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Search for leukemia-restricted targets: a new paradigm for precision immunotherapy in acute myeloid leukemia

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Abstract

Acute myeloid leukemia (AML) remains a major therapeutic challenge despite remarkable advances in genomic characterization. Although molecular profiling has transformed disease classification and risk stratification, treatment for most patients continues to rely on cytotoxic chemotherapy regimens developed more than 50 years ago. Numerous attempts to improve outcomes through treatment intensification, alternative chemotherapy approaches, and targeted agents have produced incremental benefits for selected patient populations, but have failed to fundamentally alter outcomes for many high-risk AML subtypes. A major obstacle to the broader application of immunotherapy in AML has been the absence of targets that clearly distinguish leukemic cells from normal hematopoietic tissues. Consequently, most immune-based therapies have focused on AML-associated antigens such as CD33, CD123, and CLEC12A, resulting in a narrow therapeutic window and significant hematopoietic toxicity.

Recent advances in large-scale transcriptomic profiling, integrated genomic analyses, and immunopeptidomics have enabled systematic identification of AML-restricted biomarkers linked to leukemia-defining oncogenic programs. Rather than seeking a universal AML antigen, these approaches have uncovered subtype-specific targets, including FOLR1 in *CBFA2T3::GLIS2* AML, CLEC2A in *KMT2A*-rearranged AML, mesothelin or CD7 in selected high-risk AML subsets, and intracellular targets such as WT1, PRAME, NPM1 neoantigens, and fusion-derived peptides. These discoveries have created opportunities for immunotherapeutic approaches capable of selectively targeting leukemic cells, while preserving normal hematopoiesis.

This Spotlight Review discusses the evolution of AML biomarker discovery from prognostic classification to therapeutic target identification and highlights emerging AML-restricted biomarkers that may enable a new generation of biologically-precise immunotherapies for molecularly defined AML subsets.

Introduction

Acute myeloid leukemia (AML) is a biologically heterogeneous malignancy characterized by diverse genetic, epigenetic, transcriptional, and developmental abnormalities that collectively influence leukemogenesis, treatment resistance, and clinical outcome.(1) Recent advances in genomic profiling have transformed our understanding of AML biology, leading to increasingly refined disease classification systems and the recognition of numerous molecularly defined subtypes with distinct biology, disease evolution, and prognostic significance. Despite this growing knowledge of AML as a galaxy of biologically distinct diseases rather than a single entity, therapeutic development has consistently ignored this intrinsic heterogeneity, seeking broadly applicable treatments aimed at eradicating all AML subtypes. As a result, most patients continue to receive similar therapies regardless of the underlying biology of their disease, and improvements in long-term outcomes have been limited. This disconnect between increasingly precise biological classification, and relatively non-selective therapeutic intervention remains one of the central challenges in AML treatment and highlights the need for disease-specific therapeutic vulnerabilities that can be exploited with greater precision.

One of the most striking observations in AML treatment is the persistence of standard treatment regimens developed more than half a century ago.(2) The combination of cytarabine and daunorubicin, commonly referred to as "7+3," remains the backbone of induction therapy despite decades of clinical investigation. Numerous randomized studies have evaluated intensified chemotherapy schedules, alternative anthracyclines such as idarubicin or mitoxantrone, novel cytotoxic agents, and targeted therapies to improve outcomes (Supplemental Table 1). While some have demonstrated benefit in defined patient populations, most phase III trials have failed to produce meaningful improvements in survival. This challenge has been observed across both adult and pediatric AML trials. Large cooperative group studies, including adult MRC, SWOG, ECOG and pediatric studies such as St. Jude and COG trials have failed to demonstrate improvements over established standard therapies – Supplemental Table 1. Collectively, these experiences underscore a central challenge in AML therapeutics: improvements in treatment intensity alone have not been sufficient to overcome the biologic complexity of the disease.

More striking is the observation that delivering targeted therapies in a non-directed fashion may lead to dilution and loss of efficacy signal. Three consecutive phase III trials enrolling nearly 4000 adults (MRC 15, SWOG S0106) and children (COG AAML0531), designed to evaluate impact of gemtuzumab-ozogamicin (GO), a CD33-directed antibody-drug conjugate (ADC), failed to demonstrate survival benefit to the addition of the GO to conventional therapy (Supplemental Table 1).(3-5) Subsequent analysis demonstrated that specific subsets of patients, including those with high CD33 expression or those with specific CD33 polymorphisms benefited from GO.(6-10) Notwithstanding this information, GO is still being delivered in a non-targeted fashion. This non-targeted delivery of targeted, cytotoxic therapies increases the risk of toxicity and dilutes the efficacy in the target-positive patients.

Historically, most approaches of cellular immunotherapy in AML have focused on **AML-associated** antigens, including CD33, CD123 (IL3RA), FLT3, and other lineage-related markers that are expressed on leukemic cells, but also on normal hematopoiesis. Although these targets have demonstrated some clinical activity, their expression on healthy progenitors inherently limits

therapeutic selectivity and contributes to toxicity. As increasingly potent immunotherapeutic platforms, including chimeric antigen receptor (CAR)-T cells have emerged, the limitations of AML-associated targets have become increasingly apparent. Evaluation of expression of these targets in AML vs. normal bone marrow (NBM) and CD34+ peripheral blood stem cells (PBSC) demonstrate substantial overlap of expression of these genes in AML and in normal cells (Figure 1). Clinical experience with CAR-T cells directed against AML-associated antigens including CD33, CD123, CLEC12A, and FLT3 has demonstrated the feasibility of immune targeting in AML, but has also highlighted the relevant limitations of lineage-associated antigens. Although occasional objective responses have been observed, durable remissions remain uncommon, and target expression on normal hematopoietic progenitors creates a narrow therapeutic window that may result in prolonged cytopenias, myeloablation, and high treatment-related toxicity (Figure 2). Collectively, these studies underscore the need for AML-restricted biomarkers, capable of sparing normal hematopoiesis while maintaining potent antileukemic activity.(11-14)

Recent advances in transcriptomic and molecular profiling technologies have provided the opportunity for an alternative paradigm. Rather than seeking broadly expressed lineage markers, investigators are increasingly focused on identifying AML-restricted biomarkers that distinguish leukemic cells from normal hematopoiesis and, ideally, from normal tissues more broadly (Figure 2). Importantly, many of these potential targets are associated with specific molecular subtypes and appear to be directly linked to leukemia-defining oncogenic programs. This observation suggests that the future of AML immunotherapy may not depend upon identifying a single universal AML antigen, but rather upon exploiting subtype-specific, therapeutic vulnerabilities.

In this Spotlight Review, we discuss the emergence of AML-restricted biomarkers as a new framework for precision immunotherapy. We review strategies used to identify AML-restricted targets, summarize emerging surface and intracellular biomarkers, and discuss the therapeutic platforms that are enabling their clinical translation. Collectively, these advances support a transition from lineage-directed targeting toward biologically precise immunotherapies tailored to molecularly defined AML subsets.

Discovery of AML-Restricted Biomarkers

The success of precision oncology has been driven largely by the identification of molecular abnormalities that distinguish malignant cells from their normal counterparts. These discoveries have revolutionized disease classification and risk stratification, but translating biologic insights into effective immunotherapeutic targets has proven considerably more challenging. Unlike B-cell malignancies, where targets such as CD19 are functionally dispensable and provide a favorable therapeutic window, AML lacks universal lineage-specific antigens that can be safely eliminated without significant clinical consequences. Consequently, the identification of biomarkers that distinguish leukemic cells from normal hematopoietic tissues has become a central objective of AML immunotherapy research. Historically, target discovery in AML relied primarily on candidate-based approaches where genes were selected based on biologic plausibility, or differential expression. While these studies identified clinically relevant targets such as CD33 and CD123, they were inherently limited by prioritization of prevalence over specificity. As a result, many candidate targets ultimately demonstrated substantial expression in healthy hematopoietic tissues, restricting their therapeutic applicability (Figure 2). Further, for immunotherapy, broad

expression of targets provides continuous and consistent target exposure leading to enhanced clinical symptoms and rapid effector cell exhaustion. Advances in large-scale genomic profiling of tumor and its normal counterpart have enabled a fundamentally different strategy, permitting unbiased interrogation of the AML transcriptome to identify biomarkers uniquely expressed in the malignant tissue. Such approaches are particularly attractive in AML because of the remarkable molecular heterogeneity of the disease and the increasing recognition that therapeutically actionable targets may be restricted to specific biologic subtypes.

To address this challenge, systematic discovery approaches have been developed leveraging large transcriptomic datasets (15-19), encompassing RNA sequencing data from more than 3,000 infants, children, adolescents, and adults representing a broad spectrum of cytogenetic, molecular, and clinical subtypes. A distinguishing feature of these approaches has been the use of normal hematopoiesis as the primary biologic filter. Candidate biomarkers are evaluated against extensive reference datasets including NBM, purified CD34-positive hematopoietic stem and progenitor cells, cord blood, peripheral blood, and hematopoietic populations spanning multiple stages of normal hematopoiesis. This strategy is designed to identify genes that are highly expressed in AML, while transcriptionally silent throughout normal hematopoiesis. Because destruction of normal hematopoietic stem and progenitor cells remains one of the principal toxicities associated with AML-directed immunotherapies, absence of expression within normal hematopoietic compartments represents a critical prerequisite for therapeutic target selection. Application of this framework has led to the identification of a library of **AML-restricted** biomarkers characterized by high expression in leukemic cells and little to no expression in normal hematopoietic tissues (Figure 2). Expression of several of these targets have subsequently been validated at the protein level and have advanced from discovery and preclinical validation (Figure 3) to clinical development and are discussed below.

Candidate genes identified through this initial filtering process were subsequently evaluated against large-scale, normal tissue expression resources, including the Genotype-Tissue Expression (GTEx) database. This second stage of analysis enabled systematic assessment of expression across a broad range of normal organs and tissue types and facilitated elimination of targets with substantial expression in critical non-hematopoietic tissues (Figure 4). Together, hematopoietic and non-hematopoietic filtering established a stringent framework for identifying biomarkers with the potential to provide a meaningful therapeutic window. Importantly, target discovery was performed not only across all AML, but also within molecularly defined disease subsets. This distinction proved to be critical, since analyses performed across aggregate AML populations may obscure highly selective biomarkers associated with individual leukemia subtypes.

Subtype-focused analyses revealed that some of the most promising therapeutic targets were not broadly expressed across AML, but, rather, were instead tightly linked to specific oncogenic programs. This observation represents an important conceptual shift in AML target discovery. The therapeutic value of a biomarker is determined not simply by prevalence, but by the combination of prevalence, specificity, expression intensity, stability, and biologic relevance. A highly selective target expressed in a molecularly defined subset may ultimately possess greater therapeutic value than a broadly expressed lineage marker if it provides a substantially wider therapeutic window.

Application of this discovery framework identified a growing repertoire of AML-restricted biomarkers, including FOLR1 in *CBFA2T3::GLIS2* AML,(20) CLEC2A in *KMT2A*-rearranged and monosomy 7 AML,(21) mesothelin in selected high-risk AML populations,(22, 23) and multiple intracellular targets including WT1,(24, 25) PRAME,(25, 26) NPM1-derived neoantigens, and fusion-derived peptides (Figure 2). Importantly, many of these biomarkers demonstrated expression patterns considerably more selective than conventional AML-associated antigens, while remaining linked to biologically important leukemia programs. These observations suggest that the future of AML immunotherapy may not depend upon identifying a universal AML antigen. Instead, a more effective strategy may involve the systematic identification of subtype-specific AML-restricted biomarkers that collectively cover the molecular diversity of AML, while maintaining substantially greater therapeutic selectivity. This framework shifts target discovery from a prevalence-driven paradigm toward a precision oncology paradigm in which biologic specificity is prioritized over disease-wide applicability.

Prior systematic efforts to identify suitable AML-directed CAR targets have largely sought antigens broadly expressed across AML rather than exploiting the molecular heterogeneity of the disease. In this regard, Perna et al. integrated transcriptomic and surface-proteomic datasets and evaluated 24 candidates in 30 primary AML specimens, ultimately prioritizing the transmembrane proteins ADGRE2, CCR1, CD70, and LILRB2. Although relatively enriched in AML compared with hematopoietic stem/progenitor cells, with the exception of CD70, that is highly enriched in KMT2A-rearranged AML, none is AML-restricted, as these molecules are physiologically expressed in normal myeloid and/or other immune-cell compartments, leading the investigators to conclude that no single target exhibited a CD19-like expression profile and to pursue combinatorial targeting instead.(27) More recently, Gottschlich et al. used single-cell transcriptomic data from 15 patients with AML together with a large normal-cell atlas to identify the transmembrane receptors CSF1R and CD86 as candidate CAR targets.(28) While providing greater cellular resolution, these targets likewise are not leukemia-restricted—CSF1R is physiologically expressed within the monocyte/macrophage lineage and CD86 on antigen-presenting immune cells. In contrast, our strategy does not require an antigen to be broadly expressed across AML; rather, it interrogates molecularly defined AML subsets to identify cell-surface proteins that are either absent or nearly absent from normal hematopoiesis, but highly and consistently expressed within a genetically defined leukemia subtype. This molecularly resolved approach converts AML heterogeneity from an obstacle to target discovery into an opportunity for precision immunotherapy.

The sections that follow highlight representative AML-restricted surface and intracellular biomarkers identified through this approach and illustrate how these discoveries are enabling the development of increasingly precise immunotherapeutic strategies for AML. Supplemental Table 2 provides details of AML-associated and AML-restricted biomarkers and available published data.

AML-Restricted Surface Biomarkers in Clinical Development

Cell-surface antigens remain the most immediately actionable class of therapeutic targets, because they are directly accessible to antibodies, ADCs, cellular therapies, and immune-engaging platforms. Examples of these targeted immunotherapy approaches are:

FOLR1 in *CBFA2T3::GLIS2* AML: Folate receptor alpha (FOLR1) represents one of the most compelling examples of subtype-specific target discovery in AML. Identified through transcriptomic analyses of pediatric AML cohorts, FOLR1 is highly and homogeneously expressed in AML harboring the *CBFA2T3::GLIS2* fusion gene, a high-risk leukemia subtype that occurs in infants and very young children and is associated with poor clinical outcomes.(29)

Importantly, FOLR1 expression is absent across normal hematopoiesis and demonstrates limited expression outside of known folate receptor-positive tissues, providing a potentially favorable therapeutic window. Functional studies demonstrated that FOLR1 expression is closely linked to the fusion-driven transcriptional program and is maintained across leukemic blast and progenitor populations. These findings established FOLR1 as an attractive candidate for immune-based targeting. The translational development of FOLR1 has been accelerated by extensive prior experience targeting this antigen in solid tumors. Monoclonal antibodies, and ADCs developed for ovarian carcinoma and other epithelial malignancies, have provided validated reagents and clinical experience that facilitated rapid adaptation to AML.(30) The FOLR1-directed ADC, Luveltamab Tazavibulin (LT), has shown significant efficacy in infants with *CBFA2T3::GLIS2* AML. FOLR1-directed CAR-T cells have demonstrated potent antileukemic activity in preclinical models and have advanced into early-phase clinical evaluation (NCT06609928), representing one of the first examples of a molecularly-defined AML immunotherapy developed directly from this systematic biomarker discovery.(31, 32) This trial is in active recruitment stage, 8 patients having been treated with promising results.

CLEC2A in *KMT2A*-Rearranged AML: CLEC2A provides a second example of how subtype-focused target discovery can reveal highly selective therapeutic vulnerabilities. Identified through analyses of molecularly defined AML cohorts, CLEC2A demonstrates selective enrichment in *KMT2A*-rearranged AML, an aggressive leukemia subtype associated with poor outcomes despite intensive therapy. Monosomy 7 AML is another subset where CLEC2A is specifically enriched.(21, 33-35) Unlike many conventional AML antigens, CLEC2A is entirely absent in normal hematopoiesis. Furthermore, emerging evidence suggests that CLEC2A expression is mechanistically linked to KMT2A fusion-driven transcriptional programs, raising the possibility that target expression may be maintained throughout disease evolution and less susceptible to antigen-loss escape. The favorable expression profile of CLEC2A has supported development of multiple therapeutic modalities, including CAR-T cells, ADCs, and T-cell-engaging platforms. Collectively, these studies further illustrate how biomarkers directly connected to leukemia-defining biology may provide a superior therapeutic index compared with broadly expressed lineage markers. Currently, CLEC2A-directed CARTs have advanced into early phase trials in AML in US and in Italy (Meshinchi and Locatelli personal communication).

Mesothelin in High-Risk AML: Mesothelin (MSLN) highlights a complementary strategy in which targets originally developed for solid tumors are repurposed for AML. Mesothelin is a glycosylphosphatidylinositol-anchored surface protein normally expressed at low levels on mesothelial tissues and highly expressed across multiple epithelial malignancies. Transcriptomic analyses subsequently identified mesothelin expression in selected high-risk AML populations, while demonstrating minimal expression throughout normal hematopoiesis. Mesothelin-directed CARTs are in clinical development in AML with clinical trial activation is expected in late 2026.

CD7-targeting CAR T cells with a protein expression blocker (PEBL) to avoid fratricide among CAR T cells has shown promising efficacy in T-cell acute lymphoblastic leukemia.(36) CD7 has been reported to be aberrantly expressed on AML cells in approximately 15% of pediatric patients with AML.(37) Very recently, 3 young patients with CD7pos relapsed/resistant AML have been reported to have obtained complete remission with MRD negativity after treatment with PEBL CD7-CAR T cells.(38) Therefore, this approach may represent a valuable option to be further validated in formal clinical trials in those 15% of patients whose leukemia blasts express CD7 in ≥98% leukemia blasts.

CD276/B7-H3 in AML: B7-H3 (CD276) represents another promising example of a target initially developed in solid tumors that may be repurposed for AML immunotherapy. B7-H3 is a member of the B7 family of immune regulatory molecules and is broadly overexpressed across a wide range of pediatric and adult solid tumors, where its expression has been associated with immune evasion, disease progression, and adverse clinical outcomes. Importantly, transcriptomic and proteomic studies have identified B7-H3 expression in subsets of AML, while demonstrating limited expression within normal hematopoietic stem and progenitor cell compartments, supporting its potential as a therapeutic target in myeloid malignancies (Locatelli and Meshinchi personal communication). Given its advance stage of clinical development in solid tumors, this CART-target is advancing to clinical trials in AML.

There is a library of recently discovered cell surface AML-restricted targets at various stages of pre-clinical development. Among these, thymic stromal lymphopoietin receptor (TSLPR; encoded by CRLF2), and lysosome-associated membrane protein 5 (LAMP5) have shown high expression in subsets of high-risk AML with promising results in preclinical AML models. (39-41) For all these targets, clinical trials will allow to prove the absence of their extra-medullary toxicity and their therapeutic efficacy.

AML-Restricted Intracellular Biomarkers

While surface antigens remain the most accessible therapeutic targets, many of the most biologically relevant and leukemia-specific biomarkers are intracellular proteins, including cancer-testis antigens, mutant proteins, fusion oncoproteins, transcription factors, and developmental regulators. Historically, these proteins were inaccessible to conventional antibody-based therapies. However, advances in T-cell receptor (TCR)-engineered therapies, TCR-mimic technologies, and immunopeptidomics have dramatically expanded the range of therapeutically actionable intracellular targets. By providing direct access to the molecular machinery that drives leukemogenesis, intracellular biomarkers often offer greater biologic relevance and disease specificity than conventional lineage-associated antigens.

WT1: A Foundational Intracellular Target: Wilms Tumor-1 (WT1) remains one of the most extensively studied intracellular immunotherapy targets in AML. WT1 is overexpressed in the majority of AML cases, is frequently maintained throughout disease progression and relapse, and has long been utilized as a biomarker for measurable residual disease.(42) Although not strictly leukemia-specific, WT1 expression in AML is substantially higher than in normal tissues and spans multiple molecular subtypes, providing broad applicability. WT1-derived peptides are naturally processed and presented by HLA molecules and were among the first intracellular

leukemia antigens successfully targeted using peptide vaccines and TCR-based therapies. These studies established critical proof-of-concept that intracellular leukemia antigens could be recognized and therapeutically exploited through HLA-restricted immune approaches. More recently, high-affinity WT1-specific TCRs have been incorporated into engineered cellular therapies with encouraging early clinical activity (NCT00153582, and NCT00398138).(43) Consequently, WT1 remains an important benchmark against which newer intracellular immunotherapies are measured.

PRAME: A Prototype AML-Restricted Intracellular Target: Preferentially Expressed Antigen in Melanoma (PRAME) is a cancer-testis antigen that exemplifies the next generation of intracellular immunotherapy targets. Unlike WT1, PRAME is largely absent from normal adult tissues, but is aberrantly expressed across multiple AML subtypes, including leukemic stem and progenitor populations. PRAME-derived peptides are naturally processed and presented by selected HLA molecules, and spontaneous PRAME-specific T-cell responses have been identified in patients with AML and other malignancies. These features have made PRAME one of the most clinically advanced intracellular immunotherapy programs currently under development, with multiple TCR-engineered and TCR-mimic approaches entering clinical evaluation (NCT02743611, and NCT06383572). More broadly, PRAME demonstrates how intracellular leukemia-associated antigens can be targeted with substantially greater specificity than conventional lineage-associated markers, providing a framework for the development of increasingly precise immunotherapies directed against neoantigens and fusion-derived peptides.

Multi-Antigen Targeting Strategies

A potential approach to overcoming immunotherapy antigen escape involves simultaneous targeting of multiple biomarkers. Advances in cellular engineering and antibody design now permit development of dual-targeting CARs, multispecific engagers, and combinatorial targeting approaches capable of recognizing more than one antigen simultaneously. AML-restricted biomarker discovery has generated a growing portfolio of candidate targets that may be combined rationally according to disease biology

Conclusions and Future Directions

The history of AML immunotherapy has been constrained by the absence of targets capable of distinguishing leukemic cells from normal hematopoietic tissues. As a result, most immune-based therapies have relied on lineage-associated antigens that provide limited therapeutic selectivity and are frequently associated with hematopoietic toxicity. The emergence of AML-restricted biomarkers represents a fundamental shift in therapeutic strategy. Systematic transcriptomic, genomic, and immunopeptidomic approaches have identified a growing repertoire of surface and intracellular targets linked to leukemia-defining oncogenic programs and molecularly distinct AML subsets. These discoveries have expanded the range of actionable vulnerabilities and enabled increasingly precise application of cellular therapies, antibody-based platforms, and TCR-directed approaches. Importantly, the future of AML immunotherapy may depend less on identifying a universal AML antigen and more on developing a portfolio of subtype-specific biomarkers that collectively address the molecular diversity of AML. As therapeutic platforms continue to evolve and additional AML-restricted targets are identified, biologically precise immunotherapy has the

potential to transform AML treatment from broadly applied lineage-directed therapy to molecularly tailored approaches capable of improving efficacy while preserving normal hematopoiesis.

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Figure Legends:

Figure 1. **Transcript expression of AML-associated genes in AML and in single cell fractions of normal hematopoietic cells.** A: Expression of AML-associated targets in AML, normal bone marrow (NBM) and CD34+ peripheral blood stem cells (PBSC). B: Single cell expression of AML associated targets in varied fractions from normal peripheral blood mononuclear cells (PBMC).

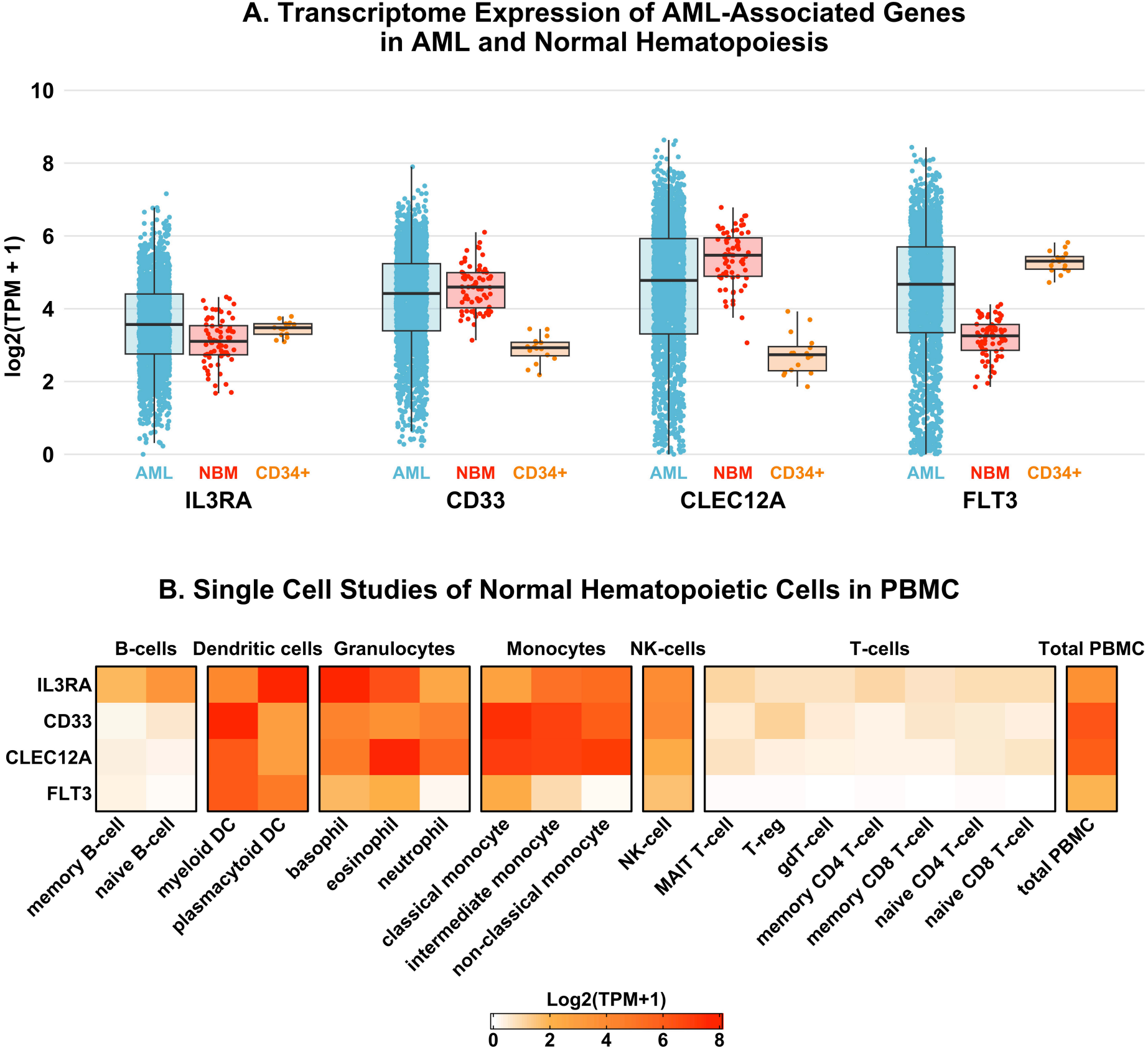
AML data extracted from TCGA, BeatAML and Target Pediatric AML data sets located in the Database of Genotype and Phenotype (dbGaP);https://dbgap.ncbi.nlm.nih.gov/ (TCGA AML: phs000178.v11.p8; BEAT AML: phs001657.v3.p1; TARGET AML: phs000465.v23.p8). Single cell data obtained from Human Protein Atlas (HPA) site https://www.proteinatlas.org/humanproteome/single+cell/single+cell+type.

Figure 2. **AML-associated and AML-restricted antigens and corresponding immunotherapeutic platforms.** AML-associated antigens (blue) are expressed on leukemic blasts, but are also present on normal hematopoietic cells, whereas AML-restricted antigens (red) demonstrate absent or highly limited expression in normal hematopoiesis and may be enriched within molecularly defined AML subsets. Surface antigens can be targeted by CAR-T cells, antibodies, antibody-drug conjugates (ADCs), bispecific engagers, and NK-cell platforms, whereas intracellular antigens can be targeted through peptide–HLA recognition using TCR-based or TCR-mimic approaches.

Figure 3. **Transcript expression of AML-restricted genes in AML and in single cell fractions of normal hematopoietic cells.** A: Expression of AML-restricted targets in AML, NBM and CD34+ PBSC. B: Single cell expression of AML-restricted targets in varied fractions from normal PBMC.

AML data extracted from TCGA, BeatAML and Target Pediatric AML data sets located in the Database of Genotype and Phenotype (dbGaP);https://dbgap.ncbi.nlm.nih.gov/ (TCGA AML: phs000178.v11.p8; BEAT AML: phs001657.v3.p1; TARGET AML: phs000465.v23.p8). Single cell data obtained from Human Protein Atlas (HPA) site https://www.proteinatlas.org/humanproteome/single+cell/single+cell+type.

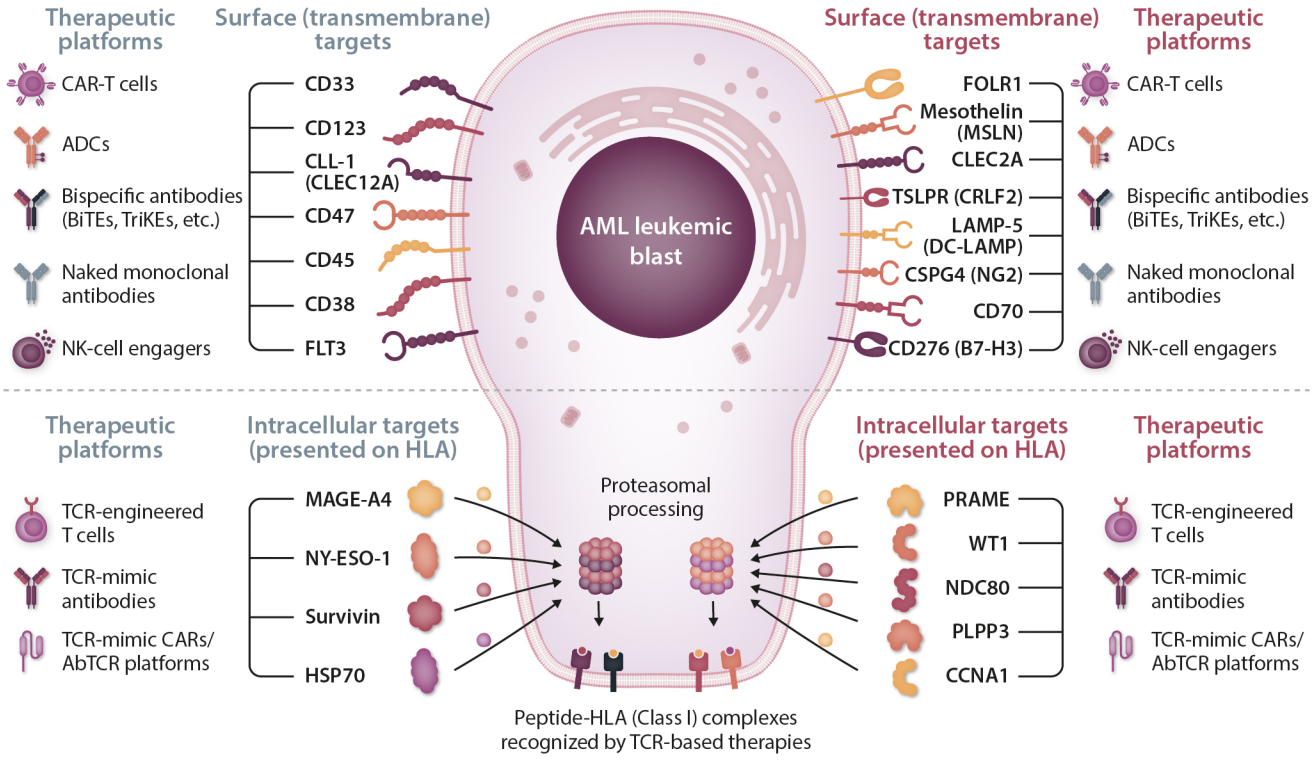
Figure 4. **Expression of therapeutic targets in normal tissues.** GTEx data for expression of AML-associated targets (upper panel) and AML-restricted targets (lower panel) in varied normal tissues. Data obtained from The Genotype-Tissue Expression (GTEx) Portal.


A. Transcriptome Expression of AML-Associated Genes in AML and Normal Hematopoiesis
log2(TPM + 1)
10
8
6
4
2
0
AML
NBM
CD34+
IL3RA
CD33
CLEC12A
FLT3
B. Single Cell Studies of Normal Hematopoietic Cells in PBMC
B-cells
Dendritic cells
Granulocytes
Monocytes
NK-cells
T-cells
Total PBMC
IL3RA
CD33
CLEC12A
FLT3
memory B-cell
naive B-cell
myeloid DC
plasmacytoid DC
basophil
eosinophil
neutrophil
classical monocyte
intermediate monocyte
non-classical monocyte
NK-cell
MAIT T-cell
T-reg
gdT-cell
memory CD4 T-cell
memory CD8 T-cell
naive CD4 T-cell
naive CD8 T-cell
total PBMC
Log2(TPM+1)
0
2
4
6
8

AML-associated and AML-restricted antigens and corresponding immunotherapeutic platforms

AML-associated antigens
Expressed on leukemic blasts and normal hematopoietic cells (variable density)

AML-restricted antigens
Absent or highly limited expression in normal hematopoietic and non-hematopoietic tissues



AML-associated antigens:
Target expression may be present on normal hematopoietic cells (variable density). On-target, off-tumor toxicity may occur.

AML-restricted antigens:
Absent or highly limited expression in normal hematopoiesis and non-hematopoietic tissues. Potential for improved therapeutic index.

CAR-T cells:
Chimeric antigen receptor T cells

ADCs:
Antibody-drug conjugates

Bispecific Engagers:
T cells or NK cells to tumor antigens

Naked monoclonal antibodies:
Direct effector functions (antibody-dependent cellular cytotoxicity [ADCC], complement-dependent cytotoxicity [CDC], and targeted blockade)

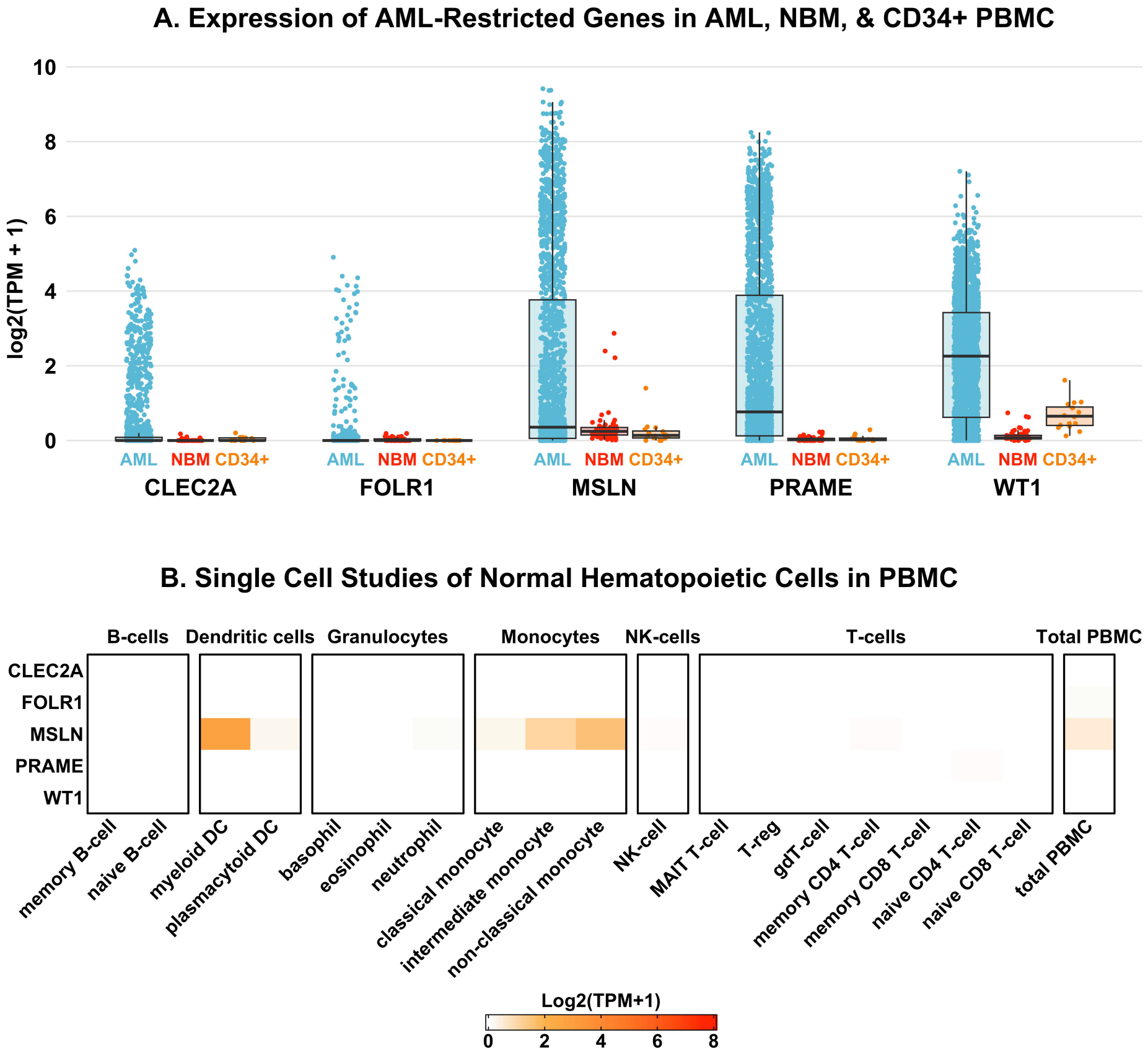
NK-cell engagers:
Activate NK cells against target-expressing blasts

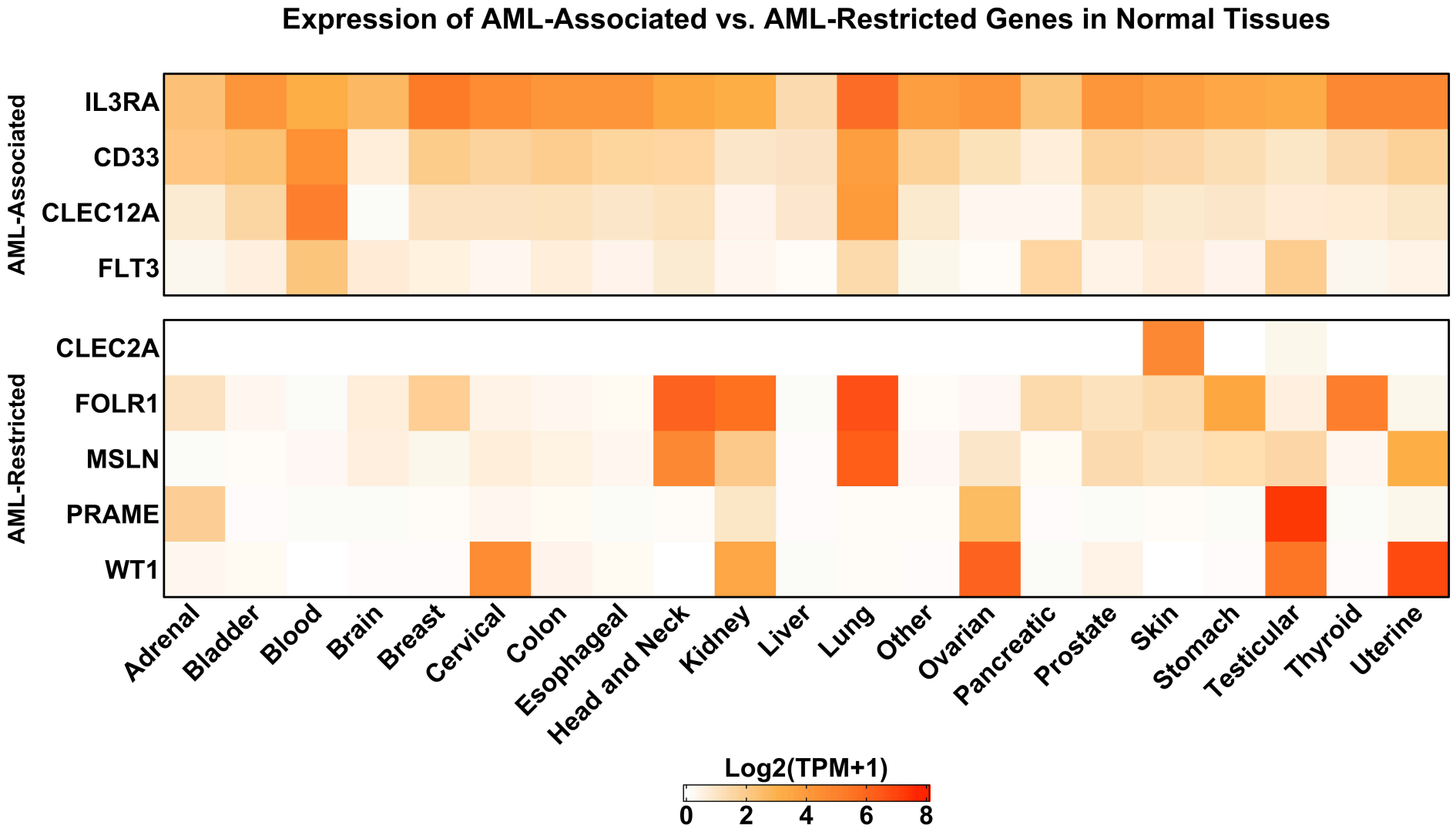
TCR-engineered T cells:
Recognize peptide HLA complexes

TCR-mimic antibodies:
Recognize peptide HLA complexes

TCR-mimic CARs/ATCR platforms: Target peptide-HLA complexes

Antigen expression is often restricted to specific AML subtypes or molecular contexts (e.g., KMTA-r, CBF-AML, RAM, NUP98-r).


A. Expression of AML-Restricted Genes in AML, NBM, & CD34+ PBMC
log2(TPM + 1)
10
8
6
4
2
0
AML NBM CD34+
CLEC2A
FOLR1
MSLN
PRAME
WT1
B. Single Cell Studies of Normal Hematopoietic Cells in PBMC
B-cells
Dendritic cells
Granulocytes
Monocytes
NK-cells
T-cells
Total PBMC
CLEC2A
FOLR1
MSLN
PRAME
WT1
memory B-cell
naive B-cell
myeloid DC
plasmacytoid DC
basophil
eosinophil
neutrophil
classical monocyte
intermediate monocyte
non-classical monocyte
NK-cell
MAIT T-cell
T-reg
gdT-cell
memory CD4 T-cell
memory CD8 T-cell
naive CD4 T-cell
naive CD8 T-cell
total PBMC
Log2(TPM+1)
0
2
4
6
8


Expression of AML-Associated vs. AML-Restricted Genes in Normal Tissues
AML-Associated
IL3RA
CD33
CLEC12A
FLT3
AML-Restricted
CLEC2A
FOLR1
MSLN
PRAME
WT1
Adrenal
Bladder
Blood
Brain
Breast
Cervical
Colon
Esophageal
Head and Neck
Kidney
Liver
Lung
Other
Ovarian
Pancreatic
Prostate
Skin
Stomach
Testicular
Thyroid
Uterine
Log2(TPM+1)
0
2
4
6
8

Supplemental Table 1. Published trials testing conventional therapies for AML.

Trial	Population	Intervention	Principal Result	Citation / PMID
MRC AML15 GO Trial	Younger adults	Addition of gemtuzumab ozogamicin to induction chemotherapy	No survival benefit; modest survival benefit in subsets	Burnett et al., 2011 — PMID: 21172891
MRC/NCRI AML15	Younger adults	DA vs ADE vs FLAG-Ida; consolidation strategies	No major overall survival improvement from intensification strategies	Burnett et al., 2013 — PMID: 23940227
NCRI AML16 GO Trial	Older adults	GO added to induction chemotherapy	Minimal improvement in survival	Burnett et al., 2012 — PMID: 22851554
SWOG S0106	Adults <60 years	GO + standard induction/post-consolidation	No improvement in CR, DFS, or OS; increased early mortality	Petersdorf et al., 2013 — PMID: 23591789
SWOG S1203	Younger adults	DA vs IA vs IA + vorinostat	No EFS advantage for IA or IA + vorinostat over DA	Garcia-Manero et al., 2024 — PMID: 37935977
EORTC/GIMEMA AML-10	Adults ≤60 years	Daunorubicin vs mitoxantrone vs idarubicin	Anthracycline substitution did not falter outcomes	Mandelli et al., 2009 — PMID: 19826132
NCRI Clofarabine Trial	Older AML / high-risk MDS	Clofarabine vs low-dose cytarabine	Higher response rate but no OS improvement	Burnett et al., 2013 — PMID: 23838349
COG AAML0531	Pediatric/AYA AML	GO added to chemotherapy	No OS improvement. Minimal EFS improvement	Gamis et al., 2014 — PMID: 25092781
COG AAML1031	Pediatric/AYA AML	Bortezomib added to chemotherapy	Increased toxicity; no improvement in survival	Aplenc et al., 2020 — PMID: 32029509
St. Jude AML02	Pediatric AML	High- vs low-dose cytarabine; MRD-directed therapy	Cytarabine intensification did not improve outcomes	Rubnitz et al., 2010 — PMID: 20451454
MRC AML12	Pediatric AML	Four versus five chemotherapy courses	Fifth chemotherapy course failed to improve survival despite greater treatment intensity	Gibson et al., 2011 — PMID: 21902686
MRC AML14	Older adults	Alternative induction approaches and maintenance strategies	Multiple modifications failed to substantially improve long-term survival	Burnett et al., 2009 — PMID: 19291085
ECOG E1900	Adults <60 years	High-dose daunorubicin (90 mg/m²) versus standard-dose daunorubicin (45 mg/m²)	Improved CR and survival in selected subsets, but did not fundamentally alter AML therapy	Fernandez et al., 2009 — PMID: 19776406

Supplemental Table 2. details of AML-associated and AML-restricted biomarkers and available published data.

Target	Category	Normal BM Expression	CD34+ PBSC Expression	Molecular Subset / Enrichment	Coverage	Clinical Development	Representative References
CD33	AML-associated	Yes	Yes	Broadly expressed across AML subtypes	**Broad AML**	Approved ADC; CAR-T and BiTE development	Burnett et al., 2011; Petersdorf et al., 2013; Pollard et al., 2016
CD123 (IL3RA)	AML-associated	Yes	Yes	Broadly expressed; enriched in leukemic stem cell populations	**Broad AML**	CAR-T, BiTE, antibody-based therapies	Mardiros et al., 2013; Gill et al., 2016
CLEC12A (CLL-1)	AML-associated	Yes	Yes/low	Broad AML expression; LSC-enriched	**Broad AML**	CAR-T and antibody therapies	Daver et al., 2026
FLT3	AML-associated	Yes	Yes	FLT3-mutated AML; also expressed in FLT3-wildtype AML	**Broad AML**	Approved kinase inhibitors; CAR-T development	Meshinchi et al., 2006; Meshinchi & Appelbaum, 2009; Wu et al., 2026
FOLR1	AML-restricted surface	No	No	Highly enriched in **CBFA2T3::GLIS2 AML**	**Highly subtype-specific**	Phase I CAR-T	Le et al., 2024; Tarlock et al., 2025; Williams et al., 2023
CLEC2A	AML-restricted surface	No	No	Highly enriched in **KMT2A-rearranged AML**; also monosomy 7 AML	**Highly subtype-specific**	IND-enabling CAR-T and T-cell therapies	Kirkey et al., 2023; Kirkey et al., 2024; Kirkey et al., 2025
Mesothelin (MSLN)	AML-restricted surface	No	No	Enriched in **KMT2A-rearranged AML**; present in ~30–40% of AML	**Intermediate**	AbTCR, CAR-T, ADC development	Le et al., 2021; Kaeding et al., 2018
CD276 (B7-H3)	AML-restricted surface	No	No	Multiple AML subtypes; stem-cell-like AML enrichment	**Intermediate**	Clinical development primarily in solid tumors; AML emerging	B7-H3 immunotherapy literature
TSLPR (CRLF2)	AML-restricted surface	No	No	Rare CRLF2-expressing AML subsets	**Rare**	Preclinical / early clinical	Russell et al., 2017; Yoda et al., 2010; Call et al., 2021
LAMP5	AML-restricted surface	No	No	Monocytic AML; KMT2A-rearranged AML enrichment	**Subtype-specific**	Preclinical	Hawkins et al., 2024
CD7	Subtype-restricted AML target	Absent from myeloid cells; present on normal T cells	No/low	CD7-positive AML (~10–20%)	**Subtype-specific**	Early clinical CAR-T	Oh et al., 2024; Coustan-Smith et al., 2018; Becilli et al., 2026
WT1	Intracellular AML-Specific antigen	Low	No/low	Broad AML expression across multiple molecular subtypes	**Broad AML**	TCR-T and vaccine studies	Ho et al., 2010; Liu et al., 2019; Blankenfeld et al., 2024
PRAME	Intracellular AML-Specific antigen	No	No	Broad AML expression; enriched in favorable-risk and fusion-driven AML	**Broad AML**	TCR-T and TCR-mimic therapies	Kirkey et al., 2023; Blankenfeld et al., 2024

WT1 + CD33 AND-gate	Combinatorial intracellular/surface target	CD33 on normal HSPCs; WT1 absent	Yes (CD33)	Broad AML expression with enhanced specificity through dual-antigen recognition	**Broad AML**	Preclinical TCRm-CAR platforms	Emerging combinatorial targeting studies
PRAME + CD33 AND-gate	Combinatorial intracellular/surface target	CD33 on normal HSPCs; PRAME absent	Yes (CD33)	Broad AML expression with enhanced specificity through dual-antigen recognition	**Broad AML**	Preclinical TCRm-CAR platforms	Emerging combinatorial targeting studies
NPM1-mutant peptides	Mutation-derived neoantigen	No	No	**NPM1-mutated AML** (~25–30% adult AML)	**Subtype-specific**	TCR-T and neoantigen-directed therapies	van der Lee et al., 2019; van der Lee et al., 2021