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

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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 pedAML 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 HSPC.[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 CAR, 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 CAR, which require the ligation of one of two or more targeted antigens (e.g., CD33/CD123/CLL1); (iii) NOT-gated CAR, 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 CAR require the exclusive ligation of the antigen cross-expressed on both tumoral and healthy cells without the recognition of the selective healthy-tissue target.[4]

Nowadays, the scientific effort aims to discover even new leukemia-associated markers, overexpressed in blasts rather than normal tissues and neoantigens, deriving from a 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 (MFC) in a cohort of 973 pediatric AML at first diagnosis enrolled in the Children's Oncology Group AAML 1031 clinical trial, aiming to identify any correlation with clinical features. All MFC analyses were performed in a Central Reference Laboratory (Seattle, WA, USA).

In diagnostics, antigen expression is routinely evaluated with a semi-quantitative approach. Here, the authors used a quantitative assessment of CD74: the expression intensity was divided into four quartiles according to the mean fluorescence intensity (MFI) value. Even if more complex than the semi-quantitative evaluation, this approach is

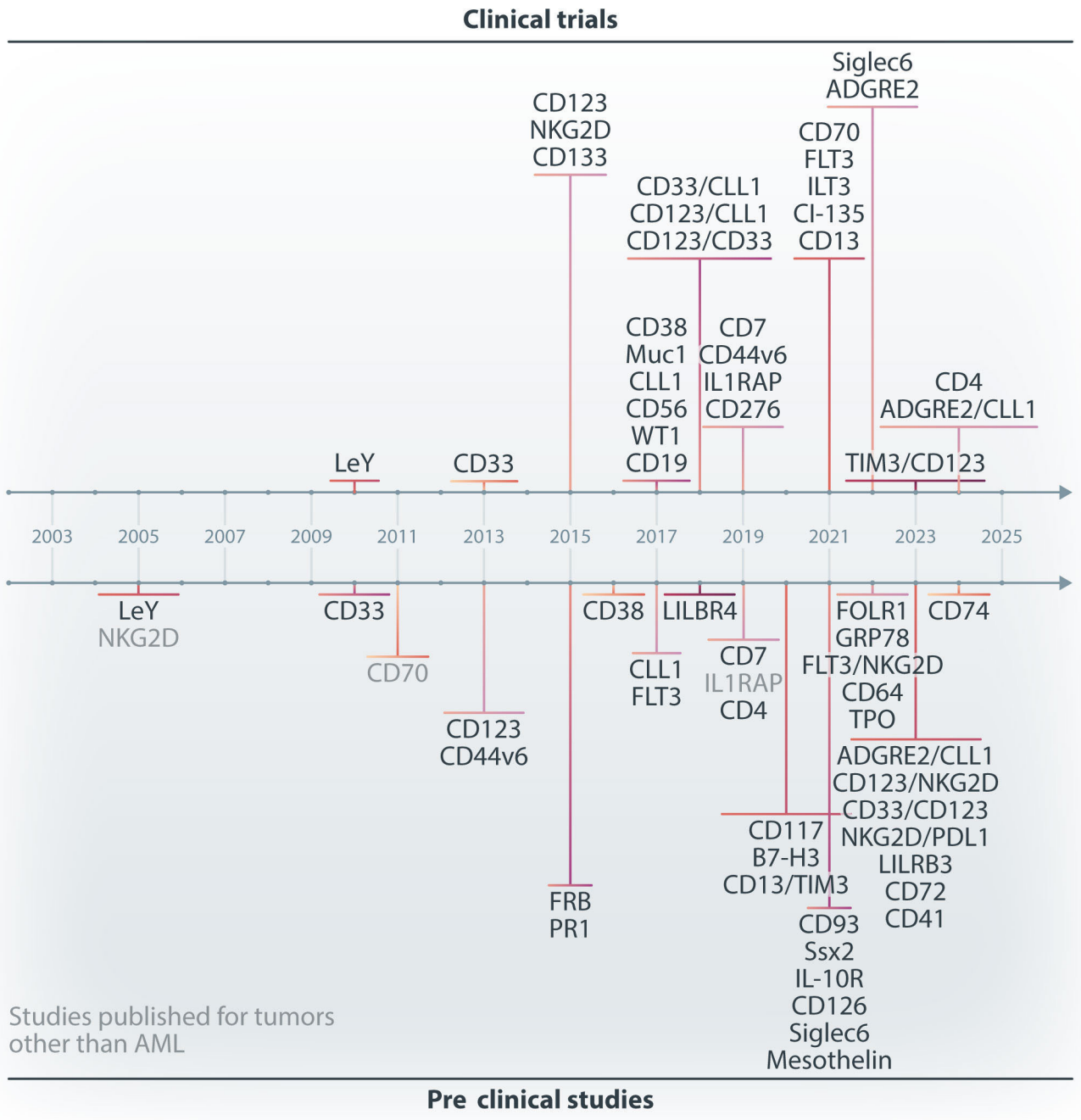

Figure 1. Principal targets for CAR T generation in preclinical and clinical studies for acute myeloid leukemia treatment. The figure was created with BioRender.com. CAR: chimeric antigen receptor; AML: acute myeloid leukemia.

critical to identify potential candidate patients for targeted immunotherapy in the discovery field.[6]

The authors here used the fourth 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*-α 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, the authors also described stable CD74 expression at relapse even if in only 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 cells than on normal hematopoietic cells: $CD34^+$ cord blood, regenerating and normal/resting bone marrow. Of note, in the bone marrow, 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 or 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 I 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 re-

fractory/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-cell development in pediatric AML, with limited toxicities on normal tissue.

Disclosures

No conflicts of interest to disclose.

Contributions

BB and VE wrote and revised the manuscript.

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