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Institute of Affiliation	Fondazione M. Tettamanti M. De Marchi Onlus
Title of the proposed project:	Advanced proteomic characterization of relapse and immunotherapy resistance in PAX5-rearranged pediatric B-cell Leukemia
Short description of the project	<p>Pediatric B-cell precursor acute lymphoblastic leukemia (BCP-ALL) harboring alterations of the PAX5 gene represents a biologically distinct subgroup associated with poor clinical outcomes, yet its molecular mechanisms remain poorly characterized. PAX5 is a key regulator of B-cell differentiation and directly controls the transcription of genes involved in B-cell identity, including CD19. Alterations affecting PAX5 may therefore contribute not only to leukemogenesis but also to immune escape mechanisms associated with CD19 loss after treatment with Blinatumomab or CD19-directed CAR-T cells, an issue of increasing clinical relevance with the progressive introduction of immunotherapy into frontline treatment protocols. Within the AIEOP-BFM ALL protocols, PAX5r cases have been identified and Whole Transcriptome Sequencing (RNA-seq) performed by our group on diagnostic samples from pediatric BCP-ALL patients enrolled across Italian centers, identifying a specific transcriptional signature in PAX5-rearranged cases. The aim of this project is to extend the analysis to sequential relapse samples in order to investigate mechanisms of clonal evolution, treatment adaptation, and relapse occurrence, even after immunotherapy. Particular attention will be devoted to understanding whether PAX5 alterations contribute to CD19 downregulation and resistance to immunotherapy. Transcriptomic analyses will be integrated with high-dimensional single-cell proteomic profiling using Cytometry by Time-Of-Flight (CyTOF). Customized metal-conjugated antibody panels will be developed to characterize signaling pathways, cellular heterogeneity, and leukemic subpopulations at the single-cell level, as well as to identify novel predictive biomarkers and potential therapeutic targets. The integration of transcriptomic and proteomic data is expected to provide novel insights into the biology of PAX5-altered BCP-ALL, identify biomarkers predictive of adverse outcomes, and uncover potential therapeutic vulnerabilities. The PhD candidate will carry out clinical activity at Tettamanti Center, located within the Pediatric Hematology Unit of IRCCS Fondazione San Gerardo dei Tintori, a leading center in pediatric hematologic malignancies and translational leukemia research.</p>
Main research area for the project	Cancer biology
5 key words for the project	Pediatric tumors, Acute Lymphoblastic Leukemia (ALL), Fusion genes, Preclinical studies, Next generation sequencing

LAB INFO	
Main topic/s of the lab	Pediatric onco-hematology, with particular focus on Acute Lymphoblastic Leukemia
Short description of the lab activity	<p>Fondazione Tettamanti is a research center within the Pediatric Clinic of Fondazione IRCCS San Gerardo dei Tintori, the teaching hospital of University of Milano-Bicocca, in Monza. The center serves as the national reference laboratory for the molecular characterization of pediatric acute lymphoblastic leukemia (ALL) within the Italian Association of Pediatric Hematology and Oncology (AIEOP) network. Alongside its diagnostic activity, the Foundation has developed extensive research programs in leukemia biology, genomics, and immunotherapy, becoming an internationally recognized center of excellence in hematology. Fondazione Tettamanti is a founding and board member of the European Study Group on Minimal Residual Disease (EuroMRD), a member of the EuroClonality-NGS consortium and of the Biology&Diagnosis Committee of the International BFM Study Group (I-BFM-SG). It also participates in the Biology Working Group of AIEOP, in the Management Committee of the COST Action "Leukemia GENetics" (LEGEND), and chairs Working Group on Familial Leukemia. The center has access to approximately 5,000 pediatric leukemia samples collected for diagnostic and research purposes, with informed consent and approval from the AIEOP Scientific Board. The scientific activities and interests of the laboratory are focused on (i)molecular pathogenesis of childhood ALL, with functional characterization of fusion genes in BCP-ALL, implementing preclinical in-vivo and in-vitro models in leukemogenesis and drug targeting; (ii)dissection of the complexity of childhood ALL by Next Generation Sequencing and proteomics, with interest on novel fusion genes/transcriptomics and Ig/TR analyses (coordinating NGS molecular activities for AIEOP-BFM treatment protocols). Furthermore, the research area includes dedicated laboratories for cytogenetics, molecular biology, cell culture, flow cytometry and manipulation of genetically modified microorganisms. The facility is equipped with automated cell analysis systems, including the Countess Automated Cell Counter and the DxH500 Hematology Analyzer, as well as Luminex and Spark platforms for ex-vivo drug screening assays. The flow cytometry core facility includes multiple instruments for high-dimensional immunophenotyping, such as FACSCantoII, FortessaX20, Cytex Aurora and FACSLyric. In addition, the center is equipped with CyTOF XT platform for single-cell analysis integrating mass spectrometry and flow cytometry technologies. This approach enables the evaluation of dozens of protein markers at single-cell resolution, allowing multidimensional characterization of leukemic and immune cells, for translational research applications. The facility also includes a certified Biosafety Level 2 laboratory authorized by the Italian Ministry of Health for the handling of genetically modified microorganisms. Importantly, the center hosts three molecular biology laboratories, pre/post-PCR rooms for next-generation sequencing workflows. The</p>

AVAILABLE POSITIONS

	<p>molecular biology core facility includes thermocyclers, multiple quantitative PCR platforms and a BioRad digital droplet PCR. The genomics facility includes Illumina MiSeq and NextSeq2000, Agilent TapeStation and the Fluent Dreamprep TECAN automated systems for library preparation. Additional platforms include Alliance Instrument Uvitec system for chemiluminescence analyses and Seahorse XF Analyzer for metabolic studies. The center also has access to a fully-equipped animal facility for immunodeficient mouse models. Overall, Fondazione Tettamanti provides the infrastructure, technological platforms and multidisciplinary expertise required to successfully accomplish the proposed project.</p>
Recent bibliography	<ul style="list-style-type: none"> - RECURRENT GENETIC FUSIONS REDEFINE MLL-GERMLINE ACUTE LYMPHOBLASTIC LEUKEMIA IN INFANTS. BLOOD 2021 Apr; 137: 1980 - PAX5 fusion genes are frequent in poor risk childhood acute lymphoblastic leukaemia and can be targeted with BIBF1120. EBioMedicine 2022 Sep; 83: 104224 - Diverse mechanisms of leukemogenesis associated with PAX5 germline mutation. LEUKEMIA 2024 Nov; 38: 2479 - PAX5::AUTS2 childhood B-ALL: a relapse-prone genetic subtype with frequent central nervous system involvement and a poor outcome. LEUKEMIA 2025 Feb; 39: 482 - Efficacy of the kinase inhibitor CHZ868 against JAK2 rearrangements in pediatric acute lymphoblastic leukemia. HAEMATOLOGICA 2026 Apr; :
Group composition	<p>The PI, project leader of a group in Genetics of Leukemia Unit and coordinating the NGS and molecular activities, currently supervises a multidisciplinary team consisting of: -1 Post-Doc (MS in Biotechnology, PhD in Translational Medicine - DIMET); -3 PhD students enrolled in the DIMET PhD program (UNIMIB), currently in their third year (MD in Pediatrics), second year (MS in Medical Biotechnology), and first year (MS in Pharmaceutical Biotechnology); -1 Research Fellow (MS in Pharmaceutical Biotechnology); -1 Master's Thesis Student (MS in Biology).</p>
Institutional page link	<p>https://fondazionetettamanti.it/</p>
Lab website link	<p>https://fondazionetettamanti.it/centro-di-ricerca/</p>
Social media links	<p>https://www.linkedin.com/company/fondazione-tettamanti-onlus/posts/?feedView=all&viewAsMember=true</p>