(PF641) RNA-GUIDED SELECTIVE DNMT1 INHIBITION RESTORES MYELOID DIFFERENTIATION AND OUTPERFORMS AZACITIDINE IN TET2-MUTANT MYELODYSPLASTIC SYNDROMES

Topic: 09. Myelodysplastic syndromes - Biology & translational research

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Background

Loss-of-function mutations in *TET2* are among the most frequent genetic alterations in myelodysplastic syndromes (MDS) and drive aberrant DNA methylation and impaired myeloid differentiation. Patients with TET2-mutant MDS commonly receive hypomethylating agents (HMAs), such as azacitidine and decitabine, which broadly inhibit DNMT activity. Although TET2-mutant patients may initially respond, most eventually relapse due to resistance, and treatment is limited by toxicity. This therapeutic gap underscores the need for selective and durable strategies to reverse TET2-driven epigenetic dysregulation. We developed aptaDiR_Ce-49_sh_v1, a DNMT1-selective RNA inhibitor designed to counterbalance TET2 mutation-driven epigenetic rewiring.

Aims

To determine whether selective DNMT1 inhibition restores myeloid differentiation in TET2-deficient MDS in vivo and and provide advantages over conventional HMAs, specifically azacitidine

Methods

A syngeneic Tet2/Asxl1 double-knockout hematopoietic transplantation model of myelodysplasia was generated by transplanting Vav-Cre^{+} Tet2$^{flox/flox}$ Asxl1$^{flox/flox}$ CD45.2^{+} bone marrow cells with wild-type CD45.1^{+} support cells into lethally irradiated congenic recipients. Following engraftment, mice developed an MDS-like phenotype characterized by myeloid skewing and HSPC expansion. Three treatment arms (n=3/group) were evaluated: vehicle, azacitidine (1 mg/kg), aptaDiR_Ce-49_sh_v1 (1 mg/kg). RNA molecules were encapsulated in myeloid-targeted lipid nanoparticles and administered intravenously three times weekly for four weeks. Engraftment and differentiation were assessed by flow cytometry, bone marrow cytomorphology, and peripheral white blood cell counts.

Results

Selective DNMT1 inhibition by aptaDiR_Ce-49_sh_v1 induced robust granulocytic maturation and restored myeloid differentiation in TET2-deficient hematopoiesis, exceeding the effects observed with azacitidine (**Fig.1**). Treatment activated myeloid lineage and innate immune transcriptional programs consistent with differentiation rescue. In contrast to azacitidine, aptaDiR_Ce-49_sh_v1 did not induce cytopenias or reduce total white blood cell counts, indicating superior hematologic tolerability.

Summary/Conclusion

RNA-guided selective DNMT1 inhibition restores myeloid differentiation in vivo in a TET2-mutant MDS model, outperforming conventional HMAs while preserving hematologic integrity. These findings identify selective DNMT1 targeting as a precision therapeutic strategy for TET2-mutant MDS.

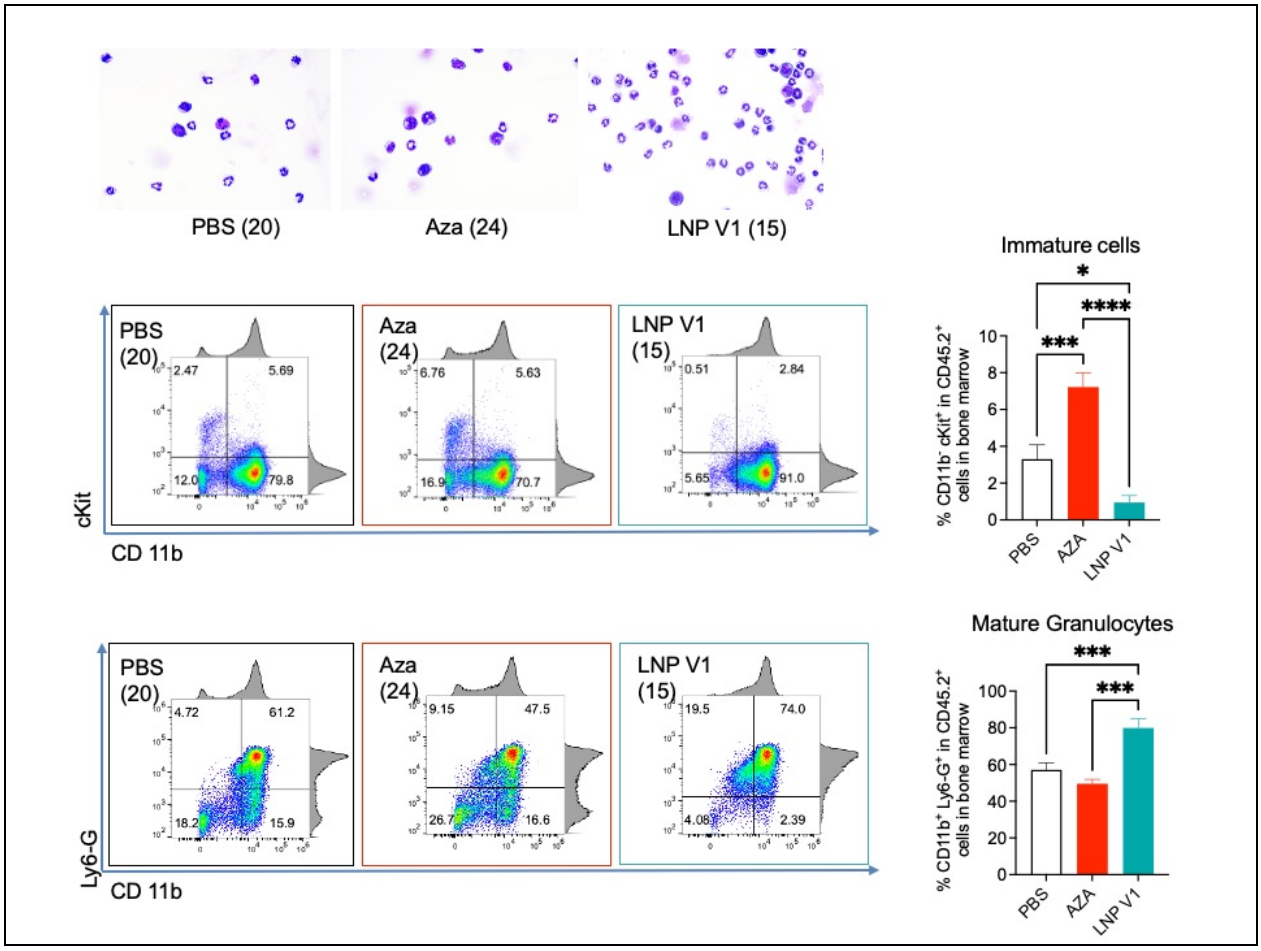

Figure 1. aptaDiR_Ce-49 sh_v1 induces myeloid differentiation. Upper panels: May–Grünwald–Giemsa staining of bone marrow cytospins after 4 weeks of treatment in Vehicle (PBS), azacitidine (1 mg/kg), and aptaDiR_Ce-49_sh_v1 (LNP V1; 1 mg/kg) groups (n=3/group), demonstrating remarkable myeloid maturation with granulocytic differentiation in LNP V1–treated mice. Middle panels: Representative flow cytometry plots showing increased mature myeloid cells ($CD11b^+/cKit^-$) and reduced immature progenitors ($CD11b^-/cKit^+$) following LNP V1 treatment. Quantification is shown on the right. Lower panels: Representative flow cytometry plots demonstrating increased granulocytic differentiation ($CD11b^+/Ly6G^+$) in LNP V1–treated mice, with corresponding quantification. Data are presented as mean ± SD (n=3). Statistical analysis was performed using one-way ANOVA (**p<0.01, ***p<0.001, ****p<0.0001).