Published in final edited form as:

Pediatr Blood Cancer. 2017 April ; 64(4): . doi:10.1002/pbc.26304.

Initial Testing of VS-4718, a novel inhibitor of Focal Adhesion Kinase (FAK), against pediatric tumor models by the Pediatric Preclinical Testing Program

Raushan T. Kurmasheva, PhD^{1,*}, Richard Gorlick, MD², E. Anders Kolb, MD³, Stephen T. Keir, PhD⁴, John M. Maris, MD⁵, Richard B. Lock, PhD⁶, Hernan Carol, PhD⁶, Jiarong Min Kang, PharmD⁷, C. Patrick Reynolds, MD, PhD⁷, Jianrong Wu⁸, Peter J. Houghton, PhD^{1,*}, and Malcolm A. Smith, MD, PhD⁹

¹Nationwide Children's Hospital, Columbus, OH

²The Children's Hospital at Montefiore, Bronx, NY

³A.I. duPont Hospital for Children, Wilmington, DE

⁴Duke University Medical Center, Durham, NC

⁵Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine and Abramson Family Cancer Research Institute, Philadelphia, PA

⁶Children's Cancer Institute Australia, Randwick, NSW Australia

⁷Texas Tech University Health Sciences Center

⁸St. Jude Children's Research Hospital, Memphis, TN

⁹Cancer Therapy Evaluation Program, NCI, Bethesda, MD

Abstract

VS-4718 was tested against the PPTP's *in vitro* cell line panel and showed a median relative IC₅₀ of 1.22 μ M. VS-4718 was tested *in vivo* against the PPTP xenograft models using a dose of 50 mg/kg administered by the oral route twice daily for 21 days. VS-4718 induced significant differences in EFS distribution compared to control in 18 of 36 of the evaluable solid tumor xenografts and in 0 of 8 acute lymphoblastic leukemia (ALL) xenografts, but no xenograft lines showed tumor regression. Future plans include further evaluating the role of FAK inhibition in combination with ABL kinase inhibitors for Ph-positive ALL.

Corresponding Author: Raushan T. Kurmasheva, PhD, Greehey Children's Cancer Research Institute, 8403 Floyd Curl Drive, San Antonio, TX 78229, Kurmasheva@uthscsa.edu, Voice: 210-562-9155.

*Current Address: Greehey Children's Cancer Research Institute, University of Texas Health Science Center, 8403 Floyd Curl Drive, San Antonio, TX 78229

Conflict of interest statement: The other authors consider that there are no actual or perceived conflicts of interest.

Supplemental Appendix S1

Supplemental Table S1

Keywords

Preclinical Testing; Developmental Therapeutics; VS-4718; Focal Adhesion Kinase

INTRODUCTION

Focal Adhesion Kinase (FAK) is a non-receptor cytoplasmic tyrosine kinase that is encoded by *PTK2* (chromosome 8q24.3). FAK is involved in many cellular processes through its role in integrin and growth factor signaling, with activation of FAK inducing recruitment of several SH2-domain and SH3-domain containing proteins that mediate signaling to downstream pathways ¹. A role for FAK in cancer has been suspected since it was described as a protein with increased tyrosine phosphorylation in viral Src transformed chicken embryo cells ²⁻⁴. Subsequent research has provided evidence for a role of FAK in promotion of cancer through effects on cell survival, angiogenesis, migration, proliferation, epithelial-mesenchymal transformation, invasion, and stem cell maintenance ⁵.

Several small molecule inhibitors of FAK have entered clinical development, including defactinib (VS-6063, PF-04554878), VS-4718, and GSK2256098 ⁵. VS-4718 (previously named PND-1186) is a selective, reversible inhibitor that has an IC₅₀ of 1.5 nM using an *in vitro* kinase assay and a cellular IC₅₀ of approximately 100 nM for FAK inhibition as measured by effects on phosphorylation at Tyr-397 ⁶. VS-4718 induced apoptosis in cells cultured in suspension but not in cells grown under adherent conditions ⁶.

Here we report the *in vitro* and *in vivo* activity of VS-4718 against PPTP cell lines and xenograft models.

MATERIALS AND METHODS

In vitro testing

In vitro testing was performed using DIMSCAN and cells were incubated in the presence of VS-4718 for 96 hours at concentrations from 1.0 nM to 10 μM and analyzed as previously described ^{7,8}

In vivo tumor growth inhibition studies

Procedures for propagation of xenografts in CB17SC *scid*^{-/-} for solid tumors, BALB/c nu/nu mice for glioma models, ⁹, and leukemia models (NOD)/*scid*^{-/-} mice have been described previously ¹⁰. All mice were maintained under barrier conditions and experiments were conducted using protocols and conditions approved by the institutional animal care and use committee of the appropriate consortium member. An in-depth description of the analysis methods is included in Supplemental Appendix S1. Statistical methods for analysis have been described previously ⁹.

Drugs and Formulation

VS-4718 was provided to the Pediatric Preclinical Testing Program by Verastem Inc., through the Cancer Therapy Evaluation Program (NCI). VS-4718 was formulated in 0.5%

carboxymethylcellulose/0.1% Tween 80, and administered P.O. twice daily for 21 consecutive days at 50 mg/kg/dose, a dose and schedule shown to be synergistic when administered with dasatinib in the ALL-4 leukemia model ¹¹. VS-4718 was provided in coded vials for blinded testing.

RESULTS

VS-4718 *in vitro* testing

VS-4718 was tested against the PPTP's *in vitro* cell line panel at concentrations ranging from 1.0 nM to 10 μ M using the PPTP's standard 96-hour exposure period. The median relative IC₅₀ (rIC₅₀) was 1.22 μ M, with a range from 0.25 μ M (CHLA-9, Ewing sarcoma) to 3.53 μ M [COG-LL-317, acute lymphoblastic leukemia (ALL)]. The median Ymin (T/C%) was 4%, with a range from 0% to 29%.

A metric used to compare the relative responsiveness of the PPTP cell lines to VS-4718 is the ratio of the median rIC₅₀ of the entire panel to that of each cell line (Table 1). Overall, there was not a large variation in rIC₅₀ by histotype. There is a trend for the Ewing cell lines (TC-71 through CHLA-258) to be more sensitive than the non-Ewing cell lines (0.85 μ M versus 1.44 μ M, $p=0.05$). The median Relative I/O% value for the PPTP cell line panel was -56%, indicating a cytotoxic effect at the highest concentrations tested for most of the cell lines.

VS-4718 *in vivo* testing

VS-4718 was well tolerated, with only a 1.2% mortality rate (5/427) in the treated groups, the same as that observed for control animals. Each of 44 tested xenograft models were considered evaluable for efficacy. Complete details of testing are provided in Supplemental Table 2. VS-4718 induced significant differences in EFS distribution compared to control in 18 of 36 (50%) of the evaluable solid tumor xenografts and in 0 of 8 (0%) of the evaluable ALL xenografts, including the Ph+ ALL xenograft, ALL-4 (Table 2). Significant differences in EFS distribution were most commonly observed for the osteosarcoma panel (6 of 6), the rhabdomyosarcoma panel (4 of 6), and the neuroblastoma panel (4 of 6). VS-4718 did not induce tumor growth inhibition meeting criteria for intermediate EFS T/C(>2) activity in either the solid tumor or ALL xenografts. Only 3 models met criteria for PD2 responses (one Ewing, rhabdomyosarcoma, and neuroblastoma xenograft each). Objective responses were not observed for the solid tumor or ALL xenografts.

DISCUSSION

VS-4718 inhibits FAK phosphorylation at Y397 in cellular assays with an IC₅₀ in the 100 nM range ⁶, and it also reduces the proportion of cancer stem cells (CSCs) in aldefluor and Hoechst dye exclusion assays with an IC₅₀ value of approximately 100 nM ¹². Other work has shown that malignant pleural mesothelioma (MPM) cell lines with mutated NF2 have IC₅₀ values in the 100 nM range, while MPM cell lines with wild-type NF2 have IC₅₀ values greater than 1 μ M ¹³. Under the assumption that these results indicate that FAK "on-target" effects are observed at 100 nM concentrations or less, then it is likely that the PPTP *in vitro* results showing IC₅₀ values generally above 1 μ M reflect "off-target" effects of VS-4718.

VS-4718 was well tolerated at the dose and schedule evaluated (50 mg/kg BID×21 days). However, there was limited *in vivo* activity observed. Significant differences in EFS distribution were noted in approximately one-half of the solid tumor models, but the magnitude of the shift in time to event was small with only 3 xenografts showing EFS T/C ratios greater than 1.5. No ALL models showed significant differences in EFS distribution.

A recent publication highlighted the potential utility for FAK inhibition against ALL that exhibits loss of Ikaros function¹⁴, a finding that is particularly common in Ph⁺ ALL expressing the *BCR-ABL1* fusion gene¹⁵. Joshi, et al., showed that loss of Ikaros function in early pre-B cells leads to a differentiation block that drives the cells into an adhesive state, promotes self-renewal, and increases the likelihood for malignant transformation¹⁴. Treatment of Ikaros-defective malignant B-cells with a small molecule FAK inhibitor (VS-6062) led to apoptotic cell death. However, it is unknown how closely leukemias developing from murine pre-B cells null for Ikaros align with the biology of Ph⁺ ALL in children. The PPTP has two ALL xenograft lines, ALL-4 and ALL-19, for which SNP array data indicate alterations in *IKZF1*. ALL-4 harbors the *BCR-ABL1* translocation while ALL-19 has been identified as harboring a *NUP214-ABL1* fusion. However, neither of these lines responded to single agent VS-4718, consistent with previous results¹¹. Churchman, et al., made the important observation that VS-4718 potentiates the activity of dasatinib both *in vitro* and *in vivo* against *BCR-ABL1* expressing Ph⁺ ALL preclinical models, including ALL-4¹¹. A promising line of future research is to further evaluate how VS-4718 can be used to more effectively treat Ph⁺ ALL. An open question is whether there are other childhood cancers for which FAK inhibition can be used to enhance the activity of established anticancer agents.

In summary, the *in vivo* activity observed for VS-4718 as a single agent against the PPTP preclinical xenograft lines is low. Similarly, the *in vitro* data are consistent with a limited effect of FAK inhibition alone 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 used VS-4718 supplied by Verastem Inc. In addition to the authors this paper represents work contributed by the following: Sherry Ansher, Catherine A. Billups, Ingrid Boehm, Joshua Courtright, Kathryn Evans, Edward Favours, Henry S. Friedman, Danuta Gasinski, Nicholas Pettit, Melissa Sammons, 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 the Sydney Children's Hospitals Network.

Abbreviations

PPTP	Pediatric Preclinical Testing Program
EFS T/C	Ratio of Event Free Survival Treated/Control
FAK	Focal Adhesion Kinase

(NOD)/*scid*^{-/-}

Non-Obese diabetic/severe combined immune deficient

References

1. Parsons JT. Focal adhesion kinase: the first ten years. *Journal of cell science*. Apr 15; 2003 116(Pt 8):1409–1416. [PubMed: 12640026]
2. Schaller MD, Borgman CA, Cobb BS, Vines RR, Reynolds AB, Parsons JT. pp125FAK a structurally distinctive protein-tyrosine kinase associated with focal adhesions. *Proc Natl Acad Sci U S A*. Jun 1; 1992 89(11):5192–5196. [PubMed: 1594631]
3. Kanner SB, Reynolds AB, Vines RR, Parsons JT. Monoclonal antibodies to individual tyrosine-phosphorylated protein substrates of oncogene-encoded tyrosine kinases. *Proc Natl Acad Sci U S A*. May; 1990 87(9):3328–3332. [PubMed: 2110361]
4. Hanks SK, Calalb MB, Harper MC, Patel SK. Focal adhesion protein-tyrosine kinase phosphorylated in response to cell attachment to fibronectin. *Proc Natl Acad Sci U S A*. Sep 15; 1992 89(18):8487–8491. [PubMed: 1528852]
5. Lee BY, Timpson P, Horvath LG, Daly RJ. FAK signaling in human cancer as a target for therapeutics. *Pharmacol Ther*. Feb. 2015 146:132–149. [PubMed: 25316657]
6. Tanjoni I, Walsh C, Uryu S, et al. PND-1186 FAK inhibitor selectively promotes tumor cell apoptosis in three-dimensional environments. *Cancer Biol Ther*. May 15; 2010 9(10):764–777. [PubMed: 20234191]
7. Frgala T, Kalous O, Proffitt RT, Reynolds CP. A fluorescence microplate cytotoxicity assay with a 4-log dynamic range that identifies synergistic drug combinations. *Mol Cancer Ther*. Mar 1; 2007 6(3):886–897. 2007. [PubMed: 17363483]
8. Kang MH, Smith MA, Morton CL, Keshelava N, Houghton PJ, Reynolds CP. National Cancer Institute Pediatric Preclinical Testing Program: Model description for in vitro cytotoxicity testing. *Pediatr Blood Cancer*. Feb; 2011 56(2):239–249. [PubMed: 20922763]
9. Houghton PJ, Morton CL, Tucker C, et al. The pediatric preclinical testing program: Description of models and early testing results. *Pediatr Blood Cancer*. Oct 25. 2006
10. Liem NL, Papa RA, Milross CG, et al. Characterization of childhood acute lymphoblastic leukemia xenograft models for the preclinical evaluation of new therapies. *Blood*. May 15; 2004 103(10):3905–3914. [PubMed: 14764536]
11. Churchman ML, Evans K, Richmond J, et al. Synergism of FAK and tyrosine kinase inhibition in Ph+ B-ALL. *JCI Insight*. 2016; 1(4)
12. Xu Q, Kolev VN, Vidal CM, et al. 400 FAK Inhibitor VS-4718 Attenuates Breast Cancer Stem Cell Function and Inhibits Tumor Growth in Vivo. *Eur J Cancer*. 2012; 48(Suppl 6):122. (Abstr #400).
13. Shapiro IM, Kolev VN, Vidal CM, et al. Merlin deficiency predicts FAK inhibitor sensitivity: a synthetic lethal relationship. *Science translational medicine*. May 21. 2014 6(237):237ra268.
14. Joshi I, Yoshida T, Jena N, et al. Loss of Ikaros DNA-binding function confers integrin-dependent survival on pre-B cells and progression to acute lymphoblastic leukemia. *Nature immunology*. Mar; 2014 15(3):294–304. [PubMed: 24509510]
15. Mullighan CG, Miller CB, Radtke I, et al. BCR-ABL1 lymphoblastic leukaemia is characterized by the deletion of Ikaros. *Nature*. May 1; 2008 453(7191):110–114. [PubMed: 18408710]

In vitro activity for VS-4718**Table 1**

Cell Line	Histotype	rIC ₅₀ (μM)	Panel rIC ₅₀ / Line rIC ₅₀	Ymin	Relative In/Out% (Observed Ymin) ^f
RD	Rhabdomyosarcoma	1.22	1.00	21%	16%
Rh41	Rhabdomyosarcoma	0.53	2.31	2%	-93%
Rh18	Rhabdomyosarcoma	1.63	0.75	22%	-50%
Rh30	Rhabdomyosarcoma	0.71	1.72	3%	-83%
BT-12	Rhabdoid	3.13	0.39	24%	17%
CHLA-266	Rhabdoid	1.16	1.05	29%	3%
TC-71	Ewing sarcoma	1.05	1.16	2%	1%
CHLA-9	Ewing sarcoma	0.25	4.92	1%	-60%
CHLA-10	Ewing sarcoma	1.03	1.19	5%	-17%
CHLA-258	Ewing sarcoma	0.66	1.84	4%	-90%
SJ-GBM2	Glioblastoma	1.68	0.73	21%	13%
NB-1643	Neuroblastoma	0.54	2.27	6%	-72%
NB-EBc1	Neuroblastoma	1.57	0.78	10%	-56%
CHLA-90	Neuroblastoma	0.64	1.92	26%	-7%
CHLA-136	Neuroblastoma	1.06	1.15	15%	-46%
NALM-6	ALL	2.67	0.46	2%	-24%
COG-LL-317	ALL	3.53	0.35	0%	-94%
RS4;11	ALL	1.23	1.00	6%	-62%
MOLT-4	ALL	2.65	0.46	1%	-88%
CCRF-CEM (1)	ALL	2.55	0.48	1%	-82%
Kasumi-1	AML	1.44	0.84	3%	-90%
Karpas-299	ALCL	0.37	3.32	2%	-73%
Ramos-RA1	NHL	2.83	0.43	1%	0%
Median		1.22	1.00	4%	-56%
Minimum		0.25	0.35	0%	-94%
Maximum		3.53	4.92	29%	17%

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

The Relative In/Out (I/O)% values compare the relative difference in final cell number compared with the starting cell number for treated cells and for control cells calculated as follows: (Observed $Y_{min} - Y_0$)/(100 - Y_0) if Observed $Y_{min} > Y_0$; and (Observed $Y_{min} - Y_0$)/(Y_0) if Observed $Y_{min} < Y_0$. Y_0 is an estimate of the starting cell number derived from determinations of the doubling time for each cell line. Relative I/O% values range between 100% (no treatment effect) to -100% (complete cytotoxic effect), with a Relative I/O% value of 0% being observed for a completely effective cytostatic agent.

Summary of *in Vivo* Activity of VS-4718**Table 2**

Line	Tumor Type	Median Time to Event	P-value	EFS T/C ¹	Median RTV/CD45 at End of Study	Tumor Volume T/C ²	Median Group Response	EFS Activity
BT-29	Rhabdoid	28.2	<0.001	1.5	>4	0.64	PD1	Low
KT-16	Rhabdoid	15.4	0.796	1.4	>4	0.82	PD1	Low
KT-14	Rhabdoid	20.7	0.378	1.3	>4	0.80	PD1	Low
KT-10	Wilms	18.6	0.089	1.4	>4	0.74	PD1	Low
KT-11	Wilms	15.2	0.367	1.1	>4	0.84	PD1	Low
KT-13	Wilms	12.0	0.598	0.9	>4	1.18	PD1	Low
SK-NEP-1	Ewing	13.5	0.110	1.4	>4	0.66	PD1	Low
EW5	Ewing	10.7	<0.001	1.9	>4	1.36	PD2	Low
EW8	Ewing	18.6	0.925	1.3	>4	0.84	PD1	Low
TC-71	Ewing	10.6	0.197	1.1	>4	0.81	PD1	Low
CHLA258	Ewing	8.0	0.351	0.8	>4	0.99	PD1	Low
Rh10	Alveolar RMS	24.1	0.351	1.2	>4	0.67	PD1	Low
Rh28	Alveolar RMS	19.2	0.015	0.7	>4	1.29	PD1	Low
Rh30	Alveolar RMS	15.1	0.013	1.5	>4	0.57	PD1	Low
Rh30R	Alveolar RMS	19.9	<0.001	1.6	>4	0.48	PD2	Low
Rh41	Alveolar RMS	12.4	0.010	1.4	>4	0.66	PD1	Low
Rh18	Embryonal RMS	16.7	0.009	1.5	>4	0.65	PD1	Low
BT-28	Medulloblastoma	16.5	0.002	1.3	>4	0.73	PD1	Low
BT-50	Medulloblastoma	33.0	0.123	1.1	>4	1.05	PD1	Low
BT-36	Ependymoma	27.0	0.074	0.8	>4	1.50	PD1	Low
GBM2	Glioblastoma	24.6	0.636	1.3	>4	0.83	PD1	Low
BT-39	Glioblastoma	6.9	0.517	0.9	>4	1.14	PD1	Low
D645	Glioblastoma	6.7	0.470	1.0	>4	1.04	PD1	Low
D456	Glioblastoma	8.1	0.004	1.4	>4	0.50	PD1	Low
NB-SD	Neuroblastoma	10.1	0.048	1.3	>4	0.87	PD1	Low

Line	Tumor Type	Median Time to Event	P-value	EFS T/C ¹	Median RTV/CD45 at End of Study	Tumor Volume T/C ²	Median Group Response	EFS Activity
NB-1771	Neuroblastoma	8.7	0.138	1.2	>4	0.88	PD1	Low
NB-1691	Neuroblastoma	10.6	0.013	1.4	>4	0.77	PD1	Low
NB-EBc1	Neuroblastoma	4.9	0.162	1.1	>4	0.90	PD1	Low
CHLA-79	Neuroblastoma	12.1	0.002	1.5	>4	0.73	PD2	Low
NB-1643	Neuroblastoma	6.7	0.020	1.1	>4	0.82	PD1	Low
OS-1	Osteosarcoma	> EP	<0.001	> 1.3	3.1	0.58	NE	NE
OS-2	Osteosarcoma	21.9	<0.001	1.2	>4	0.69	PD1	Low
OS-17	Osteosarcoma	17.0	0.024	1.3	>4	0.75	PD1	Low
OS-9	Osteosarcoma	30.3	<0.001	1.4	>4	0.54	PD1	Low
OS-33	Osteosarcoma	28.4	<0.001	1.4	>4	0.50	PD1	Low
OS-31	Osteosarcoma	20.9	<0.001	1.4	>4	0.71	PD1	Low
ALL-2	ALL B-precursor	15.5	0.940	0.9	>25	.	PD1	Low
ALL-4	ALL B-precursor	4.9	0.834	1.1	>25	.	PD1	Low
ALL-7	ALL B-precursor	7.0	0.258	0.9	>25	.	PD1	Low
ALL-8	T-cell ALL	10.0	0.718	1.0	>25	.	PD1	Low
ALL-17	ALL B-precursor	9.7	0.452	0.9	>25	.	PD1	Low
ALL-19	ALL B-precursor	5.7	0.331	0.9	>25	.	PD1	Low
ALL-31	T-cell ALL	9.0	0.598	1.0	>25	.	PD1	Low
MLL-7	Infant ALL ³	9.0	1.000	1.0	>25	.	PD1	Low

¹ EFS T/C value is defined by the ratio of the median time to event of the treatment group and the median time to event of the respective control group. High activity requires: a) an EFS T/C > 2; b) a significant difference in EFS distributions, and c) a net reduction in median tumor volume for animals in the treated group at the end of treatment as compared to at treatment initiation. Intermediate activity demonstrates criteria a) and b) above, but not having a net reduction in median tumor volume for treated animals at the end of the study. Low activity demonstrates EFS T/C < 2. ³ Objective response measures are described in detail in the Supplemental Response Definitions. PD1 = progressive disease with EFS T/C 1.5, and PD2 = progressive disease with EFS T/C > 1.5.

² *Tumor Volume T/C value*: Relative tumor volumes (RTV) for control (C) and treatment (T) mice were calculated at day 21 or when all mice in the control and treated groups still had measurable tumor volumes (if less than 21 days). The T/C value is the mean RTV for the treatment group divided by the mean RTV for the control group. High activity = T/C 0.15; Intermediate activity = T/C 0.45 but > 0.15; and Low activity = T/C > 0.45.

³ KMT2A (MLL) rearranged B-ALL.