Background: The multinational, open-label, phase 3 CLL14 trial (NCT02429492) compared fixed-dose targeted venetoclax plus obinutuzumab (VenG) treatment with chlorambucil-obinutuzumab (ClbG) treatment in previously untreated patients (pts) with chronic lymphocytic leukemia (CLL) and comorbidities.

Aims: We present endpoint analyses with particular emphasis on progression-free survival (PFS) and minimal residual disease (MRD)-negativity.

Methods: Pts with a CIRS score >6 and/or an estimated creatinine clearance <70 mL/min were randomized 1:1 to receive equal duration treatment with 12 cycles of standard Clb or Ven 400 mg daily in combination with G for the first 6 cycles. The primary endpoint was PFS, MRD-negativity in peripheral blood (PB) or bone marrow (BM) 3 months after treatment completion was a key secondary endpoint. MRD was analyzed serially from Cycle 4 every 3 months by an allele-specific oligonucleotide polymerase chain reaction assay (ASO-PCR; cut-off, 10^-4) and by next generation sequencing (NGS; cut-offs, 10^-4, 10^-5, 10^-6).

Results: In total, 432 pts were enrolled; 216 in each treatment group (intent-to-treat population). Median age, total CIRS score, and CrCl at baseline were 72 years, 8, and 66.4 mL/min respectively. After 29 months median follow-up, superior PFS was observed with VenG vs ClbG (Figure 1a). Median PFS was not reached in either group: At Month 24, PFS rates were 88% with VenG and 64% with ClbG (hazard ratio [HR] 0.35; 95% confidence interval [CI] 0.23–0.53; P < 0.0001). MRD-negativity by ASO-PCR was significantly higher with VenG vs ClbG in both PB (76% vs 35% [P < 0.0001]) and BM (57% vs 17% [P < 0.0001]) 3 months after treatment completion. Overall, 75% of VenG MRD-negative pts in PB were also MRD-negative in BM vs 49% in the ClbG group. Landmark analysis for this timepoint by PB MRD status showed that MRD-negativity was associated with longer PFS. MRD-negativity rates were more sustainable with VenG: 81% (VenG) vs 27% (ClbG) of pts were MRD-negative 12 months after treatment completion; HR for MRD conversion 0.19; 95% CI 0.12–0.30 (median time off-treatment: 19 months) (Figure 1b). MRD-negativity rates by NGS confirmed these results; 78% (VenG) vs 34% (ClbG) of pts had MRD-negative status at <10^-4, 35% vs 15% at <10^-5, and 31% vs 8% at <10^-6, respectively.

Summary/Conclusion: Fixed-duration VenG induced deep, high (<10^-4 in 3/4 of pts and <10^-6 in 1/3 of pts), and long lasting MRD-negativity rates (with a low rate of conversion to MRD-positive status 1 year after treatment) in previously untreated pts with CLL and comorbidities, translating into improved PFS.
the invasion of ALL cells was increased when ADAM28 overexpressing. In vitro, transwell chamber results showed that the migration ability of ALL cells in the presence of ADAM28 was significantly higher than that of the controls, and the migration ability was decreased when ADAM28 was knocked down or treated with ADAM28 inhibitor. In addition, when ADAM28 was knocked down, the expression of CXCR4 mRNA and protein level were decreased, and the migration ability of ALL cells was decreased when treated with CXCR4 inhibitory antibodies, which suggested that there might be a cross talk between ADAM28 and CXCR4 signaling pathway.

**Summary/Conclusion:** The incidence of CNS in ALL patients was increased. The invasion into CNS of ALL cells was increased when ADAM28 was overexpressed in xenograft mouse model. ADAM28 might promote the migration and invasion ability via cross talking with CXCR4 pathway.

**PF151**

**RAS/MAPK ACTIVATION COOPERATES WITH GAIN OF CHROMOSOME 21 IN B CELL LEUKAEMIA AND IS AN ATTRACTIVE TARGET TO IMPROVE THE OUTCOME OF DS CHILDREN WITH B-ALL**


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**Background:** B cell precursors acute lymphoblastic leukemia (B-ALL) is the most commonly diagnosed childhood cancer. Major advances in treatments protocols, allowing for the 5-year overall survival rate to reach 90%. However, many children still undergo intensive therapy and will require relapse after a complete remission. Children with Down syndrome (DS) have a 27-fold increased risk of developing B-ALL and face a dismal prognosis due to treatment-related mortality, and an increased risk of infections and relapse rate.

**Aims:** The goal of the study was to better understand the molecular bases of DS-ALL and develop specific therapies to improve outcomes.

**Methods:** Here, we comprehensively characterized DS-ALL samples, extended our study to other groups of childhood ALL carrying gains of chromosome 21 (B-ALL+21) and developed Patient Derived Xenograft (PDX) models suitable for preclinical studies. We performed exome and RNA sequencing to characterize the genetic and transcriptomic landscape of DS-ALL (n = 8), tAMP21-ALL (n = 7), HeH-ALL (n = 14) compared to other pediatric B-ALL (n = 12).

**Results:** Somatic mutations leading to RAS/MAPK pathway activation (NRAS, KRAS, c-MYC) and CBL (CBL-b1) genes were present in 75% (22/29) of B-ALL samples, confirming recent observations by other groups, and strengthening the link between RAS/MAPK pathway activation and pre-B ALL. To gain insights into the molecular basis of this cooperation, we ectopically expressed the constitutively active mutant KRASG12D in mouse bone marrow progenitors. We observed that KRASG12D expression in trisomic progenitors led to an increased number of CFU-preB colonies that presented increased self-renewal capacities compared to wild-type, trisomy 21 or KRASG12D alone. RNA sequencing showed that 261 genes were synergistically deregulated by KRASG12D+Trisome 21 in murine B cell progenitors, including 24 genes that were transcriptionally deregulated in B-ALL+21 with RAS/MAPK mutations patient samples compared to healthy B-cell progenitors. Together, these results indicated that constitutive RAS activation and XBP1-S21 functionally cooperates at the cellular and molecular level to impair an altered differentiation of B-cell progenitors.

From this cohort, we also developed 18 preclinical PDX models (13 B-ALL+21 and 5 Others) to test the efficacy of inhibitors of the RAS/MAPK pathway. We showed that the most potent compound, Trametinib (GSK1120212), significantly decreased viability of B-ALL+21 blasts presenting a constitutive RAS/MAPK activation in vitro (IC50 = 0.1 ± 0.05 µM) compared to 3.95 ± 4.74 µM for B-ALL samples without RAS/MAPK activation. In vivo treatments with Trametinib significantly decreased leukemia burden in the bone marrow and spleen compared to vehicle controls, and increased survival of DS-ALL, tAMP21-ALL and HeH-ALL PDX models. Finally, we assessed the efficacy of Trametinib in combination with conventional chemotheraphy agents. We observed that Trametinib significantly synergized with Vincristine and significantly increased leukaemia progression in vivo to enhance survival of DS-ALL PDXs.

**Summary/Conclusion:** Altogether, we built a comprehensive cohort of preclinical models of DS-ALL and B-ALL+21, and suggested that inhibition to characterize the genetics in combination with current chemotherapy may represent a promising strategy to improve the outcome of Down syndrome children with B-ALL.

**PF152**

**XP1 PROMOTES PRE-B CELL ACUTE LYMPHOBlastic LEukemia THROUGH OF IL-7 Receptor SIGNALING IN MICE**

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**Background:** Activating RAS mutations such as NRASG12D drive one third of pre-B cell acute lymphoblastic leukemia (pre-B-ALL). RAS mutations are a major cause of resistance to conventional chemotherapy or targeted therapy like tyrosine kinase inhibitors (TKIs). In addition, targeting of leukemia stem cells (LSCs) as the fundamental source of relapse remains challenging with currently available therapies. Recent published studies identified the unfolded protein response (UPR) as critical for pre-B-ALL survival and high expression of XBP1 correlates with a poor prognosis of ALL patients. In addition, clinical data demonstrated that the UPR genes are up-regulated in pre-B-ALL. However, the mechanism of dependency on the UPR-dependency and particularly its IRE-1a-XBP1 axis is not yet elucidated in RAS mutated pre-B-ALL.

**Aims:** In this study we aimed to identify the molecular signature of IRE-1a-XBP1 signaling at the different stages of RAS-mediated leukemogenesis. Subsequently, we aimed to decipher the mechanism behind of UPR-dependency of RAS-mutated pre-B-ALL.

**Methods:** We employed a TET-ON inducible NRASG12D model in conditional Xbp1 knockout mice. For this purpose, we transduced IL-7-dependent murine Xbp1fl/+ pre-B cells with a TET-ON inducible NRASG12D retrovirus. Thereafter the TET-ON NRASG12D Xbp1fl/+ pre-B-ALL cells are transduced with inducible-Cre. We performed in vitro cell cycle and apoptotic assays using propidium iodide (PI) and Annexin-V / 7-AAD staining, respectively. Furthermore, Western Blot and qRT-PCR were performed to analyze target gene expression. In a second approach to assess the efficacy of the IRE-1a inhibitor MKC-8866, we focused on the signaling events following pharmacological inhibition of XBP1 activation.

**Results:** To better understand the role of Xbp1 at the different stages of progression of pre-B-ALL, we examined Xbp1 transcript levels in three different cell types: normal pre-B cells, early pre-B ALL cells and established pre-B ALL cells. We demonstrated that Xbp1 expression increased upon activation of NRASG12D. To determine the significance of Xbp1 in pre-B-ALL, we genetically deleted the IRE-1a target Xbp1 using Cre-mediated deletion of Xbp1flox in our mouse model of pre-B-ALL. Genetic loss of Xbp1 strongly induced apoptosis (2.4-fold, p ≤ 0.0001), Pre-B-ALL cells in the absence of active Xbp1 also increased RAS downstream targets p-ERK, p-ERK and MAPK negative regulators such as Dusp6 (1.4-fold p = 0.0004) and slightly increased pro-apoptotic BIM protein and cell cycle negative regulator P21 (1.7-fold, p ≤ 0.0001). Pre-B-ALL cells in the absence of active Xbp1 also increased RAS downstream targets p-ERK and MAPK negative regulators such as Dusp6 (1.4-fold p = 0.0004) but decreased expression of Dusp1 (1.6-fold, p = 0.0011). Interestingly, pharmacological inhibition of Xbp1 activation using MKC-8866 or in combination of pre-B-ALL cells without IL-7 resulted in similar effects on target gene expression in comparison to the genetic deletion of Xbp1.

**Summary/Conclusion:** In summary, our work strongly supports the hypothesis that XBP1 is critical for progression and maintenance of RAS mutated leukemogenesis. Reduction of active transcript of Xbp1 by MKC-8866 or genetic loss of Xbp1 did reduce STAT5 as a downstream linchpin of the IL7 receptor signaling pathway and resulted in increased Trametinib sensitivity as a downstream target of RAS pathway. XBP1 expression is positively regulated by STAT5. Furthermore, loss of XBP1 might induce negative feedback on upstream STAT5 and inhibit proliferation and induce apoptosis via the ERK signaling pathway.