Efficacy and Biological Activity of Imatinib in Metastatic Dermatofibrosarcoma Protuberans (DFSP)

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Abstract

Purpose: To report on imatinib mesylate (IM) in patients with metastatic dermatofibrosarcoma protuberans (DFSP)/fibrosarcomatous (FS)-DFSP and on the impact of the treatment on tumor biology.

Experimental design: Ten consecutive patients treated with IM from 2007 to 2015 for a metastatic relapse from DFSP/FS-DFSP were identified. FISH analysis for COL1A1-PDGFB was performed. Two IM-treated and 4 naïve FS-DFSP were transcriptionally profiled by RNAseq on HiScanSQ platform. Differential gene expression was analyzed with edgeR (Bioconductor), followed by hierarchical clustering and Principal Component Analysis.

Results: All cases featured fibrosarcomatous in the metastasis and retained the COL1A1-PDGFB. Best RECIST response was: 8 partial response, 1 stable disease, and 1 progressive disease. Median progression-free survival was 11 months. Five patients received surgery after IM and all relapsed. IM was restored in 4 patients with a new response. After IM, the most upregulated genes included those encoding for immunoglobulins and those affecting functions and differentiation of endothelial cells. Pathway enrichment analysis revealed upregulation in genes involved in antigen processing and presentation, natural killer cytotoxicity, and drug and xenobiotics metabolism. Conversely, a significant down-regulation of kinase signaling pathways was detected.

Conclusions: All metastatic cases were fibrosarcomatous. Most patients responded to IM, but PFS was shorter than reported in published series which included both DFSP and FS-DFSP. All patients operated after IM had a relapse, suggesting that IM cannot eradicate metastatic cases and that the role of surgery is limited. Transcriptional profile of naïve and posttreatment samples pointed the contribution of immune infiltrates in sustaining the response to IM. Clin Cancer Res; 1–10. ©2015 AACR.

Introduction

Dermatofibrosarcoma protuberans (DFSP) is a rare sarcoma (1), arising from the skin and marked by rearrangement of chromosomes 17 and 22, which results in the COL1A1–PDGFB fusion gene (2). This chimera is responsible for constitutive activation of Platelet Derived Growth Factor Receptor Beta (PDGFRB; ref. 3), by mean of an autocrine-paracrine loop.

DFSP is characterized by an indolent and locally invasive growth, with a very low metastatic potential that is usually related to the presence of a more aggressive, fibrosarcomatous (FS) component (1, 4–6). Standard treatment consists of wide surgical excision, by which a high cure rate is achieved (4). FS aspects are detected in 5% to 15% of DFSP (1, 4–6), and may be present in the primary tumor or may occur at the time of relapse. FS transformation is related to an increased risk of metastases, in the range of 10% to 15% (5, 6), that can be located to unusual anatomic sites as pancreas (7) and brain (5). Metastases in completely resected DFSP/FS-DFSP are an extremely rare event, which, on the basis of available evidence, might be estimated to be less than 0.05 new cases per 1,000,000 each year (5, 8). Moreover, diagnosis can be really challenging in metastatic cases, with FS transformation occurring over time and sometimes loss of a classic DFSP featured component.

Imatinib mesylate (IM) is approved for treatment of advanced DFSP, where its impressive activity has been related to the inhibition of PDGFRB (9, 10). In contrast, the role of IM in the metastatic setting is not clarified. Both the prospective and the retrospective studies reported so far analyzed locally advanced and metastatic patients together, and the efficacy of IM treatment...
was not investigated specifically in FS-DFSP (11–15). We already reported on 4 patients with advanced, translocated FS-DFSP (3 metastatic, one locally advanced) who received IM (5).

If on one side the clinical response to IM of FS-DFSP is still unclear, on the other side, the biology of DFSP undergoing FS evolution has been only marginally addressed (9, 16). In addition, little is known about gene expression profile of FS-DFSP and how IM treatment affects the gene expression profile of these lesions.

To shed light on these issues, we analyzed a retrospective series of 10 patients with metastatic DFSP/FS-DFSP, molecularly confirmed by the presence of COL1A1–PDGFB fusion gene, treated with IM at our institution and within the Italian Rare Cancer Network, from 2007 to 2015. Three of these patients were among the 4 previously reported (5). We describe their clinical behavior and we report on the gene expression profile (GEP) analysis performed in few naive and IM-pretreated FS-DFSP tumor samples.

Materials and Methods

We retrospectively reviewed all consecutive adult patients treated with IM for metastatic relapse from DFSP or FS-DFSP at Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy (INT), and within the Italian Rare Cancer Network (IRCN) from 2007 to 2014. All cases treated with IM for local regional disease in the absence of metastatic lesions were excluded. Clinical data were extracted from the institutional and the Rare Cancer Network databases of all adult patients with soft tissue and bone sarcomas. The analysis was approved by the Institutional Ethics Committee.

Pathology

All cases were reviewed and reclassified applying updated criteria (17, 18). In all cases, the metastatic disease was assessed histologically and diagnosis confirmed. The primary lesion and metastases were compared in all patients, retrieving the tissue of the primary tumor that in 7 cases was resected and sampled elsewhere, whereas in 3 patients, the primary was operated at INT (Table 1). FS change was defined by the appearance in a classical storiform DFSP of at least 5% of high cellular area made up of spindle cells arranged in an herringbone pattern with a mitotic index >7/10 high power field (17); necrosis, pleomorphic features, myofibroblastic differentiation were also recorded and considered in making FS-DFSP diagnosis as well as decrease/disappearance of CD34 immunoreactivity (18).

Furthermore, in light of GEP analysis results, immunohistochemical analysis was performed using antibodies against CD20 (clone L26; Dako; 1:400; antigen unmasking 96°C EDTA 15’) and CD57 (clone TB01; Dako; 1:100; antigen unmasking 96°C EDTA 30’). The sections were stained using a Dako Flex+ Autostainer (Dako). Three additional cases have been added to the series (cases 11, 12, and 13; Table 1) in order to interrogate FS-DFSP naïve profile and to compare it with that of IM-treated cases.

FISH for detection of the COL1A1–PDGFB fusion gene

FISH analysis was performed in all the metastatic lesions. BAC clones (BACPAC Resource Center. C.H.O.R.I. Children’s Hospital Oakland Research Institute Oakland, CA) for PDGFB (RP1-506F7 and RP11-630N12) and COL1A1 (RP11-93L18 and RP11-630N12) were labeled with Spectrum Orange and Spectrum Green (Vysis), respectively. FISH experiments were carried out accordingly to the manufacturer’s instructions. At least 100 tumoral nuclei were scored for the presence of gene fusion.

Whole transcriptome sequencing

RNAseq analysis was performed on 6 cryopreserved FS-DFSP. We evaluated 4 naïve FS-DFSP samples, with tumor cellularity greater than 80% (patients 8, 11, 12,13; Table 1), and 2 post-IM FS-DFSP samples (in one case corresponding to patient 4 in Tables 1 and 2, the sample was obtained from a metastatic lesion resected in 2012; in the other case, the sample was taken from a locally relapsed tumor that appeared concomitantly to the metastatic lesions, corresponding to patient #2 in Tables 1 and 2). The hematoxylin and eosin (H&E) frozen control section of patient #4 showed a cellular area made by 70% of viable tumor, whereas in H&E-frozen control section of patient #2, the residual viable tumor accounted for 30% of the specimen. Total RNA was extracted with the RNeasy Mini Kit (Qiagen), after mechanical dissociation with a pestle. cDNA libraries were synthesized from 250 ng of total RNA with the TruSeq RNA Sample Prep Kit v2 (Illumina) according to the manufacturer’s instructions. Sequencing by synthesis was performed on HiScanSQ sequencer (Illumina) at 80 bp in paired-end mode. Approval of the present study by the Institutional Review Board was provided.

Gene expression profile analysis and variant detection

After demultiplexing and FASTQ generation (performed with Bcl2Fastq), the paired-end reads were mapped with the pipeline TopHat/ Bowtie on human reference genome HG19, collected from UCSC Genome Browser (http://www.genome.ucsc.edu). After the alignment procedure, the BAM file obtained was processed with Samtools in order to remove the optical/PCR duplicate, and to perform the sorting and indexing procedures (19). The detection of single-nucleotide variants (SNV) was performed with Mutect (20), while insertion and deletion (InDels) were called with GATK (21). The variations were filtered on dbSNP, 1000Genomes, and Exome Variant Server (EVS) in order to select the novel mutations that were then annotated with the software Annovar. SNVs and Indels were prioritized based on the predicted effect of genomic variant on protein structure and stability with a suite of computation tools, including Provean, SIFT, and Polyphen2.

The analysis of gene expression was performed in two steps: (i) the function htsseq-count (Python package HTseq) was adopted to count the number of reads mapped on known genes, included in
<table>
<thead>
<tr>
<th>Patient ID #</th>
<th>Diagnosis by year</th>
<th>Site</th>
<th>Morphology</th>
<th>IHC</th>
<th>Diagnosis by year</th>
<th>Site</th>
<th>Morphology</th>
<th>IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DFSP, 1987</td>
<td>Scalp</td>
<td>Usual</td>
<td>n.d.</td>
<td>1997</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
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<td>DFSP, 1992</td>
<td>Trunk</td>
<td>Usual</td>
<td>n.d.</td>
<td>FS-Dfsp, 2013</td>
<td>Spindle</td>
<td>CD34⁺</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>DFSP, 2002</td>
<td>Groin</td>
<td>Usual</td>
<td>CD34⁺</td>
<td>FS-Dfsp, 2008</td>
<td>Spindle</td>
<td>CD34⁺</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>DFSP; FS-Dfsp, 2003 (referred)</td>
<td>Scalp</td>
<td>Spindle</td>
<td>CD34⁺</td>
<td>FS-Dfsp, 2008</td>
<td>Spindle</td>
<td>CD34⁺</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>DFSP; FS-Dfsp, 2001 (referred)</td>
<td>Trunk</td>
<td>Spindle</td>
<td>CD34⁺ + focal</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>DFSP; FS-Dfsp, 2003 (referred)</td>
<td>Trunk</td>
<td>Myxoid</td>
<td>CD34⁺</td>
<td>FS-Dfsp, 2008</td>
<td>Myxoid</td>
<td>CD34⁺</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>DFSP; FS-Dfsp, 2006 (referred)</td>
<td>Trunk</td>
<td>Spindle</td>
<td>CD34⁺</td>
<td>FS-Dfsp, 2008</td>
<td>Myxoid</td>
<td>CD34⁺</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>DFSP; FS-Dfsp, 2008 (referred)</td>
<td>Scalp</td>
<td>Spindle</td>
<td>CD34⁺</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>DFSP; FS-Dfsp, 2012 (referred)</td>
<td>Trunk</td>
<td>Spindle</td>
<td>CD34⁺</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>DFSP; FS-Dfsp, 2008 (referred)</td>
<td>Scalp</td>
<td>Spindle</td>
<td>CD34⁺</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>DFSP; FS-Dfsp, 2010</td>
<td>Groin</td>
<td>Myxoid</td>
<td>CD34⁺</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>DFSP; FS-Dfsp, 2014</td>
<td>Thigh</td>
<td>Spindle</td>
<td>CD34⁺</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>DFSP; FS-Dfsp, 2014</td>
<td>Trunk</td>
<td>Spindle</td>
<td>CD34⁺</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Notes:**
- Abbreviations: FNA, fine needle aspiration; IHC, immunohistochemistry; n.d., not determined.
- **Imatinib treatment:**
  - FS-Dfsp, 2012 (biopsy; referred)
  - FS-Dfsp, 2014 (trucut)
  - FS-Dfsp, 2011 (referred)
  - FS-Dfsp, 2009 (referred)
  - FS-Dfsp, 2006 (referred)
  - FS-Dfsp, 2007 (FNA; referred)
  - FS-Dfsp, 2011 (treated)
  - FS-Dfsp, 2014 (trucut; referred)
  - FS-Dfsp, 2014 (trucut)
  - FS-Dfsp, 2014 (FNA)
  - FS-Dfsp, 2014 (FNA)
  - FS-Dfsp, 2014 (FNA)

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**Metastasis**

<table>
<thead>
<tr>
<th>Diagnosis by year</th>
<th>Site</th>
<th>Morphology</th>
<th>IHC</th>
<th>Imatinib treatment</th>
</tr>
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<tr>
<td>1997</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>2014</td>
<td>Lung, abdomen</td>
<td>Spindle</td>
<td>CD34⁺</td>
<td>No</td>
</tr>
<tr>
<td>2012</td>
<td>Lung (1 nodule, 3 cm)</td>
<td>Spindle</td>
<td>CD34⁺</td>
<td>No</td>
</tr>
<tr>
<td>2012</td>
<td>Lung (1 nodule, 15 cm)</td>
<td>Spindle</td>
<td>CD34⁺</td>
<td>Yes</td>
</tr>
<tr>
<td>2011</td>
<td>Lung (2 nodules, 1 and 1.2 cm)</td>
<td>Spindle</td>
<td>CD34⁺</td>
<td>No</td>
</tr>
<tr>
<td>2012</td>
<td>Lung (3 nodules, 1.5, 0.7 cm)</td>
<td>Spindle</td>
<td>CD34⁺</td>
<td>Yes</td>
</tr>
<tr>
<td>2012</td>
<td>Lung (2 cm)</td>
<td>Spindle</td>
<td>CD34⁺</td>
<td>Yes</td>
</tr>
<tr>
<td>2011</td>
<td>Lung (1 nodule, 8 cm)</td>
<td>Spindle</td>
<td>CD34⁺</td>
<td>Yes</td>
</tr>
<tr>
<td>2011</td>
<td>Abdomen, STS</td>
<td>Spindle</td>
<td>CD34⁺</td>
<td>No</td>
</tr>
<tr>
<td>2014</td>
<td>Abdomen, STS</td>
<td>Pancreas</td>
<td>Spindle</td>
<td>CD34⁺</td>
</tr>
<tr>
<td>2014</td>
<td>Pelvis</td>
<td>Spindle</td>
<td>Spindle-pleomorphic</td>
<td>CD34⁺</td>
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<tr>
<td>2014</td>
<td>Stomach</td>
<td>Spindle</td>
<td>Spindle-pleomorphic</td>
<td>CD34⁺</td>
</tr>
<tr>
<td>2014</td>
<td>Lung, pancreas, stomach, STS</td>
<td>Spindle</td>
<td>Spindle-pleomorphic</td>
<td>CD34⁺</td>
</tr>
</tbody>
</table>

**Path response to imatinib**

- 1997: Unknown
- 2001: FS-DFSP
- 2002: FS-DFSP
- 2003: FS-DFSP
- 2004: FS-DFSP
- 2005: FS-DFSP
- 2006: FS-DFSP
- 2007: FS-DFSP
- 2008: FS-DFSP
- 2009: FS-DFSP
- 2010: FS-DFSP
- 2011: FS-DFSP
- 2012: FS-DFSP
- 2013: FS-DFSP
- 2014: FS-DFSP
- 2015: FS-DFSP

**Abbreviations:**
- FNA: fine needle aspiration
- IHC: immunohistochemistry
- n.d.: not determined

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**Table 1. Pathology.**
Table 2. Patient characteristics

<table>
<thead>
<tr>
<th>ID</th>
<th>Gender</th>
<th>Age at the time of first diagnosis/IM</th>
<th>Location of primary tumor</th>
<th>Disease extent at time of starting IM</th>
<th>Site of metastases at the time of starting IM</th>
<th>Best response to IM at the time of surgery</th>
<th>Best response to surgery after IM</th>
<th>Treatment with IM</th>
<th>Site of metastases after surgery</th>
<th>Best response to surgery after IM</th>
<th>PFS (months)</th>
<th>Reason for IM discontinuation</th>
<th>Surgery after IM</th>
<th>Progression after IM</th>
<th>Best FDG-PET response</th>
<th>Reason for IM discontinuation</th>
<th>Surgery after IM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>35/61</td>
<td>Scalp</td>
<td>L, M</td>
<td>Lung, CNS</td>
<td>Yes</td>
<td>PR</td>
<td>Not assessed</td>
<td>2</td>
<td>Progression</td>
<td>NO</td>
<td>6</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>DOD</td>
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<tr>
<td>2</td>
<td>Male</td>
<td>48/70</td>
<td>Trunk</td>
<td>M</td>
<td>Lung, abdomen, soft tissue</td>
<td>Yes</td>
<td>SD</td>
<td>Not assessed</td>
<td>3+</td>
<td>Progression</td>
<td>Yes</td>
<td>4</td>
<td>Toxicity</td>
<td>No</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>Not applicable</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>37/50</td>
<td>Groin</td>
<td>M</td>
<td>Lung</td>
<td>Yes</td>
<td>PR</td>
<td>PR</td>
<td>10</td>
<td>No progression</td>
<td>No</td>
<td>5</td>
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<td>Not applicable</td>
<td>Not applicable</td>
<td>AWD</td>
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<tr>
<td>4</td>
<td>F</td>
<td>50/59</td>
<td>Scalp</td>
<td>L, M</td>
<td>Lung</td>
<td>Yes</td>
<td>PR</td>
<td>PR</td>
<td>25</td>
<td>Progression</td>
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<td>Not applicable</td>
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<tr>
<td>5</td>
<td>F</td>
<td>45/55</td>
<td>Trunk</td>
<td>M</td>
<td>Soft tissue, bone</td>
<td>Yes</td>
<td>PR</td>
<td>Not assessed</td>
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<td>No progression</td>
<td>No</td>
<td>5</td>
<td>Progression</td>
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<td>46/49</td>
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<td>PR</td>
<td>PR</td>
<td>4</td>
<td>No progression</td>
<td>Yes</td>
<td>5</td>
<td>DOD</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>AWD</td>
</tr>
<tr>
<td>7</td>
<td>Male</td>
<td>48/53</td>
<td>Scalp</td>
<td>M</td>
<td>Abdomen, soft tissue</td>
<td>Yes</td>
<td>PR</td>
<td>PR</td>
<td>22</td>
<td>Progression</td>
<td>Yes</td>
<td>1</td>
<td>Toxicity</td>
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<td>Not applicable</td>
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<td>Not applicable</td>
</tr>
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<td>8</td>
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<td>41/53</td>
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<td>Lung</td>
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<td>PR</td>
<td>PR</td>
<td>3</td>
<td>Progression</td>
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<td>Progression</td>
<td>Yes</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>

Abbreviations: AWD, alive with disease; CNS, central nervous system; DOD, dead of disease; F, female; IM, imatinib; L, local; M, metastatic; PFS, progression free survival.

The Ensembl release 72 annotation features (http://www.ensembl.org); (ii) the differential expressed genes were modeled with the negative binomial distribution using the R-Biocoridor package edgeR. Hierarchical clustering of differentially expressed genes and unsupervised Principal Component Analysis were performed with Multiple Array Viewer (MEV available at http://www.tm4.org/mev.html). Pathway analysis was performed on the KEGG database with David/EASE tool (http://david.abcc.ncifcrf.gov).

Medical therapy and efficacy assessment

Patients included in this study had to have a metastatic DFSP/FS-DFSP, a performance status (ECOG) ≤3, an adequate bone marrow, and organ function in order to receive IM. All Patients gave their written informed consent to the treatment.

Patients received oral IM 400 mg, OD, continuously till progression or planned surgical resection. In case of resistance, the daily dose of IM was tentatively increased to 800 mg/day. At baseline, all patients were evaluated with medical history and physical examination, a complete blood count and serum chemistry and cardiologic assessment. A whole-body CT scan and a CT or MRI of the sites of disease were also required. PET scan was performed in some cases. Tumor assessment was performed after 8 to 12 weeks from starting treatment, then every 2 months, in case of suspected progression and before surgery.

Response was assessed according to RECIST (version 1.1; ref. 22). PET response was evaluated according to the European Organization for Research and Treatment of Cancer (EORTC) 1999 criteria (23). Overall survival (OS) and progression-free survival (PFS) were estimated by the Kaplan–Meier method. All patients receiving at least one dose of IM were included in the analysis. Patients were censored at the last contact. Death of any cause was considered therapy failure. To estimate the PFS, patients progressing after IM discontinuation whose response was restored after restarting IM were considered progressing at the time of definitive progression on IM.

Results

Clinical findings

Out of 52 patients who requested a treatment with IM for a DFSP or an FS-DFSP from January 2007 to March 2015, 10 cases with metastatic disease were identified. Three cases had been initially operated for their primary tumor in our institution, while 7 presented to our attention at the time of relapse.

Median age at the time of treatment with IM was 53 years (range, 49–73); female/male ratio was 3/7. The ECOG performance status was ≤2 in 7 cases, 3 in 3. All patients had been pretreated with surgery. Three patients were treated with radiotherapy, 2 with chemotherapy, and 1 with IM (in the local phase of the disease). Site of metastases at the time of treatment with IM were lung (7), abdomen (4), soft tissue (4), bone (1), and brain (1; Table 2).

All patients were evaluated by CT scan.

All patients started IM 400 mg/day. In 4 cases, IM was increased to 800 mg, twice a day, after the evidence of progression (one primary resistance, 3 secondary resistance); in 2 patients, dose escalation was not feasible due to patient’s comorbidities. IM was continued until evidence of progression or complete surgical resection of the residual tumor or toxicity. Overall, IM was well tolerated. Temporary treatment interruptions due to G2-3...
neutropenia and G2 fluid retention were necessary in 3 cases; in one case, IM was definitively stopped due to toxicity (G2 anorexia and G3 anemia followed by worsening of general condition).

Efficacy assessment
All cases are evaluable for response. At the time of the present analysis, 2 patients are still on therapy, 7 stopped treatment for progression while under therapy and one for toxicity.

Best RECIST response was: 8 partial response (PR; 70%), 1 stable disease (SD), 1 progressive disease (PD). All responses were confirmed at 3 months. PET response was consistent with PR RECIST in the 5 cases that were evaluated. In the single patient (case 1, Table 2) who progressed soon after starting IM, the drug was increased to 800 mg, again with PD. This patient died in 5 months. In 3 further cases who progressed after the evidence of an initial response, IM was increased to 800 mg/day, with PD (cases 2, 5, and 7). Five patients received complete surgical excision of the residual tumor after IM, after 5, 4, 5, 9, and 5 months from starting IM, respectively. Four cases were operated at INT, the fifth patient required a neurosurgical procedure performed in another hospital. Four of them were resected while under response; the disease was located to the lung in 3 cases, and to the bone in one; in all cases, preoperative CT scan showed a single metastatic lesion, but in one of them, three nodules were discovered at the time of the surgical procedure. The fifth patient received surgery after PD. A pathologic response was evident in 3 patients (see “Pathology” paragraph), with aspects depending on surgical timing and affecting tumor cells and stroma. All the 4 patients who were operated while under response to IM relapsed, after 12, 6, 5, and 5 months, respectively. IM was restored in 4 patients with a new response. Three of those cases eventually progressed after 4 months from IM re-start, while one is still on treatment. Median PFS was 11 months (range, 2–25). Median OS has not yet been reached.

Pathology
Pathologic features of patients treated with IM are detailed in Table 1 (patients 1–10). All patients had histologic confirmation of their distant relapse. In all cases, the pathologic appearance was consistent with an FS-DFSP. The primary tumor showed classic DFSP aspects without an FS component in 3 patients, who had the longest relapse-free survival (25, 22, and 10 years). In 6 cases, the primary tumor retained focal marginal areas of classic/low-grade DFSP together with FS areas featuring spindle cells; one case showed myxoid aspects. The concomitance of a low- and high-grade component was also showed by 1 local recurrences, whereas the metastatic lesions exhibited only FS features; in addition, in one case, extensive necrotic areas were detected. No morphologic changes in the pattern of growth were observed in 9 of 10 cases comparing primary tumor, recurrence, and metastasis, whereas one case (assessed by tru-cut) exhibited additional pleomorphic area in the metastatic tissue. In 2 of 8 cases, CD34 immune-positivity was lost in the high-grade component of the primary tumor.

After IM treatment, tumor samples were available in 5 cases. They exhibited different aspects depending on surgical timing, with changes in tumor cellularity that—based on baseline—from 0% to 70%. More frequently, there were cellular-depleted areas replaced by hyalinized stroma (Supplementary Fig. S1). In other cases, we detected changes consistent with early signs of response to IM, marked by a variable inflammatory component and intermingled with scattered viable tumor cells (Supplementary Fig. S1, corresponding to patient 4, Tables 1 and 2, whose matched cryopreserved halve was used for RNAseq analysis). The presence of immune/inflammatory infiltrate is in line with the GEP analysis results described below. Immunohistochemistry failed to highlight decrease of PDGFRB expression in the viable tumor cells surrounding the regressed areas (data not shown). This is consistent with our previous reports on the lack of evidence of PDGFRB decrease/switch-off in IM-responsive DFSP patients, as assessed by both IHC and immunoblot (5).

Whole transcriptome sequencing
To gain insight into the biologic activity exerted by IM in FS-DFSP, RNAseq transcriptional profile was performed on 6 FS-DFSP for which frozen material was available, including 4 naive cases (2 localized and 2 metastatic) and 2 post-IM treatment samples. All cases retained the COL1A1–PDGFB fusion.

Naive and IM-treated samples clustered separately by unsupervised Principal Component Analysis, indicating a significantly different GEP analysis (Fig. 1A). Supervised analysis highlighted the differential expression of 250 genes, with a P value <0.01 and 2-fold difference (Supplementary Table S1; Fig. 1B).

IM-treated samples displayed an enhanced expression of genes involved in drug and xenobiotics metabolism, a finding not completely unexpected, and a significant downregulation of components of kinase signaling pathways, such as TGFB2, PDGFD, and TGFBR1 (Fig. 1B and C; Table 3). The expression of PDGFB and COL1A1 was observed both in untreated and treated samples.

Seven of the top overexpressed genes displaying a Log2 ratio equal or higher than 3.5 in IM-treated samples were related to the innate or adaptive immune system. These 7 genes included genes related to B-cell responses and encoding for constant and variable chains of the immunoglobulin family together with genes associated with natural killer (NK)–mediated functions, with internal T-cell signaling (24), and with myeloid cells differentiation (ref. 25; Table 4). IHC analysis performed in post-IM samples (Table 1, patient 4) using anti-CD20 and CD57-specific antibodies confirmed the presence of tumor-infiltrating lymphoid cells expressing B- and NK-specific markers (Fig. 2). Six genes related to endothelial cells and angiogenesis also belonged to this top list. These genes encoded for proteins regulating at different levels the endothelial/vascular permeability (26–28) and differentiation of endothelial cells (29) and included both transcription factors and adhesion molecules. Moreover, VEGF-related genes, such as the gene encoding for VEGFR3 or enzyme affecting HIF1 and VEGF expression, resulted also upregulated in IM-treated tumors (Table 4; ref. 30).

Pathway enrichment analysis confirmed the modulation of genes related to the immune response, with genes involved in antigen processing and presentation and in NK-mediated cytotoxicity being overrepresented in IM-treated samples (Table 3; Fig. 1C).

Discussion
In this series of 10 histologically confirmed, metastatic DFSP patients treated with IM, we found that all lesions were made up of FS-DFSP, and, as expected, retained the COL1A1–PDGFB rearrangement. The RECIST response rate was 80%, with only 1 patient showing a primary resistance. Neither primary nor
secondary resistance could be overcome by increasing the dose of IM to 800 mg/day. Median PFS was only 11 months. All 5 patients undergoing a complete surgical resection after IM relapsed afterwards. IM was restored in the 4 patients with no previous evidence of resistance to IM, obtaining a new transient response. Contrary to naïve FS-DFSP, RNAseq analysis of IM-treated patients revealed a strong modulation of genes involved in drug/xenobiotic metabolism and those regulating tumor-related kinase signaling. Immune-related genes were also affected by IM.

Although retrospective and limited to 10 cases, this is the largest series of metastatic DFSP patients treated with IM. In fact, metastatic relapse is an extraordinary rare event in DFSP/FS-DFSP. We confirm that IM is active in the vast majority of patients who develop metastases from DFSP/FS-DFSP. However, primary resistance is observed and median PFS is shorter than reported in series including nonmetastatic DFSP. The first two reports on IM activity in DFSP were by Rubin and colleagues (10) and by Maki and colleagues (12) in 2002 in 3 DFSP metastatic patients, with evidence of a major response in 2 cases, whereas the third patient had a transient response and eventually underwent a rapid progression (12). IM activity was further confirmed within 6 prospective phase II studies (11, 13–15, 31). The largest was reported in 2010 (31) on 25 patients treated with neoadjuvant IM, but included only localized, classic DFSP. The second largest series was obtained pulling together the phase II studies run by EORTC and SWOG (11). The analysis showed a 46% response rate by RECIST (e.g., 11 PR of 21 evaluable patients) and a 1.7-month median time to progression. Interestingly, the activity of IM was not superimposable in classic DFSP and FS-DFSP: all 11 patients carrying a classic DFSP benefited from IM, whereas 2 of 8
FS-DFSP had a progression as their best response. In addition, primary or secondary resistance was observed in 6 of 7 patients with metastatic disease. Overall, DFSP metastatic cases included in several case reports or case series (7) is less sensitive to IM.

Interestingly, all our metastatic cases were found to have a FS component in the metastatic tissue. Seven were FS from the onset of disease. In 3 cases, the review of primary samples did not show FS aspects. Thus, one cannot exclude that FS areas were already present in other areas of the primary lesions. Interestingly, however, these patients without an apparent FS component at onset had the longest previous relapse-free survival (25, 22, and 10 years). Thus, retrospective studies on surgical series with an updated pathologic review are needed to clarify to which extent classic DFSP, i.e., those without any FS aspect, do have a metastatic potential.

In this series, all patients who were operated after being treated with IM had a relapse, suggesting that IM is incapable of eradicating metastatic DFSP/FS-DFSP and the role of surgery is limited. It follows that policies of re-establishment of IM after surgery of responding metastatic disease should be pursued in DFSP. This is standard practice in metastatic gastrointestinal stromal tumor (GIST) surgically excised of IM-responding lesions (32).

To gain insights into the mechanism of action of IM in FS-DFSP, we compared the transcriptional profile of naïve versus IM-treated samples. GEP analysis confirmed that the post-IM samples obtained from patients with radiologic and pathologic evidence of response maintained the expression of DFSP-associated transcripts COL1A1 and PDGFB. Moreover, GEP analysis underscored significant changes in the transcriptional profile of IM-treated samples, which displayed a molecular signature indicative of a modulation of innate and adaptive immunity, changes in vascular permeability, and silencing of some kinase signaling pathways that mainly involve TGFβ. Among genes upregulated in IM-treated samples, we found those encoding constant and variable chains of the immunoglobulin family and genes encoding components of the NK pathway and NK-mediated lysis, such as NKG7 and GZMB. These findings correlated with the pathologic evidence in these specimens of B cells, which likely play a role in adaptive-specific immunity and in Fc receptor–dependent phagocytosis, and CD57-positive lymphoid cells, essentially consisting of finally differentiated, cytotoxic-competent NK cells (33, 34). This strongly suggests that IM-induced alterations on tumor cells in turn trigger the activation of this innate response in DFSP/FS-DFSP, as already described in GIST (35–37).

Table 4. List of immune- and vascular-related genes upregulated in IM-treated tumors and displaying FC equal or higher than 3.5 log2 ratio (log2R).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Log2R</th>
<th>P</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGH3</td>
<td>9.7</td>
<td>0.00089</td>
<td>Heavy constant gamma chain 3, Immunoglobulin, B-cell response</td>
</tr>
<tr>
<td>IGH1</td>
<td>7.1</td>
<td>0.00678</td>
<td>Heavy constant mu chain, Immunoglobuline–B cells response</td>
</tr>
<tr>
<td>IGKV3-11</td>
<td>6.4</td>
<td>0.00731</td>
<td>Kappa variable 3-11 chain, Immunoglobulin, B cells</td>
</tr>
<tr>
<td>GZMB</td>
<td>4.7</td>
<td>0.00396</td>
<td>Granzyme B, NK, and cytotoxic T-lymphocyte–associated serine esterase 1</td>
</tr>
<tr>
<td>NKG7</td>
<td>4.0</td>
<td>0.00612</td>
<td>NK cell granule protein 7</td>
</tr>
<tr>
<td>GRAP</td>
<td>3.9</td>
<td>0.00011</td>
<td>GRB2-related adaptor protein. Molecule involved in TGFβ and CD28 signaling</td>
</tr>
<tr>
<td>SLP</td>
<td>6.4</td>
<td>0.00754</td>
<td>Secretory leukocyte peptidase inhibitor, Involved in the differentiation of myeloid cells</td>
</tr>
<tr>
<td>CLDN5</td>
<td>5.5</td>
<td>0.00070</td>
<td>Claudin 5, Claudins are integral membrane proteins and components of tight junction strands</td>
</tr>
<tr>
<td>C2CD4B</td>
<td>5.5</td>
<td>0.00856</td>
<td>C2 calcium-dependent domain containing 4B, expressed by endothelial cells and regulating vascular permeability</td>
</tr>
<tr>
<td>HOXA3</td>
<td>3.5</td>
<td>0.00927</td>
<td>HOXA3 transcription factor. Accelerates wound repair by mobilizing endothelial progenitor cells and by attenuating the excessive inflammatory response of chronic wounds</td>
</tr>
<tr>
<td>FLT4</td>
<td>4.0</td>
<td>0.00106</td>
<td>Fms-related tyrosine 4, receptor for VEGFRC, known also as VEGFR3</td>
</tr>
<tr>
<td>SOD3</td>
<td>3.9</td>
<td>0.00532</td>
<td>Superoxide dismutase 3. SODs are antioxidant enzymes that catalyze the dismutation of two superoxide radicals into hydrogen peroxide and oxygen. Involved in the negative regulation of HIF1 and VEGF expression (21)</td>
</tr>
</tbody>
</table>
studies on both T-cell and NK-mediated immunity are currently ongoing, taking advantage of few fresh cryopreserved cellular suspensions of post-IM tumor samples. Moreover, the role of TGFβ signaling and the presence of nonsynonymous mutations possibly involved in response to IM are under evaluation.

We could observe 1 patient with primary resistance to IM and 5 cases with progression after response. The basis for the resistance to IM in DFSP/FS-DFSP is still a matter of discussion and does not seem to be related to PDGFRB (5). Unfortunately, the lack of samples suitable to RNAseq analyses prevented us from fully exploring the mechanisms underlying resistance. By whole-genomic sequencing, Hong and colleagues examined one case of DFSP prior and after development of resistance to IM, and identified 8 nonsynonymous somatic gene mutations, involving ACAP2, CARD10, KIAA0556, PAAQR7, PPP1R39, SAFB2, STARD9, and ZFYVE9, in the IM-resistant tumor tissue (16). This study revealed diverse possible candidate mechanisms by which IM resistance to PDGFRB inhibition may arise in DFSP. We failed to detect somatic or deleterious mutations in our RNAseq, although variant calling from RNAseq data is notoriously less efficient compared with genome/exome analyses.

Eilers and coworkers demonstrated CDKN2A deletion in a subset of FS-DFSP, providing evidence of a contribution of p16 loss to DFSP progression (9). Neither study highlighted a role for PDGFRB genetic alteration in IM resistance. Rather, results by Eilers and colleagues seem to support a role for candidates downstream of PDGFRB. Linn and colleagues (38) reported that DFSP expresses high levels of GRB2 and PRKA, both downstream to the PDGF receptor. Intriguingly, our gene expression analysis showed an upregulation of the GRAP gene, encoding the GRB2-related adaptor protein, responsible for the coupling of signals from receptor and cytoplasmic tyrosine kinases to the Ras signaling pathway. On this basis, it is tempting to speculate that this adaptor has a role in DFSP progression (39).

In conclusion, our study confirms that IM is active in the majority of metastatic DFSP, but duration of response is inferior than in published series which included both DFSP and FS-DFSP. In our series, all metastatic patients had an FS-DFSP. We failed to

Figure 2.
B- and NK-cell infiltration in a metastatic FS-DFSP lesion after treatment with IM, corresponding to patient #4 in Tables 1 and 2. H&E, CD20 (B cells), and CD57 (NK cells) stains are shown. A, the metastatic tumor after the first period of treatment with IM, followed by surgery in 2012 (this is the sample evaluated also by RNA-seq). B, the relapsed tumor retreated with IM, and again resected in 2014. B- and NK-cell infiltration was already evident in the first post-IM lesions (A) and even more pronounced in the second one (B). A, original magnification, ×100; B, original magnification, ×20 (H&E) and ×50 (CD20 and CD57 stainings).
eradicate the tumor even in those patients responsive to IM who could undergo a complete surgical resection, suggesting that the role of surgery is limited. IM reintroduction should be considered on relapse. Finally, IM treatment was found to have an impact also on tumor microenvironment, in terms of increased permeability of endothelial cells and immune infiltrates. This suggests that the elicitation of an innate and adaptive immune response may be crucial in sustaining DFSP-FS response to the treatment.

Disclosure of Potential Conflicts of Interest

S. Stacchiotti reports receiving a commercial research grant from Novartis. M. A. Pantaleo reports receiving a commercial research grant from Novartis and speakers bureau honoraria from Pfizer. A. Gronchi and P. G. Casali report receiving speakers bureau honoraria from and are consultant/advisory board members for Novartis. M. Fiore reports receiving speakers bureau honoraria from Novartis. No potential conflicts of interest were disclosed by the other authors.

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