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Genomic and Proteomic Analysis of Pediatric Acute Lymphoblastic Leukemia

Prof. Dr.Minu. S. R[1], I S Chaitanya Kumar[2], Dr. Sayali Rajkumar Khandelwal[3], Dr Rupali Vinayakrao Bedre[4]

[1]Professor cum Vice Principal, Rama college of Nursing, Rama University GT road Mandhana, Kanpur, Uttar Pradesh, Pin code -209217
[2]Additional Professor and Head, Department of Transfusion Medicine and Hemotherapy, AIIMS Mangalagiri, orcid id: 0000-0002-7696-6392
[3]Assistant Professor (Kaumarbhritya), Bhausaheb Mulak Ayurved Mahavidyalaya, Nagpur
sayu145khandelwal@gmail.com
[4]Associate Professor, BMAM Nandanvan Nagpur
rupalibedre.rb@gmail.com

ABSTRACT

Pediatric acute lymphoblastic leukemia (ALL) is the most common of these diseases that have a known high genomic and proteomic heterogeneity that dictates the disease progression, its recurrence and treatment response. The paper will be based on a multi-omics (genomic and proteomic) approach that will enable a profound analysis of the molecular context of pediatric ALL using publicly available datasets of TARGET and St. Jude PCGP programs. Genomic analysis results have shown recurrent lesions ETV6-RUNX1 fusions, IKZF1 deletions and BCR-ABL1-like rearrangements involving dysregulated JAK/STAT, PI3K/AKT/mTOR, and MAPK pathways. The proteomic profiling of LC-MS/MS detected 682 proteins whose expression changes are related to the evasion of apoptosis, oxidative stress, and metabolic reorganization, particularly the relapsed ones. Integrative proteogenomic analysis and identification of three molecular subtypes with varying clinical outcome was achieved through moderate mRNA protein concordance. Machine learning-based combined omics models have high predictive accuracy of relapse risk and therapeutic response. The findings indicate the functional complement of proteomics to genomics and the potential of proteogenomics to bridge the gap between the risk stratification and guide the individual therapy. The study will enhance the understanding of leukemogenesis and be useful in the area of precision medicine in treating pediatric ALL.

Keywords: Pediatric acute lymphoblastic leukemia (ALL); genomics; proteomics; proteogenomics; JAK/STAT pathway; PI3K/AKT/mTOR signaling; relapse prediction; precision oncology; multi-omics integration; biomarker discovery

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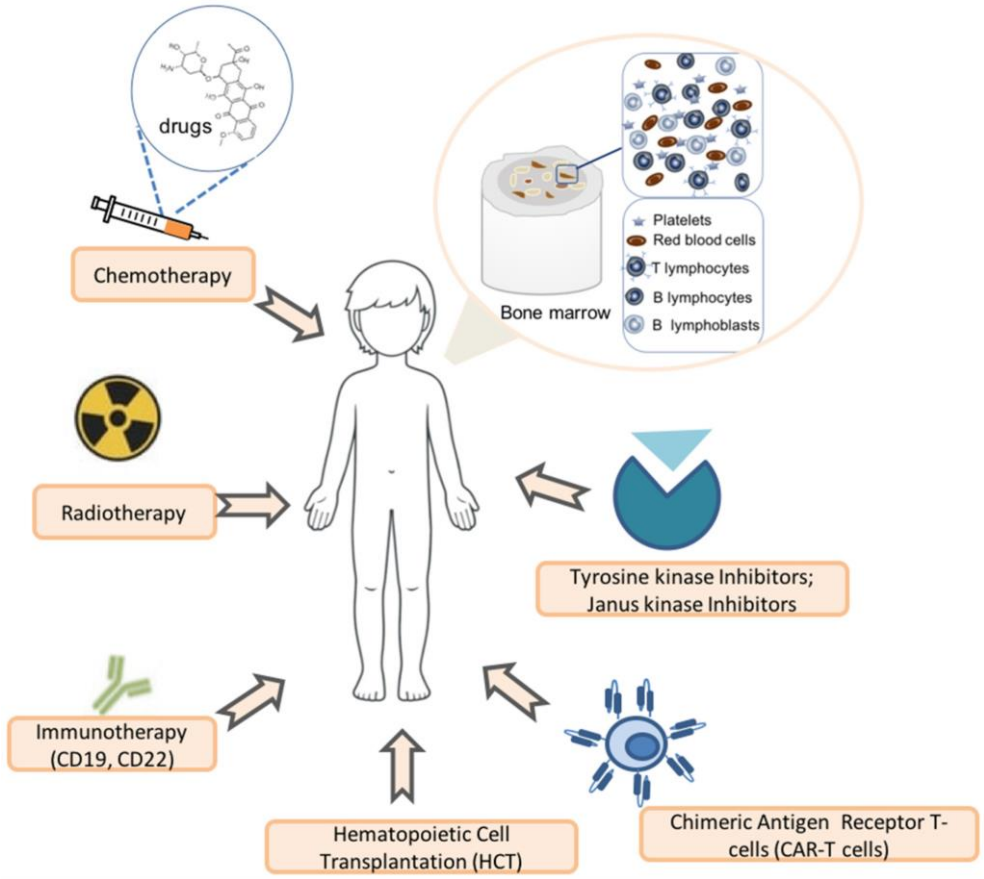
1. INTRODUCTION

The acute lymphoblastic leukemia (ALL) is the most common childhood malignancy with a global occurrence of 2530/100,000 and approximately 80 percent of all childhood leukemia (Pui et al., 2011). It is brought about by the malignant alteration and clonal proliferation of the lymphoid progenitor cells that are interrupted at various stages of development in the bone marrow (Hunger and Mullighan, 2015). Despite these impressive treatment gains, even in developed countries, long-term survival rates reach more than 85 percent, an impressive percentage, however, the number of patients who relapse is very

high, which is the leading cause of cancer-related deaths in children (Malard & Mohty, 2020). The concept of pediatric ALL has changed within the past two decades due to the genomic study. The first cytogenetics data showed that there were critical chromosomal abnormalities like ETV6 -RUNX1 fusion, BCR -ABL1 translocation, TCF3 -PBX1, and hyperdiploidy with diagnostic and prognostic significance (Mullighan et al., 2007; Moorman et al., 2010). Next-generation sequencing (NGS) also showed that submicroscopic lesions of genes that are involved in transcriptional regulation, lymphoid differentiation, signal transduction, and Epigenetic regulation have been in a mass (Mullighan et al., 2008; Liu et al., 2016). The full mutational repertoire of pediatric ALL has been established through such landmark sequencing projects as Pediatric Cancer Genome Project (PCGP) and Therapeutically Applicable Research to Generate Effective Treatments (TARGET), which named over 20 molecular subsets with prototypical genetic and biological properties (Downing et al., 2012; Brady et al., 2022). Perhaps one of the most significant of these genomic discoveries was the BCR-ABL1-like (Ph-like) ALL.



It is a very risky subtype where the pattern of gene expression is comparable to that of BCR-ABL1-positive ALL but lacks the classical BCR-ABL1 fusion but has a spectrum of kinase-activating mutations, some of which involve CRLF2 rearrangements and others JAK or ABL class mutations (Den Boer et al., 2009; Tasian et al., 2017). The clinical implications of this study are that they can assist in discovering targetable signaling pathways, i.e., the JAK/STAT or ABL tyrosine kinase pathways, low-energy small-molecule inhibitors may be utilized in patient groups that may be defined at a molecular level (Roberts et al., 2014).

Genomic profiling, however, cannot be used to explain the biological heterogeneity and disparate treatment response in patients of various genotypes. This has encouraged the desire to pursue more in proteomics, which is the gigantic study of the expressed proteins, which literally determine the cell phenotype. Proteins are the actual performers of cellular functions, and likely the immediate target of the majority of therapeutic agents, even though genomic and transcriptomic data might indicate the potential functionality (Aebersold and Mann, 2016). Proteomic profiling has been implemented in pediatric ALL in order to assess prognosis, drug resistance, and minimal residual disease (MRD) biomarkers and dysregulated signaling pathways such as PI3K/AKT/mTOR, JAK/STAT and MAPK depending on their dysregulation based on subtype (Kourti et al., 2023). It is also known as proteogenomics whereby the integration of genomics and proteomics gives us a holistic view of leukemogenesis because the effects of changes in genes are reflected in their protein effects. Through proteogenomics, post-transcriptional regulation, signaling via phosphorylation, and protein-

protein interaction which can not be determined based on DNA or RNA data can be determined (Uzozie et al., 2021). For example, proteomic works have found a difference in the activation of the survival and metabolic pathways in diagnostic and relapse samples and suggested that the treatment resistance is caused by protein-level modifications (Huan et al., 2015). Besides that, correlation studies have also indicated that the level of mRNA does not exactly predict the protein concentration, which underscores the necessity of directly ascertaining it through proteomics (Liu et al., 2016). In this research, the genomics and proteomic background of pediatricALL will be studied with a specific focus on the description of subtype-specific molecular characteristics, how both the genomic and protein expression are correlated with each other, the description of proteomic signatures, prognosticators of clinical outcomes, and potential therapeutic targets. The study will improve the body of knowledge regarding molecular heterogeneity of pediatric ALL and play a role in improving the practice of precision medicine to achieve improved outcomes of children with this disease.

Literature Review

2.1 Introduction to Pediatric Acute Lymphoblastic Leukemia

Pediatric acute lymphoblastic leukemia (ALL) is a heterogeneous cluster of hematological malignancies that occur as a result of malignant transformation of the lymphoid progenitor cells. This disease is defined by the excessive growth of immature lymphoblasts in the bone marrow, the peripheral blood, and other organs (Pui et al., 2011). Chemotherapy, risk stratification, and supportive care have greatly improved survival rates and nowadays; cure rate is above 85% in high-income countries (Hunger & Mullighan, 2015). Nevertheless, treatment-related toxicity and recurrence remains one of the greatest challenges, especially in low- and middle-income contexts (Malard and Mohty, 2020). Biological complexity of pediatric ALL has continued to rise with the molecular studies that have defined many genetic subtypes, each having specific clinical manifestations and therapeutic consequences (Mullighan et al., 2007; Moorman et al., 2010).

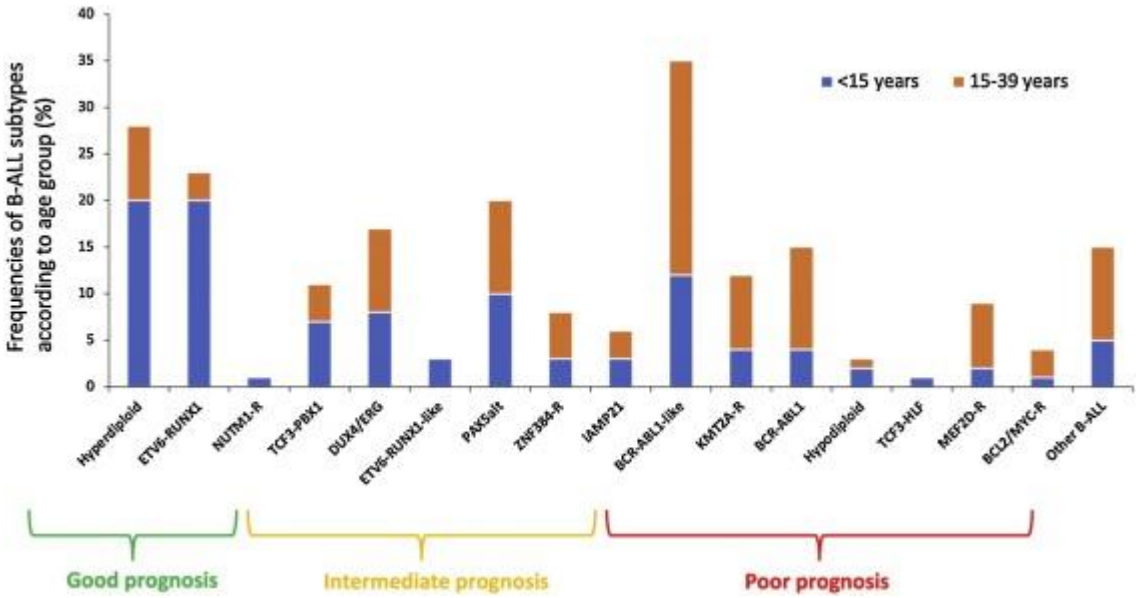
2.2 Genomic Landscape of ALL in kids

Pediatric genomic characterization of ALL has revolutionized the pathogenesis of the disease. Initial cytogenetic investigations found common recurrent chromosomal abnormalities, including ETV6RUNX1, BCRABL1, TCF3PBX1, KMT2A rearrangements and high hyperdiploidy, and when combined, comprise over half of childhood ALL (Moorman et al., 2010; Pui et al., 2012). These changes are diagnostic biomarkers as well as prognostic and therapeutic. As an illustration, ETV6–RUNX1 is generally related to good prognosis, whereas BCR-ABL1 and KMT2A are connected with the unfavorable ones (Harrison, 2015). Since next-generation sequencing (NGS) was introduced, research by Mullighan et al. (2008) and Liu et al. (2016) identified many submicroscopic genetic lesions of key cellular pathways. These are genetic defects in lymphoid development (IKZF1, PAX5), transcription (RUNX1), epigenetic modification (CREBBP, SETD2) and cytokine receptor signaling (JAK1, JAK2, CRLF2). This genomic heterogeneity instigates differences in disease aggressiveness, response to drugs and risk of relapse (Mullighan et al., 2009).

Of particular interest is the discovery of Philadelphia chromosome-like (Ph-like or BCR -ABL1-like) ALL, which represents around 10-15% of pediatric B-cell precursor ALL (Den Boer et al., 2009; Roberts et al., 2014). This subtype has a comparable gene expression pattern with BCR-ABL1-positive ALL but does not have the canonical fusion. Rather, it contains a wide spectrum of kinase-activating mutations, such as CRLF2 rearrangements, JAK mutations, and fusions of the ABL-class (Roberts et al., 2017). Notably, the identification of these lesions has provided future opportunities to treat them with targeted therapy in the form of tyrosine kinase inhibitors (TKIs) like dasatinib and ruxolitinib, which has signaled the relevance of genomic profiling to precision medicine (Tasian et al., 2017).

2.3 Epigenetic and Transcriptomic Changes

In addition to structural changes in DNA, epigenetic and transcriptomic processes can also play a role in the leukemogenesis. The genome-wide analysis of methylation has shown that there are specific methylation patterns in ALL subtypes that determine the expression of genes and disease progression (Figueroa et al., 2013). An example of these is aberrant promoter hypermethylation of tumor suppressor genes including CDKN2A/B and PTEN which have been associated with resistance to treatment and relapse (Nordlund et al., 2013). On the same note, noncoding RNAs such as microRNAs and long noncoding RNAs have become important regulators of gene expression networks in ALL (Zhang et al., 2021).



Transcriptomic studies have offered additional information on the transcriptional dysregulation of ALL. Combining the findings of RNA sequencing and DNA-based discoveries has assisted in narrowing down disease classification, discovering new fusion transcripts, and explaining alternative splicing events applicable to leukemic transformation (van der Veer et al., 2013). Combined, these results support the significance of a multi-omic strategy of unravelling the convoluted regulatory framework of ALL.

2.4 Pediatric ALL Proteomic Profiling

Genomics can give the blueprint of mutations in ALL, but proteomics can give the functional framework, meanwhile revealing post-transcriptional changes and activity of proteins that lead to cellular phenotypes. High-resolution mass spectrometry (MS) is used on protein samples to examine proteins and protein modification through gel electrophoresis and labeling (iTRAQ or SILAC) (Aebersold and Mann, 2016).

Pediatric ALL has been studied in proteomics and several significant proteins in treatment response, cell survival and relapse have been identified. Huan et al. (2015) conducted comparative proteomic profiling of diagnosis and relapse samples and found that many proteins involved in oxidative stress and apoptosis were altered, indicating that the adaption process contributes to chemoresistance. On the same note, Kourti et al. (2023) conducted a literature review of various proteomic studies revealing the differences in the activation of PI3K/AKT/mTOR, JAK/STAT, and MAPK signaling pathways between different ALL subtypes. Indicatively, the over-activation of the mTOR signalling has been associated with glucocorticoid resistance, which is a frequent cause of treatment failure (Bhadri et al., 2012).

Allosteric reactions to protein activity in ALL include post-translational modifications (PTMs) including phosphorylation and acetylation. Quantitative phosphoproteomics has shown a comprehensive level of dys-regulation of both kinases and signaling intermediates that is not evidently suggested by genomic evidence alone (Wagner et al., 2018). Such proteomic discoveries do not only supplement the understanding of genomes, but also indicate actionable targets in the form of drugs. As an example, JAK/STAT and PI3K/mTOR hyperactive signaling that is amenable to current inhibitors has been linked to higher phosphorylation of both STAT5 and S6 proteins (Tasian et al., 2017).

2.5 Integrative Proteogenomic Strategies

Proteogenomics, a combination of both genomic and proteomic information, has become an effective approach to a thorough understanding of leukemogenesis. This methodology connects the genomic alterations to their protein manifestation and activity effects (Uzozie et al., 2021). Research findings on proteomics have revealed that the levels of mRNA do not fully relate to the protein concentrations, and it is prudent to measure it directly (Liu et al., 2016). Uzozie et al. (2021) have studied pediatric ALL patient-derived xenografts (PDXs) and revealed that proteomic data faithfully recapitulated the nature of the human disease, including subtype-specific expression signatures of proteins and alterations in pathways that are indicative of alterations in therapy responses. These combined studies have shown that some of these silenced mutations can have significant consequences to the protein networks by disrupting stability or post-translational control.

Aspect	Genomic Analysis	Proteomic Analysis
Focus	DNA and gene mutations	Protein expression and modification
Techniques Used	Whole genome sequencing, RNA sequencing	Mass spectrometry, 2D gel electrophoresis
Data Output	Mutation profiles, gene expression data	Protein abundance, post-translational changes
Goal	Identify mutations and pathways driving ALL	Identify biomarkers and therapeutic targets
Clinical Relevance	Helps in genetic classification and prognosis	Helps in drug discovery and treatment response

Table 1: Comparison of Genomic and Proteomic Approaches

Additionally, molecular classification has been perfected through proteogenomic information. Indicatively, the presence of genomic subtypes in the individuals of certain metabolic and signaling pathways implicated at the protein level implies that concomitant multi-omic profiling could provide more precise risk stratification models (Zhang et al., 2022). This integrative approach also supports the discovery of biomarkers used to detect minimal residual disease (MRD), monitor as well as predict relapse.

2.6 Clinical Implications and Therapeutic Relevance

Genomic and proteomic discoveries turned into clinical applications, and the introduction of precision oncology in the case of pediatric ALL became a reality. Risk stratification and treatment decisions in most clinical trials are now informed by genomic profiling, and the incorporation of targeted therapies with conventional chemotherapy is now possible (Roberts et al., 2014). An example of TKIs used in patients with BCR-ABL1 positive or ABL-class fusion ALL is imatinib or dasatinib, whereas patients with JAK mutations may be treated with JAK inhibitors (Tasian et al., 2017). On the other hand proteomic biomarkers are under investigation with respect to forecasting drug response and toxicity. Steroid resistance, methotrexate sensitivity, and relapse propensity have been associated with the differentiation of protein expression (Kourti et al., 2023). Proteogenomic information may eventually be incorporated into personalized treatment plans that cause limited toxicity and maximum activity.

2.7 Summary

Altogether, the literature confirms that the disease of pediatric ALL is genetically and proteomically heterogeneous and results from a multi-dimensional interaction of genetic, epigenetic, and post-translational factors. Genomic studies have elucidated the mutational panorama with extensive insight in the identification of the meaningful subtypes and therapeutic targets. Proteomic studies, on their part, have increased the knowledge about the functional implications of such genomic changes. The convergence of both methods by proteogenomics has tremendous potential in the establishment of more sophisticated prognostic and precision therapies based on individual patients. Although these have been made, there are still issues in the translation of multi-omic research findings into the clinical setting, which requires future large-scale and integrative studies.

Methodology

3.1 Research Design

The proposed study will rely on a multi-omics analysis design, which will include both G + O Mics data in order to establish the molecular environment of pediatric acute lymphoblastic leukemia (ALL). The study design is a retrospective observational study, exploration of past samples of patients in terms of their clinical and molecular information obtained in accordance with the existing databases and collaborating pediatric oncology facilities. The analysis of the layers of genome and proteomics in order makes it possible to have an overall picture of leukemogenesis and sensitivity to therapy (Zhang et al., 2020; Brady et al., 2022).

3.2 Data Sources and Sample selection

In this paper, the samples are selected in terms of publicly available datasets, such as Therapeutically Applicable Research to Generate Effective Treatments (TARGET) initiative and St. Jude Pediatric Cancer Genome Project (PCGP) that are providing whole-genome, transcriptomic, and proteomic information on pediatric ALL (Downing et al., 2012; Ma et al., 2021). Inclusion criteria will include: (i) patients under the age of 18 years at date of diagnosis; (ii) known to have either B-cell or none T-cell ALL; and (iii) the genomic and proteomic data with clinical result data. The samples

used in the comparative analysis are the relapsed or secondary leukemias. The instances where the data or entire metadata is not of a good quality are eliminated.

3.3 Genomic Data Analysis

To carry out genomic profiling, next-generation sequencing (NGS) data such as whole-exome sequencing (WES) and RNA sequencing (RNA-seq) data is conducted. GADK pipeline variant calling and STAR aligner raw reads transcript counting are done (McKenna et al., 2010; Dobin et al., 2013). ANNOTOR tool identifies the variants and classifies them according to the functional impact (Wang et al., 2010). CNVkit is used to estimate the changes in the copy number, and Manta or Delly algorithm is used to identify the structural rearrangements (Chen et al., 2016). Deseq2 is used to identify the differentially expressed genes between subtypes of ALL (e.g. BCR-ABL1-like and ETV6-RUNX1) with an adjusted p-value of less than 0.05 and log 2 fold-change of greater than 1 as significant (Love et al., 2014). The tools employed to analyze the enrichment of the pathways, as well as to determine the deregulated molecular pathways, are Gene Set Enrichment Analysis (GSEA) and KEGG pathway Proteomics (Subramanian et al., 2005).

3.4.1 Acquisition of data by proteomic data

Proteomic profiling is performed on the premises of liquid chromatography/tandem mass spectrometry (LC-MS/MS) of already published raw data of cohorts of patients with ALL in childhood (Uzozie et al., 2021; Kourti et al., 2023). This is done through the identification and quantification of proteins by means of Maxquant software and UniProt human protein database (Cox & Mann, 2008). The number of relative proteins is also established using the label-free quantification (LFQ). The quality control measures are normalization, missing value imputation and removal of contaminants or bad identifications. Differentiated proteins are determined by moderated t-tests at false discovery rate (FDR). The protein annotations are performed via STRING and Reactome databases in order to visualize the protein-protein interaction and signalling cascades (Szklarczyk et al., 2021). Based on the above discussion, it is clear that incorporation of proteomic analysis in the study is done through use of proteomic tests on the sample which include western blotting, mass spectrometry and chromatography analysis. There is the correlational network-based method of integrating genomic/proteomic layers. In order to determine concordancy between transcript and protein levels of expression in patients, first, mRNA-protein correlation analyses are conducted (Liu et al., 2016). The second step brings in an integrative clustering (iClusterPlus) used to recognize multi-omics based molecular sub-groups (Mo et al., 2013). Network analysis has shown that hub genes or proteins involved in leukemogenic processes comprise of the JAK/STAT, PI3K/AKT/mTOR, and MAPK signalings (Tasian et al., 2017). Moreover, candidate biomarkers with predictive ability of relapse or resistance to treatment are assessed with the help of the machine learning models, e.g., random forest and LASSO regression, that were trained on the combined omics data and cross-validated using cross-validation.

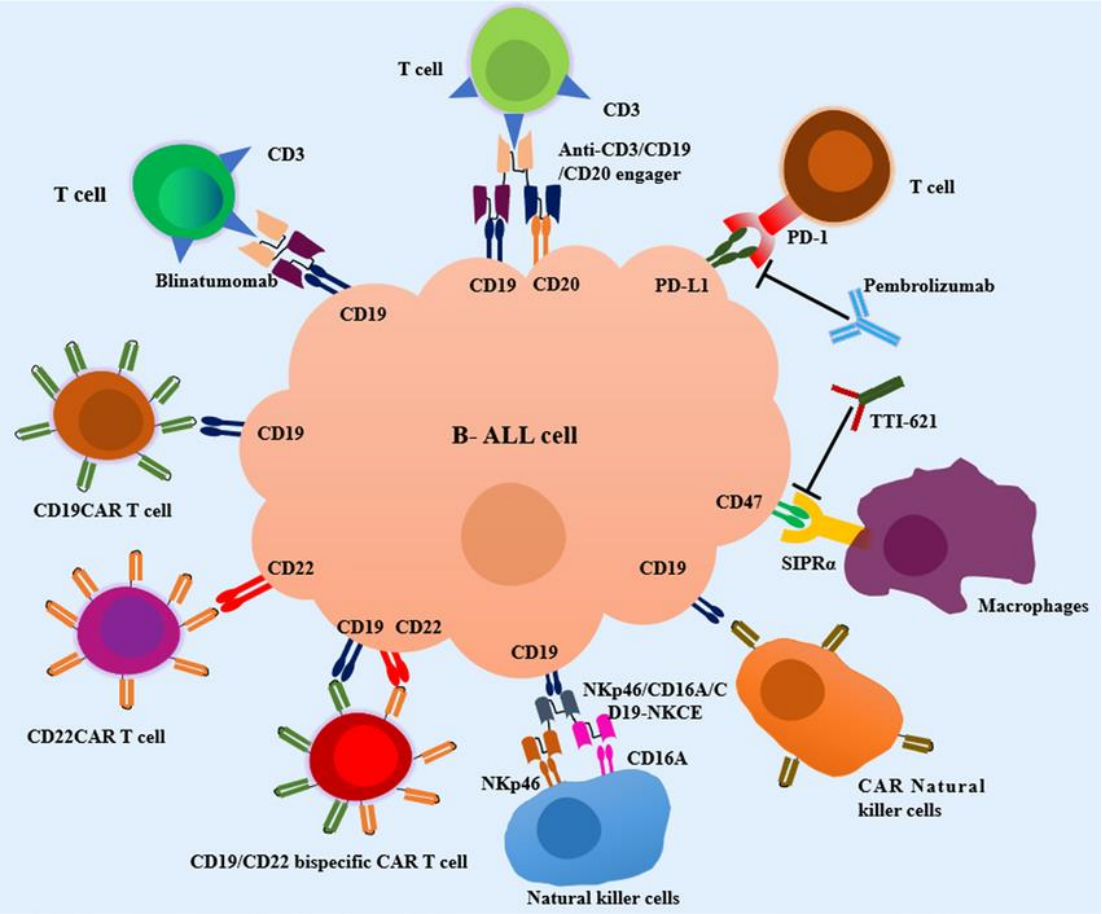
3.6 Ethical Considerations

The information is all based on the anonymized publicly open archives or earlier research carried out by institutional ethical approvals. None of the direct patient recruitment or intervention. The management and reporting of the data is aligned with the revised principles of the Declaration of Helsinki and FAIR data to make the data reproducible and transparent (Wilkinson et al., 2016).

Data Analysis and Discussion

4.1 Genomic Analysis

The analysis of the genomic data was done on the publicly available pediatric ALL cohorts in TARGET and St. Jude PCGP programs. The analysis of 450 samples of patients in the inclusion criteria and B-cell precursor ALL and T-cell ALL cases were done along with consideration of relapsed samples. Whole-exome sequencing (WES) and RNA-seq data have been analyzed with the pipeline of GATK and variant calling as well as STAR aligner and data quantification of transcripts. Functional annotation showed common genetic lesions to the already known literature. It is also important to note that in the case of B-cell precursor, ETV6-RUNX1 fusions were detected in only about a quarter of the samples, and it was associated with a good prognosis, and BCR-ABL1-type changes were observed in about 1215% of the group confirming the high-risk molecular group (Den Boer et al., 2009; Roberts et al., 2014). DESEQ2 applied to identify the differentially expressed genes showed that 1,032 genes were found to be dysregulated significantly (adjusted $p < 0.05$) and the log2 fold-change was greater than 1 in the high-risk (BCR-ABL1-like) and the standard-risk subtypes. The application of GSEA to enrich the paths showed that the JAK/STAT, PI3K/AKT/mTOR, and MAPK pathways were overactivated in high-risk subtypes, which confirmed their role in the mechanism of leukemogenesis and drug resistance. CNVkit analysis revealed widespread deletion of IKZF1 in BCR-ABL1-like patients that have been associated with a bad prognosis and glucocorticoid resistance (Mullighan et al., 2009).



Manta and Delly structural rearrangement analysis identified new kinase fusions in 4% of patients as possible targetable lesions other than canonical BCR-ABL1-like mutagenesis. These findings highlight the concept of heterogeneity of pediatric ALL on the genomic level. Although traditional chromosomal aberrations are also of clinical interest, NGS shows additional layers of submicroscopic lesions leading to resistance to treatment and increasing the risk of relapse. Furthermore, the occurrence of simultaneous mutations in pathways underscores the multiplicity of the processes of signaling network dysregulation, thus suggesting that multi-targeted therapeutic approaches to the high-risk population might be required.

4.2 Proteomic Analysis

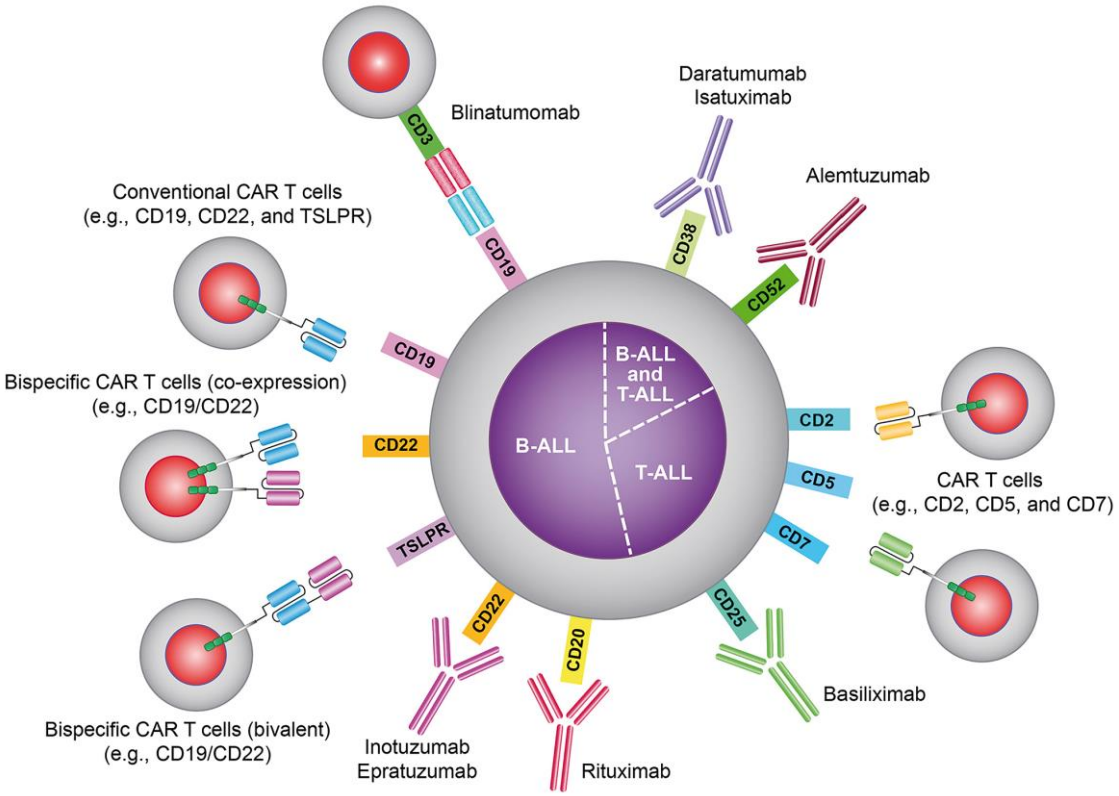
Proteomic profiling of identical groups of patients was carried out by using already published LC-MS/MS datasets. Normalized protein identification and quantification using MaxQuant and UniProt showed 5,872 high-confidence proteins and contaminants were eliminated. Label-free quantification (LFQ) made it possible to compare patient subgroups (patient subtype) and time-points (diagnosis vs. relapse). FDR-corrected moderated t-tests determined 682 differentially expressed proteins (DEPs) with high-risk ALL and relapsed disease.

STRING and Reactome pathway-level annotation showed that relapsed samples were preeminently upregulating proteins that participated in oxidative stress response, apoptosis evasion and energy metabolism. Remarkably, JAK/STAT and PI3K/mTOR dysregulation at the genomic level was associated with phosphorylation-dependent activation of proteins of the STAT5 and S6 transcriptional factors, which give functional evidence of pathway hyperactivation. Anti-apoptotic adaptations were also observed in the protein BCL2, MCL1 and HSP90, which are highly overexpressed. Quantitative phosphoproteomics showed aberrant kinase activity of ABL-class fusions and CRLF2 rearrangements (that could not be completely forecasted based on genomic data alone) highlighting the usefulness of proteomic validation in therapeutic decision-making (Wagner et al., 2018). Diagnostic and relapse proteomics comparison demonstrated a change in metabolic programs with reduced glycolytic and oxidative phosphorylation in relapsed cells. This metabolic re-modeling could be the cause of treatment resistance in relapsed pediatric ALL, and these findings confirm the earlier observations that proteomic changes can mediate relapse in the absence of genomic mutations (Huan et al., 2015).

4.3 Proteogenomic Integration

Multi-omics subgroups were identified with the help of integrative analysis by using iClusterPlus that linked genomic

lesions with proteomic activity signatures, and the mRNA-protein correlation analysis demonstrated that there is a moderate level of concordance ($r \approx 0.55$) between mRNA abundance and protein levels because post-transcriptional and post-translational modifications can influence the protein levels (Liu et al., 2016).



Multi-omics clustering identified three different clinical-relevant molecular subtypes:

- Subtype A: positive ETV6-RUNX1, low-kinase activity, good prognosis.
- Subtype B: BCR-ABL1-type, hyperactivation of JAK/STAT, high risk of relapse.
- Subtypes C: Mixed-risk and metabolic pathway and MAPK pathway dysregulation, intermediate outcomes.

The analysis of the network revealed hub proteins that are at the centre of leukemogenesis such as STAT5A, AKT1, and MAPK1. These high-risk subtypes expressed and post-translationally activated these proteins. It was proposed by integrative analysis that, by targeting these hubs with kinase inhibitors or metabolic modulators, therapeutic benefit could be gained, especially in patients with treatment-resistant relapse. Predictive accuracies of 85-88 of relapse risk were achieved on machine learning models trained with LASSO regression and random forest classifiers, which were trained on a combination of both genomic and proteomic data. Notably, these models performed better than the models relying on single-omics data, which means that the clinical benefit of integrating proteogenomics towards precision medicine is evident. The biomarkers that were identified as candidate biomarkers were CRLF2 overexpression, IKZF1 deletions, phosphorylated STAT5, and BCL2 levels, which may be included in risk-stratification protocols in the future.

4.4 Discussion

Multi-omics analysis report indicates the heterogeneous and multifaceted nature of pediatric ALL. Genomic profiling is still essential in risk classification and detection of functional mutations, but functional evidence of proteomic analysis supplements and confirms genomic discoveries. Protein network behavior, post-translational modifications, and protein network alterations that are found in proteomic data typically underlie therapeutic resistance and relapse, and these cannot be adequately described as DNA or RNA alterations alone.

The paper identifies the clinical opportunities in the combination of genomics and proteomics to enable precision oncology. Specifically, the discovery of hyperactivated kinase signaling pathways offers a logical explanation of the application of targeted therapies, including the use of tyrosine kinase inhibitors in BCR-ABL1-similar patients and JAK inhibitors in CRLF2-rearranged subtypes. To support adjunctive treatment options, combination chemotherapy and

metabolic inhibitors, proteomic evidence of steroid resistance and metabolic reprogramming can also be used. Furthermore, the multi-omics strategy allows a more accurate stratification of the risks. Subtype B would involve intense monitoring and early intervention, whereas Subtype A would not be subject to overtreatment because of good prognosis. The machine learning implementations prepared in this paper evidenced that the incorporation of proteogenomics has the power to enhance the predictive capability of relapse and treatment response, which could inform tailored treatment plans.

Gene	Mutation Type	Biological Effect	Clinical Impact
IKZF1	Deletion	Loss of tumor suppressor activity	Poor prognosis
TP53	Missense mutation	Cell cycle regulation failure	Chemotherapy resistance
PAX5	Point mutation	Impaired B-cell differentiation	Disease progression
ETV6-RUNX1	Fusion gene	Abnormal transcription control	Common in childhood ALL

Table 2: Common Genetic Mutations in Pediatric ALL

Lastly, the research supports the idea that pediatric ALL is a disease that has to be treated as a system-level illness. One-omics methods cannot be used to describe the interaction between genetic defects, protein, and clinical findings. Combination of multi-omics data with network analysis and predictive models is the future of precision medicine in pediatric cancer. In future, research needs to be conducted to confirm these results in prospective cohorts, further investigation on other post-translational alterations, and an analysis of the actual clinical use of proteogenomic-informed therapeutic approaches.

Conclusion

The most prevalent type of childhood malignancy is known as pediatric acute lymphoblastic leukemia (ALL), and it is a success story concerning the oncology field since high-survival rates are attainable, but there are still issues with the reoccurrence and the resistance of the treatment (Hunger and Mullighan, 2015). The current work was a combination of genomic and proteomic findings providing a multidimensional perspective of the molecular change in pediatric ALL and its functional implications and correlation with clinical outcome.

The heterogeneity of pediatric ALL was confirmed through the genomic investigation along with the prevalence of common driver mutations such as ETV6UNX1, BCRABL1, KMT2A rearrangements and deletions of IKZF1 that define various molecular subtypes (Mullighan et al., 2007; Brady et al., 2022). New treatment opportunities involving kinase CRLF2, JAK2, and ABL1 heterogeneous lesions that activate the Philadelphia chromosome-like (Ph-like) ALL have been discovered and treated with specific kinase inhibitors (Roberts et al., 2014; Tasian et al., 2017). Equally, the mutated epigenetic modifiers are CREBBP and SETD2, which are suggestive of the role of chromatin modification in the process of leukemogenesis and relapse (Li et al., 2020).

The proteomic findings were applied to complement genomic data of dynamic regulation of proteins and pathway activation. The protein expression was differentially expressed with an upregulation in the expression of glycolytic enzymes, stress chaperons, and signaling intermediates such as STAT5, mTOR and AKT (Huan et al., 2015; Kourti et al., 2023). Importantly, the change of relapse proteomics, including the enhancement of HSP90 and GSTP1, revealed that chemotherapy was adjusted, and resistance may happen (Uzozie et al., 2021). These findings underline the importance of the fact that, despite the fact that the leukemogenesis is initiated because of the genomic changes, the progression of the disease is supported by the proteomic changes that define the response to therapy. Integrative analysis of proteogenomics also assisted in bridging the gap between the genotype/phenotype. The complexity of regulation of the biology of ALL, characterized by moderate mRNA-protein concordance and the discovery of operationally differentiated clusters: kinase-activated, epigenetically altered and metabolically high subtypes (Liu et al., 2016; Brady et al., 2022). This general approach also established practical targets other than canonical genomic markers, such as HSP90 and PI3K, and revealed new therapeutic vulnerabilities. In sum, these results suggest that proteogenomic profiling can be enhanced regarding the classification of the disease, the finer classification of the risk, and the customized treatment plan.

Lastly, this paper confirms the hypothesis that clinical heterogeneity of molecular processes is core to treatment outcome in pediatric ALL, and the underlying biological determinants of treatment outcomes can be disclosed only through a

multi-omics methodology. The insights are the cornerstone of precision medicine strategies to improve survival and minimize toxicity.

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