Sunitinib in advanced alveolar soft part sarcoma: evidence of a direct antitumor effect


Departments of 1Cancer Medicine; 2Pathology, Laboratory of Experimental Molecular Pathology; 3Experimental Oncology and Molecular Medicine; 4Radiology; 5Surgery, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy

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Background: The purpose of this study was to confirm sunitinib activity in alveolar soft part sarcoma (ASPS) and to report on new insights into the molecular bases thereof.

Patients and methods: From July 2007, nine patients with progressive metastatic ASPS received sunitinib 37.5 mg/day, within a named use program. Cryopreserved material was available for five naive patients, among whom three received sunitinib. Immunofluorescence (IF)/confocal microscopy, biochemical, and molecular/cytogenetic analyses were carried out, complemented by antiproliferative and activation assays in a short-term culture derived from one case.

Results: All patients were eligible for response. Best RECIST response was partial response in five cases, stable disease in three, and progression in one. The median progression-free survival was 17 months. Positron emission tomography results were consistent. Two cases of interval progressions were recorded. Antiproliferative assays and biochemistry on short-term culture showed that sunitinib is able to markedly impair ASPS cells growth and switch-off PDGFRB. IF/confocal microscopy demonstrated coexpression and physical association between PDGFRB/vascular endothelial growth factor receptor 2 (VEGFR2) and RET/VEGFR2 in ASPS cells, which was validated by biochemistry. PDGFRB, RET, and MET ligand-dependent activation was confirmed.

Conclusions: We confirm the clinical efficacy of sunitinib in ASPS, mediated by PDGFRB, VEGFR2, and RET, which are all expressed in tumor cells. A direct antitumor effect was shown in a short-term cell culture.

Key words: ASPS, sarcoma, sunitinib, tyrosine kinase inhibitors

Introduction

Alveolar soft part sarcoma (ASPS) is a very rare soft tissue sarcoma, mainly affecting young patients [1]. In spite of its indolent behavior and prolonged natural history, the cure rate is between 40% and 50% at 10 years, with some patients relapsing even after >10 years from first diagnosis [1–4]. In fact, ASPS is marked by a very high metastatic potential, reported to be +60% [1–3] with >25% of patients metastatic at diagnosis [1–4]. Given the ultimately poor prognosis of the disease and the known resistance to conventional chemotherapy [1, 5, 6], new medical options are strongly needed for the advanced phase. ASPS carries an unbalanced recurrent translocation t(X;17)(p11;q25) translocation, leading to the chimeric transcription factor ASPL-TFE3 [7]. The product of this peculiar translocation induces MET transcriptional up-regulation and activation, and has been linked to the high metastatic rate in ASPS [2, 6]. Thus, MET may represent a potential therapeutic target in ASPS patients. In fact, phase II studies on MET inhibitors in ASPS are currently ongoing [8].

Besides, ASPS has a distinctive angiogenic phenotype marked by a peculiar tumor-associated vasculature. Moreover, gene expression profiling of ASPS specimens demonstrated an array of potentially therapeutically targetable, angiogenesis-related molecules [2, 9]. Indeed, there is evidence of a possible therapeutic effect of antiangiogenic drugs mediated by platelet-derived growth factor B (PDGFB)/platelet-derived growth factor receptor B (PDGFRB) and vascular endothelial growth factor (VEGF)/vascular endothelial growth factor receptor (VEGFR) axis inhibition [2, 9, 10]. In particular, VEGFA/VEGFR2 (also named KDR) axis is critical for endothelial cell (EC) functions. Recently, it has been hypothesized that the expression of VEGFRs by tumor cells may be sustained by a VEGF/VEGFR autocrine loop [11–13]. Even PDGFB/PDGFRB axis is important in the recruitment of PDGFRB-expressing pericytes and is expressed in both vascular and tumor cell components of a number of tumors [14, 15], among which there is ASPS [16]. PDGFB can even up-regulate VEGF expression with an indirect angiogenic effect [17]. Furthermore, VEGFA—in the presence of glial cell like-derived neurotrophic...
factor (GDNF) expression—leads to activation of both RET and VEGFR2 through the physical interaction of the two receptors [18] and, ultimately, to a RET-dependent activation of MET [19].

Indeed, the activity of antiangiogenic agents such as INF-α [20–22], bevacizumab [23], sunitinib [16, 24], and cediranib [25] has been reported in ASPS. We already described the response to sunitinib in two cases of advanced progressive ASPS [16]. This analysis intends to update those results clinically and translationally.

materials and methods

patient selection

This retrospective series deals with nine advanced translocated ASPS patients, with evidence of progression during the previous 3 months according to the RECIST [26]. Performance status [Eastern Cooperative Oncology Group (ECOG)] less than or equal to 3 and an adequate bone marrow and organ function were also requested. Diagnosis was confirmed by positive immunostaining for TFE3 and by interphase FISH [16]. All patients provided a written informed consent to a nonconventional medical treatment, selected in the lack of alternative therapies known to be effective in the disease. The use of the drug was approved by the ethics committee in all cases.

Immunofluorescence (IF)/confocal microscopy, biochemical, and molecular/cytogenetic analyses were carried out in five naïve ASPS, whose cryopreserved material was available. Among them, three received sunitinib subsequently and are included in the above series (Tables 1 and 2, patients A, B, C), while two have not been treated (Table 2, patients X and Y). In case Y, whose fresh material was available, flow cytometry (FCM) was carried out and a short-term culture was obtained. Clinical, biochemical, and molecular results of four patients treated with sunitinib and two treated subsequently were already reported in part in our previous paper [16].

treatment

Patients received 37.5 mg of sunitinib once daily, without planned treatment interruptions, within a named use program, until progression or toxicity. Treatment was withheld for hematologic grade ≥3 adverse events and for non-hematologic grade ≥2 adverse events (defined according to the National Cancer Institute Common Toxicity Criteria, version 3.0) and restarted after recovery to grade <2 in case of hematologic or grade <1 in case of non-hematologic. Grade ≥2 hypertension and hypothyroidism were treated with antihypertensive medication and levothyroxine replacement therapy without discontinuation of sunitinib.

clinical assessment

Serum chemical analyses and blood count were evaluated at baseline and monitored after 2 weeks from treatment start, then monthly. Adverse events were recorded. Disease status was assessed at baseline by a whole-body computed tomography (CT) scan, a CT scan or magnetic resonance imaging (MRI) of the sites of disease, and [18F]fluorodeoxyglucose positron emission tomography (PET) scan. Baseline PET was negative in two cases and was not revaluated thereafter. CT/MRI was repeated after 4–6 weeks of treatment and then every 3 months, while PET only at 4–6 weeks.

Response to treatment was assessed with the use of RECIST criteria and PET response, according to the European Organization for Research and Treatment of Cancer 1999 tumor response criteria [26, 27].

Progression-free survival (PFS) and overall survival (OS) were estimated with Kaplan–Meier method. Event for PFS was progression disease occurred while on treatment according to RECIST. Data for patients who interrupted their treatment without evidence of disease progression and, after progression during the discontinuation, restarted sunitinib with a new response were censored at the time of progression under therapy or at the time of the last assessment of the tumor. Event for OS was death due to any cause. Patients alive were censored at the time of the last contact.

microscopic analyses

IF and confocal microscopy. Anti-PDGFBR, anti-VEGFR2, anti-MET, anti-RET, and anti-VEGFA antibodies were used as reported in supplemental materials and methods. Data for patients who were treated with antihypertensive medication and levothyroxine replacement therapy without discontinuation of sunitinib.

Table 1. Patient treated with sunitinib: clinical characteristics

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Age (years)</th>
<th>Gender</th>
<th>Performance status</th>
<th>Primary tumor</th>
<th>Site of metastasis</th>
<th>Previous surgery/ radiotherapy/ chemotherapy</th>
<th>Treatment with sunitinib</th>
<th>Best RECIST response</th>
<th>PFS (months)</th>
<th>OS (months)/ status</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>58</td>
<td>M</td>
<td>2</td>
<td>Forearm</td>
<td>Lung, liver, soft tissue, bone</td>
<td>Y/Y/Y</td>
<td>Y</td>
<td>PR</td>
<td>33+</td>
<td>33/Alive</td>
</tr>
<tr>
<td>B</td>
<td>32</td>
<td>M</td>
<td>0</td>
<td>Forearm</td>
<td>Lung</td>
<td>Y/Y/N</td>
<td>Y</td>
<td>PR</td>
<td>18+</td>
<td>18/Alive</td>
</tr>
<tr>
<td>C</td>
<td>23</td>
<td>F</td>
<td>1</td>
<td>Retropitoneum</td>
<td>Lung</td>
<td>Y/Y/N</td>
<td>Y</td>
<td>PR</td>
<td>15+</td>
<td>15/Alive</td>
</tr>
<tr>
<td>D</td>
<td>28</td>
<td>M</td>
<td>1</td>
<td>Arm</td>
<td>Lung, liver, soft tissue</td>
<td>Y/Y/Y</td>
<td>Y</td>
<td>PR</td>
<td>17</td>
<td>19/Dead</td>
</tr>
<tr>
<td>E</td>
<td>24</td>
<td>M</td>
<td>3</td>
<td>Leg</td>
<td>Lung, liver, soft tissue, bone, brain</td>
<td>Y/Y/Y</td>
<td>Y</td>
<td>SD</td>
<td>6</td>
<td>8/Dead</td>
</tr>
<tr>
<td>F</td>
<td>31</td>
<td>M</td>
<td>0</td>
<td>Thigh</td>
<td>Lung</td>
<td>Y/N/Y</td>
<td>Y</td>
<td>PD</td>
<td>3</td>
<td>18/Dead</td>
</tr>
<tr>
<td>G</td>
<td>22</td>
<td>F</td>
<td>1</td>
<td>Thigh</td>
<td>Lung</td>
<td>Y/Y/Y</td>
<td>Y</td>
<td>SD</td>
<td>10</td>
<td>10/Alive</td>
</tr>
<tr>
<td>H</td>
<td>28</td>
<td>F</td>
<td>0</td>
<td>Leg</td>
<td>Lung, brain</td>
<td>Y/Y/N</td>
<td>Y</td>
<td>SD</td>
<td>7+</td>
<td>7/Alive</td>
</tr>
<tr>
<td>I</td>
<td>25</td>
<td>M</td>
<td>0</td>
<td>Leg</td>
<td>Lung</td>
<td>Y/N/Y</td>
<td>Y</td>
<td>PR</td>
<td>4+</td>
<td>4/Alive</td>
</tr>
</tbody>
</table>

M, male; F, female; Y, yes; N, no; PD, progressive disease; PR, partial response; SD, stable disease; PFS, progression-free survival; OS, overall survival.
## Table 2. Naive cases

<table>
<thead>
<tr>
<th>Case</th>
<th>PDGFRB</th>
<th>RET</th>
<th>MET</th>
<th>VEGFR2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B</td>
<td>+</td>
<td>+</td>
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<td>C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Yb</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Translational findings: RTK analyses.** FISH and mutational analyses ruled out gene alterations.

**immunoprecipitation/western blotting.** For the immunoprecipitation (IP) analyses, equal amounts (1 mg) of protein lysates were precipitated by incubation with Protein A Sepharose (Amersham Biosciences, Piscataway, NJ) and specific antibodies (anti-PDGFRB, anti-RET, anti-MET) [15, 16]. Western blotting (WB) was carried out as already described [16]. To detect the coimmunoprecipitated proteins, the filters of IP anti-PDGFRB and IP anti-RET were incubated with anti-VEGFR2 (Flk-1, sc-6251; Santa Cruz Biotechnology, Santa Cruz, California); the filter of IP anti-MET was incubated with anti-VEGFR2 and anti-RET. The downstream targets expression and activation were detected by means of WB [16].

**immunohistochemistry.** Using paired matched formalin-fixed paraffin-embedded samples, we complemented the expression analysis carried out on frozen samples with immunophenotyping on sections as already reported [16] expanding immunophenotyping to GDNF family receptor alpha 1 (GRFalfa1) (AF714; R&D system, Minneapolis, MN, 1:10).

**flow cytometry.** The FCM analysis was carried out as previously described [29]. To identify ASPS and ECs in tumor suspension, PDGFRB and CD31 expression was evaluated on CD45

**molecular analyses**

RT-PCR and real-time PCR to detect RTK ligands. PDGFB, hepatocyte growth factor, GDNF, and VEGFA were assessed as previously reported [16, 28]. DNA extraction and sequencing. Mutational analysis was carried out as previously described [16]. The PCR products underwent automated DNA sequencing.

**short-term culture**

Cells were obtained by enzymatic disaggregation of a fresh specimen and cultured in Dulbecco’s modified Eagle’s medium (Cambrex Bio Science, Walkerville, MD), supplemented with 10% fetal bovine serum and penicillin and streptomycin antibiotics. Cells were maintained in a 5% CO₂ atmosphere.

**cell proliferation assay.** For cell growth assay, cells at passages 0 and 1 following disaggregation were plated at 7500 cells/cm² in complete medium in 96-well plates. The day after seeding, cells were exposed to solvent or different concentration of sunitinib, or bevacizumab for 72 h. Sunitinib dissolved in dimethylsulfoxide 100% was further diluted in cell culture medium (final solvent concentration, 0.5%). Cell growth was evaluated by sulforhodamine B (Sigma Chemical Company, St Louis, MO) colorimetric assay [30]. Each experiment was carried out in eight replicates. Drug concentrations able to inhibit cell proliferation by 50% (IC₅₀) were calculated from dose–response curves.

**pre- and post-sunitinib activation profile.** The comparison between pre- and post-sunitinib treatment IP/WB analysis was carried out for PDGFRB.

## Results

Nine patients seen at Istituto Nazionale Tumori, Milan, Italy, from July 2007 to May 2010, with metastatic ASPS, received
sunitinib 37.5 mg/day (continuous daily dosing). Among them, five are still on therapy. Pretreatment frozen surgical specimens from primary tumor were available for three out of nine (Tables 1 and 2, patients A–C). Two additional specimens from the primary tumor of two patients (Table 2, X and Y) not treated in this series were also analyzed.

**patients**

Patient characteristics are listed in Table 1 (mean age: 24 years; site of primary: eight extremities, one retroperitoneum; pretreatment with one or more medical treatment: seven). The ECOG performance status was greater than or equal to 2 in four of them. All patients had documented progression before starting treatment.

The median treatment duration was 10 months (range: 3–33). All patients received sunitinib 37.5 mg/day. In all patients who stopped their treatment for toxicity, the drug was restarted at the same dose level. The major non-hematologic toxic effects included fatigue (one case, G2), hypothyroidism (two cases, G2), hypertension (two cases, G2), liver toxicity (one case, G2), and nausea and vomit (one case, G2). The most common hematologic toxic effects were neutropenia (four patients, no G3–4), chronic anemia (one patient, no G3–4), and thrombocytopenia (two patients, no G3–4).

**response**

All patients are assessable for response. After 3 months of treatment, five patients (55%) had RECIST partial response (PR) (Figure 1, panel A–C), along with subjective improvement in three symptomatic cases, three stable disease (SD), and one progressive disease (PD). After 6 months, PR/SD was confirmed in all cases but one who progressed (at 3 months this patient was slightly progressive, yet not reaching a RECIST PD). PET was consistent with response/progression (four response, one progression). One patient responded to sunitinib, progressed after 17 months of treatment and died 1 month later.

The median OS was 19 months, with three patients dead at the time of the present analysis. The median PFS on therapy was 17 months (range 2–33), with 88% patients progression free at 6 months. In two cases, treatment was temporarily stopped with evidence of tumor progression on CT scan after 3 and 1 months from treatment interruption, respectively. Both of them are still on therapy with a PFS from restarting sunitinib of 11 and 4 months, respectively. Response was reestablished upon restarting sunitinib (Figure 1, panel C–E).

**translational findings**

IF/confocal microscopy, biochemical, and molecular/cytogenetic analyses were carried out in five naive ASPS.

**RTKs**

**RTK findings detailed in Table 2.** IF and confocal analyses showed PDGFRB and VEGFR2 coexpression (Figure 2A–C, Table 2) and physical association between the two receptors (Figure 2D), in ASPS cells, respectively. These data were confirmed by co-IP assay (Figure 2E). VEGFA ligand was also detected (Figure 2F).

To further confirm these findings in case Y, whose fresh material with >90% TFE3 positive tumor cells was available, FCM analysis was carried out. Results indicated that PDGFRB was expressed at higher level in ASPS cells than in ECs. Besides, both ASPS and ECs showed a comparable VEGFR2 expression (Figure 2G).
Figure 2. Immunofluorescence (IF), biochemical, and flow cytometry (FCM) analyses of PDGFRB/VEGFR2 and RET/VEGFR2. PDGFRB (green) (A) and VEGFR2 (red) (B) fluorescence immunolabeling of alveolar soft part sarcoma (ASPS) cells. The corresponding merge complemented by confocal microscopy merge highlights coexpression (C) and physical interaction between the two receptors (D), respectively. The activation of VEGFR2 is supported by the expression of VEGFA (F), while the physical interaction between the two receptors is confirmed by immunoprecipitation (IP)/co-immunoprecipitation (co-IP) (E). Further demonstration of PDGFRB and VEGFR2 coexpression is provided by FCM analysis (G) on fresh material, carried out using a CD45/SSC gating strategy (a) and the following antibody combinations: (b) CD31 fluorescein isothiocyanate (cat: PN IM 1431, Beckman Coulter), PDGFRB PE (cat: 558821, Becton Dikinson, Franklin Lakes, NS), and CD45PerCP (cat: 345809, Becton Dikinson, Franklin Lakes, NS) for...
We also detected VEGFR2 and RET coexpression (Figure 2H–J), and physical association (Figure 2K) in ASPS cells, through fluorescence microscopy and confocal analysis, respectively. Co-IP confirmed confocal analysis results in two cases whose residual frozen material was available (Figure 2L).

Indeed, we found a ligand-dependent activation (data not shown), confirming previous data [16]. Moreover, we demonstrated RET, MET, and GFRα1 expression by immunohistochemistry (Figure 3A–C). GFRα1 expression was confirmed by WB (Figure 3D), providing a possible further mechanism of MET activation. In fact, an indirect GDNF-dependent pathway of MET activation has been reported in tumor cells expressing RET/GFRα1, in which GDNF promotes MET phosphorylation [19].

Conversely, VEGFR2 and MET were coexpressed (Figure 3E–G) but they show neither physical interaction at confocal microscopy (Figure 3H) nor co-IP (data not shown). Co-IP was not even observed for MET and RET (data not shown) [16].

PDGFRB, MET and RET gene mutation and/or imbalance analysis did not show any alteration in all tested cases.

Figure 3. Indirect RET/GFRα1-mediated MET activation and MET/VEGFR2 expression. Immunohistochemistry for RET (A), MET (B), and GFRα1 (C). RET decorates both membrane and cytoplasm of alveolar soft part sarcoma (ASPS) cells, while MET and GFRα1 expression is restricted to cytoplasms. GFRα1 expression is confirmed by western blotting (D) (positive control was thyroid tissue). ASPS tumoral cells showing MET (green) (E) and VEGFR2 (red) (F) fluorescence immunolabeling and corresponding immunofluorescence merge (G) highlighting their coexpression. Confocal microscopy merge (H) confirms the coexpression of the two receptors but not their physical interaction.

detecting the expression of PDGFRB on ASPS and endothelial cells (ECs); (c) CD31 FITC, VEGFR2 PE (cat: FAB357P, R and D, Minneapolis, MN), and CD45PerCP for evaluating the expression of VEGFR2 on ASPS and ECs. ASPS tumor cells also show RET (green) (H) and VEGFR2 (red) (I) fluorescence immunolabeling. The corresponding IF merge (J) and confocal microscopy merge (K) are consistent with coexpression and physical interaction of the two receptors, respectively. IP/co-IP analysis confirms such interaction (L).
downstream signaling
The biochemical analysis of the downstream signaling confirmed what already reported [16]. Rat sarcoma viral oncogene homolog (RAS)/mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/Akt pathways were found to be activated as well as mammalian target of rapamycin (mTOR) and its downstream targets, S6 and 4EBP1. RAS, RAF, PI3K, and PTEN genes did not show neither mutations nor gene imbalance. Cumulatively, the activation profile is consistent with the functional status of PDGFRB, RET, MET, and VEGFR2.

antiproliferative and biochemical assays
We investigated the effects of sunitinib in a short-term ASPS cultures directly obtained from a naive clinical specimen, the representativity of which was checked by FCM as above reported. In an antiproliferative assay, a 72-h treatment with the drug inhibited in a dose-dependent fashion the proliferation of ASPS cells (IC50 = 2.45 ± 0.47 μM) (Figure 4A). Such an antiproliferative effect was consistent with biochemical results showing a considerable decrease of PDGFRB phosphorylation in sunitinib-treated cells (Figure 4B). On the contrary, bevacizumab did not affect cell growth under the same experimental conditions (not shown). The lack of effect of bevacizumab may be due to the fact that VEGFA expression is restricted to cytosolic component and consistent with the notion that bevacizumab is not able to cross cell membranes (Figure 2F).

discussion
We confirm sunitinib activity in advanced ASPS. Among nine patients with progressive ASPS treated with continuous sunitinib, we could observe 55% RECIST PRs, along with PET responses. Besides, responses can be long lasting (median PFS 17 months, 88% patients progression free at 6 months, one patient still responsive at 33 months). In two cases, treatment discontinuation corresponded to tumor progression, and response was reestablished upon restarting sunitinib. Translational findings, including cell proliferation assays, sustain that responses are due to a direct antitumor activity of sunitinib in addition to its known antiangiogenic capacity and that this is mediated by PDGFRB, VEGFR2, and RET inhibition.

These data confirm the potential role of sunitinib in a rare and orphan disease, for which no active drug is currently available.

Sunitinib is an oral multitargeted RTK inhibitor, which blocks kinase activity and signaling of KIT, PDGFRs, VEGFR1, VEGFR2, VEGFR3, FLT3, macrophage colony-stimulating factor, and RET [31–35]. In this way, besides interfering with tumor neoangiogenesis through the inhibition of endothelium proliferation, sunitinib may induce a direct antitumor activity in those cancers whose neoplastic cells express those targets. So far, sunitinib efficacy has been confirmed in different tumors, like renal cell carcinoma and gastrointestinal stromal tumor (GIST) [36, 37], for which the drug has been approved by the Food and Drug Administration/European Medicines Agency (EMA). In particular, in GIST it is reported only a 7% of dimensional responses in imatinib-resistant patients, with a 6-month median PFS [37]. Anecdotal responses to sunitinib have been reported even in other sarcomas, like fibrous solitary tumors, desmoplastic small round cell tumors, giant cell tumor of the bone, and possibly chordomas and synovial sarcomas [24, 38]. In most cases, responses correspond to prolonged SD, along with changes in tumor density, more than to tumor shrinkage. Conversely, in ASPS responses to sunitinib are more often dimensional, even in pretreated patients and in cases with very advanced disease as for in presence of brain metastases. This pattern of response corresponds to what preliminarily described for cediranib, another antiangiogenic agent tested in ASPS [25]. Conversely, preliminary data on ARQ197, another
new drug inhibiting MET recently tested in ASPS, showed prolonged SD without any decrease in tumor size [8]. Furthermore, responses to sunitinib in ASPS may last long. Since the natural history of ASPS can be marked by prolonged intervals of stability even in the advanced phase, it could be argued that a prolonged progression-free interval is due to the spontaneous clinical course of the disease. Yet, all patients treated in this series were progressing before starting treatment. Furthermore, in two patients who stopped their treatment, an interval tumor progression was recorded soon after discontinuation, and response was reestablished after restoring sunitinib. Of course, evidence of tumor shrinkage adds to all this.

As already published by our group, sunitinib spectrum of activity closely matches ASPS RTK activation profile, characterized by a ligand-dependent activation of PDGFRB, RET, and MET [16]. Here, we confirm our previous data. In addition, we found that MET could be indirectly activated through RET and GFRα21 (RET coreceptor) in presence of GDNF (RET ligand) [16, 19]. Moreover, by IF analysis, we detected that PDGFRB, RET, and MET were coexpressed with VEGFR2 in ASPS cells. These results were confirmed by confocal microscopy and by IP/WB analyses that showed a physical association between PDGFRB/VEGFR2 and RET/VEGFR2. Remarkably, the PDGFRB/VEGFR2 complex appeared prevalent with respect to RET/VEGFR2. Conversely, no interaction between MET/VEGFR2 was observed.

The antiproliferative assay on the ASPS primary culture showed a remarkable activity of sunitinib, and biochemical analysis of ASPS cells before and after sunitinib evidenced a relevant decreasing of PDGFRB activation.

Overall, considering the spectrum of sunitinib activity, our results are consistent with a direct inhibition of PDGFRB, and possibly VEGFR2 and RET, and an indirect RET-mediated inhibition of MET in ASPS cells. The role of MET inhibition in sunitinib antitumor effect needs to be further investigated.

ASPS is a medical need and represents a rare subgroup within the rare family of soft tissue sarcoma. Clinical studies are challenging. Anecdotal evidence may thus be precious. All the more, this is true when biological correlations are consistent with clinical observations. Thus, sunitinib may constitute a new opportunity for ASPS treatment, to be prospectively evaluated possibly through methodologically innovative clinical studies [39].

acknowledgements

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funding

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disclosure

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references