Genetic Landscape in Primary Acute Myeloid Leukemia (AML) and Relation with Wilms Tumour 1 (WT-1) Gene Expression

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INTRODUCTION

- AML is a heterogeneous disease characterized by abnormal differentiation and uncontrolled proliferation of myeloid progenitor cells
- Wilms tumour 1 gene (WT-1), located at chromosome 11p1, encodes a transcriptional regulatory protein for the genes involved in cellular growth and metabolism
- Recurrent somatic mutations in WT-1 occur in about 6–15% cases of de novo AML. WT-1 mutations cluster is mainly in exons 7 & 9 and less in exons 1, 2, 3, & 8. WT1 gene expression has been shown to have significant reduction in >80% cases showing morphological remission at the end of induction therapy
- Enough characterization of these therapeutic targets and prognostic markers especially WT-1 has not been done adequately in the scientific literature. Therefore, WT-1 gene expression may have a potential role as a marker of disease progression

AIM

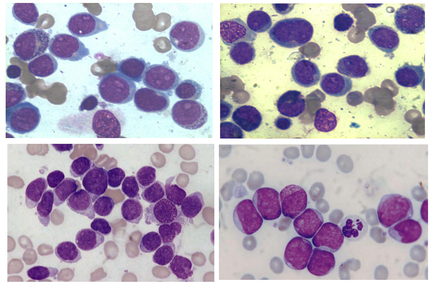
- To investigate the prognostic role of WT-1 gene expression in patients of primary AML and evaluation of molecular functions of WT-1 gene in the context of other genetic alterations in AML
- Design: Prospective clinical research
- Setting: Tertiary cancer care centre (AIIMS, New Delhi, India)

METHODS

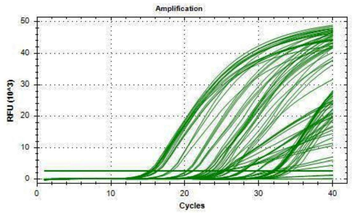
- A total of 151 diagnosed cases of AML, having blast percentage of ≥20% blasts in peripheral blood (PB) or bone marrow (BM) on Day-0 and treated with induction therapy for 28 days were enrolled
- Flowcytometric immunophenotyping (FCI) was carried out
- Detection of mutations, including AML-ETO, CBFB-MYH11, NPM1, FLT3-ITD, and PML-RARA was done and classification of AML was established
- WT-1 gene expression was assessed using RNA extracted from blood/bone marrow samples.
- Molecular functions of WT-1 were analyzed by performing gene set enrichment analysis (GSEA)
- Relationship of WT-1 expression with immune checkpoints was analyzed using the Sangerbox 3.0 database
- Kaplan–Meier survival analysis was performed to estimate the prognostic significance of WT-1 gene in AML

RESULTS

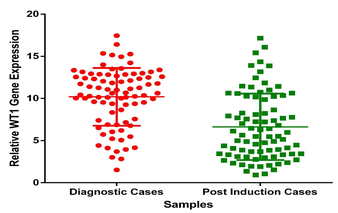
- Age ranged from 1-81 years (Mean - 26.35 years)
- Male: Female ratio - 1.9:1
- Clinically: fever, weakness, anaemia & leukocytosis
- Blast count %age in the cases: 28% to 80%.
- Mutations: AML-ETO mutation (29.6% cases); CBFB-MYH11 mutation (5.6% cases); NPM1 mutations (11.11% cases); FLT3-ITD (14.8% cases)
- 122 (80.8%) cases showed increased WT-1 gene expression at day-0 as compared to WT-1 gene expression in cases post induction (day-28)
- WT-1 expression was inversely correlated with normal hematopoiesis and positively correlated with age, high marrow blast counts, M4 subtype, and inferior outcomes compared to patients with low WT-1 expression levels.
- In GSEA, WT-1 gene displayed an important role in mis-regulating DNA-binding transcription factor activity, RNA and protein binding, as well as negative regulation of cell growth and cell population proliferation.
- In immune checkpoint analysis, WT-1 gene expression was positively correlated with CD28, CD40, CD44, CD48, CD80, CD70, CD27, CD86.
- In Kaplan-Meier survival analysis, poorer overall survival was seen in patients having higher WT-1 gene expression.



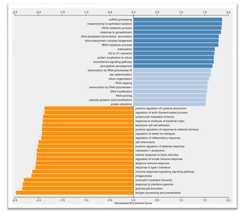
PBF showing myeloid blasts



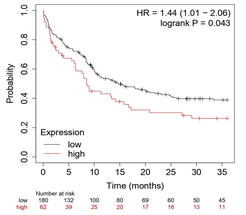
Real-time PCR Amplification of different samples



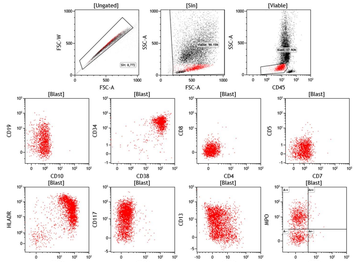
Relative WT-1 Gene Expression



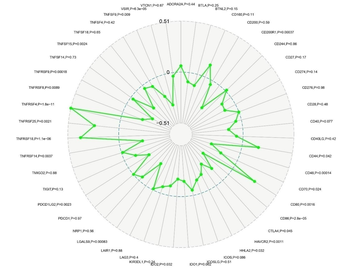
Gene Set Enrichment Analysis



Kaplan-Meier Survival Analysis



Representative Flow Cytometry of a case of AML



Immune Checkpoint Analysis

DISCUSSION

- A previous study by Dou C Y et.al (2018) revealed that WT-1 gene expression can help to divide AML patients into low-/middle-/high-risk groups & to evaluate therapeutic response in middle & high-risk AML patients.
- A study by Mashima K et.al (2018) showed that sequential monitoring of the WT-1 mRNA is of value for the early detection of haematological relapse in AML in remission after chemotherapy or stem cell transplantation.
- Another study by Valkova V et.al (2021) highlighted that WT-1 status before allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a strong prognostic factor for both overall survival & relapse risk and WT-1 is a reliable marker for MRD monitoring after allo-HSCT.

CONCLUSIONS

- Increased expression of WT-1 positively correlates with the leukemic burden in most cases of AML
- The WT-1 gene can be considered a promising molecular marker for early diagnosis, MRD detection, and a target for developing novel therapeutic approaches against AML.

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