Abstract # 4678

A NOVEL AND POTENT INHIBITOR OF E-SELECTIN, GMI-1687, ATTENUATES THROMBUS FORMATION AND AUGMENTS CHEMOTHERAPEUTIC INTERVENTION OF AML IN PRECLINICAL MODELS FOLLOWING SUBCUTANEOUS ADMINISTRATION

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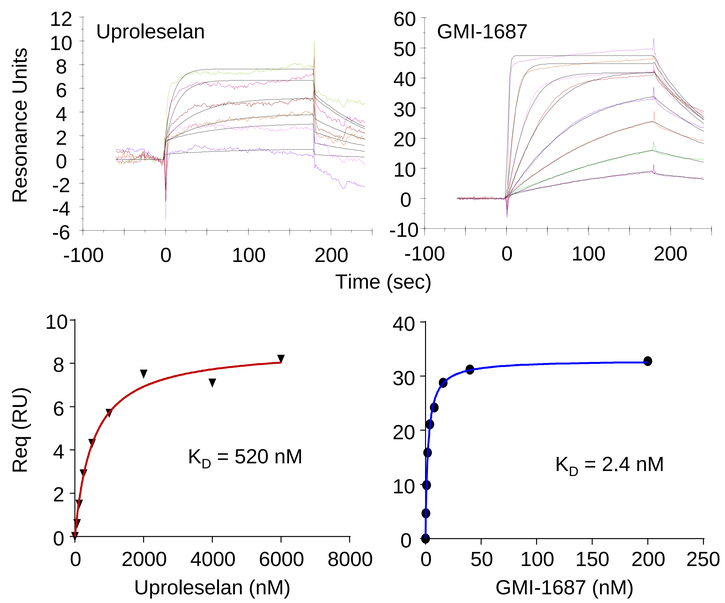
Background and Introduction

Uproleselan (GMI-1271), an E-selectin antagonist, has been shown in preclinical models to disrupt activation of cell survival pathways in acute myeloid leukemia (AML), enhance chemotherapy efficacy, and improve survival. Uproleselan received FDA breakthrough therapy designation for adult relapsed/refractory AML in 2017 and Phase III studies are ongoing. In the present studies we report on the *in vitro* and *in vivo* comparative activities of an innovative high potency E-selectin antagonist, GMI-1687, to Uproleselan.

Results

Figure 1. Direct Binding of Uproleselan and GMI-1687 to Immobilized E-selectin

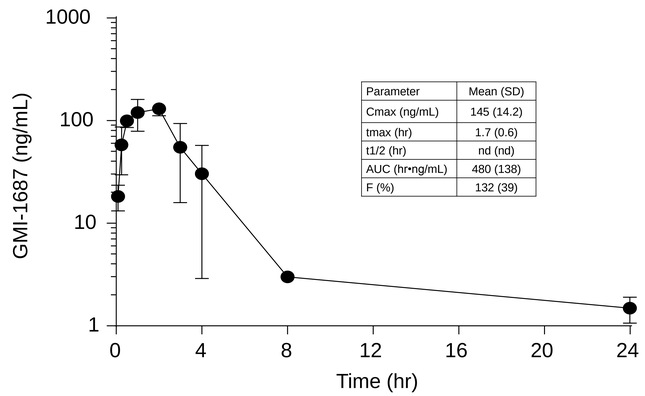
Real time binding of Uproleselan or GMI-1687 was monitored at 25ºC by Surface Plasmon Resonance (SPR) using a Biacore X100 instrument (GE Healthcare). E-selectin/Fc chimera protein was captured an anti-Fc CM5 sensor chip. The assays consisted of cycles of compound injection at a flow rate of 30 µL/min during 3 min followed by a dissociation period of 1 min and subsequent regeneration of the E-selectin surface with 1M NaCl. Uproleselan was evaluated at 62.5, 125, 250, 500, 1000, 2000, 4000, 6000 nM and GMI-1687 at 0.5, 1, 2, 4, 8, 16, 40, 200 nM. Three or more independent experiments were performed. Double referenced sensorgrams were fitted to a 1:1 binding model (Langmuir model) using the Biacore X100 evaluation software to obtain kinetic rate constants. Sensorgrams (color) and fitted curves (black) are shown for Uproleselan (top left) and GMI-1687 (top right). Steady state affinity analysis were performed using GraphPad Prism software and shown in the bottom panels.



Summary: For Uproleselan, k_{on}= 0.02x 10^6 $M^{-1}s^{-1}$ and k_{off} = 1 x10^{-2} s^{-1} ; for GMI 1687, k_{on} = 3x 10^6 $M^{-1}s^{-1}$ and k_{off} = 1 x10^{-2} s^{-1}. The K_D for Uproleselan and GMI-1687 binding to E-selectin was 520 nM and 2.4 nM, respectively.

Figure 2. Mean Blood Concentrations (ng/mL) and Pharmacokinetic Parameters for GMI-1687 After Subcutaneous Administration at 0.576 mg/kg

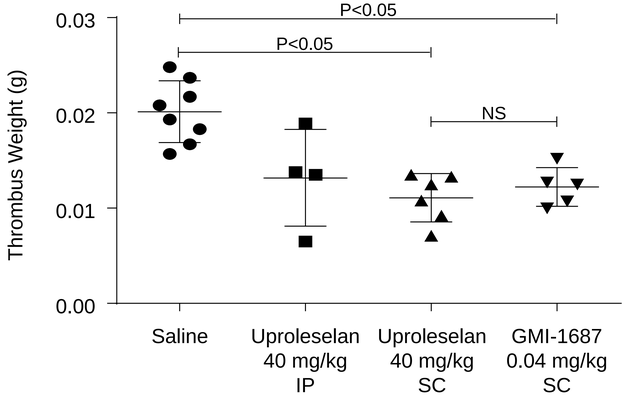
The pharmacokinetics of GMI-1687 was evaluated in fasted male CD-1 mice. Mice were fasted for a minimum of twelve hours prior to formulation administration. Food was returned at four hours post dosing. Animals had free access to water throughout the study. Each mouse received either a single bolus IV injection via a jugular vein cannula or a subcutaneous injection via an appropriate sized needle. Pharmacokinetic parameters were calculated from the time course of the blood concentration of GMI-1687 determined by LC-MS/MS methodology. Pharmacokinetic parameters were determined with Phoenix WinNonlin (v8.0) software using a non-compartmental model.



Summary: After SC dosing of GMI-1687 at 0.576 mg/kg, maximum blood concentrations (average of 145 ±14.2 ng/mL) were observed between 1 and 2 hours post dosing. The average half-life could not be determined; however, the half-life for one mouse was 4.78 hours. The average bioavailability for GMI-1687 was 132 ± 38.0%.

Figure 3. GMI-1687 Shows an Approximate 1000-fold Increase in Activity as Uproleselan in a Mouse Inferior Vena Cava Model

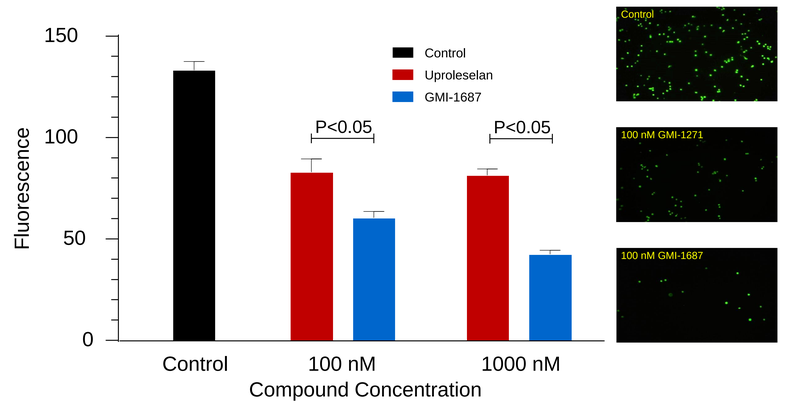
Male C57BL/6J mice underwent an electrolytic inferior vena cava (IVC) model to produce a non-occlusive thrombosis via electrical stimulation (250 µAmp). Animals were divided into four cohorts (N = 4 to 8 mice/group) and treated with: Cohort 1- saline (0.2 mL/20g SC); Cohort 2- Uproleselan (40 mg/kg IP); Cohort 3- Uproleselan (40 mg/kg SC); and Cohort 4- GMI-1687 (0.04 mg/kg SC). Mice received a single injection of saline or drug following thrombus induction on day 1. Mice were euthanized 2 days post-thrombosis for tissue harvest and thrombus weight was determined.



Summary: Subcutaneous administration of GMI-1687 attenuated thrombus formation in an IVC model similar to Uproleselan but at an approximate 1000-fold reduction in dose.

Figure 4. Uncoupling of E-selectin-adherent KG1a AML Cells with GMI-1687

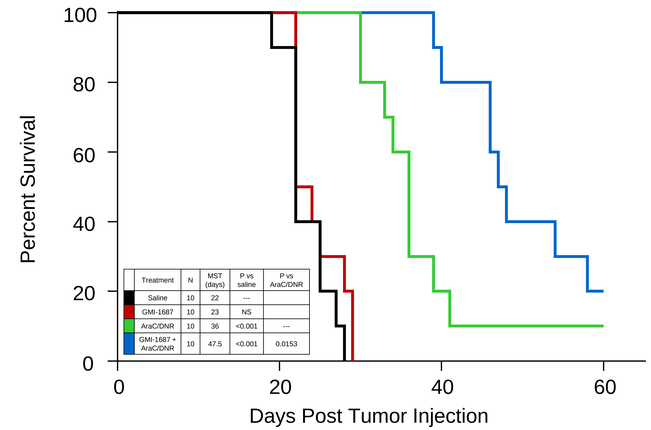
KG1a AML cells were labeled with the fluorescent, cell-permeant dye Calcein AM. The cells were added to a 96-well plate that had previously been coated with 2 µg/mL E-selectin/Fc and the cells were allowed to adhere for 45 minutes at room temperature. Adherent cells were then detached with Uproleselan or GMI-1687 (100 or 1000 nM drug for 30 min) and cell adhesion was assessed by fluorescence microscopy and by measuring fluorescence using a FlexStation3 plate reader (Molecular Probes).



Summary: Uproleselan and GMI-1687 detach AML cells from E-selectin. However, GMI-1687 was significantly more potent than Uproleselan at equivalent concentrations (P<0.05).

Figure 5. Combination of Low Dose SC GMI-1687 with Ara-C and DNR Extends Survival of KG1 AML-bearing Mice

Female NSG mice (6 wks of age) were injected iv with KG-1 human AML tumor cells modified with luciferase (5.0x10^6 cells/mouse). Beginning 7 days post injection, mice were randomized into 4 cohorts and treated with: Cohort 1- saline (0.2 mL/20g IP qdx14); Cohort 2- GMI-1687 (0.04 mg/kg SC qdx14); Cohort 3- cytarabine (300mg/kg, IP) + daunorubicin (3mg/kg, IV); and Cohort 4- the combination of GMI-1687 and chemotherapy. The efficacy of the treatment on survival was estimated by the Kaplan-Meier method, and log-rank statistics was used to test for differences in survival.



Summary: Combination of low dose SC GMI-1687 cytarabine and duanorubicin extends survival of KG1-engrafted mice beyond chemotherapy alone.

Conclusion

IND-enabling studies have initiated with a highly potent innovative antagonist of E-selectin. GMI-1687 is differentiated from Uproleselan by low dose, SC activity in Uproleselan-responsive models. GMI-1687 is well-positioned to be used in outpatient treatment settings where an E-selectin antagonist has therapeutic relevance.

GlycoMimetics, Inc.