Published in final edited form as:

Pediatr Blood Cancer. 2012 October ; 59(4): 749–752. doi:10.1002/pbc.23364.

Initial Testing (Stage 1) of SGI-1776, a PIM1 Kinase Inhibitor, by the Pediatric Preclinical Testing Program

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Abstract

The PIM kinase inhibitor, SGI-1776, was tested against the PPTP *in vitro* (1.0 nM to 10 μ M) and *in vivo* panels (148 mg/kg daily x 5 days for 3 weeks). SGI-1776 exhibited cytotoxic activity *in vitro* with a median relative IC₅₀ of 3.1 μ M. SGI-1776 induced significant differences in EFS distribution *in vivo* in 9 of 31 solid tumor xenografts and in 1 of 8 of the evaluable ALL xenografts. SGI-1776 induced tumor growth inhibition meeting criteria for intermediate EFS T/C activity in 1 of 39 evaluable models. In contrast, SGI-1776 induced complete responses of subcutaneous MV4;11 (B myeloid leukemia).

Keywords

Preclinical Testing; Developmental Therapeutics; kinase inhibitors

INTRODUCTION

Pim (Provirus Integration site for Moloney murine leukemia virus) proteins are highly conserved serine/threonine kinases (PIM1, PIM2 and PIM3) that are constitutively activated in a variety of hematopoietic and solid malignancies. [1–3] They are thought to exert an oncogenic effect primarily via regulation of Myc family protein transcriptional activity. Evidence for this comes from PIM1 transgenic mice that have been shown to develop clonal

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CONFLICT OF INTEREST STATEMENT: The authors consider that there are no actual or perceived conflicts of interest.

T cell lymphomas when infected with murine leukemia virus via increased myc activation due to proviral insertion near myc genes [4,5], indicating cooperation between MYC and PIM1 in lymphomagenesis [6]. Pim kinases also appear to function to suppresses apoptosis [7], modulate migration [8], inducing genomic instability via disruptions in mitotic spindle checkpoints [9], influencing angiogenesis [10] and modulating cell cycle regulation [11–13].

Pim kinases are over-expressed in a variety of human cancers including CLL, mantle cell lymphoma, diffuse large B cell lymphoma, FLT3/ITD AML and solid tumors including prostate, pancreas and colon [14], and aberrant expression may be associated with outcome. In view of the role in cell differentiation, angiogenesis, survival and proliferation PIM kinase inhibition provides a tractable therapeutic target for several pediatric malignancies.

Unlike other kinases, PIM proteins do not possess the typical canonical hydrogen bond donor in the hinge region (due to the presence of a proline residue instead of methionine)[15,16], a typical key binding site for many small molecules. SGI-1776 is an imidazo [1,2-b]pyridazine compound that was discovered as a potential novel inhibitor of the PIM kinases with IC₅₀ concentrations for PIM1, PIM2 and PIM3 of 7 nM, 363 nM and 69 nM, respectively. It is relatively specific with some on target activity against FLT3 and the haspin group of kinases. SGI-1776 has shown preclinical activity against leukemia and solid tumor cell line models [16] with IC₅₀ values of 0.005-11.68 μ M. Preclinical *in vivo* studies with human-derived myeloid leukemia xenograft models showed that SGI-1776 was extremely active as an orally delivered drug and induced complete tumor regression. The PPTP therefore performed preclinical testing to assess the potential activity of SGI-1776 against pediatric cancers.

MATERIALS AND METHODS

In vitro testing

In vitro testing was performed using DIMSCAN, as previously described.[17] Cells were incubated in the presence of SGI-1776 for 96 hours at concentrations from 1 nM to 10 μ M and analyzed as previously described [18].

In vivo tumor growth inhibition studies

Studies were conducted and analyzed using methods previously described [18–20]. Responses were determined using three activity measures as previously described [18]. An in-depth description of the analysis methods is included in the Supplemental Response Definitions section.

Statistical Methods

The exact log-rank test, as implemented using Proc StatXact for SASR, was used to compare event-free survival distributions between treatment and control groups. P-values were two-sided and were not adjusted for multiple comparisons given the exploratory nature of the studies.

Drugs and Formulation

SGI-1776 was provided to the Pediatric Preclinical Testing Program by Supergen, through the Cancer Therapy Evaluation Program (NCI). Powder was dissolved in sterile water for injection, titrated to pH 3.5 with 1N NaOH, and stored for up to 1 week. SGI-1776 was administered orally 5 days per week at 148 mg/kg (solid tumor models) or 74 mg/kg (ALL models) for 3 consecutive weeks. SGI-1776 was provided to each consortium investigator in coded vials for blinded testing.

RESULTS

In vitro testing

SGI-1776 demonstrated potent cytotoxic activity, with T/C% values approaching 0% for all of the cell lines at the highest concentration tested. The median relative IC₅₀ value for the PPTP cell lines was 3.1 μ M, with a range from 0.3 μ M (Kasumi-1) to 4.5 μ M (Ramos). The most sensitive cell line, Kasumi-1, is an AML cell line that has an activating KIT mutation.

A metric used to compare the relative responsiveness of the PPTP cell lines to SGI-1776 is the ratio of the median relative IC₅₀ of the entire panel to that of each cell line, Table I. Higher ratios are indicative of greater sensitivity to SGI-1776. Kasumi-1 (AML) and CHLA-9 (Ewing sarcoma) were relatively sensitive to SGI-1776 with each cell line having a relative IC₅₀ value substantially lower than the median for the entire panel. The remaining cell lines showed similar relative IC₅₀ values, with 18 of the remaining 20 cell lines tested showing values between 1 μ M and 4 μ M. These observations suggest that SGI-1776 has a relatively specific effect against a minority of pediatric cancer cell lines with selected activated kinases at SGI-1776 concentrations 0.5 μ M, whereas most cell lines require higher concentrations to respond (in the 1–4 μ M range).

In vivo testing

SGI-1776 was tested against the PPTP solid tumor xenografts using a dose of 148 mg/kg administered by the oral gavage daily for 5 days per week for 3 weeks. For the ALL panel (using NOD-SCID mice), the maximum tolerated dose was 74 mg/kg, and this dose was used for efficacy testing. The total planned treatment and observation period was 6 weeks. SGI-1776 was well tolerated with 10 of 369 treated mice dying (3%) compared to 2 of 363 control mice (0.6%). All 39 xenograft models studied were considered evaluable for efficacy. A complete summary of results is provided in Supplemental Table I.

SGI-1776 induced significant differences in EFS distribution compared to control in 9 of 31 (29%) of the evaluable solid tumor xenografts and in 1 of 8 (13%) of the evaluable ALL xenografts (Table II). For those xenografts with a significant difference in EFS distribution between treated and control groups, the EFS T/C activity measure additionally requires an EFS T/C value of > 2.0 for intermediate activity and indicates a substantial agent effect in slowing tumor growth. High activity further requires a reduction in final tumor volume compared to the starting tumor volume (see Supplemental Response Definitions). SGI-1776 induced tumor growth inhibition meeting criteria for intermediate EFS T/C activity in 1 of 31 (3%) evaluable solid tumor xenografts. For the ALL panel, 0 of 8 xenografts met criteria for intermediate activity. Objective responses were not observed in either the solid tumor panels or in the ALL panel. As a positive control we tested SGI-1776 against the Flt-3-driven B myeloid leukemia, MV4;11, growing as a subcutaneous xenograft, based on the high level of activity previously observed [16]. SGI-1776 induced complete response in this model, suggesting that pediatric models of solid tumors and ALL are relatively less sensitive to SGI-1776 than the MV4;11 model.

DISCUSSION

SGI-1776 is an imidazo [1,2-b] pyridazine compound, which has been shown to have inhibitory activity against PIM1, 2 and 3 kinases as well as FLT3 and haspin kinases. However, two recent Phase 1 studies of SGI-1776 in patients with relapsed leukemias or refractory prostate cancer /NHL were terminated due to dose limiting toxicity of cardiac QTc prolongation. In parallel, the PPTP tested this agent against a broad spectrum of pediatric cancer histotypes. There was very limited evidence for activity, except against the FLT3-driven MV4;11 model. SGI-1776 inhibits PIM1 and FLT3 kinases in the same

concentration range, hence the results *in vivo* suggest that at the drug exposures achieved PIM1 would be inhibited. Thus, our data suggest a limited role for PIM1 as a driver kinase for growth and survival for the pediatric models studied.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NO1-CM-42216, CA21765, and CA108786 from the National Cancer Institute, and AT13387 was provided by Astex Therapeutics. In addition to the authors represents work contributed by the following: Sherry Ansher, Lili T Belcastro, Edward Favours, Henry S. Friedman, Debbie Payne-Turner, Charles Stopford, Mayamin Tajbakhsh, Chandra Tucker, Amy Wozniak, Joe Zeidner, Ellen Zhang, and Jian Zhang. Children's Cancer Institute Australia for Medical Research is affiliated with the University of New South Wales and Sydney Children's Hospital.

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Table 1

Summary of *in Vitro* Activity of SGI-1776

Cell Line	Histotype	Relative IC ₅₀ (μM)	R ²	Panel Relative IC ₅₀ / Line Relative IC ₅₀	Y _{min} % (Observed)
RD	Rhabdomyosarcoma	3.88	0.91	0.77	3.0
Rh41	Rhabdomyosarcoma	3.21	0.96	0.93	0.0
Rh18	Rhabdomyosarcoma	1.97	0.95	1.52	0.3
Rh30	Rhabdomyosarcoma	3.79	0.92	0.79	0.5
BT-12	Rhabdoid	3.57	0.96	0.84	0.5
CHLA-266	Rhabdoid	2.37	0.96	1.26	1.5
TC-71	Ewing sarcoma	4.00	0.90	0.75	4.6
CHLA-9	Ewing sarcoma	0.52	0.90	5.75	0.0
CHLA-10	Ewing sarcoma	NA	NA	NA	NA
CHLA-258	Ewing sarcoma	NA	NA	NA	NA
SJ-GBM2	Glioblastoma	3.36	0.97	0.89	2.9
NB-1643	Neuroblastoma	1.33	0.92	2.25	0.0
NB-EBc1	Neuroblastoma	1.54	0.99	1.94	0.0
CHLA-90	Neuroblastoma	2.38	0.94	1.26	5.3
CHLA-136	Neuroblastoma	4.44	0.91	0.67	10.8
NALM-6	ALL	3.52	0.94	0.85	0.0
COG-LL-317	ALL	2.23	0.96	1.34	0.0
RS4;11	ALL	1.23	0.94	2.43	1.0
MOLT-4	ALL	2.81	0.92	1.06	0.0
CCRF-CEM (1)	ALL	3.61	0.85	0.83	0.0
CCRF-CEM (2)	ALL	3.46	0.95	0.86	0.0
Kasumi-1	AML	0.28	0.98	10.62	0.7
Karpas-299	ALCL	3.05	0.91	0.98	0.0
Ramos-RA1	NHL	4.53	0.94	0.66	5.1
Median		3.13	0.94	0.96	0.40
Minimum		0.28	0.85	0.66	0.00
Maximum		4.53	0.99	10.62	10.80

Table II

Summary of *in Vivo* Activity of SGI-1776

Xenograft Line	Tumor Type	Median Time to Event	P- value	EFS T/C	Median Final RTV	Tumor Volume T/C	T/C Volume Activity	EFS Activity	Response Activity
BT-29	Rhabdoid	33.6	0.553	1.3	>4	0.80	Low	Low	PD1
KT-12	Rhabdoid	10.7	0.146	0.9	>4	1.13	Low	Low	PD1
KT-10	Wilms	12.9	0.026	1.1	>4	0.46	Low	Low	PD1
KT-13	Wilms	13.1	0.002	2.1	>4	0.48	Low	Int	PD2
KT-14	Rhabdoid	22.3	0.336	1.1	<4	0.74	Low	Low	Low
SK-NEP-1	Ewing	8.5	0.920	1.2	>4	1.06	Low	Low	PD1
EW5	Ewing	8.4	0.007	0.8	>4	1.39	Low	Low	PD1
EW8	Ewing	9.8	0.887	0.9	>4	1.02	Low	Low	PD1
TC-71	Ewing	12.9	0.572	1.9	>4	0.74	Low	Low	PD2
Rh28	ALV RMS	16.3	0.400	1.0	>4	1.26	Low	Low	PD1
Rh30	ALV RMS	14.9	0.503	1.1	>4	0.80	Low	Low	PD1
Rh30R	ALV RMS	9.8	0.667	1.0	>4	0.98	Low	Low	PD1
Rh41	ALV RMS	12.8	0.086	1.2	>4	0.71	Low	Low	PD1
Rh18	EMB RMS	5.6	0.672	0.9	>4	1.17	Low	Low	PD1
Rh36	EMB RMS	10.6	0.370	0.9	>4	1.04	Low	Low	PD1
BT-28	Medulloblastoma	7.0	0.776	0.9	>4	1.25	Low	Low	PD1
BT-45	Medulloblastoma	6.4	0.843	1.0	>4	0.95	Low	Low	PD1
GBM2	Glioblastoma	13.0	<0.001	1.6	>4	0.66	Low	Low	PD2
BT-39	Glioblastoma	7.9	0.152	0.7	>4	1.34	Low	Low	PD1
D645	Glioblastoma	10.1	0.394	1.3	>4	0.76	Low	Low	PD1
D456	Glioblastoma	7.9	0.012	1.2	>4	0.70	Low	Low	PD1
NB-SD	Neuroblastoma	12.5	0.313	1.5	>4	0.88	Low	Low	PD1
NB-1771	Neuroblastoma	6.0	0.280	0.8	>4	1.10	Low	Low	PD1
NB-1691	Neuroblastoma	5.9	0.086	1.2	>4	0.67	Low	Low	PD1
NB-EBc1	Neuroblastoma	6.8	0.004	1.4	>4	0.54	Low	Low	PD1
CHLA-79	Neuroblastoma	5.2	0.207	0.9	>4	1.19	Low	Low	PD1

Xenograft Line	Tumor Type	Median Time to Event	P- value	EFS T/C	Median Final RTV	Tumor Volume T/C	T/C Volume Activity	EFS Activity	Response Activity
OS-1	Osteosarcoma	34.9	0.184	1.1	>4	0.84	Low	Low	PD1
OS-2	Osteosarcoma	23.5	0.133	1.0	>4	0.86	Low	Low	PD1
OS-17	Osteosarcoma	14.4	0.028	1.1	>4	0.87	Low	Low	PD1
OS-9	Osteosarcoma	18.1	<0.001	1.1	>4	0.81	Low	Low	PD1
OS-33	Osteosarcoma	22.9	<0.001	1.3	>4	0.67	Low	Low	PD1
OS-31	Osteosarcoma	22.8	0.012	1.1	>4	0.80	Low	Low	PD1
ALL-2	ALL B-precursor	10.7	0.305	1.0	>25	.	.	Low	PD1
ALL-3	ALL B-precursor	5.2	0.090	1.0	>25	.	.	Low	PD1
ALL-4	ALL B-precursor	5.1	0.732	1.0	>25	.	.	Low	PD1
ALL-7	ALL B-precursor	4.3	0.008	1.8	>25	.	.	Low	PD2
ALL-8	ALL T-cell	7.8	0.055	1.8	>25	.	.	Low	PD2
ALL-16	ALL T-cell	10.3	0.679	1.1	>25	.	.	Low	PD1
ALL-17	ALL B-precursor	6.2	0.594	1.2	>25	.	.	Low	PD1
ALL-19	ALL B-precursor	7.9	0.962	0.9	>25	.	.	Low	PD1
MV4;11	AML	>EP	<0.001	>2.3	1.7	0.01	High	Int	High