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POSTER ABSTRACTS

618.ACUTE LYMPHOBLASTIC LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Somatic and Germline Cohesin Genes Alterations in Pediatric Acute Lymphoblastic Leukemia

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Leukemia is a complex disease and the molecular mechanisms of malignant transformation are not fully understood yet. Cohesins are essential proteins, forming a complex which plays critical roles in various cellular processes, not only by canonical DNA binding and chromosome segregation but also in non-canonical regulation of gene expression in both proliferating and post-mitotic cells.

Mutations or gene expression alterations of genes encoding the components of the cohesins' complex may affect chromosomal stability and potentially contribute to the pathogenesis of leukemia. Somatic cohesins mutations have been already reported to occur in myeloproliferative disorders and pediatric Acute Lymphoblastic Leukemia (ALL), while on the other hand, germline mutations are causative of Cohesinopathies such as Cornelia de Lange Syndrome (CdLS). Our previous first report of a patient affected by both CdLS and ALL suggested a potential involvement of cohesin germline mutations in the pathogenesis of ALL. Moreover, we demonstrated that germline mutations in *STAG1* gene could play a crucial role in predisposition to pediatric hematological malignancies, including ALL. These findings suggest that both somatic and germline alterations of cohesin genes may be associated with the development of ALL.

This study aims to understand the molecular mechanisms affected by Cohesin genes alterations, that could potentially pave the way for the development of targeted therapies and improved treatment strategies for leukemia patients.

By employing a DNA-targeted next-generation sequencing (NGS) screening of a cohort of 120 consecutive pediatric ALL cases (enrolled in the AIEOP-BFM protocol at various Italian centers), we discovered a total of 11 germline cohesin mutations, further categorized as follows: 6 out of 11 were missense, 4 out of 11 were located in the 3' or 5' untranslated regions (UTR) and

1 out of 11 was a splice-acceptor variant. To investigate the functional implications of selected germline mutations in *STAG1* gene, we took advantage of the CRISPR/Cas9 technique to generate *in vitro* models of two Lymphoblastoid Cell Lines (LCLs) harboring specific variants already found in two patients of our cohort. Additionally, we established control LCLs from healthy donors' samples using the same CRISPR/Cas9 approach. Subsequently, we performed RNA sequencing (RNAseq) analysis on these cell lines, and our preliminary findings revealed a total of 619 differentially expressed genes (DEGs) compared to the control group. Many of them are involved in critical intracellular pathways, like ribosomal transcription, suggesting potential disruptions of essential cellular processes caused by germline *STAG1* mutations.

Moreover, we have successfully developed an advanced computational analysis pipeline with the primary objective of detecting fusion transcripts from RNAseq data. This pipeline was applied to a dataset of 529 consecutive diagnose and 95 relapse samples of pediatric B/T-ALL patients and in five B-ALL cases a fusion transcript involving the *STAG1* (n=1), *STAG2* (n=3), and *NIPBL* (n=1) genes was identified. The significance of these findings was further reinforced by additional cases from both pediatric and adult cohorts through an international collaborative effort and five more fusion transcripts of *STAG2* (n=2) and *NIPBL* (n=3) were uncovered. Investigation to clarify the underlying functional consequences of these fusion genes in pediatric ALL are ongoing.

Collectively, these observations endeavor to elucidate the role of germline and somatic cohesin events in pediatric ALL. In particular, the investigation seeks to unveil the non-canonical functions of these proteins, thus paving the way for the identification and characterization of a novel and distinct subcategory of leukemia within the pediatric population.

Disclosures Lo Nigro: *Amgen:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *Clinigen:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *Jazz Pharmaceutical:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *Novartis:* Consultancy, Membership on an entity's Board of Directors or advisory committees. **Vinti:** *Amgen / Clinigen:* Consultancy; *Amgen / Nequil / Takeda:* Other: Travel/Accommodations/Expenses; *Merck Sharp & Dohme:* Research Funding; *Amgen / Takeda:* Speakers Bureau. **Biondi:** *Colmmune:* Membership on an entity's Board of Directors or advisory committees, Research Funding; *BMS:* Membership on an entity's Board of Directors or advisory committees; *Novartis:* Speakers Bureau; *Agmen:* Speakers Bureau; *Galapagos:* Membership on an entity's Board of Directors or advisory committees.

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