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Initial Testing (Stage 1) of the Notch Inhibitor PF-03084014, by the Pediatric Preclinical Testing Program

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Abstract

PF-03084014, a γ -secretase inhibitor, was tested against the PPTP *in vitro* cell line panel (1.0 nM to 10 μ M) and against the *in vivo* xenograft panels (administered orally twice daily on days 1–7 and 15–21). PF-03084014 demonstrated limited *in vitro* activity, with no cell line achieving 50% inhibition. PF-03084014 induced significant differences in EFS distribution in 14 of 35 (40%) solid tumor xenografts, and 1 of 9 ALL xenografts (which lacked a NOTCH1 mutation), but objective responses were not observed. PF-03084014 demonstrated limited single agent activity *in vitro* and *in vivo* against the pediatric preclinical models studied.

Keywords

Preclinical Testing; Developmental Therapeutics; Notch inhibitors

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INTRODUCTION

The oncogenic role of Notch was initially described in T-ALL [5]. However the role of Notch in cancer appears highly context dependent. In addition to T-ALL, activating oncogenic mutations have been described in patients with chronic lymphocytic leukemia and mantle cell lymphoma [6]. By contrast, inactivating NOTCH1 mutations consistent with a tumor suppressor role for Notch pathway signaling have been identified in squamous cell carcinomas (including cutaneous, head and neck, and lung cancers) [6].

γ -secretase is a protease complex with several transmembrane components that plays a central role in release of the NICD and, while not being substrate specific, is amenable to pharmacological inhibition [1]. Given that several γ -secretase inhibitors (GSIs) initially developed for Alzheimer's disease treatment are available for clinical evaluation, inhibition of Notch signaling by blocking release of the NICD has become an appealing approach for cancer treatment. The selective GSI PF-03084014 has shown inhibition of T-ALL cell growth *in vitro* and *in vivo* [7,8].

Aberrant Notch signaling has been implicated in several pediatric malignancies in addition to the hallmark T-lineage ALL [5], including medulloblastoma [9], glioblastoma [10], rhabdomyosarcoma [11], Ewing sarcoma [12], and particularly osteosarcoma [13–15]. NOTCH1 mutations have also been reported for a subset of patients with ependymoma [16].

PF-03084014 has completed phase 1 testing in adults with advanced solid tumors [17]. Dose-limiting toxicities included diarrhea and rash, and early evidence of clinical activity was observed, in particular for patients with desmoid tumors. Given the potential role of Notch signaling in pediatric cancers, PF-03084014 was selected by the PPTP for evaluation *in vitro* and *in vivo*.

MATERIALS AND METHODS

In vitro testing

In vitro testing was performed using DIMSCAN, as previously described [18][19].

In vivo tumor growth inhibition studies

Solid tumors were propagated in CB17SC *scid*^{-/-} mice (Taconic Farms, Germantown NY), and glioblastoma models were propagated in BALB/c nu/nu mice [20,21]. Human leukemia cells were propagated by intravenous inoculation in non-obese diabetic (NOD)/*scid*^{-/-} mice as described previously [22]. Responses were determined using three activity measures as previously described [20]. Because of the relevance of the Notch pathway in T-cell ALL, the acute leukemia xenograft panel included 6 out of 8 T-lineage ALL (three with Notch mutations).

Statistical Methods

The exact log-rank test, as implemented using Proc StatXact for SAS®, 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

PF-03084014 was provided to the Pediatric Preclinical Testing Program by Pfizer, through the Cancer Therapy Evaluation Program (NCI). Drug was formulated in 0.5% methylcellulose, in sterile water for injection, and stored in the dark at 4°C for up to one month. PF-03084014 was administered orally (P.O.) at 150 mg/kg (112.5 mg/kg for the ALL xenografts based on toxicity testing in NOD/*scid*^{-/-} mice) using a twice-daily schedule (days 1–7 and 15–21) for one cycle, followed by 3 weeks of observation. PF-03084014 was provided to each consortium investigator in coded vials for blinded testing.

RESULTS

In vitro testing

PF-03084014 was tested against the PPTP's *in vitro* cell line panel at concentrations ranging from 1.0 nM to 10 µM using the PPTP's standard 96 hour exposure period. PF-03084014 demonstrated limited activity (Table I), with no cell line achieving 50% or greater inhibition at the highest concentration tested.

In vivo testing

PF-03084014 was generally well tolerated, with only a 2.6% toxicity rate in the treated groups, and no toxicity observed for control animals. Forty-four of 44 tested xenograft models were considered evaluable for efficacy. Complete details of testing are provided in Supplemental Table I.

PF-03084014 induced significant differences in EFS distribution compared to control in 14 of 35 (40%) of the solid tumor xenografts and in 1 of 9 (11%) of the ALL xenografts (Table II). Significant differences in EFS distribution were most commonly observed for the osteosarcoma panel (5 of 5) and the Ewing sarcoma panel (3 of 5). PF-03084014 did not induce tumor growth inhibition meeting criteria for intermediate EFS T/C (≥ 2) activity in the solid tumor and ALL xenografts. Objective responses were not observed for any of the models.

DISCUSSION

PF-03084014 showed little or no effect *in vitro* against the PPTP cell line panel using 96 hour exposure at concentrations up to 10 µM. The IC₅₀ for inhibition of Notch signaling by PF-03084014 is in the 10 nM to 150 nM range with maximal inhibition by 1.0 µM [7,23]. Therefore, the lack of *in vitro* activity observed for PF-03084014 against the PPTP cell line panel cannot be explained by use of insufficient concentrations to induce inhibition of Notch signaling.

Notch pathway signaling is activated in some of the solid tumor panels, as evidenced by expression patterns of NOTCH1-4, and transcriptional targets HES1 and HEY1/HEY2 (Supplemental Figure 1). Although there is circumstantial evidence that Notch pathway

inhibition may be an effective therapeutic strategy for selected childhood cancers, our results demonstrate that inhibition of this pathway through γ -secretase inhibition has little impact on cancer cell survival/proliferation for models across a range of histotypes.

The oncogenic role of Notch signaling is illustrated by T-cell ALL, for which NOTCH1 activating mutations are present in over 50% of cases [25]. Two types of NOTCH1 mutations are observed in T-cell ALL: mutations in the extracellular heterodimerization (HD) domain increase susceptibility to ligand-independent proteolytic cleavage, and mutations in the NICD enhance its stability. Among the PPTP T-cell ALL xenografts tested, three have NOTCH1 mutations: ALL-8 has a heterozygous mutation in the PEST domain; ALL-27 and ALL-29 have heterozygous mutations in both the PEST and HD domains. However, PF-03084014 only caused a small growth delay, although significant, in a single T-cell ALL xenograft (ALL-31) that lacks NOTCH1 mutations. Two of the three xenografts with NOTCH1 mutations showed reduced levels of human CD45 in blood at week 2 post initiation of PF-03084014 treatment compared to week 1 (Supplemental Figure 2). While the observed reductions of leukemia in the peripheral circulation are consistent with some degree of a delayed treatment effect, they are generally modest and not sustained. While the observed reductions of leukemia in the peripheral circulation are consistent with some degree of a delayed treatment effect, the effects are minor and not sustained. Results for PF-03084014 show similarities to those reported for RO4929097 [26]. For the T-cell ALL xenografts, the PPTP results for PF-03084014 with no induction of remission were consistent with those for RO4929097, as well as with clinical results for the GSI MK-0752 [27].

The lack of activity appears not to be a consequence of inadequate dosing, as doses used have been shown to produce robust modulation of NICD levels and of expression levels of Notch down stream genes and the peak drug levels that can be achieved in mice at the 150 mg/kg dose appear to be greater than those observed in humans at the PF-03084014 maximum tolerated dose [7,17]. Treatment with PF-03084014 also reduced NOTCH1 and HES1 expression in Rh10 xenografts (Supplemental Figure 3).

The activity observed for PF-03084014 against these preclinical models is low, which is similar to PPTP results previously reported for another GSI [26]. Despite a biological rationale for γ -secretase inhibition in T-ALL and some pediatric solid tumors, only minimal activity has been observed by the PPTP for agents in this class, raising questions about the clinical relevance of GSIs for pediatric cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table I*In vitro* activity for PF-03084014

Cell Line	Histotype	rIC ₅₀ (μM)	Panel rIC ₅₀ / Line rIC ₅₀	Y _{min} % (Observed)
RD	Rhabdomyosarcoma	>10	1.00	90
Rh41	Rhabdomyosarcoma	>10	1.00	86
Rh18	Rhabdomyosarcoma	>10	1.00	62
Rh30	Rhabdomyosarcoma	>10	1.00	88
BT-12	Rhabdoid	>10	1.00	87
CHLA-266	Rhabdoid	>10	1.00	69
TC-71	Ewing sarcoma	>10	1.00	83
CHLA-9	Ewing sarcoma	>10	1.00	55
CHLA-10	Ewing sarcoma	>10	1.00	66
CHLA-258	Ewing sarcoma	>10	1.00	68
SJ-GBM2	Glioblastoma	>10	1.00	100
NB-1643	Neuroblastoma	>10	1.00	85
NB-EBc1	Neuroblastoma	>10	1.00	84
CHLA-90	Neuroblastoma	>10	1.00	78
CHLA-136	Neuroblastoma	>10	1.00	85
NALM-6	ALL	>10	1.00	76
COG-LL-317	ALL	>10	1.00	82
RS4;11	ALL	>10	1.00	81
MOLT-4	ALL	>10	1.00	64
CCRF-CEM (1)	ALL	>10	1.00	76
CCRF-CEM (2)	ALL	>10	1.00	78
Kasumi-1	AML	>10	1.00	54
Karpas-299	ALCL	>10	1.00	90
Ramos-RA1	NHL	>10	1.00	75
Median		>10	1.00	80
Minimum		>10	1.00	54
Maximum		>10	1.00	100

Table II

Summary of *in vivo* Activity of PF-03084014

Xenograft Line	Tumor Type	Median Time to Event	P-value	EFS T/C	Median Final RTV or CD45%	Tumor Volume T/C	Median Group Response	EFS Activity
BT-29	Rhabdoid	35.7	0.041	1.4	>4	0.70	PD1	Low
KT-16	Rhabdoid	11.0	<0.001	1.8	>4	0.51	PD2	Low
KT-14	Rhabdoid	17.9	0.733	1.1	>4	0.99	PD1	Low
KT-10	Wilms	12.9	0.002	1.3	>4	0.72	PD1	Low
KT-11	Wilms	23.4	0.517	1.1	>4	0.73	PD1	Low
KT-13	Wilms	16.0	0.025	1.4	>4	0.73	PD1	Low
SK-NEP-1	Ewing	11.6	0.553	1.2	>4	1.02	PD1	Low
EW5	Ewing	10.2	<0.001	1.6	>4	0.61	PD2	Low
EW8	Ewing	10.6	0.034	1.6	>4	0.76	PD2	Low
TC-71	Ewing	11.6	<0.001	1.9	>4	0.40	PD2	Low
CHLA258	Ewing	22.0	0.173	1.1	>4	1.05	PD1	Low
Rb10	Alveolar RMS	18.3	0.263	1.6	>4	0.59	PD2	Low
Rb28	Alveolar RMS	14.3	0.966	1.1	>4	0.94	PD1	Low
Rb30	Alveolar RMS	10.8	0.168	1.1	>4	0.93	PD1	Low
Rb30R	Alveolar RMS	12.5	<0.001	1.5	>4	0.61	PD1	Low
Rb41	Alveolar RMS	11.9	0.052	1.4	>4	0.65	PD1	Low
BT-28	Medulloblastoma	14.2	0.525	1.1	>4	0.87	PD1	Low
BT-45	Medulloblastoma	35.8	0.148	1.1	>4	0.64	PD1	Low
BT-50	Medulloblastoma	> EP	0.647	.	2.8	0.55	PD2	NE
BT-36	Ependymoma	> EP	1.000	.	1.8	0.76	PD2	NE
BT-41	Ependymoma	> EP	1.000	.	2.3	0.64	PD2	NE
GBM2	Glioblastoma	11.3	0.214	0.8	>4	1.05	PD1	Low
BT-39	Glioblastoma	11.7	0.027	1.5	>4	0.59	PD1	Low
D645	Glioblastoma	9.9	0.114	1.3	>4	0.83	PD1	Low
D456	Glioblastoma	8.5	0.639	1.3	>4	0.69	PD1	Low
NB-SD	Neuroblastoma	11.9	0.622	1.0	>4	1.06	PD1	Low
NB-1771	Neuroblastoma	10.8	0.141	1.3	>4	0.80	PD1	Low

Xenograft Line	Tumor Type	Median Time to Event	P-value	EFS T/C	Median Final RTV or CD45%	Tumor Volume T/C	Median Group Response	EFS Activity
NB-1691	Neuroblastoma	6.9	0.131	1.1	>4	0.79	PD1	Low
NB-EBc1	Neuroblastoma	5.3	0.298	1.1	>4	0.87	PD1	Low
NB-1643	Neuroblastoma	12.3	0.268	0.9	>4	0.82	PD1	Low
OS-2	Osteosarcoma	19.8	0.047	1.2	>4	0.87	PD1	Low
OS-17	Osteosarcoma	24.3	0.005	1.3	>4	0.83	PD1	Low
OS-9	Osteosarcoma	18.4	0.042	1.1	>4	0.92	PD1	Low
OS-33	Osteosarcoma	12.6	<0.001	1.1	>4	0.85	PD1	Low
OS-31	Osteosarcoma	20.6	<0.001	1.3	>4	0.74	PD1	Low
ALL-2	ALL B-precursor	16.2	0.858	1.1	>25	.	PD1	Low
ALL-4	ALL B-precursor	4.3	0.351	0.7	>25	.	PD1	Low
ALL-8	ALL T-cell	5.0	1.000	1.0	>25	.	PD1	Low
ALL-16	ALL T-cell	23.6	0.567	1.9	>25	.	PD2	Low
ALL-27	ALL T-cell	5.0	0.913	0.6	>25	.	PD1	Low
ALL-29	ALL T-cell	5.0	0.241	1.0	>25	.	PD1	Low
ALL-30	ALL T-cell	6.2	0.620	0.8	>25	.	PD1	Low
ALL-31	Tertiary, T-cell ALL	13.6	0.015	1.8	>25	.	PD2	Low
MLL-7	ALL (Infant MLL)	6.9	0.507	0.6	>25	.	PD1	Low

In bold: P<005; NE – not evaluable