

Notch Inhibition in Desmoids: “Sure It Works in Practice, but Does It Work in Theory?”

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In a phase 1 trial of PF-03084014, an oral Notch inhibitor, 5 of 7 patients (approximately 70%) with desmoid tumors (DTs) had a partial response.¹ This was an unprecedented response in DTs. When the first DT patient enrolled in the study, there was no published evidence to support Notch inhibition in DTs. Nevertheless, this serendipitous discovery led to further studies. Biomarker studies in peripheral mononuclear cells showed a predictable decrease in *HES4* levels, a downstream target; however, pre- and posttreatment tumor biopsies were unrevealing.

Why did DT patients respond to the Notch inhibitor? Our usual approach to drug development is bench to bedside, but on occasion, bedside observations can in themselves lead to new insights into biology. In this issue of *Cancer*, Shang et al² attempt to provide preclinical insights into Notch inhibition in DTs. To get a better perspective on their important findings, it is helpful to review the biology of DTs and notch signaling.

DTs are rare with an annual incidence of 2 to 4 per million, and they affect adults in the third decade of life.^{3,4} They commonly arise in the extremities, abdominal wall, mesenteric root, and chest wall. They do not metastasize, but their natural history is variable and ranges from an asymptomatic, indolent course (with rare spontaneous regressions) to aggressive infiltration of neurovascular structures and vital organs resulting in pain, loss of function, organ dysfunction, and death. After surgical resection, they are firm, bland, and interspersed with blood vessels and infiltrate muscles and/or nerves (Fig. 1A). Microscopically, they are indistinguishable from a healing wound. They are composed of fibroblasts and blood vessels in a dense collagenous matrix (Fig. 1B).⁵ The malignant cell of origin is unknown, although evidence points to mesenchymal stem cells (MSCs).^{6,7} The hallmark of DTs is constitutive activation of the Wnt signaling pathway. Although gains and losses in chromosomes 8, 5, 6, and 20 have been infrequently reported, the DT genome is bland except for activating mutations in *CTNNB1* (>95%) or inactivating mutations in adenomatous polyposis coli (*APC*; approximately 3%), which are suspected to be the initiating events or oncogenic drivers.⁸ Therefore, in the context of a single mutation, it is difficult to explain the variable behavior of DTs. DTs respond to different classes of inhibitors that target inflammation (sulindac), estrogen (tamoxifen), cytokines (interferon- α), cell cycle (doxorubicin), multitargeted kinases (sorafenib and gleevec), Wnt (OMP-54F28), and Notch (PF-03084014).^{1,3,4,9,10} Recently, growth inhibition was seen with a hyaluronan (HA) inhibitor.¹¹ Attempts to identify biomarkers of response have been entirely unrevealing. In this context, how do we interpret the current data on Notch inhibition in DTs?

Briefly, Notch, Wnt, transforming growth factor B, and Hedgehog are critical pathways that have pleiotropic functions ranging from embryonic development to adult homeostasis. Notch is activated when one of several ligands (Delta-like 1 [DLL 1–3, Jagged 1 [JAG1], and JAG2) binds to 1 of 4 receptors (NOTCH 1–4). A 2-step proteolytic cleavage is first mediated by ADAMS10/17 and then by γ -secretase (GS) releasing Notch intracellular domain (NICD); a transcription factor, that activates *HES1* and others. PF-03084014 is a GS inhibitor that inhibits this step (Fig. 2).

In this article, Shang et al² evaluate the expression of NICD, JAG1, and Hairy and enhancer of split, induced by Notch (*HES1*) in a large collection of patient tissue microarrays. According to immunohistochemistry, there was significantly greater *HES1* staining in comparison with a normal scar, but surprisingly, this was not seen with NICD or JAG1. The status of the NOTCH1 receptor is not known, and the authors note that this is due to limitations of available NOTCH1 antibodies; however, an evaluation of the messenger RNA expression of *Notch*, *Hes1*, and *Jag1* would have

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The quotation in the article title has been attributed to the late Walter Heller, the economic advisor to President Kennedy who famously quipped, “An economist is a man who, when he finds something works in practice, wonders if it works in theory.”

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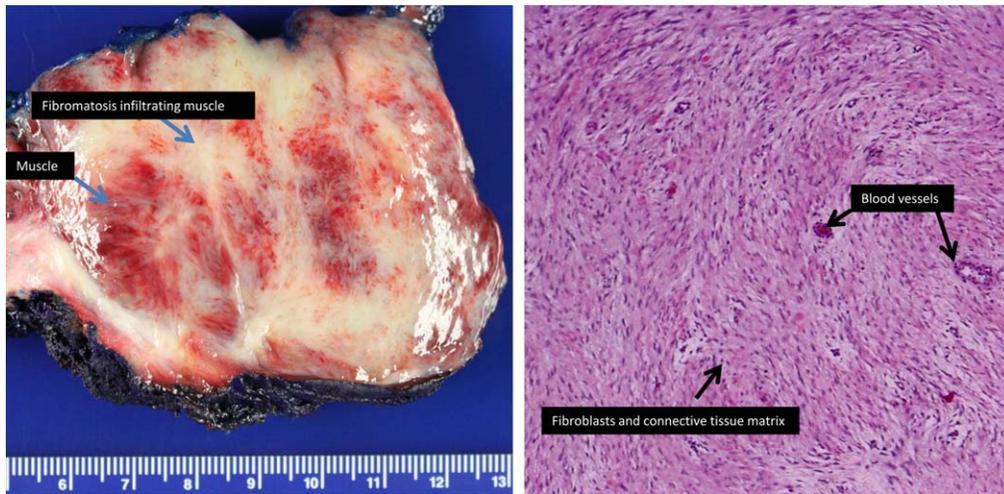


Figure 1. (A) Gross resection of a large infraclavicular desmoid tumor in a 20-year-old man with muscle infiltration and positive margins. (B) H & E stain showing monotonous fibroblasts with scattered blood vessels in a collagenous stroma. Courtesy of Dr. Meera Hameed (Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY).

been helpful. Downstream activation of *Hes1* without activation of upstream targets is a perplexing finding that raises the possibility of noncanonical pathway activation in which β -catenin can directly express *Hes1*.¹² In seven patient-derived DT cell lines, there was variable protein expression of NOTCH1 and JAG1. Although T-cell and myeloma cell lines were used as controls, expression levels in a fibroblast cell line would be helpful so that we could know whether this finding is simply reflective of a fibroblastic lineage. Although all DT cell lines showed expression of NOTCH1 and JAG1, only a subset (approximately 60%) showed expression of NICD. A convincing experiment for canonical notch activation was one determining whether *Hes1* expression was restricted to these cell lines. When cell lines were treated with PF-0384014, a smaller subset (approximately 30%) showed decreases in NICD and HES1. Given this, we would expect either growth arrest or apoptosis in both cell lines; however, there was significant growth arrest in only 1 (Desm14). Notch inhibition caused G₁ arrest in 1 cell line, which paradoxically showed low levels of NOTCH1 and JAG1 protein expression. In summary, in DT cell lines, there seems to be no clear correlation between Notch pathway activation and growth inhibition. An impressive finding is the effect of PF-0384014 on cell migration and invasion; however, the mechanism is not further explained. Finally, PF-0384014-treated cells were shown to differentially express 43 unique genes in 2 of 3 cell lines tested. With the use of analytical software, Wnt1-inducible signaling pathway protein 2 (*WISP2*), a

β -catenin target gene and putative tumor-suppressor gene, was identified as a possible link between Notch and Wnt pathways via integrins. As expected, cell lines treated with PF-0384014 showed upregulation of *WISP2* expression. We do not know the effect of altering *WISP2* expression in cell lines; this requires further validation in pre- and posttreatment biopsies from patients treated with PF-0384014.

This work by Shang et al² should be applauded for multiple reasons. First, they took a clinical observation and embarked to understand the underlying biology of a rare disease. Second, conducting research in DTs is extremely challenging and takes a unique level of commitment, as evidenced by the years of effort that it takes to construct a tissue microarray from hundreds of patients and to generate every cell line due to lack of good animal models. DT cell lines, like the disease itself, are heterogeneous and slow in growth media. The work presented here is thought-provoking and adds to the body of evidence, but it is not convincing that Notch activation is the smoking gun in all DTs.

There is a wide discordance between clinical and laboratory observations. For example, PF-0384014 resulted in tumor shrinkage (cell death) in approximately 70% of patients, whereas in the laboratory, no cell lines showed apoptosis, and only 1 (approximately 14%) showed a minimal decrease in growth inhibition. Similarly, patients treated with sorafenib inhibitor of vascular endothelial growth factor and platelet derived growth factor receptor have approximately 25% tumor shrinkage, but attempts to replicate this in cell lines have been unsuccessful. This

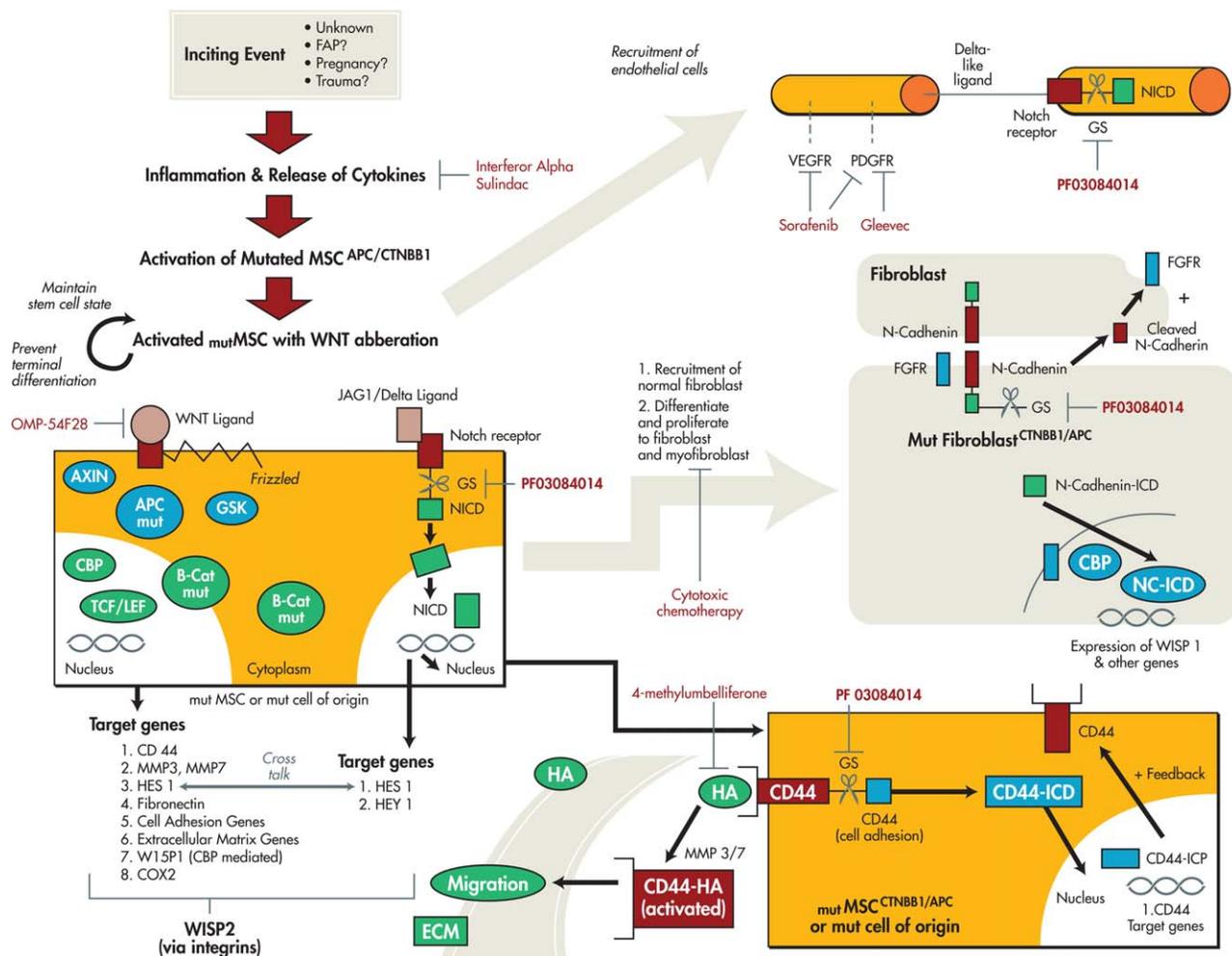


Figure 2. Proposed role of GS in DTs. An inciting event results in an inflammatory response that activates CTNNB1- or APC-mutated MSCs, which elicit the formation of a tumor stroma by 1) remaining in a stem cell state, 2) differentiating into fibroblasts harboring *CTNNB1/APC* mutations, and 3) recruiting normal fibroblasts and endothelial cells for blood vessel formation. Mutations in APC or β -catenin result in an accumulation of nuclear β -catenin and the transcription of genes in a Wnt ligand-independent manner. The Jag1 or delta-like ligand activates the Notch pathway by binding to the Notch receptor, which then undergoes proteolytic cleavage by GS and releases NICD, a nuclear transcription factor. MSCs have CD44, which is also activated by GS; this results in cleaved CD44, a nuclear transcription factor. Fibroblasts in DTs express *N-cadherin*, which together with cytoplasmic β -catenin forms a complex with actin and is involved in migration and invasion. Activation of *N-cadherin* is dependent on GS, and cleaved ectodomain can activate FGFRs in a paracrine manner; the cleaved cytoplasmic domain is a transcription factor that upregulates WISP1. Endothelial cells are part of all tumor stroma, and neoangiogenesis is dependent on vascular endothelial growth factor, platelet-derived growth factor, and Notch signaling (a GS-dependent event). PF-03084014 is a GS inhibitor that may have wide-ranging effects in DTs. APC indicates adenomatous polyposis coli; B-Cat, β -catenin; CBP, CREB-binding protein; COX2, cyclooxygenase 2; DT, desmoid tumor; ECM, extracellular matrix; FAP, familial adenomatous polyposis; FGFR, fibroblast growth factor receptor; GS, γ -secretase; GSK, glycogen synthase kinase; JAG1, Jagged 1; LEF, lymphoid enhancer factor; MMP, matrix metalloproteinase; MSC, mesenchymal stem cell; NICD, Notch intracellular domain; PDGFR, platelet-derived growth factor receptor; TCF, T-cell factor; VEGFR, vascular endothelial growth factor receptor; HA, hyaluronan; WISP, Wnt1-inducible signaling pathway protein. Courtesy of Miss Sydney Peterson, BS, Department of Medicine, Memorial Sloan Kettering Cancer Center.

raises an important question: can DTs be modeled in vitro? We know that they are composed of cells that are integral to wound healing: fibroblasts, myofibroblasts, MSCs, and blood vessels in an extracellular matrix of metalloproteinase, collagen, and hyaluronan.⁵ However, calling a DT a “scar gone wild” may be incorrect because the processes that control tumor stromal development are similar to those that

govern wound healing.¹³ Does the unknown DT cell of origin simply elicit abundant tumor stroma? Genomic studies show that only 5% to 20% of the cells have mutations in *CTNNB1* or *APC*, and this further confirms the immunohistological observation that DTs are a mosaic of tumor and normal cells. Can the activity seen with various classes of drugs in DTs be a stromal effect (Fig. 2)?

A critical aspect of this study is the recognition that PF-03084014 is a GS inhibitor. GS is an intramembrane protease that cleaves and activates a number of proteins, including amyloid precursor protein, Notch, CD44, *N*-cadherin, ErbB4, and ephrin-B2.¹⁴ Moreover, PF-03084014 has known antiangiogenic and anti-invasive effects in normal tissue, breast cancer, and chronic lymphocytic leukemia.¹⁵⁻¹⁷ In xenografts, antiangiogenic effects of PF-03084014 were confirmed by dynamic contrast-enhanced magnetic resonance imaging and Doppler ultrasound.¹⁸ The picture that emerges here is that PF-03084014 is not specific for Notch signaling and could therefore affect other signaling in DTs through CD44, *N*-cadherin, and angiogenesis.^{5,6} To firmly establish that Notch targeting is essential, studies need to be conducted with drugs that target Notch receptors or ligands in a GS-independent mechanism. If Notch signaling is a critical driver, we are unable to explain why DT also responds to estrogen, vascular endothelial growth factor, platelet-derived growth factor, cell cycle, and hyaluronan inhibitors.

One hypothesis is that an inciting event results in the activation of a mutated MSC^{CTNBBI/APC}, which initiates tumor stroma by recapitulating the wound healing process through an interplay of growth factors, cytokines, fibroblasts, endothelial cells, and extracellular matrix proteins (Fig. 2).^{13,19} In addition to recruiting normal fibroblasts and inducing neo-angiogenesis, activated MSC^{MutCTNBBI/APC} may differentiate into myofibroblasts^{MutCTNBBI/APC}, remain in a stem cell state, and avoid terminal differentiation. Some investigators have noted that MSCs (CD44+, CD168+) are likely the cells of origin.^{6,7,11,20} CD44 is an adhesion molecule first activated by HA and then by proteolytic cleavage by GS; this results in an ectodomain and a CD44 intracellular domain. The cleaved ectodomain mediates migration, and the CD44 intracellular domain mediates the transcription of CD44.^{21,22} Interestingly, CD44 is directly upregulated by β -catenin in colon cancer cell lines.²³ CD44 along with fibroblast growth factor is essential for limb-bud development in the embryo.²² *N*-Cadherin along with α , β -catenin and fibroblast growth factor receptor form an adhesion complex that is cleaved by GS, and the cleaved ectodomain stimulates fibroblast growth factor receptor in a paracrine manner.²⁴ Therefore, inhibition of proteolytic cleavage by CD44 and *N*-cadherin by PF-03084014 may additionally explain the robust inhibition of migration in DTs.²

DTs are a complex disease for which the cell of origin remains elusive. Although many therapies are active in

the clinic, the mechanism of action is unknown. This work by Shang et al² is a step in the right direction. In addition to Notch signaling, PF-03084014 may also inhibit CD44, *N*-cadherin, and angiogenesis; these processes may be critical in DTs. Determining to what extent each of these contributes to tumorigenesis requires further experimentation, and this is likely best learned from pre- and post-treatment biopsies from patients, in which both tumor and stromal effects can be best evaluated.

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The biology of inhibiting Notch and potentially other targets of γ -secretase in desmoid tumors is examined.