DECODING THE CLINICAL VALUE OF m^{6}A EPITRANSCRIPTOME IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

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Introduction: Acute lymphoblastic leukemia (ALL) represents the most prevalent malignancy in children and adolescents, accounting for approximately 25-30% of all cases up to 16 years old. Despite the high 5year survival rates, emerging drug resistance and subsequent disease recurrence continue to compromise clinical outcomes for a significant subset of patients. N6-methyladenosine (m^{6}A) has emerged as the most abundant internal epitranscriptomic modification on eukaryotic mRNAs, serving as a critical post-transcriptional regulator of RNA metabolism and fate. Growing evidence associates the dysregulation of the m^{6}A landscape directly to oncogenesis, tumor progression, and chemoresistance. The present study aims to decode the m^{6}A epitranscriptomic landscape of oncogenic drivers, specifically *ETV6* and *KMT2A*, in childhood ALL (chALL) and investigate the clinical significance in chALL prognosis and treatment response.
Methods: RNA was isolated from bone narrow aspirates (n=50) at diagnosis. m^{6}A RNA was subsequently isolated by m^{6}A RNA immunoprecipitation (MeRIP) and the quantification of m^{6}A sites on *ETV6* and *KMT2A* was performed by RT-qPCR. For the identification of m^{6}A sites, *in silico* analysis using the SRAMP platform was performed, which identified three (3) target sequences in the *ETV6* and one (1) target sequence in the *KMT2A* genes. To assess technical variability and background noise, relative enrichment was calculated as an adjusted foldchange by normalizing the data against total input RNA and non-specific IgG controls.
Results: Our analysis highlighted the association of the lower *ETV6* m^{6}A levels with ≥50.000 white blood cells/μL (WBC) at diagnosis, poor (≥1000 blasts) prednisone response (day 8) and ≥0.1% minimal residual disease at day 15 (MRD_{15}) of

chemotherapy induction in patients treated according to BFM protocols. Moreover, Kaplan-Meier and Cox regression analysis demonstrated the higher risk for short-term post-treatment progression of the patients with low *ETV6* m6A levels, compared with the high m^6A patients' subgroup. Finally, multivariate Cox models adjusted for patients' age, WBC, prednisone response (day 8), MRD and bone marrow response (day 15) highlighted *ETV6* m6A loss as an independent prediction of post-treatment survival of the patients.

Conclusions: our findings reveal that m^6A signatures represent a promising avenue for tumor heterogeneity tracking and prediction of treatment outcome in chALL.