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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Abstract #

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 designations 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 25°C by Surface Plasmon Resonance (SPR) using a Biacore X100 instrument (GE Healthcare). Uproleselan 185 uM protein was captured on an HP CM5 sensor chip. The analyte consisted of queried E-selectin coated on T2000 Resonance Units (black). Uproleselan was evaluated at 42.5, 105, 250, 500, 1000, 2000, 4000, 6000 nM and GMI-1687 at 0.5, 1, 2, 4, 5, 10, 20 nM. Three or more independent experiments were performed. Doubly antiporter antibodies were fitted to a 1:1 binding model (Langmuir model using the Biacore X100 evaluation software to obtain kinetic rate constants. Antibodies (colored) and fitted curves (black) are shown for Uproleselan (top left) and GMI-1687 (top right). Slow state affinity analysis were performed using GraphPad Prism software and shown in the bottom panels.

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 tested male CD-1 mice. Mice were bled for a minimum of twelve hours prior to formulation administration. Food was removed 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 catheter or a subcutaneous injection via a appropriate sized needle. Pharmacokinetic parameters were calculated from the time courses of the blood concentrations of GMI-1687 determined by LC-MS/MS methodology. Pharmacokinetic parameters were determined with Phoenix WinNonlin (v8.0) software using a non-compartmental model.

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 endoluminal inferior vena cava (IVC) model to produce a non-circulatory thrombus via electrical stimulation (250 kHz). Animals were divided into four cohorts (8-4 to 8-4 micrograms) and treated with: Cohort 1: saline (0.2 mL/kg SC); Cohort 2: Uproleselan (40 mg/kg IP); Cohort 3: Uproleselan (40 mg/kg SC); and Cohort 4: GMI-1687 (0.84 mg/kg SC). Mice were injected with Coumarin-6 and assessed for thrombus formation on day 1. Mice were euthanized 2 days post-thrombosis to obtain tissue harvest and thrombus weight was determined.

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

KG1a AML cells were labeled with the fluorescent, enhancement dye Calcein-AM. The cells were added to a 96-well plate that had previously been coated with 2 μg/ml E-selectin and the cells were allowed to adhere for 45 minutes at room temperature. Adherent cells were then prestimulated with Uproleselan or GMI-1687 (15 or 100 nM drug for 30 min) and cell adhesion was assessed by fluorescence microscopy and by measuring fluorescence using a FlexStation3 plate reader (Molecular Probes).

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 with KG-1 human AML tumor cells cultured with luteinase (5,6-dihydrocaffeic acid), Sephadex/Ficoll and irradiated. Mice were randomized into four cohorts and treated with: Control 1: saline (0.2 mL/kg IP); Control 2: GMI-1687 (0.84 mg/kg SC); Control 3: Calcein-AM (1 mg/mL IP) + dexamethasone (20 mg/kg SC); and Control 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.

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.