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Title: Recommendations for the Assessment and Reporting of Immunotherapy Targets in Hematologic Malignancies

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Introduction

The field of immunotherapy for hematologic neoplasms has seen rapid and sustained development. The pace at which new therapeutics are developed and introduced to clinical practice inevitably creates gaps in knowledge among healthcare providers as they try to keep up to date. Disease immunophenotype is often crucial in determining which treatments are indicated and assessing disease response. Although many clinical publications addressing the mechanism of action, indications, and efficacy of immunotherapeutic agents in the treatment of hematolymphoid neoplasms are found in current literature, few have focused on relevant laboratory testing, which can be critical to such therapeutics, and for follow-up of patients using them.

Antigens targeted by immunotherapeutic agents have been selected as therapeutic targets because they are expressed by the vast majority of neoplastic cells. Consequently, confirmation of antigen expression at diagnosis is not always required before initiating therapy. Nevertheless, assessment of these molecular targets remains good laboratory practice, as it may support the selection of the most appropriate immunotherapeutic agent, identify potential therapeutic alternatives, and help predict primary or acquired treatment resistance.

Immunophenotyping by multicolor flow cytometry represents an essential and powerful method to evaluate the expression, or lack thereof, of a number of molecules targeted on neoplastic cells by immunotherapeutics. However, there is no standardized way of reporting the results of such testing, in addition to the lack of clarity on what information the treatment team is required to know before starting therapy and during monitoring. The aim of this module is to provide recommendations based on review of seminal publications and current practices in flow cytometry for reporting of currently approved immunotherapy targets used in the treatment of some of the common types of leukemia, lymphoma, and multiple myeloma (MM).

The literature review and information on approved immunotherapeutic agents included in this module were current as of July 2026. Given the rapidly evolving landscape of immunotherapy in hematologic malignancies, new therapeutic agents and indications are expected to emerge after publication of this module. To provide updated information beyond the scope of this publication, the ICCS will maintain a

dedicated section on its website incorporating relevant updates on newly approved therapies and their laboratory assessment and reporting.

1. Immunotherapy for acute myeloid leukemia (AML)

Approved immunotherapeutics

Gemtuzumab ozagomicin (GO) is a humanized anti-CD33 IgG4 monoclonal antibody conjugated to the toxin calicheamicin. GO was initially approved in 2002, followed by retraction in 2010, based on questionable clinical benefit. Subsequent studies demonstrated benefit limited to favorable and intermediate risk patients, particularly in patients with CBF-AML and it was re-approved in 2017 (Hills et al. 2014; Döhner et al. 2022).

The addition of GO to standard AML therapy prolonged survival in patients with previously untreated CD33-positive, favorable and intermediate risk cytogenetics, and it improved event-free survival (EFS) in NPM-1 mutated/FLT3-ITD wild type AML (Shimony et al. 2025). The benefit of adding GO to induction chemotherapy in intermediate-risk patients is small and many of these patients who achieve first complete remission (CR1) receive stem cell transplantation (SCT). Considering that GO increases the risk of sinusoidal obstruction syndrome (SOS), the benefit of exposing intermediate-risk patients to GO may not be worth the increased risk of developing SOS during SCT (Shimony et al. 2025).

Venetoclax is a selective BCL-2 inhibitor that promotes apoptosis by antagonizing BCL-2–mediated anti-apoptotic signaling. It is commonly administered in combination with hypomethylating agents (HMAs) and low-dose cytotoxic chemotherapy. Venetoclax-based regimens have transformed the management of older patients and those considered unfit for intensive chemotherapy. In pediatric patients, phase I clinical trials have shown promising results in relapsed or refractory AML, and smaller studies suggest a potential role in other high-risk childhood myeloid neoplasms (Masetti et al. 2024). Although venetoclax is a targeted therapy rather than an immunotherapy, comprehensive immunophenotypic characterization of leukemic blasts before treatment initiation is essential for identifying disease subtypes associated with response or primary resistance and for guiding therapeutic decision-making.

Assessment and reporting of target antigen expression

At the time of its first approval, it was not considered necessary to demonstrate surface expression of CD33 on blasts for the use of GO. Some clinical trials included AML cases regardless of CD33 expression and suggested that GO could have clinical effect even in patients with CD33-negative blasts (Petersdorf et al. 2013; Kell et al. 2003).

In 2017, GO was reapproved by the U.S. Food and Drug Administration (FDA) and in 2018 by the European Medicines Agency (EMA) only for CD33-positive AML patients (Molica et al. 2021). Children's Oncology Group (COG) proposed a CD33 pharmacogenetics score (CD33_PGx6_score) using 6 CD33 SNPs (rs12459419, rs2455069, rs201074739, rs35112940, rs61736475, and rs1803254) to evaluate the interaction between CD33 expression and GO efficacy. Patients with a score of 0 or higher showed better disease-free survival with the use of GO, while there was no observed improvement for those with a score less than 0 (Chauhan et al. 2019).

Of particular relevance to immunophenotyping, the rs12459419 C>T splicing polymorphism promotes skipping of exon 2, resulting in a CD33 isoform lacking the IgV domain (Lamba et al. 2017). Notably, CD33 surface expression in the study evaluating the CD33_PGx6_score was assessed using the p67.6 antibody clone, which recognizes an epitope within the IgV domain. Currently, there is no evidence regarding the effect of this polymorphism on CD33 expression assessed using other anti-CD33 antibody clones.

A study from COG divided CD33 expression into quartiles and found that for patients with low CD33 expression (Q1), complete remission (CR) rates were not significantly different from those receiving and not receiving GO. However, patients with higher CD33 expression (Q2 to Q4), CR rates were significantly higher for those who received GO (Pollard et al. 2016). Nonetheless, a phase III trial in adult patients defined low CD33 expression as expression in less than 30% of blasts and found no significant association between CD33 expression and treatment response (Lambert et al. 2019). Evidence for clinical importance of reporting CD33 expression level by immunophenotyping is still unclear. We suggest that the CD33 expression intensity should be reported (Table 1), compared to residual normal internal controls: negative (lymphocytes); dim positive (mature granulocytes) and strong positive (mature monocytes).

Emerging data suggest that CD7 and CD56 expression may identify patients with inferior outcomes after frontline therapy with a combination of hypomethylating agents and venetoclax (HMA/venetoclax) and may therefore be useful to report when assessed as part of diagnostic flow cytometry (Islam et al. 2024; Sasca et al. 2019). A multi-institutional study of 279 newly diagnosed AML patients treated with frontline HMA/venetoclax reported that surface CD7 and/or CD56 positivity was associated with inferior response and survival (Lachowiez et al. 2024). Patients with CD7 or CD56 expression had lower CR/CRi rates than patients lacking both markers, and CD7-positive or CD56-positive cases had shorter overall survival. The same study reported that CD7/CD56 status further stratified patients across molecular prognostic risk groups, suggesting additive value beyond currently proposed molecular models.

Monocytic AML has been associated with primary resistance to HMA/venetoclax therapy (Pei et al. 2020). In a large multicenter study, Lachowiez et al. demonstrated that the adverse prognostic impact of monocytic differentiation identified by flow cytometry was independent of established prognostic factors, including genetic alterations (Lachowiez et al. 2025).

Based on the available evidence, we recommend reporting CD7 and CD56 expression status on leukemic blasts at diagnosis, particularly in patients who are candidates for venetoclax-based therapy. In addition, monocytic differentiation should be assessed and reported based on the expression pattern of monocytic-associated antigens, including CD11b, CD11c, CD14, CD36, CD64, and CD300e. When distinct leukemic subpopulations are identified, the presence or absence of monocytic differentiation should be described for each subpopulation (Table 1).

Pitfalls

Common to all antigen evaluation by flow cytometry is the placement of a cutoff to mark *positive* vs. *negative* expression. CD33 is negative on lymphocytes, which can be used as internal negative controls, and positive on granulocytes and monocytes, which serve as internal positive controls.

Many laboratories use CD33 in combination with CD13 conjugated to the same fluorochrome, which makes it impossible to distinguish CD13 from CD33 expression on leukemic blasts. Therefore, when CD33

expression is assessed as a potential immunotherapeutic target, CD33 and CD13 should be evaluated using separate fluorochromes to allow independent assessment of CD33 expression.

Fluorochrome intensity often affects the resolution of positive and negative populations. In general, it is advised to use a bright fluorochrome when evaluating antigen status for immunotherapy.

Investigational therapies

Several immune therapies are under development for AML, including antibody-drug conjugates, bispecific T-cell engaging antibodies (BiTEs) and chimeric antigen receptor T (CART) cells directed against different antigens, including CD33, CD37, CD47, CD70, CD123 and CLL-1 (Wang et al. 2025; Shimony et al. 2025; Jeremy et al. 2025). Reporting the percentage of cells expressing these antigens and their qualitative expression intensity (dim, normal, or bright relative to an appropriate normal cell population) will help generate data to determine if such reporting proves to be clinically relevant.

2. Immunotherapy for multiple myeloma

Approved Immunotherapeutics

Immunotherapeutics targeting specific molecules for multiple myeloma (MM) are available in diverse categories, including monoclonal antibodies, antibody-drug conjugates, bispecific T-cell engagers (BiTEs) and CART therapy.

Daratumumab, an anti-CD38 monoclonal antibody is an immunotherapeutic approved for high-risk smoldering MM, for newly diagnosed MM and for relapsed or refractory MM (Lokhorst et al. 2015, Dimapoulos et al. 2025). Isatuximab, also an anti-CD38 monoclonal antibody, and Elotuzumab, an anti-SLAMF7 (Signaling Lymphocytic Activation Molecule Family member 7) antibody, are used in combinational regimens for the treatment of relapsed or refractory multiple myeloma (Lonial et al. 2015; Attal et al. 2019).

Belantamab Mafodotin is an anti B-cell maturation antigen (BCMA) antibody conjugated to the cytotoxic agent monomethyl auristatin F (MMAF). This antibody-drug conjugate was the first of this class approved for relapsed/refractory MM (Lonial et al. 2020).

BCMA-targeted bispecific antibodies are designed to recognize BCMA on plasma cells and CD3 on T-cells, thereby promoting the interaction between plasma cells with cytotoxic T-cells, leading to tumor-plasma cell lysis. Teclistamab, elranatamab and linvoseltamab are representants of this class and have demonstrated effectiveness in the treatment of relapsed and refractory MM (Moreau et al. 2022; Lesokhin et al. 2023; Portuguese et al. 2026; Kumar et al. 2026).

There are two commercially available CAR-T cell products approved for relapsed or refractory MM, Idecabtagene Vicleucel (ide-cel) and Ciltacabtagene Autoleucel (cilta-cel), both targeting BCMA (Munshi et al. 2021; Berdeja et al. 2021).

Another therapeutic target in MM, the G protein–coupled receptor, family C, group 5, member D (GPRC5D), an orphan receptor predominantly expressed in plasma cells and hard keratinized tissues, with increased expression in malignant plasma cells. Talquetamab is a bispecific IgG4 antibody that

simultaneously binds GPRC5D and CD3, promoting T-cell recruitment and activation to mediate the killing of GPRC5D-expressing plasma cells (Chari et al. 2022). Clinical studies have demonstrated that talquetamab is an effective treatment for patients with relapsed or refractory MM (Al Hadidi et al. 2025).

Assessment and reporting of target antigen expression

The antigens targeted by immunotherapeutic agents directed to neoplastic plasma cells are typically expressed in more than 95% of cases. Consequently, confirmation of antigen expression at diagnosis is generally not required. Nevertheless, MM immunotherapeutic targeted molecules are easily identified by common laboratory methods:

- Immunohistochemistry (IHC): Used to detect protein expression of CD38, SLAMF7, and BCMA on bone marrow biopsy specimens. IHC often enables assessment of both the (estimate) percentage of positive plasma cells and staining intensity (Krista Kinneer et al. 2019).
- Flow Cytometry: Allows for more robust quantitative measurement of surface expression of CD38, SLAMF7, BCMA and GPRC5D on plasma cells obtained from bone marrow aspirates. The technique provides rapid analysis and can often distinguish neoplastic from normal plasma cells (Rawstron et al. 2016; Lee et al. 2026).

The laboratory plays a critical role in determining the suitability of immunotherapeutic agents by reporting target antigen expression. Best practices include the reporting of whether target antigen is positive or negative on neoplastic plasma cells, the percentage of positive cells (e.g., "CD38 expressed in 90% of plasma cells"), and a description of the intensity of staining (e.g., weak, moderate, strong) in comparison to the 'typical' level of expression on a normal cell.

Pitfalls

Prior therapy may reduce antigen expression, resulting in a false negative signal. This is often due to antigen masking or due to antigen internalization/downregulation. It may persist for several months after cessation of treatment (Courville et al. 2019). Camelid CD38 heavy chain antibody (VHH), clone JK36 and EuroFlow's multi-epitope CD38 antibody have been reported to detect CD38 even in post-daratumumab therapy patients (Ecker et al. 2026). Daratumumab may result in artifactual pseudo-kappa-restriction on plasma cells and hematogones (Jiang et al. 2020; Kleinot et al. 2021).

Antibodies to BCMA (CD269) need to be conjugated to bright fluorochromes because of the low level of expression of BCMA on plasma cells. Another difficulty in reporting BCMA is defining the positive/negative cutoff. Lymphocytes have been suggested and can be used as internal negative controls; however, the background (autofluorescence) of plasma cells can be significantly different than lymphocytes. Given these limitations, assessment of BCMA expression using other modalities, including IHC, may be preferable in some settings.

Investigational therapies

Numerous investigational therapies are being evaluated in clinical trials for MM. These include:

- Novel Bispecific Antibodies: Targeting antigens such as GPRC5D and FcRH5 on MM cells, aiming to recruit T cells for enhanced cytotoxicity (Lee et al. 2023).

- Next-generation CAR T-cell Therapies: Engineering has improved persistence and can target multiple antigens to overcome resistance, such as BCMA, CD19, CD38, CD44v6, CD138, GPRC5D, immunoglobulin kappa light chain (IKLC), Fc receptor-homologue 5 (FcRH5), signaling lymphocyte activation molecule member 7 (SLAMF7), integrin b7 and natural killer group 2 member D (NKG2D) (Yu et al. 2025).
- Checkpoint Inhibitors: PD-1/PD-L1 inhibitors have shown limited efficacy in MM but are being studied in combination regimens (Tremblay-LeMay et al. 2018).

3. Immunotherapy for T-cell mature lymphoproliferative diseases

Approved immunotherapeutics

In general, patients with Peripheral T-Cell Lymphomas (PTCL) have poor outcomes. Frontline treatment options are chemotherapy with anthracycline-containing regimens (Ong and Zain 2024). ECHELON-2 was a clinical trial that studied the addition of Brentuximab Vedotin (BV), an anti-CD30 antibody, to chemotherapy in patients with newly diagnosed PTCL (anaplastic large-cell lymphoma (ALCL) and other CD30-expressing subtypes). The addition of BV to chemotherapy improved progression free survival (PFS) and reduced the risk of relapse (Horwitz et al. 2019). Other studies confirmed the utility of adding BV to chemotherapy in ALCL and other T-cell lymphomas with CD30 expression in at least 1% of tumor cells (Thomas and Barta 2025).

The CD52 antigen is a protein expressed at high density on the cell surface of normal and malignant lymphocytes, including most T-cell lymphomas, except ALCL (Wulf et al. 2021). Alemtuzumab, an anti-CD52 humanized monoclonal antibody, has shown significant efficacy against T-cell lymphomas however it comes with a high cost of toxicity (Ong and Zain 2024; Wulf et al. 2021). Although Alemtuzumab is no longer available in some countries, it may still be obtained for clinical use, in special situations, for the treatment of T-prolymphocytic leukemia and T-large granular leukemia.

CCR4 is expressed in tumor cells of most patients with adult T-cell leukemia/lymphoma (ATLL) and other PTCL, such as ALK-negative ALCL, PTCL-NOS, angioimmunoblastic T cell lymphoma (AITL) and mycosis fungoides/Sézary syndrome (MF/SS). Mogamulizumab is a humanized monoclonal anti-CCR4 antibody, which has direct cytotoxic effect on CCR4-positive lymphoma cells (Ong and Zain 2024). Its effectiveness has been best demonstrated in MF/SS and ATLL (Thomas and Barta 2025).

Assessment and reporting of target antigen expression

ECHELON-2 clinical trial used the level of CD30 expression identified by immunohistochemistry at the membrane, cytoplasm or Golgi of the neoplastic cells (Horwitz et al. 2019). Extrapolating this finding to flow cytometry, CD30 may be assessed at the cell surface and/or intracellularly, and the percentage of CD30-positive neoplastic cells should be reported (Table 1). In ECHELON-2, CD30 positivity was defined as expression in at least 10% of neoplastic cells or total lymphocytes (Horwitz et al. 2019).

Recent studies demonstrated that the addition of BV to chemotherapy was active and safe in patients with expression of CD30 in at least 1% neoplastic cells (Herrera et al. 2024; Nizamuddin and Mehta-Shah 2024). It may be difficult or considerably subjective to identify and report CD30 expression in only a small proportion of neoplastic cells. In such cases, careful selection of an appropriate internal negative control

(e.g., residual normal B or T lymphocytes), use of an anti-CD30 antibody conjugated to a bright fluorochrome, and careful assessment by an experienced cytometrist are particularly important to reliably distinguish low-level CD30-positive populations from background fluorescence.

Although quantitative estimation of surface CD52 expression may identify patients with higher probability of responding to alemtuzumab (Jiang et al. 2009; Ginaldi et al. 1998), this approach is not routinely applicable outside clinical trials. However, because a subset of T-cell lymphomas lacks CD52, assessment of CD52 surface is recommended whenever alemtuzumab therapy is being considered. Both the expression status and expression intensity should be reported (Jiang et al. 2009) (Table 1).

CCR4 expression should be identified on the surface of the neoplastic cell of ATLL and MF/SS, if the patient is from a country where mogamulizumab is approved for the treatment of this disease (Yonekura et al. 2020). When present, the expression intensity of surface CCR4 should be reported in relation to internal controls (as dim, intermediate or bright) (Table 1).

Pitfalls

It may be difficult or considerably subjective to identify and report CD30 expression in only a small proportion of neoplastic cells, such as approximately 1%. CCR4 is not a commonly used antibody in leukemia/lymphoma panels, and therefore cytometrists and pathologists may not be familiar with its normal expression patterns.

Investigational therapies

CD25 is a component of the high affinity interleukin-2 receptor alpha chain (IL-2Rα). Surface CD25 expression may be identified in up to 50% of all PTCL. In clinical trials, anti-CD25 antibody-drug conjugates have shown promising results (Ong and Zain 2024). A phase II study with E7777, a recombinant cytotoxic fusion protein, composed of the active portion of the diphtheria toxin fragments and the receptor binding domain of human interleukin-2 (IL-2), demonstrated that patients with expression of CD25 in more than 20% of tumor cells had better overall response rate than those without (Kawai et al. 2021). A phase III study with Dinileukin difitox (DD-cxdl), another fusion protein composed of the human IL-2 and diphtheria toxin fragments, demonstrated the efficacy of this drug in patients with MF/SS with at least 20% CD25 positivity (Foss et al. 2025). Although in these studies the expression of CD25 was identified by IHC, if this data is extrapolated to flow cytometry, the percentage of surface CD25 positive tumor cells should be reported.

Designing CAR-T cells to target T-cell neoplasia has lagged behind B-cell malignancies due to inherent difficulty of targeting a marker expressed by the therapy (fratricide), among other challenges. There are several CAR-T products in early phase studies, targeting different antigens, such as CD5, CD7, CD30, CD37 and CD70 (Ong and Zain 2024). In addition, bispecific antibodies targeting CD30 are currently under investigation (Thomas and Barta 2025).

4. Immunotherapy for B-cell lymphoblastic leukemia

Approved immunotherapeutics

Blinatumomab is a first-in-class bispecific CD19-directed T-cell engager used in CD19 positive B-ALL in patients older than 1 month. Specific FDA indications include first or second complete remission with minimal residual disease ≥ 0.1%, relapsed/refractory B-ALL, and/or Philadelphia chromosome-negative disease in the consolidation phase of multiphase chemotherapy.

Inotuzumab ozogamicin is an antibody-drug conjugate. It links a humanized monoclonal antibody against CD22 to calicheamicin, a potent cytotoxic antibiotic. Once the antibody binds CD22 primarily on leukemic blasts, the complex is internalized, and calicheamicin is released, causing DNA double-strand breaks and apoptosis. Originally approved for relapsed/refractory B-ALL in adults, outcomes in the Children's Oncology Group AALL1621 trial recently resulted in approval for all patients aged 1 year and older (O'Brien et al. 2022).

Currently FDA approved CART therapies for B-ALL are tisagenlecleucel for pediatric/young adult patients and brexucabtagene autoleucel for adults, which both result in T-cells targeting CD19 on the cell surface.

Rituximab is a chimeric anti-CD20 monoclonal antibody that promotes antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and direct apoptotic signaling in surface CD20-expressing B cells. CD20 is expressed in nearly 50% of adult and pediatric patients diagnosed with B-ALL (Chevallier et al. 2012; Maury et al. 2016) and its expression may be enhanced by the use of steroids (Dworzak et al. 2008; Marks et al. 2022). Although Rituximab has been used for CD20 positive B-ALL in adults (Maury et al. 2016), its use in pediatric patients is still restricted to clinical trials.

Assessment and reporting of target antigen expression

Flow cytometry is the primary method used to assess CD19 expression in clinical trials of both blinatumomab and CD19-directed CAR T-cell therapies and remains the gold standard for evaluating surface CD19 expression. A phase III randomized trial comparing blinatumomab with chemotherapy for the treatment of relapsed or refractory *BCR::ABL1*-negative B-ALL led to FDA approval of blinatumumab for this indication in 2014 (Kantarjian et al. 2017). In this study, CD19 expression was reported qualitatively as positive, partial positive, weak positive or negative; however, the criteria defining these categories were not described.

Subsequent studies in both pediatric and adult patients have used heterogeneous approaches to report CD19 expression, with substantial variability in both the thresholds used to define positivity and the percentage of blasts required to be considered CD19-positive (Badar et al. 2021; Horibe et al. 2020; Topp et al. 2015; Martinelli et al. 2021; Locatelli et al. 2022). Similarly, pivotal studies evaluating CD19-directed CAR T-cell therapies have used different methodologies and thresholds for defining CD19 positivity (Brentjens et al. 2013; Maude et al. 2014; Finney et al. 2019). Despite these differences, CAR T-cell therapy has demonstrated comparable efficacy in CD19-dim and CD19-normal/bright B-ALL. Furthermore, assessment of CD19 antibody-binding capacity in a limited number of cases did not distinguish clinical responders from nonresponders (Pillai et al. 2019).

Consequently, there is currently no standardized definition of CD19 positivity or consensus threshold for expression intensity or the percentage of CD19-positive blasts. In routine clinical practice, CD19 expression is therefore generally reported qualitatively, often together with the percentage of positive blasts. When flow cytometry is unavailable, immunohistochemistry may be used to assess CD19

expression; however, no standardized criteria for positive or negative interpretation have been established.

For patients being considered for blinatumomab or CD19-directed CAR-T therapy, we recommend reporting CD19 expression status and intensity on leukemic blasts. CD19 expression intensity should be assessed relative to that of mature B cells and reported qualitatively (e.g., dim, intermediate, or bright). An appropriate internal negative control (e.g. normal residual T lymphocytes) should be used to distinguish true CD19 expression from background fluorescence.

Flow cytometry is the primary method used to assess surface CD22 (sCD22) expression in clinical trials and remains the gold standard for evaluating this antigen. The pivotal Phase III INO-VATE ALL trial, which supported FDA approval of inotuzumab ozogamicin, defined sCD22 positivity as expression on ≥20% of leukemic blasts but did not specify criteria for distinguishing positive from negative expression or thresholds for expression intensity. Importantly, the trial demonstrated clinical efficacy in patients with both high (≥90%) and lower (<90%) proportions of sCD22-positive blasts (Kantarjian et al. 2016).

The subsequent COG AALL1621 trial defined sCD22 positivity as expression above background, with background established as 1% positivity over an appropriate negative control population. In addition to qualitative assessment, this study quantified CD22 site density by measuring the number of anti-CD22 phycoerythrin-conjugated antibodies bound per cell (Rubinstein and O'Brien 2023; O'Brien et al. 2022).

In settings in which flow cytometry analysis is unavailable, immunohistochemistry may be used. In the setting of IHC, moderate to strong membranous expression in the majority of blasts is required to designate positivity (Kantarjian et al. 2021).

Patients with B-ALL who express CD20 in more than 20% of leukemic B-lymphoblasts are typically considered candidates for rituximab-based chemoimmunotherapy (Maury et al. 2016). This 20% cut off is historically based on studies demonstrating the association of CD20 expression above this threshold with poor prognosis (Jabbour et al. 2015; Borowitz et al. 1997; Aref et al. 2017; Levato and Molica 2018). However, more recent evidence suggests that even lower levels of CD20 expression, around 10% of blasts, may also correlate with inferior outcomes (Bommannan et al. 2024; Tian et al. 2022; Marks et al. 2019). Additionally, CD20 expression is often upregulated during steroid-based induction regimens for B-ALL, raising further questions about the validity of using the 20% cut-off to determine eligibility for rituximab (Dworzak et al. 2008; Marks et al. 2022).

Emerging data indicates that prognosis may be more closely related to the intensity of CD20 expression rather than simply the proportion of positive blasts (Tian et al. 2022; Marks et al. 2019; Bommannan et al. 2024; Serbanica et al. 2023). Specifically, a mean fluorescence intensity (MFI) of <19.98 for CD20 (calculated as the ratio of blast CD20 MFI to isotype control MFI) has been identified as an independent predictor of worse overall survival (OS) and progression-free survival (PFS). Similarly, a normalized CD20 intensity—defined as the ratio of CD20 expression between B-lymphoblasts and T-cells—greater than 1.85 has been associated with inferior relapse-free survival (RFS) (Bommannan et al. 2024).

Pitfalls

In patients previously exposed to CD19-targeted therapies, apparent loss or reduction of CD19 expression should be interpreted with caution, as therapeutic antibodies or other treatment-related mechanisms

may interfere with antigen detection through epitope masking (Ikoma-Colturato et al., 2025). When prior treatment is known or suspected, the potential for interference between the therapeutic agent and the antibody clone used for flow cytometric detection should be considered.

In general, there is no established or uniform practice designating positivity or percent thresholds, and reports often quantify CD22 as "bright/dim" and "% positive." If ≥90% blasts are "positive", clinicians are generally more confident about inotuzumab use, as variable responses have been reported in patients with <90% positivity (Kantarjian et al. 2021). Also confounding the establishment of uniform reporting are analytical variability and differences in anti-CD22 clone performance. In the INO-VATE trial, central laboratories used the RFB4 clone and reported higher expression levels than local laboratories (Kantarjian et al. 2016); however, interlaboratory differences may reflect multiple analytical variables, including antibody clone, fluorochrome selection, instrument configuration, and instrument settings. In a smaller study directly comparing antibody clones, RFB4 detected a higher fraction of sCD22+ leukemic cells than SJ10.1H11 (Reinert et al. 2021). The issue of sCD22dim or sCD22 negative subpopulations remains largely unstudied. There are, however, anecdotal reports of response in patients presenting with very dim to negative sCD22 expression (Fingrut et al. 2020).

Investigational therapies

Numerous immunotherapeutic agents targeting distinct antigens and employing different mechanisms of action are currently under investigation for B-ALL. These include monoclonal antibodies directed against CD20, CD22, and CD52, namely Ofatumumab, Epratuzumab, and Alemtuzumab, respectively, among others (Luskin et al. 2025; Jabbour et al. 2020; Raetz et al. 2015).

Of note, immunotherapeutic approaches are being explored for T-cell acute lymphoblastic leukemia (T-ALL). Notable examples include the anti-CD38 monoclonal antibody Daratumumab (Senapati and Jabbour 2024) and CD7-directed chimeric antigen receptor (CAR) T-cell therapy (Chiesa et al. 2026).

Table 1. Summary of recommendations for the assessment and reporting of immunotherapy targets

Disease	Target antigen	Recommended reporting*
Acute Myeloid Leukemia	CD33	Expression status and intensity
	CD7	Expression status
	CD56	Expression status
	Monocytic antigens (CD11b, CD11c, CD14, CD36, CD64, CD300e)	Monocytic differentiation status of all leukemic subpopulations
Multiple Myeloma	CD38	Expression status, intensity and percentage of positive cells
	BCMA	Routine evaluation not required
	SLAMF7	Routine evaluation not required
	GPCR5D	Routine evaluation not required
Mature T-cell Lymphoproliferative Diseases	CD30	Expression status and percentage of positive cells

	CD52	Expression status and intensity
	CCR4	Expression status and intensity
B-Cell Lymphoblastic Leukemia	CD19	Expression status and intensity
	CD22	Expression status, intensity and percentage of positive cells
	CD20	Expression status and percentage of positive cells

* Expression status refers to the classification of antigen expression as positive or negative. When assessment of expression intensity is recommended, it should be reported separately (e.g., dim, intermediate, or bright) relative to an appropriate internal reference population.

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