Comprehensive Immune Signature Profiling in AML: Cross-Dataset Analysis and Validation

a. Brief Outline

Acute Myeloid Leukemia (AML) is an aggressive hematologic malignancy with substantial genetic and clinical heterogeneity. Despite advances in chemotherapy and targeted therapies, AML remains challenging to treat, with high relapse rates and poor prognosis. AML originates and proliferates in the milieu of the bone marrow which is a primary lymphoid organ involved in the generation of the immune response. In a murine model of AML, it has been shown that intravenous administration of leukemic cells resulted in immune tolerance. Spontaneous remission, a rare event, has been reported in AML patients with bacteremia. The remission is attributed to the stimulatory effect of systemic infection on the immune system. These findings underscore the significance of the immune microenvironment and its potential as a therapeutic target in AML. In this proposal, we aim to comprehensively profile the immune signatures in AML using multi-cohort datasets and validate the findings in Indian pediatric and adult AML samples, with the goal of identifying novel biomarkers and therapeutic targets.

b. Objectives

1. To analyse immune cell signatures in AML using publicly available datasets.
2. To validate immune signature findings using RNA-seq data from 30 adult AML samples, 30 pediatric AML samples, and 10 controls.
3. To investigate correlations between immune signatures and clinical outcomes such as survival, progression, and treatment response.

c. Brief Methodology

Study Design: The proposed project will be a retrospective and prospective (ambispective) observational study using patient samples. Blood Sample collection and preliminary processing will be done at fellow's institute, and further analysis will be done at mentor's institute.

Selection of Cases: (n=60)

Inclusion criteria:

- Newly diagnosed cases of acute myeloid leukemia - paediatric (n=30) and adult (n=30)
- Patients with WBC count > 10000 with at least 20% blast cells on the peripheral smear.

Exclusion criteria:

- Patients of acute promyelocytic leukemia
- Partially treated AML
- Therapy associated AML

Selection of controls: (n=10)

- G-CSF injected healthy donors of siblings who are undergoing stem cell transplantation

(It is important to note that G-CSF injection is done as a routine procedure for the donors, there is no separate injection for the purpose of this study)

Objective wise brief Methodology

Objective 1: To analyse immune cell signatures in AML using publicly available datasets

RNA-seq data from publicly available datasets including but not limited to BEAT-AML (828 patients), TARGET-AML (2189 patients), TCGA LAML (200 patients) will be downloaded. Batch correction will be done using Combat-Seq. Immune cell deconvolution will be performed using tools like CIBERSORTx and xCell to estimate the relative proportions of immune cell types based on bulk RNA seq data. Immune cell composition will be compared across datasets to identify conserved and divergent immune signatures in AML. Weighted Gene Co-expression Network Analysis (WGCNA) will be performed on gene expression profiles associated with immune cell proportions to identify co-expressed gene modules and pathways linked to AML

progression. Statistical tools will be applied to evaluate the association between immune signatures and available clinical metadata, such as age, cytogenetic risk, treatment protocols and survival. Identified markers will be taken for validation in objective 2.

Objective 2: To validate immune signature findings using RNA-seq data from 30 adult AML samples, 30 pediatric AML samples, and 10 controls

RNA will be extracted from clinical samples followed by quality assessment at the fellow's institute. RNA-seq libraries will be prepared and sequenced using an Illumina platform at the host institute to generate high-quality transcriptomic data. Immune cell deconvolution will be performed using the same computational pipeline as in Objective 1 for consistency. Comparative analysis will be done to validate immune signatures identified in public datasets against signatures derived from local samples. Special focus will be given to pediatric versus adult AML to identify unique immune features.

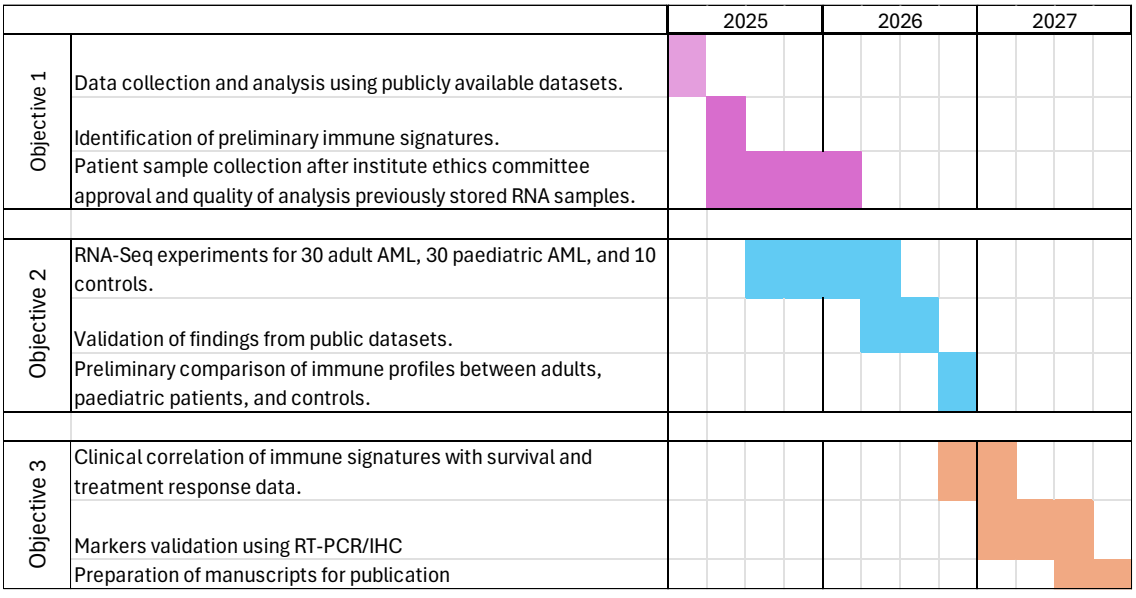
Objective 3: To investigate correlations between immune signatures and clinical outcomes such as survival, progression, and treatment response

Clinical outcomes for the 30 adult and 30 paediatric AML samples will be collected and anonymised. Survival analysis will be performed by correlating immune cell proportions and gene signatures with overall survival and progression-free survival using Kaplan-Meier curves and Cox proportional hazard models. Correlations of immune and gene signatures with therapy response (e.g., complete remission or relapse) will be evaluated using statistical methods such as logistic regression. Comparative analysis will highlight immune profiles associated with favorable or poor clinical outcomes. If possible, highly significant genes or candidate markers will be validated using RT-PCR/flow cytometry /immunohistochemistry to confirm their expression levels in AML. This step will help in developing a panel of markers which can be utilised even in the low-resource setting without the need for RNA-sequencing.

d. Anticipated Outcomes

- Comprehensive understanding of immune cell signatures in AML across different age groups in Indian population.
- Development of potential targets for immunotherapeutic interventions.
- Identification and establishment of potential prognostic markers to be used in low-resource settings.

e. Timeline



National Centre for Biological Sciences
Tata Institute of Fundamental Research

Date 28.11.2024

To,
The selection committee
Sun Pharma Science Foundation
Clinical Research Fellowship

Dear Selection Committee Members,

This letter is in reference to Dr Karthikeyan Pethusamy's application for the Clinical Research Fellowship.

My lab at the National Centre for Biological Sciences (NCBS), TIFR, Bengaluru is focused on studying cancer evolution using computational and functional genomics approaches. In particular, we are interested in understanding how cancer cells evolve in the context of immune cell infiltration. The research work proposed by Dr Karthikeyan is exciting and aligns well with the research interest of our lab. We have all the bioinformatics pipeline and infrastructure available to explore the RNA-seq data from AML cohorts and to perform the deconvolution of immune cell signatures from the bulk transcriptomics data. In addition, the analysis of data from Indian patients will help to understand the similarity of the disease mechanisms with other populations as well as the identification of biomarkers specific to the Indian population, which would be helpful for patient stratification and identification of suitable treatments. I am happy to host Dr Karthikeyan in our lab to conduct this proposed research work.

If you have any further questions, please do not hesitate to contact me.

Yours Sincerely,

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