A novel oncogenic *PDGFRB* variant in severe infantile myofibromatosis with response to imatinib utilizing therapeutic drug monitoring

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Abbreviations:

IM	Infantile myofibromatosis
PDGFRB	Platelet-derived growth factor receptor β
MAPK	Mitogen-activated protein kinase
STAT	Signal transducer and activator of transcription
TKI	Tyrosine kinase inhibitor
NGS	Next-generation sequencing

Infantile myofibromatosis (IM) is a rare benign neoplastic condition characterized by proliferation of myofibroblast cells throughout the skin, soft tissues, bones, and visceral organs.[1] Most cases are identified before the age of 2, with a median age of 3 months at diagnosis.[2] IM has historically been grouped into three main categories: 1) solitary myofibromatosis, 2) multicentric myofibromatosis without visceral involvement, and 3) multicentric myofibromatosis with visceral involvement.[3] Generally, a favorable prognosis is seen in solitary and multicentric IM without visceral involvement, with frequent spontaneous regression. However, multicentric myofibromatosis with visceral involvement is associated with multiple complications and high mortality rates.[1-3]

Activating mutations in the platelet-derived growth factor receptor beta protein (PDGRFβ, encoded by the *PDGFRB* gene) have been implicated in many cases of both sporadic and autosomal-dominant IM.[3-5] PDGFRβ belongs to the receptor-tyrosine kinase family of transmembrane proteins and is composed of 5 Ig-like extracellular domains, a helical transmembrane domain and an intracellular part that contains the tyrosine kinase domain.[6] Upon binding to its ligands PDGF-BB or PDGF-DD, PDGFRβ dimerizes, transphosphorylates, and activates downstream signaling pathways, including the RAS-mitogen-activated protein kinases (MAPK), signal transducers and activators of transcription (STATs), phosphoinositide 3-kinase (PI3K) and phospholipase Cɣ (PLCɣ).

Chemotherapy has been used for treatment of IM with visceral involvement to varying degrees of success. Reported chemotherapy regimens include prednisolone, 2-chlorodeoxyadenosine, vinblastine/methotrexate, vincristine/dactinomycin ± cyclophosphamide, antiestrogen therapies, and α-interferon. Unfortunately, significant toxicities are associated with these regimens including myelosuppression,

nausea, transaminitis, nephrotoxicity, and alopecia.[2,7,8] Therapies targeting PDGFRβ provide promising new options for these patients with the potential for higher rates of response with fewer toxicities. Several tyrosine kinase receptor inhibitors (TKIs) such as imatinib, sunitinib, and sorafenib have been reported to induce objective responses with generally tolerable toxicity profiles.[9-12] Here, we report our experience using imatinib with therapeutic drug monitoring to treat a child with multicentric IM with visceral involvement secondary to a novel *PDGFRB* variant. This novel mutation was further characterized as likely disease-causing and sensitive to imatinib.

Consent was obtained from this patient's legal guardian to publish this case report.

Case presentation

The patient was a term gestation Caucasian female who presented at 6 weeks of age to a pediatric dermatology clinic for assessment of subcutaneous nodules identified since birth. Due to ill appearance she was emergently transferred to the emergency department where she was found to have hyponatremia, hyperkalemia, leukocytosis, thrombocytosis, signs of shock, and superior vena cava syndrome requiring pediatric intensive care unit admission.

Physical exam demonstrated diffuse subcutaneous nodules on the abdomen, extremities, and labia ranging in size from 1 to 10 mm. A punch biopsy of one lesion demonstrated benign spindle cell proliferation of myofibroblasts admixed with bundles of collagen fibers, consistent with a myofibroma (Figure 1A). Fluorescent *in situ* hybridization was negative for *PDGFRB* fusions, and next generation sequencing (NGS) identified a likely pathogenic but previously unreported deletion insertion variant (p.1535_A540delinsVPSWP) in the *PDGFRB* gene.

Throughout admission, the patient experienced ongoing abdominal distension and enteral feeding intolerance with associated electrolyte derangements, requiring gastric decompression via low-intermittent wall suction.

Esophagogastroduodenoscopy (EGD) and colonoscopy were performed at two months of age demonstrating biopsy-confirmed myofibromas within the stomach, duodenum, and colon leading to proximal duodenal and distal colonic obstructions (Figure 1B-F).

Due to visceral involvement while awaiting results of *PDGFRB* sequencing, systemic treatment was initiated at two months of age with weekly methotrexate 1 mg/kg/dose and vinblastine 0.17 mg/kg/dose. At three months of age, she developed a symptomatic bowel obstruction; therefore, prior to receiving results of *PDGFRB* sequencing, and after failing two cycles of vinblastine/methotrexate, she was empirically started on imatinib 11.3 mg/kg/day.

Plasma imatinib trough levels were monitored to assess drug absorption given gastrointestinal IM involvement. A goal imatinib range of 1100-3000 ng/mL was extrapolated from published outcomes and toxicity data in gastrointestinal stromal tumor patients.[13,14] Initially, the infant had difficulty tolerating time off gastric suctioning following imatinib administration and imatinib levels were consistently low or undetectable despite dose escalation. To increase imatinib residence time for improved systemic absorption, intravenous famotidine, pantoprazole, and octreotide were initiated to decrease gastric secretions. Eventually pantoprazole was transitioned to esomeprazole for added benefit of inhibiting cytochrome P450 3A4, the primary pathway responsible for imatinib metabolism.[15] After these interventions and additional imatinib dose escalation, the infant could tolerate up to 60 minutes off gastric suction post-drug administration, allowing adequate time for drug absorption.

Goal imatinib levels were achieved and sustained on approximately 20 mg/kg/day, divided BID to minimize drug volume (Figure 2).After 6 weeks of therapeutic imatinib levels, the patient's proximal small bowel obstruction and gastric myofibromas had improved. Two months later, cytotoxic chemotherapy was discontinued due to consistently therapeutic imatinib levels. With improving GI absorption secondary to resolution of obstruction and after surgical resection and re-anastomosis of fibrotic obstructed bowel, imatinib dosing was gradually reduced to approximately 9.9 mg/kg/day with appropriate trough levels in range. She was discharged at 17 months of age after 528 days of hospitalization.

Given pronounced improvement, she was initiated on a slow imatinib wean outpatient to mitigate growth failure and other adverse effects of TKI treatment.[10,16,17] A treatment wean was initiated in consideration of the self-resolving nature of many cases after treatment, even despite visceral involvement.[2,18] Such weans of TKI have been previously attempted with variable success, however, when unsuccessful, rescue with TKI administration has been appropriate and effective.[12] There are no clear guidelines for wean or discontinuation of TKI use in IM. This patient is currently tolerating her wean under the supervision of her primary oncology team.

Molecular analyses

A solid tumor NGS panel was performed by the Genomic and Pathology Services laboratory at the Washington University School of Medicine on the formalin-fixed, paraffin-embedded tissue from the skin punch biopsy.[19] The following variant in *PDGFRB* (NM_002609.3) was identified: c.1603_1618delins13, (p.I535_A540delinsVPSWP), with the inserted nucleotides being GTGCCATCCTGGC.

To characterize the effects of PDGFRβ:p.I535_A540delinsVPSWP, we introduced the mutation in the coding sequence of *PDGFRB*. We first tested the effect of the variant on the expression of the receptor. Figure 3A showed that the mutated receptor was expressed in transfected cells, although the fully glycosylated heavier form of the receptor was reduced, suggesting a perturbation of receptor trafficking through the Golgi. We investigated the expression of the p.I535_A540delinsVPSWP mutant by flow cytometry, which showed that the mutated receptor was present at the cell surface, albeit at a lower level than the WT receptor (Figure 3C).

To characterize the signaling capacities of the p.I535_A540delinsVPSWP mutant, we assessed the phosphorylation of the receptor and STAT3 by western blot. The results showed that the mutant was constitutively phosphorylated and induced a statistically significant phosphorylation of STAT3 (Figure 3A and 3B). The activity of the p.I535_A540delinsVPSWP receptor was tested using two luciferase reporter assays, which measures activation of the MAPK and STAT pathways in response to PDGFRβ signaling.[20] Congruent with the western blot results, the variant constitutively activated the receptor in the absence of a ligand, similarly to the p.N666K substitution previously identified in a myofibroma (Figure 4A and B).[21,22] The mutated receptor remained sensitive to imatinib.

DISCUSSION

In this study, we report a novel *PDGFRB* variant identified in an infant with multicentric IM with visceral involvement. IM with visceral involvement can impart significant morbidity and mortality onto patients, particularly with gastrointestinal tract or pulmonary involvement.[1-3] Cytotoxic chemotherapy has improved outcomes in these patients. However, responses to chemotherapy can be variable, and the toxicity burden can be significant depending on the regimen selected. Given the established relationship between *PDGFRB* activating mutations and IM, targeted therapies against PDGFRβ offer promising opportunities for improved outcomes and treatment with decreased toxicity.[4,5,10,20,23]

We demonstrate that the p.I535_A540delinsVPSWP mutant is activated in a ligand-independent fashion, which corroborates its oncogenic role in disease. The mutation affects PDGFRβ expression on the cell surface by flow cytometry and induces a decrease in glycosylation compared to the WT receptor, suggesting that this variant impairs PDGFRβ maturation. Similar findings have also been reported in other class III receptor tyrosine kinases, including PDGFRA.[24-26] The p.I535_A540delinsVPSWP variant is located at the N-terminus of the transmembrane domain, which stabilizes PDGFRβ in the membrane and promotes receptor orientation and activation. Mutations in this domain remain rare oncogenic events in receptor tyrosine kinases, unlike those in the juxtamembrane and tyrosine kinase domains.[20,23] These transmembrane variants might constitutively activate receptors by altering their conformation, promoting their dimerization and activation. Further studies are needed to determine the impact of PDGFRβ transmembrane mutations on receptor localization and mechanism of activation.

Our results show that the mutant is sensitive to imatinib, which has extensive safety data in pediatric patients and has been reported in the successful treatment of infantile myofibromatosis and *PDGFRB*-associated syndromes.[10,27,28] Our study suggests the utility of careful monitoring of plasma imatinib trough levels to achieve therapeutic effect in the setting of a patient with significantly altered GI function.

Given the rarity of IM, it is difficult to perform robust studies evaluating and standardizing the use of TKIs. Little information is available describing ideal length of treatment or methods and timing for cessation or weaning of TKI therapy. Recurrence of disease in adulthood has been reported in patients affected as infants with *PDGFRB* mutant IM, leaving unanswered questions regarding surveillance after cessation of therapy.[29] While the utilization of TKIs offers promise in IM management, further investigation is required for optimizing efficacious treatment.

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FIGURE LEGENDS

Figure 1 Histology of stomach pylorus and skin with identifying features of myofibromatosis. (A) Skin biopsy demonstrating benign spindle cell proliferation in the dermis consistent with myofibroma. (**B**) Tissue from the stomach pylorus demonstrating spindle cell proliferation in the lamina propria of the pylorus (H&E x400). **(C)** Immunohistochemistry of pylorus for smooth muscle actin highlighting spindle cell proliferation in the lamina propria of the pylorus (SMA x400). Images from diagnostic esophagoduodenoscopy demonstrating significant partial obstruction in the **(D)** pylorus, **(F)** duodenum, and **(G)** rectum.

Figure 2 Imatinib dose over time with corresponding imatinib plasma trough values. Goal trough value was 1100 ng/mL. Due to poor oral tolerance and absorption, increases in dosing were required to achieve adequate imatinib trough values. With improved absorption and decrease in disease burden, final dosing to maintain appropriate levels was 9.9 mg/kg/day. Drop in dose and trough level around October 2020 correlated with drug cessation due to surgical resection of fibrotic intestinal obstruction. Maximum value for trough assay was 3000 ng/mL.

Figure 3 PDGFRβ p.I535_A540delinsVPSWP is expressed and constitutively phosphorylated. See supplemental information for methodological details. **(A)** HEK293T cells were transiently transfected with the empty vector, PDGFRβ WT or p.I535_A540delinsVPSWP. Cells were lysed and the total lysates were analyzed by western blot with the indicated antibodies. One representative experiment out of three is shown. (**B**) STAT3 phosphorylation level was quantified using Fusion Edge. The

results were normalized to the PDGFRβ WT value. The histograms represent the mean of three independent experiments, with the standard deviation indicated (Student's t-test; *$P < 0.05$). **(C)** Cells were stained with a phycoerythrin-labeled anti-PDGFRβ antibody and analyzed by flow cytometry. One independent experiment out of two is shown.

Figure 4 PDGFRβ p.I535_A540delinsVPSWP is constitutively activated and sensitive to imatinib. Porcine aortic endothelial cells were transiently co-transfected with plasmids expressing the WT or mutated receptor, and luciferase reporter controlled by STAT transcription factors **(A)** or MAP Kinases **(B)**. The oncogenic N666K variant was used as positive control. The results (mean of 3 independent experiments) were normalized on the untreated PDGFRβ WT (Student t-test; *$P < 0.05$;***$P < 0.0005$). The standard errors of the mean are shown.

Figure 1

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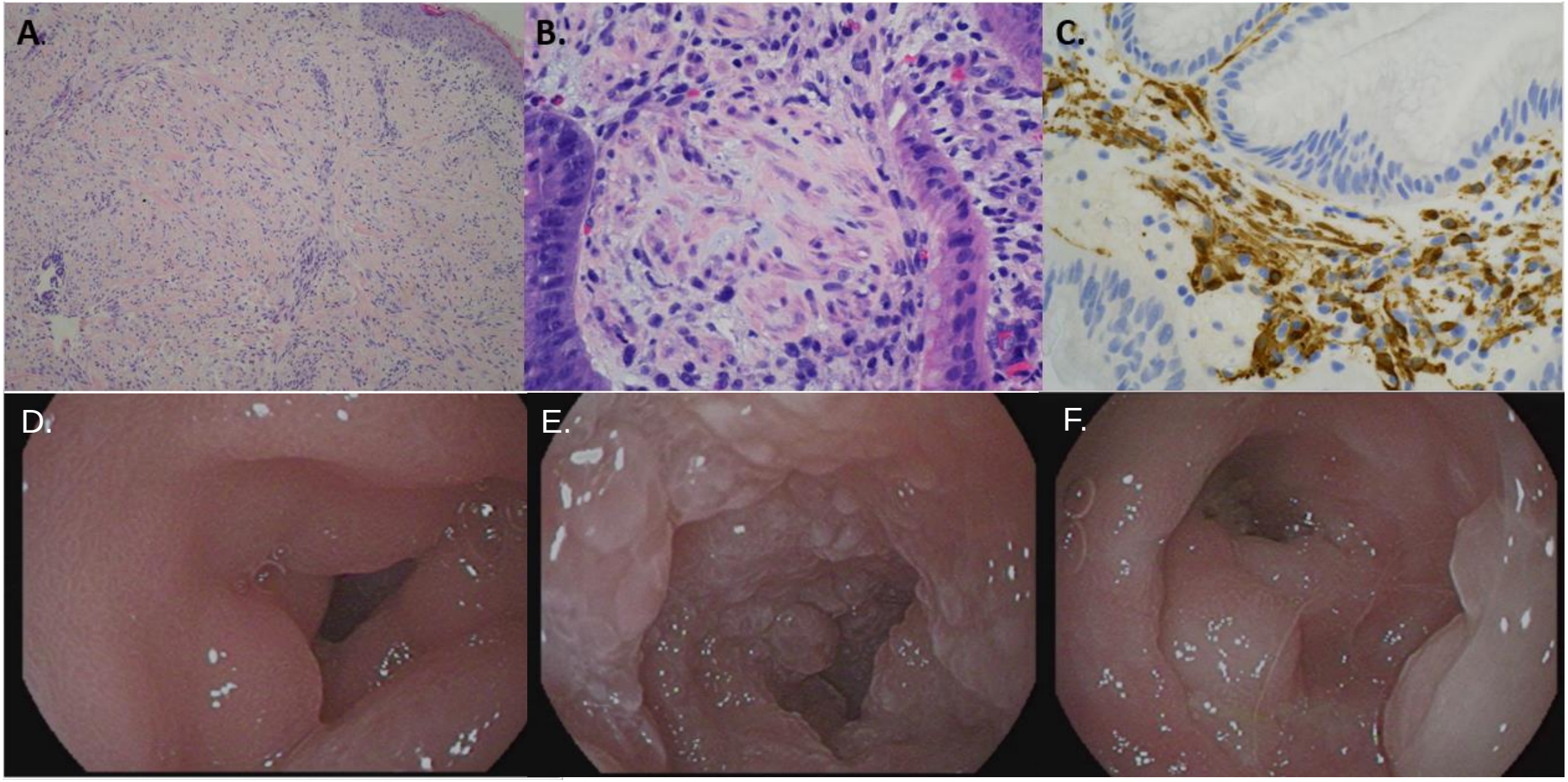

Figure 2

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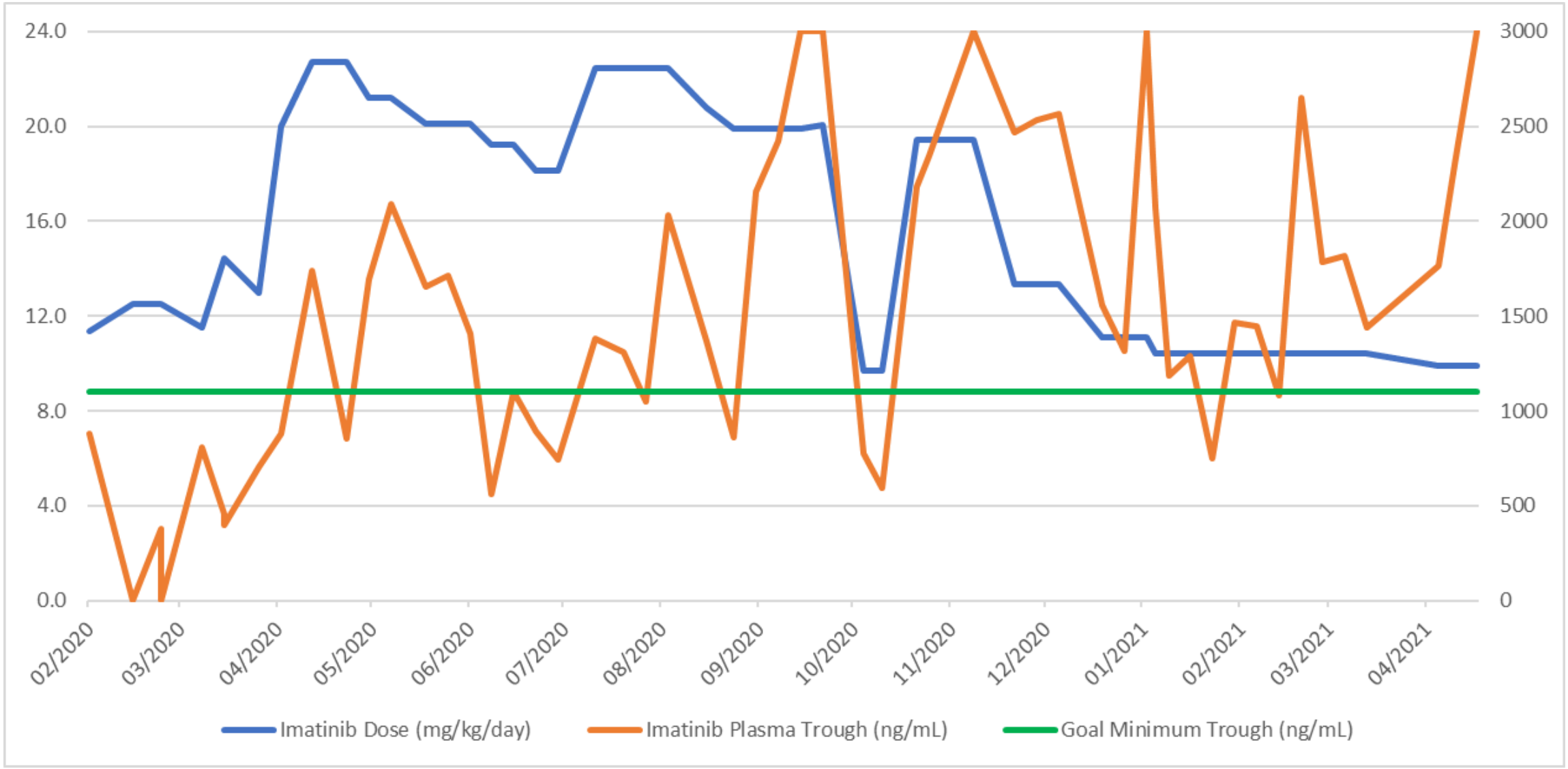

Figure 3

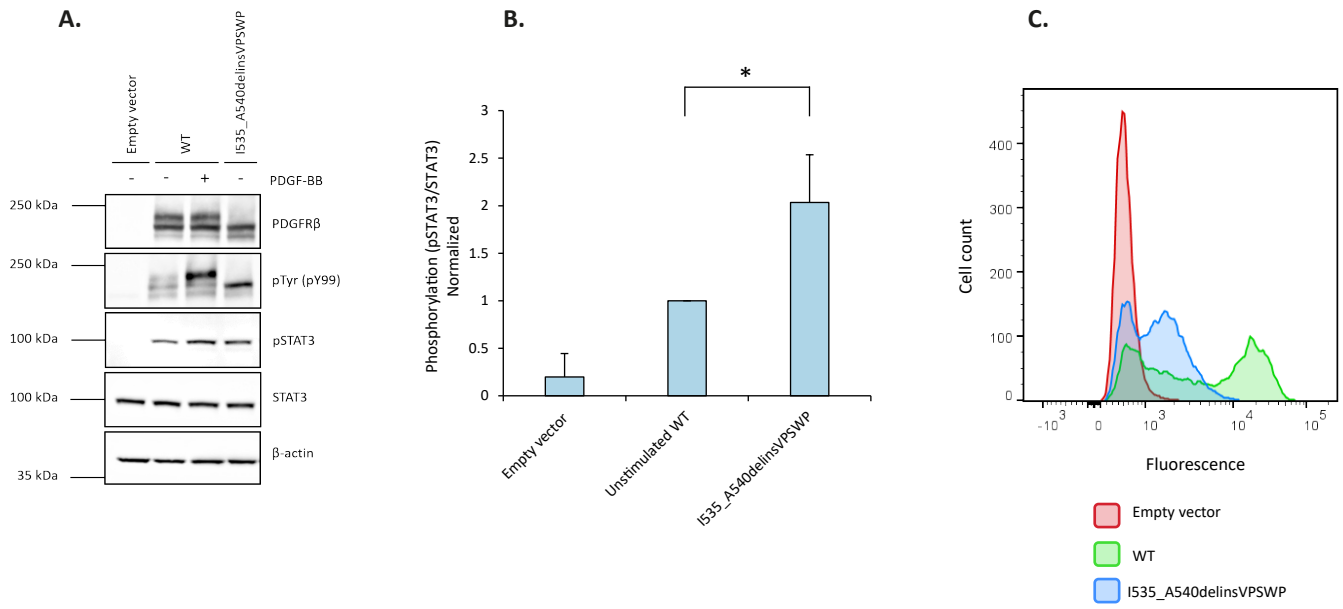

Figure 4