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Three-year follow-up of advanced melanoma patients who received ipilimumab plus fotemustine in the Italian Network for Tumor Biotherapy (NIBIT)-M1 phase II study

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Background: In the NIBIT-M1 study, we reported a promising activity of ipilimumab combined with fotemustine in metastatic melanoma (MM) patients with or without brain metastases. To corroborate these initial findings, we now investigated the long-term efficacy of this combination.

Patients and methods: This analysis captured the 3-year outcome of MM patients who received ipilimumab combined with fotemustine as first- or second-line treatment. Median overall survival (OS), 3-year survival rates, immune-related (ir) progression-free survival (irPFS), brain PFS, and ir duration of response (irDOR) for the entire population and for patients with brain metastases were assessed. Clinical results were correlated with circulating $CD3^+CD4^+ICOS^+CD45RO^+$ or $CD45RA^+$ T cells, neutrophil/lymphocyte (N/L) ratios, and tumor*BRAF*-V600 mutational status.

Results: Eighty-six MM patients, including 20 with asymptomatic brain metastases that had been pre-treated with radiotherapy in 7 subjects, were enrolled in the study. With a median follow-up of 39.9 months, median OS and 3-year survival rates were 12.9 months [95% confidence interval (CI) 7.1–18.7 months] and 28.5% for the whole study population, and 12.7 months (95% CI 2.7–22.7 months) and 27.8% for patients with brain metastases, respectively. Long-term ir adverse events consisting of G1 rush and pruritus occurred in 21% of patients. The absolute increase from baseline to week 12 in 'memory' but not in 'naïve' T cells identified patients with a better survival ($P = 0.002$). The N/L ratio correlated with a significantly better survival at early time points. *BRAF* status did not correlate with clinical outcome.

Conclusions: Long-term analysis of the NIBIT-M1 trial continues to demonstrate efficacy of ipilimumab combined with fotemustine in MM patients. Fotemustine does not seem to impair the immunologic activity of ipilimumab.

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Key words: metastatic melanoma, immunotherapy, CTLA-4, ipilimumab, fotemustine

introduction

Metastatic melanoma (MM) remains a highly fatal disease; however, the availability of immune-checkpoint blocking monoclonal antibodies (mAb) [1] and of MAP kinase inhibitors has significantly improved the therapeutic landscape of (MM) patients [2].

In spite of limited objective response rates, the anti-CTLA-4 mAb ipilimumab induced long-term survivals in MM [3, 4]: ~20% of patients treated in clinical trials and in Expanded Access Programs (EAP) survived at least 2 years. Survival rates plateaued at 3 years in different studies [3, 6] and in a pooled analysis reporting a 3-year survival rate of 22% in 1861 MM patients [7].

Spreading to the brain is the most common and serious complication of MM and, due to their poorer prognosis, MM

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patients with brain metastases have been generally excluded from clinical trials with new therapeutic agents [8, 9]. Nevertheless, a *post hoc* analysis of the pivotal, three-arm study CA184-020, comparing ipilimumab plus a gp100 vaccine to ipilimumab or to gp100 vaccine alone, provided initial proof of activity of ipilimumab as a single agent in MM patients with brain metastases [3]. The first study that has prospectively explored the potential of ipilimumab monotherapy in MM patients with brain metastases enrolled 72 subjects in a phase II multicentre trial [10]. In this study, ipilimumab was used at 10 mg/kg, and it was found to be active, particularly in patients with small and asymptomatic brain lesions: at week 12 of treatment, the global disease control rate (DCR) and the brain-only DCR were 18% and 5% and 24% and 10%, in asymptomatic and symptomatic subjects, respectively.

To begin exploring the efficacy and safety of ipilimumab in novel therapeutic associations, the Italian Network for Tumor Biotherapy (NIBIT) designed and conducted the open-label, multicentric, phase II trial NIBIT-M1 combining ipilimumab with fotemustine that is widely utilized in European countries in MM patients [11, 12]. In light of the up-coming proofs of activity of ipilimumab as single agent in brain metastases, and due to the efficacy of fotemustine also in MM to the brain, the NIBIT-M1 study allowed enrolling patients with asymptomatic brain lesions [13]. Among the 86 patients enrolled, 20 had brain metastases. The irDCR for the globally treated population and for patients with brain metastases were 46.5% and 50%, respectively; of note, patients who achieved a disease control in the brain also experienced irDCR in peripheral tissues. The median overall survival (OS) and the 1-year survival rates for the global population and for patients with brain metastases were 13.3 and 13.4 months, and 52.6% and 54.2%, respectively [14]. The combination was found to be feasible and safe, as no overlapping toxicities with the two agents were observed.

In this study, we analysed the long-term survival and safety of MM patients enrolled in the NIBIT-M1 study. Clinical results were also correlated with immunologic and molecular parameters.

patients and methods

study design and treatment

Adult patients with unresectable stage III/IV cutaneous melanoma who had received first line of therapy were eligible. Patients with asymptomatic brain metastases were allowed. All participating patients (or their legal representatives) provided signed informed consent before enrolment.

The study design and treatment regimens for study NIBIT-M1 have been described [14]. Briefly, in the induction phase, patients received 4 doses of ipilimumab at 10 mg/kg i.v. every 3 weeks and 3 doses of fotemustine at 100 mg/m^2 i.v. at weekly intervals. In the maintenance phase, fotemustine was administered every 3 weeks from week 9 and ipilimumab every 12 weeks from week 24. Treatment continued until confirmed ir progressive disease (PD), excessive toxicity, or patient refusal.

efficacy and safety assessment

We conducted a milestone analysis with a minimum follow-up of 3 years in all patients, current as of 31 July 2014.

Tumour assessments were carried out for all patients at baseline and week 12, then at weeks 20, 28, 36, and every 12 weeks from week 36 onwards for all non-progressing subjects. Tumour response was evaluated using the immune-response criteria (irRC) [15]. Efficacy end points were based on assessment carried out by investigators.

The occurrence of new ir adverse events (AEs) during maintenance therapy was evaluated in patients receiving ipilimumab at the time of analysis.

immunologic and molecular analyses

Serially collected peripheral blood mononuclear cells were available from a subset of patients and analysed by flow cytometry for $CD3^+CD4^+ICOS^+$ $CD45RO^+$ or $CD45RA^+$ T cells. The ratio between circulating neutrophils (N) and lymphocytes (L) (N/L ratio) was determined at different time points. The *BRAF*-V600 mutation status was retrospectively determined by PCR-based assay.

statistical analysis

Analyses of efficacy end points have been based on all treated subjects. However, ir duration of response (irDOR) was only estimated for subjects reaching an objective response, using ir response criteria.

Survival times were estimated using the Kaplan–Meier product-limit method; median survival together with the two-sided 95% confidence interval (95% CI) for the median were calculated using the method of Brookmeyer and Crowley and 2 and 3-year survival rates were calculated [16]. Median follow-up was evaluated with the reverse Kaplan–Meier method. Differences in survival curves were evaluated with the log-rank test. Descriptive statistics were used for patient demographic and characteristics.

results

demographics and treatment

Of the 86 patients treated, 20 (23%) had asymptomatic brain metastases that had progressed in 3 of 7 subjects who had received previous radiotherapy. Baseline patients and disease characteristics are provided in Table 1.

Twenty-eight patients (33%) had received maintenance treatment with ipilimumab beyond 2 years; at the time of analysis 8 patients (10%) were on maintenance treatment with ipilimumab.

long-term efficacy and safety

By July 2014, follow-up was considered current for all patients, except one that was lost to follow-up. Survival analysis was conducted on all the 86 treated patients enrolled. With a median follow-up of 39.9 months, median OS was 12.9 months (95% CI 7.1–18.7 months), and 2- and 3-year survival rates were 33.4% and 28.5% for the whole study population, while median OS was 12.7 months (95% CI 2.7–22.7 months), and 2- and 3-year survival rates were 38.9% and 27.8% for patients with brain metastases (Figure 1). Median immune-related (ir) progression-free survival (irPFS) was 4.5 months (95% CI 3.1–5.9 months) and 3.4 months (95% CI 2.3–4.5 months) for the whole study population and for patients with brain metastases, respectively (Figure 2); median brain PFS was 8.3 months (95% CI 4.7–11.8 months) in the whole study population and 3.0 months (95% CI 2.9–3.1 months) in patients with brain metastases at baseline. Median irDOR was 30.3 months (95% CI 15.5–46.5 months); irDOR rate at 2 and 3 years was 55.4% (95% CI 34.7–76.1) and 49.2% (95% CI 27.4–71.0), respectively.

Table 1. Patient demographics

	Study population ($n = 86$)
Age (years)	55 (43–66)
Gender	
Male	60 (70%)
Female	26 (30%)
ECOG performance status	
0	77 (90%)
1	9 (10%)
M stage at study entry	
M0	3 (3%)
M1a	10 (12%)
M1b	17 (20%)
M1c	56 (65%)
BRAF status	
Mutated	41 (48%)
Wild type	30 (35%)
Unknown	15 (17%)
Prior systemic therapy for metastatic disease	
No	44 (51%)
Yes	42 (49%)
Presence of brain metastases	20 (23%)
Number of brain lesions	
1	6 (30%)[a]
2 or 3	11 (55%)
>3	3 (15%)
Previous treatment of brain metastases	7 (35%)
Stereotactic radiosurgery	4 (20%)
Whole-brain radiotherapy	3 (15%)

[a]Percentages calculated from number of patients with brain metastases.
ECOG, Eastern Cooperative Oncology Group.

For the 28 patients who survived ≥2 years, the ir best overall response (irBOR) was irCR, irPR, irSD and irPD in 9, 14, 3 and 1 patient, respectively, with 1 patient not assessable. Among these long-term surviving patients, seven had brain metastases and their irBOR was irCR and irPR for three and four patients, respectively; two patients who achieved an irPR had received stereotactic radiotherapy for brain metastases before entering the study. Median irDOR for the 28 subjects surviving ≥2 years was not reached at the time of analysis and 2- and 3-year irDOR were 60.6% (95% CI 39.1–82.1) and 53.8% (95% CI 31.1–76.5), respectively.

As far as the long-term safety of treatment, 6 of the 28 (21%) patients who received ipilimumab beyond 2 years experienced G1 rush and pruritus irAEs.

efficacy according to immunologic correlates and BRAF mutational status

Baseline levels of circulating $CD3^+CD4^+ICOS^+CD45RO^+$ or $CD45RA^+$ T cells isolated from 31 patients did not allow discriminating subjects with a significantly different survival (data not shown). However, levels of circulating $CD3^+CD4^+ICOS^+CD45RO^+$ T cells higher or lower than the median value of 0.50 at week 12 identified patients with a significantly better (median 36.4 months) or poorer (median 12.9 months) survival, respectively ($P = 0.01$). Subjects with an increase from week 1 to week 12 in absolute values of circulating $CD3^+CD4^+ICOS^+CD45RO^+$ T cells higher or lower than the median value of 0.10 had a significantly better (median not reached) or poorer survival (median 12.9 months; 95% CI 6.8–18.9), respectively ($P = 0.002$) (Figure 3). Opposite to these findings, levels of circulating $CD3^+CD4^+ICOS^+CD45RA^+$ T cells did not discriminate patients with a significantly different survival neither when analysed at week 12 nor when their absolute increase from week 1 to week 12 was investigated (data not shown).

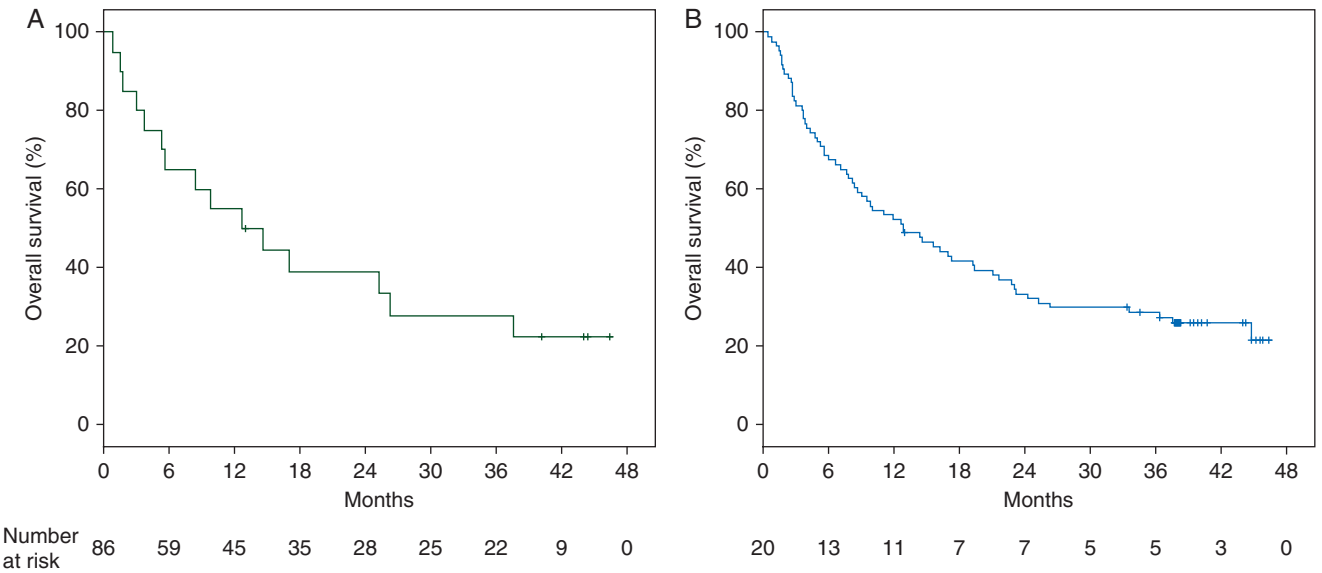

Figure 1. Kaplan–Meier plot of overall survival for all patients (A) and for patients with brain metastases (B). Vertical lines indicate censoring.

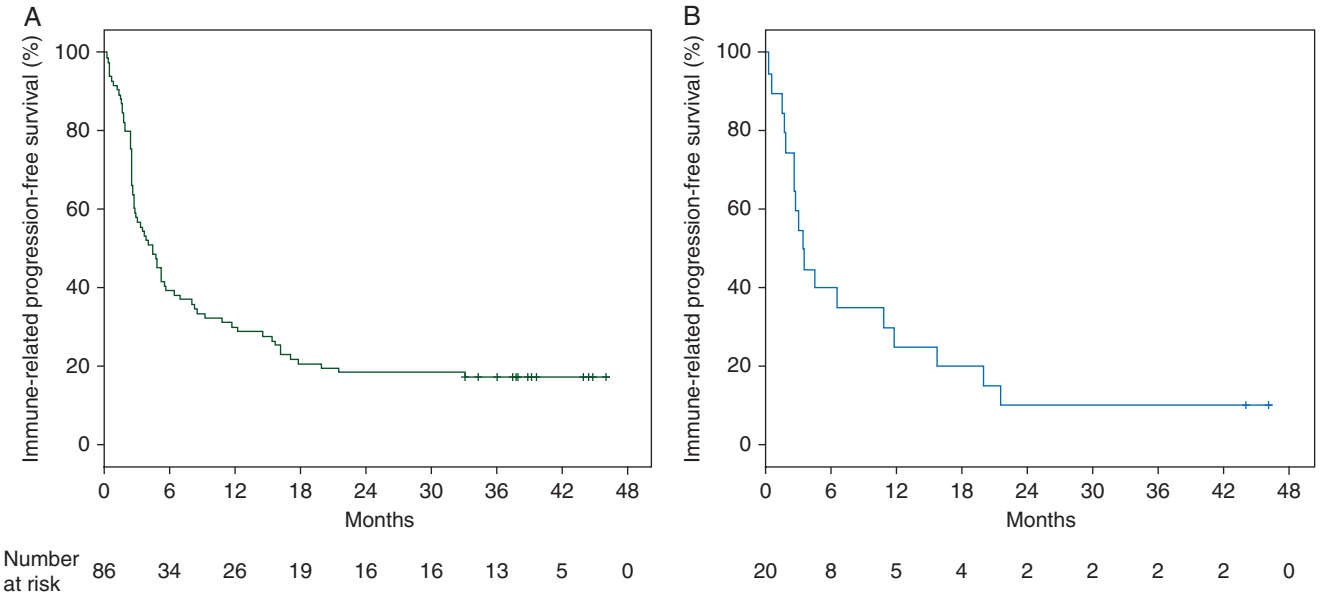

Figure 2. Kaplan–Meier plot of immune-related progression-free survival for all patients (A) and for patients with brain metastases (B). Vertical lines indicate censoring.

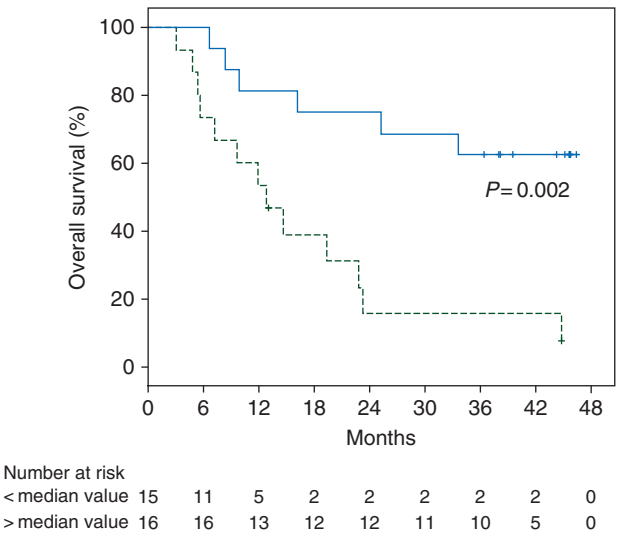

Figure 3. Kaplan–Meier plots of overall survival according to levels of circulating $CD3^+CD4^+ICOS^+CD45RO^+$ T cells. Subjects with an increase in absolute values of circulating $CD3^+CD4^+ICOS^+CD45RO^+$ T cells higher (solid line) or lower (dashed line) than the median value of 0.10 from baseline to week 12. Vertical lines indicate censoring.

The peripheral blood N/L ratio was investigated at baseline and at different time points during treatment. Subjects with N/L ratios lower than the median value at baseline, week 4 and week 7 but not at week 10 and week 12 had a significantly better survival when compared with patients with N/L ratios greater than the median value (Table 2).

Among the 71 patients in which the *BRAF* gene mutational status was investigated, 30 (34.8%) carried out a *BRAF*-V600 mutation (Table 1). The irDCR was 46.3% (19/41) and 60% (18/30) for patients with *BRAF* WT and mutated gene, respectively ($P = 0.25$). The median OS was 11.8 months (95% CI 6.4–17.2 months) and 17.3 months (95% CI 10.3–24.3 months) for *BRAF* WT and mutated patients, respectively ($P = 0.44$) (Figure 4).

discussion

In the initial report of the NIBIT-M1 study, the combination of ipilimumab and fotemustine in MM patients with or without brain metastases was found to be clinically effective [14]. These initial findings and the upcoming data on the long-term survival induced by ipilimumab in treatment-naïve and pre-treated MM patients, prompted us to investigate the long-term results of the NIBIT-M1 study.

The global 2- and 3-year survival rates of 33.4% and 28.5% for treated patients suggest for a long-term efficacy of the combination of ipilimumab and fotemustine. Though results from different clinical trials must be placed in context, these findings seem to be intriguing also in light of the 2- and 3-year survival rates of 28.5% and 21.3% observed in the phase III trial CA 184024 combining ipilimumab and dacarbazine in MM patients [4]. Along this very same line, a 3-year survival rate of 22% was recently observed in a pooled analysis of 12 different II/III studies in which ipilimumab was utilized at different doses and schedules in MM patients [7]. Supporting the notion that ipilimumab can induce long-term disease control in MM, among the 28 patients who survived ≥2 years in the NIBIT-M1 study, 96.4% had achieved an irDCR that was found to be long-lasting based on the 2- and 3-year irDOR observed.

As far as the 20 patients with brain metastases at baseline enrolled in the study, the 2- and 3-year survival rates corroborate initial evidences from the NIBIT-M1 study on the efficacy of the combination of ipilimumab and fotemustine in this highly unfavourable clinical setting; further support to this notion derives from the 2- and 3-year brain PFS rates of 35% and 25% observed. Noteworthy, 7 of the 20 patients with brain metastases

Table 2. Correlation between N/L ratio and overall survival

Week of analysis	N/L ratio (median)	Number of patients	Median OS for patients < median	Median OS for patients > median	*P*-value
1	3.02	84	22.8 (16.6–29.0)[a]	5.6 (3.3–7.9)	0.01
4	2.54	75	19.2 (9.2–29.2)	8.4 (2.4–14.4)	0.03
7	1.32	65	33.6 (12.0–55.2)	12.7 (6.6–18.8)	0.01
10	2.12	59	26.3 (7.8–44.8)	16.2 (10.3–22.1)	0.17
12	2.05	52	21.6 (16.6–26.6)	21.1 (6.6–35.6)	0.77

[a]Data are months (95% CI).
N/L, neutrophil/lymphocyte; OS, overall survival.

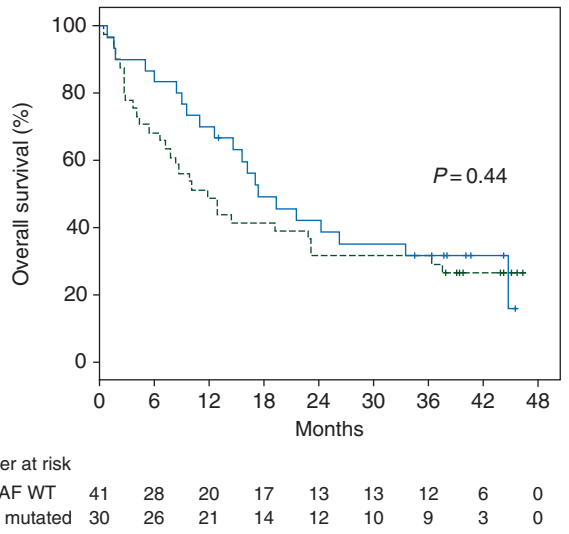

Figure 4. Kaplan–Meier plots of overall survival according to BRAF-V600 mutation status. Subjects with *BRAF* mutated (solid line) or WT (dashed line) MM. Vertical lines indicate censoring.

at baseline enrolled in the NIBIT-M1 study survived ≥2 years, among them 5 had not received previous treatment of brain disease. To the best of our knowledge, no data on the long-term efficacy of ipilimumab administered alone or in combination have been so far reported in MM patients with brain metastases. Though with the caution required by the small number of such patients enrolled in the NIBIT study and to their limited intracranial tumour burden, the 2- and 3-years survival rates of subjects with brain metastases, unexpectedly similar to the global population, provide first time evidence that long-term survivals and objective responses can also be obtained in a sizeable proportion of melanoma patients with brain disease, regardless of previous local treatment. Building on these promising initial data, and to further explore the efficacy of ipilimumab combined with fotemustine in this clinical setting, the first-line, phase III, NIBIT-M2 study is randomizing MM patients with brain metastases to receive fotemustine alone or combined with ipilimumab (EUDRACT Number 2012-004301-27). To explore more comprehensively, the efficacy of ipilimumab in melanoma patients with brain metastases, in light of its recently identified remarkable activity once combined with the anti-PD-1 mAb nivolumab, the NIBIT-M2 study has been lately amended to include the combination of ipilimumab and nivolumab as a third experimental arm [17].

The prospective analysis of circulating levels of $CD3^+CD4^+ICOS^+CD45RO^+$ T cells carried out in the NIBIT-M1 study supports retrospective data suggesting for a predictive role of $CD3^+CD4^+ICOS^+$ T cells as an early pharmacodynamic marker that identifies patients with an improved survival in the course of treatment with ipilimumab as single agent [6, 18, 19]. Furthermore, increased levels of circulating $CD3^+CD4^+ICOS^+CD45RO^+$ T cells but not of $CD3^+CD4^+ICOS^+CD45RA^+$ T cells were found to correlate with an improved OS of treated patients. This preferential expansion of the memory rather than of the naïve T-cell compartment provides evidence that treatment with ipilimumab combined with fotemustine favours the increase of antigen-primed T-cell populations [20, 21].

We previously showed that the peripheral blood N/L ratio correlated with a better clinical outcome in MM patients treated with ipilimumab as single agent [6]. In the present analysis, we found that the peripheral blood N/L ratio correlated with a significantly better survival of treated patients at early time points; this phenomenon faded at later time points, possibly due to late toxicity of fotemustine utilized in the study. These findings provide evidence that the analysis of the N/L ratio at early time points of ipilimumab treatment seems to be a useful marker to identify patients who will have a more favourable clinical outcome, in spite of its association with fotemustine. However, whether this is a more general phenomenon occurring in combinations of ipilimumab with other cytotoxic agents remains to be fully investigated.

No correlation between *BRAF*-V600 mutation and OS was observed in treated patients suggesting that the *BRAF* gene mutational status did not influence the long-term survival results, as recently postulated in a retrospective analysis of MM patients treated with ipilimumab alone at 3 mg/kg in two large EAP [22, 23].

We previously reported that the combination of ipilimumab and fotemustine is safe [14]; corroborating these finding, long-term treatment induced in a minority of patients low-grade irAEs that were confined to the skin. This evidence is consistent with the notion that limited rates of irAEs occur during protracted treatment with ipilimumab [5, 24].

Altogether, the results of this study demonstrate a long-term efficacy of ipilimumab combined with fotemustine in MM patients with or without brain metastases. Of note, the addition of fotemustine did not impair the T-cell immunomodulatory

effect of ipilimumab. The comprehensive findings of the NIBIT-M1 trial will be further explored in the ongoing randomized phase III NIBIT-M2 study.

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disclosure

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