Imatinib Mesylate and AMN107 Inhibit PDGF-Signaling in Orbital Fibroblasts: A Potential Treatment for Graves’ Ophthalmopathy

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PURPOSE. Excessive orbital fibroblast proliferation and hyaluronan production are characteristic of Graves’ ophthalmopathy (GO) and are driven by local mediators. Imatinib mesylate and AMN107 are tyrosine kinase inhibitors that inhibit fibroblast proliferation and collagen production in lungs and skin. This study was conducted to determine whether imatinib mesylate and AMN107 inhibited orbital fibroblast proliferation and hyaluronan production induced by PDGF-BB and TGF-β1, and whether expression of the genes PDGF-B and TGF-β1 (growth factors suggested to play a role in GO) are increased in GO orbital tissues.

METHODS. PDGF-B and TGF-β1 mRNA levels were determined in orbital tissues of 13 patients with GO and 5 control patients. Orbital fibroblasts were cultured from eight patients with GO and three control patients and the effect of imatinib mesylate and AMN107 on PDGF-B and TGF-β1–induced orbital fibroblast proliferation, signaling cascades, hyaluronan synthase (HAS) gene expression and hyaluronan production were determined.

RESULTS. PDGF-B and TGF-β1 mRNA levels were significantly increased in GO orbital tissues. Imatinib mesylate and AMN107 inhibited PDGF-BB–induced orbital fibroblast proliferation. HAS induction and hyaluronan production by blocking PDGF-receptor phosphorylation. TGF-β1–induced HAS expression and hyaluronan production. This induction was not inhibited by imatinib mesylate or AMN107, due to the inability of TGF-β1 to activate c-Abl kinase activity in orbital fibroblasts.

CONCLUSIONS. Imatinib mesylate and AMN107 inhibit orbital fibroblast proliferation and hyaluronan production induced by PDGF-BB; a factor highly expressed in orbital tissue from patients with GO. The drugs, however, had no effect on TGF-β1–induced HAS expression and hyaluronan production. Nevertheless, imatinib mesylate and AMN107 should be considered as treatment candidates for GO. (Invest Ophthalmol Vis Sci. 2009;50:3091–3098) DOI:10.1167/iovs.08-2443

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eration as well as hyaluronan synthesis through inhibition of PDGF-R phosphorylation. TGF-β1-induced HAS expression and hyaluronan production was not inhibited by imatinib mesylate or AMN107, because of the inability of TGF-β1 to activate c-Abl kinase activity in orbital fibroblasts. Smad signaling was activated by TGF-β1. This study provides evidence that imatinib mesylate and AMN107 should be considered candidates for the treatment of patients with therapy-resistant GO, especially those with severe periorcular edema and ocular motility dysfunction who are not responding to common therapies.

**Methods**

**Reagents**

Dulbecco’s modified Eagle’s medium (DMEM), fetal calf serum (FCS), penicillin, streptomycin, and trypsin/EDTA were purchased from Cambrex BioWhittaker (Verviers, Belgium). The human fetal lung fibroblast cell line (HFL-1) was obtained from ATCC (Manassas, VA). Recombinant human PDGF-BB, recombinant human TGF-β1, and a hyaluronan ELISA were obtained from R&D Systems (Abingdon, UK). Anti-PDGFRα antibody (SC-339) was purchased from Santa Cruz Biotechnologies (Heidelberg, Germany). Anti-phospho-tyrosine (cat no. 9411), anti-c-Abl (cat no. 2862), anti-Smad3 (cat no. 9513), and anti-phospho-Smad3 (cat no. 9520S) antibodies were purchased from Cell Signaling (Danvers, MA). Anti-β-actin (AB6276) was purchased from Abcam (Cambridge, UK). Anti-c-Abl antibody (8E9) was kindly provided by Dynomics (Rotterdam, the Netherlands). Imatinib mesylate and AMN107 were kindly provided by Novartis Pharma (Basel, Switzerland). SB431542 was obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands). GST-CRK was a kind gift of Edward B. Leof (Mayo Clinic, Minneapolis, MN).

**Patients and Control Subjects**

The patients’ characteristics are given in Tables 1A and 1B, and clinical scores were determined as described before. Orbital tissue was obtained from 15 patients with GO undergoing orbital decompression surgery. Six of the orbital tissues were used for mRNA detection and yielded orbital fibroblast strains, seven of the tissues were only used for mRNA detection, and from two tissues only fibroblast strains were established. Of the patients, 3 had active and 12 inactive disease. All patients undergoing orbital surgery were euthyroid. Furthermore, orbital tissue was obtained from seven control patients without thyroid dysfunction who are not responding to common therapies.

<table>
<thead>
<tr>
<th>Patients with GO</th>
<th>Control</th>
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<tbody>
<tr>
<td><strong>A. Orbital Tissues Used for mRNA Determination</strong></td>
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<tr>
<td><strong>Patients with GO</strong></td>
<td><strong>Control</strong></td>
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<tr>
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<tr>
<td>RAI</td>
<td>12/13</td>
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<tr>
<td>Surgery</td>
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<tr>
<td>Surgery</td>
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<td>TPO antibodies</td>
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<td><strong>B. Orbital Fibroblast Strains</strong></td>
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<td><strong>Patients with GO</strong></td>
<td><strong>Control</strong></td>
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<tr>
<td>NO-SPECS (range)*</td>
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* NO-SPECS score: as described elsewhere.

**Detection of PDGF-B and TGF-B1 mRNA Levels in Orbital Tissue**

RNA was isolated (RNeasy columns; Qiagen, Hilden, Germany) and reverse transcribed into cDNA. PDGF-B and TGF-B1 transcript levels were determined by real-time quantitative PCR (QqPCR; 7700 PCR system; Applied Biosystems [ABI], Foster City, CA). Transcript levels were normalized to the control gene abelson. Primer–probe combinations used are listed in Table 2.

**Orbital Fibroblast Culture**

Fibroblast strains were established from orbital tissues, as described previously. Once fibroblast monolayers were obtained, cultures were serially passaged after gentle treatment with trypsin/EDTA. Eight GO fibroblast strains were obtained, of which three were from patients with active GO and five from those with inactive GO. Three control fibroblast strains were obtained. Fibroblast strains used for experiments were between the 6th and 15th passages.
tion was expressed as the percentage change in mean absorbance from that of cells exposed to DMEM 1.0% FCS alone.

**HAS mRNA Expression in Orbital Fibroblasts**

Fibroblasts were seeded at 4 x 10^5 cells/well into six-well plates in DMEM 10% FCS and allowed to adhere. They were then incubated in DMEM 1.0% FCS in the presence or absence of imatinib mesylate or AMN107 for 16 hours. Subsequently, the cells were cultured in DMEM 1.0% FCS with or without TGF-β1 (10 ng/mL) or PDGF-BB (50 ng/mL) for 6 hours. RNA was isolated and reverse transcribed into cDNA. HAS1, HAS2, and HAS3 expression was determined by RQ-PCR and normalized to the control gene abelson. Because of variability in basal HAS mRNA, the results are expressed as x-fold induction relative to basal HAS expression. Primer–probe combinations used are listed in Table 2.

**Hyaluronan Production by Orbital Fibroblasts**

Fibroblasts were seeded at 1.5 x 10^5 cells/well into 24-well plates in DMEM 10% FCS and allowed to adhere. They were then incubated in DMEM 1.0% FCS in the presence or absence of imatinib mesylate or AMN107 for 16 hours. Subsequently, the cells were cultured in DMEM 1.0% FCS with or without TGF-β1 (10 ng/mL), PDGF-BB (50 ng/mL), or a combination of TGF-β1 and PDGF-BB for 24 hours. The supernatants were harvested, and hyaluronan levels were determined by ELISA. Because of variability in basal production levels, the results are expressed as x-fold induction relative to the basal hyaluronan production.

**Detection of PDGF-R Phosphorylation**

To assess PDGF-R activation, the cultures were stimulated with PDGF-BB (50 ng/mL) for the indicated times (see Fig. 3) and lysed (20 mM Tris [pH 8.0], 137 mM NaCl, 10 mM EDTA, 100 mM NaF, 1% NP-40, 10% glycerol, and protease inhibitors). Equivalent amounts of protein (~50 μg) were loaded onto gels for SDS-PAGE. Western blots were stained with either anti–PDGF-R or anti-phosphotyrosine antibodies. To determine the effect of imatinib mesylate and AMN107 on PDGF-BB-induced PDGF-R phosphorylation, immunoprecipitation using an anti–PDGF-R antibody was performed on cell lysates (~500 μg protein). Immune complexes were collected with a mix of protein A- and G-Sepharose (Sigma-Aldrich, Zwijndrecht, The Netherlands) and the level of PDGF-R phosphorylation was determined with an anti-phosphotyrosine antibody, as described.

**Detection of c-Abl Kinase Activity**

c-Abl kinase assays were essentially performed as described. Briefly, cultures were stimulated for the indicated times (see Fig. 6) and lysed at 4°C. Immunoprecipitation was performed with an anti-c-Abl antibody (cat no. 2862; Cell Signaling). Immune complexes were collected with a mix of protein A- and G-Sepharose and washed in lysis buffer and three times in kinase buffer (10 mM HEPES [pH 7.4], 50 mM NaCl, 5 mM MgCl2, 5 mM MnCl2, 0.5 mM DTT, and protease inhibitors). Kinase reaction was performed in 10 μL kinase mix containing 2 μg GST-CRK, 100 μM ATP, and 0.5 μCi γ-32P-dATP for 15 minutes at 37°C. The reaction was stopped by adding loading buffer, and samples were loaded on gels for SDS-PAGE. Total c-Abl and actin protein were detected using anti-c-Abl (8E9) and anti-β-actin antibodies.

**Detection of Smad Activation**

To assess Smad activation, the cultures were treated with TGF-β1, for the indicated times (see Fig. 7) and lysed. Equivalent amounts of protein were loaded on gels for SDS-PAGE, and Western blots were subsequently stained with antibodies to phosphorylatedSmad3, Smad-3, and β-actin.

**Statistical Analysis**

PDGF-B and TGF-β1 mRNA levels in orbital tissue were analyzed with the Mann-Whitney test. Data concerning the effect of imatinib mesylate
and AMN107 on orbital fibroblast proliferation, HAS expression levels and hyaluronan production were analyzed with the paired Student’s t-test. *P < 0.05 was considered significant.

**RESULTS**

**PDGF-B and TGF-β1 mRNA Levels in GO Orbital Tissue**

PDGF-B mRNA levels were significantly higher (approximately threefold: P < 0.05; Fig. 1) in GO compared with control orbital tissue. Also, TGF-β1 mRNA levels were significantly higher (approximately twofold: P < 0.05; Fig. 1) in GO compared with control orbital tissue. No difference in PDGF-B and TGF-β1 mRNA expression was observed between patients with active or inactive GO, and no correlations were observed between expression levels and clinical activity scores (CAS).18

**Effect of Imatinib Mesylate and AMN107 on PDGF-BB–Induced Orbital Fibroblast Proliferation, and of TGF-β1 on Orbital Fibroblast Proliferation**

PDGF-BB equally stimulated proliferation of orbital fibroblasts derived from patients with GO and control patients with a mean induction of 57% and 45% proliferation above control, respectively (Fig. 2). No difference in PDGF-BB–induced proliferation was observed between fibroblasts from patients with active or inactive GO. Initial studies revealed that imatinib mesylate and AMN107 dose-dependently (range, 0.16–2.5 μg/mL) inhibited PDGF-BB–induced orbital fibroblast proliferation (data not shown). At the dose of 2.5 μg/mL, imatinib mesylate and AMN107 inhibited PDGF-BB–induced proliferation of GO and control orbital fibroblasts up to basal proliferation levels (GO: imatinib and AMN107 both 5% proliferation above control; P < 0.05, controls: imatinib 6% and AMN107 8% proliferation above control, P < 0.05; Fig. 2).

With regard to the mechanisms involved, PDGF-BB stimulated PDGF-R phosphorylation in a time-dependent manner (Fig. 3A). Imatinib mesylate and AMN107 both reduced PDGF-BB–induced PDGF-R phosphorylation (Fig. 3B), indicating efficient inhibition of PDGF-R tyrosine kinase activity in orbital fibroblasts by both tyrosine kinase inhibitors.

In contrast, stimulation with TGF-β1 (range, 2.5–40 ng/mL) did not induce orbital fibroblast proliferation and was not further explored.

**Effect of PDGF-BB and TGF-β1 on HAS Expression**

Hyaluronan synthesis is regulated by three hyaluronan synthases, encoded by individual genes: HAS1, -2, and -3.22 We determined whether PDGF-BB and TGF-β1 influence the expression of these HAS genes in orbital fibroblasts. PDGF-BB stimulation increased HAS1 and -2 expression by approximately sixfold in GO orbital fibroblasts (P < 0.01), but did not influence HAS3 expression (Fig. 4). In control orbital fibroblasts PDGF-BB stimulation increased HAS2 expression approximately threefold, but did not affect HAS1 and -3 expression. TGF-β1 stimulation increased HAS1 expression ~60-fold (P < 0.05) in both GO and control fibroblasts, but did not affect HAS2 and -3 transcript levels (Fig. 4). No differences were observed between GO orbital fibroblasts from active or inactive disease with regard to PDGF-BB and TGF-β1–induced HAS expression.

**Figure 1.** Relative mRNA expression for PDGF-B (A) and TGF-β1 (B) in orbital tissue from patients with GO and control subjects. mRNA expression levels were determined by RQ-PCR and normalized to the expression levels of the abelson gene. Each data point represents a single individual. Horizontal bars: mean values. Data were analyzed with the Mann-Whitney test. *P < 0.05.

**Figure 2.** The effect of imatinib mesylate (IM; 2.5 μg/mL) and AMN107 (AMN; 2.5 μg/mL) on PDGF-BB (50 ng/mL)–induced proliferation of GO (A) and control (B) orbital fibroblasts. Each data point represents a fibroblast strain obtained from a single individual. Horizontal bars: mean values. Data were analyzed using the Student’s t-test. *P < 0.001.

**Figure 3.** Orbital fibroblasts were stimulated with PDGF-BB (50 ng/mL) with or without imatinib mesylate (2.5 μg/mL) and AMN107 (2.5 μg/mL), for the indicated times. (A) Western blot analysis was performed on lysates with antibodies against PDGF-R (top row) and phosphorylated tyrosine residues (bottom row). (B) Top row: Western blot analysis was performed with PDGF-R antibody on an aliquot before immunoprecipitation. Bottom row: after PDGF-R immunoprecipitation, phosphorylated PDGF-R was determined with an antibody against phosphorylated tyrosine residues. Data depicted are from a representative experiment.
Effect of Imatinib Mesylate and AMN107 on PDGF-BB–Induced HAS1 and -2 Expression and Hyaluronan Production

Imatinib mesylate reduced PDGF-BB–induced expression of HAS1 and -2 in GO orbital fibroblasts up to basal expression levels ($P < 0.05$; Fig. 4). AMN107 reduced PDGF-BB–induced HAS1 expression in GO orbital fibroblasts by approximately threefold ($P < 0.05$) and HAS2 expression up to basal expression levels ($P < 0.05$; Fig. 4). Both imatinib mesylate and AMN107 inhibited the PDGF-BB–induced HAS2 expression in control orbital fibroblasts (Fig. 4).

To examine whether treatment with imatinib mesylate and AMN107 reduced hyaluronan production, we selected five GO orbital fibroblast strains (two from active and three from inactive GO) and three control orbital fibroblast strains. PDGF-BB stimulated the production of hyaluronan by orbital fibroblasts derived from both patients with GO (approximately threefold induction; Fig. 5) and control patients (approximately fourfold induction; Fig. 5). Imatinib mesylate and AMN107 treatment efficiently reduced the PDGF-BB–induced hyaluronan production by GO and control orbital fibroblasts up to basal levels (both $P < 0.05$; Fig. 5).

Effect of Imatinib Mesylate and AMN107 on TGF-β1–Induced HAS1 Expression and Hyaluronan Production and of TGF-β1 on c-Abl Kinase Activity in Orbital Fibroblasts

Although TGF-β1 clearly upregulated HAS1 expression in orbital fibroblasts and although it also stimulated hyaluronan production by both GO (~4-fold induction; Fig. 5) and control (~14-fold induction; Fig. 5) orbital fibroblasts, imatinib mesylate and AMN107 neither affected the TGF-β1–induced increase of HAS1 expression (Fig. 4) nor reduced the TGF-β1–induced hyaluronan production (Fig. 5). It must be noted that TGF-β1–induced HAS1 expression was blocked by the selective TGF-β1 receptor 1 kinase inhibitor SB431542 in a dose-
Orbital fibroblast proliferation and hyaluronan accumulation are key features of GO and are thought to be driven by mediators present within the orbital tissue.23–26 PDGF-BB, a homodimeric protein consisting of two PDGF-B chains, is regarded as one of the most potent mitogens for fibroblasts and increased PDGF-BB production plays a key role in fibrosis of a variety of organ systems.9 We report, for the first time to our knowledge, increased levels of PDGF-B mRNA in orbital tissues from patients with GO. In line with previous effects in orbital fibroblasts further, we studied the effects of TGF-β1 stimulation on c-Abl kinase activity. It appeared that TGF-β1 stimulation did not increase c-Abl kinase activity in orbital fibroblasts but was associated with a decrease in total c-Abl protein, in contrast to the effect of TGF-β1 on lung fibroblasts, where it induced c-Abl kinase activity without affecting total c-Abl levels (Fig. 6). TGF-β1 did induce Smad3 phosphorylation in orbital fibroblasts (Fig. 7A), and the induction was not inhibited by imatinib mesylate and AMN107 (Fig. 7B). These data indicate that in orbital fibroblasts the Smad pathway is activated by TGF-β1, whereas the c-Abl pathway is not.

**DISCUSSION**

Orbital fibroblast proliferation and hyaluronan accumulation are key features of GO and are thought to be driven by mediators present within the orbital tissue.23–26 PDGF-BB, a homodimeric protein consisting of two PDGF-B chains, is regarded as one of the most potent mitogens for fibroblasts and increased PDGF-BB production plays a key role in fibrosis of a variety of organ systems.9 We report, for the first time to our knowledge, increased levels of PDGF-B mRNA in orbital tissues from patients with GO. In line with previous effects in orbital fibroblasts further, we studied the effects of TGF-β1 stimulation on c-Abl kinase activity. It appeared that TGF-β1 stimulation did not increase c-Abl kinase activity in orbital fibroblasts but was associated with a decrease in total c-Abl protein, in contrast to the effect of TGF-β1 on lung fibroblasts, where it induced c-Abl kinase activity without affecting total c-Abl levels (Fig. 6). TGF-β1 did induce Smad3 phosphorylation in orbital fibroblasts (Fig. 7A), and the induction was not inhibited by imatinib mesylate and AMN107 (Fig. 7B). These data indicate that in orbital fibroblasts the Smad pathway is activated by TGF-β1, whereas the c-Abl pathway is not.

**FIGURE 5.** The effect of imatinib mesylate (IM; 2.5 μg/ml) and AMN107 (AMN; 2.5 μg/ml) on (A) PDGF-BB (50 ng/ml)-, (B) TGF-β1 (10 ng/ml)-, and (C) PDGF-BB/TGF-β1-induced hyaluronan production by orbital fibroblasts. Hyaluronan levels in culture supernatants were determined by ELISA. The first row depicts hyaluronan production in GO and control fibroblasts after PDGF-BB stimulation, the second row after TGF-β1 stimulation and the third row after stimulation with both PDGF-BB and TGF-β1. Each data point represents an orbital fibroblast strain obtained from a single individual. Data are presented as x-fold induction relative to the unstimulated control. Horizontal bars: mean values. Data were analyzed with Student’s *t*-test. *P < 0.05.

**FIGURE 6.** Orbital fibroblasts (left) and HFL-1 cells (right) were stimulated with TGF-β1 (10 ng/ml) for indicated times. Top row: after c-Abl immunoprecipitation, c-Abl kinase activity was determined by using GST-CRK as a substrate. Remaining rows: Western blot analysis was performed with antibodies against c-Abl and β-actin on an aliquot before immunoprecipitation. Data are from a representative experiment.

Th2 and TGF-β1 activate the c-Abl pathway in orbital fibroblasts.

**FIGURE 7.** Orbital fibroblasts were stimulated with TGF-β1 (10 ng/ml) for the indicated times (A). Western blot analysis was performed on lysates with antibodies against phosphorylated-Smad3, Smad3, and β-actin. Data depicted are from a representative experiment. Orbital fibroblasts were stimulated with TGF-β1 (10 ng/ml) for the indicated times, with or without imatinib mesylate (2.5 μg/ml) and AMN107 (2.5 μg/ml) for the indicated times (B). Western blot analysis was performed on lysates with antibodies against phosphorylated-Smad3, Smad3, and β-actin. Data are from a representative experiment.

**TABLE 1.** Effects of TGF-β1 on c-Abl activity in orbital fibroblasts. Data are from a representative experiment.
observations,\textsuperscript{15} we also demonstrate that PDGF-BB is capable of inducing proliferation of orbital fibroblasts. We also found elevated levels of TGF-B\textsubscript{1} mRNA in GO orbital tissue. However, in our study, TGF-B\textsubscript{1} did not induce orbital fibroblast proliferation, which is opposed to the study by Heufelder and Bahn,\textsuperscript{15} who found TGF-B\textsubscript{1} to be mitogenic for GO orbital fibroblasts. Unfortunately, we were unable to examine the actual orbital PDGF-BB and TGF-B\textsubscript{1} protein levels because of a lack of tissue. However, several studies in fibrotic diseases showed clear correlations between PDGF-B and TGF-B\textsubscript{1} mRNA and protein levels.\textsuperscript{27–30} PDGF-B and TGF-B\textsubscript{1} mRNA levels did not correlate with disease activity. Although the reason and underlying mechanism for this are unclear so far, the data suggest that PDGF-BB and TGF-B\textsubscript{1} are involved in both active and inactive GO.

Next to proliferation, excessive hyaluronan production by orbital fibroblasts plays an important role in GO.\textsuperscript{1,5} Both TGF-B\textsubscript{1} and PDGF-BB are powerful stimulators of hyaluronan production by fibroblasts from a variety of organs and diseases characterized by tissue remodelling.\textsuperscript{16,31,32} In our study, PDGF-BB enhanced HAS1 and \textsuperscript{2} mRNA expression in GO orbital fibroblasts, whereas TGF-B\textsubscript{1} increased HAS1 mRNA expression, in contrast with previous reports demonstrating that PDGF-BB does not induce HAS expression in orbital fibroblasts.\textsuperscript{55} We take this discrepancy to be due to differences in methodologic approaches and consider the PDGF-BB-induced HAS\textsuperscript{1} expression to be genuine, since we found it to co-exist with an increased hyaluronan production. In sum, our combined findings of increased orbital expression of PDGF-BB and TGF-B\textsubscript{1} mRNA and in vitro stimulation data strengthen the case for the involvement of PDGF-BB and TGF-B\textsubscript{1} in the increased hyaluronan synthesis in GO.

So far, the fibroblast has not been a target for therapy in GO. Imatinib mesylate and AMN107 are tyrosine kinase inhibitors known to block PDGF- and TGF-B\textsubscript{1}-related tyrosine kinase activity in fibroblasts, making the drugs potential candidates to inhibit fibrosis and hyaluronan production associated with peri-orbital edema in GO.

Our studies show that imatinib mesylate and AMN107 inhibit the in vitro PDGF-BB-induced orbital fibroblast proliferation as well as the in vitro PDGF-BB-induced HAS expression and hyaluronan production by orbital fibroblasts by interfering with the PDGF-BB-induced autophosphorylation of its receptor. These observations are in line with previous observations on these drugs in lung fibroblasts from patients with systemic sclerosis.\textsuperscript{11} However, in contrast to lung (and skin) fibroblasts,\textsuperscript{6,10,11} the tyrosine kinase inhibitors had no effect on TGF-B\textsubscript{1}-induced extracellular matrix production from orbital fibroblasts, since TGF-B\textsubscript{1} did not induce c-Abl kinase activity in orbital fibroblasts. This finding suggests that TGF-B\textsubscript{1}-induced hyaluronan production by orbital fibroblasts is regulated by a c-Abl independent signaling pathway and underscores previous notions that orbital fibroblasts are distinct from fibroblasts from other anatomic sites\textsuperscript{34} and originate from a different embryonic site.\textsuperscript{35}

Another major route by which TGF-B\textsubscript{1} regulates cell activation is the Smad signaling cascade. c-Abl-independent Smad signaling has been shown to be involved in hyaluronan production in different cell types.\textsuperscript{31,36,37} We indeed found this pathway to be activated in TGF-B\textsubscript{1}-stimulated orbital fibroblasts. Drugs targeting this pathway should be considered in GO.

Thus far, treatment with imatinib mesylate and AMN107 has been widely applied to BCR-ABL-positive chronic myeloid leukemia.\textsuperscript{9} In addition, imatinib mesylate has been successfully used to treat patients with gastrointestinal stromal tumors and mastocytosis by targeting c-Kit kinase activity.\textsuperscript{38,39} Recently, we and others have shown that treatment with imatinib mesy-


