EVALUATION OF TARGETED BONE MARROW ARREST BY G1T28, A CDK4/6 INHIBITOR IN CLINICAL DEVELOPMENT TO REDUCE CHEMOTHERAPY-INDUCED MYELOSUPPRESSION

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BACKGROUND

- Chemotherapy-induced myelosuppression continues to represent the major dose-limiting toxicity of cytotoxic chemotherapy
  - manifested as neutropenia, lymphopenia, anemia, and/or thrombocytopenia
  - source of many important side effects of cancer treatment such as infection, sepsis, bleeding, and fatigue leading to the need for hospitalizations, growth factor support and transfusions (red blood cells or platelets)
  - clinical concerns raised by myelosuppression commonly lead to chemotherapy dose reductions and limit therapeutic dose-intensity
- G1T28 (formerly G1T28-1) is a highly potent, selective, and reversible CDK4/6 inhibitor being developed for intravenous (IV) administration to cancer patients to reduce chemotherapy-induced myelosuppression
- G1T28 acts by transiently producing a G1 cell cycle arrest of hematopoietic stem and progenitor cells (HSPCs) in the bone marrow
  - G1-arrested HSPC are more resistant to the DNA damaging effects of chemotherapy, thereby preserving bone marrow and immune system function
- In preclinical animal models, administration of G1T28 prior to myelosuppressive chemotherapy resulted in:
  - improved complete blood cell count (CBC) recovery
  - preservation of immune system and long-term bone marrow function
  - ability to tolerate more cumulative chemotherapy
- To rationally design a schedule for administering G1T28 prior to chemotherapy, it is critical to precisely understand the magnitude and duration of G1 cell cycle arrest of HSPCs at a given dose level of G1T28 to ensure that:
  1. the effect of G1T28 lasts long enough to avoid releasing HSPCs into the S (DNA synthesis) phase of the cell cycle in the presence of high concentrations of chemotherapy, which could potentially exacerbate myelosuppression
  2. the effect of G1T28 does not last too long such that prolonged G1 cell cycle arrest of HSPCs could potentially contribute to myelosuppression

OBJECTIVES

The objective of this study was to characterize the magnitude and duration of G1T28-induced G1 cell cycle arrest in human bone marrow HSPCs, in order to rationally design tolerable and active chemotherapy combination regimens with reduced myelosuppression and maximized anti-tumor activity.

METHODS

PHARMACOKINETIC/PHARMACODYNAMIC (PK/PD) MODEL DEVELOPMENT

- Preclinical and clinical data were used to evaluate dose response relationships for HSPC G1 cell cycle arrest and to construct a cross-species, allometrically-scaled PK/PD model (Representative data from dogs are shown in Figure 1)
- The multi-compartment model was constructed in two stages (Table 1)
  1. PK data were compiled (doses, routes of administration, observed concentrations, etc) and PK parameters were determined.
  2. PD data were layered onto the PK data set, various physiological compartments were created and specific assumptions were entered
- The multi-compartment model was built to simulate the effect of G1T28 on hematopoiesis, with cell proliferation originating at the hematopoietic stem cell and continuing through the various stages of differentiation and ultimately culminating in the release of a mature neutrophil into peripheral circulation (Figure 2)
- Model assumptions included transit time through the cell cycle, the number of cells in G1 versus S phase for each level of differentiation, and the life span of mature circulating cells

TABLE 1. LIST OF STUDIES USED TO BUILD THE PK/PD MODEL

Data Set	Species	Route	Endpoint
1 -2	Mouse	Oral/IP	PK
3	Rat	IV	PK
4-7	Dog	IV	PK
8	Human	Oral	PK
9-12	Mouse	Oral/IP	BM/EdU/PMNs
13	Rat	IV	PMNs
14-15	Dog	Oral/IV	BM/EdU/PMNs

BM, bone marrow; EdU, 5-ethynyl-2'-deoxyuridine; IP, intraperitoneal; IV, intravenous; PK, pharmacokinetic; PMNs, polymorphonuclear cells

EVALUATION OF BONE MARROW PROLIFERATION IN HUMANS FOLLOWING G1T28 ADMINISTRATION

- In the first-in-human Phase 1a safety, PK, and PD study of the CDK4/6 inhibitor G1T28, 12 subjects (Cohort 7) were enrolled to confirm the predicted biologically effective dose (BED) of G1T28 (G1T28-1-01; NCT02243150; Abstract #2527)
- PK/PD model simulations and human PK/PD data from the Phase 1a trial suggested the BED of G1T28 was 192 mg/m^2
- A single bone marrow aspirate was obtained from all subjects enrolled in the BED cohort (192 mg/m^2, n=12) to determine the effect of G1T28 on the cell cycle phases (i.e. G1 or S/G2/M) of various bone marrow progenitor lineages
- The cell surface markers used to identify the specific HSPC populations are shown in Table 2.
- HSPC proliferation was measured prior to dosing (n=5), 24 hours after G1T28 dosing (n=3), or 32 hours after G1T28 dosing (n=4)
- Two bone marrow samples (one at pre-dose and one at 32 hour post-dose) were heavily contaminated with peripheral blood, technically inadequate, and removed from the cell cycle analysis datasets

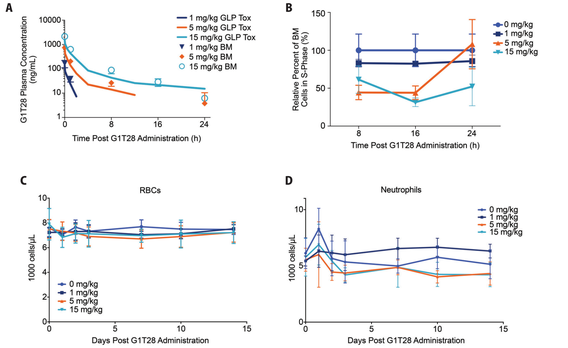
TABLE 2. HSPC FLOW CYTOMETRIC STRATEGY

Cell Population	Surface Marker Expression
Hematopoietic stem and multipotent progenitor cells (HSC and MPP)	CD45^{dim}/CD34$^+$/CD38$^-$
Oligopotent progenitors (OPPs)	CD45^{dim}/CD34$^+$/CD38$^+$
Monocyte progenitors	CD45$^+$/CD14$^+$/CD11b$^+$
Granulocyte progenitors	CD45$^+$/CD14$^-$/CD11b$^+$
Erythroid progenitors	CD45$^-$/CD71$^+$
Megakaryocyte progenitors	CD45$^+$/CD61$^+$

RESULTS

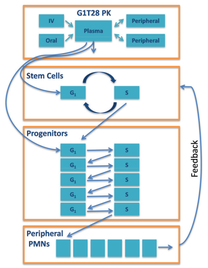
PK/PD MODEL

FIGURE 1. G1T28 INDUCES A DOSE DEPENDENT BONE MARROW ARREST IN DOG BONE MARROW



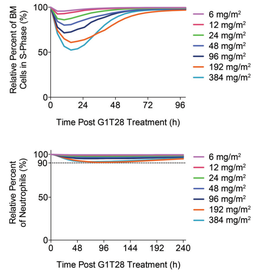
(A) PK time course of G1T28 in dogs from GLP toxicology study (solid lines, mean only) and spot PK verification from bone marrow EdU experiment (symbols). (B) EdU incorporation in dog whole bone marrow following a single dose of G1T28 at 0, 1, 5 and 15 mg/kg. (C) Red blood cell (RBCs) and (D) neutrophil counts from dogs who received a single G1T28 dose of 0, 1, 5, or 15 mg/kg. Data shown are mean ± SD. GLP, Good Laboratory Practices; BM, bone marrow

FIGURE 2. PK/PD MODEL OF G1T28 INHIBITION OF BONE MARROW PROLIFERATION



Preclinical and clinical observations suggest there is a "persistence effect" where G1-arrest is maintained for some period of time beyond G1T28 exposure. This effect is included as a compartment in the model, but for simplicity is not depicted here.

FIGURE 3. SIMULATION OF TOTAL BONE MARROW ARREST AND CHANGE IN NEUTROPHIL COUNTS FOLLOWING A SINGLE DOSE OF G1T28 IN HUMANS

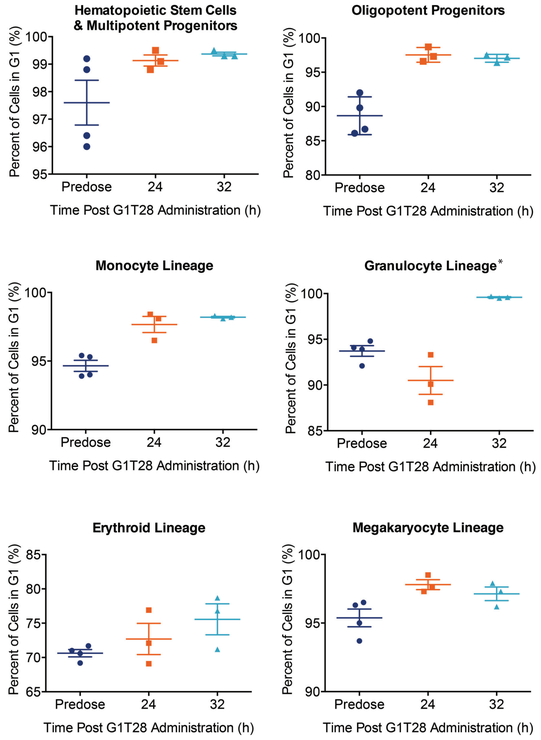


Simulations represent mean values from 500 individuals. BM, bone marrow

- G1T28 demonstrated 2-compartment linear PK, with rapid distribution and nearly linear scaling of clearance with weight
- The model predicted a dose of 192 mg/m^2 or greater would induce a 40-50% decrease in total bone marrow proliferation with return to baseline at approximately 48 hours (note that the simulation was for total bone marrow which includes CDK4/6-independent cell populations)
- The model predicted little change in peripheral neutrophil counts following a single dose up to 384 mg/m^2

EFFECT OF G1T28 ON HUMAN BONE MARROW PROLIFERATION

FIGURE 4. G1T28 CAUSES A G1 CELL CYCLE ARREST IN BONE MARROW HEMATOPOIETIC STEM AND PROGENITOR CELLS UP TO 32 HOURS POST DOSE



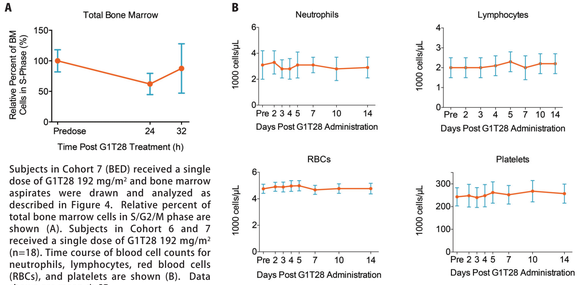
Subjects in Cohort 7 (BED) received a single dose of G1T28 192 mg/m^2 and bone marrow aspirates were drawn at various time points (predose [n=5], and 24 [n=3] or 32 [n=4] hours post G1T28 dose). One pre-dose bone marrow sample and one 32 hour post-dose sample were inadequate and were excluded. White blood cells were isolated using a Ficoll gradient and stained for specific bone marrow lineage markers (CD45, CD71, CD61, CD38, CD11b, CD14). Cells were then treated with Draq5 (DNA staining dye), and cell cycle analysis was completed using flow cytometry. Percent of cells in each phase of the cell cycle (G1 vs. S/G2/M) were calculated for specific lineage populations (also see Table 2). *Draq5 analysis may have been confounded in the granulocyte lineage due to an unexpectedly low percent of viable cells in some samples, particularly the 24 hour samples. Data shown are mean ± SD.

TABLE 3. PERCENT OF BONE MARROW LINEAGE POPULATIONS IN G1 OR S/G2/M PHASE OF THE CELL CYCLE FOLLOWING G1T28 ADMINISTRATION

	G1			S/G2/M		
	Predose	24 hours	32 hours	Predose	24 hours	32 hours
HSC and MPP	97.60	99.13	99.37	2.55	0.93	0.63
OPP	88.65	97.53	97.03	11.78	2.57	2.97
Granulocyte Lineage	93.73	90.50	99.60	6.85	10.37	1.80
Monocyte Lineage	94.65	97.67	98.20	5.58	2.73	1.53
Erythrocyte Lineage	70.63	72.70	75.57	29.28	27.30	24.53
Megakaryocyte Lineage	95.38	97.80	97.13	4.75	2.30	2.93

HSC, hematopoietic stem cells; MPP, multipotent progenitors; OPP, oligopotent progenitors

FIGURE 5. G1T28 INDUCED TRANSIENT BONE MARROW ARREST WITHOUT ALTERING PERIPHERAL BLOOD CELL COUNTS



Subjects in Cohort 7 (BED) received a single dose of G1T28 192 mg/m^2 and bone marrow aspirates were drawn and analyzed as described in Figure 4. Relative percent of total bone marrow cells in S/G2/M phase are shown (A). Subjects in Cohort 6 and 7 received a single dose of G1T28 192 mg/m^2 (n=18). Time course of blood cell counts for neutrophils, lymphocytes, red blood cells (RBCs), and platelets are shown (B). Data shown are mean ± SD.

- G1T28 induced a G1 cell cycle arrest in most lineages assayed at 24 hours, with some populations maintaining the arrest while others were further arrested at 32 hours
- Concomitantly, the percentage of cells in the S/G2/M cell cycle phase was decreased
- Analysis of total bone marrow demonstrated an approximate 40% arrest at 24 hours, with partial recovery at 32 hours, which is consistent with both the dog total bone marrow data and the simulations from the PK/PD model
- Despite robust inhibition of HSPC proliferation, a single dose of G1T28 did not alter peripheral blood cell counts

SUMMARY

- Following a single IV infusion of 192 mg/m^2 G1T28, a clear increase was observed in the percentage of bone marrow progenitor subsets in the G1 cell cycle phase up to 32 hours post dose
- No changes were noted in the peripheral blood counts, indicating that the bone marrow arrest is transient, reversible, and consistent with the effects seen in animals and PK/PD model simulations
- Based on the observed PK, PD and safety profile, G1T28 200 mg/m^2 IV (rounded up from the BED of 192 mg/m^2) was selected as the starting dose for further development in patients with CDK4/6-independent cancers
- Two Phase 1b/2a studies in SCLC will be initiated in Q3 2015 to evaluate the potential of G1T28 to protect the bone marrow/immune system, preserve cell function, and enhance cancer treatment outcomes
  - 1st line with G1T28 administration prior to etoposide/carboplatin
  - 2nd/3rd line with G1T28 administration prior to topotecan

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