Annals of Oncology 27: 1138–1142, 2016
doi:10.1093/annonc/mdw134
Published online 8 March 2016

Targeted treatment and immunotherapy in leptomeningeal metastases from melanoma

M. H. Geukes Foppen[1], D. Brandsma[2], C. U. Blank[1], J. V. van Thienen[1], J. B. Haanen[1] & W. Boogerd[2*]

Departments of [1]Medical Oncology; [2]Neuro-Oncology, Netherlands Cancer Institute, Amsterdam, The Netherlands

Received 24 December 2015; revised 3 February 2016; accepted 3 March 2016

Background: Historically leptomeningeal metastases (LM) from melanoma have a poor prognosis, with a median survival of only 2 months despite treatment. Targeted therapy and immune checkpoint inhibitors are promising new treatment options in advanced melanoma. We sought to determine the impact of targeted therapy and immunotherapy on the outcome of melanoma patients with LM and to evaluate the influence of prognostic factors.

Patients and methods: We analyzed a series of 39 consecutive patients diagnosed with LM from melanoma between May 2010 and March 2015 treated at the Netherlands Cancer Institute. Thirty-four of these patients also had brain metastases (BM). Statistical analyses assessed the influence of clinical and biological characteristics on survival.

Results: Median overall survival of the entire cohort was 6.9 weeks (95% confidence interval 0.9–12.8). Due to a poor performance status or rapidly progressive disease, 14 patients received no treatment. Median overall survival of untreated patients after the diagnosis of LM was 2.9 versus 16.9 weeks for treated patients ($P < 0.001$). The median survival of 21 patients treated with systemic targeted therapy and/or immunotherapy, with or without RT was 21.7 weeks (range 2–235 weeks). Five patients had LM without BM. Three of these patients died within 3 weeks before any treatment was given, whereas 2 patients are in ongoing remission for 26 weeks (following dabrafenib) and 235 weeks (following WBRT and ipilimumab). Elevated serum lactate dehydrogenase and S100B at diagnosis of LM were associated with shorter survival.

Conclusion: LM from melanoma still has an extremely poor prognosis. As observed in extracranial metastatic disease, new treatment modalities such as systemic targeted therapy and immune checkpoint inhibitors seem to increase overall survival in LM, and may result in long-term remission. These new treatment options should be considered in patients with LM.

Key words: melanoma, leptomeningeal metastases, survival, BRAF inhibitors, immune checkpoint inhibitors

introduction

Leptomeningeal metastases (LM) are one of the most devastating complications of solid tumors. It is clinically detected in ~5% of patients with cancer, mainly in breast cancer, lung cancer and melanoma [1]. Higher numbers are reported in autopsy series of patients with brain metastases (BM) [2, 3]. Difficulties to differentiate symptoms of LM from those caused by BM may contribute to this underestimation, but limited sensitivity of diagnostic tests may also play a role. Besides, specific clinical signs are absent in at least 25% of patients at the diagnosis of LM [4]. The golden standard for the diagnosis of LM is demonstration of tumor cells in the cerebrospinal fluid (CSF). Sensitivity of CSF cytology is 50% on first lumbar puncture and increases to 80% after repeated punctures [5]. The diagnosis can also be made by magnetic resonance imaging (MRI). MRI has a sensitivity and specificity of ~75% [6]. On clinical suspicion of LM, typical leptomeningeal contrast enhancement on MRI is considered diagnostic. Median survival of untreated patients with LM from solid tumors is only 4–6 weeks, usually due to progressive neurologic dysfunction [7]. Focal radiotherapy (RT) can relieve neurologic symptoms, but has no significant effect on survival [8]. Intrathecal chemotherapy (IT) is considered the mainstay of treatment of LM but its efficacy remains uncertain [5]. In LM from breast cancer, systemic treatment appeared at least as effective but less toxic than IT chemotherapy, suggesting that the blood–CSF barrier is not the crucial factor in LM [9]. Only a few series of patients with LM from melanoma have been published with reported median overall survival of 8–10 weeks [10, 11].

Two new treatment modalities have significantly improved survival in patients with advanced melanoma. Vemurafenib and dabrafenib, inhibitors of the mutated BRAF protein (evident in 50% of melanoma patients) have shown impressive albeit temporary responses, also in BM [12, 13]. The second new treatment strategy is the application of immune checkpoint inhibitors, like

*Correspondence to: Dr Willem Boogerd, Department of Neuro-Oncology, Netherlands Cancer Institute, Plesmanlaan 121, Amsterdam 1066 CX, The Netherlands. Tel: +31-20-512-2570; Fax: +31-20-512-2572; Email: w.boogerd@nki.nl

© The Author 2016. Published by Oxford University Press on behalf of the European Society for Medical Oncology.
All rights reserved. For permissions, please email: journals.permissions@oup.com.

ipilimumab and nivolumab that enhance the antitumor T-cell response and, importantly, induce long-lasting responses in a subset of patients. A complete response in a patient with LM from melanoma treated with RT and ipilimumab was reported earlier [14]. In this study, we sought to determine the influence of new treatment modalities and of prognostic factors on outcome in patients with LM.

materials and methods

A cohort of 39 consecutive patients diagnosed with LM from melanoma at The Netherlands Cancer Institute between May 2010 and March 2015 was analyzed. Diagnosis was based on MRI and/or CSF cytology.

Data collected included age, gender, date of diagnosis of melanoma, date of diagnosis of LM, performance status (PS) at diagnosis of LM, presence of BM, number (1, 2–5 or >5) and volume (< or >2 cm diameter) of BM, neurological signs and symptoms at diagnosis of LM, use of corticosteroids, CSF results [leukocyte count, protein, glucose, lactate dehydrogenase (LDH)], treatment for BM and/or LM, date of death or last follow-up, serum blood LDH and S100B levels at diagnosis of LM.

statistical analysis

Survival was measured from the date of diagnosis of LM to death, or last follow-up. Kaplan–Meier curves were made to estimate survival percentages. A P value <0.05 was considered statistically significant. Statistical analysis was carried out using SPSS version 22 (IBM Corp., Armonk, NY). The log-rank test was used to assess the influence of baseline characteristics on survival.

results

patient characteristics

Patient characteristics at time of diagnosis of LM are summarized in Table 1. Median time from diagnosis of melanoma to LM was 3.2 years (range 0–29 years). At time of data analysis (June 2015), four patients were still alive. At diagnosis of LM, 10 patients (26%) had a WHO PS of 2 (26%) and 6 patients (15%) a PS of 3. The diagnosis LM was established in 36 patients (92%) by MRI and in 3 patients (8%) by CSF cytology. Thirty-three patients (85%) had neurological symptoms. The most common LM symptoms at diagnosis were headache (46%), nausea and vomiting (44%), gait difficulty (39%) and seizures (31%). In six asymptomatic patients, diagnosis of LM was an incidental finding at screening or follow-up MRI. Thirty-four patients (87%) also had BM. Ten patients (29%) were not treated for their BM. Thus, 24 patients (71%) were treated for BM; 16 patients received RT and 21 patients systemic therapy.

Table 1. Patient characteristics and univariate analysis of factors associated with survival

	No. of patients (%)	Median OS (95% CI)	P value
Age (range), years	52.9 (range, 26–84)		
Sex			
Male	23 (59)	6.4 (1.5–11.3)	0.8
Female	16 (41)	8.0 (0–17.5)	
WHO performance status			
0–1	22 (56)	18.6 (9.8–27.9)	<0.001
2–3	16 (41)	3.6 (2.7–4.4)	
Unknown	1 (3)		
Lactate dehydrogenase			
0–248 U/l (normal)	19 (49)	18.6 (10.8–26.9)	<0.001
>248 U/l (elevated)	14 (36)	3.1 (1.6–4.7)	
Unknown	6 (15)		
S100B			
0–0.10 μg/l (normal)	9 (23)	24.9 (15.7–34.0)	0.03
>0.10 μg/l (elevated)	23 (59)	5.1 (1.8–8.5)	
Unknown	7 (18)		
Brain metastases			
Yes	34 (87)	6.9 (1.1–12.6)	0.43
No	5 (13)	3.1 (1.3–5.0)	
Number of brain metastases[a]			
None	5 (13)	HR 0.5 (0.1–1.7)	0.24
1	2 (5)	HR 1.6 (0.4–6.8)	0.54
2–5	9 (23)	HR 0.5 (0.2–1.1)	0.09
>5	23 (59)	1 (ref)	
Treatment for LM			
Yes	25 (64)	16.8 (11.6–22.1)	<0.001
No	14 (36)	2.9 (0–6.0)	
Treatment for LM[a]			
No treatment	14 (36)	1 (ref)	
RT	4 (10)	HR 0.53 (0.2–1.7)	0.28
Systemic	10 (26)	HR 0.17 (0.06–0.5)	0.001
RT + systemic	11 (28)	HR 0.07 (0.02–0.2)	<0.001
Symptoms of LM			
Yes	33 (86)	6.4 (2.6–10.3)	0.45
No	6 (14)	11.0 (0–40.0)	

HR, hazard ratio; LM, leptomeningeal metastases; OS, overall survival; RT, radiotherapy; WHO, World Health Organization.
[a]Hazard ratio.

treatment and survival

Twenty-five patients (64%) were treated for LM (for characteristics of treated patients see Table 2). Treatment for LM included cranial or spinal RT in 15 patients and systemic therapy in 21 patients. No IT chemotherapy was given. Of the 21 systemically treated patients, eight patients were treated with a BRAF inhibitor (vemurafenib or dabrafenib), 3 patients were treated with a BRAF inhibitor in combination with an MEK inhibitor (dabrafenib and trametinib), 6 received ipilimumab (a CTLA-4 monoclonal antibody), 2 patients were treated with ipilimumab followed by a BRAF inhibitor, 1 patient was treated with dabrafenib in combination with trametinib followed by ipilimumab and 1 patient was treated with dacarbazine followed by ipilimumab. Thus, a BRAF inhibitor was given in 14 patients, and ipilimumab in 10 patients. Fourteen patients (36%) did not receive any therapy after the diagnosis of LM due to rapid disease progression or poor performance. Of the 16 patients with a PS of 2 or 3, only 6 (38%) received treatment for LM (3 RT and 3 systemic treatment). Patients with a PS of 2 or 3 had a significantly worse median overall survival compared with patients with a PS of 0 or 1 (3.6 versus 18.8 weeks, $P < 0.001$). There was no significant difference in median survival between

Table 2. Characteristics of the treated patients at time of diagnosis of LM

Patient no.	PS at diagnosis	Age at diagnosis (years)	Symptoms of LM	Treatment	Time from LM to death (weeks)
1	0	50	Cerebral	Vemurafenib, WBRT	21.7
2	1	66	Cerebral	WBRT, ipilimumab	235.1[a]
3	2	61	Cerebral	Dabrafenib + trametinib	3.1
4	1	39	Cerebral and cranial nerves	Ipilimumab, WBRT	15.1
5	1	44	Cerebral	Vemurafenib, WBRT	15.3
6	0	59	Cerebral	Dabrafenib + trametinib, WBRT	24.9
7	1	64	None	Vemurafenib, ipilimumab	26.0
8	0	64	Cerebral	WBRT, vemurafenib	18.9
9	1	47	Cerebral	WBRT	2.3
10	0	65	Cerebral and cranial nerves	Ipilimumab	6.0
11	0	48	None	Vemurafenib	48.4
12	1	49	Cerebral	Ipilimumab, WBRT	10.0
13	0	50	None	WBRT, DTIC, ipilimumab	68.6
14	3	51	Cerebral	WBRT	3.6
15	0	50	Cerebral and cranial nerves	WBRT, dabrafenib + trametinib, ipilimumab	47.0
16	0	52	Cerebral	Vemurafenib, WBRT	33.6
17	0	49	Spinal	Spinal RT, dabrafenib + trametinib	61.9[a]
18	3	67	Cerebral	WBRT	15.9
19	2	26	Cerebral and cranial nerves	Vemurafenib	3.9
20	2	49	Cerebral and cranial nerves	SRT	5.1
21	3	73	Cerebral	Vemurafenib	16.9
22	1	57	Cerebral	Ipilimumab	6.4
23	1	60	Cerebral and cranial nerves	Ipilimumab	2.0
24	1	52	Cerebral, cranial nerves and spinal	Dabrafenib, ipilimumab	16.4[a]
25	0	77	Spinal	Dabrafenib	26.4[a]

LM, leptomeningeal metastases; PS, performance status; SRT, stereotactic radiotherapy; RT, radiotherapy; WBRT, whole-brain radiotherapy.
[a]Patient alive at the time of analysis.

untreated patients with a PS of 2 or 3 and the six patients who received treatment (1.9 versus 3.9 weeks $P = 0.075$). Median overall survival for all patients was 6.9 weeks [95% confidence interval (CI) 0.9–12.8] (Figure 1). There was no significant difference in survival in patients with or without neurological symptoms ($P = 0.45$). There was also no difference in survival in patients with or without corticosteroids ($P = 0.85$). Volume of BM was not significantly related to overall survival ($P = 0.54$). Of the 14 patients who did not receive any therapy for their LM, median survival was 2.9 weeks (95% CI 0–6.0) versus 16.9 weeks for treated patients (95% CI 11.6–22.1) ($P < 0.001$). Median survival of the 21 patients treated with a BRAF inhibitor and/or ipilimumab was 21.7 weeks (range 2–235 weeks). Median survival of the 14 patients in which treatment included a BRAF inhibitor (with or without an MEK inhibitor) was 24.9 weeks (range 3–62 weeks) (with RT 25 weeks, without RT 16 weeks). Median survival of the 10 patients in which treatment included ipilimumab was 15.8 weeks (range 2–235 weeks) (with RT 47 weeks, without RT 6 weeks). Median survival of the 4 patients treated with RT only was 4.3 weeks (range 2–16 weeks).

Serum LDH at diagnosis of LM was available from 33 patients (85%); 14 of these (42%) had an increased LDH (>248 U/l). Patients with LM and an increased LDH had a significant shorter survival of 3.1 weeks (95% CI 1.5–4.7) compared with 18.9 weeks for patients with normal LDH (95% CI 10.8–26.9, $P < 0.001$, Figure 2). Patients with increased LDH were less likely to receive any treatment modality for LM; 4 of 14 patients with increased LDH were treated versus 18 of 19 patients with a normal LDH ($P < 0.001$). Serum S100B values were available from 32 (82%) patients at time of LM diagnosis. Nine patients (28%) had a normal serum S100B level, and 23 (72%) had an increased serum S100B level. Patients with a normal serum S100B level had a

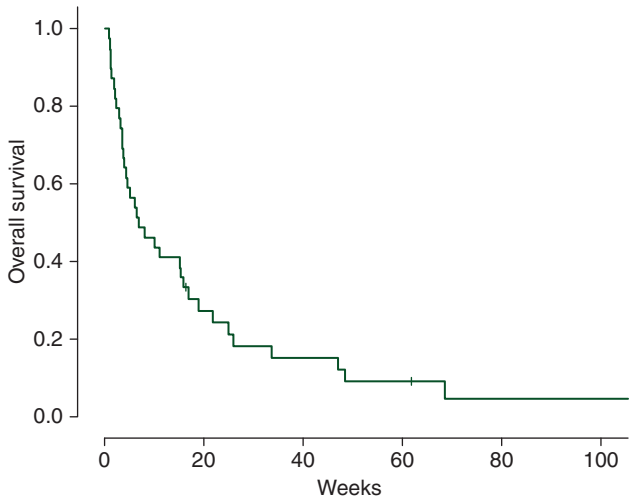

Figure 1. Kaplan–Meier curve for overall survival in weeks. Median overall survival = 6.9 weeks.

median overall survival of 24.9 weeks (95% CI 15.7–34.0) versus 5.1 weeks (95% CI 1.7–8.5) for patients with an increased S100B level ($P = 0.04$).

Thirty-five patients had died at time of analysis. Twenty-four patients (68%) died primarily of neurological progression, 8 patients (23%) of both intracranial and extracranial progression, while 3 deaths (9%) were not directly tumor related. Of the 24 patients who primarily died of neurological progression, 2 patients died of progression of BM, 11 patients due to progression of LM while in 11 patients cause of death could not be attributed to LM or BM with certainty. Of the four patients still alive at time of analysis, one patient was treated with local RT at L2-S5 (1 × 8 Gy) followed by dabrafenib and trametinib for widespread spinal LM causing a Cauda equina syndrome (Figure 3). An ongoing response of 62 weeks was achieved of LM and of asymptomatic BM.

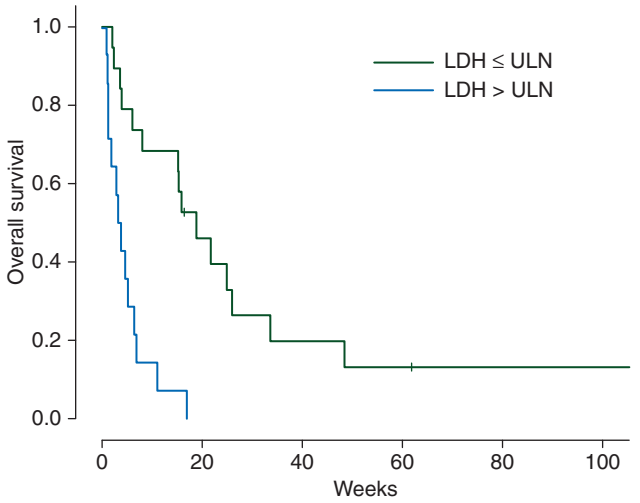

Figure 2. Kaplan–Meier curve for LDH. Median overall survival in patient group with LDH ≤ ULN = 18.9 weeks ($n = 19$), median overall survival in patient group with LDH > ULN = 3.1 weeks ($n = 14$). LDH, lactate dehydrogenase; ULN, upper limit of normal.

patient characteristics and treatment characteristics in patients with LM only

Five patients had LM without BM. Three of these five patients presented with headache, vomiting, seizures and cranial nerve involvement with rapid clinical deterioration, and died within 3 weeks before any specific treatment was given.

The fourth patient presented with weight loss, fatigue and pain in both legs 28 years after resection of a melanoma on his back. A PET–CT scan showed metastases in lymph nodes, kidneys, peritoneum, small bowel and subcutaneously and pathologic FDG activity in the lumbar spinal canal. Additional MRI of the lumbar spine showed diffuse LM. He is currently being treated with dabrafenib, resulting in a neurological and radiological partial response for 6 months now. He did not receive local RT.

The fifth patient presented with progressive nausea and vomiting. Cerebral MRI showed multifocal enhancement of the leptomeninges consistent with the diagnosis of LM. She also had lymph node and lung metastases. She was treated with WBRT and four cycles of ipilimumab, resulting in a complete radiological and clinical remission (see also [14]). She is free of disease for 4.5 years now.

discussion

This retrospective study confirms the well-known dismal outcome of LM, and shows that for patients with melanoma, outcome is even worse compared with patients with LM from other solid tumors. More than one-third of our patients had a PS too poor for antitumor treatment and died in a median time of less than 3 weeks. The typical steep decline in the survival curve for about one-third of the patients is consistent with data from literature [5, 8, 11]. A remarkable and encouraging new

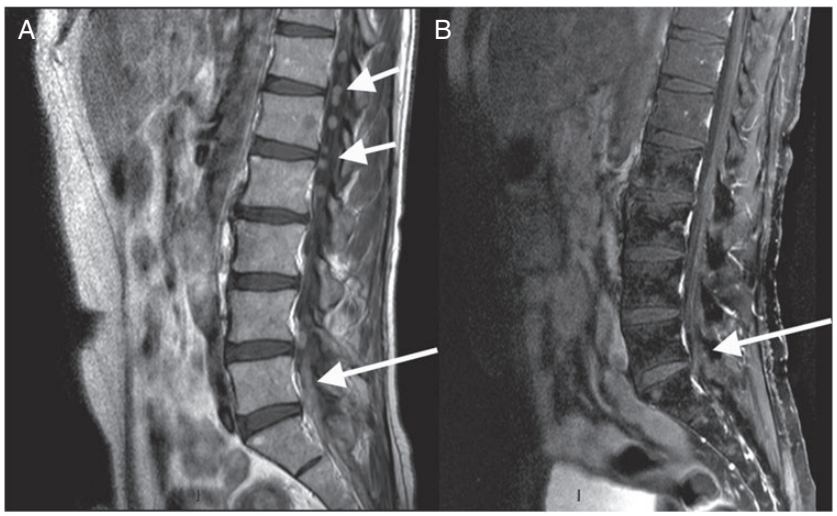

Figure 3. Post-gadolinium sagittal MRI T_1-weighted images of Th11-S2, demonstrating thickening and enhancement of the cauda equine nerve roots (long arrows) and enhancing intradural nodules (short arrows) in December 2013 before RT L2-S5 and start of dabrafenib and trametinib (A), and only slight enhancement of lumbosacral nerve roots in January 2015 during treatment with dabrafenib and trametinib (post-gadolinium sagittal MRI T_1 with fat-suppression) (B).

finding in our study is the long-term survivors when patients are being treated with targeted treatment or immunotherapy. Moreover, the median survival of 22 weeks following these new therapies compares favorably to reported results of IT chemotherapy for LM from melanoma [10, 11]. Earlier studies on immunotherapy for LM from melanoma included IT interleukin-2, which showed incidental responses, but also marked toxicity [11, 15]. The new checkpoint inhibitor ipilimumab has shown impressive responses in patients with advanced melanoma with a 4-month increase in median survival and, importantly ~20% long-term survival [16]. Ipilimumab enhances antitumor T-cell activation in the lymph nodes. As activated T cells can cross the blood–brain barrier or blood–CSF barrier, these barriers seem less relevant for a response within the CNS. In patients with BM not requiring steroids, the intracranial response after ipilimumab approximated the extracranial response (RR 24% versus 27%) [17]. Combination with RT may increase the response by the so-called abscopal effect, i.e. increased release of tumor antigen by RT can increase antigen presentation to T cells [18]. Responses to immune checkpoint inhibitors can be delayed as first an increase of activated T cells at the tumor location is needed. In contrast, the response of metastasized melanoma to BRAF inhibitors is prompt. The response rate is ~50% in advanced BRAF-mutated melanoma [19]. Although vemurafenib does not cross an intact blood–brain barrier, vemurafenib has shown to be effective in BM from melanoma, but also high rates of intracranial relapse during extracranial disease control were observed [20]. Dabrafenib also does not cross an intact blood–brain barrier but similar intracranial and extracranial responses (±40%) were reported after first-line treatment with dabrafenib [12, 21]. A response of LM to BRAF inhibitors as single agent has not been reported yet. In the present study, an ongoing response of 62 weeks of LM outside the RT portal was documented following dabrafenib and trametinib treatment, again demonstrating that the blood–CSF barrier does not exclude successful systemic treatment of overt CNS metastases. Upregulation of the MEK pathway causes BRAF inhibitor resistance, so combination with the MEK inhibitor trametinib probably prolonged the duration of response in our patient.

At univariate analysis, elevated serum LDH and S100B levels, both markers for tumor burden in melanoma, were associated with shorter survival. Most of the patients with elevated LDH were not treated after the diagnosis LM because of poor PS and rapid clinical deterioration. Other possible prognostic factors, like the presence and kind of neurologic symptoms, use of corticosteroids, and presence, volume and number of BM were not associated with survival.

conclusion

LM from melanoma still have an extremely poor prognosis. As observed in extracranial metastatic disease new treatment modalities, such as systemic targeted therapy and immunotherapy seem to increase median survival with a few months, and may result in long-term remissions. Combining these therapies with RT might enhance their efficacy. Especially in LM patients with a good performance score and low serum LDH and S100B levels, these treatment options should be considered.

disclosure

The authors have declared no conflicts of interest.

references

1. Taillibert S, Laigle-Donadey F, Chodkiewicz C et al. Leptomeningeal metastases from solid malignancy: a review. J Neurooncol 2005; 75: 85–99.
2. Amer MH, Al-Sarraf M, Baker LH, Vaitkevicius VK. Malignant melanoma and central nervous system metastases: incidence, diagnosis, treatment and survival. Cancer 1978; 42: 660–668.
3. Rosen ST, Aisner J, Makuch RW et al. Carcinomatous leptomeningitis in small cell lung cancer: a clinicopathologic review of the National Cancer Institute experience. Medicine (Baltimore) 1982; 61: 45–53.
4. Beauchesne P. Intrathecal chemotherapy for treatment of leptomeningeal dissemination of metastatic tumours. Lancet Oncol 2010; 11: 871–879.
5. Le Rhun E, Taillibert S, Chamberlain MC. Carcinomatous meningitis: leptomeningeal metastases in solid tumors. Surg Neurol Int 2013; 4: S265–S288.
6. Straathof CS, de Bruin HG, Dippel DW, Vecht CJ. The diagnostic accuracy of magnetic resonance imaging and cerebrospinal fluid cytology in leptomeningeal metastasis. J Neurol 1999; 246: 810–814.
7. Wasserstrom WR, Glass JP, Posner JB. Diagnosis and treatment of leptomeningeal metastases from solid tumors: experience with 90 patients. Cancer 1982; 49: 759–772.
8. Boogerd W, Hart AA, van der Sande JJ, Engelsman E. Meningeal carcinomatosis in breast cancer. Prognostic factors and influence of treatment. Cancer 1991; 67: 1685–1695.
9. Boogerd W, van den Bent MJ, Koehler PJ et al. The relevance of intraventricular chemotherapy for leptomeningeal metastasis in breast cancer: a randomised study. Eur J Cancer 2004; 40: 2726–2733.
10. Pape E, Desmedt E, Zairi F et al. Leptomeningeal metastasis in melanoma: a prospective clinical study of nine patients. In Vivo 2012; 26: 1079–1086.
11. Harstad L, Hess KR, Groves MD. Prognostic factors and outcomes in patients with leptomeningeal melanomatosis. Neuro Oncol 2008; 10: 1010–1018.
12. Long GV, Trefzer U, Davies MA et al. Dabrafenib in patients with Val600Glu or Val600Lys BRAF-mutant melanoma metastatic to the brain (BREAK-MB): a multicentre, open-label, phase 2 trial. Lancet Oncol 2012; 13: 1087–1095.
13. Dummer R, Goldinger SM, Turtschi CP et al. Vemurafenib in patients with BRAF (V600) mutation-positive melanoma with symptomatic brain metastases: final results of an open-label pilot study. Eur J Cancer 2014; 50: 611–621.
14. Bot I, Blank CU, Brandsma D. Clinical and radiological response of leptomeningeal melanoma after whole brain radiotherapy and ipilimumab. J Neurol 2012; 259: 1976–1978.
15. Chamberlain MC. A phase II trial of intra-cerebrospinal fluid alpha interferon in the treatment of neoplastic meningitis. Cancer 2002; 94: 2675–2680.
16. Schadendorf D, Hodi FS, Robert C et al. Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma. J Clin Oncol 2015; 33: 1889–1894.
17. Margolin K, Ernstoff MS, Hamid O et al. Ipilimumab in patients with melanoma and brain metastases: an open-label, phase 2 trial. Lancet Oncol 2012; 13: 459–465.
18. Grimaldi AM, Simeone E, Giannarelli D et al. Abscopal effects of radiotherapy on advanced melanoma patients who progressed after ipilimumab immunotherapy. Oncoimmunology 2014; 3: e28780.
19. McArthur GA, Chapman PB, Robert C et al. Safety and efficacy of vemurafenib in BRAF and BRAF mutation-positive melanoma (BRIM-3): extended follow-up of a phase 3, randomised, open-label study. Lancet Oncol 2014; 15: 323–332.
20. Peuvrel L, Saint-Jean M, Quereux G et al. Incidence and characteristics of melanoma brain metastases developing during treatment with vemurafenib. J Neurooncol 2014; 120: 147–154.
21. Mittapalli RK, Vaidhyanathan S, Sane R, Elmquist WF. Impact of P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2) on the brain distribution of a novel BRAF inhibitor: vemurafenib (PLX4032). J Pharmacol Exp Ther 2012; 342: 33–40.