A Single Arm, Open Label, Phase 1 Study of Novel BET inhibitor PLX51107 and Corticosteroids for Treatment-Refractory Acute GVHD

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Synopsis

Primary objectives	To evaluate the safety and tolerability of PLX51107 and corticosteroids for allogeneic transplant recipients with treatment-refractory acute GVHD To assess the PK and PD of orally administered PLX51107 in treatment-refractory acute GVHD patients
Secondary objective	To evaluate the preliminary efficacy of PLX51107 and corticosteroids in treatment-refractory acute GVHD patients
Study design	This is a Phase 1, open-label, single-center, dose-finding study to evaluate the safety, tolerability, and biologically effective and tolerable dose (BETD) of PLX51107 in combination with corticosteroids in treatment-refractory acute GVHD patients. We will determine the dose of PLX51107 that both shows biological activity (BA) and is tolerable when used in combination with corticosteroids. BA is assessed by pharmacokinetics (PK) for target exposure of $AUC_{0\text{-}24}$ 8300 ng•hr/mL at Day 1 based on preclinical in vivo pharmacological efficacy studies (PLX51107 IB). All diseases, transplant and donor types are included. PLX51107 will be administered orally daily as tolerated. All patients will concurrently receive prednisone 1mg/kg orally daily (may be divided into 2 doses) or MP IV equivalent. Corticosteroids may be tapered per institutional guidelines, but taper may not be initiated sooner than 7 days after Study Day 1 and to no less than prednisone 0.25mg/kg daily before Study Day 28. We will further

assess biological activity as compared across clinical responses at different dose levels to determine the recommended Phase 2 dose (RP2D) for additional evaluation.

The observation period for DLT and BA will be the first cycle of 28 days. A dose level will be deemed the BETD if 5 or more out of 6 patients demonstrate a BA with 0-1 DLTs. This dose will be declared biologically effective and tolerable. The starting dose level is at Dose Level 1 (40 mg/day). Refer to Table 1 for Dose Levels.

This study design combines a standard rule-based Phase 1 3+3 trial design that simultaneously evaluates tolerability (DLTs) and evidence of biological activity at each dose level. Three patients are entered at each dose level and expanded to six patients if sufficient tolerability and BA is observed. See Figure 5 for a study schematic. If 2 or more patients out of 6 at a given dose level experiences DLT, accrual at that dose level will stop, and the dose level will be de-escalated. If at the lowest dose level, accrual will be suspended and the study terminated.

After BETD is determined, we will review the tolerability, biological activity, and clinical responses observed to justify a RP2D.

Table 1. Dose levels

Cohort	Dose level (mg/day)
0	20
1	40
2	80
3	120

DLTs are defined as AEs or laboratory abnormalities occurring during the first cycle (28 days) of study drug administration that are at least possibly related to PLX51107, deemed to be clearly unrelated to disease progression, concurrent illness or concomitant medication and that meet one of the following NCI CTCAE v5.0 criteria below.

- **Hematological toxicities**
  - Grade >3 neutropenia lasting >7 days
  - Grade ≥3 febrile neutropenia
  - Grade 4 thrombocytopenia lasting >14 days despite study drug discontinuation
- **Non-hematological toxicities**
  - New onset non-hematological clinical and laboratory toxicity grade ≥3 lasting >72 hours with the exception of the following events:

	▪ Grade ≥3 nausea, vomiting or diarrhea that resolves to grade ≤2 within 72 hours, with maximal medical intervention ▪ Grade 3 fatigue, asthenia, anorexia, fever or constipation that resolves to grade ≤2 within 14 days ▪ Grade ≥3 asymptomatic changes in alkaline phosphatase, hypomagnesemia, hyperglycemia or hypophosphatemia ▪ Grade 3 increases in transaminases confirmed upon repeat testing lasting ≤ 5 days ▪ Any Grade ≥3 electrolyte abnormality that is corrected to Grade ≤2 within 24 hours ▪ Non-hematologic laboratory Grade 3 AE that is asymptomatic and/or rapidly reversible (returned to baseline or to Grade ≤ 1 within 7 days) unless identified as clinically relevant by the Investigator; and (in non-diabetic patients) singular or non-fasting elevations in blood glucose • Any case of Hy's law defined as AST or ALT >3 x ULN with concurrent total bilirubin >2 x ULN, absence of cholestasis (elevated ALP >2 x ULN), and no alternative etiology, which can explain the combination of increased AST or ALT and total bilirubin, such as viral hepatitis A through E, other preexisting or acute liver disease, liver acute GVHD, or another drug capable of causing the observed injury • Any dose reduction required during Cycle 1 due to grade ≥3 adverse events • Any treatment delay of >7 consecutive days during Cycle 1 due to study drug-related Grade ≥3 adverse events that fails to resolve to baseline or grade ≤1 • Any Grade ≥3 gastrointestinal, genitourinary or central nervous system hemorrhage • Any grade 4 or 5 nonhematological adverse reaction • Any death unless unequivocally due to underlying disease (or extraneous cause) Any adverse reaction unrelated to active GVHD that leads to the study drug PLX51107 dose reduction or withdrawal should be considered a DLT. Grade 3 or higher adverse events occurring past 28-days must be recorded and reviewed for final analysis and for Phase 2 dose evaluation.
Eligibility	Patients with a diagnosis of treatment-refractory acute GVHD are eligible. Patients must be refractory to first-line steroid therapy and failed one or more salvage therapy regimens. Patients with a contraindication to the use of steroids are not deemed steroid-refractory and are ineligible. Patients

	who meet eligibility criteria, sign informed consent, and dose with PLX51107 are enrolled. **Inclusion criteria** 1. Age ≥ 18 years 2. Treatment-refractory acute GVHD as defined as patients who failed steroids (as defined as (a) progressed after 3 days of treatment with methylprednisolone (MP) 2 mg/kg/day equivalent, (b) did not improve after 7 days of treatment with MP 2 mg/kg/day equivalent, (c) progressed to a new organ after treatment with MP 1 mg/kg/day equivalent for skin and upper gastrointestinal (GI) GVHD, or (d) recurred during or after a steroid taper[2]) plus one or more salvage regimens with no limit to the number of prior lines of salvage therapies 3. Recipients of ablative and reduced-intensity conditioning regimens 4. Recipients of HLA-matched related and unrelated, 1-allele mismatched, haploidentical, or umbilical cord blood donor grafts 5. Prior lines of therapy for treatment of steroid-refractory acute GVHD are allowed. There is no minimum or maximum to the number of prior therapies. However, exposure to investigational therapies for the treatment of GVHD must be > 14 days or 5 half-lives (whichever is longer) of first administration of study drug. Prior cell therapy trial exposure must be >14 days where no known half-life applies. For patients treated with ruxolitinib for the treatment of acute GVHD, ruxolitinib must be discontinued by at least one day prior to initiation of PLX51107. 6. ECOG performance status 0-3. 7. Absolute neutrophil count ≥1.0 x 10^9/L for 3 consecutive days). Use of growth factor support is allowed. 8. Platelet count ≥50 x 10^9/L without transfusion support for 2 consecutive days. 9. Women of child-bearing potential must have a negative serum pregnancy test at Screening and must agree to use an effective form of contraception from the time of the negative pregnancy test up to 6 months after the last dose of study drug. Effective forms of contraception include abstinence, hormonal contraceptive in conjunction with a barrier method, or a double barrier method. Women of non-child-bearing potential may be included if they are either surgically sterile or have been postmenopausal for ≥1 year. 10. Fertile men must agree to use an effective method of birth control during the study and for up to 6 months after the last dose of study drug **Exclusion criteria** 1. Prior exposure to a bromodomain inhibitor 2. Evidence of active relapse of underlying disease 3. Exposure to other investigational or anti-cancer therapies (not for GVHD) within 28 days or 5 half-lives (whichever is shorter) of first administration of study drug

	4. Active, uncontrolled bacterial, fungal, or viral infection 5. Known or suspected allergy to the study drug 6. Clinically significant cardiac disease, defined as: A. Clinically significant cardiac arrhythmias, including bradyarrhythmia, and/or a need for anti-arrhythmic therapy (excluding beta blockers or digoxin). Individuals with controlled atrial fibrillation are not excluded. B. Fridericia-corrected QT interval (QTcF) ≥ 470 ms (male and female) at Screening. C. History of clinically significant cardiac disease or congestive heart failure greater than New York Heart Association Class II. Subjects must not have unstable angina (angina symptoms at rest) or experienced either new-onset angina within the last 3 months or myocardial infarction (MI) within the last 6 months unless it was due to the underlying disease and there has been appropriate revascularization. Individuals with ambiguous troponin levels that are not diagnostic of an MI should be discussed with the PI prior to enrollment. D. Arterial or venous thrombotic or embolic events such as cerebrovascular accident (including transient ischemic attacks), deep vein thrombosis, or pulmonary embolism within the 6 months before start of study medication (except for catheter-related venous thrombosis. 7. Inability to take oral medication or significant small bowel resection that, in the opinion of the Investigator, would preclude adequate absorption. 8. Active thrombotic microangiopathy (TMA) 9. Women who are either pregnant or breast feeding 10. Measured or calculated (Cockcroft-Gault formula) creatinine clearance (CrCl) <45 mL/min 11. Prothrombin time or international normalized ratio >1.5 × ULN 12. Activated partial thromboplastin time >1.5 × ULN 13. Requiring mechanical ventilation or vasopressor support 14. Subject is participating in any other therapeutic clinical study (observational or registry studies are allowed). 15. Contraindication to steroids. Steroids may be concurrently weaned per institutional practice.
Study endpoints	The primary endpoints include: • To determine the biologically effective and tolerable dose (BETD) • Safety endpoints (adverse events) • PK analysis The secondary endpoints include: • Preliminary efficacy (CR at Day 28) among all patients treated and among all patients treated at BETD • Overall response rate (ORR at Day 28) among all patients treated and among all patients treated at BETD

	• NRM at 6 months
Data analysis	To assess the safety and tolerability, adverse events by grade will be summarized. The occurrence of Grade 3+ adverse events according to CTCAE will be summarized as well. Adverse events will initially be reviewed regardless of attribution, but also according to whether adverse events are possibly, probably, or definitely related to treatment. Complete response (CR) at Day 28 among all patients treated and among all patients treated at BETD will be estimated. The proportion of CR with a 95% CI will be reported, assuming a binomial distribution. The overall response rate (ORR) will include CR and partial response (PR), while mixed response (MR) and no response (NR) will be classified as no response. The ORR will be similarly analyzed as CR. To determine 6-month non-relapse mortality (NRM), the event will be death due to reasons other than disease and the time will be measured from the date of starting PLX51107 to date of death with the competing risk as death due to disease (relapse). The cumulative incidence curve accounting for competing risks will be generated to estimate the cumulative incidence of NRM rate at 6 months. The comparison in NRM between patient subgroups may be explored graphically.

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Background & Significance

Acute GVHD is the most common cause of morbidity and mortality post-SCT, affecting 30-70% of transplant recipients.[3–5] However, despite a high incidence of acute GVHD post-SCT, treatments options are severely limited. The primary initial treatment of acute Graft Versus Host Disease (GVHD) is uniformly high dose steroids, from which only 24-40% of patients have durable responses. Those that do not respond by four weeks after the initiation of systemic therapy have higher non-relapse mortality (48% vs. 16%).[6] The majority (>90%) of those non-responsive to steroids die with an estimated overall survival at 2-years of only 10%.[7,8] Thus, treatment-refractory acute GVHD patients present a desperate need for new therapies. There is no standard therapy for steroid-refractory aGVHD due to limited response rates. Various treatment modalities have included cytokine signaling blockade via JAK/STAT inhibition, anti-IL-6[22], anti-TNF-α[6] monoclonal antibodies (mAbs), T-cell trafficking blockade[23], and immunomodulation with alpha-1 antitrypsin[24]. Recently FDA approved oral JAK1/2 inhibitor, ruxolitinib, reported an overall response rate (ORR) of 62% and a modest complete response (CR) of 34% by Day 28 with no aGVHD survival benefit after treatment in combination with steroids[25].The therapeutic potential of other more immunosuppressive agents such as anti-thymocyte globulin and anti-CD52 mAb (alemtuzumab) is hindered by an increased risk of lethal infections and increased risk of relapse, negating improvement in survival[26, 27]. Thus, there is a need for more effective and novel therapies with improved tolerability.

Bromodomain and extraterminal domain (BET) inhibitors have received high profile attention for their epigenetic antitumor activity seen across solid tumor and hematologic malignancies. BET proteins (including BRD2, BRD3, BRD4, and BRDT) modulate histone acetylation involved in the recruitment of positive transcription elongation factor complex (P-TEFb) and RNA Pol II to specific locations on chromatin to control target pro-tumor gene expression, such as MYC and BCL2, which are highly up-regulated in malignancies such as AML. Most recently, the Byrd and Lapalombella labs at OSU published their findings of PLX51107 *in vitro* and *in vivo* in CLL models in *Cancer Discovery.*[9] There is also an ongoing Phase 1b trial of PLX51107 in advanced malignancies, including AML and high-risk MDS (NCT02683395).

Previous *in vitro* and *in vivo* studies of BET inhibition of key inflammatory genes have demonstrated that BET inhibition with iBET or JQ1 decrease cytokine and chemokine expression of NF-kB dependent genes IL-6, IFNb, IL-1b, IL-12a, CXCL9 in murine macrophages[10] and decrease the differentiation of naïve T cells to Th17 cells without affecting Treg cell differentiation[11] thus exhibiting powerful anti-inflammatory effects. Sun et al. additionally demonstrated that BET inhibition with iBET-151 decreased cytokine secretion of activated dendritic cells and decreased activated T cell proliferation by disrupting the interaction of BRD4 and the acetylated component important to NF-kB activation, RelA.[12] NF-kB is an important transcriptional regulator of IL-2 expression, which is produced by activated T cells and is key to the development of a T cell alloantigen response, as seen in acute GVHD.[13] Thus, NF-kB signaling blockade in activated T cells reduces the pathologic hyper-responsiveness seen in acute GVHD. Given these anti-inflammatory properties via NF-kB suppression, Reddy et al. successfully tested iBET-151 in an *in vivo* acute GVHD mouse model, demonstrating for the first time that BET inhibition decreased GVHD severity and prolonged survival[12].

Unfortunately, the first generation BET inhibitors, including iBET, JQ1, and OTX015 have had disappointingly poor tolerability in human clinical trials due to hematologic dose-limiting toxicities and gastrointestinal adverse effects. However, unlike the first generation BET inhibitors that

share a benzodiazepine scaffold structure, PLX51107 is a novel, potent non-benzodiazepine structured small molecule BET inhibitor with a unique binding mode selective for BRD4 inhibition and a more tolerable side effect profile. Importantly, in its first published description of its structure and binding mode by the Byrd and Lapalombella labs at OSU, the conformation of BRD4 bound by PLX51107 was confirmed to resemble the acetylated Lys310 binding conformation of RelA[9] – pertinent to the hypothesis that PLX51107's inhibitory effects would similarly mimic that of iBET-151 by NF-kB modulation but with an improved pharmaceutical profile. Thus, we propose the evaluation of the novel small molecule inhibitor PLX51107 for its anti-inflammatory and activated T cell dampening properties in clinical trial for treatment-refractory acute GVHD patients that otherwise have no other therapeutic options.

Pre-clinical data of PLX51107 *in vitro* and *in vivo*

At American Society of Transplant and Cellular Therapy conference 2018, the Ranganathan lab at OSU presented the results from mirrored iBET-151 studies with PLX51107, analyzing the *in vitro* effects of activated dendritic cells and activated T-cell proliferation. In Figure 1, we show the analysis of supernatant for pro-inflammatory cytokine secretion of B6 T cells with PLX51107, demonstrated decreased IFN-g, IL-6, and TNF-a secretion. Figure 2 demonstrates the dose-response decrease in activated T cell proliferation with continuous *in vitro* culture with PLX51107. T cells were activated with both allogeneic bone marrow derived cell and microbead CD3/CD28 activation with similar results.

Figure 1.

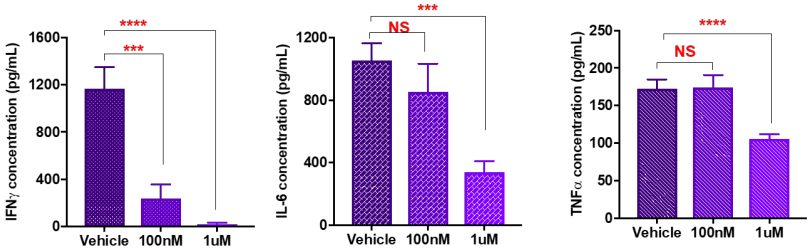
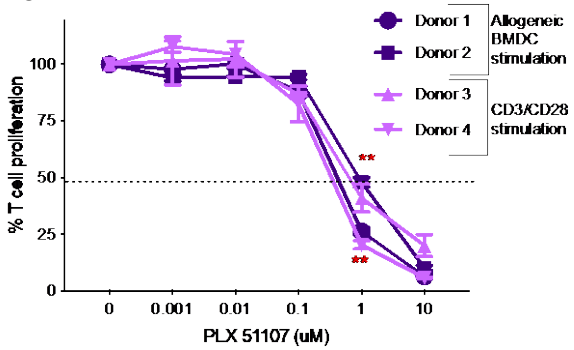


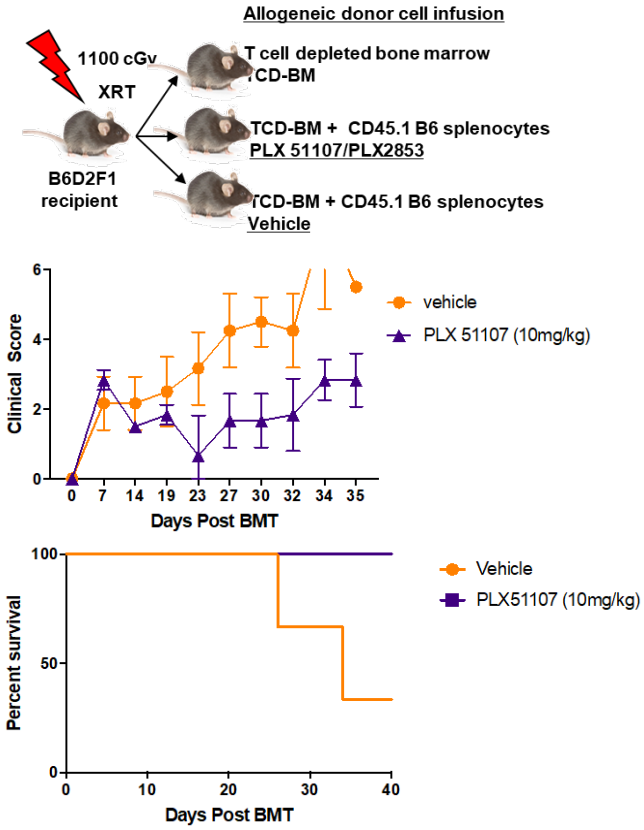
Figure 2.



Early pre-clinical murine acute GVHD model studies of PLX51107 are currently ongoing with promising results. Unpublished data from the Ranganathan lab comparing GVHD severity and survival in a GVHD murine model of PLX51107 10mg/kg oral three times weekly dosage are below in Figure 3. Lethally irradiated F1 recipient mice received T cell depleted bone marrow cells (TCD-BM, $10x10^6$) along with CD45.1 B6 splenocytes (15×10^6) transferred intravenously. Recipient mice were treated with PLX 51107 (10mg/kg) or vehicle by oral gavage three times

weekly starting at Day +7 post-transplant administered continuously until death or compassionate sacrifice. Studies evaluating outcomes at Day +1 treatment with dose-finding and tolerability are ongoing.

Figure 3.



The Mann-Whitney U-test was used for comparison of clinical scores between groups, and the log-rank test was used to compare survival. These results are based on the same formulation of PLX51107 as used in the MV4-11 AML xenograft study (EXP 14 AC8862 PLX51107 IB). In that study, continuous daily dosing of PLX51107 10mg/kg resulted in $AUC_{0\text{-}24}$ = 29300 ng•hr/mL. The mean $AUC_{0\text{-}24}$ for Figure 3 (three times weekly) is estimated to be ~12,000 ng•hr/mL, not significantly different from the target exposure ($AUC_{0\text{-}24}$ = 8300 ng•hr/mL). This pre-clinical data supports the use of PLX51107 for acute GVHD given the reduced activated T cell proliferation and decreased cytokine secretion seen in vitro as well as the reduced clinical GVHD severity and improved survival in the mouse.

PLX51107 was evaluated in a number preclinical efficacy models with PK analyses (PLX51107 IB). The Ba/F3 splenomegaly model, a murine bone marrow-derived pro-B cell line, is most relevant for GVHD as NF-κB plays a role in the survival of Ba/F3 cells. When injected into the tail veins of nude mice, Ba/F3 cells home to the spleen and proliferate, leading to marked splenomegaly. PLX51107 treatment resulted in dose-dependent reduction in splenomegaly. The exposure required to produce approximately 50% efficacy is $AUC_{0\text{-}24}$ 8300 ng•hr/mL. This value defines the target exposure required for PLX51107 to be pharmacologically effective in vivo

(PLX51107 IB) and defines biological activity in the clinical trial design.**Early phase human trials of PLX51107**
PLX51107 related adverse reaction that occurred in >15% of subjects based on study PLX122-01 (A Phase 1b dose escalation study to assess the safety, pharmacokinetics, pharmacodynamics, and preliminary efficacy of PLX51107 in subjects with relapsed or refractory solid tumors (Group A) and hematological malignancies (Group B)) was nausea, fatigue, vomiting, diarrhea, blood bilirubin increased and decreased appetite. The one and only subject enrolled in Group B developed fatal, suspected acute differentiation syndrome that was assessed as probably related to PLX51107. The subject had European LeukemiaNet (ELN) high risk AML with complex karyotype at relapse along with 17p deletion and TP53 mutation. The subject's WHO classification was therapy-related AML. Please refer to the PXL51107 IB for additional safety information.

In solid tumor patients, PLX51107 exhibited nearly dose-proportional increase in exposure from 20 mg to 160 mg (MTD). At steady state, the daily exposures (AUC_{0-24}) are 3820 and 9150 ng•hr/mL at 40 and 120 mg doses, respectively. Refer to Table 2 below from PLX51107 IB. The exposure at the 120 mg dose (the proposed top dose in this study – Dose Level 3) is expected to be very close to the target (AUC_{0-24} is 8300 ng•hr/mL). However, patients with treatment-refractory acute GVHD treated with PLX51107 may have a different safety profile compared with solid tumor patients. To ensure an adequate safety margin, a lower starting dose of PLX51107 at 40 mg/day is recommended in this study. The proposed starting dose of 40 mg/day is 4x lower than the MTD for solid tumor patients. At the proposed starting dose (40 mg) in solid tumor patients, PLX51107 demonstrated a minimal pharmacodynamic effect in modulating the expression of BET target genes in peripheral blood cells (Figure 4 below from PLX51107 IB).

Table 2: Summary of Pharmacokinetic Data to Date

Cohort (Dose)	N[a]	Geometric Mean								
		Day 1				Day 15				Accumulation Ratio
		T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-24} (ng•hr/mL)	$T_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-24} (ng•hr/mL)	$T_{1/2}$ (hr)	
Group A (solid tumors)										
1 (20 mg/day)	6/6	1.00	596	1070	0.985	0.794	660	1300	1.19	1.21
2 (40 mg/day)	3/3	2.29	721	3460	2.55	1.59	1070	3820	2.29	1.10
3 (60 mg/day)	4/4	1.19	1520	3460	1.49	1.19	1510	3810	1.63	1.10
4 (90 mg/day)	4/3	1.19	3170	7560	1.78	1.26	2330	6300	1.50	0.83
5 (120 mg/day)	11/9	1.41	3960	11300	1.71	1.61	2990	9150	2.10	0.81
6 (160 mg/day)	11/7	1.51	4950	16200	1.97	1.17	4760	11300	1.99	0.70
7 (120 mg/day)[b]	3/3	1.00	2980	6970[c]	1.63	1.44	2310	9220[d]	1.72	0.66
8 (240 mg/day)	3/3	1.82	7610	29100	2.69	1.71	4190	18900	4.53	0.65
9 (20 mg/day)	4/1	2.21	8470	38700	3.28	2.00	4600	32200	2.63	0.83
Group B (AML)										
1 (120 mg/day)	0/1	ND	ND	ND	ND	2.00	2990	11500	1.54	ND

AUC_{0-24} = area under the concentration-time curve from time 0 to 24 hours postdose;
$AUC_{0-\infty}$ = area under the concentration-time curve from time 0 extrapolated to infinite time; BID = twice daily;
C_{max} = maximum concentration; T_{max} = time of maximum observed concentration;
$T_{1/2}$ = terminal elimination half-life; ND = not determined

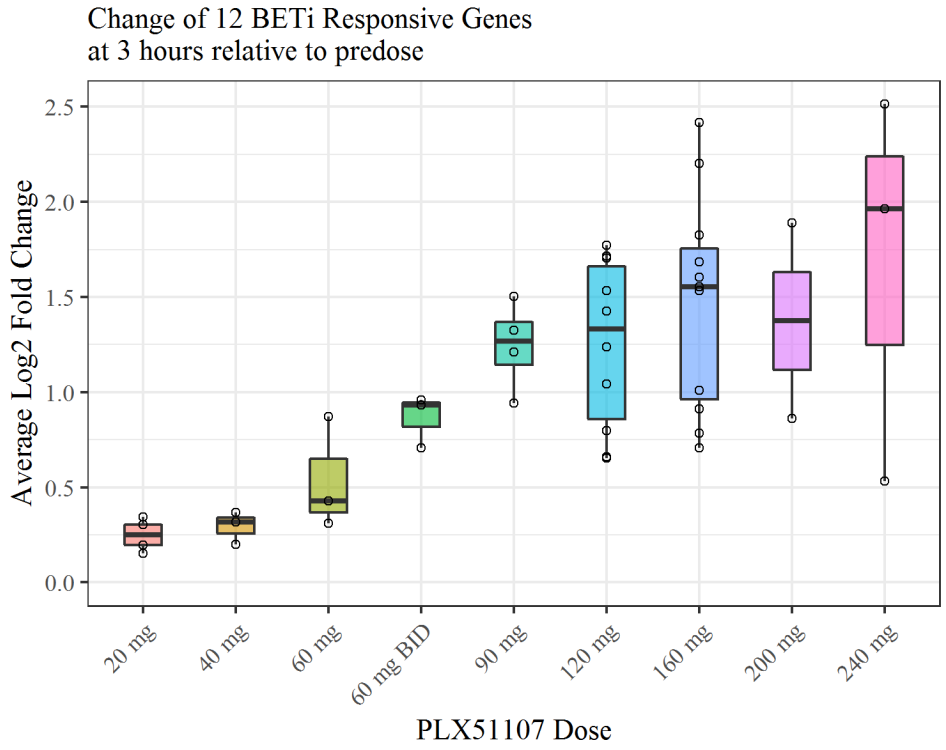
[a] N: number of patients with Day 1 PK/number of patients with Day 15 PK.

[b] 60 mg BID.

[c] AUC_{0-12}.

[d] AUC_{0-24} = 2 x AUC_T because of BID dosing schedule.

Figure 4: PLX51107 Pharmacodynamic Effect on Peripheral Blood Cells



Hypothesis: PLX51107 is safe and effective in combination with corticosteroids in allogeneic stem cell transplant recipients with treatment-refractory acute GVHD

Objectives

Primary objectives: To evaluate the safety and tolerability of PLX51107 and corticosteroids for allogeneic transplant recipients with treatment-refractory acute GVHD

To assess the PK and PD of orally administered PLX51107 in treatment-refractory acute GVHD patients

Secondary objective: To evaluate the preliminary efficacy of PLX51107 and corticosteroids in treatment-refractory acute GVHD patients

Eligibility

Patients with a diagnosis of treatment-refractory acute GVHD are eligible. Patients must be refractory to first-line steroid therapy and failed one or more salvage therapy regimens. Patients with a contraindication to the use of steroids are not deemed steroid-refractory and are ineligible. Patients who meet eligibility criteria, sign informed consent, and dose with PLX51107 are enrolled.

Subject Registration Procedures

For subsite patients, sites must send the signed consent form, documentation of the consent process, and the Screening Form (refer to Supplemental Forms Document) within 2 business days of initial consent.

Patients will be registered after meeting all entry requirements and signing of the informed consent document.

OSU patients will be registered by the OSU research coordinator, as per CTO standard practice.

Subsite patients will have eligibility verified and will be entered on study centrally at The Ohio State University by the Multi-Center Trial Program (MCTP). All subsites must email the MCTP to verify slot availabilities prior to consenting patients. Once a patient signs consent, the signed consent document and documentation of the consenting process must be faxed or securely emailed to the MCTP. The required forms, including Eligibility Criteria Checklist and Registration Form, can be found in the Supplemental Forms Document.

To register a subsite patient, the following documents must be completed by the subsite research team and faxed or securely e-mailed to the MCTP:

- Copy of all baseline tests required per the protocol calendar. Tests must be within the specified window.
- Signed Patient Consent Form, if not previously sent
- Signed Patient HIPAA Authorization Form (if separate), if not previously sent
- Consent Documentation Note, if not previously sent
- Completed & Signed Eligibility Checklist (refer to Supplemental Forms Document)
- Registration Form (refer to Supplemental Forms Document)
- Source documents verifying every inclusion & exclusion criteria

Upon receipt of registration documents, the MCTP will send an email confirming receipt. If confirmation of receipt is not received within 24 hours of submission, please contact the MCTP by phone and/or pager to confirm receipt.

Upon receipt of all required registration documents and upon verification the subsite patient meets all eligibility criteria, the MCTP will:

- Assign the patient a study sequence ID, if not already provided at time of consent
- Register the patient on the study
- Fax and/or e-mail the subsite the completed Registration Form with the assigned study sequence ID and registration date as confirmation of patient registration

Following registration, patients should begin protocol treatment within ***5*** business days. Issues that would cause treatment delays should be discussed with the Principal Investigator and MCTP as soon as possible. If a patient does not receive protocol therapy following registration, the PI and MCTP must be notified immediately within 1 business day.

Each participating institution will order study agents directly. Agents may be ordered by a participating site only after the required regulatory documents, including the initial IRB approval for the site, have been forwarded to the MCTP and all other study-specific requirements have been met (as outlined during site activation).

Inclusion criteria

1. Age ≥ 18 years
2. Treatment-refractory acute GVHD as defined as patients who failed steroids (as defined as (a) progressed after 3 days of treatment with methylprednisolone (MP) 2 mg/kg/day equivalent, (b) did not improve after 7 days of treatment with MP 2 mg/kg/day equivalent, (c) progressed to a new organ after treatment with MP 1 mg/kg/day equivalent for skin and upper gastrointestinal (GI) GVHD, or (d) recurred during or after a steroid taper[2]) plus one or more salvage regimens with no limit to the number of prior lines of salvage therapies
3. Recipients of ablative and reduced-intensity conditioning regimens
4. Recipients of HLA-matched related and unrelated, 1-allele mismatched, haploidentical, or umbilical cord blood donor grafts
5. Prior lines of therapy for treatment of steroid-refractory acute GVHD are allowed. There is no minimum or maximum to the number of prior therapies. However, exposure to investigational therapies for the treatment of GVHD must be > 14 days or 5 half-lives (whichever is longer) of first administration of study drug. Prior cell therapy trial exposure must be >14 days where no known half-life applies. For patients treated with ruxolitinib for the treatment of acute GVHD, ruxolitinib must be discontinued by at least one day prior to initiation of PLX51107.
6. ECOG performance status 0-3
7. Absolute neutrophil count ≥1.0 x 10^9/L for 3 consecutive days). Use of growth factor support is allowed.
8. Platelet count ≥50 x 10^9/L without transfusion support for 2 consecutive days.
9. Women of child-bearing potential must have a negative serum pregnancy test at Screening and must agree to use an effective form of contraception from the time of the negative pregnancy test up to 6 months after the last dose of study drug. Effective forms of contraception include abstinence, hormonal contraceptive in conjunction with a barrier method, or a double barrier method. Women of non-child-bearing potential may be included if they are either surgically sterile or have been postmenopausal for ≥1 year.
10. Fertile men must agree to use an effective method of birth control during the study and for up to 6 months after the last dose of study drug

Exclusion criteria

1. Prior exposure to a bromodomain inhibitor
2.
3. Evidence of active relapse of disease

4. Exposure to other investigational or anti-cancer therapies (not for GVHD) within 28 days or 5 half-lives (whichever is shorter) of first administration of study drug
5. Active, uncontrolled bacterial, fungal, or viral infection
6. Known or suspected allergy to the study drug
7. Clinically significant cardiac disease, defined as:
   A. Clinically significant cardiac arrhythmias, including bradyarrhythmia, and/or a need for anti-arrhythmic therapy (excluding beta blockers or digoxin). Individuals with controlled atrial fibrillation are not excluded.
   B. Fridericia-corrected QT interval (QTcF) ≥ 470 ms (male and female) at Screening.
   C. History of clinically significant cardiac disease or congestive heart failure greater than New York Heart Association Class II. Subjects must not have unstable angina (angina symptoms at rest) or experienced either new-onset angina within the last 3 months or myocardial infarction (MI) within the last 6 months unless it was due to the underlying disease and there has been appropriate revascularization. Individuals with ambiguous troponin levels that are not diagnostic of an MI should be discussed with the PI prior to enrollment.
   D. Arterial or venous thrombotic or embolic events such as cerebrovascular accident (including transient ischemic attacks), deep vein thrombosis, or pulmonary embolism within the 6 months before start of study medication (except for catheter-related venous thrombosis.
8. Inability to take oral medication or significant small bowel resection that, in the opinion of the Investigator, would preclude adequate absorption.
9. Active thrombotic microangiopathy (TMA)
10. Women who are either pregnant or breast feeding
11. Measured or calculated (Cockcroft-Gault formula) creatinine clearance (CrCl) <60 mL/min
12. Prothrombin time or international normalized ratio >1.5 × ULN
13. Activated partial thromboplastin time >1.5 × ULN
14. Requiring mechanical ventilation or vasopressor support
15. Subject is participating in any other therapeutic clinical study (observational or registry studies are allowed).
16. Contraindication to steroids

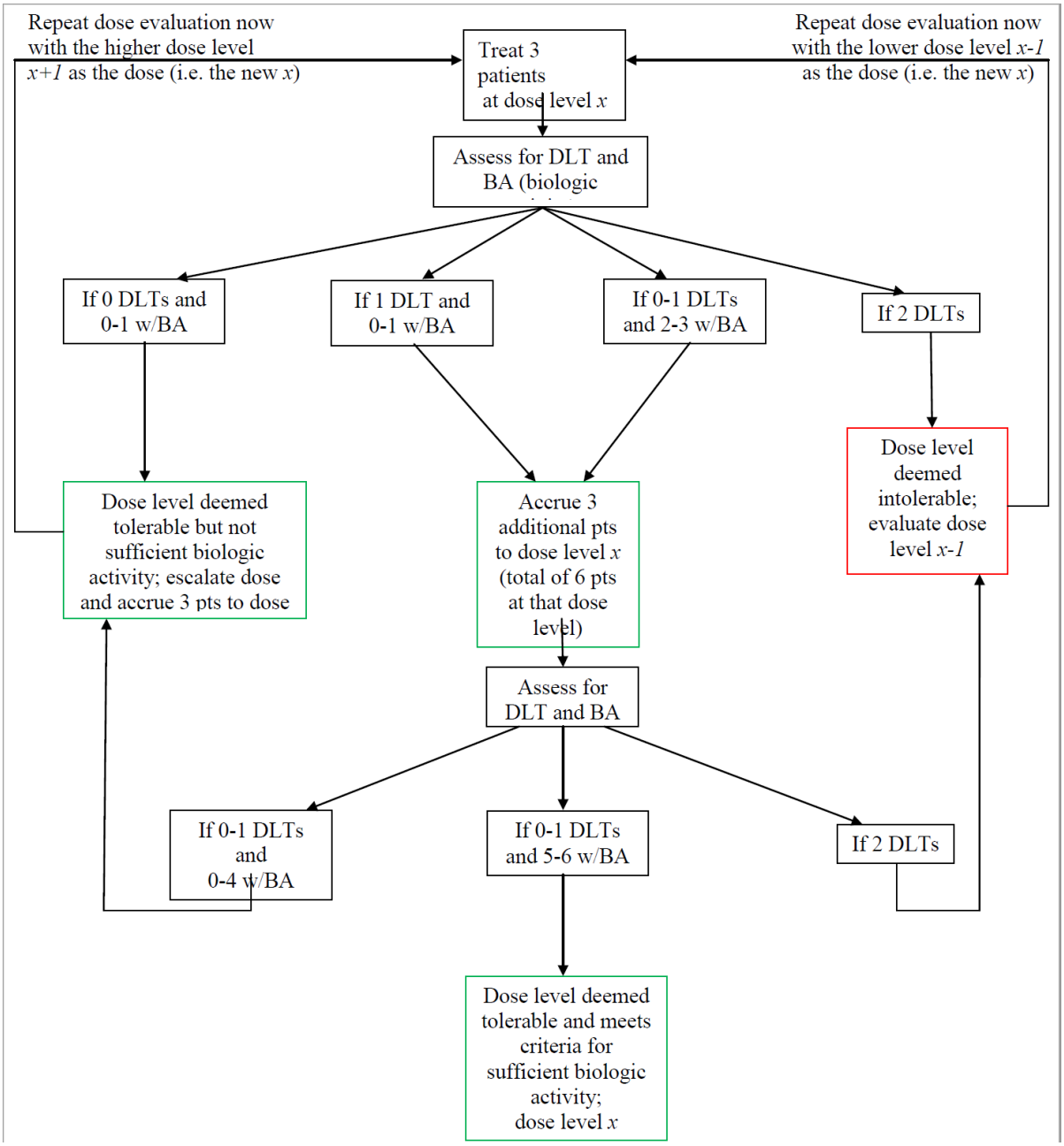
Steroids may be concurrently weaned per institutional practice.

Statistical Considerations
Study Design
This is a Phase 1, open-label, single-center, dose-finding study to evaluate the safety, tolerability, and biologically effective and tolerable dose (BETD) of PLX51107 in combination with corticosteroids in treatment-refractory acute GVHD patients. The dose cohorts are as outlined in Table 1. We will determine the dose of PLX51107 that both shows biological activity (BA) and is tolerable when used in combination with corticosteroids. BA is assessed by pharmacokinetics (PK) for target exposure of $AUC_{0\text{-}24}$ 8300 ng•hr/mL at Day 1 based on preclinical in vivo pharmacological efficacy studies (PLX51107 IB).

All diseases, transplant and donor types are included. PLX51107 will be administered orally daily as tolerated. All patients will concurrently receive prednisone 1mg/kg orally daily (may be divided into 2 doses) or MP IV equivalent. Corticosteroids may be tapered per institutional

guidelines, but taper may not be initiated sooner than 7 days after Study Day 1 and to no less than prednisone 0.25mg/kg daily before Study Day 28. We will further assess biological activity as compared across clinical responses at different dose levels to determine the recommended Phase 2 dose (RP2D) for additional evaluation.

Figure 5. Study schematic



The observation period for DLT and BA will be the first cycle of 28 days. A dose level will be deemed the BETD if 5 or more out of 6 patients demonstrate a BA with 0-1 DLTs. This dose will be declared biologically effective and tolerable. The starting dose level is at Dose Level 1 (40 mg/day). Refer to Table 1 for Dose Levels.

This study design combines a standard rule-based Phase 1 3+3 trial design that simultaneously evaluates tolerability (DLTs) and evidence of biological activity at each dose level. Three patients are entered at each dose level and expanded to six patients if sufficient tolerability and BA is observed. See Figure 1 for a study schematic. If 2 or more patients at a given dose level

experiences DLT, accrual at that dose level will stop, and the dose level will be de-escalated. If at the lowest dose level, accrual will be suspended and the study terminated.

After BETD is determined, we will review the tolerability, biological activity, and clinical responses observed to justify a RP2D.

Table 1. Dose levels

Cohort	Dose level (mg/day)
0	20
1 (initial)	40
2	80
3	120

Statistical analysis plan:
To assess the safety and tolerability, adverse events by grade will be summarized. The occurrence of Grade 3+ adverse events according to CTCAE will be summarized as well. Adverse events will initially be reviewed regardless of attribution, but also according to whether adverse events are possibly, probably, or definitely related to treatment. Complete response (CR) at Day 28 among all patients treated and among all patients treated at BETD will be estimated. The proportion of CR with a 95% CI will be reported, assuming a binomial distribution. The overall response rate (ORR) will include CR and partial response (PR), while mixed response (MR) and no response (NR) will be classified as no response. The ORR will be similarly analyzed as CR. To determine 6-month non-relapse mortality (NRM), the event will be death due to reasons other than disease and the time will be measured from the date of starting PLX51107 to date of death with the competing risk as death due to disease (relapse). The cumulative incidence curve accounting for competing risks will be generated to estimate the cumulative incidence of NRM rate at 6 months. The comparison in NRM between patient subgroups may be explored graphically.

Study endpoints
The primary endpoints include:

- To determine the biologically effective and tolerable dose (BETD)
- Safety endpoints (adverse events)
- PK analysis

The secondary endpoints include:

- Preliminary efficacy (CR at Day 28) among all patients treated and among all patients treated at BETD
- Overall response rate (ORR at Day 28) among all patients treated and among all patients treated at BETD
- NRM at 6 months

GVHD Response Definitions

Acute GVHD response will be in comparison to the acute GVHD staging upon Screening. The Mount Sinai Acute GVHD International Consortium (MAGIC) criteria[14] will be used to assess staging and grading. Response will be defined as below (as previously used in BMT-CTN 1802 clinical trial: https://web.emmes.com/study/bmt2/protocol/1802_protocol/1802_protocol.html)

Complete Response (CR) is defined as complete resolution of all signs and symptoms of acute GVHD equivalent to staging 0 in all organs.

Partial Response (PR) is defined as improvement in one or more organs involved at diagnosis/screening and without progression or new acute GVHD symptoms in any organ.

Mixed Response (MR) is defined as improvement in one or more organs involved at diagnosis/screening but with progression or new symptoms in any organ.

No Response (NR) is defined as no improvement and/or progression of acute GVHD symptoms in any organ.

Number of Subjects

The minimum number of patients accrued to the Phase 1 portion is 6 patients (3 patients with >=2 DLTs at Dose Levels 1 and 0) with maximum of 18 patients (6 patients each at Dose Levels 1-3 .

Duration

We anticipate a screen failure rate of approximately 40% and enrolling a maximum 18 patients. The expected time to screen 45 patients at our center would span approximately 2 years. Patients will require at least 6 months of follow-up (6 months after initiation of study drug or 30 days after discontinuation of study drug). Total duration of this trial is expected to take approximately 2.5 years.

Study Calendar

	Cycle 1					Cycle 2				†Cycle 3+	SAE/DLT	*6-mo F/U	EOT
	Screening	Day 1	Day 8	Day 15	Day 22 ±3	Day 1	Day 8 ±3	Day 15 ±3	Day 22 ±3	Day 1 ±3	±3 days	±7 days	±7 days
Informed Consent/Eligibility Review	X												
Concomitant Medication Review	X		X	X	X	X	X	X	X	X	X	X	X
Physical Exam with Vital Signs	X		X	X	X	X	X	X	X	X	X	X	X
ECOG Performance Status	X		X	X	X	X	X	X	X	X	X	X	X
PLX51107		Daily											
Adverse Event Evaluations		X	X	X	X	X	X	X	X	X	X	X	X
Complete Blood Count (CBC)	X		X	X	X	X	X	X	X	X	X	X	X
Comprehensive Metabolic Panel (CMP)	X		X	X	X	X	X	X	X	X	X	X	X
PT/aPTT/INR	X		X	X	X	X	X	X	X	X	X	X	X
Serum Pregnancy Test	X												
GVHD Staging	X		X	X	X	X	X	X	X	X	X	X	X
Relapse, Survival			X	X	X	X	X	X	X	X	X	X	X
Peripheral Blood for PK		X	X	X									
Peripheral Blood for PD		X	X								X		
‡Peripheral Blood for MAP		X	X	X	X	X							
Peripheral Blood for Immune Reconstitution		X	X	X	X	X				X	X	X	X

†Applies to C3D1, C4D1, C5D1, and C6D1

*6 month follow-up may overlap with EOT. In this case, these will be deemed EOT days.

‡MAP refers to MAGIC Algorithm Probability

Please note: CnD28=C(n+1)D1

Dose limiting toxicity (DLT)

DLTs are defined as AEs or laboratory abnormalities occurring during the first cycle (28 days) of study drug administration that are at least possibly related to PLX51107, deemed to be clearly unrelated to disease progression, concurrent illness or concomitant medication and that meet one of the following NCI CTCAE v5.0 criteria below.

- **Hematological toxicities**
  - Grade >3 neutropenia lasting >7 days
  - Grade ≥3 febrile neutropenia
  - Grade 4 thrombocytopenia lasting >14 days despite study drug discontinuation
- **Non-hematological toxicities**
  - New onset non-hematological clinical and laboratory toxicity grade ≥3 lasting >72 hours with the exception of the following events:
    - Grade ≥3 nausea, vomiting or diarrhea that resolves to grade ≤2 within 72 hours, with maximal medical intervention
    - Grade 3 fatigue, asthenia, anorexia, fever or constipation that resolves to grade ≤2 within 14 days
    - Grade ≥3 asymptomatic changes in alkaline phosphatase, hypomagnesemia, hyperglycemia or hypophosphatemia
    - Grade 3 increases in transaminases confirmed upon repeat testing lasting ≤ 5 days
    - Any Grade ≥3 electrolyte abnormality that is corrected to Grade ≤2 within 24 hours
    - Non-hematologic laboratory Grade 3 AE that is asymptomatic and/or rapidly reversible (returned to baseline or to Grade ≤ 1 within 7 days) unless identified as clinically relevant by the Investigator; and (in non-diabetic patients) singular or non-fasting elevations in blood glucose
  - Any case of Hy's law defined as AST or ALT >3 x ULN with concurrent total bilirubin >2 x ULN, absence of cholestasis (elevated ALP >2 x ULN), and no alternative etiology, which can explain the combination of increased AST or ALT and total bilirubin, such as viral hepatitis A through E, other preexisting or acute liver disease, liver acute GVHD, or another drug capable of causing the observed injury
  - Any dose reduction required during Cycle 1 due to grade ≥3 adverse events
  - Any treatment delay of >7 consecutive days during Cycle 1 due to study drug-related Grade ≥3 adverse events that fails to resolve to baseline or grade ≤1
  - Any Grade ≥3 gastrointestinal, genitourinary or central nervous system hemorrhage
  - Any grade 4 or 5 nonhematological adverse reaction
  - Any death unless unequivocally due to underlying disease (or extraneous cause)

Any adverse reaction unrelated to active GVHD that leads to the study drug PLX51107 dose reduction or withdrawal should be considered a DLT.

Grade 3 or higher adverse events occurring past 28-days must be recorded and reviewed for final analysis and for Phase 2 dose evaluation.

Hold Parameters

		Occurrence	Resume	Dose
Neutropenia	Grade 4 (ANC <500/mm3)	1st	Upon improvement to Grade ≤2 (ANC >1000/mm3). GCSF support may be used.	If improved within 7 days, continue same dose. If no improvement within 7 days, permanently discontinue drug.
		2nd	Upon improvement to Grade ≤2. GCSF support may be used.	If improved within 7 days, dose reduce by one level. If no improvement within 7 days, permanently discontinue drug.
		3rd	Permanently discontinue study drug.	
Thrombocytopenia	Grade 4 (platelet count <25,000/mm3)	1st	Upon improvement to Grade ≤2 (platelet count >50,000/mm3) within 14 days	If improved within 14 days, continue same dose. If no improvement within 7 days, permanently discontinue drug.
		2nd	Upon improvement to Grade ≤2 (platelet count >50,000/mm3) within 14 days	If improved within 14 days, dose reduce by one level. If no improvement within 7 days, permanently discontinue drug.
		3rd	Permanently discontinue study drug.	
Other non-hematologic	Grade 3 adverse events	1st	Hold until improved to Grade ≤1	If improved within 5 days, continue same dose. If >5 days, dose reduce by one level.
		2nd	Hold until improved to Grade ≤1.	If improved within 5 days, dose reduce by one

				level. If >5 days, permanently discontinue.
Other non-hematologic	Grade 4 adverse events	1st	Permanently discontinue study drug	

Treatment Plan

Investigational product

PLX51107 will be supplied by Plexxikon in 20 mg tablet formulations. Each participating site must have sufficient supply for one cycle (28 days) available prior to enrollment of each patient. Additional months' supplies will be ordered by the site accordingly.

All patients will receive prednisone 1-2 mg/kg orally daily (may be divided into 2 doses) or MP IV equivalent. Corticosteroids may be tapered per institutional guidelines but initiated no sooner than 7 days after Study Day 1 and to no less than prednisone 0.25mg/kg dailiy before Study Day 28. The minimum dose of prednisone at Study Day 1 is 1mg/kg (or MP IV equivalent).

Treatment Dosage and Administration

The first day of administration is deemed Study Day 1. The dosage administered is determined by dose level in Table 1 and in observance of DLT or SAE considerations.

Treatment will be administered orally daily. The first day of administration is Study Day 1. PLX51107 should be taken at approximately the same time of day. On Study Days 1, 8, and 15 when PK/PD sample collections are drawn, patients should fast 8 hours prior and 1 hour after daily dose administration. On other days when, there are no scheduled PK/PD sample collections, patients should fast 2 hours prior and 1 hour after daily dose administration.

Dosing Delays or Missed Doses

Doses delayed by more than 2 hours should be skipped. Doses obscured by vomiting should not be re-administered. If more than 7 doses of the first 28 are missed due to noncompliance, the patient will be considered inevaluable and study subject replaced. Patients with missed doses removed from evaluation will continue to be monitored for safety.

Duration of Therapy

Dosing is continued until

- 6 months from initiation of study drug. If dose is greater than 20 mg at 6 months, drug should be tapered over maximum one additional month for a maximum of 7 months.
- CR is reached with daily prednisone equivalent ≤ 10mg/day. If CR is reached within <6 months, study drug is then tapered by 20 mg over one month.
- toxicity
- relapse of underlying malignancy
- progression of GVHD
- lack of response as defined by No Response in any organ system by Study Day 28
- voluntary withdrawal from study

Duration of Follow-Up

All patients will be followed for up to 6 months from initiation of study drug or 30 days from drug discontinuation (end of treatment, aka EOT) whichever is longer except in the cases of voluntary withdrawal from study or death.

For subjects who withdraw consent, there must be clear documentation of whether the subject withdraws consents to treatment only (i.e. agrees to follow-up) or withdraws consent to treatment and follow-up.

Follow-up

Follow-up appointments will entail review of concomitant medications, physical exam with vital signs, assessment of ECOG performance status, adverse event evaluation, GVHD staging, and assessment for relapse/survival. Labs will also be collected for complete blood count (CBC), comprehensive metabolic panel (CMP), coagulation labs (PT, aPTT, INR). Peripheral blood for PK, PD, ELISA, and immune reconstitution will be collected as above in the Study Calendar and in the Correlative Studies section (below). Patients will be followed weekly for the first two cycles of study drug and monthly thereafter until study drug discontinuation. Additional evaluations will occur at time of SAE or DLT, scheduled 6-month follow-up from initiation of study drug, and end of treatment (30 days from drug discontinuation). All follow-up will be done in-person with the exception of Cycle 2 Days 8, 15, and 22, which may optionally be done via secure video, per the physician's discretion.

Concomitant medications

Of the 5 major CYP isoforms, 3A4 and 2C8 may be involved in Phase I metabolism of PLX51107, with possibly CYP2B6 and CYP2D6 playing a minor role. Until information regarding exposure-toxicity and exposure-response relationships are available with PLX51107, concomitant CYP3A4 and CYP2C8 inhibitors and/or inducers should be administered with caution in the event they alter the systemic exposure to PLX51107 (see Appendix for a list of strong CYP3A4/2C8 inhibitors and inducers). In general, strong inhibitors or inducers of CYP3A4 and CYP2C8 should be avoided unless absolutely necessary. These include anticonvulsants (phenytoin, carbamazepine, phenobarbital), mycin antimicrobials, azole antifungals and anti-retrovirals. Strong CYP3A4 and CYP2C8 inducers or inhibitors should be discontinued at least 14 days or 5 drug half-lives, whichever is longer, prior to the initiation of dosing with PLX51107, unless there is no alternative and the medication is absolutely necessary.

Should treatment with a strong CYP3A4 or strong CYP2C8 modulator be necessary, study drug should be discontinued during this time, and for at least 14 days or 5 drug half-lives afterwards, whichever is longer.

Concomitant medications are recommended as prophylaxis for nausea, vomiting, and infections, and are allowed for managing myelosuppression.

Initiation of QTc prolonging drugs should be performed with caution. QTc should be monitored closely with any new initiation or dose adjustment. Electrolytes should be monitored and repeated.

The concurrent use of systemic immunosuppressants commonly used for GVHD prophylaxis (calcineurin inhibition (tacrolimus, cyclosporine), mycophenolate, mofetil, and sirolimus) may enhance the immunosuppressant effects of PLX51107. Because the interactive effects of these drugs with PLX51107 are as yet unknown, proceed with caution and monitor drug

levels (tacrolimus cyclosporine, sirolimus) at minimum twice weekly and dose adjust as necessary to desired concentration

Steroids may be tapered based on starting steroid dose according to the below recommended schedule or per institutional guidelines:

% of Starting dose	Steroid Dose	Days	Number of days	% Decrease from previous dose
100%	1 mg/kg/day	Days 1-7	7	
75%	0.75 mg/kg/day	Days 8-14	7	25%
50%	0.5 mg/kg/day	Days 15-21	7	33%
25%	0.25 mg/kg/day	Days 22-28	7	50%
10%	0.2 mg/kg/day	Day ≥29 and onwards		20%

% of Starting dose	Steroid Dose	Days	Number of days	% Decrease from previous dose
100%	2 mg/kg/day	Days 1-7	7	
75%	1.5 mg/kg/day	Days 8-14	7	25%
50%	1 mg/kg/day	Days 15-21	7	33%
25%	0.5 mg/kg/day	Days 22-28	7	50%
13%	0.25 mg/kg/day	Days 28-35	7	50%
10%	0.2 mg/kg/day	Day ≥36 and onwards		20%

Preparation, Reconstitution, and Dispensation

PLX51107 is an anticancer drug and, as with other potential toxic compounds, caution should be exercised when handling PLX51107.

Study Drug Packaging and Labeling

PLX51107 tablets are manufactured, packaged, and labeled according to current Good Manufacturing Practice (cGMP) at the following address:

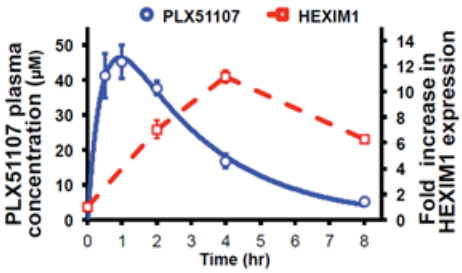
BioDuro LLC (Formerly known as Formex LLC)
11011 Torreyana Road, #100
San Diego, CA 92121

Storage and Handling
PLX51107 tablets should be stored at room temperature 20°C to 25°C (68°F–77°F); excursions permitted between 15°C and 30°C (59°F and 86°F). Subjects will be requested to store the study drug at the recommended storage conditions noted on the label.

The study drug provided in accordance with this protocol will be kept in a secure place and will only be supplied to subjects participating in this study. The Principal Investigator is accountable for all study drug in accordance with this protocol. In addition, the Principal Investigator must keep accurate and up-to-date dispensation records. Any study drug accidently or deliberately destroyed must be recorded in a timely fashion, including an explanation for the destruction in writing. Any discrepancies between the amounts of study drug dispensed and returned must also be explained in writing.

All unused and partially used study drug must be sealed and returned to the Sponsor or designee, or destroyed on site in accordance with the established procedures for drug destruction. Details of destruction, including, but not limited to, the number of boxes destroyed, batch number, and the date and method of destruction must be recorded on the study drug destruction logs.

Correlative Studies
Plasma will be collected for PK on Cycle 1 Days 1, 8, and 15 and assessed pre-dose and at hours 1, 3, 5, and 7 post-dose on Days 1 and 8 and pre-dose and at 3 hours post-dose on Day 15. PD for markers of BRD4 target engagement will also be collected on Days 1 and 8 (pre-dose and 3 hours post-dose). Notably, despite the short half-life of PLX51107, pre-clinical studies have shown that BRD4 target engagement persists despite drug metabolism (Figure 4).[9] A separate PK and PD for GI GVHD patients will be sub-analyzed given possible malabsorption complications of this oral drug. Additional samples for PK and PD (with pre-dose and 3 hours post-dose times) may be collected if a subject experiences a DLT, serious adverse event (SAE), AE of special interest, dose modification, or at the PI's request.



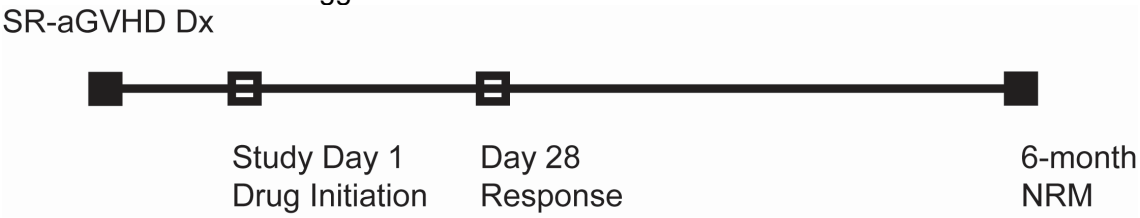
Whole blood samples for immune reconstitution analyses by flow cytometry and epigenetic markers by mass cytometry will be collected on all patients from start of treatment and then weekly thereafter for 4 weeks total and monthly for up to 6 months after initiation and at time of an SAE, DLT, and end of treatment.

Serum samples for GVHD biomarkers for Ann Arbor risk stratification (ST2 and REG3α ELISA) will be collected at time of study enrollment and weekly for four weeks after treatment initiation[48]. Ann Arbor scores will be correlated with response to therapy and NRM[48].

Study procedure and methods

Screening

Patients who meet eligibility criteria and consent will begin treatment (Study Day 1) with PLX51107 per the designated dose level. Those who are ineligible per criteria are deemed screen failures and logged as such.



Follow-up

Due to the studied disease characteristics, patients are anticipated to be hospitalized initially, during which time close assessments may be made as standard of care. However, at a minimum, patients must be assessed weekly for GVHD or toxicity throughout study drug treatment.

Withdrawal

A patient may request at anytime and for any reason to be removed from the study, to have his or her data no longer be collected, to have his or her data removed from any data collection databases, and to stop further sample collection or drug administration. This request can be made through any member of the clinical team to the PI or study team or made directly to the study team members, including PI, co-Is, and CRCs. Based on the nature of the request, study drug administration, sample and data collection may cease, and/or samples and data may be destroyed, per the patient's wishes.

Differentiation Syndrome

PLX122-01 was a Phase 1b dose-escalation study which assessed the safety, pharmacokinetics, pharmacodynamics, and preliminary efficacy of PLX51107 as single agent in subjects with advanced solid tumors (Group A) and hematological malignancies (Group B). Group B (hematologic malignancies) enrollment was initiated after the MTD for group A had been determined and with a starting dose of 120 mg/d, one dose level below the group A MTD. The first and only patient enrolled in Group B (120 mg/d) (with relapsed AML) developed suspected acute differentiation syndrome (DS) requiring hospitalization after being treated with PLX51107 120 mg/day for 22 days during cycle 1. The 73yo patient deteriorated quickly and expired after being made DNR/DNI. The Sponsor initially made a decision to halt further enrollment until additional safety measures to inform, monitor, and treat potential cases of differentiation syndrome were implemented in a protocol amendment. The FDA agreed and placed the study on clinical hold pending submission of amended documents. Plexxikon later elected not to pursue further enrollment in Group B of PLX122-01, and has terminated the study. In subjects with relapsed or refractory AML or high risk MDS being treated with PLX51107, FDA requires implementation of appropriate risk management measures for potential complications such as differentiation syndrome and tumor lysis syndrome.

In an acute GVHD patient population with no concern or evidence of active disease or suspicion of relapse, there is no known basis for the development of differentiation syndrome. Subjects with active disease/relapse are excluded from enrolling (exclusion criterion 3), and all enrolled subjects undergo a weekly physical examination with vital sign assessment and laboratory monitoring. In the event that an enrolled patient develops relapse of disease, PLX51107 must be discontinued immediately and the patient withdrawn from study.

Adverse Events and Adverse Event Management

Safety and tolerability will be monitored and determined by serial physical examinations, vital signs, hematology and chemistry laboratory studies, and reported AEs (including deaths and other SAEs and treatment-emergent AEs). The Investigator will monitor the laboratory test findings. If any laboratory test is abnormal during the course of the study, it will be followed at the discretion of the Investigator. Abnormalities of laboratory tests are evaluated by the Investigator and assessed as either clinically significant or not clinically significant. Abnormal laboratory values deemed by the Investigator to be clinically significant and, thus, constitute or are associated with an AE, must be reported on the AE form. Abnormal laboratory values that require intervention must be reported on the AE form whether or not deemed clinically significant.

Additionally, for this study, events as previously identified as resulting from administration of a stem cell transplant and/or related to the underlying disease will be considered as expected. The transplant regimen is well known to commonly affect multiple systems such as the hematologic, immunologic and gastrointestinal systems. The following are known complications of transplant: disseminated intravascular coagulation, febrile neutropenia, hemolysis, thrombotic thrombocytopenic purpura, adrenal insufficiency, colitis, constipation, diarrhea, enterocolitis, gastrointestinal pain, malabsorption, mucositis, nausea, pancreatitis, vomiting, dyspepsia, fever, pain, cholecystitis, allergic reaction, serum sickness, upper respiratory infection, urinary tract infection, weight gain/loss, acidosis, alkalosis, anorexia, dehydration, glucose intolerance, iron overload, generalized muscle weakness, reversible posterior leukoencephalopathy syndrome, bladder spasms, cystitis, hematuria, urinary frequency, urinary urgency, irregular menstruation, pruritis, hypertension, purpura, and petechiae.

An AE is any untoward medical occurrence in a subject administered a pharmaceutical product, which does not necessarily have a causal relationship with the treatment. An AE can be any unfavorable and unintended sign (e.g., including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the study drug, whether or not it is considered to be study drug related. This includes any newly occurring event or previous condition that has increased in severity or frequency since the administration of study drug. An AE includes, but is not limited to, the following:

- Any clinically significant worsening of a preexisting condition except for events clearly consistent with progression of disease under study
- An AE occurring from overdose (i.e., a dose higher than that indicated in the protocol) of a study drug, whether accidental or intentional
- An AE occurring from abuse (e.g., use for nonclinical reasons) of a study drug
- An AE that has been associated with the discontinuation of the use of a study drug

Any treatment-emergent abnormal laboratory result which is clinically significant, i.e., meeting 1 or more of the following conditions, should be recorded as an AE**:**

- Accompanied by clinical symptoms
- Leading to a change in study medication (e.g., dose modification, interruption or permanent discontinuation)
- Requiring a change in concomitant therapy (e.g., addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment)

AEs will be graded in severity according to CTCAE v5.0 criteria.

The Investigator will review each event and assess its relationship to study events to determine whether the event is unrelated or, unlikely, possibly, probably or definitely related to the study therapy. In the event that any of these known complications of transplant are thought to be at least possibly attributable to vitamin A supplementation, the event will be recorded and subsequently reported to the IRB and the PI according to current reporting requirements. All serious and medically significant adverse events considered related to PLX51107 by the Investigator will be followed until resolved or considered stable.

Medical conditions/diseases present before starting study drug are only considered adverse events if they worsen after starting study drug. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy as determined by the PI.

Serious Adverse Event Definition
An SAE is any AE occurring at any dose and regardless of causality that:

- Results in death.
- Is life-threatening. Life-threatening means that the subject was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires in-subject hospitalization longer than 24 hours or prolongation of existing hospitalization (see clarification in the paragraph below on planned hospitalizations). An emergency room visit without hospitalization is not considered a hospitalization.
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly/birth defect.
- Is an important medical event. An important medical event is an event that may not result in death, be life-threatening, or require hospitalization but may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed in the definitions for SAEs. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in in-subject hospitalization, or the development of drug dependency or drug abuse.

Clarification should be made between the terms "serious" and "severe" because they ARE NOT synonymous. The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as a severe headache). This is NOT the same as "serious," which is based on subject/event outcome or action criteria described above and are usually associated with events that pose a threat to a subject's life or functioning. A severe AE does not necessarily need to be considered serious. For example, persistent nausea of several hours' duration may be considered severe nausea but not an SAE. On the other hand, a stroke resulting in only a minor degree of disability may be considered mild but would be defined as an SAE based on the above noted criteria. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

Plexxikon will be notified of all SAEs (initial and follow-up) regardless of causality and expectedness within 24 hours of awareness by emailing Plexxikon.Safety@premier-research.com.

Subsite/External Participating Site SAE Reporting Requirements

NOTE: External participating sites are not permitted to report directly to the OSU IRB or FDA. All external site SAEs are to be reported to the OSU Principal Investigator and Multi-Center Trial Program (MCTP). The MCTP will facilitate submission of external site SAEs to the OSU IRB and FDA.

All serious adverse events (SAEs) and other adverse events must be recorded on case report forms. In addition, all SAEs must be reported to the OSU Principal Investigator and MCTP within 24 hours of knowledge of the event using the FDA MedWatch 3500A mandatory reporting form. External participating sites must also submit the "SAE Submission Form" cover sheet (refer to the Supplemental Forms Document).

Copies of de-identified source documentation pertaining to the SAE must be submitted to OSU. If a patient is permanently withdrawn from the study because of a SAE, this information must be included in the initial or follow-up SAE report form.

All SAEs must be submitted to the local IRB per local IRB and institutional policy.

Upon request of additional data or information that is deemed necessary must be reported to OSU as soon as possible but no later than 5 calendar days.

Sample Handling/Processing/Shipping Protocols
Biologic samples will be collected in the James Cancer Center inpatient or outpatient units and facilitated by the Clinical Trials Processing Lab (CTPL).

Central laboratory kits will be provided for sample collection, shipment and storage for PK analyses. Samples will be shipped to a central laboratory for forwarding to an analysis laboratory, which has been contracted by the Sponsor to process these samples. Refer to the associated laboratory manual for further information regarding sample handling for PK samples.

Samples for PD, including BRD4 target engagement and RNA-sequencing, will be collected in EDTA purple top tubes (1 x 4 ml), gently mixed, and stored at RT. Samples for mass cytometry and immunophenotyping will be collected in ACD yellow top tubes (2 x 8 ml), gently mixed, and

stored at RT. All PD, mass cytometry, immunophenotyping, and biomarker samples will be transferred same -day to the Choe laboratory in the Biomedical Research Tower at The Ohio State University. Samples for immunophenotyping may then be transferred to the laboratory of Gerard Lozanski (Dept of Pathology) for analysis by the flow cytometry immune reconstitution panel.

Data and Safety Monitoring Plan

The data and safety monitoring plan will involve the continuous evaluation of safety, data quality and data timeliness. Monitoring and auditing procedures will be followed to ensure that the study is conducted, documented, and reported in accordance with the IRB approved protocol, all applicable federal regulations and guidelines, and applicable regulatory requirements of The Ohio State University Wexner Medical Center. Serious adverse events and responses will also be reviewed by the OSUCCC Data and Safety Monitoring Committee (DSMC). The PI will also submit a progress report (quarterly for Phase I) that will be reviewed by the committee per the DSMC plan. All reportable Serious Adverse Events (SAE) will be reported to the IRB of record as per the policies of the IRB. Verification of eligibility will be performed and appropriate documentation of informed consent will be documented for all subjects enrolled into the study. The timeliness of Adverse Event and Serious Adverse Event reporting will be monitored to ensure regulatory compliance.

The PI of the trial will review toxicities and responses of the trial where applicable and determine if the risk/benefit ratio of the trial changes. The PI will conduct continuous review of data and patient safety at a regular protocol review meeting at least monthly. Frequency and severity of adverse events will be reviewed by the PI and compared to what is known about the agent/device from other sources; including published literature, scientific meetings and discussions with sponsors or collaborators, to determine if the trial should be terminated before completion.

Mandatory safety and trial review teleconferences will be scheduled and moderated by the Multi-Center Trial Program (MCTP). All sites involved in the study are expected to have a representative present for every call to review and discuss patients on study and other applicable agenda items. Meeting minutes will be used to document each teleconference. The minutes will be stored in the MCTP protocol files. Teleconferences must minimally be held monthly and may be held more frequently, as needed. For studies closed to accrual with patients expected to remain on long-term treatment and/or follow-up, teleconferences may be extended to occur every two months or quarterly. Decreasing frequency of teleconferences requires OSU PI and MCTP approval.

Data Submission

The study will be managed per the Multi-Center Trial Program (MCTP) policies. Subsite data must be submitted to the MCTP as outlined in the protocol-specific monitoring plan. The protocol-specific monitoring plan will be provided by the MCTP to external participating sites prior to site activation. Data will be submitted using case report forms and the Data Submission Form cover sheet (refer to Supplemental Forms Document) supplied by the MCTP. Access to the OSU OnCore database may be provided to external participating sites for direct electronic data entry.

All data submitted must be accompanied by supporting source documents, where applicable and as outlined in the protocol-specific monitoring plan.

Informed consent
Study team members will consult with the patient to explain the procedures, risks, and benefits of the study at the patient's level of understanding. Opportunity will be given to consider the study and have questions answered. Information will be given in a written format in the form a description of the study, which will include a signature space for consent to be given. Prior to the initiation of the study, acknowledgement of the receipt of this information and the subject's freely tendered offer to participate will be obtained in writing from each subject in the study. Participation is voluntary, and all subjects will give informed consent to participate. Information will also be given verbally, and all patients will have an opportunity to ask questions.

Non-English speaking patients are not excluded from this study. When possible, a short form or fully translated consent form will be provided.

Patient confidentiality
Data collected is treated in strictest confidence. No information provided from individual patient's records may be discussed with anyone other than those individuals designated study staff or clinical care providers. In order to protect patient confidentiality, patients are identified by patient ID and case ID numbers, which are generated for each patient and stored separately. Clinical information is accessed, according to HIPAA requirements, by study personnel to complete study documents. All records generated are stored in a locked office area, only accessible by the PI and specified designees only. Study records are maintained in a secure database with access restricted to only PI and specified designees. Personalized data will only be viewed by the PI and specified designees and the IRB if needed. The key matching codes to patient identification numbers is kept on password-protected computers in limited-access network folders by the PI and study research staff or in locked office cabinets if paper. All results from the analysis will be stored on password protected computers in laboratories and offices which are locked when not occupied. Identifying information will not be used in any publication of results.

Institutional Review Board
This study will be submitted to the OSU Cancer IRB.

Auditing

As the study sponsor, The Ohio State University Comprehensive Cancer Center (OSUCCC) will audit each site as per OSU policies. Audits will be performed by the OSUCCC Clinical Research Audit Team. For sites with an auditing mechanism in place that are able to share documentation of their auditing standards and processes followed, an agreement may be requested for the site to perform local auditing and provide formal audit reports to the OSUCCC Multi-Center Trial Program (MCTP) and the Quality Assurance Oversight Committee.

Potential risks for study participants
There is a potential risk of breach of confidentiality, patient identity, and privacy during the study. Study personnel will employ standard measures to protect patient privacy and the confidentiality of all patient information. Study subjects will be assigned a unique identification number not related to PHI, and the code will be securely maintained by the PI or designee. All data collected will be stored on a secure, password-protected computer, and any paper copies or reports will be stored in a secure location. No research findings will be reported to individual patients or treating physicians. No identifiable data will be reported. Clinical study lab results may be shared with subjects.

Potential benefits of study participation
By study participation, patients are eligible to receive a novel therapy. PLX51107 may offer a therapeutic option for treatment-refractory acute GVHD, thereby improving non-relapse mortality.

Costs and payments
There are no direct costs to patients for participation in the study. Subjects or their insurance companies will be charged for hospitalizations including tests and treatments of any side effects. In the event that subjects' insurance does not cover these costs, subjects may incur additional costs because of treatment side effects.

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APPENDIX 1: CYP3A4/CYP2C8 INHIBITORS AND/OR INDUCERS

Strong CYP3A4 and/or CYP2C8 inhibitors and/or inducers and CYP2D6 substrates with a narrow therapeutic index

CYP3A4 and/or CYP2C8 Strong Inhibitors

- Protease inhibitors
  - Ritonavir
  - Indinavir
  - Nelfinavir
- Macrolide antibiotics
  - Erythromycin
  - Telithromycin
  - Clarithromycin
- Azole antifungals
  - Voriconazole
  - Ketoconazole
  - Itraconazole
- Chloramphenicol (antibiotic)
- Nefazodone (antidepressant)
- Bergamottin (constituent of grapefruit juice)
- Aprepitant (antiemetic)
- Verapamil (calcium channel blocker)
- Gemfibrozil (hypolipidemic)
- Thiazolidinedione (antidiabetic)
- Montelukast (Leukotriene receptor antagonist)
- Quercetin (anti-inflammatory)

CYP3A4 and/or CYP2C8 Strong Inducers

- Anticonvulsants, mood stabilizers
  - Phenytoin
  - Carbamazepine
  - Oxcarbazepine
- Non-nucleoside reverse transcriptase inhibitors
  - Efavirenz
  - Nevirapine
  - Etravirine
- Phenobarbital (barbiturate)
- Rifampicin (bactericidal)
- Modafinil (stimulant)
- Hyperforin (constituent of St John's Wort)
- Cyproterone (antiandrogen, progestin)

CYP2D6 Substrates

- Amiodarone
- Dosulepin
- Flecainide
- Sotalol
- Pimozide
- Procainamide
- Theophylline

This list is not comprehensive and subject to change and all medications should be reviewed prior to administering.