Aplidin in patients with advanced dedifferentiated liposarcomas: a French Sarcoma Group Single-Arm Phase II study

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Background: Preclinical data have suggested a therapeutic role of JUN pathway activation in dedifferentiated liposarcoma (DDLPS) tumorigenesis. Aplidin® is a drug inducing apoptosis through a strong, sustained activation of c-Jun NH2-terminal kinase.

Methods: This phase II trial included patients with progressive advanced DDLPS. They received Aplidin® 5 mg/m² days 1–15, 28-day cycle until disease progression or unacceptable toxicity. The primary end point was the 3-month nonprogression rate (PFS3) defined as the proportion of patients with nonprogressive disease at 3 months. A PFS3 of 40% considered as a reasonable objective to claim drug efficiency.

Results: Between August 2012 and May 2013, 24 patients were included. Sixteen had received prior chemotherapy. Twenty-two were assessable for efficacy. The PFS3 was 9.1% [95% confidence interval (CI) 1.1–29.2]. Median progression-free and overall survivals were 1.6 months (95% CI 1.4–2.6) and 9.2 months (95% CI 6.6–). The most frequent adverse events of any grade were nausea, fatigue, anorexia, vomiting and diarrhea.

Conclusion: Aplidin® did not meet the primary end point of this trial and do not deserve further investigation in DDLPS.

ClinicalTrials.gov Identifier: NCT01876043.

Key words: dedifferentiated liposarcoma, JUN, treatment, Aplidin, plitidepsin

introduction

Accounting for up to 25% of all sarcomas in adults [1], liposarcoma (LPS) is the most common soft-tissue sarcoma (STS). well-differentiated liposarcoma (WDLPS)/dedifferentiated liposarcoma (DDLPS) are characterized by the consistent presence of supernumerary rings and/or giant rod chromosomes that contain amplified sequences of the region 12q14-15 [2] with an overexpression of MDM2, HMGA2 and CDK4 resulting in uncontrolled proliferation through combined effects on p53 and the cell cycle. The detection of MDM2 and CDK4 genes amplification is a useful ancillary diagnostic tool [3, 4] that provides strong support in distinguishing DDLPS from other sarcomas such as undifferentiated pleomorphic sarcomas [5, 6]. Moreover, the better understanding of these specific molecular aberrations

in WDLPS/DDLPS has recently led to the development of several promising targeted therapeutic agents.

DDLPS are biphasic neoplasms occurring in the same age group as WDLPS, with one component being a WDLPS and the other a nonlipogenic sarcoma of variable histological grade. About 90% of DDLPS arise de novo, while 10% occur in recurrence. This histological subtype is found mostly in the retroperitoneum [7, 8] and has a more aggressive behavior than WDLPS with an estimated 5-year disease-specific-survival of 44% versus 93% [9]. The local recurrence rate for retroperitoneal tumors can reach 80% and distant metastatic relapse is observed in up to 30% of cases [10, 11].

Conventional cytotoxic agents are generally considered as poorly effective in DDLPS patients with metastatic or locally unresectable disease [12, 13].

Besides MDM2 amplification, DDLPS are also characterized, in contrast to WDLPS, by a consistent co-amplification of the 1p32 or the 6q23 region. It has been recently demonstrated that 1p32 and 6q23 amplicons target MAP3K5 and JUN, respectively [14, 15]. Both genes encode proteins involved in the c-Jun NH2-terminal kinase pathway.
terminal kinase (JNK) signaling pathway. Moreover, it has been suggested that JNK overexpression, which is present in tumors with JUN or MAP3K5 amplification is responsible for the aggressive undifferentiated phenotype of DDLPS by interfering with adipocytic differentiation [15].

Aplidin® is a cyclic depsipeptide originally isolated from a Mediterranean marine tunicate, Aplidium albicans, which currently is manufactured by total synthesis. The recommended dose was established at 5 mg/m² every 2 weeks [16]. It is currently in phase II clinical trials for solid and hematological malignant neoplasias like T-cell lymphoma and in phase III clinical trials for multiple myeloma.

Several lines of evidence indicate that Aplidin® induced apoptosis of tumor cells as the result of a sustained activation of JNK. Mouse embryo fibroblasts lacking JNK isoforms are less sensitive to Aplidin® when compared with wild-type cells [17]. Moreover, treatment of tumor cells with JNK inhibitors such as SP600125 partially rescued them from Aplidin®-induced cell death [18] and tumors cells that are at least partially resistant to the drug show only weak, transient JNK activation [19]. The phosphorylation by JNK of JUN appears to be an important mechanism involved in the proapoptotic action of Aplidin® [17, 18]. A functional JNK pathway is essential for Aplidin®-induced apoptosis to occur in vitro [20, 21].

Preclinical data from our DDLPS cell lines have shown that Aplidin® induces cell death in DDLPS cell lines. Therefore, Aplidin® may be an agent of choice for DDLPS by interfering with the JNK signaling pathway. Based on these data and the clear unmet need for patients with advanced DDLPS, the French Sarcoma Group has conducted a multicenter phase II trial of assessing safety and efficacy of Aplidin® in patients with advanced DDLPS.

patients and methods

patients

Patients had to be aged 18 years or older and histologically confirmed metastatic and/or unresectable DDLPS; with documented disease progression (as per RECIST 1.1) [22] within 3 months before study entry. Detailed eligibility criteria are described in supplementary data, available at Annals of Oncology online.

study design and treatment

This was a single-arm phase II multicenter clinical trial based on a two-stage Simon’s design [23] and conducted in accordance with the Declaration of Helsinki and Good Clinical Practices. The sponsor was Institut Bergonié. All patients provided written informed consent before enrollment in the study.

Patients received Aplidin® as an i.v. 3-h infusion of 5 mg/m²/day on days 1 and 15 every 4 weeks (q4wk). Patients discontinued Aplidin® if one of the following occurred: patient decision to withdraw, unacceptable toxicity, disease progression as per RECIST 1.1 [22], intercurrent illness or general or specific changes in the patient’s condition preventing further treatment in the judgment of the investigator.

No more than two dose reductions per individual patient were allowed during the trial under any circumstances. Dose reductions were based on the worst treatment-related toxicity found since the last dose administration. Patients who continued to experience the treatment-related toxicity and/or frequent dose delays or omissions after two dose reductions had to be withdrawn from the study (except in case of obvious patient’s clinical benefit and upon Sponsor agreement). Once the dose was reduced for an individual patient, dose re-escalation was not allowed under any circumstances. Dose reduction were not required in case of not optimally treated grade ≥3 nausea and/or vomiting, grade 3 asthenia lasting ≤5 days or not optimally treated grade 3 diarrhea lasting ≤2 days.

response assessment and toxicity

Tumor assessment was carried out every 6 weeks. Response was determined per RECIST 1.1 [22] after blinded central imaging review. Toxicities were assessed continuously per Common Terminology Criteria for Adverse Events 4.0.

correlative studies

Molecular analyses were carried out for consenting patients. Archival tumor tissue was analyzed by comparative genomic hybridization to assess JUN and MAPK35 amplification status. Protocol is available on request.

statistical analysis

The primary end point was the 3-month nonprogression rate (PFS3) defined as the percentage of patients remaining alive and progression free at 3 months (i.e. week 12 ± 1) after the first infusion of Aplidin® as per RECIST 1.1 [22]. Nonprogression is a worldwide recognized end point for STS phase II trials based on the European Organization for Research and Treatment of Cancer results who demonstrated that, in case of progressive disease following a first-line chemotherapy, a drug can be considered active in soft-tissue sarcoma patients if the 3-month nonprogression rate is at least ≥40% [24].

A two-stage Simon’s design [23] with 37 eligible patients (first step: 17 patients) was used to distinguish a favorable true nonprogression rate (PFS3) of 40% from a null rate of 20% with 90% power and 10% type I error. Following the inclusion of the first 17 assessable patients, if three or less patients were progression free (complete response, partial response or stable disease) at 3 months, the study would be terminated early. Otherwise, the second group of 20 subjects will be recruited. If at the end of recruitment, 11 patients or more were progression free (of the first 37 assessable patients), Aplidin® would be considered worthy of further testing in this disease.

To be evaluable for the first efficacy end point, a subject had to meet the eligibility criteria, received at least one complete or two incomplete cycles of Aplidin® and underwent at least one disease measurement recorded not less than 6 weeks after treatment onset. In order to account for not assessable patients ±10%, 41 recruitments were planned.

Secondary end points included the best overall response as per RECIST 1.1, 1-year progression-free survival (PFS), 1-year overall survival (OS), safety and correlations with molecular characteristics of tumors. PFS was defined as the duration of time from start of treatment to time of progression or death (from any cause), patients alive and progression free were censored at the date of last follow-up, death, or last patient contact. OS was defined as the duration of time from start of treatment to the time of death, or last patient contact. All enrolled patients who received at least one infusion of Aplidin® were eligible for safety analyses.

Descriptive statistics were used to characterize patients at study entry. Ninety-five percent two-sided exact binomial confidence intervals (CI) were computed for PFS3. PFS and OS were estimated using the Kaplan–Meier method. The data reported here represent the study database as of 3 February 2014 and were submitted to an Independent Data Monitoring Committee for review. All analyses were conducted with SAS 9.2 (SAS Institute, Cary, NC).

results

patient enrollment

Between 3 August 2012 and 27 May 2013, 24 patients were enrolled across six centers. Twenty-two patients were assessable
for the first efficacy end point (supplementary Figure S1), available at *Annals of Oncology* online) and 24 for safety. Baseline patient characteristics are listed in Table 1. The majority of patients had locally advanced disease. Eleven patients (46%) had distant metastasis, lung being the most frequent metastatic site. Fifteen patients (63%) had received prior lines of chemotherapy including anthracyclines for 8 of them.

**Patient disposition**

After a median follow-up of 9.8 months, none of the patients were still on treatment. Twenty-four patients discontinued Aplidin. Discontinuation was related to disease progression for 17 patients, toxicity for 5 patients, patient refusal in 1 patient, and death of unknown origin in 1 patient.

**Safety**

Twenty-four patients were included in the safety analysis. At the time of analysis, 35 cycles of Aplidin® had been administered, with a median of 1 cycle administered per patient (range 0–5). The most commonly observed toxicities of any grade were nausea, fatigue, anorexia, vomiting and diarrhea, occurring in 19 (79.2%), 18 (75%), 12 (50.0%), 7 (29.2%) and 7 (29.2%) patients, respectively (Table 2). Eleven (46%) patients had at least one grade 3 or 4 toxicity. The most frequent grade 3 or 4 toxicity was nausea, vomiting, anorexia, fatigue and Troponin T increase occurring in 4 (16.7%), 4 (16.7%), 4 (16.7%) and 3 (12.5%) patients respectively. Three patients (12.5%) had reversible grade 3–4 Troponin T increase. Four patients stopped treatment because of an adverse event related to the treatment: two because of grade 3 Troponin T increase, one because of an episode of grade 3 hypersensitivity reaction and one because grade 3 nausea and vomiting despite optimal antiemetics. One patient stopped treatment because of a sepsis not related to the study drug.

**Correlative molecular analyses**

The genomic status of the *JUN* and of *MAP3K5* was obtained by using array-comparative genomic hybridization. We observed an amplification of *JUN* in one case and an amplification of *MAP3K5* in one another case. The two patients with SD at 3 months had no amplification of *JUN* or *MAP3K5*.

**Discussion**

The primary end point requiring a 3-month nonprogression rate ≥40% to suggest that Aplidin® was active in DDLPS was not met. Only 2 patients of 22 eligible for efficacy analysis had disease stability at 3 months and the median PFS was poor (1.6 months).

Thirty-seven percent of patients included in this study were not previously treated with chemotherapy in the advanced

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**Table 1. Patient characteristics (N = 24)**

<table>
<thead>
<tr>
<th>Gender</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>12 (50.0)</td>
</tr>
<tr>
<td>Female</td>
<td>12 (50.0)</td>
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<table>
<thead>
<tr>
<th>Age</th>
<th>Median in years (range)</th>
<th>ECOG performance status</th>
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</thead>
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<tr>
<td></td>
<td>63 (48–83)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 (42)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14 (58)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of metastatic sites</th>
<th>0</th>
<th>1</th>
<th>≥2</th>
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<tbody>
<tr>
<td></td>
<td>13</td>
<td>5</td>
<td>6</td>
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<table>
<thead>
<tr>
<th>Stage</th>
<th>Locally advanced</th>
<th>Metastatic</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>13 (54)</td>
<td>11 (46)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Prior lines of chemotherapy</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>≥2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9</td>
<td>9</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

**Table 2. Adverse events of any grade (related to Aplidin)*

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**Clinical events**

<table>
<thead>
<tr>
<th>Event</th>
<th>Grades 1 and 2</th>
<th>Grades 3 and 4</th>
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<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>15 (62.5)</td>
<td>3 (12.5)</td>
</tr>
<tr>
<td>Nausea</td>
<td>16 (66.7)</td>
<td>4 (16.7)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>4 (16.7)</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>7 (29.2)</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td>Anorexia</td>
<td>8 (33.3)</td>
<td>4 (16.7)</td>
</tr>
<tr>
<td>Weight loss</td>
<td>4 (16.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>4 (16.7)</td>
<td>4 (16.7)</td>
</tr>
</tbody>
</table>

**Laboratory investigations**

<table>
<thead>
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<th>Event</th>
<th>Grades 1 and 2</th>
<th>Grades 3 and 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td>2 (8.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Aspartate aminotransferase</td>
<td>2 (8.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>CPK increased</td>
<td>1 (4.2)</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td>Cardiac Troponin T increased</td>
<td>0 (0)</td>
<td>3 (12.5)</td>
</tr>
<tr>
<td>Hypoalbuminemia</td>
<td>2 (8.3)</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td>Anemia</td>
<td>6 (25.0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*Number of patients with at least one adverse event. In case, a patient presented more than one event of the same type, the highest grade was retained. Rates were calculated out of the 24 patients.

**Efficacy**

Of the first 17 patients assessable for efficacy analysis, only one patient was progression free at 3 months indicating that the first stage of the Simon’s design was not met. Twenty-two patients were assessable for final efficacy analysis. Median follow-up was 9.8 months (95% CI 6.0–12.2). Fourteen patients (63.6%) had progressive disease. No objective responses were observed. Stable disease (SD) ≥3 months was documented in two patients. The PFS3 was 9.1% (95% CI 1.1–29.2). Median PFS was 1.6 months (95% CI 1.4–2.6 months) (Figure 1). The 3-month and 6-year PFS rates were 22.7% (95% CI 8.3–41.4) and 9.1% (95% CI 1.6–25.1), respectively. There were 11 deaths, 10 of them due to disease progression. Median OS was 9.2 months (95% CI 6.6–12.2).
setting. All of them had disease progression at 3 months. In a large retrospective multicentric study, our research group showed that chemotherapy was associated with a median PFS of 4 months in the first-line setting for 171 patients with advanced DDLPS [12]. The majority of them were treated with an anthra-cycline-containing regimen. Our results suggest that Aplidin® is even less effective than doxorubicin in patients with advanced DDLPS.

One explanation for the poor efficacy of Aplidin in DDLPS may be related to an overestimated role of the JNK pathway in DDLPS tumorigenesis. Apoptosis induction by Aplidin® has been described as occurring through a strong, sustained activation of JNK [17]. Cells resistant to Aplidin show lesser extent of JNK activation [19]. Previous studies have suggested that dedifferentiation of WDLPS likely occurs via an upregulation of the JNK pathway that directly block adipocytic differentiation. This upregulation resulted from an amplification of the JUN or the MAP3K5 genes [14, 15]. MAP3K5 encodes a MAP3K upstream of JUN which trigger a phosphorylation cascade that activates JNK, which then phosphorylates and stabilizes JUN. Importantly, JUN overexpression was shown to block adipogenesis and to regulate the activity of transcription factors involved in such as C/EBPβ and PPARγ [15, 25]. We found here that JUN or MAP3K5 amplifications are infrequent events in

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**Figure 1.** Kaplan-Meier curves of progression-free (A) and overall survivals (B) of patients with advanced DDLPS treated with Aplidin.
DDLPS (7% of cases, respectively). This result is in agreement with more recent data showing JUN amplification in only up to 17% of DDLPS and that adipocytic differentiation in LPSS is not always inhibited by JUN [25]. Indeed, copy number increase of JUN had been observed in both the DD and the WD components of LPSSs [26, 27]. Moreover, the most prominent effect of JUN knockdown in LPSS cells has been shown to result more in an inhibition of proliferation and tumor formation rather than in the induction of adipogenesis [26]. Altogether, these results show that JNK pathway is not dispensable to DDLPS tumorigenesis and suggest that other genetic or epigenetic events may be involved in dedifferentiation of LPSS cells.

The overall toxicity profile of single-agent Aplidin* observed in this study agrees with that previously reported for this same schedule with nausea, fatigue, vomiting being among the most frequent events [28, 29]. Of note, three patients had reversible grade 3–4 Troponin T increase without any sign of ischemia. Two of them were withdrawn from the protocol based on the investigator decision. Myocardial injury events were relatively rare in previous studies investigating Aplidin* (<3%) and were not correlated as in our study to prior anthracycline treatment [30]. The rapid accrual in this study demonstrates that histology-driven clinical research is feasible in sarcomas. Our results should serve as a reference for response and outcome in the assessment of new investigational drugs. Indeed, further efforts are needed to identify effective therapies in DDLPS. Besides JUN, other oncogenes such as MDM2 and CDK4 can represent relevant targets. For instance, in vitro data have shown that the MDM2-antagonist nutflin-3A can efficiently induce apoptosis in WDLPS/DDLPS cell lines [31]. Strikingly, preliminary data from a phase 0 study including 20 patients with WD/DDLPS have shown that the MDM2-antagonist RG7112 is able to increase the expression of p53 and p21 and decrease the expression of Ki-67 in human tumors [32]. Early signs of clinical activity have also been observed but need further confirmatory studies [33]. CDK4 inhibitors have also shown some kind of clinical activity [34] even if the amplification of this oncogene may represent only a secondary event in LPSS tumorigenesis [35]. Combination of these new agents between them or with cytotoxic drugs deserves further investigations.

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funding


disclosure

The authors have declared no conflicts of interest.

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Plasma vemurafenib concentrations in advanced BRAFV600mut melanoma patients: impact on tumour response and tolerance†

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Background: Vemurafenib improves survival in advanced BRAFV600mut melanoma patients, but tolerance is often poor and resistance frequently occurs, without predictive factor. Our aim was to investigate for the first time a relationship between plasma vemurafenib concentration (PVC) and efficacy or tolerance.

Methods: Plasma samples from unresectable metastatic BRAFV600mut melanoma patients treated with vemurafenib monotherapy were prospectively collected at each tumour response evaluation (RECIST 1.1) or when adverse event occurred (CTCAE 4.0). PVC was measured with liquid chromatography–tandem mass spectrometry. Herein, we report on PVC at steady state (≥14 days after vemurafenib introduction or dose modification). Samples collected after first...

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