th17 cell expansion in nonresponders	NCT02397720
Vadakekolathu et al[9] and Uy et al[10]	Flotetuzumab	R/R AML	88 (15 with *TP53* mutations)	TIS higher at baseline in responders	NCT02152956
Zeidner et al[73]	High-dose cytarabine + pembrolizumab	R/R AML	37	Higher frequency of $CD8^+$ T_{PEX} expressing TCF-1 at baseline in responders	NCT02768792
Abbas et al[37]	Azacitidine + nivolumab	R/R AML	8	Higher abundance of $CD8^+GZMK^+TCF7^+$ T cells in responders Loss of chr7/7q associated with NR	NCT02397720
Goswami et al[74]	Decitabine + pembrolizumab	R/R AML	10	irAEs linked to clonal expansions of $CD8^+PD1^+$ effector T cells	NCT02996474
Rutella et al[55]	Azacitidine + pembrolizumab	R/R AML	33	Dysfunctional NK-like $CD8^+$ T cells associated with NR to pembrolizumab	NCT02845297
Rimando et al[75]	Azacitidine + pembrolizumab High-dose cytarabine + pembrolizumab	Newly diagnosed and R/R AML	31 18	Differentiated blasts (promonocytic profile) expanded in responders	NCT02845297 NCT02768792
Garcia et al[76]	Decitabine + ipilimumab	R/R AML (after HSCT and HSCT naive)	54	Immune activation (irAEs) associated with survival benefit	NCT02890329
Penter et al[77]	Decitabine + ipilimumab	R/R AML (after HSCT and HSCT naive)	18	Altered $CD4^+$ T-cell gene expression after ipilimumab. Increased infiltration with antigen–experienced resident memory T cells in leukemia cutis samples from responders.	NCT02890329

irAEs, immune-related adverse events; NR, no response; ORR, overall response rate; TCF-1, T-cell factor 1; TIS, tumor inflammation signature.

and *PRF1* in tissue biopsies, decrease of circulating Treg cells, and increase of T-cell–attracting chemokines in association with clinical response. The Experimental Therapeutics Clinical Trials Network (ETCTN) 9204 trial demonstrated that ipilimumab can induce regression of post-HSCT relapsed AML, possibly through recruitment of cytotoxic $CD8^+$ T cells to leukemic sites.[71,77] However, immune-related adverse events, including 1 death, were observed in 6 patients (21%) and graft-versus-host disease precluding further administration of ipilimumab was documented in 4 patients (14%). Integrative transcriptomic analyses of clinical samples from the ETCTN/Cancer Therapy Evaluation Program (CTEP) 10026 study testing the combination of decitabine and ipilimumab for AML/myelodysplastic syndrome either after HSCT or in the HSCT-naive setting unveiled a strong association between a high baseline ratio of T-to-AML cells and response, and showed evidence of immune activation after ipilimumab exposure.[77] In responders, including patients with leukemia cutis, $CD8^+$ T cells were recruited to extramedullary sites,[83] which harbored *ZNF683*$^+$ antigen–experienced tissue-resident memory T cells. These findings suggest that ipilimumab may counteract the establishment of protective, antiapoptotic extramedullary AML niches.[1]

A first-in-human clinical trial of pembrolizumab and decitabine for R/R AML has identified changes in TCR sequences and immune transcriptomes of patients who develop immune-related adverse events.[74] Clonal expansions occurred at irAE onset and largely involved $CD8^+$ effector memory T cells with high expression of PD-1 and transcriptional features reflective of an activation/cytotoxic state. Another study leveraging paired scRNA-seq and TCR profiling of BM samples in R/R AML has documented TCR repertoire expansion and higher abundance of stem-like $CD8^+GZMK^+TCF7^+$ T cells in responders to azacitidine and nivolumab and in patients having a stable disease.[37] In contrast, lack of response to nivolumab was correlated with TCR repertoire contraction and with inferred loss of chr7/7q in the malignant cells. One patient who responded to immunotherapy had an expansion of mucosal-associated invariant T cells after treatment. Importantly, mucosal-associated invariant cytotoxic T lymphocytes displayed the highest fraction of expanded clones, suggesting that $CD8^+$ cells in AML can be effectively reinvigorated by CPIs. Interestingly, a Th1-like state in pretreatment BMs correlated with better outcomes, highlighting a previously unappreciated role for $CD4^+$ T cells in mediating response of AML to CPIs.[84] We showed that

IED multigene signatures encompassing CD8/NK markers correlate with resistance to pembrolizumab, pointing to T-cell exhaustion and senescence as targetable circuits to overcome immune dysfunction.[55] IED scores also correlated with shorter OS in patients with melanoma who received no previous systemic therapy, as well as with lack of response to CPI treatement,[55] suggesting that AML may share immunological hallmarks with solid tumor types.

Deconvolution of transcriptomic data from >1000 pediatric and adult patients with AML has allowed the establishment of a novel cellular hierarchy framework from single-cell reference profiles of leukemia stem, progenitor, and mature cell types.[85] Leukemia hierarchy composition varied across functional and genomic subtypes of AML and was associated with response to chemotherapy, survival, and drug sensitivity profiles of investigational targeted therapies. Genes predicting shorter survival were enriched for HSC-specific programs, whereas genes associated with longer survival were enriched for granulocyte-macrophage progenitor–specific programs. By relapse, malignancies in most patients were classified as primitive with significant expansion of total leukemia stem and progenitor cell populations and quiescent leukemia stem and progenitor cells. Recent observations indicate that AML stem cell hierarchies could also affect response to CPIs. Compared with nonresponders, patients achieving CR to pembrolizumab in combination with either azacitidine[86] or high-dose cytarabine[73] had higher inferred proportions of differentiated AML blasts, including cells with a promonocyte-like transcriptional profile.[75] Conversely, AML blasts from nonresponders had higher stemness scores at baseline. Finally, CPI efficacy could be improved by increasing NK recognition of AML targets. LSCs lack expression of NKG2D ligands, which is reversed by genetic or pharmacological inhibition of PARP1.[87,88] Importantly, a stimulatory DC–NK axis has been associated with melanoma response to CPIs and with prolonged OS.[89]

Chimeric antigen receptor (CAR) T cells

CAR T cells are genetically modified autologous T cells equipped with a synthetic antigen-binding domain and additional costimulatory domains, enabling MHC-independent target recognition.[90] In 2011, second-generation CAR T cells targeting CD19, which is expressed in B-cell malignancies, emerged as the lead paradigm for engineered T-cell therapies in cancer.[91] However, CAR T-cell therapy is challenging in AML, owing to lack of an ideal target and concerns over prolonged myelosuppression.[92] AML antigens, which are not significantly expressed on normal BM progenitor populations, such as B7-H3,[93] are being evaluated with the aim to minimize hematopoietic toxicity. A high-resolution single-cell expression approach has identified colony-stimulating factor 1 receptor and CD86 as candidate targets for CAR T-cell therapy, with minimal off-target toxicity in preclinical models of AML.[94]

Novel insights into the basis of clinical resistance to CAR T cells are surfacing. CAR T-cell exhaustion has been proposed as a mechanism of tumor escape in patients with large B-cell lymphoma.[95] Failure to achieve an early molecular response to CD19 CAR T cells has been associated with $CD8^+$ T-cell exhaustion and high frequency of $LAG3^+TIM3^+CAR^+CD8^+$ T cells.[96] Chronically stimulated mesothelin-directed $CD8^+$ CAR T cells upregulate exhaustion markers and acquire a dysfunctional phenotype with high levels of NK receptors and checkpoint molecules.[97] Furthermore, genes upregulated in CAR T cells collected on day 28 after infusion partially overlapped with genes expressed by intermediate and terminally exhausted T cells from LCMV-infected mice. Disruption of the transcription factors ID3 and SOX4 using CRISPR–CRISPR-associated protein 9 translated into a reduction in the frequency of dysfunctional NK-like T cells compared with wild-type cells and into enhanced tumor killing.[97] In this respect, in AML-bearing mice, pretreatment of AML cells with hypomethylating agents has been shown to augment CD123 expression and to expand CTLA-4^{neg} anti-CD123 CAR T cells with superior cytotoxic activity.[98] This study suggests that epigenetic modifiers could be combined with CD123-targeting CAR T cells for AML treatment.

Immune landscape of AML relapsing after allogeneic HSCT

Treatment of relapse of AML after HSCT remains a challenge. Only 20% of patients who respond to further chemotherapy achieve long-term remissions with a second HSCT or with chemotherapy followed by donor lymphocyte infusions.[3] AML blasts at relapse express low/undetectable MHC-II, ultimately favoring immune escape.[99-101] In this respect, flotetuzumab may reactivate alloreactive T cells by upregulating MHC-II on AML cells through local release of IFN-γ.[102] Inhibitory receptors (IRs) and other immune-related genes are more highly expressed on purified AML blasts from patients with post-HSCT relapse compared with those from patients with post-chemotherapy relapse,[101] highlighting the potential for CPIs to reinvigorate T cells and restore beneficial anti-AML immune responses also in this setting, as further discussed hereafter. In patients with AML relapsing after a long phase of post-HSCT clinical remission, functionally impaired but leukemia-reactive $CD8^+$ T cells expressing IRs CTLA-4 and TIM-3 accumulate in the TME.[103] Importantly, the exhausted phenotype of IR^+ T cells could be partly reverted by in vitro exposure to high doses of IL-2, with T cells recovering their polyfunctionality (IFN-γ and TNF-α secretion) but not the ability to produce IL-2.[103]

It is now established that tumor cells impose metabolic constraints on T cells through glucose depletion and generation of large amounts of lactate and other immunosuppressive byproducts in the TME.[104] In this respect, blood T cells isolated from patients with post-HSCT relapse of AML exhibit reduced glycolysis and IFN-γ production compared with matched specimens collected at time of disease remission.[105] AML-derived lactic acid negatively affected T-cell–mediated control of AML in a humanized MOLM-13 xenograft model. T-cell dysfunction was reversed after in vitro treatment of $CD8^+$ T cells with sodium bicarbonate.[105] A synergism between sorafenib and donor lymphocyte infusions has been documented in patients with *FLT3*-ITD^+ AML who relapse after HSCT.[106] Serum IL-15 and IFN-γ levels, as well as blast *IL15* and *IRF7* messenger RNA, were increased in patients who achieved a complete hematological remission. Concomitantly, metabolic rewiring of blood $CD8^+$ T cells was documented.[106] This study suggests that sorafenib might promote a strong graft-versus-AML effect in patients with *FLT3*-ITD^+ AML relapse after HSCT, who would otherwise have a dismal prognosis. Finally, machine learning–informed analyses of longitudinal, paired BM samples have

revealed novel intriguing associations between proinflammatory signaling and AML progression, including overexpression of CD6, which encodes a lymphoid–associated surface glycoprotein involved in immune synapse formation, in adult patients at time of relapse.[107]

Conclusions and perspectives

CPI therapy has been particularly challenging in AML. However, recent advances in multimodal omics technologies have considerably deepened our knowledge of the dynamic cellular interactions at the AML immune interface, highlighting both AML-associated and conserved, pan-cancer pathways that could be modified to overcome immune resistance. We propose that immune gene expression profiling be integrated with clinically validated prognosticators, including ELN risk category, 17-gene LSC score, and molecular lesions (*TP53*, *RUNX1*, *IDH1/2*, and *TET2* mutational status), to accurately stratify AML into subgroups with substantially different survival probabilities (Figure 1). Patients with an IFN-γ–dominant, immune-enriched TME could be allocated immunotherapies that aim to reinvigorate dysfunctional T cells, including T-cell engagers and CPIs. Conversely, patients with a "cold," immune-depleted transcriptional profile could benefit from other therapeutic strategies, such as increasing T-cell trafficking to the BM TME and/or vaccines, adoptive T-cell transfer, and allogeneic HSCT. Immunotherapies targeting immunological hallmarks, such as type I (tumor-cell intrinsic) and type II (immune-cell intrinsic) IFN signaling, and combinatorial approaches incorporating CPIs and molecularly targeted agents that might enhance T-cell–mediated anti-AML activity such as venetoclax[108,109] should be explored in future AML clinical trials. It will also be essential to identify and validate immune correlates of response, resistance, and toxicity in patients with AML who are treated with CAR T cells, to devise strategies to antagonize CAR T-cell exhaustion and senescence, and to neutralize metabolic barriers in the TME.[110]

The observation that chemotherapy reduces the expression of T-cell checkpoints[111] raises important questions regarding optimal timing of immunotherapy and leads to the conjecture that CPIs could be more effective as a first-line treatment option rather than after multiagent chemotherapy and/or HSCT. Recent data showing an increase of IED scores after cytotoxic chemotherapy, irrespective of the achievement of CR,[55] lend additional credence to the concept that immunotherapy, either alone or in combination with orthogonal cytoreduction strategies,[112] could yield superior results if patients were treated earlier in their disease course rather than in the R/R setting. Further research is needed to elucidate whether chemotherapy-induced changes of the immune TME, including heightened inflammation and senescence, affect response to CPIs and other immunotherapeutic modalities. Studies addressing the intricate balance between antitumor immunity and type I/II IFN signaling, and whether an epigenetically poised state of responsiveness to IFN-I favors AML resistance to CPIs by constraining T-cell function, are also warranted.

In conclusion, broader adoption of systems-level approaches, including spatial transcriptomics,[113] underpinned by machine learning and advanced computational pipelines,[114] and responsible sharing of immunotherapy clinical trial and biomarker data[115] will shed further light onto T-cell functional states and their coordinated changes under immune selection pressure in the AML TME. The collection of pretreatment, on-treatment, and postprogression BM samples should complement clinical efforts, because the availability of longitudinal specimens would enable high-dimensional analyses of response in relation to immune evolutionary dynamics, and potentially suggest personalized treatment options.

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Footnote

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