


A new insight updates in diagnosis and management of acute lymphoblastic leukemia, cytogenetics, immunophenotyping, and proteomic profile

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ABSTRACT

Acute lymphoblastic leukemia (ALL) is a severely invasive hematological cancer that results from the rapid increase and accumulation of lymphoid blasts in the blood, bone marrow (BM), and other organs. The pervasiveness of ALL has made it the prevalent childhood acute leukemia making up approximately 80% of leukemia in children and about 20% in adults. Due to the prevalence of ALL, more accurate diagnosis and prognostic methods are required. Proteome analysis uses a variety of analytical techniques, including protein sequencing, structural or expression proteomics, protein modification, sub-cellular protein localization, protein-protein interactions, and biological functional proteomics. Analysis of cell-signaling pathways and activation/deactivation are crucial to follow up the development, remission, or relapse of ALL. As a result, this review emphasizes cytogenetics and immunophenotyping while also highlighting the proteomic profile, clinical symptoms, diagnosis, and management of ALL. Also, it evaluates the procedures and techniques for the testing of bodily fluids (peripheral blood, cerebrospinal fluid, and BM) from ALL patients at various stages of disease, as well as the use of proteomic platforms in discovering sensitive and specific biomarkers for ALL.

Keywords: acute lymphoblastic leukemia, cytogenetics, immunophenotyping, proteomics, biomarkers

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a class of cancer in which bone marrow (BM) loses its control and tends to produce excessive and immature lymphocytes. In both adults and children, ALL affects all of the red and white blood cells as well as the platelets [1, 2]. Approximately 80% of childhood acute leukemia cases impact children, but only 20% of adult instances of leukemia are caused by this common type of childhood acute leukemia. Children make up for 75% of ALL positive patients [3]. One-third of pediatric malignancies are ALL, which is one of the most common juvenile malignant cancers with a peak incidence between two and six years of age [4, 5]. About 30% of ALL patients relapse, nevertheless the improvement in therapeutic treatment for leukemia in the least 20 years [6]. Isolated extramedullary relapse occurs in about 20% of relapse cases, while the central nervous system (CNS) accounts for 65% of relapse cases [6, 7]. ALL covers a cluster of onco-hematological fast developing medically and biologically varied entities, typified by unrestrained increase in undeveloped white blood cells in the BM and blood, and permeation of these cells into surrounding tissues. The prevalent signs of ALL are fever, extreme tiredness or feeling of exhaustion, anemia, bleeding and easy bruising, bone or arthralgia, ecchymosis and petechiae [1]. More severe clinical manifestations include shortness of breath, enlarged liver,

enlarged spleen, swollen lymph nodes, mediastinal and testicular infiltration [1, 8]. The symptoms are frequently randomized, and the medical prognosis is performed according to pathologic, immunochemical, and molecular assessment of the BM aspirate and biopsy material, with the standard of a minimum of 20% of BM lymphoblasts for the determinative diagnosis of ALL.

ALL is thought to result from the interaction of endogenous or exogenous stressors with genetic vulnerability and likelihood. Infections, ionizing radiation, and chromosomal translocations that arise in utero during fetal hematopoiesis and postnatal genetic events are regarded as primary causes and derivative contributor to ALL [9]. These genetic modifications dislocate the genes that control normal hematopoiesis and lymphoid growth. This could lead to initiate the activation of oncogenes or instigate the activation of tyrosine kinases. Patients with Klinefelter's syndrome, trisomy 21, and hereditary ailments with extreme chromosomal frailty such as Fanconi's anemia, ataxia-telangiectasia and Bloom's syndrome are more susceptible to ALL [3, 10]. The risk of developing ALL may be further exacerbated by chemotherapeutic treatment and exposure to radiation. Nonetheless, no gross chromosomal modification was observed in the bulk of ALL patients, indicating that further submicroscopic genetic modifications possibly account for leukemogenesis [9].

The time between the first diagnosis and the relapse, locations of the relapse, and the immunophenotype of the leukemic cells, which has a worse prognosis for T-cell phenotype, are three important predictive criteria that are often analyzed for the clinical outcome of first ALL-relapse patients [11, 12]. The treatment of ALL relapse cases is rely on the aforementioned predictive aspects, and they include chemotherapy and BM transplant in patients with high threats of early on and delayed relapses, which are evident by weak responses to chemotherapy treatment [13]. The polymerase chain reaction (PCR) of markers, such as immunoglobulins and reorganizations of T-cell receptor genes, is used to describe the relapse leukemic blasts at an extramedullary site [14, 15].

Exploring biomarkers for different hematological malignancies has drawn a lot of attention as a result of recent developments in proteomic-related technology. The conventional proteomic platforms' extensive protein analysis of proteins from cells, fluids, tissues, and organs has provided information on the complexity and heterogeneity of the malignancy at different phases of development and progression [16, 17]. Proteomics is a cutting-edge technique for improving the detection of protein-based markers in ALL relapses and is a useful tool for creating customized and altered treatments [16, 18]. Proteomics has thus steadily become the main method in cancer research for characterizing aberrant protein expressions that may be used as disease biomarkers [17, 19]. These biomarkers might be used in conjunction with more conventional diagnostic techniques, such as hematology and cytogenetics, to improve the accuracy of the diagnosis and offer prognoses for ALL with the least number of intrusive biopsies [20]. Understanding how ALL originates, remits, or returns depends on a detailed understanding of cell-signaling routes and activation/deactivation. This is because cell-signaling processes control the leukemic cell's rapid growth, separation, apoptosis, and survival [20, 21].

A perusal of relevant and current literature on ALL indicates randomization of vital knowledge on proper profiling of the cancer. The existing literature is inundated with repetitive reportage of the diagnosis and treatment of ALL. Therefore, this review strives to concisely highlight the pertinent details on ALL by comprehensively compiling information on the proteomic profile, clinical manifestations and diagnosis, and cure of ALL with emphasis on cytogenetics and immunophenotyping. The review also assesses the work-flow and protocols for the examination of body fluids (peripheral blood, cerebrospinal fluid, and BM) from ALL patients at various stages of pathology, significance of proteomic platforms in identifying ALL-specific and sensitive biomarkers.

MATERIALS AND METHODS

Based on a thorough computerized search of the PubMed database using the phrases "acute lymphoblastic leukemia", "cytogenetics of ALL", "immunophenotyping of ALL", "proteomics", and "proteomic profile of ALL". The search results were narrowed down to studies published in English. Similarly researched materials include practice guidelines, conference proceedings, clinical trials, books, systematic reviews, and meta-analysis. Publications from different related oncology based societies such as American Society of Clinical Oncology, American Society of Hematology, and European

Society of Hematology were also assessed. Papers published between 2005 and 2022 were searched up in the appropriate databases. Citations for research articles on ALL are included in this paper.

Diagnosis and Clinical Manifestations of ALL

ALL embodies a class of onco-hematological fast developing biologically and clinically diverse entities, differentiated by uninhibited increase in immature WBCs in the blood and BM, and permeation of these cells into other tissues. Fever is among the most prevalent signs and symptoms (induced by leukemia or derived from severe infections in the case of abnormally low count of neutrophils), tiredness, lack of or dysfunction in red blood cells, bleeding, bone or articular pain, and blood disorders such as ecchymosis and petechiae [1, 22, 23]. Additional critical clinical expressions comprise shortness of breath (dyspnea), hepatomegaly (enlarged liver), splenomegaly (enlarged liver), mediastinal and testicular infiltration, and swelling of the lymph node [1, 23].

Assessment of the peripheral blood film to determine the presence of blasts is the first step in ALL diagnosis. The manifestation of only growths in the mediastinum or swelling of the lymph nodes (lymphadenopathy) implies that the patient may require tissue samples for biopsy. However, every patient requires a BM test. Based on the classification developed by World Health Organization (WHO), contrary to myeloid malignancies, no consensus has been reported on the minimum limit for the fraction (%) of blasts needed to launch a prognosis of lymphoblastic leukemias [24]. Regardless, several standard procedures propose that the assessment of ALL demands the expression of >20% blasts in the BM aspirate [25]. Conventionally, 'lymphoma' is utilized in a case, where the progression is restricted to a mass lesion with no or negligible indication of peripheral blood and BM contribution. According to morphology, lymphoblastic lymphoma exhibits similar inherent and Immunophenotypic characteristics with ALL.

The disparity in prognosis of ALL is anchored in the attributes of routine Romanowsky stains of BM and peripheral blood smears i.e., cytochemical staining (negative myeloperoxidase, alpha-naphthyl acetate esterase, Sudan black B) and immunophenotyping of leukemic cells. Until lately, the classification of ALL using Cooperative French-American-British (FAB) Group was derived from the shape and size of leukemic cells (subclass L1, L2, and L3); nonetheless, the real categorization is according to immunophenotype [24]. Pre-B ALL is largely discriminated via expressions of cytoplasmic immunoglobulins (cIg) and indicators that include CD79a, CD19, HLA-DR and CD10; B cells ALL is distinguished by the expression of surface immunoglobulins (sIg) and heavy chains; while T-cell ALL is typified by the cytoplasmic expression of CD3, CD7, CD5, or CD2. In addition, a subclass of ALL referred to as pre-B transitional is discriminated by the expression of cytoplasmic ion heavy chains μ in immunoglobulins, and poor expression of these surface chains, in the absence of light chains λ or k [1]. A minute group (<5%) of ALL cases possesses indistinct genetic makeup given that they can manifest as lymphoid and myeloid markers (biphenotypic) or are characterized by two cell populations.

Clinical manifestations of ALL are extremely inconsistent. The patients may manifest a wide range of symptoms at presentation, such as bleeding, ecchymosis, shortness of breath, dizziness, and infections resulting from anemia, low platelet level and abnormally low count of neutrophils in

Table 1. Frequently identified cytogenetic aberrations & ALL prognosis [30]

Cell line	Cytogenetic abnormalities	Genes	Frequency (%): Reported populations (%)	Prognosis
B cells	Hyper ploidy		20-30 [1, 23, 28]	Favorable
	Hyper ploidy		5-6 [1, 23]	Unfavorable
	t(12;21) (p13;q22)	TEL-AML1	25 [1, 23]	Favorable
	t(1;19) (p23;q13)	E2A-PBX1	13 [23, 28]	Unfavorable
	t(9;22) (p34;q11)	BCR-ABL1	3-5 [23, 28]	Unfavorable
	t(4;11) (p21;q23)	MLL-AF4	6 [1, 23]	Unfavorable
T cells	9q34	NOTCH1HOX11	60 [23, 28]	Unfavorable
	10q24		20 [23, 28]	Unfavorable

Table 2. Cytogenetic prognostic factors in ALL

Aberration: Cytogenetics	Clinical effect	Remarks
MLL rearrangements	Unfavorable prognostic indicator	Use of pediatric protocols in adult patients is important. Immature immunophenotype, co-expression of myeloid antigens, B-cell lineage, & elevated WBC counts [35].
Philadelphia chromosome	Unfavorable prognostic indicator	8-10% adolescents, 15-30% adults, & 50% elderly [36, 37].
iAMP of chromosome 21	Unfavorable prognosis	About 2% of older children with B-ALL [37, 38].
t(1;19) [TCF3- PBX1]	Unfavorable prognostic indicator	Constitute about 30% of childhood ALL; unfavorable prognosis can be overcome with exhaustive chemotherapy treatment in both adults & children, higher risk of CNS relapse [39]. Adults exposed to hyper CVAD treatment exhibited higher CR & OS than other patients [40].
t(12;21) [ETV6- RUNX 1]	Positive prognosis	Apparent in an estimated 18-25% of children [41, 42].
Complex karyotype	Unfavorable prognosis	Over five chromosomal abnormalities [43].
Hypodiploidy	Unfavorable prognosis	5-6% of ALL cases; near haploid & low hypodiploid possess the worst perspectives [44].
Hyper diploidy	Positive prognosis	25-30% of cases; non-random gain of chromosomes X, 4, 6, 10, 14, 17, 18, & 21; A higher tendency for the cells to undergo caspase-induced cell death may be cause of good prediction [45].

Note. WBC: White blood cells; ALL: Acute lymphoblastic leukemia; iAMP: Intrachromosomal amplification; CNS: Central nervous system; CVAD: Combination of cyclophosphamide, vincristine, dexamethasone, doxorubicin, methotrexate, & cytarabine; CR: Complete remission, & OS: Overall survival

addition to extremity and arthritis or arthralgia [25]. Swollen lymph nodes enlarged spleen and/or enlarged liver are observed on physical assessment in roughly 20% of patients [25]. Abdominal masses from gastrointestinal contribution or numb chin syndrome from the involvement of cranial nerve are observable, although they are more indicative of mature B-ALL. About 10% of patient's exhibit indicative CNS involvement. T-lineage ALL with a mediastinal mass can initiate muscular breathing/extra thoracic airway obstruction and wheezing, superior vena cava syndrome (SVCS), and pericardial effusions. Testicular contribution is atypical in adults [26]. It was confirmed that Philadelphia chromosome-positive ALL cells has elevated level expressions of the non-DNA-binding isoform I_k6, which was produced subsequent to IKZF1 genomic deletions and aberrant splicing resulting in diverse non-DNA-binding Ikaros cDNA transcripts [27].

Cytogenetics

With regard to genetics, ALL is a heterogeneous and intricate entity, characterized by genetic alterations that include the presence of abnormal number of chromosomes i.e., aneuploidies (hyper diploid and hypodiploidy of > 50 chromosomes and <44 chromosomes, respectively) and chromosomal translocations. For pediatric leukemia, B-cell lineage translocations t(12;21) (TEL-AML/ETV6-RUNX1), t(1;19) (E2A-PBX1 /TCF3-PBX1) and t(9;22) (BCR-ABL) and fusions that entail the MLL (principally MLL-AF4) gene are subjected to the most explanations and most common genetic incongruities. In general, ETV6-RUNX1 is detected in 3-45% of cases, accompanied by TCF3-PBX1 (13%), BCR -ABL (3-5%) and MLL-AF4 (6.0%) [1, 28]. For instance, for ALL patients in Mexico, the initial three alterations are the most prevalent and comprise

between 17.7% to 28.8% of all genetic anomalies though BCR-ABL reached up to 19% in certain populations [29]. However, MLL gene reorganizations were noted in 1.4% of the entire cases and in 23% of patients <26 months. In addition, T-cell ALL is differentiated by mutations in NOTCH1 (equal to two-third of cases) and reorganizations in TLX1-HOX11 (5-10%), TLX3-HOX11L2 (20%) (Table 1) [1, 23].

Karyotype is a vital predictive factor with several cytogenetic defects being related to distorted diagnosis in ALL (Table 2). The occurrence of cytogenetic anomalies differs between pediatric and adult ALL and may partly clarify the disparities in experimental results among patients [25]. The importance of cytogenetic aberrations as the utilization of pediatric procedures in adult patients increases is not known [31]. About 90% of pediatric Philadelphia chromosome-positive (Ph+) ALL patients possess the typical ALL-type p190 translocation [32]. It has been reported that BCR-ABL1 translocation exists in about 8-10% of adolescents and is the main recurrent and clinically pertinent genetic aberration in adult ALL, i.e., from 15% to 30% with rising incidence, reaching 50% in the aged [26]. Till lately, the BCR-ABL1 was identified as the most unfavorable subclass of adult ALL. Even though CR rate varied from 75%-80%, median DFS was approximately 10 months and five years survival lower than 10-20% through only chemotherapy [33]. As early as 1995, it was reported that the CALGB 8811 medical assessment of t(9;22) identification exhibits an unfavorable impact on survival of statistical significance, with only an estimated 16% of patients with either a Ph chromosome or a BCR-ABL reorganization surviving for 36 months, in contrast to 62% of patients who showed negative results for both genetic tests (p<0.001) [34]. On the other hand, majority of studies have demonstrated a higher survival rate

with HSCT than only chemotherapy [33]. Combining chemotherapy with tyrosine kinase inhibitors (TKIs) have generated prospective outcomes, even though with ambiguous effect on lengthy DFS.

The t(4;11) occurs in approximately 60% of infants aged below one year, although it is seldom detected in adults. It was reported that the reorganized MLL gene is related to lower RFS and OS in adult patients with the use of a pediatric procedure [31]. The t(1;19) reportedly exists in 30% of childhood ALL. For example, it was assessed the effect of modern therapeutic treatment on children with t(1;19)/TCF3/PBX1 who were handled at St Jude Children's Research Hospital, where the patients exhibited similar EFS and a lesser collective occurrence of several hematological relapse. Data have shown that t(1;19) is an autonomous risk parameter for isolated CNS relapse [39]. Similarly, it was reported that adults subjected to hyper-CVAD treatment or regimen exhibited a considerably higher CR and OS than all other patients put together, and showed similarities with patients that displayed Ph+, t(4;11), and lymphoma-like aberrations (deletion 6q, addition q14q, t[11;14], and t[14;18]). The study inferred that adults with ALL and t(1;19) had an exceptional prognosis when treated with the hyper-CVAD regimen [40]. However, an earlier report discovered that this aberration is often related to early treatment breakdown, thus suggested that E2A-PBX1+ adult ALL should be taken into consideration for aggressive therapy [46]. The t(12;21) aberration resulting in ETV6-RUNX1 fusion is evident in approximately a quarter of children and 3% of adults with B-ALL. Patients normally exhibit a positive prognosis [47].

Hyper diploidy (>50 chromosomes) is observed in about 25%-30%, and 7% of pediatric and adult cases, respectively, and is the most frequently detected chromosomal anomaly in children. As presented in **Table 1**, hyper diploidy is related to a positive prognosis (20-30%) irrespective of leukocyte count and age [48]. Its distinctive genetic attribute is the uniform gain of chromosomes X, 4, 6, 10, 14, 17, 18, and 21, with individual trisomies or tetrasomies that are observed in more than three-quarter of cases. The individual structural aberrations show no effect on the result in patients with hyper diploidy with the exception of t(9;22), which is related to unfavorable prognosis.

The favorable prognosis may reveal a higher tendency of these cells to experience caspase induced cell death [39]. On the contrary, around 5 to 6% of ALL patients, regardless of age, lose different chromosomes, leading to a hypodiploid clone with less than 44-46 chromosomes. These patients usually have an unfavorable prognosis, particularly cases with near-haploid and low-hypodiploid clones [47]. Current data infer that composite karyotypes (≥ 5 chromosomal aberration) arise more regularly as persons aged and may be related to inferior survival [33]. Intrachromosomal amplification of chromosome 21 (iAMP21) takes place at a frequency that reaches 2% in grown-up children with B-cell precursor ALL [38]. iAMP21 is related to poor result and prognosis when patients are subjected to conventional treatment, since it is linked to a higher threat of both early and late relapses [47].

Diagnosis: Immunophenotyping

The evaluation of immunophenotype using flow cytometry is crucial component of the WHO classification of neoplastic diseases of hematopoietic and lymphoid tissues [26]. The preliminary immunophenotyping sheet needs to be sufficiently detailed to ascertain a leukemia associated phenotype (LAP), which enables its utilization in minimal disease monitoring

(MRD). The surveillance, epidemiology and end results (SEER) repository displayed a superior diagnosis with B cell in contrast to T cell immunophenotype in patients <20 years old, whereas in patients ≥ 20 years old, T cell immunophenotype showed more positivity [49]. This was demonstrated in a quantitative, epidemiological study [50] that comparatively analyzed the therapeutic effect of chemotherapy treatment for T- and B-lineage ALL and found relatively better survival in patients with T-lineage ALL, even though the addition of patients with Ph+ ALL possibly predisposed the outcome.

T-cell ALL in adults makes up majority of adult ALL and is considered a positive diagnosis. In both the LALA 87 and the UKALL/EGOG 2993 trials, T-ALL was related to male sex, age range of <35-39 years old, high WBC count and CNS involvement. A comparatively higher incidence of mediastinal mass and anemia were also noted in the LALA-87 trial [51]. Patients aged <40 who were treated with only chemotherapy, three years DFS demonstrated better efficacy in the group with a T-cell phenotype (59%) compared to a B-ALL phenotype (20%). No disparity in disease free survival (DFS) was observed between patients with B- and T- ALL treated with all or auto HSCT. Likewise, OS was found to improve in patients with T-cell antigen expression compared to B-lineage antigens [52].

Application of the protocol developed by the pediatric Dana Farber Cancer Institute (DFCI) showed a trend in the direction of enhanced clinical outcomes in both adolescents and adults with T-ALL [31, 33, 53]. The GRAALL study group [54] discovered that after 42 months of implementing a pediatric protocol, event free survival (EFS) rate was determined to be 62% and 52% in T-ALL and in BCP-ALL patients, respectively. Within the T-cell ALL subset, the prognosis is better for CD1a+ cortical/thymic phenotype compared with the pro-, pre-, and mature-T subtypes (CD1a-, CD3-/CD3+). The early T-cell precursor ALL that preserves stem cell-like characteristics is related to a gloomy prognosis with traditional chemotherapy [55] in both adult and childhood T-ALL patients [56].

As aforementioned, numerous research have indicated that patients with B-cell phenotype exhibit a rather poor prognosis compared to T-ALL patients. CD10-negative pro-B phenotype patients are regarded as high-risk, especially when linked with t(4;11)/abn q23 [33, 57]. The pre-B subtype that expresses cytoplasmic heavy chains has a dire viewpoint when retaining MLL rearrangements. The CD20 antigen is expressed in almost half of patients with B-cell ALL and its influence on clinical results is contentious. CD20 expression has been shown not to impact the attainment of total remission when implementing the childhood GRAALL 2003 protocol in adults within the age bracket of 15 to 60 years with Ph-negative ALL, although it was related to a higher collective occurrence of relapse (CIR) and lesser EFS at three and half years (42% vs. 29%) in patients with a WBC $\geq 30 \times 10^9/L$ ($p=0.006$).

Besides, a lower survival in CD20 positive patients using the adult-based hyper CVAD procedure was reported [58]. On the contrary, a retrospective study [59] reported no relationship between CD20 expression and clinical results amongst a cohort of adult patients, who were administered a pediatric-based regimen. There is a tendency towards positive EFS in patients displaying CD20 positivity. CD13 positivity is a self-regulating weak prognostic marker for OS, EFS and RFS in a group of patients subjected to the DFCI treatment approach. The CD13 prognostic value was largely observed in patients with regular or intermediate risk cytogenetics.

Table 3. WHO immunophenotypic classification of ALL [24]

ALL-subtype	Characteristics
B-cell ALL NOS	
Early pre-B ALL/pro B-ALL	CD19+, cCD79a+, cCD22+, nuclear TdT positive, & CD10-
Common ALL	CD10
Pre-B ALL	Cytoplasmic IgM, CD19+, CD79a+, CD22+, & CD10+
B-ALL with recurrent cytogenetic abnormalities	
t(9;22) (q34;q11.2); BCR-ABL1	CD10, CD19, TdT positive, & may express CD13/CD33. CD25 highly associated with t(9;22). p190 transcript (most pediatric cases) p210 transcript (50% of adult cases).
t(v;11q23); MLL	Highly frequent type of leukemia in infants. Short latency phase. High WBC & CNS involvement. Pro-B immunophenotype CD19+, CD10-ve, CD24-ve, CD15+ve neuroglial antigen-2 (NG2) relatively specific.
t(12;21) (p13;q22); TEL-AML1 (ETV6- RUNX1)	Rare in adulthood CD19+ve, CD10+ve, CD34 +ve (frequently). May have close to or total lack of CD9/CD220/CD66c myeloid antigens (CD13) commonly expressed? Essential but adequate for leukemic translocation?
Hyper diploidy	More than 50 & typically <66 chromosomes devoid of structural aberrations. Non-random: Chromosomes 21, X, 14, & 4 are most frequent; chromosomes 1, 2, & 3 being least widespread. CD19+ve, CD10+, CD34+ (most cases), & CD45-ve (frequent).
Hypodiploidy	<44-46 chromosomes. Structural abnormalities are infrequent. CD19+, CD10+ diagnosis may not be recognized by normal karyotyping due to endo-duplication
t(5;14) (q31;q32);IL3-IGH	Constitutive expression of IL-3 gene. Linked with eosinophilia, consider reactive & not part of leukemic performed. Diagnosis may be anchored in immunophenotype & genetic results even though BM blast count is low. CD19+ & CD10+.
t(1;19) (q23;p13.3); E2A-PBX1 (TCF3-PBX1)	CD19+, CD10+, cytoplasmic M heavy chain. Increased expression of CD9, lack of CD34 or very restricted CD34 expression.
T-cell ALL	
Precursor T- ALL	CD1a, CD2, CD3, CD4, CD5, CD7 and CD8. CD7 and cCD3 are most often positive. CD3 is lineage specific CD4 /CD8 are commonly co-expressed; CD10 may be positive; CD79a (10% of cases) CD13/CD33 (19-32% of cases).
Pro T-cell ALL	cCD3+, CD7+, CD2-, CD1a-, CD34+/-; CD4-, & CD8-
Pre T-cell ALL	cCD3+, CD7+, CD2+, CD1a-, CD34+/-; CD4-, & CD8-
Cortical T-cell ALL	cCD3+, CD7+, CD2+, CD1a+, CD34-; CD4+, & CD8+
Medullary T-cell ALL	cCD3+, CD7+, CD2+, CD1a-, CD34-, sCD34+; CD4+, or CD8+
Early T-cell precursor*	Lack of CD1a/CD8, weak expression of CD5 Presence of 1 or more myeloid or stem cell markers (CD117, CD34, HLA-DR, CD13, CD33, CD11b, or CD65).

Note. NG2: Neuroglial antigen-2; TdT: Terminal deoxynucleotidyl transferase; MLL: Mixed-lineage leukemia; BM: Bone marrow; & HLA: Human leukocyte antigen

Immunophenotype and Genotype Aberrations in T-Cell Acute Lymphoblastic Leukemia (T-ALL)

ALL is a tumor of immature B- or T-cells (lymphoblasts). There are several diverse ALL entities, majority of which are discernible solely with the use of immunophenotyping in addition to contemporary cytogenetic and molecular biology methods to identify particular chromosomal reorganizations and/or genetic modifications. Specifically, the t(9;22) translocation derived from a specific fusion between the ABL1 and BCR genes (leading to the supposed Philadelphia chromosome) arises in an estimated quarter of recorded cases and delineates a distinct B-ALL entity. The pathogenesis and differential-diagnosis of ALL are not well understood, although subjecting a person to or exposure to emitted radiation and various chemical agents were demonstrated to be related to an amplified risk of contracting the ailment. T-ALL is a biologically heterogeneous malignancy, which reveals characteristic phases of T-cell segregation arrest. It was analyzed a cohort of childhood T-ALL, with the aim of investigating whether the association of immunophenotypes with molecular modifications could forecast a patient's clinical outcome [60]. To identify genetic mutations, translocations and copy number alterations, RT-PCR, Sanger sequencing, MLPA and FISH were utilized. By means of multiparametric flow cytometry, eight immunophenotypic T-ALL subtypes: early T-cell precursor (ETP, n=27), immature (n=38), early cortical (n=15), cortical (n=50), late cortical (n=53), CD4/CD8 double negative mature (n=31), double positive mature (n=35) and simple positive mature (n=31) T-ALL was identified. The most recurring gene modifications were CDKN2A/Bdel (71.4%), NOTCH1mut (47.6%) and FBXW7mut (17%). The eight T-ALL subclasses are

distinguished by distinctive molecular profiles. The mutations in NOTCH1/FBXW7 and STIL-TAL1 reorganization had a prognostic effect that is not based on immunophenotype.

It was applied Immunophenotypic categorization of T-ALL subtypes based on earlier reported parameters [60]. Immunophenotyping was carried out via six color mix of monoclonal antibodies, implementing the criteria developed in [55] (as well as the score scheme from [61]) to recognize ETP-ALL cases. The classification of ALL is greatly dependent on morphological, Immunophenotypic, cytogenetics and molecular markers. Based on WHO categorization, there are 3 wide categories of ALL: precursor B-cell ALL, mature B-cell ALL and T-cell ALL. The immunophenotypic classification of ALL is presented in **Table 3**.

Treatment of ALL

The treatment of ALL is exceptionally intricate. It consists of several rounds of chemotherapy and, for certain patients, transplantation of stem cells [57]. It comprises three stages: induction, consolidation, and maintenance, with an array of available regimens and treatment courses. Historically, ALL treatment was anchored in chemotherapy and in certain cases, hematopoietic stem cell transplantation, frequently with high rates of mortality and relapses. Nonetheless, the recent categorization of ALL, which also considers cytogenetic and clinical multiplicity of various ALL entities, and the surfacing of new targeted therapeutic treatments, particularly TKIs, have radically transformed the treatment strategies and enhanced diagnosis and prognosis for patients with a number of ALL subclasses [62, 63]. Current developments in elucidating the biology of leukemia, modification in risk stratification and huge

international cooperative group clinical trials have remarkably improved in the outcome of pediatric ALL. In general, existing treatment regimens can cure over 80% of patients, given that it is now possible to identify a subset of patients who can be successfully cured with less rigorous treatment strategies [64]. Although approximately 20% of patients will eventually relapse, which makes relapsed leukemia a foremost cause of cancer-related mortality? Endeavors are currently in development to innovate strategies to properly recognize patients with high risk of relapsing in order to optimize their treatment [64].

Notwithstanding the significant developments in the treatment of ALL, the bulk of ALL cases succumb to the disease or treatment-related side effects. Thus, the identification of novel therapeutic targets may facilitate the production of new substitute treatment strategies such as suppression/induction of modified genes, immunotherapy [65, 66]. In ALL patients that display an expression profile analogous to BCR-ABL and which embody NUP214-ABL1 or EBF1-PDGFB gene fusions, imatinib, an inhibitor of ABL and PDGFB, is in preclinical assessment [65].

Given that T cells ALL is an uncommon and particularly invasive entity; carriers of mutations in the gene NOTCH1 is unresponsive to traditional therapies and exhibit high mortality and relapse rates. Hence, NOTCH1 inhibitors are being developed as gamma secretase inhibitors (GSIs) For ALL treatment, although they have not reached clinical stage since there is experimental proof of the resistance of leukemic cells to therapy with GSIs, a process that is possibly modulated by epigenetic incidents [66]. BDR4 and BCL2 are also target genes for the cure of T cells ALL. BDR4 is an over expressed protein in the formation of neoplasms and is labeled a possible activator for the expression of MYC in cancer and the anti-apoptotic molecule Bcl2. Actually, NOTCH1, BDR4 and BCL2 inhibitors can be combined as a substitute treatment for T cells ALL [67].

As an unconventional treatment, the recent interest in immunotherapy requires that the surface antigens of leukemic blasts are therapeutic targets. Therefore, bare antibodies and immunotoxins are in development, in addition to receptors for chimeric antigens and single chain bispecific antibodies that combine with T lymphocytes [68].

Adult Multidrug regimens have been implemented by a number of medical centers since the 1980s. It involves the design of treatments that combines accessible anti-leukemia drugs that can be administered in a succession of extensive treatments [26]. The multidrug combinations are based on a mixture of vincristine, anthracycline and prednisone, in the presence or devoid of cyclophosphamide and asparaginase. The M.D. Anderson model comprises the combination of hyper fractionated cyclophosphamide, vincristine, dexamethasone, and doxorubicin, varied with high-dose methotrexate and cytarabine (hyper-CVAD) [57]. The management involves eight courses: four courses of hyper-CVAD (courses 1, 3, 5, and 7) alternated with four courses of MTX and HIDAC (courses 2, 4, 6, and 8). The intended median survival time was 35 months with a five-year estimated survival of 39%. The pediatric approach, which is quickly being deployed globally, is the implementation of pediatric procedure or pediatric motivated course of therapy especially for young people and young adults roughly described as patients aged 15 to 35-45 [57, 69]. These courses of therapy involves considerably enhancing the non-myelosuppressive agents such as vincristine and steroids using asparaginase at significantly higher cumulative dosages for

prolonged asparagine depletion and providing very early and extended intrathecal methotrexate combined with systemic methotrexate (high-dose). This approach is tolerable in a large segment of adults.

Outline of Proteomics Techniques

Proteomes are the results of thousands of gene expressions. Posttranslational modifications (PTMs) and a substantial discrepancy in dynamic ranges are responsible for the complexities and confusion surrounding proteome identification. Thus, elucidating and making simpler the mechanism of proteomes would improve the effectiveness of the proteomic profiling. For that reason, the bulk of proteomic research focuses on enhancing separation techniques with the aim of improving the resolution of complex proteomes. Irrespective of the progress in liquid chromatography, the comprehensive evaluation of proteomics (top-down and bottom-up proteomics) remains the focus. This is to be expected since it is very hard to solely rely on a single technique or equipment for the identification and quantification of aspects of a complex protein sample in a straightforward, single-step procedure. A number of tools and measures are needed to separate and identify polypeptides as well as data analysis and integration. The foremost separation tools utilized to identify proteomes include gel-based proteomics, ionization methods, mass spectrometry equipment, and quantification techniques [40, 70]. The two-dimension electrophoresis (2-DE) is anchored in the process of protein separation based on the existing charges and molecular mass in an electric field. 2-DE is the most effectual and high-resolution protein differentiation technique for composite proteomic combination. These techniques are currently well-tested and are frequently utilized in proteomic analysis [71].

Proteomic mass spectroscopy-based techniques are utilized for the identification of considerable amounts of novel proteins that are prospectively biomarkers. The approaches involved in the development of proteomic biomarkers include the detection of indicators or identifiers by combining mass spectroscopy with multidimensional protein detection equipment [72]. Fluorescence in situ hybridization (FISH) is utilized for monitoring ailments and identifying particular aberrations. The recent use of computational systems and bioinformatics for data analysis related to biomolecules on an extensive level has become a critical and well established field in molecular biology, which is important due to its use for the management of huge volume of records and analysis of multifaceted and intricate dynamic process [73].

Using Proteomic Strategies to Decipher ALL Pathways

Protein biomarkers can discriminate between high- and low-risk ALL in a tissue specific way. The activation, propagation and survival of B and T cells for the duration of ALL is regulated by a number of signaling pathways that include PI3K/AKT/mTOR, JAK/STAT, ABL tyrosine kinase, or NOTCH1 or SRC family of tyrosine kinases [74]. Leukemogenesis is modulated by regulating and interfacing of those signaling cascades as a network. The mTOR activity is elevated at ALL-relapse, and thus recommended as the therapeutic target for the formulation of novel drugs to treat human solid cancers or lymphoid malignancies that include ALL. Recently, studies have reported the up-regulation of cyclin E in patients in the initial phase of relapse [75]. This has shown that signaling pathways are related to the progression of ALL [76]. However,

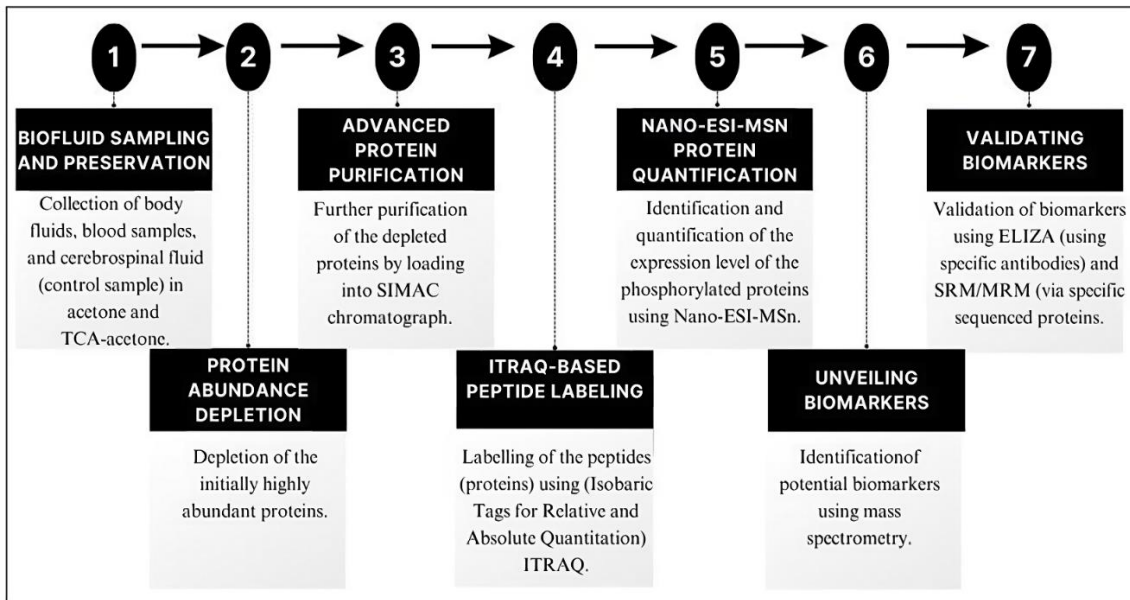


Figure 1. Feasible flow-through proteomic strategies [74-79]

there is an inadequate explication of the crucial function of proteins related to the stimulation of signaling pathways. Moreover, the role of proteins in network-based interaction in the course of diagnosing and predicting ALL-relapses has not been well elucidated. Nonetheless, phosphoproteomics are utilized for the identification and determination of protein based biomarkers of ALL-relapse and to investigate roles of targeted proteins in cells, tissues, or organs to enhance treatment strategies [77, 78].

Flow-through proteomic approaches may be valuable for ALL-relapse studies by providing a clear outline of the use of protein biomarkers to discriminate between high and low risk ALL. The strategy begins with the critical requirement of sample preparation of body fluids to obtain efficient data for proteomics [79]. For instance, cerebrospinal fluid (CSF) holds a smaller quantity of proteins that that of blood, which can be drastically reduced at the conclusion of the therapy, given the absence of leukemic cells in CSF after the treatment. Thus, CSF can serve as control. Afterwards, the proteins must be purified/isolated from the body fluids using precipitations of acetone and TCA-acetone, followed by depleting of the initially high abundant proteins as they can affect or serve as artifacts in the identification of biomarkers for ALL relapses. The resultant purified/depleted proteins are subsequently loaded into sequential elution of immunoaffinity chromatography (SIMAC) to further purify the phosphorylated proteins. Afterwards, isobaric tag for relative and absolute quantitation (iTRAQ) is utilized to label the peptides so as to recognize and quantify the expression level of the phosphorylated proteins using ElectroSpray Ionization and tandem mass spectrometry (nano-ESI-MSn) for a specific sample. The resulting likely biomarkers are recognized using mass spectrometry, which are then validated by means of ELISA and SRM/MRM. SRM or MRM is a technique of MSn that facilitates the picking of an ion with a specific mass in the initial phase of a triple quadrupole. The flow-through proteomic strategy is outlined below (Figure 1).

In addition, for the different stages of pathology (diagnosis, for the duration and at the conclusion of chemotherapy) of ALL patients suffering relapses, there is schedule for analyzing the body fluid samples (peripheral blood, CSF, BM). The samples

are analyzed using replicates placed in different vials to guarantee reproducibility of the data. The steps entailed in the analysis are outlined in Figure 2. First, replicates of the samples obtained from a repository of biodata are tested to enable reproducibility and guarantee the optimal analytical conditions are adhered to. This is imperative so as to achieve efficiency in data collection. Secondly, the lymphocytes acquired from the peripheral blood and BM samples can be extracted using Ficoll, whereas the cells from the CSF can be isolated by means of centrifugation. The cells from the three sample groups are required to be listed via kits for human cells (i.e., RIPA), and the resultant proteins can be dissolved or digested by the use of trypsin to obtain the composite combination of peptides from the samples. Lastly, the resultant peptides can be loaded into sequential elution of IMAC (SIMAC) chromatograph for isolation of the phosphorylated peptides (or phosphopeptides). Afterwards, the isolated/purified phosphopeptides are subjected to the processes of desalting, cleaning and concentration (i.e., via POROS R3 reverse-phase chromatography) before injecting them into the mass spectrometer. The mass spectrometry data allows improvement of the data on signaling networks associated with ALL relapses, which facilitates the acquisition of the phosphoproteome reference map of ALL-relapses, thereby unraveling vital indications of ALL-relapses. The use of 150 µg of proteins for each sample (individualized or pool of samples) is sufficient to perform the entire proteomic approach and biomarker recognition for ALL-relapses [11].

The illustrated issues would untangle the key indications that distinguish between stable remission ALL cases and relapses. The aforementioned approach can be deployed for determination of up and down-regulated (and post-translationally modified) proteins due to ALL-relapse. This can improve diagnosis and enhance the efficiency of therapeutic treatments. As hospitals, universities, research centers, and the industry implement this strategy and collaborate, information from different patients can be collated; consequently improving the statistical significance of data and advance the clinical benefits.

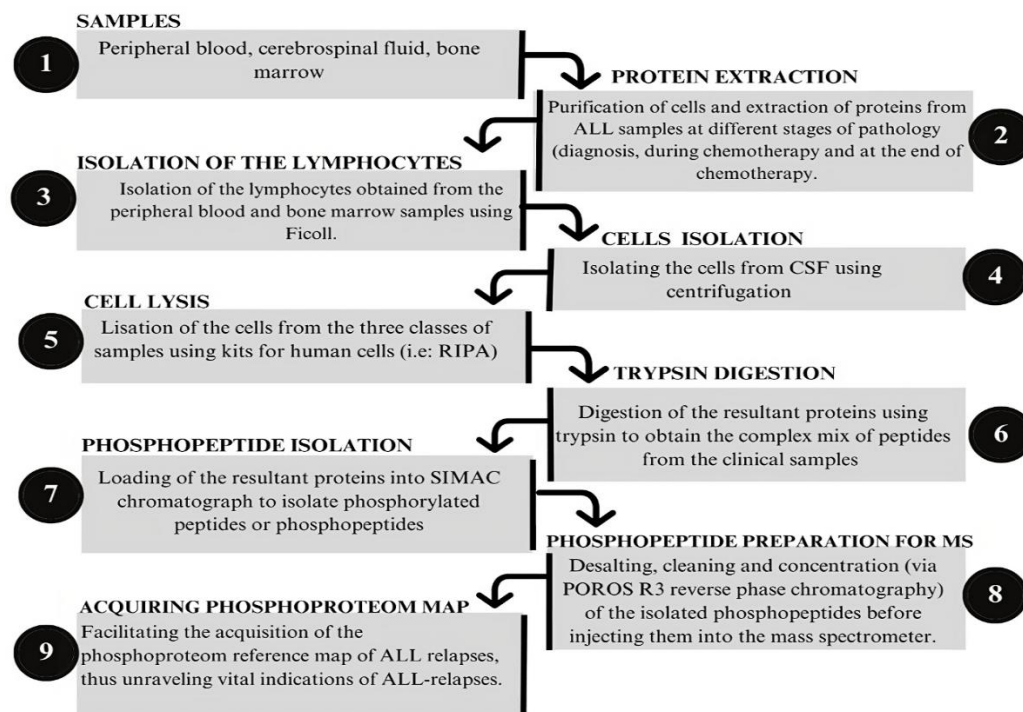


Figure 2. Workflow for examination of body fluid samples for various stages of pathology for ALL patients [11]

In summary, the illustrated proteomic tools indicate developments in clinical studies on paediatric ALL-relapses. Although 2DE-gels enable advances in clinical research, 2DE-electrophoresis mainly visualizes high abundant proteins; hence, protein biomarkers with low expressions can be missed or overlooked using this tool. Nonetheless, 2DE is an appealing instrument for performing ALL studies on isoform-proteins. By directly utilizing LC-MS/MS in preference to, or avoiding 2DE-gels, the accuracy of the data can be improved with more refinement. The appropriate proteomic strategy is dependent

on the research objectives and kinds of clinical samples to be analyzed. By effectively deploying the work-flow presented in **Figure 1** and **Figure 2**, the actual protein biomarkers of ALL-relapses can be identified, which will certainly contribute to innovating effectual treatments for ALL patients that succumb to relapses. An overview and significant proteomic flow-through of a number of leukemia research are mentioned in **Table 1**, presenting the sample characteristics and study objectives. Case studies of leukemia research utilizing proteomics are outlined in **Table 4**.

Table 4. Synopsis of leukemia studies using proteomics

Study	Methodology	Samples/targets/study objectives	Findings
[11]	Used SELDI-TOF-MS. Subsequently, selected candidates were submitted to LC-MS/MS & validated via protein chip immunoassays.	Measured the proteomic serum profiles.	Identified as potential protein biomarkers of pediatric ALL: platelet factor 4 (PF4), connective tissue activating peptide III (CTAP-III), & two fractions of C3a, to differentiate pediatric ALL patients from healthy controls & pediatric AML patients.
[12]	Applied global chromatin analyses via MS coupled with proteomics to detect & quantify levels of histone modifications in bulk chromatin to visualize histone alterations related to cancer.	Performed proteomic approaches to detect distinct molecular chromatin signatures profiling global histone modifications in human cancers.	Identified specific chromatin finger-prints when comparing 115 cancer lines. NSD2p.E1099K alteration was identified in 14% of t(12;21) ETV6-RUNX1-containing ALL.
[80]	Surface-enhanced laser desorption/ionization time-of flight mass spectrometry (SELDI-TOF-MS).	Pretreatment of leukemic bone marrow arising from pediatric leukemia cases & analysis of cell lysates from juvenile leukemia cell lines were both performed.	Proteome analysis has shown ability to efficiently differentiate between different types of juvenile leukemia.
[81]	Applied proteomics techniques using DIGE combined to mass spectrometry (MS) with a MALDI-TOF.	Studied proteins whose expression level was affected via comparing between leukemia cell line HL-60 & adriamycin-resistant HL-60 (HL-60/ADR).	Outcome showed that proteins directly involved in drug resistance include nucleophosmin/B23 (NPM B23) & nucleolin C23 (C23) are
[82]	Applied 2DE coupled to MS & bioinformatics tools.	Created novel molecule-hybrids of spirocyclic ketones with antiproliferative, pro-apoptotic, & differentiating action in leukemia cell lines.	After proteome comparisons, proteins involved in cellular metabolism, chaperone function, cytoskeletal structure, & RNA synthesis were found to be differently expressed. MEL-S3-treated leukemia cells showed a marked expression of glycoprotein IIb/IIIa (CD41) & glycoprotein Ib (CD42).

Table 4 (Continued). Synopsis of leukemia studies using proteomics

Study	Methodology	Samples/targets/study objectives	Findings
[83]	Utilized the medication prednisolone to find effective prednisolone-drug regulators and possible prognostic biomarkers (2DE coupled to MS) and specific Leukemia cells of REH 697, Sup-B15 and RS4 11 via a mass spectrometer (MALDI-TOF/TOF).	Performed to identify prognostic protein biomarkers in acute lymphoblastic childhood leukemia (ALL).	Identified 77 proteins. 17 showed modified protein-level expression when comparing prednisolone cell lines sensitive versus resistant. Examples of the identified proteins included PCNA, cofilin 1, VDAC1 and proteasome activator subunit 2 (PA28b).
[84]		Compared 94 pediatric ALL patients with 84 healthy controls.	Emphasized significance of using three protein biomarkers: platelet factor, connective tissue activating peptide III, & two fragments of C3a to distinguish pediatric ALL patients from healthy controls & pediatric AML patients when analyzing protein profile of serum.
[85]	Used phosphoproteomics to identify & quantify around 2000 phosphorylated residues in acute myeloid leukemia (AML), lymphoma (LPH) and multiple myeloma (ML) cell lines.	Performed phosphoproteomics to classify haematological cancer cell lines based on the type of tumor and the sensitivity to kinase inhibitors.	Identified and quantified around 2000 phosphorylated residues in AML, LPH and ML cell lines. They reported that cell lines within these diseases can be differentiated according to their specific phosphoprotein content.
[86]	A review study.	Presented a review showing several proteomic tools which obtained refined clinical data from ML cells and proteins via: SILAC, iTRAQ, & DIGE, for biomarker discovery; & via RPPA (reverse-phase protein array: RPPA is an antibody-based assay, which detects & quantifies protein production-) & MRM, for biomarker validation.	Detailed that studies on myeloid leukemia -cell proteome allow the identification of a large number of new biomarkers. They also indicated that the prediction of response to therapy - through identifying these markers- is an interesting avenue for future personalized medicine.
[87]	Used 2DEgels coupled to MALDI, to identify proteins that serve as potential biomarkers.	Identified these proteins: CLUS, CERU, APOE, APOA4, APOA1, GELS, S10A9, AMBP, ACTB, CATA and AFAM, which play vital roles in Leukemia prognosis, possibly acting as distinctive biomarkers for Leukemia aggressiveness, or as suppressor proteins in HR (high risk)-ALL pediatric cases.	Majority of proteins were found to be up-regulated in HR & LR (low risk)-ALL BM & PB from pediatric patients at diagnosis when compared to non-leukemic patients (control). Recommended that detection of BICR1 in ALL pediatric-patients can be an indicator of early telomere dysfunction in children. CLUS, CERU, APOE, APOA4, APOA1, GELS, S10A9, AMBP, ACTB, CATA, & AFAM proteins were shown to be pertinent regulators in differentiating between HR & LR-ALL via bioinformatic tools. Vitronectin & plasminogen can partially account for leukemogenesis, whereas bicaudal related protein 1 could possibly be an important biomarker for pediatric ALL therapeutics.

CONCLUSIONS AND FUTURE PERSPECTIVES

In recent times, emphasis has been given to studies of leukemia via proteomic strategies. Proteomics is a clear approach to profiling ALL since proteins can be altered in cellular reactions to internal and external stimuli, and progression in disease. Transformation of fundamental findings into comprehensive treatment strategy has led to numerous studies on proteomic profiling of potential biomarkers. Proteome analysis is capable of relating gene expressions to cellular functions, which can then be utilized for assessment of progression in ALL, diagnosis and prognosis, and response to treatment. Hence, this review highlights proteomic profile, clinical manifestations and diagnosis, and treatment of ALL with emphasis on cytogenetics and immunophenotyping. With implementation of flow-through proteomic strategies and the wide-ranging workflow for the examination of body fluid samples for various stages of pathology for ALL patients, relapse cases can be drastically reduced. Also, by effectively charting sites of proteins in cells via proteome analysis; it is feasible to study molecular and biological mechanisms that control manifestation and growth of ALL. It is evident from review that proteome analysis should be utilized for viable profiling of ALL.

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Data sharing statement: Study data is available upon request from the author.

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