DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

National Institutes of Health
National Cancer Institute
Bethesda, Maryland 20892

REQUEST FOR PROJECT TEAM MEMBER APPLICATIONS FOR CONDUCTING PRECLINICAL STUDIES USING GMI-1271 (NSC# 801708)

The National Cancer Institute (NCI) Cancer Therapy Evaluation Program (CTEP) is accepting Project Team Member Applications to **develop pre-clinical/translational science to support clinical development of GMI-1271 (uproleselan), an E-selectin antagonist being developed by CTEP as an anticancer agent in collaboration with GlycoMimetics, Inc**. GMI-1271 is currently being developed for the treatment of hematological malignancies such as acute myeloid leukemia (AML) and multiple myeloma (MM) and has been granted breakthrough therapy designation for the treatment of adults with relapsed/refractory AML.

GMI-1271 binds E-selectin, a vascular cell adhesion molecule that is expressed by endothelial cells (Borsig, 2017). High expression of E-selectin and its carbohydrate ligand (sialyl Lewis A or X [$sLe^{a/x}$]) correlates with metastatic disease (Diamandis *et al.*, 2013). GMI-1271 inhibits adhesion of $sLe^{a/x}$-expressing cancer cells to E-selectin-expressing endothelial cells (Chien *et al.*, 2012), causing cancer cells to mobilize out of bone marrow niches and increasing their susceptibility to chemotherapy (Natoni *et al.*, 2017; Winkler *et al.*, 2014). GMI-1271 also reduces chemotherapy-associated side effects, such as mucositis (Winkler *et al.*, 2013), and enhances hematopoietic stem cell (HSC) quiescence (Winkler *et al.*, 2014). Evidence suggests that GMI-1271 downregulates activated cancer survival pathways (Fogler *et al.*, 2017) such as phosphatidylinositol 3-kinase-related kinase (PI3K)-nuclear factor kappa B (NFKB) (Porquet *et al.*, 2011).

In preclinical models of AML or MM, GMI-1271 in combination with chemotherapy or other agents was more cytotoxic and improved survival more than the chemotherapy or other agents alone (Chien *et al.*, 2012; Fogler *et al.*, 2017; Muz *et al.*, 2017; Natoni *et al.*, 2017). In a phase 1/2 study of adult patients with relapsed/refractory AML (n=47), GMI-1271 at the recommended phase 2 dose (RP2D) of 10 mg/kg in combination with chemotherapy demonstrated encouraging preliminary clinical efficacy with a remission rate of 41% and overall response rate (ORR) of 50%; a higher remission rate and ORR at 68% and 80%, respectively, were observed in a group of elderly treatment naïve AML patients (n=25) (DeAngelo *et al.*, 2017). Induction mortality and the rate of mucositis were also low, suggesting improved tolerance of chemotherapy.

At the present time, the preliminary CTEP drug development plan for GMI-1271 is to conduct preclinical studies with GMI-1271 in combination with proteasome inhibitors, immunomodulatory imide drugs (IMiDS), and melphalan using myeloma and solid tumor models. Preclinical evaluation of uproleselan in combination with HDAC and hypomethylating/demethylating agents for the treatment of elderly AML patients. Further preclinical exploration of chemotherapy combination would also be of interest. The role of the preclinical project team is to evaluate all available evidence to modify and refine this initial plan, as well as generation of new data on combinations if existing data is insufficient.

The project team will include:

1. **Translational scientists** with an interest in the development of prognostic and predictive E-selectin biomarkers using flow cytometry and immunohistochemical (IHC) assays (fill out **Part A** of the attached Application and see the submission instructions at the end of this letter); and
2. **Basic scientists** with expertise in the inhibition of E-selectin in hematological malignancies (fill out **Part B** of the attached Application and see the submission instructions at the end of this letter).

Prospective team members may apply for multiple roles using a single application form by completing all the appropriate Parts. The preclinical project team will be recruited nationally and will prioritize the research

questions regarding GMI-1271 in preclinical studies, including prioritization and development of a variety of biomarker studies. It is anticipated that the scientists on the preclinical project team will be tasked with writing the Preclinical Letters of Intent or Research Plans describing the study design, based upon the team's recommendations, for CTEP approval, and that these scientists will ultimately lead the preclinical studies. It is also anticipated that other extramural members of the preclinical project team will stay involved in the subsequent design and execution of the proposed preclinical studies. Once the preclinical projects are defined and have been reviewed by the Investigational Drug Steering Committee, the investigators will be informed if supplemental NCI-funding is available or it is suggested that the investigators seek grant or other funding. It is anticipated that the preclinical project team will complete its work in three months or less.

Background/Rationale

One of three members of the selectins family, E-selectin (also known as CD62E) is a vascular cell adhesion molecule that is expressed by endothelial cells in blood vessels and vascular niches of the bone marrow (Borsig 2017; Dimitroff *et al.*, 2001). Other family members, P-selectin (also known as CD62P) and L-selectin (also known as CD62L), are expressed on platelets and endothelial cells and on leukocytes, respectively (Borsig 2017). The selectins are transmembrane glycoproteins with extracellular C-type (Ca^{2+}-dependent) lectin domains to bind carbohydrates, specifically the sialylated, fucosylated glycans $sLe^{a/x}$. Physiologically, selectins are involved in inflammation, immunity, and hemostasis, but they are also involved in cancer metastasis. Aberrant glycosylation and altered carbohydrate structures have been linked to malignant transformation, and enhanced expression of $sLe^{a/x}$ is frequently associated with cancer progression and poor prognosis. E-selectin has been implicated in an initiating adhesion event during metastasis (Laubli and Borsig, 2010); its binding to carbohydrate ligands on cancer cells enhances their adhesion to endothelium, including in bone marrow niches (Ernst and Magnani, 2009), which prevents the cancer cells from entering circulation and shields them from chemotherapy. Cytotoxic chemotherapy, however, not only targets malignant cells, but also mucosal cells lining the respiratory and gastrointestinal tracts and normal HSCs (Winkler *et al.*, 2013). E-selectin expression increases in the intestines 10-fold after cytotoxic chemotherapy. In the bone marrow, E-selectin expression increases 10- to 20-fold in the bone marrow after cytotoxic chemotherapy, and dormant HSCs are activated to replenish blood and the immune system (Winkler *et al.*, 2014). E-selectin binding to cancer cells may also alter the gene expression of cancer cells (Laubli and Borsig, 2010) and confer survival advantages to cancer cells by activating survival pathways (Fogler *et al.*, 2017) such as the PI3K-NFKB pathway (Porquet *et al.*, 2011). By disrupting various E-selectin mediated events, GMI-1271 prevents adhesion and localization of cancer cells, which mobilizes cancer cells into the blood circulation and increases chemotherapy sensitivity (Winkler *et al.*, 2014); protects from chemotherapy-induced mucositis by preventing recruitment of inflammatory macrophages to damaged intestines while enhancing neutrophil recovery (Winkler et al., 2013); enhances HSC quiescence (Winkler *et al.*, 2014); and downregulates activated cancer survival pathways (Fogler *et al.*, 2017).

Mechanism of Action

GMI-1271 is a small molecule antagonist that specifically binds E-selectin, thereby blocking the binding of the native carbohydrate $sLe^{a/x}$ ligand (Culmer *et al.*, 2017). Rationally designed to mimic the bioactive conformation of $sLe^{a/x}$, GMI-1271 binds to E-selectin about 1000 times stronger than $sLe^{a/x}$ (average disassociation constant [K_D]=450 nM).

In a mouse xenograft model of AML, GMI-1271 (20 mcM) inhibited adhesion of primary human AML cells to E-selectin by 45.0%±9.1% standard deviation (SD) (Chien *et al.*, 2012). For at least 24 hours after a single injection of GMI-1271 at 40 mg/kg, leukemic blasts mobilized into the blood (Winkler *et al.*, 2014). In a mouse model of MM, an anti-human CD138 antibody was used to determine by flow cytometry the number of MM cells in the peripheral blood; a persistent increase in the number of $CD138^+$ cells in the peripheral blood was observed for at least 24 hours after an intraperitoneal (IP) injection of GMI-1271 at 40 mg/kg compared to saline (2.37% versus 0.03%; $p<0.001$) (Natoni *et al.*, 2017).

Nonclinical Studies of GMI-1271

In a mouse model of AML, GMI-1271 (40 mg/kg twice daily) for 10 days in combination with standard mouse version of 7+3 induction chemotherapy (cytarabine 100 mg/kg for 5 days; doxorubicin 1 mg/kg for 3 days) significantly (p=0.0054) doubled mouse survival compared to chemotherapy alone (median survival: 41 days, and 32 days, 25 days for GMI-1271 plus chemotherapy, chemotherapy alone, and saline respectively; n=8 mice/group) (Winkler *et al.*, 2016). GMI-1271 also demonstrated *in vivo* antitumor activity in combination with azacitidine (Fogler et al., 2017). Mice (n=10/group) injected with AML cells were treated with GMI-1271 alone (40 mg/kg IP once daily for 14 days), azacitidine alone (5 mg/kg IP every 3 days); the combination of GMI-1271 and azacitidine; and saline. Median survival of mice treated with azacitidine was 88 days, which was statistically significant (p<0.005) compared to GMI-1271-treated (69 days) and saline-treated (69.5 days) groups. All treatments were well tolerated. The activity of azacitidine was significantly enhanced when combined with GMI-1271 (median survival of 110 days; p=0.0217) compared to azacitidine alone, and 70% of mice survived to study conclusion. The 50% inhibitory concentration (IC_{50}) of azacitidine alone or in combination with GMI-1271 was 637 nM and 711 nM, respectively, suggesting that the enhanced antitumor activity observed *in vivo* was not due to a simple shift in the cytotoxicity profile of azacitidine.

In a mouse xenograft model of MM, GMI-1271 (40 mg/kg IP daily for 21 days) in combination with bortezomib (0.75 mg/kg IP once weekly [QW] for 3 weeks) significantly improved survival than bortezomib alone (p=0.0363) (Natoni *et al.*, 2017). In a model with bortezomib chemoresistance, GMI-1271 disrupted the chemoresistance and significantly restored and enhanced bortezomib activity (p=0.0123). Another study using a mouse xenograft model of MM demonstrated that GMI-1271 and lenalidomide delayed tumor growth compared to vehicle by 40% and 35%, respectively; however, the combination of GMI-1271 and lenalidomide more significantly delayed tumor growth than vehicle by 64% (p=0.04) (Muz *et al.*, 2017).

In a murine retroviral transduction/transplantation model of chronic myelogenous leukemia (CML), mice treated with imatinib plus GMI-1271 had prolonged survival compared to vehicle-treated animals; approximately 20% of mice treated with GMI-1271 alone or in combination with imatinib exhibited long-term low-burden disease despite discontinuation of treatment 28 days post-transplant (Aggoune *et al.*, 2014).

Administration of GMI-1271 at 20 mg/kg twice daily for 5 days to mice after rounds of chemotherapy enhanced neutrophil recovery and protected mice from weight loss and mucositis (Winkler *et al.*, 2013). GMI-1271 administration effectively blocked secondary migration of inflammatory $F4/80^+$ $Ly\text{-}6C^+$ macrophages to intestines of mice after chemotherapy or irradiation.

Clinical Studies of GMI-1271

GMI-1271 is currently under evaluation in a phase 1 trial of MM and a phase 1/2 trial of AML. A list of ongoing and completed studies on ClinicalTrials.gov is provided in Table 1 below.

Table 1: GMI-1271 Clinical Studies on ClinicalTrials.gov

Study NCT	Phase	Agents	Disease/ Indication	Study Start-End	Status/Sponsor	Accrual Target
NCT02168595	1	GMI-1271	Healthy Adult Subjects	6/14 – 4/15	Completed/ GlycoMimetics, Inc.	28
NCT02271113	1	GMI-1271	Healthy Volunteers	10/14 – 3/16	Completed/ GlycoMimetics, Inc.	32
NCT02703051	1	GMI-1271; Filgrastim	Healthy Volunteers	7/15 – 8/16	Completed/ GlycoMimetics, Inc.	54

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Study NCT	**Phase**	**Agents**	**Disease/ Indication**	**Study Start-End**	**Status/Sponsor**	**Accrual Target**
NCT02811822	1	GMI-1271; Bortezomib; Carfilzomib	Multiple Myeloma	6/16 – 7/18	Recruiting/ GlycoMimetics, Inc.	48
NCT02744833	1/2	GMI-1271; Enoxaparin Sodium	Deep Venous Thrombosis	7/16 – 11/16	Completed/ GlycoMimetics, Inc.	16
NCT02306291	1/2	GMI-1271; Mitoxantrone, Etoposide; Cytarabine; Idarubicin	AML	3/15 – 12/18	Active, not recruiting/ GlycoMimetics, Inc.	91

Clinical Pharmacokinetics/Pharmacodynamics

GMI-1271 administered intravenously (IV) at single doses up to 20 mg/kg exhibited a favorable pharmacokinetic (PK) profile in two first-in-human (FIH) phase 1 studies (Devata *et al.*, 2015). Plasma levels, maximum plasma concentration (C_{max}), and area under the plasma concentration-time curve (AUC) increased in a dose-related manner. Clearance (CL), apparent volume of distribution at the terminal phase (Vz), and half-life ($t_{1/2}$) were not dose-dependent, with $t_{1/2}$ averaging approximately 2.3 hours. Approximately 66% of the GMI-1271 dose was excreted unchanged in the urine independent of dose level, and renal clearance (CLr) averaged 86 mL/min, less than estimated creatinine clearance (CrCL), suggesting tubular reabsorption is one component of CLr.

After administration of GMI-1271 at doses from 2 to 10 mg/kg in the initial FIH study, no dose-response increase in peripheral $CD34^+$ counts was observed, indicating no mobilization of HSCs (Devata *et al.*, 2015). Plasma concentrations of soluble E-selectin were relatively constant in cohorts receiving GMI-1271 at 2 mg/kg and placebo; however, a significant reduction (p=0.012) of soluble E-selectin compared to baseline was observed 48 hours after the 2 mg/kg dose of GMI-1271. In the 5 and 10 mg/kg GMI-1271 cohorts, soluble E-selectin decreased significantly post-dose (p<0.0001) and returned to baseline by 24 to 48 hours, respectively. In the second FIH study, significant reduction in soluble E-selectin was also seen after GMI-1271 treatment versus baseline (p=0.05), and in soluble P-selectin versus baseline (p=0.04) and Day 2 (p<0.01). Soluble intercellular adhesion molecule 1 (ICAM1) also decreased after GMI-1271 treatment versus baseline (p=0.05), indicating decreased leukocyte adhesion.

Pharmaceutical Information

GMI-1271 is being developed as an IV dosage formulation. The drug product, GMI-1271 Injection, is available as a sterile solution supplied in single-use vials at a concentration of 50 mg/mL. GMI-1271 Injection should be stored either frozen (-10°C to -25°C) or refrigerated (2°C to 8°C) prior to administration.

CTEP's Plans for GMI-1271 Preclinical Development

Clinical studies of interest to CTEP

Clinical studies will be requested in a separate clinical PTMA and will include Pediatric patients with AML, Down's Syndrome and AML, as well as adults/AYA with MDS or RLPS/Refractory AML. These studies will focus on GMI-1271 and 7 and 3 chemotherapies in addition to GMI-1271 combination therapy with novel agents.

Correlative Studies of Interest to CTEP

- Preclinical studies designed to analyze biomarkers on AML blasts from peripheral blood and from aspirates of bone marrow (when available) from all patients including Adults with MDS, AYA and adults with relapsed or refractory AML including post SCT and Pediatric AML patients (newly diagnosed, relapsed and refractory patients). Study of pediatric patient specimens from pediatric biorepositories or collections to evaluate similarities and differences of predictive and prognostic biomarkers related to response to GMI-1271for use in a PIP to apply for Pediatric Exclusivity.
    - E-selectin expression, amplification or mutation.
    - Also, of interest, would be pediatric patients with AML and Down's Syndrome (Trisomy 21).
    - Biomarkers for very poor outcome phenotypes to be explored include HECA-452, E-selectin ligand, CD56 and other markers including those for glycosylation. Any correlations with response, clinical outcome and survival should be evaluated. Similarity to adult AML expression patterns for E-selectin ligand will be reported.
    - Study of very poor prognosis phenotype AML in collaboration with Hematologic in Seattle to evaluate post-treatment those patients with poor response rates.
    - Finally, interest in genotyping/phenotyping of patients with very poor risk AML particularly those with very poor outcomes such as those with changes in glycosylation.

Pre-clinical Studies of Interest to CTEP

- Further preclinical evaluation of GMI-1271 in context dependent models in combination with standard of care treatment backbone chemotherapies or other investigational agents if indicated using appropriate models of acute myelogenous leukemia (pediatric and adult) and MDS context dependent AML/MDS models.
- Suggested combinations that may be of interest to study in models of MDS include but are not limited to GMI-1271 with HDACi, de- and hypomethylating agents or other novel agents,
- Suggested combinations of GMI-1271 including, but not limited to quizartinib, venetoclax, decitabine, other Flt3 inhibitors and IDH1/2 inhibitors and E-selectin mutated or amplified, as well as mylotarg combinations.
- Study of GMI-1271 in models of relapsed refractory AML/MDS including AYA patients and patients less than 65 years old (non-elderly populations).

GMI-1271 Project Team Selection, Composition, and Tasks

The GMI-1271 drug project team will meet regularly by WebEx to review preclinical study proposals for the project team. The project team will be composed of intramural and extramural members. The extramural members will include translational scientists with expertise in biomarker development and basic scientists with expertise in the inhibition of selectins in hematological malignancies.

Questions regarding this request for applications may be addressed to S. Percy Ivy, M.D., Medical Officer, Investigational Drug Branch, CTEP, DCTD, NCI (phone: 240-276-6565; FAX: 240-276-7894; e-mail: ivyp@ctep.nci.nih.gov).

CTEP recognizes the importance of encouraging and supporting young investigators as they embark upon a clinical cancer research career. CTEP highly encourages Career Development Applications (CrDAs) from these investigators and their mentors to participate as Preclinical Project Team members and to develop Career Development Letters of Intent (CrDLs) after conclusion of the Preclinical Project Team activities.

Project Team Member Applications (PTMAs) should contain a clear indication of the applicant's desired role on the GMI-1271 Project Team (translational scientist or basic scientist). The PTMA should also be accompanied by an NIH Biosketch containing a personal statement customized to this project. The PTMAs

should be sent to the Protocol and Information Office (PIO) at the address below by **5:00 PM Eastern Time (ET), October 26, 2018**. The most recent version of the PTMA form, which has been distributed with this communication, must be used. PTMAs should be submitted electronically to:

PIO, CTEP/DCTD/NCI
E-mail: CTEPPTMASubmissions@mail.nih.gov

Basic and **Translational Scientists** who belong to a participating ETCTN institution (Lead Academic Organization [LAO] or Affiliated Organization [AO]) **must** submit applications through your LAO's Coordinating Center. Please allow sufficient time for your organization's review. Basic and Translational Scientists from non-ETCTN-affiliated institutions may submit their applications directly to PIO.

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