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Acute Leukemias

FLT3 AS A NEW THERAPEUTIC TARGET IN PRECLINICAL MODELS OF PEDIATRIC BCP-ALL WITH PAX5 REARRANGEMENT

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Introduction: B-Cell Precursor Acute Lymphoblastic Leukemia (BCP-ALL) is the most common pediatric cancer, with survival rates exceeding 85%. However, prognosis remains poor in refractory or relapsed cases. PAX5, a transcription factor essential for B-cell development, is frequently altered in BCP-ALL (*PAX5*-alt), including rearrangements (*PAX5r*). These lead to repression of genes typically activated by wild-type PAX5 and the upregulation of genes normally repressed by PAX5. Fms-like tyrosine kinase 3 (*FLT3*) gene was previously reported among PAX5-repressed genes. We aim to investigate FLT3 expression in pediatric *PAX5r* BCP-ALL and design an effective molecularly targeted treatment in this molecular ALL subgroup.

Methods: RNA-seq was used to identify *PAX5* fusion genes. *FLT3* expression was assessed in pediatric BCP-ALL patients and PDX samples derived from *PAX5r* cases (AIEOP-BFM ALL protocols 2000, 2009, 2017) by RNA-seq and confirmed with RT-qPCR. HTP screening platform strategy has been applied to test FLT3 inhibitors. Among them, gilteritinib was selected and tested in monotherapy and combined with chemotherapy on NALL-1 cells (*PAX5::ETV6*) and n=4 *PAX5r* samples using Annexin V/7-AAD FACS staining. Phosphoflow analysis was performed to evaluate FLT3 activation in NALL-1 cells.

Results: *FLT3* expression was profiled in 599 consecutive pediatric BCP-ALL patients at diagnosis enrolled in the AIEOP-BFM ALL 2017 study. *PAX5r* patients (n=26) showed significantly higher median *FLT3* expression compared to the whole cohort of patients, and levels comparable to *KMT2Ar*, *ZNF384r*, and high-hyperdiploid ALL cases, all known to have high *FLT3* expression profiles. These results were confirmed by RT-qPCR in primary *PAX5r* samples and *PAX5r* PDXs. None of 10 *PAX5r* with the highest *FLT3* expression levels resulted positive for the most common *FLT3*-ITD mutation by RT-PCR. 16 *PAX5r* PDXs were used to perform a high-throughput screening of six FLT3 inhibitors to assess drug sensitivity. Among these, gilteritinib, demonstrated a promising efficacy and toxicity profile. *In vitro* experiments with NALL-1 cells and *PAX5r* PDX blasts (n=4) revealed gilteritinib's potent cytotoxicity at nanomolar concentrations, both as a single agent and in combination with chemotherapy (dexamethasone and asparaginase). Phosphoflow analysis further confirmed the high FLT3 expression and constitutive basal activation as measured by phospho-FLT3 levels in NALL-1 cells. Following gilteritinib treatment, a significant reduction in FLT3 phosphorylation was observed (-57.5%, $p<0.05$).

Conclusions: FLT3 is highly expressed in *PAX5r* BCP-ALL, as already known in *KMT2Ar* and *ZNF384r* ALL cases. *Ex vivo* treatment with gilteritinib induced apoptosis in leukemic cells, both as single-agent and in combination with chemotherapeutics, demonstrating synergistic (dexamethasone) or additive (asparaginase) effects. Further *in vivo* studies are needed to confirm its efficacy in this molecular ALL subgroup.