PDGF Receptor Alpha (PDGFRα) Mutations in GIST

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1. What are the roles of PDGF receptors alpha and beta in normal tissues?

The platelet-derived growth factor (PDGF) family stimulates the proliferation, survival, and motility of connective tissue cells and certain other cell types.

GROWTH FACTOR	FUNCTION/ROLE	MEMBERS	RECEPTORS
PDGF	Cell growth and division, blood vessel formation, pericyte and smooth muscle recruitment and proliferation	PDGF-A, -B, -C, and -D, which combine to form the 5 active homodimers and heterodimers: PDGF-AA, -AB, -BB, -CC, and -DD	PDGF-α and -β

PDGF is expressed in variable levels in normal human tissues and organs. For instance, the highest expression of PDGF-A is found in the heart, skeletal muscle and pancreas. PDGF-B is expressed with the highest amounts in the heart and placenta, and moderate levels in other organs. PDGF-C is expressed with high levels in the heart, kidney, adrenal gland, and pancreas, and low levels in liver and ovary. No expression of PDGF-C can be detected in spleen or colon. The highest expression of PDGF-D is found in the heart, pancreas, and ovary and no detectable expression in the brain, lung and skeletal muscle.

PDGFRα is expressed in certain nervous system precursor cells (glial precursor), particularly in the myelinating support cells (oligodendrocytes) of the central nervous system. PDGFRβ is expressed on vascular smooth muscle cells and in pericytes, cells that surround and support developing vessels. The beta isoform of PDGFR plays an important role in angiogenesis (formation of blood vessels) including blood vessel maturation, recruitment of support cells surrounding blood vessels (pericytes) and pericyte survival.

2. Are abnormalities in PDGF receptors linked to GIST or other human diseases?

Mutations caused by amplification, over-expression, deletion, fusion, translocation, and point mutation in PDGF receptors have been directly implicated in the pathogenesis of solid tumors such as GIST as well as blood malignancies.

Focal amplifications of the genes harboring *PDGFRA* and resulting in overexpression of PDGFRα protein are frequently observed in adult patients with glioblastoma (GBM), an aggressive brain tumor and also in pediatric high grade gliomas including diffuse intrinsic pontine gliomas (DIPG), Table 1.

Table 1. PDGFRA (Chromosome 4q12) amplification.

In adult gliomas		**In pediatric gliomas**		
Study	PDGFRA gene amplification in GBM	Study	Glioma	PDGFRA gene amplification
Joensuu et al., 2005	29%	Zarghooni et al., 2010	DPIG	36%
TCGA publication, 2008	13%	Paugh et al., 2010	HGG	12%
Martinho et al., 2009	21.1%	Paugh et al., 2010	DIPG	29%
Verhaak et al., 2010	17%	Bax et al., 2010	HGG	15%
		Warren et al., SNO, 2009	DIPG	20%

Aside from brain tumors, amplification of the PDGF receptor gene has also been reported in about 7% of lung adenocarcinomas and 11% of lung squamous cell carcinomas. Fusion of the PDGF receptor gene with other genes has been found to be responsible for hematological malignances like hypereosinophilic syndrome and implicated in the development of chronic myelomonocytic leukemia (CMML).

PDGFRβ is important for angiogenesis and is over expressed in the supportive tissue of a number of solid tumors, including breast cancer, ovarian cancer and lung cancer leading to tumor progression. In contrast, tumor growth is restricted when messaging signals between the PDGFRβ and PDGF-B are disrupted as pericytes are not recruited to vessels and blood vessel cells grow in an unregulated manner thereby restricting tumor growth.

The development of most gastrointestinal stromal tumors (GISTs) is a result of mutations which result in excess activity of the KIT or platelet-derived growth factor receptor α (PDGFRA) genes. More than 80% of GISTs express mutated, unregulated active KIT, and another 5% to 7% express mutated PDGFRA; 10% to 15% of tumors have no associated mutations in these genes (Fig. 1). Mutations which result in activation of the PDGF receptor α mainly include point mutations and deletions.

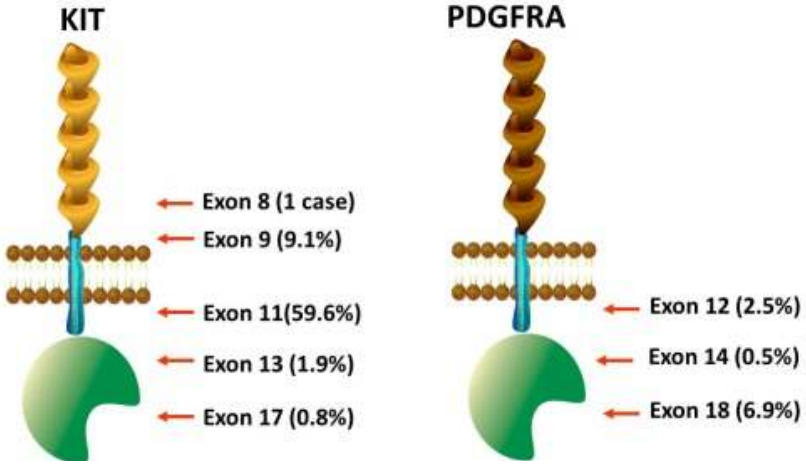

Figure 1. Mutations of KIT or PDGFRA found in GISTs are localized to certain exons (exons 8, 9, 11, 13 or 17 in KIT; 12, 14, or 18 in PDGFRA).

3. **Please compare PDGFRA-mutant GIST versus KIT-mutant GIST.**

For GIST, Surveillance, Epidemiology and End Results (SEER) analyses (2010) suggest an annual incidence of ~1,020 new cases of surgically resectable localized GIST in the US and a prevalence of ~4,260. Of these, the majority (>80%) express mutated KIT, about 5-7% have mutated PDGFRA, and up to 15% of tumors have no mutations in either KIT or PDGRFA.

KIT and PDGF display extensive structural similarity and are members of the receptor family called type III tyrosine kinase (TK) receptors. Activation of TK type III receptors occurs by binding of a receptor-specific target protein called a ligand which leads to phosphorylation of other target proteins and subsequently activates networks of other pathways that regulate important cell functions. Pathologic activation of TK receptors correlates with enhanced proliferation of cells and development of cancer. Specifically inhibiting such receptor pathways is considered an important therapeutic approach in modern oncology.

The mutational activation of KIT or PDGFRA is mutually exclusive as they represent two different alternative cancer-causing events leading to similar biological consequences. Recent studies have demonstrated that although there are similarities between PDGFRA-mutant and KIT-mutant GISTs at the molecular level, a number of clinicopathologic differences exist between these tumors. GISTs with PDGFRA mutations represent a subset of tumors almost exclusively occurring in the gastric (stomach) location Most of these tumors show a so-called "epithelioid" morphology usually have low cell division activity and most follow a more indolent clinical course. Microscopically, PDGFRA-mutant tumors are usually detected because of weak or negative KIT (CD117) staining by immunohistochemistry (IHC) on the tumor specimen although IHC detection of PDGFRA mutation does not correlate well with mutational status as suggested by recent analyses (ASCO 2009, abs 10558 and Path Intl 60(11):707, 2010). Furthermore, the gene expression profiles of PDGFRA mutant tumors cluster separately from most KIT-mutant tumors.

4. **What is the significance of the PDGFRA D842V mutation in GIST? Is there an analogous mutation in KIT linked to human disease?**

The D842V mutation in exon 18, which accounts for ~60% of all PDGFRA mutations known in GISTs, has shown primary resistance in vitro to all commercially available TK inhibitor drugs imatinib, sunitinib, and nilotinib. The D842V mutation in PDGFR is homologous to the D816V mutation in KIT which is well established as being resistant to imatinib in vitro. For both of these imatinib-resistant mutations the same aspartic acid residue is conserved in the kinase activation loop, suggesting a common basis for imatinib resistance in KIT and PDGFRA oncoproteins activated by this mechanism.

Several large trials have analyzed the results of responsiveness to TK inhibitor therapy in GIST patients with the D842V mutation.

- In the U.S-Finnish B2222 phase II trial, three patients with *PDGFRA* D842V mutations were identified. Two had progressive disease, and one was not assessable for response.
- In the EORTC phase III trial 4 patients with known *PDGFRA* D842V mutations had no response to imatinib.
- In the US SWOG/CALGB phase III study, four patients were treated with imatinib, three of whom progressed within 2 months and a fourth who had stable disease.
- At ASCO 2009 an international survey of several GIST referral centers summarized data for patients with advanced D842V mutated GIST who were treated with imatinib therapy. Of 19 assessable patients, none had complete or partial response, 5 patients (26%) had stable disease and 14 patients (74%) progressed. Median progression-free survival was short at 2.8 months, with a median overall survival of 12.7 months.

Thus, the D842V mutation confers primary resistance to available tyrosine kinase inhibiting drugs.

GISTs with secondary D842V mutation have been found to develop resistance to standard therapy probably as the result of accumulated genetic events. For instance, in a patient with imatinib sensitive GIST caused by exon 12 V561D mutation that developed a secondary D842V mutation, further treatment with imatinib was ineffective. In the phase I/II trials of sunitinib, there were no responses in the 3 patients with primary *PDGFRA* D842V mutations, or in the one patient with a primary exon 12 mutations who had a secondary exon 18 D842V mutation.

5. What is the current clinic management protocol for D842V mutant GIST and how does this compare to protocols used for KIT-mutant GIST?

Surgery remains the treatment of choice for localized GIST with no effective role for standard chemotherapy (excluding TKI drugs) or radiation. Tumors which are advanced, recurrent and unresectable require consideration of mutational analysis to select effective therapy. Tumor analysis for KIT mutation either by IHC or tissue genotyping is usually performed at the time of excision or biopsy to confirm the diagnosis of GIST. For such KIT mutant tumors imatinib is used as first line systemic therapy. When disease progresses on imatinib, sunitinib is used as a second line treatment.

However, 5-7% of patients with GIST harbor the D842V mutation in PDGFR and will demonstrate primary (<180 days of therapy) resistance to imatinib therapy or secondary (>180 days of therapy) resistance such as in GIST with primary exon 12 V561D mutation with subsequent D842V mutation. This latter scenario of secondary mutations may be observed in ~80% of progressing cases.

No effective systemic options are currently available as therapy for this group of PDGFR mutant patients. The current use of imatinib and sunitinib for most GIST is not an effective approach for patients with known D842V mutation. A new drug, crenolanib (CP-868,596), is just entering clinical trials to assess its effects for this drug resistant population.

6. **Why is the PDGFRA D842V mutation resistant to most of the current TKIs?**

It is not explicitly known the mechanism by which PDGFRA exon 18 D842V mutation is resistant to TKI therapy. It is thought that the D842V mutation interferes with a swinging movement of the activation loop that is associated with a conformational shift of the adenosine triphosphate (ATP) binding pocket from an ‘‘open’’, or active, conformation to a ‘‘closed’’ , or inactive, conformation. As imatinib binds selectively to the closed conformation of the kinase, substitutions at the PDGFRA gene at exon 18 842 (D842V) effectively reduce the accessibility of the ATP pocket and thereby confer relative resistance to the TKI drugs.

Interestingly, not all amino acid substitutions at PDGFRA 842 are equivalent at inducing primary drug resistance. For instance, the D842Y isoform of PDGFRA is more sensitive to imatinib than the D842V isoform.

7. **What are emerging experimental strategies that may benefit patients with PDGFRA D842V driven GIST?**

Crenolanib (CP-868,596) is a highly selective and potent inhibitor of PDGFR-α which is 100-500 times more potent as an inhibitor of PDGFR-α and PDGFR-β as compared to existing tyrosine kinase inhibitors like imatinib or sunitinib. Crenolanib (CP-868,596) has demonstrated preclinical activity against both primary and secondary D842V mutations in experiments conducted by Dr. Michael Heinrich at Oregon Health Sciences University.

There is currently no treatment available for GIST patients with PDGFRA D842V mutation. The phase II clinical trial (NCT01243346) is designed to evaluate the antitumor efficacy of crenolanib (CP-868,596) in patients with advanced gastrointestinal stromal tumors characterized by the D842V mutation in the PDGFRA gene. Both subsets of patients with GIST due to primary (D842V) mutation or secondary mutation (V561D + D842V) may participate to assess the drug's therapeutic benefit.

8. **When should a patient suspect that he/she may have a PDGFRA driven GIST, and what steps should he/she take?**

Suspicion of a PDGFRA mutant GIST can arise on multiple levels. Anatomically, approximately 90% of PDGFRA-mutant GISTs arise in the stomach whereas GISTs harboring mutations in KIT exon 9 are almost exclusively found in the small intestine. Histologically such tumors have been observed to have a more characteristic epithelioid

morphology, while spindle cells dominate most KIT-mutant tumors. In addition, KIT (CD117) expression as detected by immunohistochemistry is strongly present in approximately 95% of KIT-mutant tumors. In contrast, up to 40% of PDGFRA-mutant tumors are weak or negative for KIT which should lead to additional testing for PDGFRA. Mutational analysis is strongly recommended to define the specific type of PDGFRA mutation as the appropriate treatment for GIST varies according to the mutation status of the tumors.

9. What preclinical results suggest that the drug crenolanib (CP-868,596) may be effective against D842V mutant GIST?

Dr. Michael Heinrich at Oregon Health & Science University has established a preclinical model to study the biochemical consequences of somatic PDGFRA mutations by transient expression of wild-type and mutant PDGFRA cDNA constructs in Chinese hamster ovary (CHO) cells. In this model system the expression of PDGFRA D842V kinase has been associated with resistance of CHO cells to growth inhibition by commercially available TK inhibitors imatinib, sunitinib, nilotinib and sorafenib. These data thus parallel the clinical data which describe the lack of response to imatinib and sunitinib of patients with D842V mutant GIST.

In the same system, crenolanib (CP-868,596) has also been evaluated using imatinib as a comparator. Consistent with previous results, imatinib at clinically achievable serum concentrations had little to no effect on the activity of D842V mutant PDGFRA kinase, as assessed by measurement of PDGFRA autophosphorylation. In contrast, crenolanib (CP-868,596) was extremely potent at inhibiting autophosphorylation in PDGFRA D842Vmutant CHO cells with an IC_{50} of few nanomolars. Crenolanib (CP-868,596) was also similarly effective against the double mutants (cells containing two point mutations) PDGFRA V561D + D842V and PDGFRA T674I + D842V in inhibiting cell growth while imatinib had no activity against these particular double mutant PDGFRA kinases.

The preclinical data described above demonstrate the unique activity of crenolanib (CP-868,596) at inhibiting the kinase activity of D842V mutant PDGFRα, a GIST subset that is resistant to treatment by the approved drugs imatinib and sunitinib. It is hypothesized that the inhibition of PDGFRα phosphorylation will prevent downstream signaling and block tumor cell proliferation and survival. Based on these encouraging preclinical data and previously performed phase I trials, AROG Pharmaceuticals is in process of initiating a phase II trial in patients with metastatic GIST harboring the D842V mutation in the PDGFRA gene.

This Phase II trial will be conducted at Fox Chase Cancer Center, Philadelphia, PA (Dr. Margaret von Mehren) and Oregon Health & Science University (Dr. Michael Heinrich). The anticipated start date for these studies is April, 2011.