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Common Targeted Therapies in Acute Leukaemia's Requiring Modification of Practice in Hematopathology

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Abstract

In recent years, advancements in the therapeutics of acute leukemias have particularly focused on targeted therapy. Therapy against several immunophenotypic and molecular targets has become the standard of care. Pathologists need to stay informed and modify practices accordingly to support the implementation of these therapies. Here, we discuss some of the common therapeutic targets and corresponding best pathology practices in acute Leukemia.

Keywords: *Targeted therapy; Acute leukemia; Pathology practice*

Introduction

Acute leukemias, once a rapidly fatal disease, now has improved prognosis due to advancements in the therapeutic strategies that include chemotherapy, targeted therapy, and hematopoietic stem cell transplantation. In recent years, numerous targeted therapies against molecular markers and surface antigens have been approved for clinical use. Successful implementation of these therapies would be impossible if not accompanied by required modification in the practice by pathologists. In this article, common targeted therapies for acute leukemias and corresponding best pathology practices are discussed.

Investigations for Targeted Therapies in Acute Myeloid Leukemia

In the past, a new diagnosis of acute myeloid leukemia did not require extensive investigation. Most laboratories would only perform bone marrow cytochemistry to confirm the myeloid nature of leukemia and to identify monocytic differentiation. Today, identification of the lineage is only a preliminary step in the workup. A complete workup involves flow cytometry, cytogenetics, FISH analysis, and molecular studies (Table 1). Cytochemistries are rarely in this era of flow cytometry. Myeloid panels for flow cytometry are similar across laboratories and essentially include CD45, CD34, CD117, CD38, HLA-DR, CD13, CD33, cMPO, CD4, CD11b, CD14, CD64, CD56, CD123, CD7 and CD15. While the immunophenotype of the blasts along with its intensity of expression is important to assign lineage and to detect minimal residual disease on a later date, it is now important to particularly assess for certain antigens like CD33 and CD123.

Gemtuzumab ozogamycin (GO) is an antibody-drug conjugate that targets CD33 on the surface of myeloid cells including leukemic myeloid blasts [1]. CD33 is desirable as a target as it is not expressed by normal hematopoietic stem cells but is positive on the majority of the leukemic myeloid blasts and cells of myeloid origin. GO was first approved for clinical use in elderly patients who could not tolerate other chemotherapies. Although it was initially withdrawn, over the years and with more definite evidence, GO is now approved for newly diagnosed adult and pediatric acute myeloid leukemia and is evolving to be a standard of care. Studies show that there is a 2-log fold variation in the expression levels of CD33. Lower levels of CD33 expression are seen in Core Binding Factor AML (CBF) and AML with adverse karyotype. Higher levels are found in AML with FLT3-ITD, MLL, and NPM1 mutations. Cases with high expression of CD33 appear to benefit more with the addition of GO in the treatment regimen [2]. Although a cut-off threshold is not definitively established, cases with more than 70% blasts expressing CD33 showed better response to GO in one study [3].

Therefore, it is important to determine the percentage of blasts positive for CD33 and report it along with the intensity. If there are distinct subsets that are negative for CD33, it is worth mentioning them in the report.

Target	Approved therapy	Preferred method of detection	Things to consider
CD33	Gemtuzumab ozogamycin	Flow cytometry	Report intensity of expression
CD123	CAR-T cell therapy	Flow cytometry	
FLT3	Type 1 inhibitors- effective against both ITD and TKD mutation	PCR/NGS	NGS panels take longer to result. PCR is preferred. Testing recommended in new diagnosis and at relapse
	Type 2 inhibitors- effective against ITD mutation only		
IDH	IDH1 and IDH2 inhibitors	PCR/NGS	Testing recommended in new diagnosis and at relapse

Table 1: Investigation for targeted therapies in acute myeloid leukemia.

CD123 is another important antigen that is currently being explored as a target for blastic plasmacytoid dendritic cell neoplasm (BPDCN) and AML4. Although conflicting opinions exist among hematopathologists about the level of expression of CD123 in acute myeloid leukemias, it is often expressed than not by the leukemic stem cells and differentiated blasts in AML. Many laboratories do not include CD123 in their primary panel and often use it on their add-on tubes when BPDCN is suspected. Given that various monoclonal antibodies and CAR-T cell therapies against CD123 are being extensively investigated, including them in the primary panel is highly recommended [4].

Mutations in FLT3 are common and are seen in approximately one-third of patients with acute myeloid leukemia. FLT3-ITD mutation in particular is associated with an inferior prognosis. FLT3-inhibitors are approved for clinical use in acute myeloid leukemia since 2017. Type I FLT3 inhibitors are effective against both FLT3 ITD and TKD mutations while type 2 is effective only against ITD mutations. FLT3 inhibitors are used in induction along with chemotherapy [5-8]. Therefore, it is essential to perform FLT3 mutational analysis on all new diagnoses and relapse/refractory cases. In recent years, many practices routinely perform AML next-generation sequencing panels for new diagnosis and relapse/refractory cases. These panels include FLT3-ITD and TKD in addition to several others. However, next-generation sequencing takes longer to report and FLT3 inhibitors are typically added on day 8 of the induction regimen. Therefore, many laboratories perform a separate PCR test for FLT3 mutation in addition to the NGS panel to ensure rapid turnover of the results. When reporting it is essential to include if the mutation is ITD or TKD to enable the oncologist to choose between type I and type II inhibitors. It is also a good practice to report the variant allele frequency along with any mutation to identify possible sub clones.

IDH mutations are yet another targetable mutation in AML and are seen in 15-20% of newly diagnosed AML. Both IDH1 and IDH2 inhibitors are available and are recently approved for clinical use in relapse/refractory AML [9]. Smaller laboratories often perform only FLT3, NPM1, and CEBPA mutation analysis routinely. It is time that IDH mutations are also tested routinely along with these. Similarly, DNMT3 inhibitors are being explored for clinical use, and soon may be necessary to add those as well.

Investigations for Targeted Therapies in B-lymphoblastic Leukemia/Lymphoma

Immunotherapies are gaining popularity in the treatment of relapsed/refractory B-lymphoblastic leukemia/lymphoma (B-ALL) and many cases, the only hope. A range of immunotherapies from monoclonal antibodies to chimeric antigen receptor (CAR) T-cell therapies exist. These therapies often pose a challenge in diagnosing and distinguishing small neoplastic blasts and hematogones (Table 2).

Target	Approved therapy	Preferred method of detection	Things to consider
CD19	Bispecific T-cell engagers and CAR T-cell therapy	Flow cytometry	Post therapy gating strategies need modification
CD20	Monoclonal antibody	Flow cytometry	Post therapy gating strategies need modification
CD22	Monoclonal antibody and CAR T-cell therapy	Flow cytometry	
BCR-ABL1	Tyrosine kinase inhibitors	FISH, PCR	CRLF2 flow cytometry can be a starting point to identify cases that need JAK mutation analysis
JAK	Ruxolotinib`	PCR, NGS	

Table 2: Investigation for targeted therapies in B-lymphoblastic leukemia/lymphoma.

CD19 is a pan B-cell marker that is consistently expressed on all stages of B-cell differentiation and therefore makes it a reliable immunophenotypic marker for B-lymphoblastic leukemia/lymphoma (B-ALL). This property of CD19 also makes it a desirable target for immunotherapy in B-ALL. Blinatumomab is a bispecific T-cell engager that binds to both CD3 and CD19 acts as a link between the patient's T-cells and B-lymphoblasts. Subsequently, the cytotoxic T-cells lyse the B-lymphoblasts. Blinatumomab is particularly employed in the treatment of relapsed/refractory B-ALL [10]. Anti CD19 CAR-T cell therapy is also often used in relapsed/refractory B-ALL. CD19 is invariably reported in flow cytometry as it is one of the lineage-specific markers for B-ALL. It is important to recognize the fact that CD19 may not be useful in the follow-up of these cases that received blinatumomab or anti-CD19-CAR-T cell therapy. Relapse in such cases may have either CD19 positive or CD19 negative blasts. Therefore, gating strategies need to be modified accordingly especially for investigating minimal residual disease. Some laboratories use a combination of CD22, CD24, and CD66b to create a rough B-cell gate in such scenarios. CD22 cannot replace CD19 for primary gating as CD22 is not consistently positive on lymphoblasts. Similarly, targeted therapy for CD22 exists in the form of monoclonal antibodies and CAR-T-cell therapy. Inotuzumab Ozogamicin is a monoclonal antibody that targets CD22 on B-lymphoblasts. Certain clinical trials also use a combination of blinatumomab and inotuzumab for relapsed/refractory B-ALL which hypothetically complicates gating even further. Relapses in such cases are not usually negative for both CD19 and CD22 although possible. To combat such a situation, laboratories use multiple tubes with various combinations to avoid missing a small population of neoplastic blasts. Markers such as CD9, CD58, and CD13+33 also play role in distinguishing normal B-cell precursors (hematogones) from neoplastic blasts [11].

Rituximab, an anti-CD20 monoclonal antibody commonly used in the treatment of mature B-cell neoplasms, is also used occasionally in the treatment of B-ALL. B-lymphoblasts may express variable expression of CD20 and a threshold of expression in 20% blasts is required for therapy with rituximab [12]. Therefore, while reporting, it is essential to include the percentage of blasts positive for CD20 expression.

B-ALL with BCR-ABL translocation accounts for 25% of adult and 2-4% of pediatric B-ALL cases. Several generations of tyrosine kinase inhibitors are widely used along with standard chemotherapy for B-ALL with t(9;22). Flow cytometry, although not specific, sometimes offers subtle clues with an expression of CD25 and myeloid markers such as CD13 and CD33 on the B-lymphoblasts [13]. FISH studies, conventional karyotyping, and PCR studies are modalities for diagnosis and follow-up. Many laboratories perform molecular studies as reflex testing if FISH studies are negative.

BCR-ABL like B-lymphoblastic leukemia/lymphoma is a recently described entity in the WHO classification of hematopoietic and lymphoid tissues. These neoplasms lack BCR-ABL1 translocation but show a gene expression pattern similar to B-ALL with BCR-ABL1. Almost half of these leukemias have translocations involving CRLF2 that juxtaposes CRLF2 to the promoter of the P2RY8 gene. The incidence of CRLF2 translocation is higher among pediatric patients and young adults and is rare in adults. Lymphoblasts with this translocation show very high surface expression of CRLF2 and can be detected by flow cytometry. Flow cytometry is a sensitive screening tool to detect CRLF2 translocation as cases without high levels of surface expression never show the translocation. Half of B-ALL with CRLF2 translocation show JAK1 or JAK2 mutation and may benefit from JAK inhibitors like ruxolotinib [14]. Some laboratories routinely perform flow cytometry for CRLF2 expression in pediatric B-ALL cases. It may be beneficial to include this test in flow cytometry as these translocations are cryptic and can be missed by conventional karyotyping unless FISH studies are performed.

Conclusion

Cancer therapeutics in hematologic malignancies is constantly evolving and in recent years targeted therapy has been the focus. It is, therefore, necessary for pathologists to stay updated on these developments and alter practice as the treatments evolve.

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