Phase I–II study of plitidepsin and dacarbazine as first-line therapy for advanced melanoma

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Background: This phase I–II trial compared plitidepsin 1-h infusion alone or combined with dacarbazine (DTIC) 1-h infusion as front-line therapy for advanced melanoma.

Methods: The recommended dose (RD) for plitidepsin/DTIC was defined in the first stage. In the second stage, patients were randomised to receive single-agent plitidepsin 3.2 mg m⁻² (n = 20) on days 1, 8 and 15 every 4 weeks (q4wk) or plitidepsin 2.4 mg m⁻² on days 1, 8 and 15 q4wk combined with DTIC 800 mg m⁻² q4wk (n = 38).

Results: The overall response rate with plitidepsin/DTIC was 21.4%; all responders had normal serum lactate dehydrogenase (LDH) levels and performance status ≤1 at baseline. Median progression-free survival (PFS) with plitidepsin/DTIC was 3.3 months in all patients, and 4.3 months in those with baseline normal LDH. No responses occurred with single-agent plitidepsin and median PFS was 1.5 months. Both regimens were well tolerated. Haematological abnormalities were more common and transaminase increases more severe with plitidepsin/DTIC. Treatment-related transaminase increases leading to infusion omission on day 8 were relatively common. No drug–drug pharmacokinetic interactions were found.

Conclusion: This plitidepsin/DTIC schedule has antitumour activity and manageable toxicity in advanced melanoma. Further evaluation of plitidepsin 2.4 mg m⁻² fortnightly and DTIC 800 mg m⁻² q4wk is recommended.
human malignant cell lines, including melanoma. Plitidepsin triggers apoptosis in vitro and blocks VEGF secretion in different tumour models. The finding that DTIC increases protein expression of VEGF and promotes tumour growth and metastasis in vivo in human melanoma cells (Lev et al, 2003, 2004) suggested that a combination of plitidepsin and DTIC might have synergistic antineoplastic effects in metastatic melanoma. Plitidepsin showed sustained clinical antitumour activity in a melanoma patient in a phase I study (Anthoney et al, 2000). In a phase II trial, plitidepsin 3-h intravenous (i.v.) infusion administered fortnightly as second-line therapy induced durable partial response (PR) in 5% of 35 patients with unresectable melanoma resistant to prior DTIC-based chemotherapy (Eisen et al, 2009).

This open-label clinical trial was divided into two stages. The first stage was a nonrandomised, dose-finding, phase I study that evaluated the maximum tolerated dose (MTD) and the recommended dose (RD) of plitidepsin (1-h infusion on days 1, 8, and 15) combined with DTIC (1-h infusion on day 1) every 4 weeks (q4wk) as front-line treatment for unresectable advanced and 15) combined with DTIC (1-h infusion on day 1) every 4 weeks (q4wk) as front-line treatment for unresectable advanced melanoma. This weekly plitidepsin schedule was chosen over the q3wk) for single-agent DTIC when adapted to a q4wk schedule (Eggermont and Kirkwood, 2004). The starting plitidepsin dose (1.8 mg m−2) was 58% of its single-agent RD, 3.2 mg m−2 (Izquierdo et al, 2008). The starting DTIC dose (800 mg m−2) was ~60% of the RD intensity (1000 mg m−2 q3wk) for single-agent DTIC when adapted to a q4wk schedule (Eggermont and Kirkwood, 2004).

Dose escalation during the first stage is summarised in Table 1. A standard dose-escalation design for conventional cytotoxic agents was used, with cohorts of 3–6 patients treated at each dose level. Dose-limiting toxicities (DLTs) were evaluated during cycle 1 and were defined as follows: ANC < 0.5 × 10^9 per l for >5 days; grade 3 neutropenia with fever (≥ 38.5 °C), sepsis or other severe infection; platelet count < 25.0 × 10^9 per l; any other grade 3/4 nonhaematological adverse event (AE) suspected to be treatment related (except for nausea/vomiting without an optimal antiemetic regimen, hypersensitivity reactions and nonclinically relevant biochemical abnormalities); and any delays in the administration of a subsequent plitidepsin dose exceeding 2 weeks, or omissions of the infusions scheduled on days 8 and 15 because of treatment-related AEs. The MTD was defined as the dose level at which at least 2 patients had DLTs, and the RD was defined as the highest dose level at which less than one-third of patients experienced DLTs.

| Dose level | Plitidepsin/DTIC dose (mg m−2) | No. of patients treated | No. of cycles administered | No. of patients with DLTs/no. of patients evaluable for DLTs | Description of DLTs |
|------------|--------------------------------|------------------------|---------------------------|-----------------------------------------------------------|----------------------|
| 1          | 1.8/800                        | 7                      | 25                        | 1/6                                                       | Grade 3 ALT increase |
| 2          | 2.4/800 (RD)                   | 8                      | 20                        | 1/5                                                       | Grade 3 ALT increase |
| 3          | 3.0/800                        | 8                      | 23                        | 0/6                                                       | —                    |
| 4          | 2.4/1000 (MTD)                 | 5                      | 8                         | 2/4                                                       | Grade 3 ALT increase |

Abbreviations: ALT = alanine aminotransferase; DLT = dose-limiting toxicity; DTIC = dacarbazine; MTD = maximum tolerated dose; RD = recommended dose.

*Overall, 21 of 28 patients were considered evaluable for DLTs. Seven patients were not evaluable because of being withdrawn from the study before completing cycle 1 (n = 3), the presence of confounding factors (n = 2), a wrong diagnosis of metastatic melanoma (n = 1) or having bone marrow sensitivity to DTIC (n = 1).

This episode of grade 4 pancytopenia consisted of grade 4 leucopenia, grade 4 neutropenia and grade 4 thrombocytopenia.
During the second stage, eligible patients were randomised without stratification to receive either single-agent plitidepsin 3.2 mg m⁻² as 1-h infusion on days 1, 8 and 15 q4wk, or plitidepsin/DTIC at the RD previously determined in the first stage. A two-stage Simon design was used in this stage. An interim analysis was conducted on 17 evaluable patients and the MTD was declared at dose level 4 (plitidepsin 2.4 mg m⁻² and DTIC 1000 mg m⁻²). No patients treated at this dose level had DLTs, but dose omissions on day 8 or 15 occurred in 57% of each patient. Dose-limiting toxicity (grade 3 ALT increases) was observed in one of five patients treated at dose level 2 (plitidepsin 2.4 mg m⁻² and DTIC 800 mg m⁻²; Table 1). Nine patients had DLTs, but dose omissions on day 8 or 15 occurred in 57% of patients treated at dose level 3 (plitidepsin 3.0 mg m⁻² and DTIC 800 mg m⁻²). At dose level 2 (plitidepsin 2.4 mg m⁻² and DTIC 800 mg m⁻²) the RD was declared.

At the RD, median plitidepsin dose intensity was 1.2 mg m⁻² per week (range, 0.6–2.0 mg m⁻² per week) and median relative plitidepsin dose intensity was 67% (range, 33.1–99.2%). For DTIC, median dose intensity at the RD was 189.5 mg m⁻² per week (range, 151.0–199.8 mg m⁻² per week) and median relative dose intensity was 94.8% (range, 75.5–99.5%).

**Efficacy**

**Phase I stage.** In all, 19 treated patients were evaluable for efficacy. Antitumour activity in this phase consisted of one confirmed PR, two unconfirmed partial responses (PRu) and four disease stabilisations ≥ 3 months.

**Phase II stage.** Eight patients treated with plitidepsin/DTIC and four treated with single-agent plitidepsin were evaluable because they either withdrew from the study before receiving the minimum treatment required or did not have disease assessment at least 8 weeks after treatment onset. Reasons for discontinuation comprised toxicity (n = 8), AEs unrelated to treatment (n = 2) or early disease progression (n = 2).

**Plitidepsin/DTIC as first-line therapy in melanoma**

**RESULTS**

**Patient characteristics**

**Phase I stage.** A total of 28 patients were enrolled (Table 2). Of these, 26 (96%) had metastatic disease and 16 (59%) had ≥ 3 sites of disease. At baseline, 27 patients (96%) had an ECOG PS of 0–1. The median lactate dehydrogenase (LDH) level was 0.8 × ULN (range, 0.4–3.4 × ULN).

**Phase II stage.** Plitidepsin/DTIC: In all, 38 patients were enrolled, all with metastatic disease (Table 2). Of these, 24 (65%) had ≥ 3 sites of disease. At baseline, 37 patients (97%) had an ECOG PS of 0–1. The median LDH level was 0.8 × ULN (range, 0.4–8.2 × ULN). Single-agent plitidepsin: A total of 20 patients were included (Table 2). All had metastatic disease and 16 (80%) had ≥ 3 sites of disease. Nineteen patients (95%) had an ECOG PS of 0–1. The median LDH level was 1.7 × ULN (range, 0.5–3.9 × ULN).

**Treatment exposure**

**Phase I stage.** Dose ranges during escalation are shown in Table 1. A median of two cycles (range, 1–8 cycles) was administered to each patient. Dose-limiting toxicity (grade 3 ALT increases) occurred in one of five patients treated at dose level 2 (plitidepsin 2.4 mg m⁻² and DTIC 800 mg m⁻²; Table 1). No patients treated at dose level 3 (plitidepsin 3.0 mg m⁻² and DTIC 800 mg m⁻²) had DLTs, but dose omissions on day 8 or 15 occurred in 57% of cycles administered at this dose level, all because of grade 2/3 transaminase increases. At dose level 4 (plitidepsin 2.4 mg m⁻² and DTIC 1000 mg m⁻²), DLTs (grade 3 ALT increase, grade 4 pancytopenia and febrile neutropenia) occurred in two of four evaluable patients. Consequently, this dose level was declared the MTD for the plitidepsin/DTIC combination, and dose level 2 (plitidepsin 2.4 mg m⁻² and DTIC 800 mg m⁻²) was considered the RD.

At the RD, median plitidepsin dose intensity was 1.2 mg m⁻² per week (range, 0.6–2.0 mg m⁻² per week) and median relative plitidepsin dose intensity was 67% (range, 33.1–99.2%). For DTIC, median dose intensity at the RD was 189.5 mg m⁻² per week (range, 151.0–199.8 mg m⁻² per week) and median relative dose intensity was 94.8% (range, 75.5–99.5%).

**Efficacy**

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**Phase II stage.** Eight patients treated with plitidepsin/DTIC and four treated with single-agent plitidepsin were evaluable because they either withdrew from the study before receiving the minimum treatment required or did not have disease assessment at least 8 weeks after treatment onset. Reasons for discontinuation comprised toxicity (n = 8), AEs unrelated to treatment (n = 2) or early disease progression (n = 2).

**Plitidepsin/DTIC: Of the 28 evaluable patients, 6 showed objective confirmed responses (1 CR and 5 PRs; ORR = 21.4%, 95% CI, 8.3–41.0%; Table 3). The CR occurred in a 21-year-old female with multiple metastatic lesions (3 in the lungs) who received a total of 9 cycles. Tumour lesions decreased in size after cycle 2 and were undetectable after cycle 9, with no subsequent disease progression at last follow-up (18.7 months after treatment onset). All patients responding to plitidepsin/DTIC had LDH ≤ 1.1 × ULN and an ECOG PS ≤ 1 at baseline. Median duration of response was 4.5 months (range, 1.4–16.5 + months). In addition,
9 patients (32.1%) had disease stabilisation (including 1 PRu) for a median of 3.9 months (range, 3.1–13.2 months). In the whole cohort of 28 evaluable patients, median progression-free survival (PFS) was 3.3 months (95% CI, 1.6–4.6 months) and median OS was not reached before the database lock (Figure 1). Median PFS was 4.3 months (95% CI, 2.0–7.9 months) in 20 patients with baseline LDH ≤ 1.1 × ULN (Table 3).

Single-agent plitidepsin: None of the 16 evaluable patients achieved objective response. Two (12.5%) had disease stabilisation (median duration of 2.9 months; range, 2.8–3.0 months; Table 3). Median PFS for all 16 patients was 1.5 months (95% CI, 0.9–1.9 months; Figure 1) and median OS was 4.1 months (95% CI, 1.5–7.7 months). Median PFS was 1.6 months (95% CI, 1.6–2.8 months) in 3 patients with LDH ≤ 1.1 × ULN (Table 3).

Toxicity profile

Phase I stage (at the RD). All five patients treated at the RD were evaluable for safety. Most toxicities were grade 1/2. Clinically relevant toxicities comprised grade 3 fatigue (n = 2, concomitant with grade 4 neutropenic sepsis in one case), grade 3 vomiting, grade 3 diarrhoea, grade 3 hypersensitivity, grade 3 respiratory tract infection, grade 3 weakness and grade 4 pancytopenia (n = 1). Transient grade 3 ALT increase occurred in 2 patients.

Table 2. Patient characteristics at baseline

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|---------------------------------------------|

|                  | Phase I stage (n = 28) | Plitidepsin (n = 20) | Plitidepsin/DTIC (n = 38) |
|------------------|------------------------|----------------------|--------------------------|
| **Gender**       |                        |                      |                          |
| Male             | 16 (57%)               | 10 (50%)             | 21 (55%)                 |
| Female           | 12 (43%)               | 10 (50%)             | 17 (45%)                 |
| Median age (years) (range) | 48.0 (20–77) | 51.5 (36–78) | 55.5 (21–76) |
| **ECOG performance status** |                      |                      |                          |
| 0                | 16 (57%)               | 4 (20%)              | 21 (55%)                 |
| 1                | 11 (39%)               | 15 (75%)             | 16 (42%)                 |
| 2                | 1 (4%)                 | 1 (5%)               | 1 (3%)                   |
| Median plasma LDH (× ULN) (range) | 0.8 (0.4–3.4) | 1.7 (0.5–3.9) | 0.8 (0.4–8.2) |
| ≤ 1.1 × ULN      | 20 (71%)               | 4 (20%)              | 23 (61%)                 |
| > 1.1 × ULN      | 8 (29%)                | 16 (80%)             | 15 (39%)                 |
| **Disease**      |                        |                      |                          |
| Metastatic       | 26 (96%)               | 20 (100%)            | 38 (100%)                |
| Locally advanced | 1 (4%)                 |                      |                          |
| **Sites of disease** |                      |                      |                          |
| Lung             | 19 (70%)               | 11 (55%)             | 25 (68%)                 |
| Lymph node       | 18 (67%)               | 17 (85%)             | 27 (73%)                 |
| Soft tissue      | 9 (33%)                | 5 (25%)              | 11 (30%)                 |
| Liver            | 8 (30%)                | 11 (55%)             | 17 (46%)                 |
| Bone             | 7 (26%)                | 7 (35%)              | 6 (16%)                  |
| Peritoneum       | 4 (15%)                | 4 (20%)              | 6 (16%)                  |
| Pelvis           | 3 (11%)                | 1 (5%)               | 5 (14%)                  |
| Skin             | 2 (7%)                 | 3 (15%)              | 1 (3%)                   |
| Spleen           | 2 (7%)                 | 4 (20%)              | 3 (8%)                   |
| Kidney           | 1 (4%)                 | 1 (5%)               | 5 (14%)                  |
| Otherb           | 5 (19%)                | 5 (25%)              | 12 (32%)                 |
| **Number of sites of disease** |                      |                      |                          |
| ≤ 1 Sites        | 11 (41%)               | 4 (20%)              | 13 (35%)                 |
| > 3 Sites        | 16 (59%)               | 16 (80%)             | 24 (65%)                 |
| **Previous anticancer therapy** |                      |                      |                          |
| Surgery          | 28 (100%)              | 20 (100%)            | 36 (95%)                 |
| Radiotherapy     | 10 (36%)               | 10 (50%)             | 7 (18%)                  |
| Biological therapy | 2 (7%)                | 1 (5%)               | 2 (5%)                   |
| Chemotherapyd    |                        |                      | 1 (3%)                   |

Abbreviations: DTIC = dacarbazine; ECOG = Eastern Cooperative Oncology Group; LDH = lactate dehydrogenase; ULN = upper limit of normal.

*a* One patient in the phase I stage had hepatic haemangioma instead of melanoma (wrong diagnosis), and has been excluded.

*b* Adrenal, bladder, breast, central nervous system (CNS), pancreas, parotid gland, pericardial, pleura, stomach and thyroid sites.

*c* Missing data for one patient treated with plitidepsin/DTIC in the phase II stage.

*d* In the neoadjuvant setting.
Phase II stage. Of the 58 enrolled patients, 56 were treated and were evaluable for safety. The most common nonhaematological toxicities were ALT/AST increases, AP increases, fatigue, nausea and vomiting (in both arms), plus CPK increase with single-agent plitidepsin, and total bilirubin increase with plitidepsin/DTIC (Table 4). Severe AEs occurred rarely, mostly reaching grade 3 at worst, and were properly managed with dose adjustments. All transaminase increases in both arms were transient. Grade 3 ALT increases were more frequent with plitidepsin/DTIC (28% vs 10% of patients); grade 3 AST increases were rarer and only occurred with plitidepsin/DTIC (6% of patients). It is noteworthy that more patients treated with the combination skipped at least one plitidepsin infusion (77% vs 47%) and more omissions were because of treatment-related transaminase increases (65% vs 25%) as compared with plitidepsin alone. Most omissions involved the plitidepsin infusion on day 8. In contrast, grade 3/4 CPK increase occurred more frequently with single-agent plitidepsin (15% vs 3% of patients).

The most common haematological abnormalities were anaemia and lymphopenia (Table 4). Most were mild or moderate. Severe abnormalities occurred in 2 patients (10%) treated with single-agent plitidepsin and 4 (11%) treated with plitidepsin/DTIC. All other haematological abnormalities were mild and had no effects on treatment compliance. Notably, no cases of febrile neutropenia were observed.

Hypersensitivity reactions (n = 14) were more frequent with plitidepsin/DTIC (n = 9 patients, 25% vs n = 3 patients with single-agent plitidepsin, 15%) and resulted in the treatment discontinuation of 7 patients (5 vs 2, respectively). Nevertheless, one patient in each arm had grade 2/3 hypersensitivity reactions but continued treatment safely without reoccurrence of the events.

Pharmacokinetics. Complete PK data were available from 67 patients (21 during the phase I stage and 46 during the phase II stage) for plitidepsin, and from 49 patients (17 and 32, respectively) for DTIC (Table 5). The mean maximum plasma concentrations (Cmax) area under the curve (AUC) and terminal half-life (t1/2) of plitidepsin were similar when the drug was administered at 3.2 mg m–2 as single agent or at 2.4 mg m–2 combined with DTIC 800 mg m–2. Maximum plitidepsin concentrations were typically observed during or immediately before the end of infusion and then decreased in a multieponential manner, with an initial rapid decline followed by a more prolonged
Plitidepsin inhibits cell cycle progression, induces apoptosis and shows antiangiogenic activity. These effects are related to the induction of early oxidative stress, the activation of Rac1 GTPase and the inhibition of protein phosphatases, which together cause sustained activation of two members of the MAPK family: the serine/threonine kinases JNK and p38 MAPK (Cuadrado et al, 2009). Sensitivity to plitidepsin is inversely correlated with the level of expression of the Cdk inhibitor p27kip1 in a panel of low-passage human sarcoma cell lines. Furthermore, the elimination of p27kip1 in these cells (by siRNA) or in mouse embryo fibroblasts (MEFs; p27kip1 knockout) increases their sensitivity to plitidepsin (Moneo et al, 2007). Phosphorylated JNK has recently been described as a pharmacodynamic biomarker of plitidepsin in xenografted tumours as well as in normal surrogate tissues (Munoz-Alonso et al, 2013).

Plitidepsin is a compound with high pleiotropicity, affecting multiple cell signalling pathways and targets (Munoz-Alonso et al, 2009). Plitidepsin inhibits cell cycle progression, induces apoptosis and shows antiangiogenic activity. These effects are related to the induction of early oxidative stress, the activation of Rac1 GTPase and the inhibition of protein phosphatases, which together cause sustained activation of two members of the MAPK family: the serine/threonine kinases JNK and p38 MAPK (Cuadrado et al, 2003). Sensitivity to plitidepsin is inversely correlated with the level of expression of the Cdk inhibitor p27kip1 in a panel of low-passage human sarcoma cell lines. Furthermore, the elimination of p27kip1 in these cells (by siRNA) or in mouse embryo fibroblasts (MEFs; p27kip1 knockout) increases their sensitivity to plitidepsin (Moneo et al, 2007). Phosphorylated JNK has recently been described as a pharmacodynamic biomarker of plitidepsin in xenografted tumours as well as in normal surrogate tissues (Munoz-Alonso et al, 2013).

The first stage of this phase I–II clinical trial found plitidepsin 2.4 mg m⁻² plus DTIC 800 mg m⁻² as the RD for plitidepsin 1-h infusion (days 1, 8 and 15) plus DTIC 1-h infusion (day 1) q4wk regimen in chemo-naïve adult patients with advanced malignant melanoma. Unsurprisingly, transient grade 3 ALT increase was the most common DLT during dose escalation, which was similar to the findings of prior plitidepsin studies (Anthoney et al, 2000; Ciruelos et al, 2002; Faivre et al, 2005; Izquierdo et al, 2008; Gomez-Roca et al, 2010; Stein et al, 2012). Dose-limiting grade 4 febrile neutropenia and grade 4 pancytopenia occurred in one patient at the MTD, which was the only level in which DTIC was administered at 1000 mg m⁻². Haematological toxicity is more likely to be linked to DTIC (Chapman et al, 1999) rather than to plitidepsin, as it is seldom reported in single-agent studies in patients with nonhaematological tumours (Faivre et al, 2005; Maroun et al, 2006; Izquierdo et al, 2008).

The second stage of this study explored the efficacy of plitidepsin alone or in combination with DTIC. The ORR of 21.4% found for patients treated with the combination is within the ranges (11–31%) reported for DTIC combined with other active agents in metastatic melanoma, including ipilimumab, for which an ORR of 15.2% was reported in a recent phase III trial (Robert et al, 2011). Vemurafenib has resulted in a higher response rate (55%) in advanced melanoma patients with a BRAF V600E mutation (Chapman et al, 2012); however, this mutation is only present in ~50% of all melanoma patients. Much lower response rates have been reported for single-agent DTIC (range, 5–15%), thus suggesting that the combination of plitidepsin plus DTIC may be more effective than either agent alone.

| Table 4. Worst all-cycle toxicities during the phase II stage |
| --- |
| **Plitidepsin (n = 20)** | **Plitidepsin/DTIC (n = 34)** |
| | Grade 1/4 | Grade 3 | Grade 4 | Grade 1/4 | Grade 3 | Grade 4 |
| **Nonhaematological** | | | | | | |
| ALT increase | 18 (90%) | 2 (10%) | 1 (5%) | 33 (92%) | 9 (25%) | 1 (3%) |
| Anorexia | 2 (10%) | | | 6 (17%) | | |
| AP increase | 11 (55%) | | | 14 (39%) | | |
| AST increase | 15 (75%) | | | 25 (69%) | 2 (6%) | |
| Constipation | 3 (15%) | | | 4 (11%) | | |
| CPK increase | 7 (35%) | 2 (10%) | 1 (5%) | 7 (19%) | | 1 (3%) |
| Creatinine increase | 4 (20%) | | | 5 (14%) | | |
| Diarrhoea | 3 (15%) | | | 7 (19%) | 1 (3%) | |
| Electrocardiogram T wave abnormal* | | 4 (11%) | | | | |
| Fatigue | 8 (40%) | 1 (5%) | | 15 (42%) | 4 (11%) | |
| Hypersensitivity | 3 (15%) | 1 (5%) | | 9 (25%) | 3 (8%) | |
| Muscle cramps | 2 (10%) | | | 3 (8%) | | 1 (3%) |
| Muscle weakness | 1 (5%) | 1 (5%) | | 5 (14%) | 2 (6%) | |
| Myalgia | 4 (20%) | | | 4 (11%) | | |
| Nausea | 9 (45%) | 2 (10%) | | 25 (69%) | 1 (3%) | |
| Total bilirubin increased | 5 (25%) | | | 19 (53%) | | |
| Vomiting | 6 (30%) | 1 (5%) | | 13 (36%) | 2 (6%) | |
| Weight decrease | | | | 4 (11%) | | |
| **Haematological** | | | | | | |
| Anaemia | 11 (55%) | | 1 (5%) | 23 (64%) | 1 (3%) | |
| Leukopenia | | | 1 (5%) | 9 (25%) | 1 (3%) | |
| Lymphopenia | 11 (55%) | 1 (5%) | | 19 (53%) | 1 (3%) | |
| Neutropenia | | | 1 (5%) | 10 (28%) | 2 (6%) | |
| Thrombocytopenia | 5 (25%) | | | 5 (14%) | 2 (6%) | |

Abbreviations: ALT = alanine aminotransferase; AP = alkaline phosphatase; AST = aspartate aminotransferase; CPK = creatine phosphokinase; DTIC = dacarbazine; n = number of patients evaluable for safety. Data shown are number (%) of patients. Only toxicities found in ≥10% of patients with either treatment are included. Haematological and laboratory abnormalities are shown regardless of their relationship to treatment.

*Two patients enrolled into this cohort were withdrawn before receiving the first infusion and thus were not evaluable for safety.

**b**These comprised grade 2 electrocardiogram T wave inversion (n = 2), grade 2 electrocardiogram T wave abnormal, and grade 1 electrocardiogram T wave amplitude decreased (n = 1 each).

distribution. Both whole blood clearance (CL) and volume of distribution at steady state (Vss) of plitidepsin were higher when administered alone. The mean PK parameters observed for DTIC in this study do not show significant changes between different plitidepsin dose levels. No interaction between plitidepsin and DTIC was found.

**DISCUSSION**

Plitidepsin has demonstrated clinical activity, particularly in patients with advanced melanoma. The combination of plitidepsin and DTIC has shown promising results in phase I and II trials, with an ORR of 21.4% and a DLT of grade 3 ALT increase. Further studies are needed to explore the optimal dose regimen and combination with other agents to improve outcomes.
indeed have had a synergistic antitumour effect. Moreover, as the DTIC dose given (800 mg m\(^{-2}\) q4wk) is lower than the one usually administered to patients with metastatic melanoma (Mouwad et al, 2010), this might have also resulted in a much lower haematological toxicity compared with full-dose DTIC. The finding that all six responders to plitidepsin/DTIC in the second study stage had serum LDH levels ≤ 1.1 × ULN and an ECOG PS ≤ 1 at baseline is intriguing, but may reflect an imbalance for known prognostic factors in both arms, and that these patients were in better condition or had better prognosis. No responses occurred with single-agent plitidepsin. This lack of response may be partly related to the patients’ poor prognosis at baseline because of lack of stratification. Known prognostic factors, such as high serum LDH (an independent and highly significant predictor of survival in advanced melanoma) (Balch et al, 2003; Bedikian et al, 2008; Neuman et al, 2008), ECOG PS or the presence of visceral metastases, were not taken into account owing to the small number of patients planned to be included in this small phase I–II study. During the second stage, more patients in the combination arm had baseline LDH ≤ 1.1 × ULN (61% vs 20%) and ECOG PS = 0 (55% vs 20%), and fewer had ≥ 3 sites of disease at baseline (65% vs 80%). These differences suggest, in a post hoc analysis, that a better prognosis at baseline favoured patients included in the combination arm, and might have contributed towards the different response to treatment achieved in each arm.

Small differences were found between the safety profiles of single-agent plitidepsin and plitidepsin/DTIC. Nevertheless, both regimens were generally well tolerated and showed manageable toxicity. The overall toxicity profile of single-agent plitidepsin agrees with that previously reported for this same schedule (Ferme et al, 2008; Izquierdo et al, 2008). The addition of DTIC might have contributed towards the slightly greater incidence of nausea, fatigue, vomiting and transaminase increases found with the combination, although these toxicities are also frequently reported with DTIC monotherapy (Patel et al, 2011). The hypersensitivity reactions related to plitidepsin/DTIC were more common and more severe than expected, based on the toxicity profiles reported for either drug alone (Chapman et al, 1999; Ferme et al, 2008; Izquierdo et al, 2008; Patel et al, 2011); the reasons underlying this finding remain to be elucidated.

Biochemical abnormalities occurred at similar frequencies in both regimens, although transaminase increases were slightly more frequent with the combination. Most patients (77%) treated with the combination skipped at least one plitidepsin infusion, generally because of treatment-related ALT or AST increases and involving the Day 8 infusion. Skipping the Day 8 infusion generally resulted in resolution and did not compromise further dosing. Thus, a fortnightly schedule might be more convenient for plitidepsin when combined with DTIC in the treatment of metastatic melanoma.

As expected, haematological abnormalities were more frequent with plitidepsin/DTIC than with plitidepsin alone. Neutropenia only occurred with the combination. However, only 11% of patients treated with the combination had severe haematological abnormalities resulting in treatment delay or dose reduction. Severe bone marrow depletion has been reported as a frequent reason for discontinuation in DTIC-based chemotherapies (Jungnelius et al, 1998; Agarwala et al, 1999; Chapman et al, 1999; Chiarion Sileni et al, 2001; Jelic et al, 2002). Overall, these results suggest that addition of plitidepsin may improve the antitumour efficacy of DTIC in metastatic melanoma while not increasing its known haematological toxicity.

The overall whole blood PK parameters obtained for plitidepsin alone or combined with DTIC were similar to those found in a previous phase I study (Izquierdo et al, 2008). The profile was characterised by a long half-life, low clearance and a high volume of distribution. Drug levels peaked during or immediately before the end of infusion, and then decreased in a multiexponential manner. The mean plasma PK parameters found for DTIC were also similar to those reported in previous studies (Buesa and Urrechaga, 1991). Therefore, no drug–drug PK interactions were apparent.

### Table 5. Pharmacokinetic parameters of plitidepsin and DTIC in cycle 1

| Plitidepsin/DTIC dose level (mg m\(^{-2}\)) | \(n\) | \(C_{\text{max}}\) (ng ml\(^{-1}\)) | AUC (h × ng ml\(^{-1}\)) | \(t_{\frac{1}{2}}\) (h) | CL (l h\(^{-1}\)) | \(V_{ss}\) (l) |
|-------------------------------------------|------|-------------------------------|---------------------------|----------------|----------------|----------------|
| **Phase I stage**                         |      |                               |                           |                |                |                |
| 1.8/800                                   | 6    | 41.7 (25.6–50.4)              | 540 (240–824)             | 42.7 (32.2–56.5) | 7.1 (3.8–12.1) | 261 (175–401)  |
| 2.4/800 (RD)                              | 6    | 42.4 (30.0–49.9)              | 508 (262–727)             | 39.5 (16.3–53.6) | 10.4 (7.6–17.0) | 408 (163–590)  |
| 3.0/800                                   | 7    | 38.1 (24.6–49.0)              | 323 (212–523)             | 38.8 (15.4–69.1) | 19.0 (11.9–28.3) | 750 (316–1920) |
| 2.4/1000 (MTD)                            | 2    | 36.0 (35.5–36.4)              | 277 (184–369)             | 16.1 (15.1–17.0) | 16.7 (11.1–22.2) | 299 (245–353)  |
| **Phase II stage**                        |      |                               |                           |                |                |                |
| 3.2                                        | 17   | 38.3 (16.1–48.4)              | 444 (205–848)             | 45.1 (14.2–131) | 15.4 (7.5–24.5) | 652 (198–1380) |
| 2.4/800                                   | 29   | 40.3 (26.2–50.2)              | 425 (215–1130)            | 43.0 (13.7–85.5) | 12.0 (4.0–24.9) | 456 (158–826)  |
| **DTIC**                                   |      |                               |                           |                |                |                |
| **Phase I stage**                         |      |                               |                           |                |                |                |
| 1.8/800                                   | 5    | 19.9 (15.1–23.9)              | 46.0 (30.3–69.8)          | 1.3 (0.7–2.4)   | 35.2 (22.4–47.8) | 50.6 (37.1–66.1) |
| 2.4/800 (RD)                              | 7    | 18.2 (5.0–24.4)               | 42.3 (24.5–66.8)          | 1.4 (0.5–1.9)   | 41.8 (22.3–68.6) | 76.7 (28.4–185) |
| 3.0/800                                   | 4    | 16.1 (4.2–22.9)               | 48.2 (14.8–84.9)          | 2.5 (2.2–2.9)   | 41.3 (19.8–86.6) | 94.8 (63.7–156) |
| 2.4/1000 (MTD)                            | 1    | 4.6 (–)                       | 15.7 (–)                  | 3.5 (–)         | 114 (–)         | 385 (–)        |
| **Phase II stage**                        |      |                               |                           |                |                |                |
| 2.4/800                                   | 32   | 15.4 (4.3–22.6)               | 38.3 (13.6–60.8)          | 2.6 (1.2–6.7)   | 42.8 (22–124)   | 96.2 (45.8–249) |

Abbreviations: AUC = area under the concentration–time curve from time zero to infinity; CL = total body clearance; \(C_{\text{max}}\) = maximum plasma concentration; DTIC = dacarbazine; MTD = maximum tolerated dose; RD = recommended dose; \(t_{\frac{1}{2}}\) = terminal half-life; \(V_{ss}\) = volume of distribution at steady state. Values are expressed as mean (range).
This study was initiated before the availability of BRAF inhibitors and targeted immune therapies. It is unclear now how to assess a precise role for the plitidepsin/DTIC combination in the rapidly changing treatment options for advanced melanoma. Patient selection by BRAF status has led to high response rates but not durable responses in BRAF V600E-mutated melanoma and overall survival is improved with treatment with ipilimumab. Despite these improvements, most patients with advanced melanoma will ultimately progress and die of their disease; thus, new treatment options are still needed. The results of this study are promising, taking into account the novel mechanism of action of plitidepsin, which allows the drug to act synergistically with DTIC without adding harmful toxicity.

In conclusion, this phase I–II study shows plitidepsin 2.4 mg m⁻² 1-h infusion (days 1, 8 and 15) plus DTIC 800 mg m⁻² 1-h infusion (day 1) q4wk as an active, well-tolerated, first-line chemotherapy against metastatic melanoma. Further evaluation of plitidepsin/DTIC as a fortnightly schedule might be warranted in non-BRAF-mutated advanced melanoma patients.

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