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POSTER ABSTRACTS

506.BONE MARROW MICROENVIRONMENT

Modeling Gilteritinib Resistance By Enforced cxcl8a Expression in Zebrafish HSPCs

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In Acute Myeloid Leukemia (AML), the FMS-like tyrosine kinase III Internal Tandem Duplication (FLT3-ITD) is common and increases the risk of relapse which is only partially mitigated by tyrosine kinase inhibitor (TKI) therapy. Gilteritinib is the current mainstay for treating relapsed FLT3-ITD AML, but therapy is non-curative, and the duration of remission is transient. Mutations in the RAS pathway are known to promote gilteritinib resistance, however non-mutational mechanisms of resistance remain under-explored and could provide new avenues for combination therapy. Our previous work has associated AML cell-autonomous expression of chemokines such as CXCL8 (IL-8) as a mechanism of resistance to gilteritinib therapy. Here we enforce expression of *cxcl8a* (*cxcl8*, ENSDARG00000104795) by hematopoietic stem and progenitor cells (HSPCs) in transgenic zebrafish embryos using a defined Runx1 enhancer element. In the absence of gilteritinib, Runx1:cxcl8(+) animals had greater numbers of HPSCs compared to Runx1:cxcl8(-) animals (4.0 vs 2.1, $p = 0.04$). When treated with gilteritinib at 100 nM from 48-72 hours post-fertilization (hpf), HSPC numbers were decreased in both groups; however, Runx1:cxcl8(+) animals still had more HSPCs compared to Runx1:cxcl8(-) animals (1.6 vs 0.4, $p = 0.02$). This suggests that *cxcl8* may act as a resistance factor for gilteritinib *in vivo* and that understanding regulators of *cxcl8* expression may provide insight into gilteritinib resistance. Reanalysis of published single cell RNA-seq data in AML patients revealed an association between expression of CXCL8 and BTK and BMX but not other Tec-family kinases such as ITK and TEC itself. Zebrafish do not have an annotated BMX gene; BTK is expressed in HSPCs and neutrophils but not the erythroid lineage. CHMFL is a specific and potent inhibitor of human BMX with an unknown spectrum of activity in zebrafish. In Runx1:cxcl8(-) *Runx1:GFP* zebrafish, CHMFL treatment reduced HSPC numbers, but this was not rescued in Runx1:cxcl8(+) animals (untreated vs treated, cxcl8(-): 2.9 vs 1.5, $p = 0.02$, untreated vs treated, cxcl8(+): 4.0 vs 1.4, $p = 0.0006$). This contrasts with our prior work with the non-specific Tec family kinase inhibitor, ibrutinib, where HSPC numbers were rescued in Runx1:cxcl8(+) animals. Ongoing studies are focused on understanding the targets of ibrutinib and CHMFL in the zebrafish and how these pathways may affect gilteritinib resistance.

Disclosures No relevant conflicts of interest to declare.

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