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Moving forward in target antigen discovery for immunotherapy in acute myeloid leukemia

by Barbara Buldini and Elena Varotto

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In the last few decades, outstanding results have progressively been obtained in pediatric acute myeloid leukemia (pedAML) outcomes[1] due to improvements in risk stratification, response-to-therapy assessment, supportive care, and chemotherapy and hematopoietic stem cell transplantation management[2]. Despite this, refractory/relapsed disease still occurs in a significant proportion of patients and is associated with a dismal prognosis[3]. Therefore, the effort to develop new therapeutic strategies is critical. Among those, targeted immunotherapy, especially chimeric antigen receptor (CAR) T cells, is an intriguing approach, but, differently from B-lineage acute lymphoblastic leukemia, efficacious and safe targets to be used against ped AML have yet to be discovered. This is mainly due to two main reasons: (i) challenges in identifying targets expressed on both AML blast and leukemic stem cells but lacking on normal hematopoietic stem and progenitor cells (HSPC) to avoid irreversible myeloablation; (ii) potential disease relapses without target antigen on blast surface, due to antigen internalization or AML heterogeneity[4]. Up to now, myeloid lineage antigens, especially CD33, CD123, and CLL1, represent the most investigated targets for CAR-T development in AML, being widely expressed on myelomonocytic blasts. Regardless, their use is potentially burdened by severe on-target off-tumor toxicity since they are also present on normal myelomonocytic counterpart, including HSPCs[5]. To go beyond this side effect, combinatorial logic gating CAR-T cells have been proposed, whose activation mainly relies on three different mechanisms: (i) AND-gated CARs, which require the ligation of at least two of the targeted antigens for full activation and cytotoxicity (e.g., CD33/CD123, CD33/CLL1, CD123/CLL1); (ii) OR-gated CARs, which require the ligation of one of two or more targeted antigens

(e.g., CD33/CD123/CLL1); (iii) NOT-gated CARs, based on the co-targeting of two antigens, the first potentially expressed only on healthy tissues, the second both on healthy tissues and on malignant cells. The full activation and cytotoxic activity of NOT-gated CARs require the exclusive ligation of the antigen cross-expressed on both tumoral and healthy cells without the recognition of the selective healthy-tissue target, that consequently acts as CAR [4].

Nowadays, the scientific effort aims to discover even new leukemia-associated markers, overexpressed in blasts rather than normal tissues and neoantigens, deriving from leukemia-specific mutation, usually intracytoplasmic or presented on the cell surface by HLA complex. A list of potential targets for the development of CAR T cells against AML is summarized in Figure 1.

In this context, in the current issue of *Haematologica,* Menssen and colleagues presented the antigen CD74 as a potential new target for immunotherapy in pedAML. CD74, also known as an invariant chain of the major histocompatibility complex (MHC)-II, was initially described as a chaperone of the MHC-II, and more recently it has been involved in monocyte/macrophage activation, stem cell maintenance, B cell differentiation and T-cell function. CD74 is expressed on both normal hematopoietic cells (B-cells, macrophages, and dendritic cells) and the surface of several hematopoietic tumors (AML, B cell lymphomas, and multiple myeloma), increasing the interest in considering it as a potential tumor-associated antigen.

Remarkably, Menssen and colleagues investigated the cell surface expression of CD74 by multidimensional flow cytometry (MFD) in a cohort of 973 pediatric AMLs at first diagnosis enrolled in the Children's Oncology Group AAML 1031 clinical trial, aiming to identify any correlation with clinical features. All the MFD analyses were performed in a Central reference Laboratory (Seattle WA, USA).

In diagnostics, antigen expression is routinely evaluated with a semiquantitative approach. Here, the authors used a quantitative assessment of CD74: the expression intensity was divided into 4 quartiles according to the mean fluorescence intensity (MFI) value. Even if more complex than the semi-quantitative evaluation, this approach is critical to identify potential candidate patients for targeted immunotherapy in the discovery field[6].

The authors here used the 4th quartile (Q4) as previously published for other AML surface markers to assess the association between antigen expression and clinical variables in AML[7].

CD74 turned out to be positive at any level in 38% of analyzed pediatric AML samples, with a subset of patients (25%) being at Q4.

Increased CD74 expression was associated with clinical (older age, lower white blood cell count and blasts on peripheral blood), genetic [lower frequency of inv(16) but an enrichment of CEBP-alpha mutations, t(8;21), and trisomy 8], immunophenotypic (increased expression level of CD34, CD117, HLA-DR, and CD38 and increased rate of lymphoid antigens CD56, CD7, and CD19) features at diagnosis.

Interestingly, authors also described stable CD74 expression at relapse even if in a few patients. Future studies with an enlarged number of relapsed patients are necessary to strengthen this observation and increase the interest for this new biomarker in relapsed/resistant diseases.

CD74 showed a higher expression on blast than normal hematopoietic cells: CD34+ cord blood, regenerating and normal/ resting BM. Of note, in the last ones, CD74 was more dimly expressed than CD13 and CD33, confirming it as a potential effective marker.

Finally, Menssen and colleagues showed the cytotoxicity of STRO-001 on primary AML cells inducing the killing of AML cells expressing CD74, whereas no effects were observed on CD74-negative AML nor CD34-positive cells isolated from cord blood.

Similar findings were published in 2023 by Le and colleagues, some of them being co-authors also of the present manuscript.[8]

Phase 1 clinical trials testing STRO-001 in mature B cell malignancies are ongoing, and preliminary reports have indicated that the drug is well tolerated.[9]

In conclusion, target discovery for immunotherapy in AML is a critical and urgent need especially in treatment of refractory/relapse forms. As demonstrated by Menssen and colleagues in their elegant work, CD74 is a new intriguing effective marker to be potentially used for CAR T cells development in pediatric AML, with limited toxicities on normal tissue.
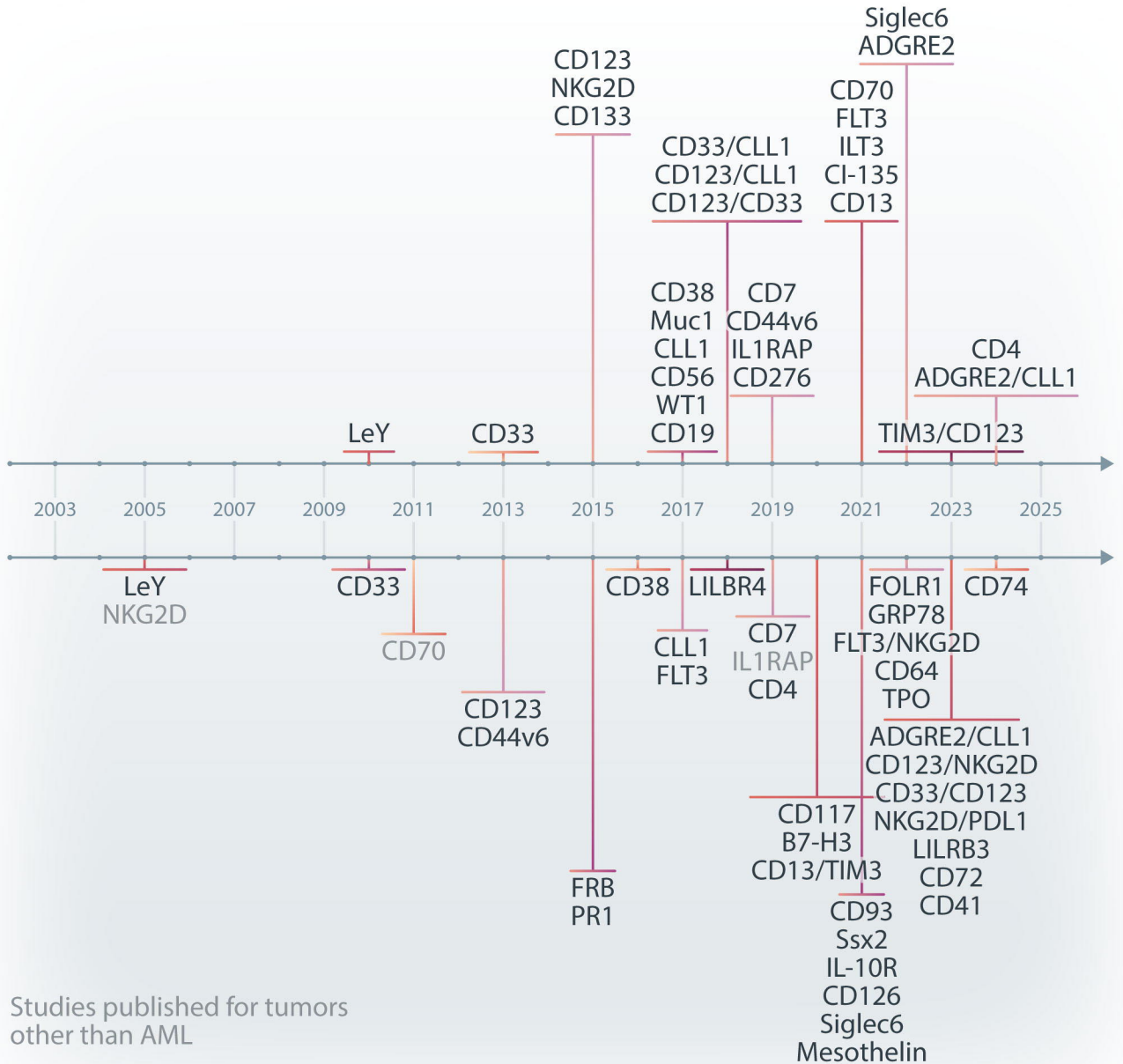
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Figure legend

Figure 1: Principal targets for CAR-T generation in preclinical and clinical studies for acute myeloid leukemia treatment. (The Figure was created with BioRender.com)

Clinical trials

CD123
NKG2D
CD133

Siglec6
ADGRE2

CD70
FLT3
ILT3
CI-135
CD13

CD33/CLL1
CD123/CLL1
CD123/CD33

CD38   CD7
Muc1   CD44v6
CLL1   IL1RAP
CD56   CD276
WT1
CD19

CD4
ADGRE2/CLL1

LeY   CD33

TIM3/CD123

2003   2005   2007   2009   2011   2013   2015   2017   2019   2021   2023   2025

LeY
NKG2D

CD33

CD38   LILBR4

FOLR1
GRP78

CD74

CD70

CLL1   CD7
FLT3   IL1RAP
         CD4

FLT3/NKG2D
CD64
TPO

CD123
CD44v6

ADGRE2/CLL1
CD123/NKG2D
CD33/CD123
NKG2D/PDL1
LILRB3
CD72
CD41

CD117
B7-H3
CD13/TIM3

CD93
Ssx2
IL-10R
CD126
Siglec6
Mesothelin

FRB
PR1

Studies published for tumors
other than AML

Pre-clinical studies