

EFFICACY OF CRENOLANIB AGAINST THE PDGFRA ACTIVATING MUTATION, D842V, ASSOCIATED WITH GASTROINTESTINAL STROMAL TUMORS



M. Heinrich¹, D. Griffith¹, A. McKinley¹, A. Presnell¹, A. Ramachandran², C. Muralidhara³, M. von Mehren³
¹Portland VA Medical Center and OHSU Knight Cancer Institute; ²AROG Pharmaceuticals, LLC, Dallas, TX;
³Fox Chase Cancer Center, Philadelphia, PA.



PDGFRA MUTATIONS ACCOUNT FOR 5-8% OF GISTS

- The D842V mutation (encoded by exon 18), is found in up to two-thirds of GIST patients with primary PDGFRA mutations, but can also develop as a secondary drug resistance mutation. This gain-of-function mutation results in auto-phosphorylation and constitutive activation of PDGFRA kinase activity.
- Current drug therapies for GIST such as imatinib, sunitinib, sorafenib and nilotinib have no effect on GIST with the D842V mutation at clinically achievable concentrations.
- An international survey of GIST referral centers for patients with the PDGFRA D842V mutation, documented that none of the nineteen assessable patients had an objective response to imatinib. The median progression-free survival was only 2.8 months. The median survival was only 12.7 months, which is much shorter than the median survival of imatinib-sensitive GIST patients which is greater than 3 years.

PATIENTS WITH D842V MUTATIONS IN GIST DO NOT RESPOND TO IMATINIB OR SUNITINIB

Therapy	Trial	Patients who responded
Imatinib	B222 phase II	0/3
Imatinib	EORTC phase III	0/4
Imatinib	US phase III	0/4
Sunitinib	Phase I/II	0/4*

*3 patients with primary PDGFRA D842V mutations, 1 patient with a primary exon 12 mutation and a secondary exon 18 D842V mutation

Table 1. Clinical responses to imatinib or sunitinib in patients with D842V mutation

CRENOLANIB BESYLATE (CP-868,596-26)

- Oral, mutant specific inhibitor of PDGFR α
- Crenolanib has demonstrated activity in inhibiting the phosphorylation of PDGFR α in murine glial cells retrovirally mediated to overexpress PDGFR α .⁵
- Crenolanib has been evaluated in Phase I⁶ (single agent) and Phase Ib⁷ (in combination with axitinib and docetaxel) trials.

RECOMBINANT PDGFR α ASSAY

The activity of crenolanib against recombinant PDGFR D842V kinase was determined using a commercially available kinase screening service (Millipore IC50 profiler).

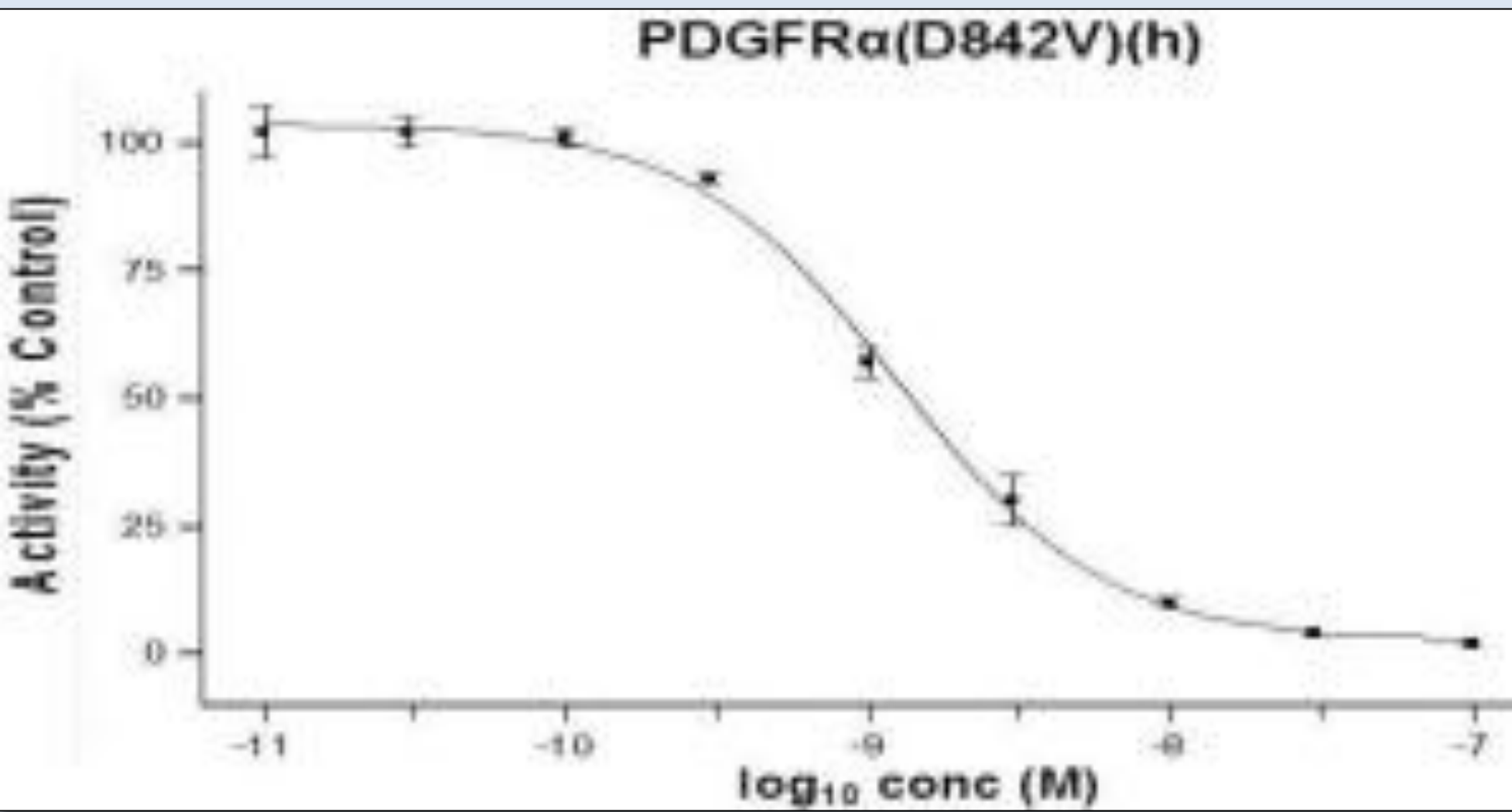


Figure 1. IC₅₀ Profiler results from Millipore demonstrate that crenolanib has an IC₅₀ of 1nM against recombinant human PDGFR α D842V kinase, Data are expressed as a percentage of the residual kinase activity compared with mock treated kinase.

CELLULAR ASSAY WITH TRANSIENTLY TRANSFECTED CHO CELLS

PDGFRA mutations were cloned by site-directed mutagenesis and all mutations were confirmed by bidirectional sequencing. CHO cells were transiently transfected with plasmids encoding cDNAs for wild-type or mutant proteins. Transfected cells were treated with either imatinib or crenolanib for 90 minutes in concentrations ranging from 0 to 1000 nM, in media containing 15% fetal bovine serum. The activation status (phosphorylation) of the PDGFR α protein was assayed by immunoprecipitation using an anti-PDGFR α antibody, followed by sequential immunoblotting for phospho-PDGFR α (using anti-phosphotyrosine antibody) or total PDGFR α (anti-PDGFR α monoclonal antibody).

PDGFR α Mutation	Crenolanib (nM)		Imatinib (nM)	
	IC ₅₀	IC ₉₀	IC ₅₀	IC ₉₀
D842V	9	44	>1000	>1000
V561D+D842V	40	100	>1000	>1000
T674I+D842V	70	205	>1000	>1000
V561D+T674I	>1000	>1000	>1000	>1000

Table 2. IC₅₀ and IC₉₀ values of crenolanib and imatinib in transient transfected CHO cells with various PDGFR α mutations.

CRENOLANIB INHIBITS THE ACTIVITY OF PDGFRA D842V MUTATION IN:

Transiently Transfected CHO cells

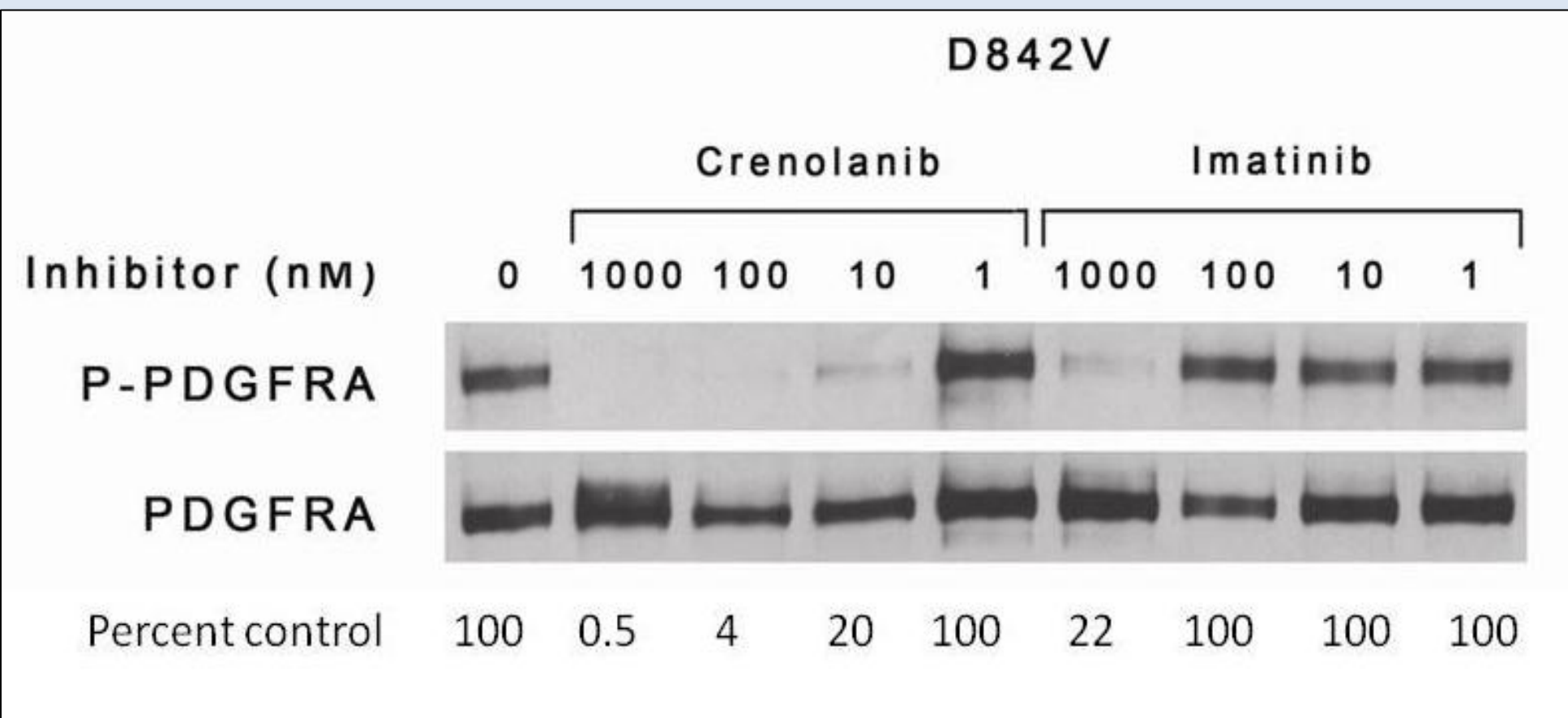


Figure 2. Inhibition of autophosphorylation of D842V mutant PDGFR α transiently expressed in CHO cells by crenolanib or imatinib. The biochemical IC₅₀ for inhibition of PDGFR α D842V transiently expressed in CHO cells by crenolanib was 10 nM.

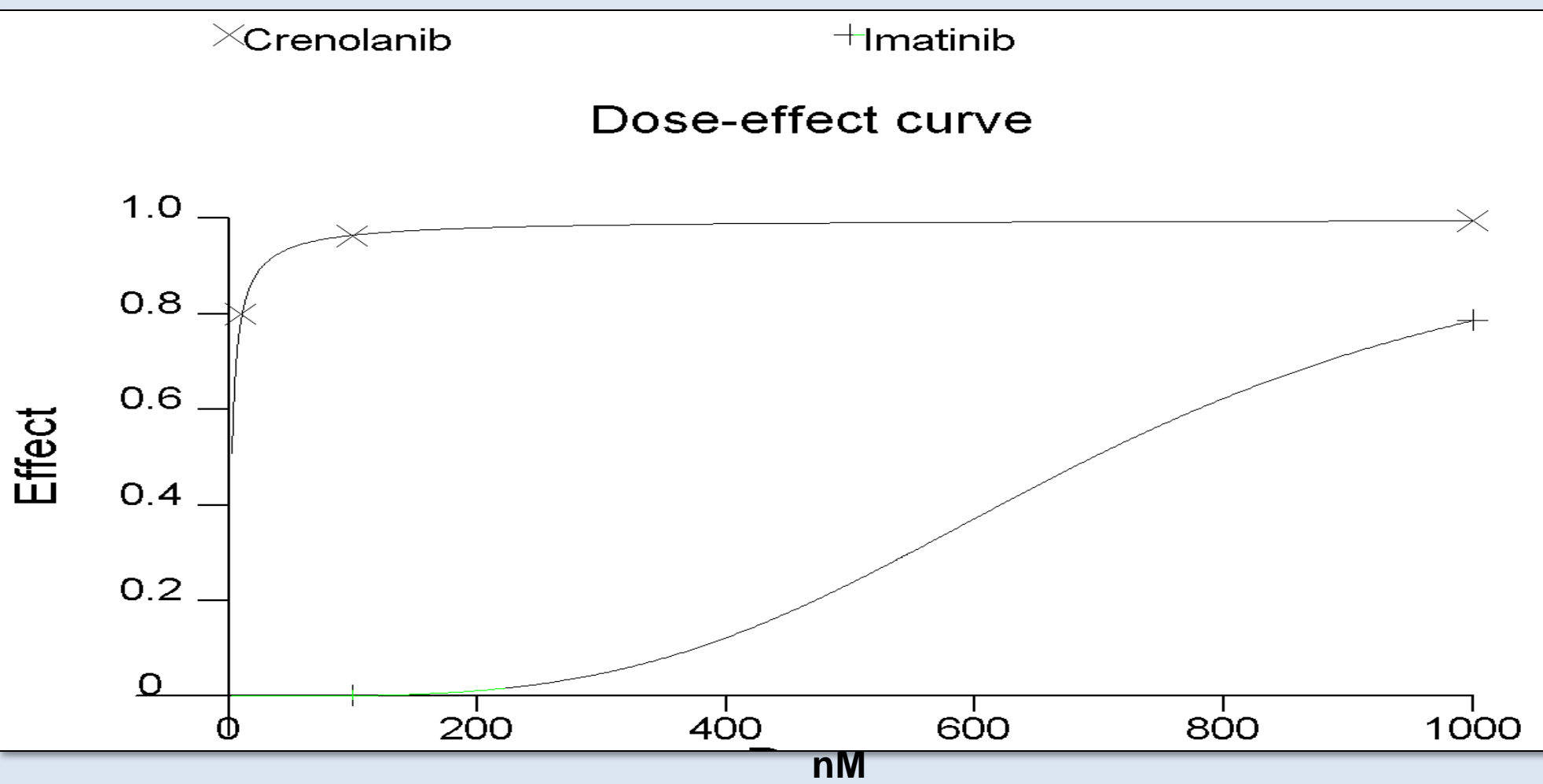


Figure 5. Representative data demonstrating that crenolanib is highly potent inhibitor of phosphorylation of D842V mutant PDGFR α in cells. The biochemical IC₅₀ for inhibition of PDGFR α D842V transiently expressed in CHO cells was 7 nM.

Stably Transduced Ba/F3 cells

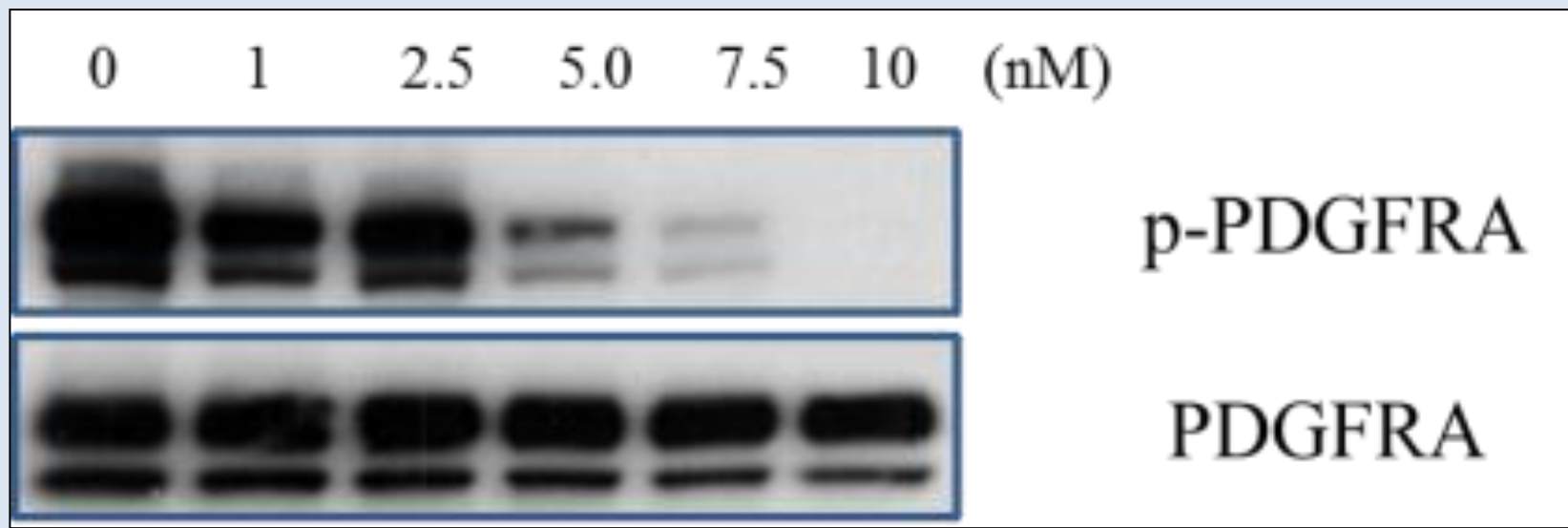


Figure 3. Western blot expression of PDGFR and p-PDGFR of PDGFR α D842V-transduced Ba/F3 cells after treatment with crenolanib. The IC₅₀ of crenolanib in PDGFR α D842V stably transduced Ba/F3 cells was 10 nM.

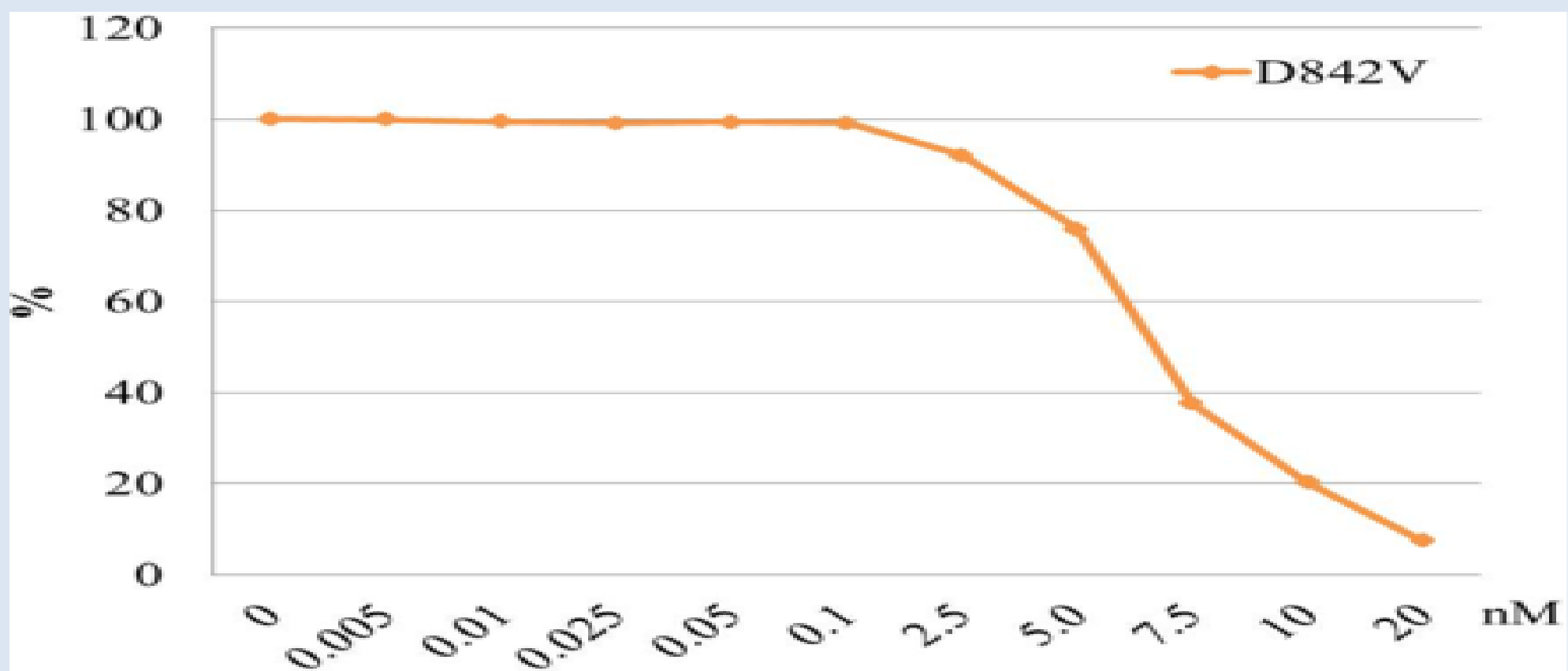


Figure 6. Proliferation assay of PDGFR α D842V-transduced Ba/F3 cells after treatment with crenolanib. Crenolanib has an IC₅₀ of 7.5 nM in these cells.

PRIMARY GIST PATIENT CELL LINES

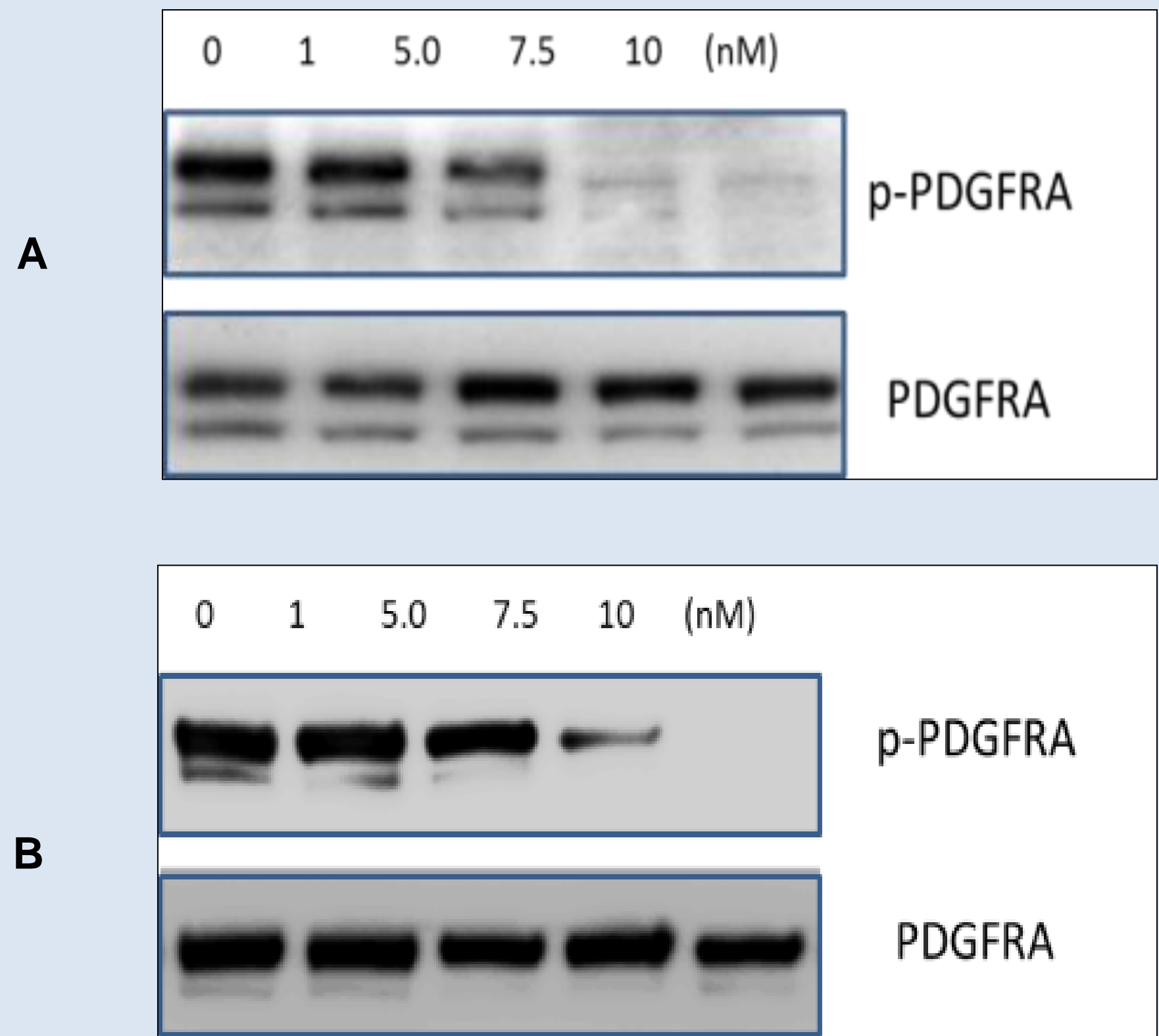


Figure 4. Western blot expression of PDGFR and p-PDGFR of two Imatinib-resistant primary GIST cell lines (A and B) after treatment with crenolanib. Inhibition of auto-phosphorylation is seen at 7.5-10 nM.

CONCLUSIONS

- Crenolanib inhibits PDGFR α phosphorylation at nanomolar concentrations in transiently transfected CHO cells, stably transduced Ba/F3 cells and primary GIST patient cell lines.
- Crenolanib is a unique TKI that blocks the kinase activity of PDGFR α D842V mutant at clinically achievable concentrations.
- Crenolanib may provide the first effective systemic therapy for GIST patients with primary or secondary PDGFR α D842V mutations as these activating mutations are clinically resistant to imatinib, sunitinib, and other commercially available tyrosine kinase inhibitors.
- A Phase II clinical study of crenolanib in patients with advanced gastrointestinal stromal tumors (GIST) with the D842V mutation in the PDGFR α gene has been initiated at Fox Chase Cancer Center and Oregon Health Sciences University (NCT01243346).

REFERENCES

- Debiec-Rychter et. al., Eur J Cancer 2006;42:1093-103.
- Heinrich et. al. J Clinical Oncol 2008;26:5352-9.
- Biron et. al. J Clin Oncol (Meeting Abstracts) 2010;28:10051.
- Heinrich et. al. J Clinical Oncol 2003;21:4342-9.
- AROG Pharmaceuticals, LLC. Crenolanib Investigator's Brochure, 2011.
- Lewis, N.L., et al., J Clinical Oncol, 2009. 27:5262-5269.
- Michael, M., et al., Br J Cancer, 2010. 103: 1554-1561.