Therapeutic Targeting of FLT3 gate keeper mutation with E2082-0047 in traditional and a novel Immunocompetent murine adoptive transfer model of AML

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BACKGROUND

Acute myeloid leukemia (AML) is the most common adult leukemia in USA. Genetic aberrations in Fms-like tyrosine kinase 3 (FLT3), typically internal tandem duplication (FLT3-ITD) and tyrosine kinase domain (FLT3-TKD) mutations, occur in 30% AML patients and correlates with poor overall survival. Despite the generation of potent FLT3 inhibitors (FLT3i) such as gilteritinib which exhibit strong initial response, resistance often occurs via the acquisition of an intrinsic F691L gatekeeper mutation or the development of adaptive resistance mechanisms such as enhanced IRAK4 or MAPK/RAS/PTPN11 growth activating mutations. Therefore, the need for novel FLT3i overcoming some of the current vulnerabilities are urgently needed. We have developed E2082-0047, a novel FLT3 inhibitor with nanomolar *in vitro* potency against FLT3-ITD and TKD mutations including the gatekeeper mutation (Elgamal OA et al., ASH 2022). Herein, we extended our studies to validate *in vivo* efficacy against the gatekeeper mutation using human xenograft and murine adoptive transfer models.

METHODS

Given that the acquisition of the F691L gatekeeper mutation represents a major hurdle for FLT3 targeted therapies such as gilteritinib, we utilized the human AML MOLM-14 cell line with F691L mutation to evaluate the ability of E2082-0047 *in vivo*. E2082-0047 was extremely potent in the MOLM-14-ITD-F691L cell line derived xenograft (CDX) model, however this model doesn't include an intact immune system which may contribute to response. Therefore, we evaluated the effect of the FLT3 inhibitors using an immunocompetent AML model where *Npm1^{cA};Flt3^{ITD}* AML tumor cells (Vassiliou et al., 2011, Lee et al., 2007) which harbor a F692L mutation (Dovey et al., 2016) are engrafted into syngeneic host animals. More detail regarding the development of the AML immunocompetent adoptive transfer model will be presented in abstract 1386, session 602 on Saturday, December 9, 5:30-7:30 PM.

A healthy volunteer clinical trial is ongoing to determine the safety and human pharmacokinetics properties to inform the future anticipated AML trial.

RESULTS

E2082-0047 exhibits superior efficacy in the MOLM-14-ITD-F691L CDX model

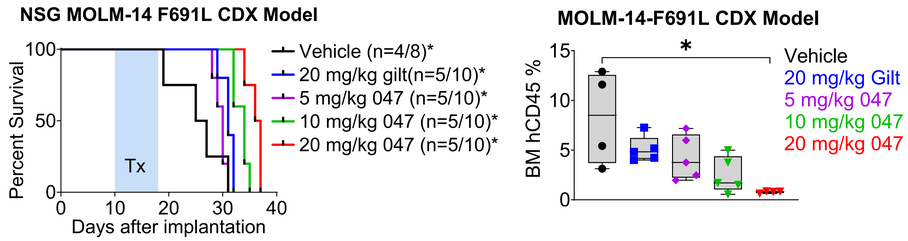

Fig. 1. **Left**, Survival Percentages of MOLM-14 F691L cells after treatment. 23 animals were engrafted with MOLM-14 F691L cells and treated with vehicle, gilteritinib (gilt), or E2082-0047 (047) from day 10 till 18. * On day 19, 4-5 mice per group were euthanized for tumor burden assessment while remaining mice monitored without treatment until they showed signs of AML disease. The day of euthanasia was recorded and graphed as a survival curve using Mantel-cos test. **Right**, Flow cytometry analysis of bone marrow human CD45 percentage.

E2082-0047 shows stronger selectivity against mutant FLT3

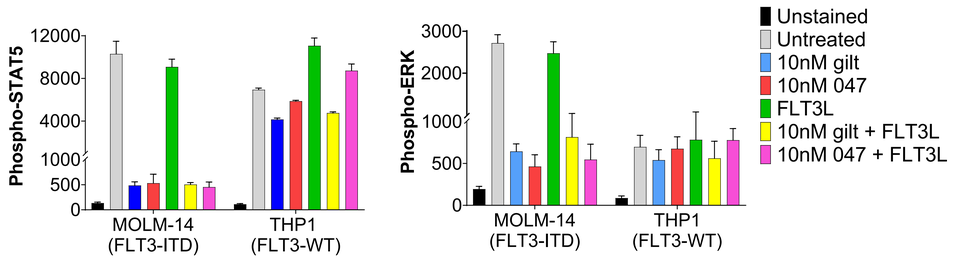

Fig. 2. Using flow cytometry, the FLT3 signaling was assessed using downstream phospho-ERK and phospho-STAT5 targets. To enhance the sensitivity to FLT3 ligand, cells were subjected to overnight serum starvation and subsequently either left unstimulated or stimulated with 100 ng/ml of FLT3 ligand followed by treatment with varying concentrations of gilteritinib or E2082-0047 for 4 hours. Both gilteritinib and E2082-0047 demonstrate potency in the MOLM-14 FLT3-ITD cell line compared to the THP1 cell line with FLT3-WT yet, E2082-0047 exhibits a higher degree of selectivity in inhibiting FLT3-ITD when compared to gilteritinib. Data are representative of 3 independent experiments (error bars, mean with 95% CI).

E2082-0047 significantly prolong survival in an AML adoptive transfer murine model

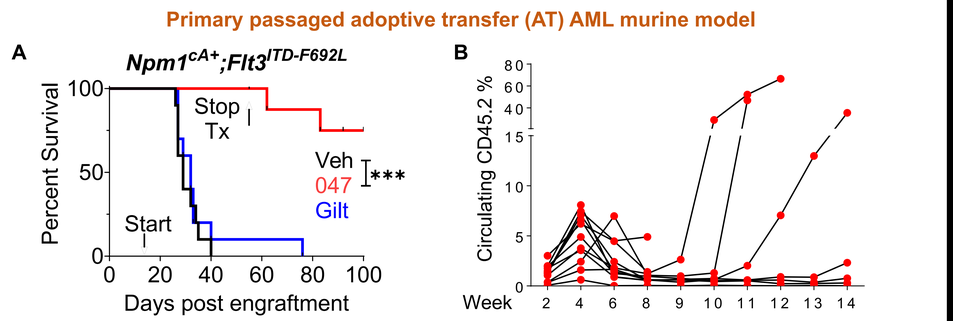

Fig. 3. **A**) CD45.2+ *Mx1^{Cre};Npm1^{CA};Flt3$^{ITD-F692L}$* splenocytes harvested from spontaneous murine AML model were injected intravenously into CD45.1+ immunocompetent mice. Treatment with daily oral vehicle (n=10) , 20 mg/kg E2082-0047 (n=8/11)* or 30 mg/kg gilteritinib (n=10) was initiated 14 days post engraftment. **B**) Mice are bled every two weeks to monitor circulating CD45.2% tumor burden by flow cytometry. *Dosing was stopped at day 55, three mice from the 047 arm were euthanized for tissue harvest and eight mice were kept for monitoring till end of study day 100.

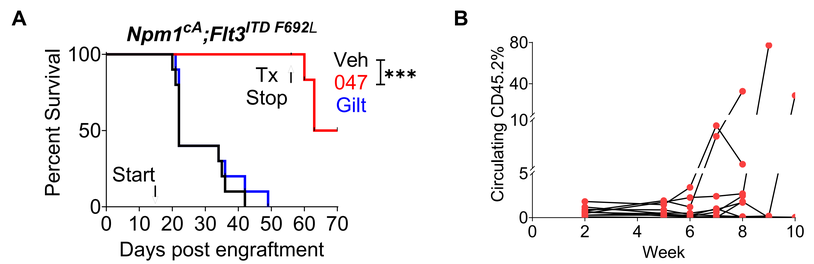

Fig. 4. **A**) CD45.2+ *Mx1^{Cre};Npm1^{CA};Flt3$^{ITD-F692L}$* splenocytes harvested from primary passaged AML model were injected intravenously into CD45.1+ immunocompetent mice (n=10/arm). Treatment with daily oral vehicle (n=10) , 20 mg/kg E2082-0047 (n=6/10)* or 30 mg/kg gilteritinib (n=10) was initiated 14 days post engraftment. *Dosing was stopped at day 56, four mice from the 047 arm were euthanized for tissue harvest and six mice were kept for monitoring till end of study day 70.

CONCLUSIONS

We previously demonstrated that E2082-0047 has favorable PK, ADME, and safety profile and synergize with venetoclax. We further reveal that:

- E2082-0047 exhibits potent sensitivity against gatekeeper resistant FLT3 mutant AML
- E2082-0047 shows potential selectivity towards mutant FLT3 signaling compared to wild type FLT3 signaling
- E2082-0047 shows compelling and superior monotherapy survival benefit in a fully immunocompetent Npm1^{CA};Flt3$^{ITD-F692L}$ AML adoptive transfer model supporting its clinical development
- A healthy volunteer study with E2082-0047 has demonstrated dose-dependent increases in plasma exposure, a long half-life, and target engagement with FLT3-ITD starting from a daily 10mg dose. Our data supports a phase 2 clinical trial in FLT3 inhibitor relapsed FLT3 mutant AML

ACKNOWLEDGEMENTS

- Eilean Therapeutics
- ChemDiv
- Viriom
- Molsoft
- Expert Systems
- Reaction Biology

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