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Zebrafish Model of MLL-Rearranged Acute Myeloid Leukemia

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ZEBRAFISH MODEL OF MLL-REARRANGED ACUTE MYELOID LEUKEMIA

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Abstract

Acute myeloid leukemia (AML) is the second most common type of leukemia and accounts for 80% of adult acute leukemia cases, and is characterized by the accumulation of poorly or un-differentiated myeloid blast cells. Furthermore, AML patients harboring a chromosomal rearrangement involving Multiple Lineage Leukemia (MLL) that results in the expression of an MLL fusion protein exhibit far worse prognoses than patients without. In recent years, Danio rerio (zebrafish) has emerged as a powerful model organism for investigating human blood malignancies due to the conservation of hematopoiesis between humans and zebrafish. The first objective of this study was to develop a transient transgenic AML model in zebrafish, and the second objective was to determine if co-treatment with two medications currently in human trials for AML, Venetoclax and Flavopiridol, would be more effective than using either drug individually. In order to develop a transient transgenic AML model, we first developed a DNA construct encoding a known mixed lineage leukemia (MLL) fusion protein associated with human AML, MLL-ENL, driven by the zebrafish lysozyme C (*lyz*) promoter, which drives myeloid specific expression in zebrafish. We then microinjected single-cell zebrafish embryos with DNA encoding *lyz* driven MLL-ENL along with transposase mRNA to facilitate the genomic integration of MLL-ENL. Injected embryos were first tested for MLL-ENL expression, and subsequently tested for AML phenotypic characteristics, via whole mount in-situ hybridization (WISH) at 48 and 72 hours post fertilization (hpf). First, WISH analysis utilizing a human MLL riboprobe verified MLL-ENL expression in injected embryos, and WISH analysis utilizing the same MLL riboprobe revealed an expansion and clustering of MLL positive cells in injected embryos, characteristic of an AML phenotype. We then characterized the cell type expressing MLL-ENL by conducting WISH with other myeloid marker probes, and also characterized the AML phenotype by showing overexpression of *Bcl2* and *Cdk9* in MLL-ENL expressing embryos, both of which are overexpressed in human AML. Finally, embryos injected with MLL-ENL DNA were treated with either DMSO (vehicle), 200 nano-Molar (nM) Venetoclax, 200 nM Flavopiridol, or 200 nM Venetoclax and 200 nM Flavopiridol from 24 hpf to 72 hpf. MLL WISH analysis of injected and treated embryos revealed a reduction in MLL positive cells in both Venetoclax treated embryos and Flavopiridol treated embryos, and an even greater reduction in MLL positive cells in embryos treated with both Venetoclax and Flavopiridol, compared to controls. Although further analysis is required to be confident, this data suggests that we successfully developed an AML transient transgenic model in zebrafish. Furthermore, these data suggest that Venetoclax and Flavopiridol co-treatment could yield better outcomes for AML patients than treatment with either drug individually.

MLL⁺ Cell Expansion in *Lyz* Driven MLL-ENL Embryos

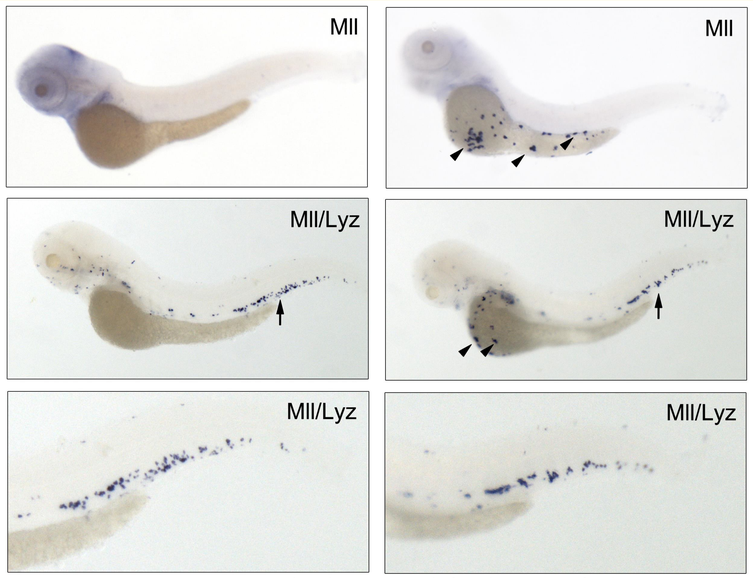


Figure 1. MLL-ENL expression in injected embryos. At 72 hpf, embryos were fixed, WISH analysis was conducted, and positive cell counts were taken on MLL-ENL injected embryos using human *MLL* and zebrafish *lyz* probes, with uninjected embryos used as controls. In injected embryos, human *MLL* expression can be observed in clusters of cells on the yolk, and endogenous *lyz* expression is not significantly reduced in the caudal hematopoietic tissue (CHT), where *lyz* is predominantly expressed at 72 hpf, compared to controls. Furthermore, the average heart rate of injected embryos was not significantly different than controls, suggesting that the increased number of of MLL positive cells on the yolk of injected embryos is not due to insufficient circulation (data now shown).

Spi1⁺ Cell Expansion in *Lyz* Driven MLL-ENL Embryos

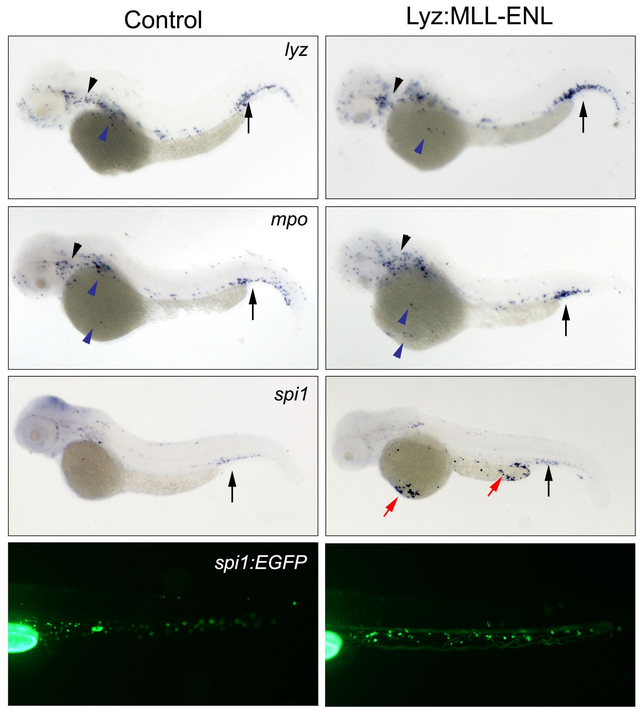


Figure 2. *Lyz, mpo, and spi1* expression in MLL-ENL expressing embryos. Embryos were fixed at 48 hpf and WISH was conducted using *lyz, mpo, and spi1* riboprobes. While there is a slight increase in *lyz* and *mpo* expressing cells in the CHT of MLL-ENL expressing embryos compared to controls, a clustered expansion on the yolk sac is not observed. However, a clustered expansion of *spi1* expressing cells can be observed on the yolk sac of MLL-ENL expressing embryos, suggesting that the cells expressing MLL-ENL are *spi1*⁺ and not *lyz*⁺ or *mpo*⁺.

Bcl2⁺ and *Cdk9*⁺ Cell Expansion in *Lyz* Driven MLL-ENL Embryos

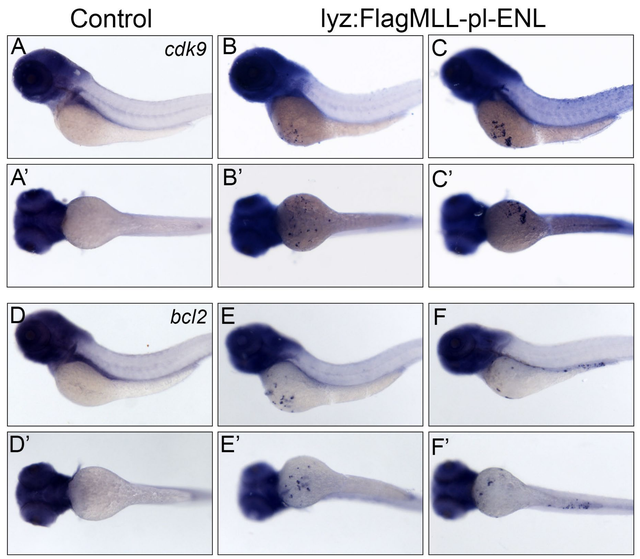
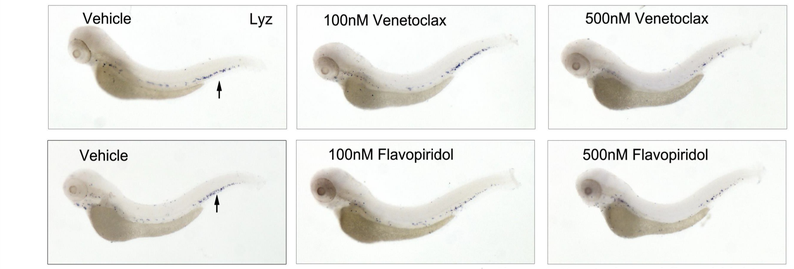


Figure 3. *Bcl2 and cdk9* overexpression in MLL-ENL expressing embryos. As both BCL2 and CDK9 are overexpressed in human AML, we designed *bcl2* and *cdk9* riboprobes to further characterize the AML phenotype of our model. Conducting WISH analysis at 48 hpf using these probes revealed a clustered expansion of both *bcl2* and *cdk9* expressing cells on the yolk sac of MLL-ENL expressing embryos. This further suggests that the expression of MLL-ENL induces an AML phenotype in zebrafish.

Venetoclax and Flavopiridol Dose Response



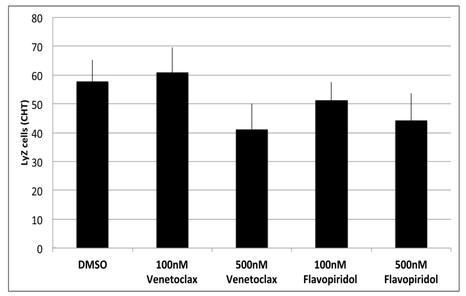
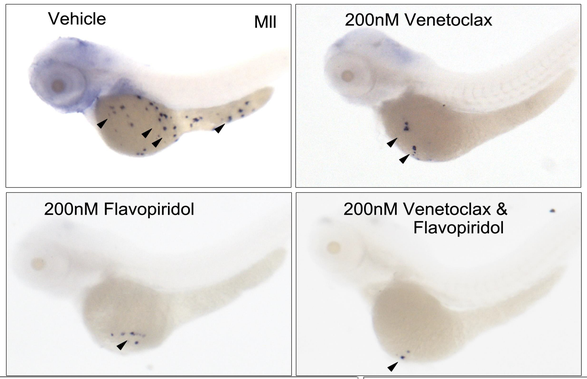


Figure 4. Venetoclax and flavopiridol treatment does not affect embryogenesis or myeloid development at low concentrations. Dr. Steven Grant provided our lab with Venetoclax and Flavopiridol to perform studies on MLL- ENL injected Embryos in conjunction with studies in his lab. To determine if drug treatment altered embryogenesis or myeloid development in zebrafish embryos we treated wild type embryos with varying concentrations from 0 nM- 500 nM of Venetoclax and Flavopiridol, and conducted WISH analysis with an *lyz* probe. Lyz⁺ cell counts in the CHT were reduced with treatment of either drug at 500 nM, but no significant difference was observed with 100 nM treatment.

Venetoclax and Flavopiridol Co-Treatment Inhibits MLL-ENL Cell Expansion



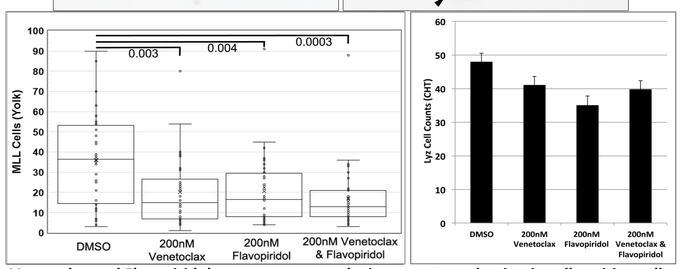


Figure 5. Venetoclax and Flavopiridol co-treatment results in a greater reduction in *mll* positive cell expansion than either individual treatment. Before fixing at 72 hpf and conducting subsequent WISH analysis, MLL-ENL injected embryos were treated with DMSO, 200 nM Venetoclax, 200 nM Flavopiridol, or 200 nM of both drugs beginning at 24 hpf. Embryos were fixed at 72 hpf, WISH analysis was performed using a human *MLL* probe, MLL⁺ cells on the yolk were counted. In a separate experiment, an *lyz* probe was used to assay *lyz*⁺ cell counts in the CHT of MLL-ENL injected embryos after one of the 4 treatments listed above.

Acknowledgements

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