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Review article

Immunoediting in acute myeloid leukemia: Reappraising T cell exhaustion and the aberrant antigen processing machinery in leukemogenesis

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A B S T R A C T

Acute myeloid leukemia (AML) establishes an immunosuppressive microenvironment that favors leukemic proliferation. The immune-suppressive cytokines altered antigen processing, and presentation collectively assist AML cells in escaping cytotoxic T-cell surveillance. These $CD8^+$ T cell dysfunction features are emerging therapeutic targets in relapsed/refractory AML patients. Besides, $CD8^+$ T cell exhaustion is a hotspot in recent clinical oncology studies, but its pathophysiology has yet to be elucidated in AML. In this review, we summarize high-quality original studies encompassing the phenotypic and genomic characteristics of T cell exhaustion events in the leukemia progression, emphasize the surface immunopeptidome that dynamically tunes the fate of T cells to function or dysfunction states, and revisit the biochemical and biophysical properties of type 1 MHC antigen processing mechanism (APM) that pivots in the phenomenon of leukemia antigen dampening.

1. Introduction

Acute myeloid leukemia (AML) is the most common form of adult leukemia that accounts for approximately 10,000 deaths each year in the United States. Treatment of most AML subtypes typically involves induction chemotherapy using a combination of cytarabine and daunorubicin, followed by a consolidation therapy with high-dose cytarabine and maintenance therapy using either methotrexate or 6-mercaptopurine. In addition to cytogenetic classification, molecular markers such as *FLT3-ITD*, *FLT3-TKD*, *IDH1/2*

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mutations, and CD33 positivity are incorporated into targeted therapies for relapsed/refractory patients. Immune therapy has emerged as a promising approach for treating hematological malignancies. While checkpoint inhibitors for innate or adaptive immune cells lack specificity for AML blasts, cancer-associated or cancer-specific antigens are used to develop adoptive T cell-based therapy or personalized vaccines that enhance adaptive immunity by promoting T cell longevity, infiltration, and docking affinity into the AML tumor microenvironment (TME), which consists of pro-tumor or anti-tumor cells, metabolites, cytokines, and chemokines. Previous studies have identified defective antigen presentation, immune checkpoint, pro-tumor macrophage, and T cell synapses as immune evasion mechanisms in AML. However, the interplay between AML blasts, antigen processing, and tumoricidal cells in the TME, especially CD8 T cells, remains poorly understood.

The peptide-major histocompatibility complex class I (pMHC-I)-T cell receptor (TCR) axis plays a crucial role in the recognition and tumoricidal effect of cytotoxic T cells against AML blasts. The AML MHC1 antigen presentation machinery (APM) comprises several proteins, including the MHC-I complex, proteasome, TAP1/2, tapasin, ERp57, calreticulin, ERAP, LMP2/7, and others. Current efforts to address aberrant AML MHC1 APM focus on selective downregulation of HLA alleles in allogeneic hematopoietic stem cell (HSC) transplantation. Alteration of MHC1 antigen presentation, defined as an alteration rate exceeding 75 % among all reactome pathways [1,2], has been quantified in previous studies. It was suggested that newly diagnosed AML patients with TP53 mutations and complex karyotypes are more likely to have an altered MHC1 APM and less susceptible to immune checkpoint inhibitor (ICI) administration. However, there is still a lack of mechanistic demonstration of the dysregulated MHC1 APM and its effect on autologous $CD8^+$ T cell cytotoxicity against AML blasts.

Aside from MHC1, MHC2 dysregulation during AML relapse following hematopoietic cell transplantation has been extensively discussed in recent decades. Approximately 29 % of relapse patients who receive donor T cell infusion after haploidentical HSC transplantation show loss of heterozygosity (LOH) at their HLA loci (chromosome 6p), resulting in decreased T cell-specific lysis of AML blasts [3]. This is primarily due to the alloreactivity of donor T lymphocytes that depends on the mismatched HLA allele on AML blasts, which is diminished at post-BMT relapse. Genome-wide SNP-array analysis further supports this theory by observing increased somatic genomic aberrations, including copy-neutral loss of heterozygosity (CN-LOH) at 6p21(HLA loci) in 18 % of patients at relapse [4]. Toffalori et al. recently provided more precise immune signatures on the surface of post-BMT relapse AML cells, including a reduction of MHC2 molecules (HLA-DP, -DQ, and -DR) and an elevation of checkpoint ligands (e.g., PD-L1, B7-H3, CD80.etc) [5].

Several approaches have been proposed to improve patient prognosis after allo-HSCT by maintaining T cell-mediated graft-versus-leukemia (GvL) while suppressing graft-versus-host disease (GVHD), such as pro-GvHD T cell depletion, donor lymphocyte infusion (DLI), antagonists for immunoediting phenotypes, designing adaptive T cells targeting minor histocompatibility antigens/neo-antigens/LAAs, and more. Additionally, the escape from GvL events contributes to post-allo-HSCT relapse. In matched allo-HSCT, the allorecognition of host minor histocompatibility antigens (miHAs) is crucial for clearing AML blasts [6]. Notably, a tractable GvL platform has been established for blast crisis chronic myelogenous leukemia (BC-CML). At relapse after allo-HSCT [7], both decreased miHA (e.g., H60) and miHA-specific CD8 T cells with exhausted phenotypes (increased PD-1, Eomes, Blimp-1, Tim-3, TIGIT, LAG3; decreased IFNγ) contribute to GvL resistance.

Meanwhile, novel bioinformatics methodologies are being applied to evaluate HLA allele diversity and binding affinity to epitopes to build a more personalized scheme upon AML diagnosis. These approaches include HLA evolutionary divergence (HED) and peptide-binding promiscuity (Pr). Based on data from the immune epitope database (IEDB), a low binding specificity, noted as high Pr of HLA1 allele, is linked to unsatisfied outcomes after immune checkpoint therapies and co-occurrence with some T cell dysfunction markers in some cancers. T cell dysfunction is a chronic antigen-induced decrease in the effectiveness of T cells. Given the correlation between T cell dysfunction and disease progression in AML, the significance of underlying workflows manipulating epitope genesis in AML, or even under a T cell dysfunction status, is an intriguing topic.

2. T cell dysfunction and its potential link to a dynamic antigen processing machinery in AML

2.1. When $CD8^+$ cytotoxic T lymphocytes (CTL) take on AML blasts: A spatiotemporal profile of T cell dysfunction in AML

AML blast cells evade pro-inflammatory cells and attract several pro-tumor immune cells in the tumor microenvironment [8–10]. For instance, AML blasts escape from immune surveillance by increasing inhibitory receptor (IR) ligands to inactivate effector T cells (Teff) and inhibit the cytotoxicity of natural killer cells (NK); additionally, they facilitate fatty acid oxidation to promote the polarization of pro-tumor M2 macrophages. Notably, $CD8^+$ T cells exhibit impaired anti-neoplastic properties in AML, including reduced cell counts [11], poor proliferation, decreased longevity and reduced antigen recognition.

To overcome this immunosuppressive environment, various treatments have been developed, including immune checkpoint inhibitors (ICIs)(Table 1), allo-HSCT, TCR-engineered T cell therapy, CAR-T therapy, and bispecific antibodies. ICIs, when combined with chemotherapy or administered during the maintenance phase after allo-HSCT, have shown promising clinical responses [12]. To expand the usefulness of the leukemic microenvironment as a breakthrough for additional immune-modulating therapies, it is necessary to mitigate the immune-related adverse events associated with all ICIs and the substantial GVHD induced by anti-PD1 antibodies.

T cell exhaustion, which refers to the decline in the effectiveness of $CD8^+$ T cell under chronic antigen stimulation, has previously been observed in solid cancer models, and can partially explain the impaired anti-tumor response in certain clinical contexts of AML. In the past decade, dysfunctional T cell populations in AML patients after chemotherapy and allo-HSCT have been reported to have elevated T cell exhaustion surface inhibitory receptors (e.g., PD-1, TIGIT, Tim3) and intracellular markers (e.g., Eomes); however, how AML impaired the hierarchy of effector T cells along disease progression needs further investigation. Herein, we attempt to establish a

Table 1
Summary of the ongoing clinical trials regarding T cell-based immune checkpoint inhibitors on AML.

Target	ICI	Regimens	Patient profile	Phase	Reference
PD-L1	Durvalumab	Azacitidine, Durvalumab	Elderly AML	2	NCT02775903
PD-1	Nivolumab	Nivolumab VS. Ipilimumab VS. N + I	AML s/p HSCT	1	NCT02846376
		Nivolumab + Decitabine, Venetoclax	TP-53 mutated AML	1	NCT04277442
		5-azacitidine, nivolumab, relatlimab	r/r AML, Elderly pAML	2	NCT04913922
	Pembrolizumab	pembrolizumab, decitabine, venetoclax	r/r AML	1	NCT03969446
		Azacitidine, Venetoclax, ± Pembrolizumab	pAML	2	NCT04284787
		cytarabine, idarubicin, daunorubicin, HSCT ± Pembrolizumab	pAML	2	NCT04214249
		Pembrolizumab	relapse AML s/p HSCT	early 1	NCT02981914
		Galinpepimut-S, Pembrolizumab	AML ≤ PR s/p first line tx (w/HMA)	1/2	NCT03761914
		Lymphodepletion, Pembrolizumab	unfavorable risk AML ≥ CR-1 s/p chemo t/x	2	NCT02771197
	Camrelizumab (SHR-1210)	Camrelizumab, Decitabine	Elderly r/r AML	2	NCT04353479
	Visilizumab	Visilizumab, Azacytidine, Homoharringtonine, Cytarabine, G-CSF	r/r AML	3	NCT04722952
	PDR001	Decitabine + PDR-001+ MBG-453	r/r AML; pAML	1b	NCT03066648
TIM-3	MBG-453	HDM201+MBG453VS. HDM201+venetoclax	r/r AML MDS s/p HMA	1b	NCT03940352
		Decitabine + PDR-001+ MBG-453	r/r AML; pAML	1b	NCT03066648
		MBG-453, Magrolima ± Azacitidine	1L unfit AML, r/r AML s/p venetoclax + azacitidine	1b/2	NCT05367401
		MBG-453, Azacitidine	AML MRD + s/p alloHSCT	1b/2	NCT04623216
	TQB2618 (mAb)	TQB2618, AZA, decitabine (DAC)	r/r AML, MDS	1	NCT05426798

Abbreviation: ICI, immune checkpoint inhibitor; s/p, status post; HSCT, hematopoietic stem cell transplantation; r/r AML, relapsed/refractory AML; pAML, newly diagnosed AML; PR, partial remission; tx, treatment; w/HMA, with hypomethylating agents; s/p chemo t/x, status post chemotherapy; MDS, Myelodysplastic syndromes; MRD, minimal residual disease.

model explaining the progression and treatment response of $CD8^+$ T cell exhaustion in AML TME based on $CD8^+$ T cell differential hierarchy and the general knowledge of cancer-induced T cell dysfunction.

2.1.1. Conventional $CD8^+$ T cell differential hierarchy (As summarized in Fig. 1.)

The hierarchy of $CD8^+$ T cells is characterized by a sequential shift in phenotypic signatures from the naive $CD8^+$ T cells to effector or memory cells [13]. Generally, short-lived effector T cells (SPEC) and memory precursor effector cells (MPEC) are two direct descending subsets of naïve T cells [14,15]. Upon acute foreign antigen stimulation, the naive $CD8^+$ T cells ($CD45RA^+$ $CCR7^+$ $CD45RO^-$ $CD27^+$) undergo a transformation into fast-acting antigen-specific cells known as SLECs, which display phenotypic characteristics such as $KLRG1^{High}$ IL-7Rα$^-$ $TCF1^-$. SLECs make up approximately 90 % of all effector cells and initiate apoptosis once the target is cleared. The remaining 10 % of effector cells differentiate into memory precursor cells (Tmp), also called MPECs ($KLRG1^{Low}$ IL-7Rα$^+$ $TCF1^+$). MPECs [16–18] differentiate further into effector memory T cells (Tem), secondary lymphoid organ-homing ($CCR7^+$) long-lived central memory T cells (Tcm), and multipotent stem central memory T cells (Tscm) [19–21]. The process of Tscm ($CD62L^+$ $CD45RO^+$ IL-7Rα$^+$ $CD45RA^+$ $CCR7^+CD95^+$) generation from naïve T cells takes place upon the stimulation by cytokines (IL-7, IL-15) and activated antigen presentation cells (APCs) to establish a memory T cell hierarchy following allo-HSCT [22]. Tscm can self-renew and is multipotent, thus expanding into either Tem or Tcm in specific contexts. Tem ($CCR7^-$ $CD45RA^-$ $CD45RO^+$ $CD62L^-$ $CD27^-$) represents a CCR7 negative cytotoxic $CD8^+$ population, exhibiting a lesser homing tendency towards secondary lymphoid organs, and is similar to exhausted T cells due to its dependence on TOX (thymocyte selection-associated high-mobility group box protein) expression. Additionally, the development of MPEC to Tcm necessitates the presence of TCF1 to guarantee its superior stemness. Therefore, Tcm can be activated upon secondary antigen exposure to repopulate SLECs. To summarize, antigen exposure primes the naive CD8 T cell, leading to the clonal expansion of SLECs to eliminate pathogens. Following the clearance of non-self-antigens, SLECs undergo an apoptotic "contraction phase," leaving long-lived MPECs (Tem, Tcm, Tscm) for secondary antigen exposure.

2.1.2. $CD8^+$ T cell exhaustion in cancer

$CD8^+$ T cell exhaustion is a specific form of T cell dysfunction defined by reduced cytotoxicity and increased inhibitory markers (i.e., PD-1) following chronic antigen challenge under infection or malignancy. CTL's unresponsiveness to cancer cells is classified into four mechanisms: tolerance, ignorance, anergy, and exhaustion [23]. First, T cell "tolerance" refers to the untriggered T cell effectiveness or even T cell deletion upon the negative selection process, which eliminates self-reactive T cells during thymic education. Second, "ignorance" implies an early malignant stage where tumor-specific/foreign antigens are below the detectable threshold for $CD8^+$ T cells. Third, even when cancer neoantigens prosper and are recognized by naive T cells, T cells might convert to an "anergy" state with low IL-2 production if poorly stimulated by innate immune cells such as $CD4^+$ T cells. Lastly, T cell "exhaustion" refers

explicitly to cancer antigen-specific and previously well-primed $CD8^+$ T cells. Persistent cancer antigen promotes tumor-specific effector T cells (Teff) to express terminal differentiated signatures, escalated inhibitory receptors, and decreased longevity [24]. Theoretically, reversing dysfunctional $CD8^+$ T cell commitments will restore anti-tumor events and result in better clinical outcomes; however, immunotherapies aiming at boosting the "$CD8^+$T cell: cancer cell" cytolytic axis are intensively hampered by exhausted T cells (Tex), which increases the difficulty of minimal residual disease (MRD) suppression or graft-versus-leukemia events development in AML patients [25].

Phenotypically(Table 2), the major differences between exhausted and normal $CD8^+$ T cells are: exhausted $CD8^+$ subsets have higher expression of inhibitory receptors (e.g., PD-1, LAG-3), decreased stemness markers (e.g., TCF1 [26]), and increased nuclear proteins regulating terminal memory T cell differentiation and severity of T cell exhaustion(e.g., TOX). Aborted TCF1 expression is a crucial indicator of self-renewal inability, leading to irreversible exhaustion. Accordingly, this antigen-guided T cell impairment, or "tumor-specific T cell dysfunction [27]," can be chronologically arrayed into the early, reversible phase ($PD\text{-}1^{High}$ $LAG\text{-}3^{High}$ $CD38^{Low}$ $CD39^{Low}$ $TIM3^{Low}$ $TCF1^{High}$ TOX^{Mid}) and the late, irreversible phase ($PD\text{-}1^{High}$ $LAG\text{-}3^{High}$ $CD38^{High}$ $CD39^{High}$ $TIM3^{High}$ $TCF1^{Low}$ TOX^{High}). Additionally, the early, reversible phase ($TCF1^{High}$ TOX^{Mid}) can be further divided into two subsets according to Ly108 and CD69 expression [28]: precursor exhaustion T cells (Tpcex: $Ly108^+$ $CD69^+$) and progenitor exhaustion T cells (Tpex: $Ly108^+$ $CD69^-$); similarly, the late, irreversible phase ($TCF1^{Low}$ TOX^{High}) can be divided into transitory exhausted T cells (Ttrex: $Ly108^-$ $CD69^-$) and terminally differentiated exhausted T cells (Ttdex: $Ly108^-$ $CD69^+$). Functionally, T cell exhaustion is a progressive course marked by gradual loss of proliferative potentials (e.g., Ki-67, CD-27) and increase of inhibitory networks (e.g., PD-1, TIM-3) on cancer antigen-experienced $CD8^+$ T cells (Table 2). Accordingly, the decreasing secretion of pro-inflammatory cytokines and cytolytic enzymes and escalating expression of surface inhibitory regulators are two classical exhausted T cell phenotypes [29]. Furthermore,

Table 2
Dynamics of $CD8^+$ T cell phenotypes upon acute/chronic antigen stimulation.

Marker/Cell set	Tn	Teff (SLEC)	MPEC	Tscm	Tcm	Tem	Temra	Tpcex	Tpex	Ttrex	Ttdex	Ref.
Conventional T cell markers												
CD45RA	+			+	–	–	+					[13]
CD45RO	–	–		–	+	+						[20]
CCR7	+	–			+	–	–	+/–			–	[30]
CD28	+				+	+	–	+			+/–	[30]
CD27	+	–		+	+	+/–	–	+*	+	+	+**	[17, 31]
CD127 (IL-7R α) [32, 33]	+	+#	+#	+	+/–	+/–	+/–	+/–			–	[30]
CD95 (Fas)	-			+	+				+			[34]
CD62L	+			+		–		+/–			–	[30]
KLRG1		+,High [35]	+,Low [35]					+	–	+	–	[36]
Ki67 [2,37]	+/–	+/–		+	+/–	+/–	+	+	+	+* [28]	+	[36]
CD57	+/–	+/–			+/–	+/–	+	+*	+	+	+**	[38]
Exhausted T cell markers												
Ly108								+	+	–	–	[28]
CD69 [39]	+/–	+/–			+/–	+/–		+	–	–	+	[28]
T cell cytotoxicity												
GrznB [40]	+	+	+	- [34]	- [34]	+ [35]		- [30]	+ [34]		+	
Perforin	+, Low	+,High			+,Low	+, High	+, Low					[20]
Bcl-2								+			–	[30]
Surface inhibitory receptor												
PD-1 [41]	+	+	+			+	+	+	+	+	+*	[36]
TIM-3								–	–	+	+	[36]
TIGIT	+	+						+*	+	+	+**	[42]
Transcription Regulator [43]												
TOX	+	+,Low	+,High		+	+	+	+*	+	+	+*	[44, 45]
TCF1 [45,46]		+/–	+		+ [35]	+	+	+#	+#	–	–	[28, 36]
Tbet [47]	–	+			+	+		+	+	+	+	
Eomes	–	+,Low	+,High		+	+		+	+	+	+	[35, 47]
BLIMP1 [46]		+,High	+,Low		+, High					+	+	[48]

#, Phenotypic traits that lack concrete human evidence; *, highest expression level over the displayed exhausted T cell lineage; **, lowest expression level over the displayed exhausted T cell lineage; Tn: Naive T cell; Tpex: progenitor exhausted T cell; SLEC: short-lived effector cell; MPEC: Memory precursor effector cell; Tcm: Central memory T cell; Tem: Effector memory T cell; Tscm: Stem central memory T cell; Temra: Terminal effector; Tex: Exhausted T cell; Tem: effector memory; Tpcex: Precursor Tex; Tpex: progenitor Tex; Ttrex: Transitory Tex; Ttdex: terminally dysfunctional Tex; +: Presence; -: Absence; +/–: Equivocal; High/Low: Relative expression level between the adjacent cell types; Blank: data not available.

several cell-intrinsic regulators (TOX, Tbet, Eomes, TCF1) transcriptionally or epigenetically mediate the expression of inhibitory receptors (IRs) and tune the fates of exhausted T cells.

Mechanistically, T cell differentiation, proliferation, cytokine production, and surface marker expression are modulated by several nuclear proteins such as TOX, TCF1, Tbet, and Emoes. In tumor-specific $CD8^+$ T cells (TST), TOX [49], an HMG DNA sequence binding protein (DBP), represses self-renewal transcription factors (TFs) (e.g., TCF1) and sustains several IRs (e.g., Pdcd1, Entpd1, Havcr2, Cd244, and Tigit) whereas knocking out TOX in TST impairs T cell functionalities, including reduced IFNγ, INF, CD107, granzyme B, tumor-specific lytic activity. TCF1 [50], a DBP encoded by the Tcf7 gene, is a stemness marker for some $CD8^+$ T cell subsets that secures their self-renewal and progenitor memory status. Also, TCF1 suppresses B lymphocyte-induced maturation protein-1 (Blimp-1), a transcriptional regulator contributing to T cell exhaustion [51,52]. Nuclear Tbet [53] can suppress the expression of IRs and promote T cell effectiveness by enhancing KLRG1 and Ki67. Of note, the supportive evidence for TCF1(tcf7) in T cell exhaustion was mostly demonstrated in murine models; the generalizability of its master regulatory role to human T cell fate remains debatable.

However, another DBP, Eomesodermin (Eomes), competes with TCF1 for the same T-box binding sequence and reduces TCF1's inhibition on T cell exhaustion. Accordingly, Eomes serves as a TF, committing T cells and NK cells into distinct fates. In HCC patients, lower Eomes transcript level contributes to tumor-infiltrating $CD8^+$ T cells with higher IRs (PD-1, CD39, CTLA4) expression [54] via negative transcriptional regulation. Nevertheless, in murine lymphoma, overexpression of Eomes reduced T cell memory (ccr7) and stemness (tcf7) signatures while upregulating T cell exhaustion genes (e.g., cd244, havcr2, and il10ra) [55]. In contrast, loss of one Eomes allele increases $CD8^+$ T cell counts, promotes stemness marker TCF1, boosts proinflammatory cytokine production (e.g., IFNγ, TNF-α, and IL-2), represses surface IRs (PD-1, Tim-3), and decreases TOX expression, which contributes to better tumor elimination. Furthermore, complete loss of Eomes consistently represses IRs and TOX expression. Intriguingly, the absent Eomes expression also downregulates effector T cell markers (IFNγ, TNF-α, IL-2, CD107a, and Granzyme B), suggesting a complex role Eomes represent in the $CD8^+$ T cell dynamics. Collectively, despite the discrepancy upon addressing the trajectory of serial Tex evolution via these nuclear markers, high TOX, low TCF1, and high Eomes/T-bet ratio generally pointed to a terminally differentiated Tex state for TST [24].

2.1.3. Phasic discrepancies of $CD8^+$ T cell in AML microenvironment: phenotypic signature and differential hierarchy

The existence of diverse $CD8^+$ T cell subsets in AML, as characterized by the aforementioned T cell exhaustion signatures, may offer prognostic insights. Firstly, AML patients with a higher proportion of $Eomes^+$ T-bet^{low} $CD8^+$ T cells at diagnosis exhibit a worse overall survival rate [56]. Secondly, a lower frequency of "PD-1^+ $TIGIT^+$" $CD8^+$T cells predict future remission after chemotherapy [57], while a higher frequency of "$Eomes^+Tbet^{low}$ [56]" or "$TIGIT^{high}$ [58]" $CD8^+$ T cells correlates with resistance to induction chemotherapy. Lastly, the presence of Tscm cells with the "PD-1^+ $Eomes^+$ T-bet$^-$" signature in the bone marrow early after HLA-matched HSCT is associated with a higher relapse rate [59]. Although recent evidence suggests that chronic exposure of cancer antigens to naïve $CD8^+$ T cells is a prerequisite for Tex development, a chronological process of AML-specific T-cell exhaustion has not been firmly established. As a result, two factors must be considered when observing the chronological changes in $CD8^+$ T cell signatures in AML patients [60].

(1) Phasic discrepancies in $CD8^+$ T-cell signature in the AML microenvironment.
(2) Phasic discrepancies in the differential hierarchy of $CD8^+$ T cells in the AML microenvironment

2.1.3.1. Phasic discrepancies of $CD8^+$ T cell signature in AML microenvironment (Fig. 3).

1 Pre-malignant AML patients

The linear differentiation of exhausted T cells is widely recognized as a gradual loss of proliferative potential, an increase in the expression of inhibitory receptors, and the gain of intrinsic transcriptional/epigenetic drivers (such as TOX and TOX2) that help maintain the exhaustion phenotype [44]. While early T cell dysfunction has been proposed to promote a pre-malignant tumor microenvironment, and irreversible T cell exhaustion corresponds to the complete establishment of a solid tumor, it may not be appropriate to fit the currently observed T cell dysfunction phenotypes of AML into this model, as the pre-malignant status of newly diagnosed AML patients is still under debate. There is no available data on pre-malignant AML T cell signatures.

2. Non-AML and newly diagnosed AML patients

On the level of surface inhibitory regulators:

Newly diagnosed AML (pAML) patients have a higher percentage of $TIGIT^+$ $CD8^+$ T cells in their peripheral blood mononuclear cells (PBMCs) compared to healthy donors (HD) [58,60]. Additionally, among the increased $TIGIT^+$ $CD73^-CD8^+$ T cells, an elevated proportion that expresses $CD39^+$ or PD-1^+ is also found in pAML patients [57,60].

On the level of intracellular transcriptional factors:

The PBMCs of pAML patients have a higher percentage of TOX^+ $CD8^+$ T cells compared to HCs, while there is a lower frequency of $TCF1^+$ $CD8^+$ T cells in pAML [60]. Furthermore, blimp-1, a reciprocal repressor on TCF1, is elevated in the $CD8^+$ T cells of pAML at both the mRNA and protein levels. Blimp-1 expression also co-occurs with surface PD-1 and TIGIT elevation, and reduced cytotoxicity of pAML-derived T cells. Moreover, co-expression analysis reveals a higher frequency of $Eomes^+$ $Tbet^{low}$ $CD8^+$ T cells in pAML than

HC. This high intrinsic Eomes level in pAML directly leads to the elevation of surface TIGIT expression by activating its promoter region (Fig. 2) [56]. The evidence above suggests that aberrant intracellular features orchestrate the expression of surface inhibitory regulators of pAML CD8$^+$ T cells, which should be given excessive attention when attempting to reverse the dysregulated T cell progeny of AML patients.

On the level of CD8$^+$ T cell cytotoxicity:

Notably, TIGIT $^+$ CD8$^+$ T cells from pAML patients display an level of perforin compared to HCs, while their pro-inflammatory cytokine secretion is decreased, and their susceptibility to apoptosis is increased. These results suggest an early stage of T cell exhaustion where some pro-inflammatory roles are lost, but part of the T cell functionality, such as cytotoxicity, is retained. On the other hand, blimp-1$^+$ CD8$^+$ T cells from pAML patients show a reduction in both perforin and granzyme B secretion [Fig. 2] [58]. According to the T cell exhaustion model, blimp-1 expression is relatively low at the stem cell-like Tex state [46], and it can transcriptionally suppress IL-2 production and epigenetically hamper long-lived central memory T cell formation [61]. Thus, the blimp-1$^+$ CD8$^+$ T cells may correspond to the later phases of Tex progenies (Ttrex or Ttdex) of AML patients. However, while attempting to reverse the exhaustive status of CD8$^+$ T cells in AML patients, blimp-1 might not be an ideal therapeutic target due to its multi-faceted role in ordinary CD8$^+$ T cell differentiation [62].

In summary, these CD8$^+$ T cell signatures in pAML resemble the late, irreversible phase (PD-1^{High} LAG-3^{High} CD38^{High} CD39^{High} TIM3^{High} TCF1^{Low} TOX^{High}) of T cell exhaustion model in solid malignancy or the terminally exhausted T cell (CXCR5$^-$ PD1^{High} TIM3^{High} EOMES^{High} T-bet^{Low} TOX^{High} TCF1^{Low}) in chronic infection. These exhausted CD8$^+$ T cell signatures in pAML suggest a potential role of chronic AML antigen stimulation that ultimately reduces the effectiveness of the host CD8$^+$ T cell population and contributes to the establishment of malignancy.

A mechanistic illustration of these intrinsic regulators is necessary to seek ideal targets for reshaping the T cell fate.

3. Post induction chemotherapy

Induction chemotherapy is the primary initial treatment for most newly diagnosed AML patients. This treatment imposes chemotoxicity on AML cells, which weakens the immunosuppressive milieu. Here, we will describe current observations of Tex signatures in responders and non-responders to induction chemotherapy for AML.

First, in patients who achieve AML remission after induction chemotherapy, there is a significant decrease in TOX expression in CD8$^+$ T cells from the BMMC (bone marrow mononuclear cells) compared to pAML patients [63]. The concentration of granzyme B and

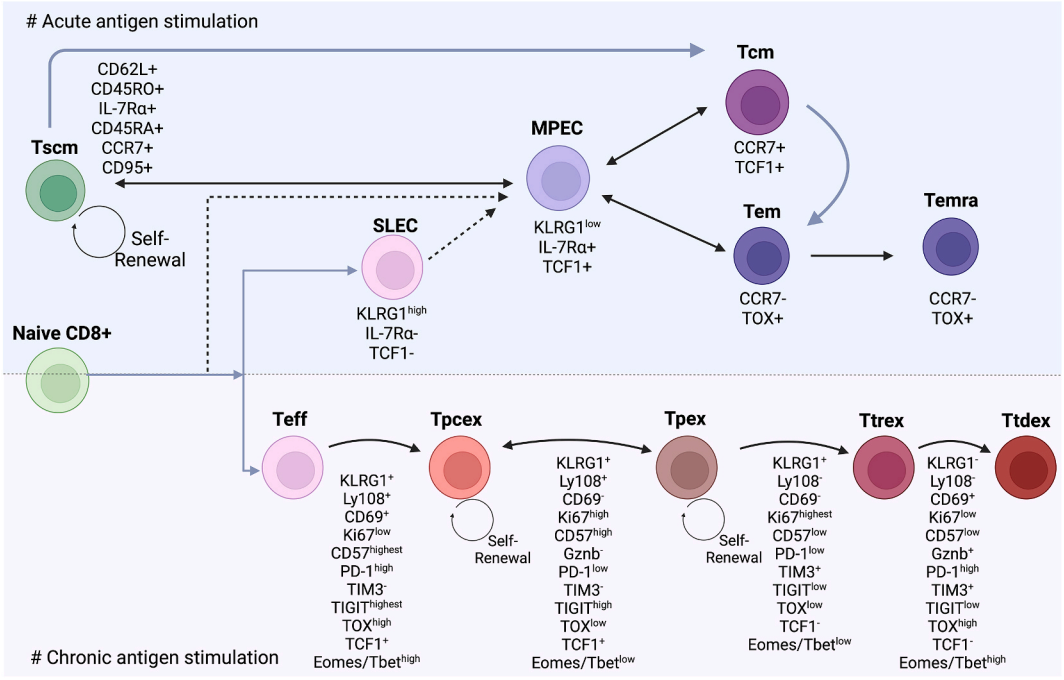

Fig. 1. CD8$^+$ T cell differential hierarchy upon antigen exposure. This schematic review summarized the current understanding of CD8$^+$ T cell progeny upon antigen exposure. Specifically, the figure displayed how the serial acquisition/loss of biomarkers tunes the CD8$^+$ T cell hierarchy upon acute (upper panel)/ chronic (lower panel) antigen stimulation. Lavender line: Canonical T cell differentiation pathway; Black line:Terminally-differentiation or T cell exhaustion-related pathway. Dashed lines: Debatable pathways. Tscm, memory stem T cell; SLEC, short-lived effector T cell; MPEC, memory precursor effector cell; T cm, central memory T cell; Tem, effector memory T cell; Temra, terminal effector T cell; Teff, effector T cell; Tpcex, precursor exhausted T cell; Tpex, progenitor exhasuted T cell; Ttrex, transitory exhausted T cell; Ttdex, terminal differentiated exhausted T cell.

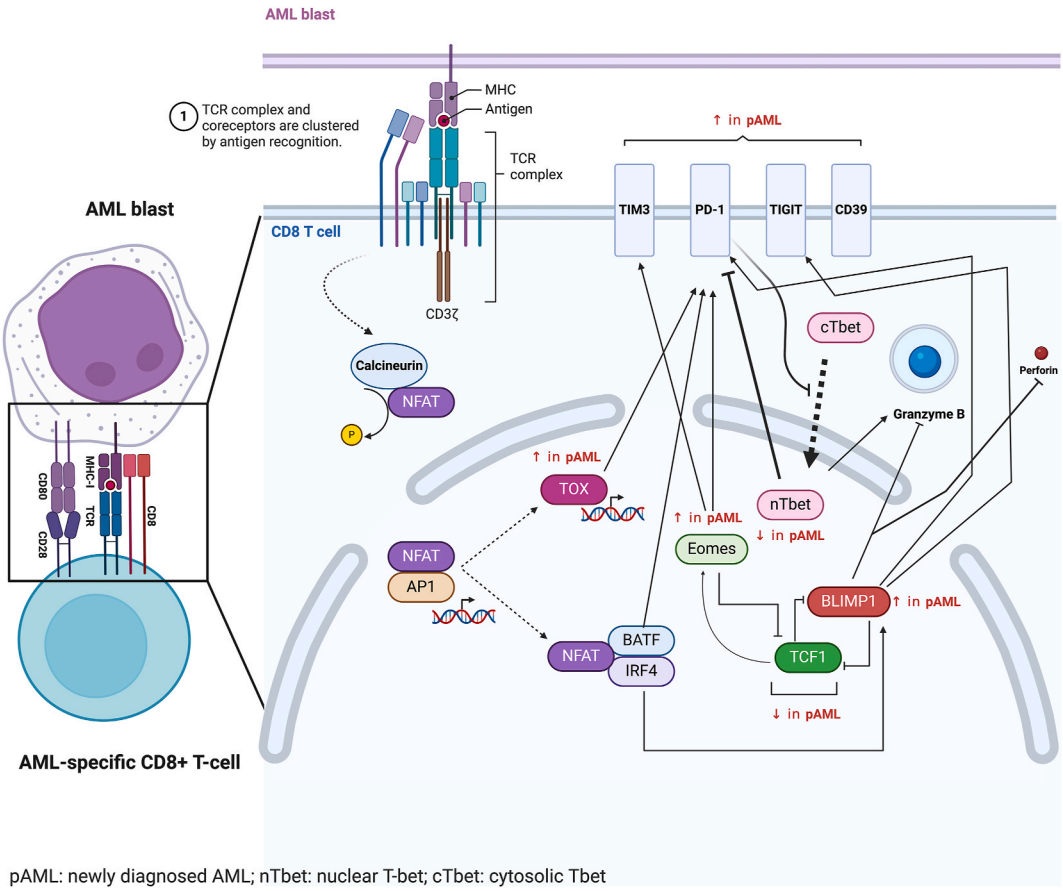

Fig. 2. Intrinsic regulatory pathway of AML T cell exhaustion.

perforin also increases in $CD8^+$ T cells of IrAML compared to pAML, indicating a functional restoration of cytotoxicity coinciding with the reverse of the early T cell dysfunction signature.

Second, in patients with primary refractory after induction chemotherapy [60], a higher proportion of TOX^+ cells were found in the $TIGIT^+$ $CD73^-$ $CD8^+$ T cells of both bone marrow and peripheral blood AML niches compared to pAML patients. The co-expression of the inhibitory receptor TIGIT and increased cell intrinsic TOX levels implied T cell exhaustion features in primary refractory AML. This exhaustion probably stems from the constant exposure of cancer antigens overwhelming the naive $CD8^+$ T cells by chemo-resistant AML cells. Besides, the granzyme B and perforin levels were similar between pAML and relapsed (rAML) patients, implying the re-emerged Tex progeny in IrAML, which also corresponds to poorer control of AML blasts and poorer prognosis [63].

4. Post-allo-HSCT: Complete Remission or Relapse

After undergoing myeloablative treatments, the $CD8^+$ T cell signatures following allo-HSCT are initially expected to resemble those of healthy donors due to successful engraftment of donor hematopoiesis. However, in patients who experience relapse following transplantation, there is an increased frequency of $CD8^+$ T cells expressing $TIGIT^+$ or $Tim3^+$ in the PBMC compared to those in remission after transplantation [58,64]. The role of graft T cell exhaustion in the mechanisms of post-transplant relapse remains to be fully elucidated, and further investigation is needed to understand the dynamics of other surface inhibitory regulators and exhaustion-specific transcription factors.

2.1.3.2. Phasic discrepancies of $CD8^+$ T cell differential hierarchy in the AML microenvironment. Other than analyzing T cell signatures among the entire $CD8^+$ T cell progenies, the T cell differential hierarchy fluctuation during the treatment-related disease phases of AML and its impact on developing T cell exhaustion remain unclear.

In addition to analyzing T cell signatures among the entire $CD8^+$ T cell progenies, it is important to understand the differential T cell hierarchy changes. Five $CD8^+$ T cell subsets can be recognized: naive ($CD45RA^+$ $CD62L^+$ $CD95^-$, Tnaive), memory stem ($CD45RA^+$ $CD62L^+$ $CD95^+$, Tscm), central memory ($CD45RA^-$ $CD62L^+$, Tcm), effector memory ($CD45RA^-$ $CD62L^-$, Tem) T cells, and terminal effectors ($CD45RA^+$ $CD62L^-$, Temra).

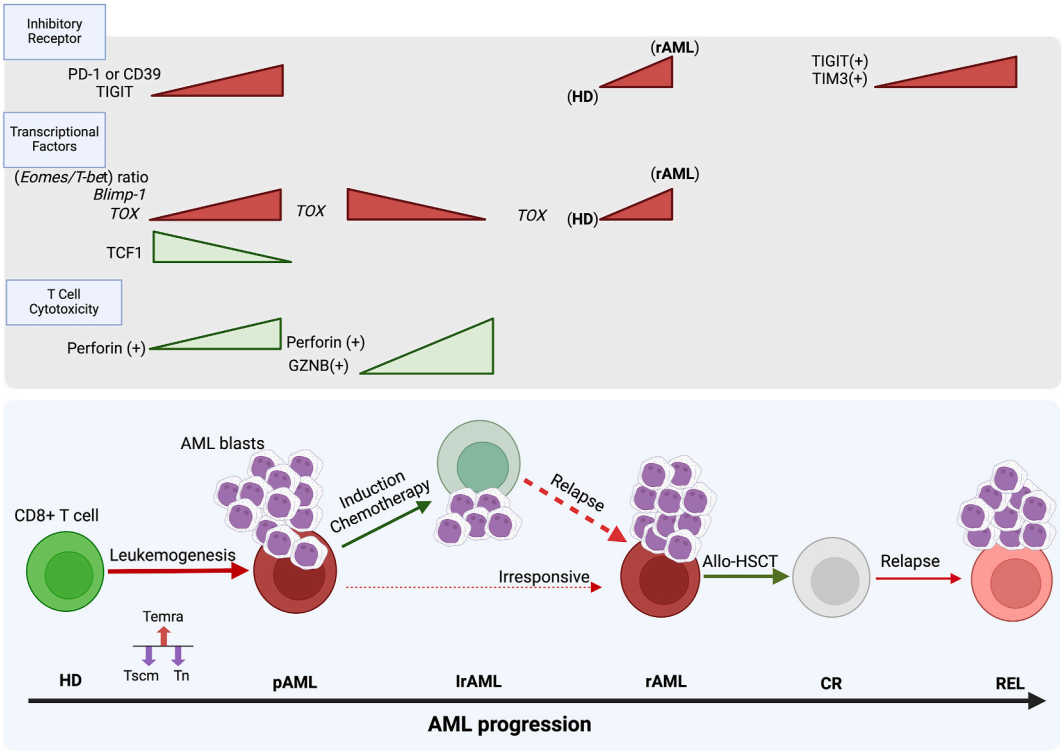

Fig. 3. Phasic discrepancies of CD8$^+$ T cell signatures in AML microenvironment: HD, Healthy donor; pAML, Newly-diagnosed AML; IrAML, AML at remission after induction chemotherapy; rAML, Relapse/Primary Refractory after induction chemotherapy; CR, Complete Remission after Allo-HSCT; REL, Relapse after Allo-HSCT; Temra, terminal effector; Tscm, stem central memory T cell; Tn, naïve T cell.

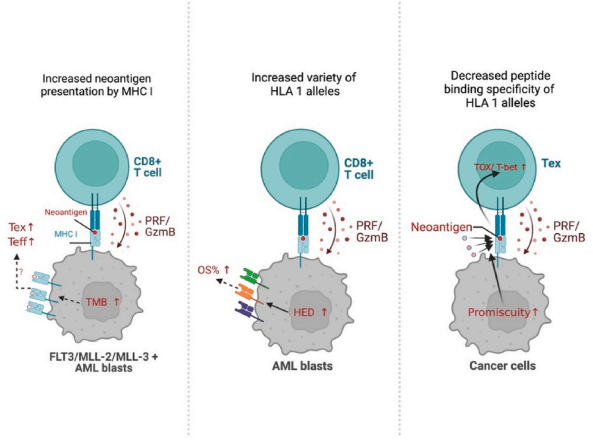

Fig. 4. HLA-1 immunopeptidome on the dysregulated T cell functionality (Section 2.2). Left panel: AML patients with driver gene mutations (FLT3/MLL-2/MLL-3) have higher tumor mutation burden (TMB), which potentially increases the recognizable neoantigens; Middle panel: For HSCT recipients, a higher HLA evolutionary divergence (HED), defined as the polymorphism of the HLA peptide binding dominas, is associated with better AML prognosis; Right panel: In melaloma, a higher peptide-binding promiscuity(Pr), defined as an estimation on the MHC1 immunopeptidome abundnace produced by each HLA allele, might lead to noisy antigenic stimulation on CD8$^+$ T cell and associate with T cell exhaustion. PRF, perforin; Gzmb, granzyme B; Teff, effector T cell; Tex, exhausted T cell; OS, overall survival.

At diagnosis, AML patients have an increased frequency of Temra and reduced frequencies of naive T cells [60], memory stem cells, and central memory cells [65]. In contrast, the frequencies of effector T cells and effector memory T cells are not distinctive [65] compared to healthy donors. Furthermore, the Tem and Temra subsets in pAML have a higher expression rate of TIGIT [60] than healthy donors. To be specific, among other CD8$^+$ T cell subsets, TIGIT expression most prominently rises in the Temra [58] during the HD-to-pAML transition.

Tnaive and Tscm subsets play a crucial role in repopulating other memory $CD8^+$ T cell subsets, including Tscm, Tem, and Tcm [31]. Their absence in the overall $CD8^+$ T cell hierarchy may indicate a potential mechanism for leukemogenesis. After allo-HSCT, $CD8^+$ T cell signatures in patients with relapse skew towards a more dysfunctional pattern, but the ratio of $CD8^+$ T cells at each differential level remains unchanged [59] between the complete remission and relapse groups. Notably, one post-BMT relapse mechanism that has been widely debated is the loss of a haploidentical HLA-allele on AML blasts, which reduces the MHC immunopeptidome, leading to poorer priming of naive T cells, creating a less-exhausting niche with fewer cancer antigens. Therefore, any slight skew towards more terminally differentiated subsets should be analyzed carefully and interpreted with caution.

Several conventional T cell exhaustion features manifest in various phases of AML. Therefore, it is imperative to investigate the fundamental cause of chronic antigen stimulation by AML blasts. The activation of naïve $CD8^+$ T cells necessitates the processing and presentation of cancer antigens as peptide: MHC1 complexes on the plasma membrane of AML cells. In addition to the distorted $CD8^+$ T cell fates, disease progression is also associated with the downregulation of mismatched type HLA1 alleles. Nevertheless, there is a lack of diversity and regulation of antigen presentation in AML patients. The flexibility of the antigen-derived T cell response depends on the co-evolution of the host TCR repertoire and AML HLA-1 immunopeptidome. Thus, we aim to elucidate the establishment of the HLA1 immunopeptidome, focusing on the AML-specific antigenic peptide formation and the intracellular antigen processing machinery (APM) of AML blasts, with the objective of linking these concepts to the antigen-induced T cell exhaustion module in the next section.

To expand our horizon of the dysfunctional T cell status in AML, we highlight the current knowledge of $CD8^+$ T cell signatures involving dysfunctional T cell markers and try to establish an explanatory scheme about the progressive transformation of T cell dysfunction during each phase-to-phase transition.

2.2. The role of HLA-1 immunopeptidome on the dysregulated T cell functionality (Fig. 4)

AML blasts evade immune cell recognition by defective HLA-mediated antigen presentation and inducing T cell dysregulation. Functionally, the HLA-1 allele encodes the MHC class 1 protein, which forms complexes with intracellular peptides for surface presentation, thereby enabling immune cells to distinguish normal cells from transformed cells in AML. AML patients typically have dysfunctional T cells and a detrimental bone marrow microenvironment. Despite the potential for allo-HSCT graft T cells to eliminate leukemic blasts, they may still escape the Graft-versus-Leukemia event by tuning down the expression of mismatched MHC1 [3,66,67]. This process is also known as loss of heterozygosity [3,4], and is achieved by substituting a homologous sequence with a host-specific haplotype without deletion in the short arm of the HLA-encoding chromosome 6 region in AML cells. In short, aberrant transcript profiles of HLA alleles in AML blasts reduce the expression of surface MHC1 complexes, thereby impairing tumoricidal T cell response and leading to post-transplantation relapse.

The expression patterns of HLA transcripts in the population of immune cells highly correlate with the activity of innate immune response. Supposing HLA alleles present more cancer-associated or cancer-specific antigens, it might increase the probability of successful recognition and elimination of cancer cells by host T cells. There are several factors that determines the expression profile of the HLA allele.

(1) The polymorphism of the HLA binding domain in cancer cells,
(2) The peptide binding specificity of a single HLA allele,
(3) The tumor mutation burden of cancer cells, which reflects the abundance of cancer-specific antigens.

The polymorphism of the HLA peptide binding domain is defined by HLA evolutionary divergence (HED), which is quantified by Grantham distance. In solid tumors, late-stage melanoma and metastatic NSCLC patients with high HED had better overall survival (OS) after therapy with immune checkpoint inhibitors anti-CTLA4 or anti-PD-1/PD-L1 [68]. For AML patients, a higher MHC class 1/MHC class 2 HED ratio of a transplant recipient is positively correlated with the prognosis of the allo-HSCT therapy, including increased disease-free-survival (DFS) and overall survival (OS) [69].

Second, a new criterion for predicting cancer prognosis, the peptide-binding promiscuity (Pr), has emerged. This criterion estimates the MHC1 immunopeptidome abundance produced by each HLA allele. Unlike HED, HLA allele peptide binding promiscuity negatively predicts prognosis in certain solid malignancies, especially after immune checkpoint inhibitor (ICI) treatment. High Pr HLA can bind to a broader but less specific spectrum of antigenic peptides, increasing the diversity of antigens presented on the surface. However, this expanded promiscuity of the HLA allele makes cancer cells less distinguishable from host cells and can facilitate T cell exhaustion, collectively dampening host immune surveillance. High HLA promiscuity boosts a more homogeneous presentation of tumor antigens and self-antigens. This process decreases the difference of binding affinity between cancer neo-peptides and wild-type peptides (predicted by DAI, Differential Agretopicity Index [70]), which leads to escaping from alloreactive T cell recognition and thereby decreasing the efficacy of ICI therapy. For example, melanoma patients with high Pr HLA allele treated with either CTLA-4 or PD-1/PD-L1 blockade had poorer overall survival and reduced clinical benefit (defined by Complete Remission or Partial Remission). Furthermore, a high genotype promiscuity [71] calculated from the mean Pr of tumor HLA-1 allele (21 HLA-A, 31 HLA-B, and 15 HLA-C alleles) is related to a higher expression of several cell-intrinsic T cell exhaustion mediators (such as TOX, TOX2, T-bet, and BLIMP1) and the tumor immune dysfunction and exclusion (TIDE) T cell dysfunction score. This partially explains the failure of ICI therapy in high HLA promiscuity cancer patients. Although the peptide binding promiscuity has not yet been applied to AML, the significance of its impact on fostering dysfunctional T cells is worth further investigation to delineate the detailed mechanism of T cell exhaustion in AML.

Third, the diversity of antigens also serve as prognosticator of cancer. Tumor mutation burden (TMB), which indicates the potential number of tumor-associated antigens generated in a cancer patient [72], has previously been employed as a predictor of immune checkpoint inhibitors (ICI) outcomes in solid tumors. Similar outcomes have been proposed for AML. The initial AML epidemiological study that employed TMB (Mayo Clinic AML Epidemiology Cohort, MCAEC) discovered higher TMB levels in patients with driver gene mutations (Fms-like tyrosine kinase 3 (FLT3), MLL2, MLL3), and a reduction in TMB after receiving specific treatments. However, the usefulness of TMB as a sole determinant for clinical outcomes is still a subject of debate [73]. TMB should be analyzed with caution since only a few somatic mutations eventually form MHC1-neoantigen complexes, which are identified by tumor-specific $CD8^+$ T cells. This suggests that further subcellular processing on the initial translated mutant peptides should not be ignored when evaluating the cancer immunopeptidome and its clinical significance. The tumor mutational burden and HLA-allele diversity (HED and promiscuity) have different effects on AML patients' clinical outcomes and potential connections to antigen-induced T cell exhaustion. The antigen processing machinery (APM) acts as a bridge between the newly generated peptide and the eventual antigen-MHC1 complex on the AML blasts. With the exception of MHC1 alleles, the effect of other components of the MHC class 1 APM on the immunopeptidome of AML blasts and their clinical significance are not well understood. Therefore, we would like to draw attention to the present understanding of this interface underlying the AML antigen presentation pathway in the following section.

2.3. Antigenesis: probing the early stages of the cancer immunity cycle in AML

The "Cancer immunity cycle [74]" is a series of necessary steps to achieve CTL-to-cancer cytotoxicity. A functional cancer immunity cycle involves the synthesis and release of short peptide sequence from cancer neoantigen, HLA-restricted ligand, and T cell reactivity. The anti-leukemia process has primarily been studied in the context of post-transplantation graft-versus-leukemia (GvL) [6]. However, little is known about the initial host-versus-leukemia events in newly diagnosed AML patients. Theoretically, the downregulation of MHC2 found in AML patients implies a poor costimulatory immune microenvironment, loss of cross-presentation from dendritic cells, and failure of $CD4^+$ T cell-mediated immunity. However, at diagnosis, AML patients with surface HLA-DR^{High} or HLA-DR^{Low} blasts have similar 5-year OS and DFS [75]. The impaired immunogenicity in HLA-DR^{Low} blasts can be offset by an elevated diversity of HLA-DQ-restricted peptides that maintain a constant pooled ligandome diversity of HLA-DR and HLA-DQ.

In the previous sections, we have presented evidence of a significant $CD8^+$ T cell exhaustion profile in pAML patients. Thus, akin to the generation of Tex in chronic antigen stimulation, early exposure of leukemic antigens to $CD8^+$ T cells in pAML may also contribute to the subsequent T cell dysfunction. However, it is unclear whether the CD8-MHC 1 axis plays a role in the uncontrolled outgrowth of leukemia and directly induces AML-specific antigen stimulation, predisposing T cell to exhaustion. Therefore, integrating multiple emerging analytical tools may provide a more comprehensive understanding of the dynamics of the AML MHC1 ligandome and Tex cell generation during leukemogenesis. Mechanistically, increased HLA complex promiscuity (decreased binding specificity) results in expanded antigen presentation on the cancer cell surface, priming more naive T cells. Moreover, reduced peptide-HLA1 binding specificity leads to poor discrimination between self-antigen and alloantigen, diminishing $CD8^+$ anti-tumor reactivity and fostering a microenvironment that promotes T cell exhaustion. Despite HLA 1 peptide promiscuity having not been analyzed in AML, high HLA1 evolutionary divergence (HED) is correlated with better overall survival in AML patients receiving immune checkpoint therapy. However, HED is not positively associated with T cell exhaustion phenotypes [71], indicating that promiscuity is a more critical index for evaluating the Tex cell strength and explaining the mechanism of MHC class 1 antigen-driven T cell exhaustion. Given that the excessive or dysregulated antigen stimulation can alter T cell activity, exploring the regulations among antigen processing machinery, T cell dysfunction profile, and the disease evolution of AML is warranted. Herein, we will emphasize the potential causality of aberrant AML blasts immunopeptidome in newly diagnosed AML patients and evaluate $CD8^+$ T cell exhaustion signatures.

2.3.1. Neoantigens of AML blasts: mutagenesis and immunogenicity (Table 3)

Generally, cancer cells can elicit an immune response through three mechanisms: somatic mutation, overexpression, or post-translational modification of normal self-proteins. Of these, somatic mutation-derived cancer-specific antigens (CSAs), also known as neoantigens, are exclusive to cancer cells. Therefore, CSAs are considered attractive therapeutic targets, as they can induce anti-tumor effects with minimal harm to normal cells. In AML, only a limited number of neoantigens have been well-characterized, such as NPM1, FLT3-ITD, and IDH1/2. These neoantigens are generated through frameshift mutation, point mutation, internal tandem duplication, etc. Specifically, both NPM1 and FLT3-ITD mutagenesis are driven by the terminal deoxynucleotidyl transferase (TdT), according to the "replication slippage" model proposed by Julian Borrow [76,77]. In this model, TdT adds nucleotides randomly to the 3′-OH end of single-stranded DNA during V(D)J recombination in a template-independent manner, potentially expanding the MHC 1 ligandome.

NPM1 heterozygous mutations have been observed in 30 % of AML patients [78], with the most common mutation being a 4-bp insertion at exon 12 of the NPM1 gene(wild type sequence aa287-aa294: LWQWRKSL). This mutation has been subdivided into 3 types: Type A (accounting for 75–80 % of NPM1-mutated AML cases), Type B (10 %), and Type D (5 %). Type A mutation is defined by a TCTG insertion at c864-c867, which generates a LCLAVEEVSLRK sequence from aa287 to aa298. The TCTG duplication is initiated by TdT, which inserts a deoxythymidine triphosphate (dTTP) at the 3′-end of c.863, aligning it with the adenosine at c.860. Type B mutation is generated by a CATG insertion at c864-c867, resulting in a LCMAVEEVSLRK sequence from aa287 to aa298. TdT adds a 2-bp non-templated deoxynucleotides, CA, to c.863, followed by a 1-bp T addition for occult microhomology. Type D mutation is defined by a CCTG insertion at c.864-c.867, which generates the same (287–298) LCLAVEEVSLRK sequence as a Type A mutation. Two CC additions and subsequent TG polymerization are initiated by TdT-catalyzed non-templated C addition and another C addition for occult homology. The digested versions of these mutated peptide sequences can be presented by specific type 1 HLA alleles of AML

blasts, which can be recognized and lysed by $CD8^+$ T cells. Mutated peptide sequences shared by Type A and type D mutation generate several 9-mer epitopes for HLA class1 binding, confirmed by in silico prediction [79,80], such as CLAVEEVSL and AIQDLCLAV. An epitope shared by Type A, B, and D NPM1 mutants (AVEEVSLRK) is recently found to be presented on HLA-A*11:01 and capable of inducing *in vitro* $CD8^+$ T cell lysis of primary AML cells. Another Type A/D NPM1 mutant ligand, CLAVEEVSL, is presented by HLA-A*02:01 [81] on primary AML blasts, which can be successfully recognized and lysed by AML patient-derived CD8 T cells and TCR-T cells transfected with modified neopeptide-specific TCR. In summary, NPM1 mutations, particularly Type A, B, and D, generate several mutated peptide sequences that can be presented by specific HLA alleles and recognized by $CD8^+$ T cells, indicating their potential as targets for immunotherapy in AML patients.

FLT3-ITD translocation is found in 30–40 % of AML patients, which leads to continuous activation of the membrane tyrosine kinase receptor, myeloid differentiation blockade, and reduced overall survival. The FLT3-ITD translocation has seven subtypes, with type A, B, and C accounting for 93 % of all FLT3-ITDs. Mechanistically, the sequence duplications are primed by TdT in a microhomology-mediated manner [76,77], and TdT expression is higher in the FLT3-ITD + than FLT3-ITD- AML subgroups [82]. One short FLT3-ITDs peptide, YVDFREYEYY, was found to be immunogenic and elicit autologous $CD8^+$ T-cell responses [83].

Missense point mutations of isocitrate dehydrogenase (IDH1/2) are found in 20 % of AML patients. Functionally, normal IDH catalyzes isocitrate to α-ketoglutarate (αKG); however, mutant IDH1(R132H) produces 2-HG, which suppresses Tet methylcytosine dioxygenase 2 (TET2) and histone demethylase, leading to DNA [84] and histone hypermethylation in AML. IDH1 R132H mutant not only causes early hematopoietic progenitor accumulation in bone marrow niche in mice, but also show neo-antigenic potential. In gliomas, the vaccination [85] of IDH1 15-mer neoantigens specifically presented on HLA-DRB1*0101 elicits antigen-specific proliferation of IFN-γ -secreting CD4 T cells. However, it is unclear whether IDH1-mut AML blasts present any antigenic epitope from any isoforms of IDH1 mutation. IDH2 has similar mutation rates to IDH1, and two IDH mutants, R140 and R172 [86], lead to an accumulation of oncometabolite 2-HG, as in IDH1-mutated AML cells. A 10-mer SPNGTIQNIL epitope [87] of IDH2R140Q-mutant has been revealed to have an exclusively higher affinity to HLA-B*07:02 than other HLA alleles, and this pHLA-complex has been utilized to design a TCRm-based chimeric antigen receptor (CAR)-T cell. The therapeutic approaches on IDH1/2-mutated AML patients are centered on enzymatic inhibitors (Ivosidenib and Enasidenib) [88], but little is known regarding the mutagenesis mechanism of IDH1/2 and its role in shaping the exhausted $CD8^+$ T cell population in AML patients.

Neoantigens presented on AML cells are ideal targets for alloreactive $CD8^+$ T cell recognition. We emphasize that TdT is a potential mutagen for AML neoantigens, such as NPM1 and FLT3-ITD. Notably, TdT also involves in the generation of other AML neoantigens, such as RUNX1, as there is a likely correlation between the presense of TdT (33 %) and RUNX1 (35 %) in the minimally differentiated AML subgroup (FAB class M0 AML) [89]. Despite the immunogenicity of NPM1 mutants are leveraged in immunotherapy design, more discussions and adoptions of NPM1 cytosolic distribution, "gatekeeper" roles in leukemogenesis, and their co-occurrence with CD33 have been conducted as therapeutic modalities [90]. Similarly, FLT3-ITD mutation represents high tumor mutation burden but lacks effective immunopeptidome for T cell recognition. Therefore, the current focus is on developing tyrosine kinase inhibitors for this

Table 3
Potential targets of AML-presented neoantigens.

Protein	Mutation rate in AML	Mutagen	Mutant sequence	Predicted antigenic sequence	Predicted HLA binding	T cell assay	Ref.
NPM1	30 %	TdT	75-80%TypeA	CLAVEEVSL	HLA-A[a]02:01	HD-derived $NPM1^{mut}$-specific CTLs	[81]
				AIQDLCLAV	HLA-A[a]02:01	CAR-T	
				AVEEVSLRK	HLA-A[a]11:01; HLA-A[a]03:01	Patient-derived $NPM1^{mut}$-specific CTLs	[78]
				CLAVEEVSLRK	HLA-A[a]11:01; HLA-A[a]03:01	HD or Patient derived $NPM1^{mut}$-specific CTLs	[98]
			10 % TypeB	AVEEVSLRK	HLA-A[a]11:01; HLA-A[a]03:01	HD or Patient derived $NPM1^{mut}$-specific CTLs	[78]
			5 % TypeD	CLAVEEVSL	HLA-A[a]02:01	HD-derived $NPM1^{mut}$-specific CTLs	[81]
				AIQDLCLAV	HLA-A[a]02:01	CAR-T	
				AVEEVSLRK	HLA-A[a]11:01	Patient-derived $NPM1^{mut}$-specific CTLs	
				CLAVEEVSLRK	HLA-A[a]11:01; HLA-A[a]03:01	HD or Patient derived $NPM1^{mut}$-specific CTLs	[98]
FLT3	30–40 %	TdT	84 % Type A (Exon14 ITD) 6 % Type B (Exon 14+ Part Intron 14) 5 % Type C (Exon 14-Exon 15 Spans Intron 14)	YVDFREYEYY	HLA-A[a]01:01	Auto-CTL	[83]
IDH1	6.6 %-8.7 %	N/A	R132H	N/A	N/A	N/A in AML	[99]
IDH2		N/A	R140Q	N/A	N/A	N/A in AML	[87]
			R172	N/A	N/A		

TdT, Terminal deoxynucleotidyl transferase; Auto-CTL, Autologous cytotoxic T lymphocyte.
HD, Healthy donor; N/A, Not available.
[a] Tables may have a footer.

FLT-ITD AML subset. Recently, an FDA-approved multifocal tyrosine kinase inhibitor, midostaurin, has been incorporated into the front-line induction chemotherapy of newly diagnosed FLT-ITD AML subsets [91]. Importantly, FLT3-ITD are detected in approximately 43 % [92] of NPM1-mutated normal karyotype AML patients, and co-occurrence of FLT-ITD translocation and NPM1 mutation has poor prognosis and high relapse rate. Their shared mutagen, TdT, highlights the need for further exploration of the contextual incidence of these two AML driver mutations. Additionally, as these built-in AML-specific mutations only present a limited MHC1 ligandome, we will describe the potential obstacles between nuclear neoantigen mutagenesis and eventual surface peptide: HLA1 complex expression that makes AML cells visible to $CD8^+$ T cells.

2.3.2. *The Antigen processing machinery (APM) in acute myeloid leukemia [93,94]*

The presentation of HLA1-bound neoantigen on cell surfaces renders AML blasts more vulnerable to $CD8^+$ T cells, which orchestrate anti-leukemic responses. Surface neopeptide-HLA1 complex detection attests to the successful catalysis and matching of AML-generated neopeptides with specific HLA1 molecules with specific peptide binding domains. However, the mechanism underlying the catalysis and transportation of surface immunopeptides from intracellular proteins requires elucidation. As previously mentioned, a correlation between decreased diversity (as measured by HED) and binding specificity (as measured by promiscuity, Pr) of HLA1 alleles and poor tumor control by $CD8^+$ T cells has been highlighted. This suggests that the accommodation of antigenic peptides in the HLA1 complex influences the functional outcomes of $CD8^+$ T cell-AML blasts interaction. Intracellular post-translational modifications of AML-associated antigens theoretically contribute to the final HLA1 immunopeptidome. Although the "loss of heterozygosity" of mismatched HLA1 alleles is a possible immune evasion mechanism of AML blasts after relapse following haploidentical allo-HSCT, the baseline characteristics of HLA1 alleles in newly diagnosed AML patients are not reported.

An extensive description of post-translational antigen processing pathways for MHCI and MHCII is provided by Janice S. Blum et al. (2012) [95]. The machinery underlying antigen presentation are fractionalized into individual components including phagosome, proteasome (LMP2/7), transporter associated with antigen processing (TAP1/2), TAP binding protein (TAPBP, tapasin), protein disulfide isomerase (PDIA3), calreticulin (CRT), and ER aminopeptidase (ERAP). An initial assessment of the APM of AML tissue microarray revealed that all patients had lost at least one of the HLA1 APM components (Table 4) [96]. Defects in proteasome and antigen transporter expression in 14 % and 31 % of AML patients, respectively, suggest a potentially limited cancer immunopeptidome due to impaired antigen digestion and transportation efficacy. Notably, defects of MHC1 APM in AML blasts can be restored by appropriate cytokine stimulation. Following a 24-h IFNγ incubation, transcript and protein levels of LMP2, LMP7, TAP1, TAP2, and β2m in $CD34^+$ AML blasts elevated, indicating that the downregulation of AML MHC1 APM is reversible with an appropriate pro-inflammatory environment. Moreover, the surface expression levels of MHC1 complexes (HLA-A, B, C) were not significantly altered in AML patients at diagnosis or relapse prior to further treatment compared to healthy leukocytes [97]. This suggests that the quantity of MHC1 complex remains unchanged even with a general defect of APM components. However, whether the quality of the MHC1 ligandome of AML blasts is affected requires further exploration. As mentioned earlier, lower peptide binding specificity (high promiscuity) can lead to a poor cancer prognosis, which is correlated with T cell exhaustion. Therefore, the following paragraphs will discuss the current knowledge of AML APM components, including the mechanistic defects and correlations with disease progression or treatment (as summarized in Fig. 5).

2.3.2.1. Proteasome. Antigenic polypeptides require proper proteasomal digestion to fit into the peptide binding cleft of the MHC1 complex. There are two proteasome subtypes - the constitutive proteasome (cP) and the immunoproteasome (iP) - that share the same core unit but have distinct catalytic subunits. Upon IFN- γ induction, three de novo synthesized subunits - β1i (encoded by the PSMB9 gene in humans and LMP2 in mice), β2i (encoded by PSMB10/MECL1), and β5i (encoded by PSMB8/LMP7) - replace β1, β2, and β3 subunits, catalyze endogenous proteins into appropriate substrates of HLA-1 complex [101], and facilitate subsequent cancer-specific

Table 4
Expressions of central MHC1 antigen presentation machinery in AML.

Type of APM/Reference	In situ tissue microarray [96]	MILE study [100]**
Sample status	At diagnosis	At diagnosis (NK-AML)
HLA-A	N/A	↓***
HLA-B	N/A	↓***
HLA-C	N/A	↓***
PSMB8(LMP7)	**High**	N/S
PSMB9(LMP2)	**Low**	↓
PSMB10	N/A	↑
TAP1	**Low**	N/S
TAP2	**Low**	N/S
Tapasin	N/A	↑
PDIA3(ERp57)	N/A	N/S
Calreticulin	N/A	N/A
ERAP1	N/A	↑
ERAP2	N/A	↑

APM: Antigen presentation machinery; N/S: Not significant; N/A: Not available; Low: >70 % partial or faint staining; High: >30 % high staining; NK-: Normal Karyotype ** Compared normal karyotype AML to healthy bone marrow ***Pooled HLA-A,B,C expression is insignificant in pAML [97].

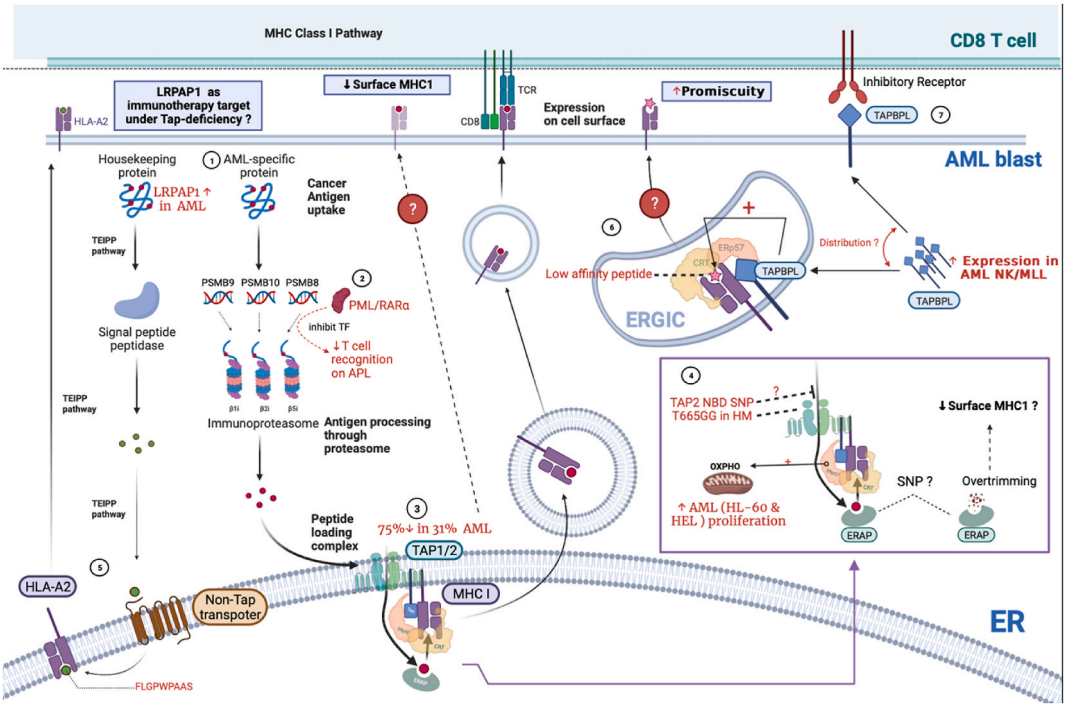

Fig. 5. The aberrant type 1 MHC antigen presentation machinery of AML. This figure denileates the multifaceted mechanisms of MHC class I-mediated antigen presentation and their deviations in AML, which potentially compromise CD8+ T cell immunity. Key processes and associated aberrations are numerically indicated: (1) AML-specific antigen level: As summarized in (Table 3). (2) Proteosomal level: PML/RARA impedes PSMB 8/9/10 transcription and results in the absence of surface HLA molecules in patients with APL. (3) Peptide loading complex level: Significantly reduced TAP 1/2 expression in 31% of patients with AML. (4) SNPs of TAP2 NBD found in hematological malignancies and their potential roles on type I MHC immunopeptidome abundance through dynamic peptide trimming. (5) Non-Tap transporter scenario: Upregulated LRPAP1 in AML and their downstream product, one kind of TEIPP, potentially serve as a HLA-A2-restricted epitope that servers as a marker of AML cells. (6) Alternate chaperone: TAPBPR/TAPBP promotes low-affinity epitope expression and increases immunopeptidome promiscuity. (7) TAPBPL probably acts as an immune checkpoint inhibitor in patients with AML.

T cell infiltration [102]. Mechanistically, iP can generate short peptides with a more hydrophobic C-terminus than cP, enhancing cytosol-to-ER transportation efficiency and accounting for up to 50 % of murine surface MHC1 expression. The clinical relevance of iP to AML involves at least two contradictory aspects: promoting MHC1 antigen presentation and maintaining leukemia cell viability.

Role 1 Maintaining MHC-1 antigen processing and presentation

Deficiencies in any iP components can result in significant CD8 T cell response loss. For instance, in acute promyelocytic leukemia (APL), PML/RARα fusion protein impede the transactivation function of PU.1, a transcription factor of PSMB 8/9/10, leading to nearly absent surface HLAs (Fig. 5. Mechanism 2) [103]. This observation suggests a possible immune evasion mechanism due to transcriptional defects of iPs. Notably, the *in vitro* treatment of human APL cells with a DNA methyltransferase (Dnmt) inhibitor, 5-azacytidine, can restore β2i and β5i expression [104], which promotes T cell infiltration against cancer cells. This restoration can be achieved as 5-AZA reduces mRNA and protein levels of DNMT1 and DNMT3A, indicating that immunoproteasomes in APL are under transcriptional and epigenetic regulation.

Role 2 Maintaining cancer cell survival

Remarkably, the transcript levels of three iPs appear to have no apparent correlation with the overall survival of AML patients. However, PSMB9 and PSMB10 are upregulated in several AML subtypes, suggesting that the clinical impact of iPs may result from a blend of both pro-leukemia and immunogenic roles. In addition to their immunogenic functions, iPs, as a pivotal component of the ubiquitin proteasome system (UPS), can eliminate the accumulated oxidized proteins through mTORC1 binding under oxidative stress [105]. This makes immunoproteasomes an attractive target in cancer due to the high demand for protein metabolism in the fast-expanding tumor microenvironment. Proteasome inhibitors (PI: Bortezomib, Carfilzomib, Ixazomib) targeting cP and/or iP have profound therapeutic effects on hematological malignancies [101], particularly multiple myeloma (MM). Administration of proteasome inhibitors in AML treatment, such as anthracycline-based chemotherapy and histone deacetylase inhibitors [106], synergistically

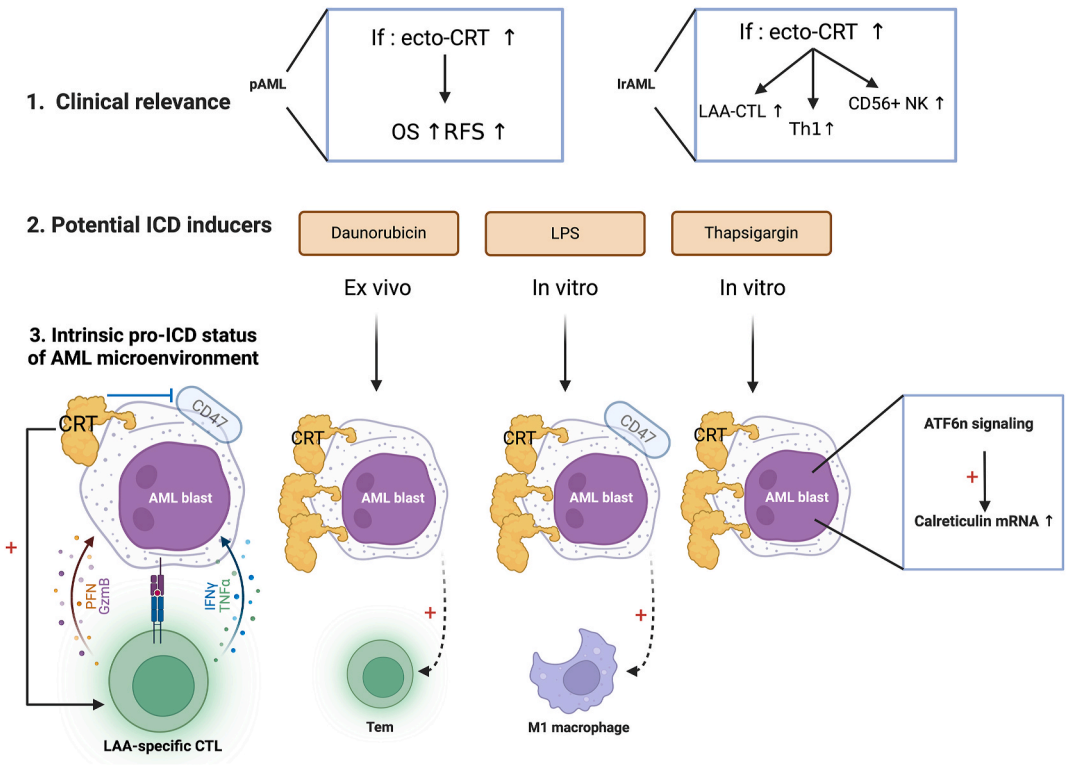

Fig. 6. Summary of calreticulin as a pivot in immunogenic cell death (ICD) of AML microenvironment:1. In patients with newly-diagnosed AML, a higher ecto-calreticulin (ecto-CRT) level is associated to improved overall survival (OS), and relapse-free survival (RFS). At the stage of complete remission after induction chemotherapy (IrAML), patients with initially high ecto-CRT level before chemotherapy were transcriptionally associated with the activation of several types of anti-tumoral immune cells. 2. (1) *Ex vivo* daunorubicin induced ecto-CRT expression of AML blast and enhanced the abundance of effector memory T cell(Tem). (2) A well-known ICD-inducer, LPS, increased AML blast engulfment through promoting M1 macrophage, while inhibiting CD-47 expression on AML blasts. (3) An endoplasmic reticulum (ER) stress inducer, thapsigargin, transcriptionally enhanced ecto-CRT expression in AML blasts. 3. Compared to healthy individuals, an intrinsic, chemotherapy-independent ecto-CRT upregulation existed in AML patients, potentially acting as a damage-associated molecular pattern (DAMP) to elicit leukemic-associated antigen-specific cytotoxic T lymphocye (LAA-specific CTL), and meanwhile combat the "do-not-eat me" signal, CD47 on AML blasts.

alter the inhibition of NF-κB signaling, inhibition of AKT signaling, and down-regulation of antiapoptotic proteins. Patients with FLT-ITD mutation are more susceptible to bortezomib, a reversible inhibitor that binds to both constitutive and immunoproteasomes.

The heterogeneous roles of IPs among different subsets of AML should be emphasized for a more precise selection of PI recipients [104]. For instance, the MLL^{+} subset of AML-M5 has a higher iP expression, while the M3 subset expresses fewer iP coding genes. High iP expression in the M5 subset is ontologically associated with cell metabolism, stress response, and mitochondria networks. In contrast, the prominent iP co-clustered genes are immune process-related in the non-M5 AML subset. In short, current evidence of iP profile in AML encompasses at least two functions: (1) Functioning as immunogenic machinery in M3 or non-M5 AML, and; (2) an upregulation in M5 AML is interpreted as an endogenous stress responder of leukemia cells.

2.3.2.2. Transporter associated with antigen presentation (TAP). The antigen presentation in AML critically depends on the TAP proteins, which facilitate the transport of tumor-derived peptides to MHC class I molecules, a process pivotal for eliciting a $CD8^{+}$ T cell response.

2.3.2.2.1. TAP in cancer and AML. Downregulation of MHC-1 antigen presentation machinery, including TAP, has been reported in several cancers. Loss of TAP 1/2 expression can result from the downregulation of their transcription factor NLCR5 and epigenetic silencing through DNA hypermethylation or H3K27me3 histone modification. A combined expressional defect of TAP1 and TAP2 (positively stained blasts below 25 % of the overall observed blasts) was found in 31 % of AML patients' bone marrow biopsies (Fig. 5. Mechanism 3) [96]. Moreover, the TAP1(ABCB2) gene was relatively homogeneous and overexpressed in pediatric AML samples but did not correlate with any definite clinical feature, such as responses to induction therapy or chemotherapy sensitivity [107]. Similarly, the gene expression profiles of both TAP1/2 of AML patients had an insignificant effect on the overall survival rate, according to the TCGA database.

In addition to quantitative reduction, partial dysfunction mediated by SNPs represents an additional route for TAP1/2-related immune evasion in cancer. For instance, an SNPs analysis in NSCLC patients from the Han Chinese population [108] revealed that the A allele of TAP2 SNP rs2228396 (NBD, alanine-to-threonine) and the C allele of TAP2 SNP rs241441(synonymous variant) were

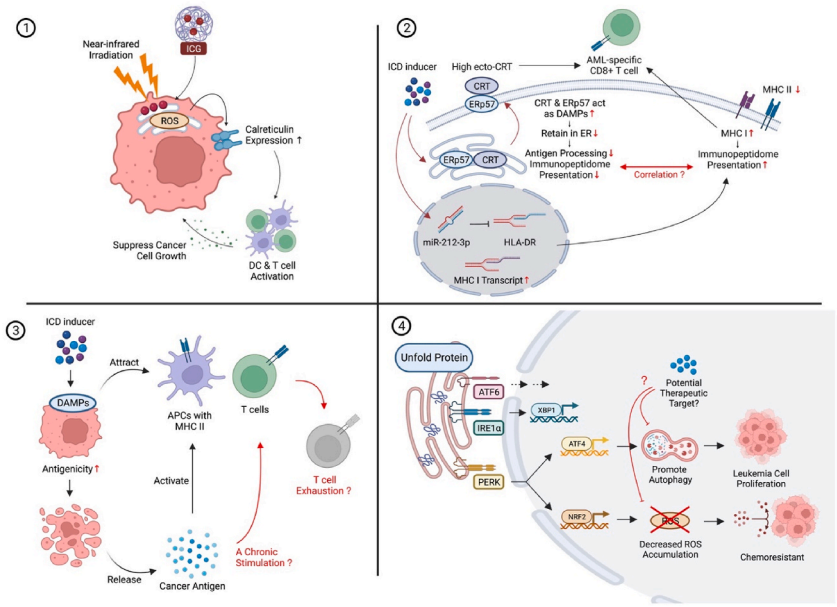

Fig. 7. Future aspects of ICD-related therapeutic targets in AML.

associated with higher risks of NSCLC (OR = 1.30 and 1.57, respectively). The pathophysiology of TAP SNPs may be closely related to an altered ATP-binding motif [109], as rs2228396 is located in the NBD of TAP2. Other cancer-related TAP SNPs include TAP2 SNP rs241447(TAP2 NBD-T665A) in some non-Hodgkin lymphomas(NHL) [110], TAP2-rs4148876 in cervical cancer [111–113], and TAP1 I333V and D637G in high-grade CIN, which have been found to be correlated with cancer risk.

Despite the potential for polymorphisms of TAP1/2 in solid tumors to modify the specificity of peptide epitopes and induce immune evasion, it is presently unclear whether these SNPs are present in hematological malignancies(HM) or AML, and if they have any clinical relevance. The foremost investigation of TAP1/2 polymorphisms in hematological malignancies, covering CML, CLL, and MM [112], shows a 2.04-fold surge in the occurrence of TAP2-665 (at TAP2 NBD) G allele in HM samples, along with a 15.1-fold escalation in the risk of HM in samples with TAP2-665 GG genotype (Fig. 5 mechanism 4). Notably, the examination of TAP polymorphisms in human melanoma uncovered unaltered trafficking activities from cytosol to ER, as well as similar levels of surface MHC1 expression among all TAP variants examined when compared to wild-type TAP [114]. However, the TAP2-665 GG SNP that is pertinent to HM was not analyzed in this study. These findings suggest that TAP polymorphisms may alter cancer susceptibility by modifying surface MHC1 abundance, with varying effects that could be dependent on allotype, disease type, or both. Unfortunately, the extent to which abnormal TAP transporter expression affects neoantigen presentation in AML blasts is not yet fully understood.

2.3.2.2.2. Non-TAP transporter of antigenic peptide. The redundancy of TAP in APM has been confirmed as TAP-deficient cells retain partial capacity to present MHC1 antigen and provoke a CD8 T cell response. T-cell epitopes associated with impaired peptide processing (TEIPP), referring to an epitope repertoire processed and presented via a TAP-independent pathway, have been first reported by Van Hall et al. [115]. These TEIPPs comprise non-mutated self-antigens originating from housekeeping proteins, with approximately 30 % being derived from the N-terminal signal peptides of their native proteins. This structural feature results in higher binding affinity of TEIPPs to the HLA-A2 allele than to other HLA1 molecules in the absence of TAP (Fig. 5. Mechanism 5). Thus, TEIPPs can act as surrogates of endogenous immunity when the conventional proteasome-TAP-mediated transportation of MHC1 epitope is hindered. Notably, TEIPPs differ from the conventional APM in at least three aspects: First, proteasome-independent degradation of antigenic peptides; Second, tap-independent trans-ER peptide loading machinery; Third, involvement of non-conventional MHC1 complexes. Furthermore, in the absence of proteasome and TAP, signal peptide peptidase (SPP) [116] or furin (a protein convertase) takes on the responsibility of protein digestion and peptide loading.

In TAP-deficient mice, ER SPP trims the C terminus of Trh4 (also known as Lass5 protein), producing an MHC1-restricted TEIPP "MCLRMTAVM." Structurally, the binding of Trh4 TEIPP requires the MHC1 molecules Kb, Db, and Qa1b. Trh4-specific cytotoxic T lymphocytes (CTLs) can recognize and eliminate RMA-S cells, demonstrating the immunogenicity of specific tumor epitopes in a TAP-deficient manner [115].

In human lung carcinoma cell also deficient in TAP, the Preprocalcitonin signal peptide [117] can be processed by signal peptidase and signal peptide peptidase to produce a VLLQAGSLHA (ppCT16-25) 10-mer epitope, presented by HLA-A2 and recognized by Heu161 CTLs. Furthermore, human TEIPP-specific CD8 T cells remain naive and likely to respond once they encounter TEIPPs, as they do not experience negative thymic selection. Consequently, TEIPP serve as a potent neoantigen selectively presented in TAP-independent conditions, such as tumors with decreased TAP expression.

To identify potential immunotherapeutic TEIPP targets for TAP-deficient cancer, Van Hall et al. [118] developed a platform to filter high-affinity human proteome TEIPP (signal peptide and tail peptide included) on HLA1 alleles overlapping with cancer-specific

peptidomes. They discovered a healthy donor HLA-A*02:01 restricted "FLGPWPAAS" TEIPP (termed p14), an epitope generated from LRPAP1, which is highly expressed in certain cancers such as lymphoma, melanoma, colon carcinoma, and renal cell carcinoma. The profound CD8 T cell reactivity specific to FLGPWPAAS occurred exclusively in a TAP-deficient context of these cancer cell lines.

Although the significance of the TEIPP pathway in the AML microenvironment is unclear as none of the HLA-restricted TEIPP of AML cells has been reported, it is worth noting that compared to healthy bone marrow, LRPAP1 gene expression was elevated in AML t (15; 17) but insignificant in normal karyotype AML or complex AML. Given the downregulated immunoprotease profile in t(15; 17) rearrangement in APL, the LRPAP1-derived epitope can be exploited as a proficient therapeutic target to enhance $CD8^+$ T cell immunity in AML subsets with an impaired MHC1 APM.

2.3.2.3. Tapasin. TAP binding protein (TBP), or Tapasin, a key component of the peptide loading complex, is crucial for the presentation of neoantigens by MHC1 in AML. It enhances the stability and affinity of peptide-MHC1 complexes, facilitating the immune system's recognition of AML cells. The process involves tapasin binding to the MHC1 heavy chain, which allows for the exchange of low-affinity peptides with those of higher affinity, thus optimizing the immune response [119]. Although intricate, these molecular interactions underscore the potential of targeting tapasin in AML therapy to enhance antigen presentation and T cell-mediated elimination of leukemic cells [120].

The significance of tapasin in hematological malignancies remains elusive. Co-incubation of CD40L with t(9; 22) ALL cells generates dendritic-like cells with elevated subcellular tapasin levels but unchanged surface HLA1 (HLA-A, B, C) expressions, rendering them highly immunogenic to induce CD8 T cell cytotoxicity regardless of the MHC1 abundance [121]. In addition to Philadelphia chromosome-positive t(9; 22) ALL, several MHC1 APM components, including LMP10, TAP1, TAP2, and tapasin, were upregulated at the mRNA level in dendritic-like cells derived from CD40L-incubated Philadelphia chromosome-negative children B-cell precursor ALL [122]. This suggests that CD40L stimulation can entitle specific lymphoblastic leukemia cells for targeting by $CD8^+$ T cell targets through stimulating MHC1 APMs. However, whether up-regulating tapasin is sufficient to elicit anti-leukemic CTL response needs further confirmation. In mice harboring AML C1498.SIY cells, agonistic anti-CD40 [123] mAb increases overall survival and alleviate T cell dysfunction by restoring the proliferation and accumulation of antigen-specific CD8 T lymphocytes. Nevertheless, the role of tapasin or other MHC1 APMs this circumstance has yet to be demonstrated. Thus, it remains unclear whether tapasin is a controllable factor by the CD40:CD40L axis for better immunogenicity of AML blasts.

TAP-binding protein-related(TAPBPR) is an alternative chaperone that is PLC-independent and can still transport antigenic peptides to the MHC1 complex on the ER membrane without binding to TAP as tapasin does [124]. TAPBPR shares about 20 % sequence similarity with tapasin, but it operates differently. Mechanistically, TAPBPR can alter the conformation of the MHC-I complex to a "peptide-receiving state" by expanding its peptide-binding groove in the region of the F-pocket [125,126]. Additionally, TAPBPR possesses a conserved MHC1 binding motif, similar to that of tapasin, which functions as a peptide editor. TAPBPR's peptide editing process [127] includes stabilizing an MHC1 complex without peptide binding in a peptide-receptive conformation, discarding suboptimal peptides via broadening the peptide binding groove of MHC1 in the α2-1 region, and regulating a dynamic switch located in the MHC1 groove. Importantly, TAPBPR and tapasin are not interchangeable since TAPBPR has a unique functional partner, UDP-glucose: glycoprotein glucosyltransferase (UGT1) [128–130]. The interaction between TAPBPR and UGT1 facilitates the re-glycosylation of MHC1, allowing the MHC1 complex to be recycled to calreticulin within PLC for better peptide optimization [131]. The choice of using either tapasin or TAPBPR as an MHC1 chaperone depends on the peptide concentration in the surrounding environment. In a peptide-rich environment, tapasin is responsible for loading high-affinity peptides onto MHC1. In contrast, TAPBPR tends to function in a low peptide concentration environment, facilitating high and moderate affinity peptide binding to the MHC1 complex. Due to their selectivity for antigenic peptides, Tapasin and TAPBPR can produce distinct pMHC1 repertoires that are not identical (Fig. 5. Mechanism 6) [127].

TAPBPL/TAPBPR, in addition to its role in antigen presentation, is constitutively expressed on the plasma membrane of T cells, antigen-presenting cells (such as B cells, macrophages, and DCs), and certain cancer cells. When expressed on the cell surface, TAPBPL acts as an immunosuppressive signal due to its peptide sequence similarity [132] with some members of the B7 protein family (e.g., B7-H5/HHLA2, with which it shares 15 % amino acid sequence similarity). TAPBPL expression levels are higher in liver, lung, and prostate cancer tissues than in their corresponding normal tissues. Moreover, upon anti-CD3 and anti-CD28 stimulation, the TAPBPL receptor is upregulated on CD8 T cells. In this context, co-incubation with recombinant TAPBPL can inhibit the activation, proliferation, and cytokine production of CD69-mediated CD8 T cells. Notably, the receptor for TAPBPL is not a conventional T cell inhibitory receptor such as PD-1, BTLA, CTLA-4, or ICOS. Thus, given its inhibitory effects on CD8 T cells and its heterogenous expression in several immune cells and cancer tissues, TAPBPL may act as the PD-1:PD-L1 axis in an autocrine or paracrine manner (Fig. 5. Mechanism 7). Additionally, TAPBPL transcript levels are upregulated in AML with normal karyotype and AML with t(11q23)/MLL abnormalities, as analyzed by Blood Spot. Depending on the cellular localization of the increased TAPBPL in AML blasts, this phenomenon could have two distinct interpretations. Specifically, TAPBPL could be a novel surface inhibitory ligand that fosters a pro-leukemia microenvironment for certain AML subtypes.

Conversely, intracellular TAPBPL elevation can skew surface MHC1 immunopeptidome toward a group of lower affinity epitopes. However, neither the transcript level of TAPBPL nor tapasin independently influences the overall survival of AML patients. This likely implies that altering the quality control mechanisms of the MHC1 immunopeptidome is insufficient to elicit a profound anti-tumor effect.

2.3.2.4. PDIA3 (ERp57). Protein disulfide isomerase family A member 3 (PDIA3) is an oxidoreductase participating in oxidative folding of glycoproteins by catalyzing and remodeling disulfide bonds, while working in conjunction with calreticulin and calnexin. PDIA3 has four domains [133], of which the a and a' domains contain a thioredoxin-like active site with a C-G-H-C sequence. Meanwhile, the b and b' domains possess binding sites for calreticulin and calnexin, which are two glycoprotein-binding lectins in the ER. The calreticulin-PDIA3 complex is crucial in the construction of MHC1-PLC, due to its role in recruiting the MHC1 complex and facilitating high-affinity peptide loading [134,135].

In AML bone marrow samples, the PDIA3 transcript level is higher than that in healthy donors. PDIA3 is notably a pro-leukemic factor in the HL-60 and HEL AML cell lines, as it supports proliferation, anti-apoptotic events, and the migratory ability of specific AML subsets with promyelocytic or erythroid features [136]. A possible explanation for this pro-AML benefit is that the upregulated PDIA3 in AML partially enhances the oxidative phosphorylation machinery, thereby contributing to the leukemogenesis of AML. Additionally, the mechanistic aspects of PDIA3 in the above contexts could be partly explained by its coordination [137] with high mobility group proteins 1 and 2 (HMGB1 and HMGB2), which act as complex components to protect cancer cells from DNA damage-inducing drugs. However, based on the TCGA database, PDIA3 expression does not reflect the overall survival of AML patients. Hence, functional alterations rather than quantitative impairments of PDIA3 may need to be explored further to expand our knowledge of its role in AML.

2.3.2.5. Calreticulin (CALR). Calreticulin [138], an ER-luminal resident lectin chaperone, possesses a mono-glycosylated glycoprotein binding site and a calcium ion binding site. Its proline-rich P-domain adopts a β-hairpin to interact with co-chaperones PDIA3 [139], cyclophilin B [140], and PDIA1 [141]. The C-terminal region, which is rich in acidic amino acids, contains calcium ion binding sites [142] and an ER-retrieval KDEL motif that is crucial for retaining calreticulin within the ER lumen [143,144]. As a constituent of the MHC1 peptide-loading complex (PLC), calreticulin [145] binds tapasin-bound ERp-57 and brings the TAP/tapasin complex to the mono-glycosylated glycan of the MHC1 complex. Within the PLC, calreticulin aids the folding and stabilization of tapasin via glycan-dependent interactions. Additionally, calreticulin participates in the quality control of surface p-MHC1 complexes in the ER-Golgi intermediate compartment (ERGIC). After loading with antigenic peptide, the MHC1 complexes accumulate in the ERGIC before being transported to the plasma membrane [146]. In the ERGIC, the KDEL sequence on calreticulin guides the retrieval of sub-optimal peptide-bound MHC1 complex and transports them back to the ER. Calreticulin deficiency may lead to immune evasion, as it may result in the expansion of the immunopeptidome of specific MHC1 alleles, thereby resembling the high peptide promiscuity condition that correlates with poor cancer prognosis and some T cell exhaustion features.

The influence of calreticulin on MHC1 antigen presentation in cancer has been demonstrated in at least two ways [147]. Firstly, the downregulation of the PLC components, including calreticulin, correlates with reduced MHC1 expression in colorectal and bladder cancer, suggesting a potential mechanism of the immunosuppressive microenvironment in solid tumors. Secondly, a frameshift mutation of calreticulin (CRT-FS) in myeloproliferative malignancies results in the loss of its original KEDL sequence, leading to constitutive activation of the JAK-STAT cascade and subsequent tumorigenesis of essential thrombocythemia (ET) or primary myelofibrosis (PMF) with non-mutated JAK2. Furthermore, CRT-FS has an altered electrostatic distribution that impairs its incorporation into the PLC and reduces the surface MHC1 complex. However, information on the impact of mutant CRT on the MHC1 antigen presentation of myelodysplastic hematological malignancies, such as MDS and AML, requires further information.

2.3.2.6. Endoplasmic reticular aminopeptidase (ERAP). The initial partially degraded cytosolic polypeptide needs to be further trimmed into an appropriate length of 8–10 amino acids for binding to MHC1 molecules. Upon encountering an Endoplasmic Reticulum aminopeptidase (ERAP), the N-terminal residues of the pre-antigenic-polypeptide or peptides bound to MHC1 complexes will be cleaved to stabilize the p-MHC1 complex. In humans, ERAP1 and ERAP2 are homologs with distinct catalytic specificities due to a higher level of polymorphism in the ERAP1 allele [148,149]. Interestingly, ERAP can also "over-trim" MHC1 complex-bound precursor peptides and cause them to fall apart, which involves the partial dissociation of the N-termini of precursor peptide while its C-termini is buried in the F pocket region of the MHC1 complex [150]. The switch between these two functions is influenced by the polymorphic ERAP allotypes, and distinct SNPs can lead to hypoactive or hyperactive functional subsets of ERAP. Furthermore, SNPs in ERAP alleles can also alter the trimming specificity of selective peptide substrates into immunogenic epitopes [151]. In cancer, the knockdown of murine ERAP (ERAAP) enhances the immunogenicity of CT-26 cells [152]. This is because ERAAP in CT-26 tends to destruct rather than properly digest the CTL reactive epitope GSW11. Additionally, a small molecule inhibitor of ERAAP can increase the cytokine released by antigen-specific $CD8^+$ T cells and the *in vivo* survival rate of CT-26 challenged mice. In AML patients, the transcript levels of both ERAP1/2 are upregulated, but this is not correlated with any discrepancy in overall survival. However, it is worthwhile to examine the exact allotypes of this upregulation and their potential links to assist AML blasts in evading specific antigen-specific $CD8^+$ T cell recognition.

2.4. Known MHC-1 APM components simultaneously participating in the immunogenic ER-stress responses: immunogenic cell death of AML blasts

ERp57 and calreticulin play essential roles in preserving the function and stability of the MHC1 peptide loading complex. Although their clinical significance in AML blasts APM has yet to be demonstrated, the ecto-ER forms of ERp57 and calreticulin in ER stress have been exhaustively discussed in AML. Specifically, ERp57 and calreticulin levels positively correlate with the survival of chemotherapy-treated AML patients via immunogenic cell death (ICD). Besides conventional pathogen-host recognition, ICD represents a contextual

cancer-cell-specific killing event mediated by the treatment-induced expression of host molecules. Thus, cancer cell surface- or extracellular-expressed "Damage-Associated Molecular Patterns (DAMPs)," such as calreticulin, attract the collateral anti-tumor networks such as type-I interferon, DCs, or CTLs. Consequently, the induced mobilization of ER-localized chaperones to the cell surface in an ICD event can be considered a prelude to eliminating AML blasts. Known ICD inducers include chemotherapy (Actinomycin D, Anthracyclines, Bleomycin, Cyclophosphamide, Lurbinectedin, Oxaliplatin, PT-112, Taxanes, Teniposide), endocrine therapy (Mifepristone), extracorporeal photochemotherapy (8-MOP), oncolytic virotherapy, photodynamic therapy, radiotherapy (γ irradiation), and targeted therapy (Belantamab mafodotin, Bortezomib, CDK4/CDK6 inhibitors, Cetuximab, Crizotinib) [153]. These interventions not only operate on their original targets but also yield considerable perturbation on the ER homeostasis, trigger the ICD cascade, and increase the immunogenicity of cancer cells.

The signaling cascade underlying ICD partially overlaps with a relatively well-characterized ER-stress regulatory mechanism: the Unfolded Protein Response (UPR). UPR is a conserved eukaryotic stress response network that maintains protein homeostasis within the ER compartment [154–156]. Generally, the UPR pathway is constitutively repressed unless sufficient misfolded or unfolded proteins occur and displace the UPR repressor, such as GRP78(BiP). Subsequently, UPR sensors on the ER surface will orchestrate the downstream molecular events to maintain protein quality control. In particular, there are three classical human UPR transmembrane sensors: IRE1α, PERK, and ATF6α3 [155].

Inositol-requiring transmembrane kinase/endonuclease-1 (IRE1α) undergoes a conformational change into an active oligomer state when its chaperone GRP78(BiP) [157] selectively binds to and releases unfolded protein peptides, thus enabling IRE1α to exhibit RNase activity for unconventional spling of XBP-1 mRNA [158]. The transcription factor, XBP-1, regulates genes responsible for protein quality control and endoplasmic-reticulum-associated protein degradation (ERAD), although the RNase has numerous substrates [159] that can lead to distinct cell fates, either stress-endurance or pro-apoptotic [160–163]. XBP-1 is currently used as a UPR activation marker in AML, but the clinical significance of non-coding RNA targets of IRE1α in AML require further examination. PERK, another UPR sensor, initially phosphorylates eIF2α and activates two downstream signaling pathways, temporarily suppressing protein synthesis and selectively enhancing stress gene expression, such as ATF4 [164]. The third UPR sensor, ATF6, is activated by the accumulation of unfolded proteins and transferred to the Golgi apparatus, where it is trimmed to become ATP6p50, a basic leucine zipper containing transcription factors for ER proteostasis gene expression. The surface activation of ATF6 under ER stress is mediated by the displacement of unfolded protein-bound BiP and structural interactions between ATF6 and ER enzymes such as ERp18 and/or PDIA5. Cancer cells can use the UPR pathway as a pro-tumorigenic process to maintain their survival in the malignant microenvironment featuring intrinsic dysregulated protein homeostasis [165–169]. However, if ER stress overwhelms UPR tolerance, PERK activation can induce downstream effectors, including ATF4, and C/EBP homologous protein (CHOP), leading to a pro-apoptotic state in cancer cells.

2.4.1. MHC 1 APM chaperone-mediated ICD event in AML (summarized in Fig. 6)

2.4.1.1. Calreticulin. Anthracycline-based regimen [170] have shown potential as inducers of ICD in AML blasts. AML treatment by daunorubicin ex vivo has been found to increase ecto-CRT expression and result in a higher ratio of $CD8^+$ memory cells (Tem) to effector T cells, which is associated with better overall survival. However, current induction therapy regimens for AML have not significantly increased the release of several DAMPs, including ecto-CRT, in real-world patients [171].

Moreover, treatment-naïve pAML patients have higher ecto-CRT expression on AML blasts than PBMCs of healthy donors. This suggests a non-standard calreticulin trafficking in AML blasts that is endogenously generated during leukemogenesis but is unresponsive to specific ICD inducers. Interestingly, this chemotherapy-independent elevation of ecto-CRT on AML blasts is still a prognostic factor, as AML patients with higher ecto-CRT expression before chemotherapy have better relapse-free survival (RFS) and OS. Additionally, patients with higher-than-median calreticulin mRNA also have a better overall survival rate [171]. This implies that higher ecto-CRT, transcriptionally and translationally, is more of a pre-treatment predictor for future prognosis than a marker of the magnitude of chemotherapy-induced ICD events in AML patients. However, the regulatory mechanisms of this intrinsic high ecto-CRT expression require further clarification.

Several molecules have been found to promote CRT externalization in AML blasts. *In vitro* studies using K562 or U937 cell lines have demonstrated a clear pathway for UPR-induced calreticulin expression [172]. Upon exposure to the ER stress inducer thapsigargin, a nuclear form of ATF6(ATF6n) binds and activates the calreticulin promoter through two sequences, ERSE (ER-stress response element) 1 and 2, which belong to the promoter region of the CALR gene. Three transcriptional factors, YY1, NF-Y, and ATF6n, directly interact with ERSE and are essential for this pathway. On the other hand, co-culturing THP-1 macrophages with lipopolysaccharide (LPS) and several AML cell lines (HL-60, NB4, THP-1) has been found to increase the engulfment of AML blasts by promoting the development of M1-like macrophages from THP-1 macrophages, while simultaneously increasing ecto-CRT and decreasing CD47 expression on these AML cancer cell lines [173].

Remarkably, despite the fact that current chemotherapy modalities cannot induce high ecto-CRT level in pAML patients, ecto-CRT still functions as a chemoattractant for several anti-tumor immune cells, which in turn are linked to improved disease outcomes in AML. Specifically, the protein level of ecto-CRT in AML positively correlates with the expansion of tumor-associated-antigen-specific T cells and the reduction of the "do-not-eat-me" signal, CD47, thereby promoting the engulfment of AML blasts by macrophages [174]. Moreover, constitutive surface calreticulin expression on murine AML C1948 cells improves T cell functions, including an increase in IFN-γ secretion by $CD4^+$ T cells and an enhancement of the frequency of $CD8^+$ T cells exhibiting the degranulation marker CD107a [175]. Additionally, the proportion of IFN-γ -secreting CD8 and CD4 lymphocytes specific to leukemia-associated antigens(LAAs)

(BIRC5, CCNB1, PRAME, and WT1) is higher in patients with high ecto-CRT expression prior to chemotherapy or during recovery compared to those with low ecto-CRT expression [171]. Interestingly, expression levels of certain LAAs (BMI1, PRAME, and HMMR) are significantly decreased in ecto-CRT^{High} pAML blasts, potentially reflecting the elimination of AML blasts by LAA-specific effector cells. In addition to adaptive immune cells, ecto-CRT on AML blasts also invigorates the quantity and effectiveness of natural killer (NK) cells [175]. Patients with initially high ecto-CrT levels exhibit higher levels of CD45^{+} CD3^{-} CD56^{+} NK cells in their peripheral blood but not in bone marrow samples following complete remission after anthracycline-based chemotherapy. Furthermore, the cytotoxicity of NK cells is indirectly enhanced by a group of myeloid CD11c^{+} CD14^{High} cells expresseing higher IL-15Rα and CCR7 in CRT^{High} AML patients [175].

On a more comprehensive scale, high ecto-CRT levels contextually modulate the immunosuppressive transcriptional profile of AML patients' PBMCs as they reach complete remission and restore normal hematopoiesis following induction chemotherapy [171]. At these time points, ecto-CRT^{High} AML patients exhibit upregulated gene clusters associated with immune cell populations, TH1 polarization, CD8^{+} T cell cytotoxicity, NK cell effectiveness (CD158 gene family, KLRF1, KLRC2, NCR1), and the peripheral immune cell population of circulating CD33^{-} CD3^{-} CD56^{+} NK cells. Meanwhile, the differential hierarchy of CD8^{+} T cells skews towards naive T cells and central memory T cells, which preserve a better repopulation capacity of the entire CD8^{+} T cell progeny. Conversely, high ecto-CRT levels in newly diagnosed AML patients are not correlated with the upregulation of immunosurveillance gene clusters. The activated UPR branch XBP1 in 17.4 % of newly diagnosed AML patients upregulates the calreticulin level [172], suggesting that the pre-treatment high calreticulin expression may partially result from autoregulation of AML blasts to withstand ER stress and maintain leukemic protein homeostasis.

By comparison, post-treatment high calreticulin tends to act as an antigenic DAMP that facilitates the degradation of AML blasts. Furthermore, the increase in immune surveillance against AML blasts and the survival benefits associated with high ecto-CRT upon complete remission can be attribute to a less exhausted and expanded population of naive CD8^{+} T cells observed at this stage. In contrast, high ecto-CRT on pAML blasts fails to generate sustainable anti-tumor T cell cytotoxicity due to the underlying exhausted CD8^{+} T cell signature of newly diagnosed AML patients (See 2.1.3).

In summary, despite the well established anti-tumor benefits of calreticulin as an inducer of ICD in several cancers, pre-treatment AML blasts appear to express ecto-CALR at levels as high as those treated with anthracycline compared to healthy donors. The initial high ecto-CRT of AML blasts may stem from an overall upregulated transcript level of calreticulin via the UPR and dysregulated intracellular trafficking of this chaperone. Moreover, in newly diagnosed AML patients, high ecto-CRT does not lead to an overall activation of the anti-cancer transcriptome, but does play immunogenic roles to the adaptive and innate immune cells. Lastly, the clinical benefit of a high initial ecto-CRT level still serves as a favorable prognostic predictor for AML patients, even though its expression is not inducible under specific conventional ICD inducers, such as anthracycline. Whether there are other potent ICD inducers for AML ecto-CRT expression or any alternative pathway to initiate the integrated stress response (ISR) in AML blasts requires future investigation.

2.4.1.2. ERp57. ERp57, a co-chaperone for calreticulin and an additional MHC1 APM component, has also been extensively implicated in immunogenic cell death (ICD) following ER stress induced by specific chemotherapy or irradiation [176]. ERp57 plays an essential role in translocating calreticulin [177], via protein-protein interactions, from the ER to the plasma membrane, thereby triggering ICD events in response to anthracycline induction. This translocation is actin-dependent and enables DAMPs recognition by innate or adaptive immune cells, thus facilitating the elimination of residual tumor cells following anthracycline-based chemotherapy. Nevertheless, in AML HL-60 cells subjected to the ICD inducer Shikonin, ERp57 acted as a protective factor against Shikonin-induced apoptosis [178]. Moreover, ERp57 is known to negatively regulate the PERK signaling pathway by reversing the oxidative status of protein disulfide isomerase-A1(PDIA1) [179]. Consequently, ERp57 can indirectly suppress the activation of the shared pathway of UPR and ICD. Therefore, the inhibitory role of ERp57 on ICD partially explains the Shikonin resistance observed in HL-60 cells, as well as why calreticulin does not serve as the primary DAMP under Shikonin-induced ICD [180]. In short, ERp57 can travel and function through the ER, cytoplasmic actin scaffold, and plasma membrane under ICD pressures. Moreover, overexpression of ERp57 has been observed in AML HL-60 cells before any treatment. In combination with its role in the PERK cascade, current knowledge suggests that ERp57 serves as a superior controller of ICD in AML cells, an spatial dynamics can be a pivot for switching between conventional ER-mediated antigen processing and stress-induced immunogenicity.

2.4.2. Future aspects (summarized in Fig. 7)

In summary, the MHC1 APM, UPR, and ICD courses in AML demonstrate high sensitivity to stress events involving calreticulin and ERp57. There are several future aspects that require elaboration.

(1) Cytotoxic therapies such as chemotherapy, irradiation, and proteasome inhibitors trigger ICD, but also damage host immune cells. Approaches have been developed to more efficiently and directly trigger a tumor-specific/regular cell-sparing exposure to DAMPs. For instance, ER-directed ICD induction can be accomplished by delivering an ER-targeting nanosystem carrying a photosensitizer, ICG, and triggering intracellular ROS accumulation by near-infrared irradiation on the ER-localized ICG. This approach led to a robust increase in calreticulin expression, immune cell activation (Dendritic cell/CD4^{+} Tcell/CD8^{+} Tcell), and suppressed cancer cell growth in the B16 murine melanoma tumor model [181]. Further exploration is required to determine if this nanosphere-delivery model could promote anti-leukemic events by increasing surface calreticulin expression in AML.

(2) If chaperones that act as DAMPs (e.g., calreticulin, ERp57) originate from the same protein pool shared with PLC components, the capacities of ER-localized calreticulin or ERp57could potentially decrease, deteriorating the quality of intracellular antigen processing workflow and resulting in an ill-presented immunopeptidome on a single cancer cell. A single-cell analysis is necessary to consolidate this hypothesis and compare the surface immunopeptidome of ecto-CRT^{High} and ecto-CRT^{Low} cancer cells. A recent study examined a similar viewpoint: In breast cancer and B-cell lymphoma cells exposed to ICD inducers such as the combination of retinoic acid (RA), interferon-alpha (IFN-α), and doxorubicin, MHC1-associated transcripts were upregulated, while MHCII-associated transcripts were downregulated [182]. To specify, miR-212-3p overexpression represses the surface expression of HLA-DR, a MHCII allele. This suggests the plasticity of antigen presentation potential upon ICD inducer treatment, and microRNAs may be the pivot mediator of APM fluctuation in this context. However, the correlation between the subcellular localization of calreticulin (or other ER chaperones) and the alteration of MHCI/MHCII transcript levels remains unclear. For AML patients, the ecto-CRT level cannot be induced by standard induction chemotherapeutic agents of AML. Nonetheless, a high calreticulin transcript level and ecto-CRT level before chemotherapy positively correlate with improved overall survival. Additionally, the high ecto-CRT on AML blasts can prime some LAA-specific CD8$^+$ T cells. The survival benefits of AML blasts with increased calreticulin transcript and steady expression of surface ecto-CRT may be attributed to an expanded quantity of ER-localized calreticulin, which facilitates a more robust HLA1 immunopeptidome of LAAs and better disease control by AML-specific CD8$^+$ T cells.

(3) Despite the fascinating discovery that DAMPs under ICD appear to enhance the antigenicity of AML blasts, it is crucial to cautiously evaluate whether a more drastic acceleration of T cell dysfunction occurs as these antigenic DAMPs surge. A possible explanation for this scenario is that the ICD inducers may not cause excessive T cell priming due to a simultaneous downregulation of MHCII complexes that suppress the co-stimulatory signals delivered by APCs. Furthermore, ICD is expected to induce cancer cell apoptosis, which may not provide chronic antigen stimulation but only serves as only a short-term stress response event that likely preserves CD8$^+$ T cells' function without inducing exhaustion.

(4) One of the three canonical UPR branches, X-box binding protein 1 (XBP1), is activated in pre-treatment AML patients, indicating that AML cells experience moderate rather than severe ER stress at the baseline. At the end of an activated PERK branch, two transcription factors, ATF4 and NRF2, help restore protein homeostasis. It is worth noting that ATF4 can also promote autophagy in FLT3-ITD AML cells, leading to AML blast proliferation and poor overall survival of xenografted mice [183]. On the other hand, NRF2 protects non-M3 AML cells from daunorubicin and cytarabine (Ara-C) treatment by reducing ROS accumulation and inhibiting early AML cell apoptosis [184]. Although ther is no direct evidence regarding the activation of PERK and ATF6α3 UPR branches in AML, these downstream effectors that sustain chemoresistance can help us more comprehensively understand the significance of UPR in AML and serve as therapeutic targets to enhance the mainstay of I3A7 induction chemotherapy.

3. Concluding remarks

Engineered T cell-based therapy, such as CAR-T, TCR-T, and donor lymphocyte infusion (DLI), have been investigated for treating relapsed/refractory or post-HSCT cases of AML. Concurrently, checkpoint inhibitors (ICIs) have been used as adjuvant to sustain efficacy and reduce exhaustion in leukemia-specific CTLs. AML is recognized as a highly heterogeneous hematological malignancy that employs various immunosuppressive networks on metabolic and cell-cell interactions levels [185]. These immune evasion networks often account for the failure of autonomous CTL-versus-leukemia events and impaired MHC1 antigen presentation, but their detailed mechanisms remain unclear.

We focus on the transcriptional landscape of pAML and attempt to explain the endogenous imbalance between host CD8$^+$ T cell and leukemia MHC1 antigen presentation workflow by the antigen-mediated T cell exhaustion theory. We observe that the CD8$^+$ T cell signatures from a healthy donor to pAML skew to an exhausted status with an elevation of some conventional exhausted T cell (Tex) markers. These Tex markers can be further described as surface inhibitory regulators (IRs), intracellular transcriptional factors, and T cell cytotoxic effectors. Central overexpression of the Tex key transcription factor, TOX, upregulates the IRs (TIGIT, PD-1). However, the definition of Tex as an isolated T cell progeny is not yet clear. A modern classification using Ly108 CD69 to partition four Tex sets with distinct differential trajectories might better resolve the future AML T cell exhaustion issue.

Furthermore, the origin of this impaired T cell population in AML could be attributed to an endogenous impaired hematopoietic program during leukemogenesis and an AML antigen-directed event. Although induction chemotherapy remains the mainstay of pAML treatment, the significance of CD8$^+$ T cell exhaustion is less emphasized in this group of patients. Notably, in the Tex development model in solid tumors, the gradual loss of CD8$^+$ T cell functionality and tumor consolidation are interdependent. Reshaping the deteriorating T cell fate with the aid of early detectable markers, such as TOX, TCF1, etc., might reverse the tumorigenesis process. However, applying this theory to AML is challenging due to the undetermined premalignant stage of the disease and the heterogenous subclones in AML patients. In addition to the overall trend of CD8$^+$ T cell phenotypes, the differential hierarchy is also tilted toward a more terminally differentiated distribution (less naive T and stem-cell-like memory T cells, more Temra) in pAML patients. This offers another perspective to intervene. Recent work has shown that maintaining the population of CAR-Tscm among overall CAR-T pools by γ-chain family cytokines in Hodgkin's lymphoma can prolong the cancer cell lysis effects and survival duration of the engrafted mice [186]. Other strategies to promote a robust GvL while mitigating GVHD include selective depletion of αβ-TCR T cells in the haploidentical donor grafts and cyclophosphamide-mediated depletion of proliferative alloreactive T cells [6].

Concerning antigen-driven T cell exhaustion, MHC 1 immunopeptidome may be one of the major contributors that should be examined from multiple perspectives, including TMB, HED, and peptide promiscuity. The primary constraint of our work is the lack of

data on the promiscuity of the AML HLA1 allele, which is a critical link between antigen processing and T cell exhaustion development. Despite the negligible alteration of HLA1 allele transcriptome in AML, the immunopeptidome can still be modified by post-translational modifications of the MHC1-bound epitope during disease progression. Our work underscores several possible modifications that contribute to MHC1 antigen presentation impairment, such as low transcript levels of APMs, PML/RARα-mediated immunoproteasome transcription suppression in APL, low-affinity peptide binding promoted by TAPBPL in the ERGIC, excessive trimming of the bound epitope by high-affinity ERAP allotypes, and so on. It is worth noting that overactive ERAP may account for the decreased peptide binding specificity of the HLA allele regulated by post-translational peptide digestion. To unravel the antigen presentation process and devise personalized strategies for AML patients, SNPs and their structural consequences on the ER-localized APMs such as TAP1/2 and ERAP1/2 should be more comprehensively considered in future studies.

Furthermore, the tap-independent MHC1 ligandome, called TEIPP, could broaden the scope of the current exploratory platform of the identification of AML neoantigen and immunogenic epitopes. Conversely, the well-established ICD event and its overlap with MHC1 APM raise several intriguing questions. With a delineation of the interplay between AML blasts and host $CD8^+$ T cells, the approach to addressing impaired autologous anti-leukemic events will become more powerful in the near future.

CRediT authorship contribution statement

Ching-Yun Wang: Writing – review & editing, Writing – original draft, Visualization, Validation, Conceptualization. **Shiuan-Chen Lin:** Writing – review & editing. **Kao-Jung Chang:** Writing – review & editing, Validation, Data curation. **Han-Ping Cheong:** Validation, Resources. **Sin-Rong Wu:** Validation, Methodology. **Cheng-Hao Lee:** Writing – review & editing. **Ming-Wei Chuang:** Writing – review & editing, Validation, Supervision. **Shih-Hwa Chiou:** Project administration, Investigation. **Chih-Hung Hsu:** Resources, Investigation, Conceptualization. **Po-Shen Ko:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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