## Abstract: P325

# Title: APPLICATIONS OF HIGH-THROUGHPUT DRUG SCREENING AS DRUG REPURPOSING STRATEGY FOR POOR OUTCOME SUBGROUPS OF PEDIATRIC B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

**Abstract Type: Poster Presentation**

**Session Title: Acute lymphoblastic leukemia - Biology & Translational Research**

**Background:**

15% of pediatric ALL patients are unresponsive to conventional chemotherapy and relapse, raising the need for novel therapeutic schemes. Preclinical high-through put (HTP) drug screening enables monitoring of personalized responses to a collection of clinically approved and novel agents. This approach effectively suggests drug repurposing opportunities concerning single and drug combinations both specific to patient subgroups and across multiple groups.

**Aims:**

Purpose of this study is to apply HTP drug screening to identify effective drugs (as a single agent or in combination) for three subgroups of poor prognosis pediatric BCP-ALL: CRLF2r Down Syndrome (DS), PAX5r, KMT2Ar.

**Methods:**

Primary cells from 34 BCP-ALL patients of three distinct ALL subgroups (9 CRLF2r DS, 15 PAX5r, 10 KMT2Ar) expanded as Patient-derived Xenografts (PDX), leukemic cell lines and healthy controls were seeded in plates pre-coated with a library of 174 compounds under a 6-point concentration range (8nM-25uM) and CellTiter-Glo assay evaluated viability upon a 3-day culture. Additionally, the CRLF2-r BCP-ALL cell line MHH-CALL-4 was pre-treated for 6 hours with vehicle or Givinostat, an HDAC inhibitor with proven high efficacy against CRLF2r ALL to dissect compounds standing as synergistic partner for combination targeting in CRLF2r cases with or without DS. In either case, a quantitative drug sensitivity score (DSS) for each drug was computed and selected efficient compounds statistically identified by Mann Whitney U-test were further interrogated for their apoptotic potential using Annexin/7AAD cytofluorometric approach.

**Results:**

With this approach, we were able to identify 9 compounds with a statistically significant profound anti-leukemic action for all ALL subgroups tested (DSS>50, p-value<0.05), accompanied by a minimum effect on healthy cells (DSS<10). These consist of the Bcl-2 inhibitor ABT-199 (Venetoclax), the HSP90 inhibitors AUY922 (Luminespib), EC144, PF-04929113, NVP-HSP990, the BET bromodomain inhibitor JQ1, the microtubule polymer stabilizer Paclitaxel, as well as two agents of the classical chemotherapy for BCP-ALL, the glucocorticoid Dexamethasone and the antimitotic Vincristine. ABT-199 (Venetoclax) was revealed as the most promising among them, not affecting healthy hematopoietic stem cells and already approved for clinical use for other haematological settings. Further in vitro validation in our ALL samples confirmed its potency in nanomolar concentrations. Interestingly, we observed an NF-kB inhibitor to selectively target DS-ALL cases irrespective of additional leukemia characteristics (mean rank difference 13.26, p-value<0.0001). In the combination setting, we managed to couple Givinostat, our previously established compound active for CRLF2r ALL cases, with Trametinib (ZIP synergy 7.04 and 16.83 for MUTZ-5 and MHH-CALL-4 respectively) or Venetoclax (ZIP synergy 9.23 and 5.03), thus providing a successful synergistic targeting further confirmed in CRLF2r ALL blasts, whose synergistic mechanism of action is currently investigated.

**Summary/Conclusion:**

This study has highlighted the emerging benefit of HTP drug screening applications guiding the early design of

therapies for multiple or specific patient subgroups in an approach of repurposing drugs available in the pharmacological landscape.

**Keywords:** Drug sensitivity, Targeted therapy, B cell acute lymphoblastic leukemia, Down Syndrome