G1T38, A NOVEL, ORAL, POTENT AND SELECTIVE CDK 4/6 INHIBITOR FOR THE TREATMENT OF RB COMPETENT TUMORS

JESSICA A. SORRENTINO, JOHN E. BISI, PATRICK J. ROBERTS, JAY C. STRUM
G1 THERAPEUTICS, INC., RESEARCH TRIANGLE PARK, NC

BACKGROUND

- The development of therapeutically effective inhibitors of the cyclin dependent kinase (CDK) family has been challenging due to a poor understanding of target and structural biology leading to the development of drugs that are cytotoxic with limited efficacy.
- Recently, the first highly selective CDK4/6 inhibitor, palbociclib, has been approved for the treatment of ER+/HER2- breast cancer in combination with the aromatase inhibitor, letrozole, and the selective estrogen receptor (ER) degrader, fulvestrant.
  - While highly efficacious, daily treatment has been shown to induce severe neutropenia resulting in at least a 7-day treatment holiday in each 28-day cycle to allow for recovery of neutrophil counts.
  - The treatment holiday may allow for renewed tumor growth and the potential for emergence of drug resistance.
- CDK4/6-induced neutropenia is the result of on-target inhibition of hematopoietic stem and progenitor cell proliferation resulting in a narrow therapeutic window between tumor efficacy and neutropenia.

OBJECTIVE

To develop a compound which is highly efficacious against CDK4/6-dependent tumors while minimizing the undesirable on-target activity of myelosuppression thus obviating the need for a treatment holiday.

Here, we describe the development of G1T38, a novel, potent and selective CDK4/6 inhibitor, that demonstrates unique pharmacokinetic and pharmacodynamic properties when compared to palbociclib.

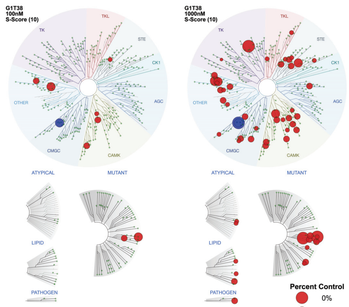
RESULTS

FIGURE 1. G1T38 IS A POTENT AND SELECTIVE INHIBITOR OF CDK4/6

	CDK4/ CyclinD1	CDK6/ CyclinD3	CDK9/ CyclinT	CDK5/ p35	CDK5/ p25	CDK2/ CyclinA	CDK1/ CyclinB1	CDK7/ CyclinH/Mat1	CDK2/ CyclinE
Mean (μM)	0.001	0.002	0.028	0.832	1.2	1.5	2.4	2.4	3.6

Biochemical profiling of G1T38 against various CDKs and their binding partners (Nanosyn, Santa Clara, CA). The assays were completed using microfluidic kinase detection technology (Caliper Assay Platform). The compounds were tested in 12-point dose response format in singlicate at Km for ATP. Results are displayed as micromolar concentrations for IC_{50} curves against putative target.

FIGURE 2. HIGH SELECTIVITY OF G1T38 IN DISCOVERX KINOMESCAN PANEL



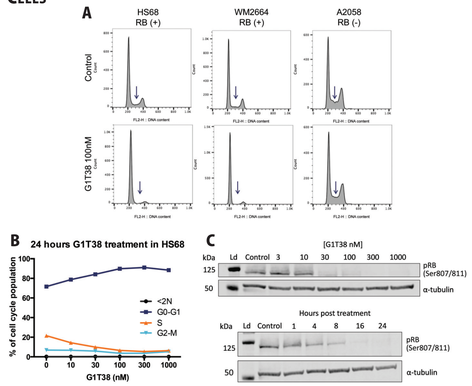
Assessment of G1T38 at 100x and 1000x the biochemical IC_{50} across 468 kinases in the DiscoveRx KINOMEscan kinase panel.

Target	Kd (nM)
CDK4/CyclinD1	0.15
CDK4/CyclinD3	0.05
FLT3 (D835V)	6.5
FLT3 (ITD, D835V)	22
NEK10	0.18
PRKD2	10
PRKD3	5
SLK	11
SNARK	0.69
TTK	4.2
ULK2	5.5

KdELECT (DiscoveRx) was used as a follow-up to quantify binding affinity of G1T38-kinase interactions identified in the primary screen. Inhibitor binding constants (Kd values) were calculated from duplicate 11-point dose-response curves for targets that elicited >90% inhibition at 100nM.

RESULTS

FIGURE 3. G1T38 INDUCES PRECISE G1 ARREST IN RB COMPETENT CELLS



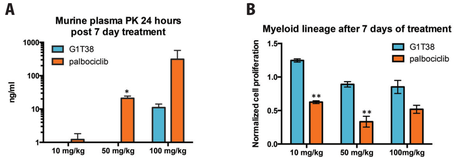
(A) 24 hour treatment with G1T38 causes a loss of S-phase (indicated by arrow) in CDK4/6-dependent cell lines (HS68 and WM2664), but not in the CDK4/6-independent cell line (A2058) as measured by propidium iodide staining. **(B)** HS68 cells show a precise G1 arrest with corresponding percent decrease in the number of cells in S-phase. **(C)** WM2664 cells treated with 30 nM of G1T38 for 24 hours exhibited an 80% decrease in RB phosphorylation and complete inhibition of RB phosphorylation with 300 nM G1T38 treatment compared to vehicle controls (top). After treatment with 300 nM G1T38 for the indicated time period, WM2664 cells had reduced RB phosphorylation within 1 hour post-treatment and generated near complete inhibition of RB phosphorylation by 16 hours post-treatment. α-tubulin was used as the loading control.

FIGURE 4. G1T38 INHIBITS CELL PROLIFERATION ONLY IN RB COMPETENT CELL LINES

Cell Line	Tumor Type	Rb Status	G1T38 EC_{50} (nM)
VCaP	Prostate	+	12
MV4-11	B cell leukemia	+	23
22RV1	Prostate	+	29
LNCaP	Prostate	+	32
PC3	Prostate	+	40
MCF7	ER+ breast	+	52
SupT1	Lymphoma	+	57
ZR75-1	ER+ breast	+	61
CCRF-CEM	Leukemia	+	183
TT	Carcinoma	+	222
Tom1	Ph1+ leukemia	+	232
BV173	Ph1+ leukemia	+	296
Daudi	Lymphoma	+	784
A2058	Melanoma	-	2691
H69	SCLC	-	2915
NALM1	Leukemia	-	>10000

Both CDK4/6-dependent and CDK4/6-independent cell lines were treated with 9 different concentrations of G1T38 (ranging from 1 nM to 10 μM) for 96 or 144 hours using CellTiterGlo. In most CDK4/6-dependent cell lines, G1T38 induced a robust proliferative arrest at a concentration <300nM. Treatment with G1T38 had minimal efficacy in CDK4/6-independent tumor lines.

FIGURE 5. G1T38 IS CLEARED MORE RAPIDLY FROM MURINE PLASMA THAN PALBOCICLIB LEADING TO REDUCED MYELOSUPPRESSION IN BONE MARROW

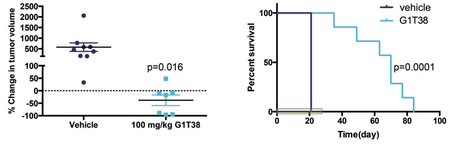


C

	Mouse ($T_{1/2}$) hrs	Rat ($T_{1/2}$) hrs	Dog ($T_{1/2}$) hrs	Human ($T_{1/2}$) hrs
G1T38	2.4-5.1	3.1	10.5-14.7	18#
Palbociclib	2.5-4.2	2.3-4.9	20.7	29

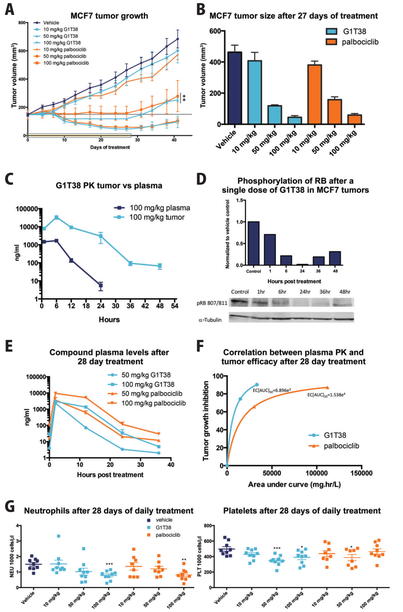
C57Bl/6 mice were treated for 7 days with daily oral doses of 10, 50, or 100 mg/kg G1T38 or palbociclib (n=3) (ILS, Research Triangle Park, NC). **(A)** 24 hours post treatment, peripheral blood was harvested and drug concentrations were measured. Palbociclib-treated mice had significantly more compound in the plasma when compared to mice treated with G1T38. **(B)** 12 hours post treatment, bone marrow was harvested and proliferation was measured. Bone marrow was stained for cellular markers to select for myeloid progenitors (Mac1+Gr1+) and EdU was measured within this population. G1T38-treated mice showed no differences in myeloid progenitor proliferation in any treatment cohort compared with vehicle, while palbociclib treatment led to more than a 50% reduction in proliferation in both the 50 and 100 mg/kg cohorts. All cohorts were normalized to vehicle control. **(C)** Comparison of palbociclib and G1T38 oral half lives in different species. Error bars represent SEM. *p≤0.05, **p≤0.01. #Based upon human PK simulation.

FIGURE 6. G1T38 CAUSES TUMOR REGRESSION AND INCREASES SURVIVAL IN HER2NEU BREAST CANCER GEMM



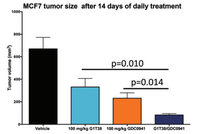
Female mouse mammary tumor virus (MMTV)-Neu GEMM mice spontaneously develop mammary tumors similar to human HER2+ luminal breast cancer. Mice were given 100 mg/kg of G1T38 in their diet for 28 days with tumor measurements at treatment initiation and every 3 to 4 days during treatment (MP1U, Chapel Hill, NC). **(A)** Mice treated with oral G1T38 (n=7) showed 38% tumor regression after 21 days of treatment while vehicle-treated controls (n=9) had a 577% increase in tumor volume relative to treatment initiation. **(B)** This resulted in improved survival of G1T38-treated animals relative to control. Additionally, G1T38 was well tolerated and did not cause significant weight loss after 28 days of G1T38 treatment (data not shown). Yellow bar represents duration of treatment. Error bars represent SEM.

FIGURE 7. WHEN ADJUSTED FOR EXPOSURE, G1T38 IS MORE EFFICACIOUS THAN PALBOCICLIB IN HUMAN ER+ BREAST CANCER MODEL



An ER+ human breast tumor xenograft model, MCF7, was implanted in estrogen-supplemented athymic nude mice (START, San Antonio, TX). Mice were given once daily doses of G1T38, palbociclib, or vehicle by oral gavage at 10, 50, or 100 mg/kg for 28 days. **(A)** G1T38 has similar (100 mg/kg and 10 mg/kg) or better (50 mg/kg) efficacy than palbociclib after 28 days of oral treatment. **(B)** MCF7 tumor size after 27 days of the indicated treatment. **(C)** G1T38 concentration in tumors is 17x higher than in plasma of C57Bl/6 mice after a single dose of oral 100 mg/kg of G1T38. **(D)** MCF7 tumors from mice treated with a single oral dose of vehicle or 100 mg/kg of G1T38 were harvested and RB phosphorylation was assessed. Phosphorylated RB decreased 98% 24 hours post-treatment, with rebound by 36 hours post-treatment. **(E)** Drug concentration levels of G1T38 and palbociclib after 28 days of treatment in C57Bl/6 murine plasma. **(F)** G1T38 is more than 2x efficacious in the MCF7 tumors than palbociclib. **(G)** G1T38 and palbociclib have similar neutrophil and platelet counts after 28 days of treatment. The differential effect on the bone marrow shown in Figure 5A and B suggests the slight difference in rodent pk/pd is not sufficient to further reduce circulating neutrophils in palbociclib treated mice. However, as shown in Figure 5C, the half-life of palbociclib is significantly longer in dogs and humans which results in the 2-4 fold accumulation of drug seen in both species. In contrast, G1T38 does not accumulate in mice, rats or dogs on repeat dosing and modeling predicts no drug accumulation in humans, suggesting continual dosing will produce less neutropenia in humans while maintaining tumor efficacy. Dotted line indicates size of tumor at start of treatment. Error bars represent SEM. Yellow bar represents duration of treatment. Statistics were completed using linear regression analysis of time during treatment (28 days). *p≤0.05, **p≤0.01, ***p≤0.001.

FIGURE 8. G1T38 COMBINATION TREATMENT WITH PI3K INHIBITOR, GDC0941, IS HIGHLY EFFICACIOUS



MCF7 tumor bearing mice were treated with daily oral doses of 100 mg/kg G1T38, 100 mg/kg GDC0941, or a combination of both for 14 days (START, San Antonio, TX). Combination treatment causes significantly more tumor growth inhibition than either compound given as a single agent. Error bars represent SEM.

FIGURE 9. G1T38 MEETS ALL TARGET CANDIDATE PROFILE CRITERIA

	Target candidate profile: criteria	G1T38
Potency	CDK 4/6 biochemical potency: IC_{50} < 10 nM	✓
	Phospho-RB in cells: IC_{50} < 50 nM	✓
	Tumor cell proliferation: EC_{50} < 500 nM	✓
Kinase selectivity	> 1,000-fold vs. CDK2 Highly selective vs. other kinase families	✓
DMPK	F%: >50% in rat and dog; appropriate $T_{1/2}$ for QD dosing	✓
	CYP IC_{50} (1A2, 2C9, 2C19, 2D6, 3A4): > 10 μM	✓
Safety	GLP hERG: IC_{50} > 3 μM; no QT prolongation in dog	✓
	GLP *in vitro* genotoxicity: clean in Ames and Chrom Ab	✓
	No significant off-target activity (non-kinase screen)	✓
	Reversible *in vivo* toxicities in GLP 28 day repeat-dose rat and dog oral studies; appropriate safety margins	✓
In vivo efficacy	Dose-dependent, on mechanism oral efficacy in multiple mouse tumor models	✓
Chemistry	Drug-like properties: soluble, rule-of-five compliant	✓
	CMC: ≤ 5 step synthesis, crystalline salt form	✓

SUMMARY

- G1T38 is a novel, potent and selective CDK4/6 inhibitor that induces a precise G1 cell cycle arrest in an RB-dependent manner in a variety of tumor types.
- G1T38 has improved *in vivo* efficacy and decreased inhibitory effect on myeloid progenitors than palbociclib.
- G1T38 causes tumor regression in a model of human ER+ breast cancer when given alone or in combination with a PI3K inhibitor.
- Preclinical studies show ER and AR antagonists are optimal combination therapies with G1T38. Phase I/II clinical studies in hormone receptor positive breast cancer will begin later this year. Also see Abstract #2820.

Contact email address: jcs@g1therapeutics.com