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Isabella C. Glitza Oliva

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LONG-TERM SURVIVAL IN METASTATIC MELANOMA PATIENTS WITH LEPTOMENINGEAL DISEASE TREATED WITH INTRATHECAL INTERLEUKIN-2

by

Isabella Claudia Glitza Oliva, M.D., Ph.D.

APPROVED:

_____
Patrick Hwu, M.D.
Advisory Professor

_____
Michael A. Davies, M.D., Ph.D.

_____
Robert Bast, M.D.

_____
Chantale Bernatchez, Ph.D.

_____
Filip Janku, M.D., Ph.D.

APPROVED:

_____
Dean, The University of Texas
Graduate School of Biomedical Sciences at Houston

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LONG-TERM SURVIVAL IN METASTATIC MELANOMA PATIENTS WITH LEPTOMENINGEAL DISEASE TREATED WITH INTRATHECAL INTERLEUKIN-2

A

THESIS

Presented to the Faculty of

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In Partial Fulfillment

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for the Degree of

MASTER OF SCIENCE

By

Isabella Claudia Glitza Oliva, M.D., Ph.D.

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Long-term survival in metastatic melanoma patients with leptomeningeal disease treated with intrathecal interleukin-2

Isabella Claudia Glitza Oliva, M.D., Ph.D.

Advisory Professor: Patrick Hwu, M.D.

BACKGROUND: Metastatic melanoma patients with leptomeningeal disease (LMD) have an extremely poor prognosis and a paucity of effective treatment options. We assessed the safety and efficacy of intrathecal interleukin-2 (IT IL-2) in metastatic melanoma patients with LMD.

METHODS: We reviewed the outcomes of 43 consecutive metastatic melanoma patients with LMD who were treated with IT IL-2 from 2006 to 2014 in a Compassionate Investigational New Drug Study. All patients had evidence of LMD based on cerebrospinal fluid (CSF) cytology, radiology, and/or surgical pathology. IL-2 at a dose of 1.2 mIU was administered intrathecally via Ommaya reservoir up to 5 times per week in the inpatient setting for 4 weeks; patients with good tolerance and clinical benefit received maintenance IT IL-2 every 1 to 3 months thereafter.

RESULTS: The median age of the patients was 46.7 years (range 18-71); 32 (74%) were male; 31 (72%) had positive CSF cytology, and 39 (91%) had radiographic evidence of LMD. Median overall survival (OS) from initiation of IT IL-2 was 7.8 months (range, 4.7-16.3 months), with 1-, 2-, and 5-year OS rates of 36%, 26%, and 13%. The presence of neurological symptoms (HR 2.1, p=0.03), positive baseline CSF cytology (HR 4.1, p=0.001) and concomitant use of targeted therapy (HR 3.0, p=0.02) were associated with shorter OS on univariate analysis. All patients developed symptoms due to increased intracranial pressure. There were no treatment-related deaths.

CONCLUSION: IT IL-2 treatment is safe and achieves long-term survival in a subset of metastatic melanoma patients with LMD.

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Dedication

This thesis is dedicated to my parents, who have supported me wholeheartedly throughout my life and all my academic endeavors; to my husband, who is the kindest and most patient person I have ever met; to my mentors, Dr. Patrick Hwu, Dr. Michael A. Davies and Dr. Nicholas Papadopoulos, all of whom have empowered me with the knowledge and belief in my quest to become the best oncologist that I can be; and most of all to patients afflicted with metastatic melanoma, specifically the ones afflicted with LMD, who inspire me to strive for new discoveries every day.

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I would like to thank our patients and their families for their trust in us and for allowing us to compile and present this data. Special thanks to our allied health professional providers, Michelle Rohlfs, Jessie Richard, Donna Gerber, Carol Lacey, Rinata Simien, Teresa Rodgers, Masood Iqbal, Ida John and Lindsay Blair, who have assisted with the treatment and management of our patients.

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Chapter I

INTRODUCTION

Background:

The spread of cancer to the central nervous system (CNS) is a devastating complication of multiple tumor types. The solid tumors that most often metastasize to the CNS are lung cancer, breast cancer, and melanoma. As melanoma has a much lower incidence compared to breast or lung cancer, melanoma has the highest risk of CNS metastasis of the common cancers. CNS metastases are diagnosed clinically in up to 60% of metastatic melanoma patients, and evidence of CNS involvement is identified in up to 80% of patients at autopsy. [1]

The CNS is a frequent site of treatment failure for melanoma, including treatment involving recently approved targeted and immune therapies. Multiple studies have demonstrated an average of 4 months of median survival time after the diagnosis of melanoma CNS metastasis. [1-2] Patients with parenchymal brain metastases have a number of treatment options including surgery, whole-brain radiotherapy (WBXRT) and stereotactic radiosurgery (SRS). New systemic therapies, including the BRAF inhibitor dabrafenib and the immune checkpoint inhibitors ipilimumab and pembrolizumab, have also demonstrated efficacy in clinical trials for metastatic melanoma patients with parenchymal brain metastases. In contrast, there are very few treatment options for patients with **leptomeningeal disease (LMD)**, and these patients have extremely poor outcomes. The leptomeninges consist of a total of 3 layers of meninges that cover the parenchymal brain and spinal cord. The first layer is called dura mater, which is made of connective tissue. The second layer in the middle is referred to as the arachnoid, which is a fairly thin layer that resembles a spider web, with multiple strains attaching to the inner layer, named pia mater. This space formed between the most inner layers, the arachnoid and

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the pia mater, contains cerebrospinal fluid (CSF) and is comprises the subarachnoid space which alos contains and houses all the blood vessels.

Any cancer can metastasize to the leptomeninges, but certain cancer types are more likely to do so. In breast cancer, the reported incidence of LMD has been up to 5%, in lung cancer in up to 25%, and in melanoma in 22-46%. [3] No effective treatment options for these patients, and that the NCCN guidelines only recommend palliative radiotherapy and best supportive care, the development of more effective treatments for LMD from melanoma is a critical unmet need.

Diagnosis of LMD

Recent reports have suggested that the incidence of leptomeningeal disease is rising due to the fact more effective systemic therapies are available for patients with metastatic disease, leading to a longer overall survival. [4] However, as patients live longer there can be subsequent metastatic spread to the central nervous system including the leptomeninges.

It is possible that the increased incidence of LMD is also due to general higher awareness of the clinician, combined with increased use of neuroimaging, specifically gadolinium-enhanced MRI of the brain and spine, which is one of required test for disease confirmation. The MRI should consist of a postcontrast T1 weighted image, as well as postcontrast FLAIR imaging. These two sequence combined are felt to be the most sensitive to detect LMD. [5-6] LMD can present on MRI imaging in different ways, and can vary from a diffuse leptomeningeal enhancement to bulky or nodular tumor foci (Fig 1). The most common presentation though is the more diffuse enhancement seen within the horns of the ventricles, as sometimes referred as zuckerguss (German for sugar coating). It is important to note that a normal MRI never excludes the diagnosis of LMD. Furthermore, the sensitivity of an MRI has been reported to range greatly

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from 22 to over 90 %, but any irritation of the leptomeninges can result in an enhancement observed on the MRI, therefore should be obtained prior to lumbar puncture. [7]

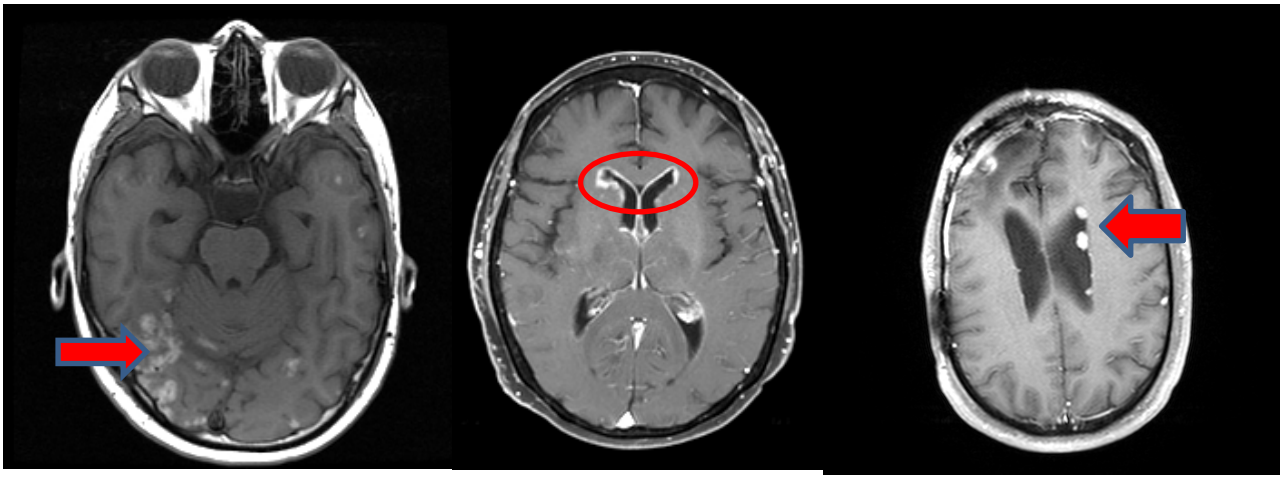

Fig 1: Radiographic findings of LMD: diffuse leptomeningeal enhancement, “zuckerguss” of the ventricle horns and nodular tumor foci within the ventricle

The gold standard for diagnosis of LMD remains CSF analysis showing tumor cells, which is termed “positive CSF cytology.” CSF analysis has several limitations. While the specificity is reported to be above 80%, the sensitivity of just 1 CSF analysis remains low and is often quoted to be around only 50%. In addition, CSF sampling is normally fairly low-volume, and improper specimen handling can again lead to inaccurate results due to cell degeneration. Other abnormalities observed in the CSF can be generally seen in the majority of cases with LMD. [8] These range from an increased white blood cell count, increase protein levels, decreased glucose levels to increased opening pressures.

A significant number of patients report symptoms and signs of neurological impairment at time of LMD diagnosis. Depending on the location and extent of LMD, these symptoms can include headaches, mental status changes, and nausea and vomiting. If cranial nerve involvement is present, patients most often describe diplopia, visual loss, and hearing loss as well as facial

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numbness, or other focal neurological deficits. [3] LMD affecting the spinal axis can lead to lower motor neuro weakness, paresthesias, back and/or neck pain, radicular pain and bladder and bowel dysfunction. Given that up to 80% of patients have concurrent parenchymal brain metastases, it is important to distinguish the symptoms caused by LMD from those due to brain metastases, as well as complications of any other treatments the patients are currently receiving. [9-10]

Finally, due to the fact that the diagnosis of LMD remains challenging, new approaches are being tested. Cell free DNA (cfDNA) and circulating tumor cells are being used to monitor treatment response in the blood, and are an exciting approach to diagnose metastatic disease prior to evidence on imaging. This approach was recently translated into the monitoring of levels of cfDNA in the CSF of a patient with metastatic BRAFV600E mutant melanoma and LMD.[11] The patient was initiated on dabrafenib and trametinib and responded to therapy with resolution of his previously reported headaches, nausea, and vomiting after 2 months of treatment. Unfortunately, his symptoms returned 3 months later, and he required neurosurgical intervention. However, he underwent 9 CSF samplings over a period of six-month. Interestingly, the fraction of mutant cfDNA gradually decreased from 53% at time of diagnosis to being undetectable at time of being symptom-free. When the patient developed recurrent symptoms, the mutant cfDNA was attempted again at high levels. Therefore it was felt that this approach could be used to diagnose and monitor therapeutic response after treatment initiation.

Survival and LMD

Patients with CNS metastasis in general have a median overall survival that is typically measured in months, but patients with evidence of LMD have the worst prognosis among all melanoma patients who suffer from metastatic disease. A cohort of 743 stage IV patients was

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reviewed, and found that 330 were diagnosed with brain metastasis at one point during their melanoma treatment history. The median survival of all patients with CNS metastasis was 4.65 months. Patients diagnosed after 1996 had a slightly increased OS with 5.92 months (HR 0.6-0.94, p+0.01), but age >or < 65 did not have an impact on outcome. Patients who developed LMD (n=11) had the worst overall survival with a median of only 1.22 months, and fared even worse than patients who were diagnosed with >3 brain metastases (3.52 months median OS). [1]

Other studies have confirmed this poor outcome and therefore underline the need for clinical trials and better treatment strategies for these patients. A recent study including 39 patients with LMD from melanoma (diagnosed 2010-2015) reported a median OS for the entire cohort of 6.9 weeks. [12] Patients who received no further treatment for LMD had a median OS of only 2.9 weeks, with an increase to 16.9 weeks in patients who received treatment. A second retrospective analysis of 55 patients described a median OS of 9.7 weeks. [13] Researchers at MD Anderson Cancer Center reported a median OS of 10 weeks in a large retrospective study of 110 patients diagnosed between 1944 and 2002, with a 1-year survival of 7%, and a 2-year OS of only 3%. [14]

I recently reviewed the clinical features, treatments, and OS of 178 melanoma patients diagnosed with LMD by CSF cytology or MRI between 2000 to 2015 at the UT MD Anderson cancer center, which represents the largest and most temporary patient cohort available for analysis. The diagnosis was based positive on positive CSF cytology in 48% of patients, highlighting that in a large number of cases the diagnosis of LMD is made by CNS imaging. The reported median age at diagnosis was 51 , with a high male predominance (74%). 39% had elevated LDH, and most patients had concomitant brain (76%) and extracranial (74%) metastases. Neurological deficits were reported in 47% patients. Median OS from LMD diagnosis was 3.0 months.

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Prognostic factors for survival in LMD patients

While it is known that patients with LMD from melanoma have the worst prognosis of all metastatic melanoma patients, very few studies have identified prognostic markers for survival. A previous retrospective study of 110 patients at MD Anderson collected the following information for analysis: [14] Patient specific demographics as well as tumor specific factor like thickness, subtype of primary lesion and its location, time interval between diagnosis of primary melanoma and LMD, serum lactate dehydrogenase (LDH), symptoms and signs of LMD, CSF specific information, location and number of concomitant parenchymal brain metastasis and treatments received. This study identified elevated serum LDH to be associated with poorer survival, but elevated LDH was probably mainly a marker of significant extracranial disease burden. Of note, number of extracranial metastasis and their location were not included in the analysis due to differences in reporting of the extracranial tumor burden. Interestingly, patients with concomitant brain metastasis did not have significantly worse outcomes than patients without other CNS metastasis, and the amount of LMD tumor burden was not associated with survival (p=0.11). Radiographic evidence of LMD or the presence of positive cytology had no impact on overall survival (10 weeks). Patients who received therapy directed at their LMD derived benefit, and this was reported for all three treatment modalities evaluated [radiation (HR = 0.5, p = 0.0015), systemic chemotherapy (HR = 0.6, 95% CI = 0.4, 0.9, p = 0.028), and direct Intrathecal therapy (HR = 0.5, 95%, p = 0.0001)]. All these patients were diagnosed between 1944 and 2002, and therefore not treated with currently available targeted therapies and immunotherapies. After multivariate analysis, primary melanoma located on the trunk of the patient appeared to be associated with worse outcome (hazard ratio [HR] = 2.0, 95% CI = 1.0-3.8, p = 0.035), which is consistent with reports associating the location of the primary tumor with the development of metastatic CNS disease.

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As with extracranial disease, poor performance status as time of LMD diagnosis has been associated with significantly worse outcomes. [3, 15] Other studies found that an elevated LDH at LMD diagnosis, time between primary tumor and development of LMD were also associated with worse survival. [12, 14, 16]

Elevated CSF protein levels correlated with poor prognosis in multiple studies (Table 1). This is defined as a CSF protein concentration >50mg/dL. These findings are often in conjunction with a low CSF glucose count and elevated opening pressures.

Table 1: Factors associated with survival in patients with LMD

Author	Number of patients	Melanoma only	Diagnosed	OS	Associated with poor survival
Foppen[12]	39	Yes	2010-2015	6.9 weeks (2.9 for untreated vs 16.9 for treated). If treated with systemic therapy +/- RT, 21.7 weeks	• Elevated serum LDH and S100B at diagnosis
Harstad[14]	110	Yes	1944-2002	10 wks	• Location of primary melanoma on the trunk (HR 2.0 (p = 0.035) • Intrathecal chemotherapy had positive effect on survival, while radiation and systemic therapy did not impact either survival nor were reported to have detrimental effects.
Oechsle[17]	2/135	No	1989-2005	10 wks overall, 0.9 months melanoma	• Differences in type of solid tumor, with lung an melanoma having the highest risk of death • Time interval between initial tumor diagnosis and the development of LMD being less than 1 year, patient s younger than 50, decreased performance status Karnofsky, use of systemic therapy and lack of clearing of CSF tumor cells observed.
Waki[18]	2/89	No	1997-2005	51 days	• Decreased overall performance status (HR: 1.72 (95% CI, 1.04-2.86) P = 0.04) • MRI evidence of leptomeningeal diseae (HR: 1.82 (95% CI, 1.11-2.98) P = 0.02)
Herrlinger[16]	21/155	No	1980-2002	4.8 months, 4.7 months for melanoma	• Advanced patient age of over 60 • Increased CSF protein levels

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Palma[19]	0/50	No	2001-2010	For patients who received specific treatment, 21.2 weeks vs. 6.38 weeks for patients receiving supportive care only	• Decreased overall performance status • Increased CSF protein levels • Time from diagnosis of primary tumor to diagnosis of LMD
Hyun[15]	0/519	No	2005-2014		• Decreased overall performance status • Increased CSF protein levels (>50 mg/dl) • lung cancer patients with LMD in general had a better outcome

While LMD directed treatment in general is felt not be very beneficial for these patients, there is some evidence that therapy can improve OS, and better survival was observed in patients receiving intrathecal and systemic therapy. [13, 17] The largest and most contemporary analysis of prognostic factors also showed that the median OS was improved for treated patients (8.2 months for patients treated with targeted therapy, 7.1 months for IT, 4.7 months for chemotherapy, and 3.7 months for IMT), while Positive CSF cytology (HR 2.26, CI 1.27-4.00, p=0.006), presence of neurological deficits (HR 2.21, CI 1.60-3.05, p<0.0001), uncontrolled systemic disease (HR=1.68; CI1.16-2.44, p-0.006) and elevated LDH (HR 1.44, CI 1.04-2.00 p= 0.03) were associated with shorter OS per Cox proportional hazard regression analysis.

The NCCN recommends that patients with leptomeningeal disease are being stratified based on several factors. Patients which are considered “poor risk” have a low KPS score, multiple and potentially serious neurological deficits, uncontrolled systemic disease or significant overall disease burden, bulky CNS disease and encephalopathy. Patients who were categorized as “good risk” have a good performance status, no major neurological deficits, and either controlled or limited systemic disease, for which treatment options are available.

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Treatment for leptomeningeal metastasis from melanoma

Treatment for leptomeningeal disease is palliative in nature, with the main goal of providing symptom relief. As per NCCN guidelines, the main treatment modalities include systemic therapy or radiation. Surgery is seldom considered due to the disseminated nature of the disease but neurosurgery is required for the placement of an intraventricular catheter or further symptom management associated with hydrocephalus or increased intracranial pressure.

Whole brain radiation (WBRT), with the average dose of 30 Gy, can be used for symptom management and is part of the NCCN recommendations for patients with LMD and good performance status. It has to be noted, that it rarely leads to significant neurological improvement. On occasion, involved field radiotherapy is being used to treat symptomatic disease or bulky disease, specifically if it is associated with significant pain. [3] In an attempt to develop a prognostic index aiding in predicting outcomes for melanoma patients with brain metastasis, 112 patients with CNS involvement who had received WBRT were analyzed. Presence and extent of extracranial disease had significant impact on survival, but patients with LMD present (n=7) had also significantly worse outcomes (HR 3.13 (1.41–6.95)). To date, it remains unclear whether WBRT has any impact on OS, even when LMD is present or in tumors that are more radiosensitive than melanoma. [17, 20]

Historically, systemic chemotherapy has not demonstrated consistent clinical benefit in patients with LMD. Case reports have noted one melanoma LMD patient with a complete response on the combination of temozolomide (TMZ) with cisplatin, and another patient showing a complete radiological response as well as a dramatic improvement in quality of life for about 10 months after initiation of TMZ. [21-22] The largest prospective clinical trial using oral TMZ in 19 patients with LMD associated with solid tumors included 1 patient with melanoma, who did not benefit. [23] In a small prospective clinical trial of nine melanoma patients, IT liposomal cytarabine was used initially, and three patients went on to receive IT thiotetraethylenepentamine, with concurrent

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systemic treatment (TMZ (n=3), fotemustine (n=5) or carboplatin (n=1). [24] The median OS was 8 weeks (range=1-168 weeks), with two patients responding and achieving prolonged survival (104 weeks). Survival of over 1 year has also been reported in a melanoma LMD patient treated with the combination of IT liposomal cytarabine, systemic TMZ, and WBXRT. [25]

Targeted therapy in the management of leptomeningeal melanoma metastasis has not been assessed in a prospective clinical trial, but there is increasing evidence that these drugs can provide benefit to patients with parenchymal CNS metastasis. [26] It has been reported that the distribution of vemurafenib, a BRAF inhibitor, was 3-logs lower in the CSF when compared to plasma concentration. Dabrafenib, a second BRAF inhibitor with regulatory approval for the treatment of metastatic melanoma appears to have higher penetration. [27-28] Case reports have shown that some patients appear to derive benefit from this treatment. [29]

The check-point inhibitor ipilimumab and pembrolizumab have demonstrated clinical responses and favorable survival outcomes in prospective clinical trials in melanoma patients with parenchymal brain metastasis. [30-33] In a non-randomized phase II trial ipilimumab administration led to a response rate of 15.7% for Cohort A (neurologically asymptomatic, no concurrent corticosteroids) and 5% for Cohort B (neurological symptoms and requiring a stable dose of corticosteroids). The overall response rate, 2-year survival rates (Cohort A 26%, Cohort B 10%) and safety in this study were similar to what has been reported in melanoma patients without brain metastases who were treated with ipilimumab. [34] Konstantinou and colleagues reported a brain control rate of 16% and a response rate of 8% in 38 melanoma patients with CNS metastases who received with ipilimumab as part of the extended access program. [35] Pembrolizumab, an anti-PD1 antibody, showed a 22% brain metastasis response rate in a recently reported open-label phase-2 trial. [33]Additional clinical trials are ongoing with other-FDA-approved targeted therapy and immunotherapy combinations (NCT02308020, NCT00338377).

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[34] However, all of those completed and most of the planned trials have excluded patients with LMD (Appendix, Table 7, 8).

Intrathecal immunotherapy

Intrathecal immunotherapy approaches have been tested in patients with melanoma LMD since the early 1990s. The idea of using an intrathecal approach stemmed from observation that many drugs do not cross the brain blood or brain-CSF barrier in a meaningful way, and that intrathecal administration was required to achieve meaningful CSF drug levels. Furthermore, long term remissions were observed in a subset of patients treated with intravenous interleukin-2, providing initial evidence that immunotherapy can result in significantly improved outcomes. One of the first intrathecal trials used interferon alfa. In this phase II trial, 20 patients with LMD from different solid and hematologic tumors received recombinant interferon alfa via Ommaya every other day for 4 weeks, which was considered an induction. [36] Only 2 of the patient's and this cohort had melanoma LMD, while the majority of patients had other solid tumors. The 2 patients treated with LMD for melanoma both progressed very fast, with progression seen after only to 4 weeks of treatment. Interestingly in the other treated patients, responses lasted up to 40 weeks, with a median duration of 16 weeks. Patients with hematologic malignancies and LMD head is significantly better outcome then patients with solid tumors. No other predictive factors for response were found, with age, performance status at treatment initiation, the addition of radiotherapy, systemic chemotherapy not altering outcome. An additional case report suggested stability of LMD in a patient receiving IT INF, but the patient passed after 3 months of IT treatment due to malignant pericarditis. [37]

IT interferon alpha-2b was reported to improve symptoms in one patient, while others progressed during treatment. [36-37] Previously published data from our institution and single-

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patient reports from other centers have shown that IT IL-2 can achieve prolonged OS in some patients, associated with significant toxicities. [38-41]

Interleukin-2 (IL-2)

The cytokine interleukin 2 (IL-2) is mainly secreted by antigen-stimulated CD4+ T cells. It supports the proliferation and activation of CD8+ T cells as well as increasing the cytolytic capacity of natural killer cells (NK). [42] When given systemically, IL-2 administration leads to release of cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin- 6 (IL-6) and interferon- gamma (INF-γ). These cytokines are responsible for the symptoms mimicking influenza infection and contributing to the capillary leak syndrome, leading to fluid retention and hypotension. The earliest clinical trial dates back to the 1980s, when HD IL-2 was tested in generally small cohorts of patients. The largest trial included 270 patients, with an overall response rate of 16.5%, including a 6% complete response (CR) rate. Importantly, durable responses were reported, which were observed in patients who had a complete response to treatment or who were converted to “complete response” by surgery for their residual disease. [43] Subsequent trials showed response rates between 5-27%, and attempts to improve the response rates with either interferon or lymphokine activated killer cells did not result in better outcome. While highly toxic, with the most common side effects being nausea/vomiting, diarrhea, hypotension, thrombocytopenia, renal and hepatic dysfunction, some patients never experienced melanoma re-occurrence. [44] These “cures” were particularly seen in patients in who did not experience progression of disease, or who had an ongoing response for more than 30 months. [45] While HD IL-2 has been mainly used in patients without active CNS metastases, some case reports hint that its use might be safe in a patient population with active

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brain metastasis. Eight patients were treated with HD interleukin-2 and radiation therapy, and the median OS was 6.7 months. [46]

Intrathecal IL-2

The use of intrathecal IL-2 (IT IL-2) dates back to the 1980s. This was based on data evaluating the ability of recombinant IL-2 to permeate the CSF- Blood barrier. [38, 40, 47-49] Twelve cancer patients without CNS involvement received I.V. administered IL-2, and changes in the levels of CSF IL-2 were monitored via lumbar punctures. IL-2 was first identified in the lumbar CSF 4-6 hours after the systemic dose, and a subsequent increase of the observed levels were observed over the next 2-4 hrs until a stable level of 3 to 9 U/ml was reached. After the last systemic dose, levels returned to less than 0.1 U/ml by 10 hours. While these levels were believed to be possibly sufficient for lymphokine-activated killer (LAK) cells activity, it also gave rise to giving IL-2 directly into the ventricle. Serum levels of intravenous injection with IL-2 showed a rapid increase in the concentration, with a reported mean peak value of 55U/ml. Clearance was shcieved rapidly, consistent with a previously reported 7 minute half-life.

In these early case reports, IT IL-2 was used in combination with LAK cells and given to a patient with malignant glioma. [48] In 1991, another case report described the use of continuous intraventricular infusion of IL-2 for 5 days, again combined with LAK. [50] Intracranial pressures rose to a maximum to 80 mmHg, but it was also noted that within 2 days after last dose of IT IL-2, the CSF pressures normalized. Interestingly, the leukocyte concentration and composition changed during the course of treatment. The initial cell count after LAK infusion and IL-2 was 202/mm^3, with 15% being monocytes, 19% lymphocytes, and 66% neutrophils. The cell count diminished after 4 days to just 8/mm^3. Prior to the second dose of IL-2, the CSF cell count was 5/mm^3, with 40% of lymphocytes and 60% monocytes. During the second cycle of

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interventricular ventricular IL-2, the CSF cell count rose to 16/mm^3, with 76% being lymphocytes, 21% granulocytes, and 3% monocytes.

Eleven patients with LMD from melanoma and adenocarcinoma of the lung were treated with intraventricular injection of IL-2. [51] All patients had an Ommaya reservoir in place, and CSF samples were collected at different intervals between 0 and 24 hours. It was found that IL-2 levels gradually decreased during the first 24 hours after injection, with an average half-life between 4 and 8 hours. It was also observed that IT IL-2 administration led to an increase in TNF- α, IL-1β, IL-6, INF-γ, as well as soluble IL-2 receptor (IL-2R). These cytokines also had different peaks after IT IL-2 administration. For example, the earliest cytokines peaked at 2-4 hours and at 4-6 hours, respectively, TNF- α and IL-6. IL-1β, INF-γ and IL-2R peaked between 6-12 hours. Of note, all cytokines returned to nearly baseline between 12 and 24 hours; however, a slightly elevated level of the IL-2 receptor was observed.

As in the previously described case reports, IT IL-2 led to an increase in CSF leukocyte and lymphocyte count. Neutrophils were the predominant cell population during the first 24 hours, and a decrease of the overall cell count in the CSF was observed at 36-48 hours. Simultaneously, an increase in total lymphocyte count was observed. Most patients also continued to have an elevated CSF lymphocyte count for 7-10 days during the subsequent treatment courses. The authors felt that the late appearance of lymphocytes and the lack of cytokine-producing cells at the initial time from CSF analysis suggested that there must be a local source for the observed cytokine production.

The early intrathecal IL-2 case reports did notice significant toxicities. For example, one patient experienced significant tachycardia, hypertension and changes in mental status 11 hours after the first dose of IT IL- 2. When an Ommaya tap was performed, his intracranial pressure was elevated, but his symptoms were improved after removal of CSF. After 4 days, all his

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neurological toxicities had resolved. Similar toxicity was seen despite 50% of dose reduction. Patient did not achieve a response to therapy. [38] Another patient overall was felt to tolerate the continuous IL-2 well, but had increased intracranial pressures that responded to CSF removal. While they did not mention in detail the side effects, it has to be noticed that his intracranial pressures were as high as 80. In regards to efficacy it appeared that the patient's neurological status did not improve, with ongoing paraplegia, but systemic disease progression, finally leading to the patient passing. [50] In the larger study of 12 patients evaluating the penetration of IL-2 across the blood CSF barrier patients received IL-2 via IV, but still experienced mentation change during treatment. Interestingly, CSF IL-2 levels in the patient with the highest level did not result in any change in mental status, and elevated albumin quotient did not seem to be associated with change in mental status or toxicity. [52] No efficacy was recorded. The most detailed side effect monitoring was performed in a 4 patient's study suffering from LMD in 1988. [47] Only one patient experienced significant side effects, which were mainly bitemporal headaches during administration of IL-2. During the last administration he also suffered from a seizure, which was only focal, and stopped with the termination of the injection. His CSF analysis at this time did not show major disturbances of the blood CSF barrier. Specifically, fever chills or anaphylactic reactions were not observed in this trial. All patients did experience an increase in their CSF leukocyte count.

Patient characteristics and biomarkers associated with response to immunotherapy

While encouraging results have been seen with the use of immunotherapy for patients with metastatic melanoma, predicting response remains difficult, and little is known about robust clinical characteristics and biomarkers associated with response to immunotherapy. The first immunotherapy regularly used for metastatic melanoma patients was high dose interleukin- 2

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(HD IL-2), which was FDA approved in 1998. While the treatment with HD IL-2 is highly toxic, requiring hospitalization and ongoing cardiac monitoring, the success rate is fairly low compared to the era of targeted therapies and immune checkpoint inhibitors. Therefore, it is even more important to find prognostic or predictive biomarkers for treatment response.

Retrospective analyses have shown a correlation between degree of thrombocytopenia and clinical benefit from HD IL-2 in patients with either stage IV renal cell cancer or melanoma. [53] In addition, the total number of HD IL-2 doses received (33.0 versus 18.0, p =0.001), number of total cycles of HD- IL- received (6.0 versus 2.0, p <0.001) and development and frequency of autoimmune-based side effects (p = 0.049) were associated with better outcomes. Another group uncovered that a cluster of 11 soluble biomarkers were associated with survival. [54] Patients with higher VEGF and fibronectin levels were much likely not to respond to HD IL-2, and represented independent predictors of non-response. Baseline elevated absolute lymphocyte count (ALC) at the start of cycle 1, as well as the ALC peak during therapy was not associated with an improved outcome. [53]

However, another clinical report showed that clinical responses in melanoma patients treated with the immune checkpoint blocker ipilimumab were associated not only with an increase in ALC, but also the absolute T cell count and the absolute number of activated T cells in peripheral blood. [55] Neutrophil to lymphocyte count ratio after 2 cycles of CTLA4 blockade was associated with outcome, and used in the development of a prognostic model for overall survival after 2 doses of ipilimumab. Correlation between the development of immune-related adverse events (irAE) and greater likelihood of achieving an objective tumor response (p=o.ooo4) was described in the early reports of ipilimumab. [56] The clinical characteristic of elevated LDH was also associated with worse prognosis in CTLA_4 treated patients.[57]

Recent advances have shed some light into biomarkers of response for patients receiving checkpoint blockade. For example, it has been shown that in increase in neoantigen burden is

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correlated with significant improved outcomes in adenocarcinoma of the lung. [58] Furthermore, gene expression profile varied between patients who respond and don't respond in this study. Genes that were different included PDL 1, pro-inflammatory IL-6 as well as genes linked to antigen presentation, T-cell migration and affect her T-cell function, specifically INF-γ, granzymes as well as LAG-3.

With the event of anti-PD1 and anti-PDL-1 agents used as the mainstay of anti- melanoma therapy, biomarkers for appropriate patient selection are needed. It appears that PDL-1 expression is required for therapeutic activity, and some clinical trials suggested that positive or high PDL-1 expression in the tumor was associated with improved response and OS.[59-60] PDL-1 expression analysis faces though multiple technical challenges due to a multitude of different antibodies and cut-off % used to score the cells. Furthermore, it is important to mention that patients without appreciated PDL-1 expression still derived benefit from treatment with anit-PD1 agents, though at a lower rate.[61,62]

Another report evaluated a cohort of metastatic melanoma patients who received either checkpoint blockade with CTLA 4 or anti-PD1. These patients were then separated into groups who were considered non-responders to therapy and responders.[63] Patients who were considered a responder were defined as having radiographic evidence of absent disease, stable disease, or decreased tumor volume for at least 6 months. Interestingly, early on- treatment biopsies evaluated by IHC found a difference in the density of CD8+ T cells in responders versus nonresponders receiving CTLA 4. For patients responding to PD1 blockade, a statistically significant difference in the density of CD8+, CD3+, and CD45RO+ T cells in the treatment samples were observed. To monitor for similar differences in the invasive tumor margins, IHC evaluation was done to compare tumor center versus tumor periphery. While no significant difference in the density of CD8+ T cells and responders versus nonresponders, was seen, a higher ratio of CD8+ T cells in the tumor center versus the tumor margin observed. The

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same group of researchers also evaluated the difference in gene expression profiling of the longitudinal biopsies, and showed 411 significantly differentially expressed genes and responders, mostly upregulated, as compared to nonresponders. It appeared that CTLA4 blockade and PD1 blockade varied slightly in which genes were upregulated. Taken together, these studies offered new understanding of the mechanisms of therapeutic resistance involved an immune checkpoint blockade treated patients. This will be specifically important when developing new strategies to overcome resistance.

While this represents exciting data for extracranial disease, these tests cannot be performed in a similar fashion for leptomeningeal disease, where no tumor tissue is readily available, and testing has to be performed on few melanoma cells found in the CSF. However, cfDNA, flow analysis and NGS sequencing are all new approaches that might increase our understanding of the CSF as a microenviroment. To date, for patients with LMD, the only CSF finding associated with poor survival is an elevated CSF protein level at diagnosis, but virtually nothing else is known about how other factors, like opening pressure at time of Ommaya tap and CSF WBC or ALC affect prognosis, or how these can be used to predict patients with poor survival.

Hypothesis:

In this proposal, *we will test the hypothesis that intrathecal immunotherapy with interleukin-2 can be a safe and effective therapy for patients with LMD from melanoma, and that clinical outcomes will correlate with clinical and immunological features.*

In order to test this hypothesis, and ultimately to develop more effective therapies for patients with LMD based on these results, we will:

AIM 1 Evaluate the safety and efficacy of IT IL-2.

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AIM 2 Examine the clinical patient characteristics and evaluate how these factors are associated with outcomes.

AIM3: Examine CSF specific features at baseline and evaluate how the observed changes with IT IL-2 treatment correlate with clinical benefit.

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CHAPTER II

METHODS

Patient selection:

This analysis included the outcomes of 43 consecutive metastatic melanoma patients with LMD treated with IT IL-2 at The University of Texas MD Anderson Cancer Center under an ongoing Compassionate Investigational New Drug Study. All patients started IT IL-2 treatment after August 2006; the last three patients initiated treatment in July 2014. The time of data cutoff was November 15, 2015. The diagnosis of LMD was established by cytopathological analysis of CSF, neuroradiological imaging with MRI, surgical/pathology report, or a combination of these diagnostic modalities. All patients were required to have a baseline MRI of the brain and entire spine and placement of an Ommaya reservoir (or shunt) with flow confirmation via radionuclide study prior to the start of treatment. IT IL-2 therapy was approved by the U.S. Food and Drug Administration (FDA) as a Compassionate Investigational New Drug (CIND), and all patients provided written consent for treatment. Previous systemic therapies, including chemotherapy, immunotherapy, or radiation therapy to the brain or spine, were allowed. Patients who were believed to be deriving clinical benefit from such therapies prior to the initiation of IT IL-2 could receive them concomitantly with the treatment at the discretion of their treating physician.

Ommaya Placement

All patients required the placement of an Ommaya reservoir prior to the first dose of IT IL-2. Briefly, the placement of an Ommaya, typically performed in less than an hour, is performed as following: After appropriate positioning the area of the scalp is cleaned and shaved, and a small round incision is made. The Ommaya reservoir is inserted and is locked safely between the bone and the scalp (Fig 2.). Then, a catheter is threaded into the ventricle in the brain. Upon confirmation of placement, the scalp incision is closed. Intraoperative CT scans confirm

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positioning and postoperative flow study must confirm unrestricted CSF flow prior to the first use of the reservoir.

Confirmation of Ommaya Catheter

Placement by flow study. Following intrathecal injection of 0.5 mCi of indium 111 DTPA, serial whole-body anterior and posterior images are acquired at 30 minutes, 4 hours, 20 hours and 24 hours.

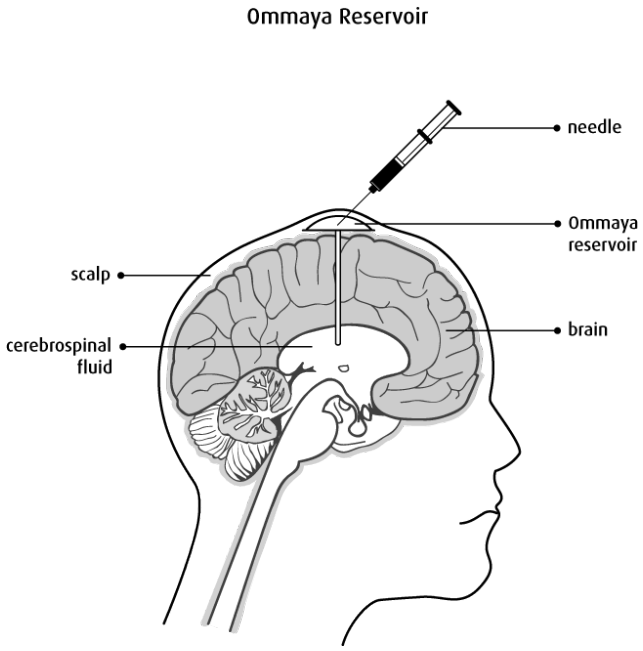

Fig 2: Ommaya reservoir

Intrathecal IL-2 Treatment

Treatment with IT IL-2 consisted of an induction and a maintenance phase for each patient. The planned induction phase was defined as a 4-week treatment period from the first IT IL-2 injection. Recombinant IL-2 at1.2 million international units (mIU) in preservative-free water (volume ~ 0.3ml) was used and injected over less than 1 minute via Ommaya reservoir into the ventricle. Treatments were administered daily for 5 days during the first week, and 2-3 times per week as tolerated for the additional 3 weeks. Patients remained hospitalized throughout the induction phase. If side effects consistent with increased intracranial pressure (ICP) (*e.g.*, severe headache, uncontrolled nausea or vomiting, confusion, or other change in mental status) developed, CSF was removed via the Ommaya reservoir for symptomatic relief. The maximum

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CSF amount removed at any time was 20 cc, and when possible, CSF was removed at least 6 hours after IT IL-2 instillation to avoid decreasing CSF IL-2 levels.[64]

Scheduled IT IL-2 treatments were delayed or omitted if clinically indicated by uncontrolled symptoms or significant decline in performance status, and in such cases subsequent doses were reduced to 1.0 or 0.6 mIU at the treating physician's discretion. Other supportive medications were provided scheduled (Tab. 2). Other as needed medication included tramadol, stool softener, especially if opioid analgesia was required, loperamide, hydromorphone, meperidine, prochlorperazine and lorazepam.

Tab. 2 Scheduled medication during IT IL-2 induction

Scheduled Medications	**Dose**	**Route**	**Dosing interval**
Acetaminophen	650mg	By mouth	Start with IT IL-2, every 4 hrs
Odansetron	8mg	intravenous	Start with IT IL-2, every 4 hrs
Diphenhydramine	12.5mg	intravenous	Start with IT IL-2, every 6 hrs
Metoclopramide	10 mg	intravenous	Start with IT IL-2, every 6 hrs

Patients continued on a maintenance therapy schedule if they derived clinical benefit from treatment and were able to tolerate treatment. Maintenance treatment consisted of a single dose of IT IL-2 initially given weekly, then biweekly, and eventually extended to every 4-8 weeks. Patients were admitted to the inpatient setting for these treatments, and were generally observed for 24 hours for signs/symptoms of elevated ICP, which were treated with CSF removal.

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Assessments

A comprehensive neurological examination was conducted prior to each administration of IT IL-2. MRI of the brain and/or the entire spine was required every 4-8 weeks. MRI brain and spine imaging was repeated approximately 4-8 weeks after the first dose of IT IL-2, and positron emission tomography (PET) and/or computed tomography (CT) scans of the chest, abdomen, and pelvis were used to evaluate extracranial disease. CSF samples underwent cytopathology evaluation for malignant cells, and cell counts, glucose and protein content were measured by the laboratories of the Division of Pathology and Laboratory Medicine at MD Anderson Cancer Center. If suspicious cells were seen on cytopathology review, immunocytochemistry with anti-melanoma monoclonal antibody HMB-45 was used to confirm the presence of melanoma cells in the CSF.

Statistical Methods and Analysis

Data were collected retrospectively for each patient, including treatment characteristics, neurological symptoms, CSF characteristics, radiographic studies, extracranial disease status, and overall survival. The Kaplan-Meier method was used to estimate the distribution of OS duration from the first IT IL-2 treatment. The log-rank test was used to compare distributions. Cox proportional hazards regression was used to assess the association between OS duration and disease and demographic covariates of interest.

For data analysis, the following patient and tumor characteristics were recorded:

Demographics

- DOB
- Gender
- Last Follow up

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Tumor Melanoma information

- Melanoma mutation status, including type of mutation
- Cause of death related to LMD
- Prior or concurrent treatments, disease status
- Type of previous systemic therapies (chemotherapy, immunotherapy, targeted therapy)
- Previous radiation to brain and/or spine
- Concomitant therapies at start of IT IL-2 treatment
- Sites of disease at start of IT IL-2 treatment
- Previous brain metastasis present
- Systemic disease controlled at start of IT IL-2 treatment
- Parenchymal disease controlled at start of IT IL-2 treatment.
- Performance status at start of IT IL-2 treatment
- LDH level at start of IT IL-2 treatment
- Neurological deficits present at start of IT IL-2 treatment
- Patient receiving steroids at start of IT IL-2 treatment
- CSF positive before induction period
- CSF clearing during induction in a previously positive CSF
- CSF turning positive during induction in a previous CSF negative

Day of first IT IL-2 treatment

- Opening pressure
- CSF WBC with differential including histiocytes, lymphocytes, eosinophiles, basophiles)
- CSF RBC
- CSF protein and glucose levels
- CSF sodium level

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Other

- Lowest CSF sodium levels during induction
- Total number of IL-2 doses during induction
- Dose reduction during IT IL-2 induction period

For survival analysis

- Date of initial LMD diagnosis
- Diagnosis based on CSF, MRI brain and/or spine, operating report
- Date of last follow up
- Date of death, if applicable

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CHAPTER III

RESULTS

Patient Characteristics

The outcomes of 43 MM patients with LMD initiated on IT IL-2 from August 2006 to July 2014 were reviewed (Table 1). Twenty-eight (65%) of the patients had both positive CSF cytology and radiographic evidence of LMD, three (7%) had positive cytology only and 11 (26%) had radiographic findings only. One patient was diagnosed with LMD based on histopathological analysis of surgical specimen showing metastatic melanoma in the brain parenchyma as well as the dura without positive radiographic findings or CSF cytology.

The median age at the start of IT IL-2 treatment was 46.7 years (range, 18.8-71.0), and the median serum LDH level was 500 (262-1826) IU/L (Table 3). A total of 33 of the 43 patients (77%) had an ECOG performance status of 0-1. Neurological deficits attributable to LMD were present in 21 (49%) patients. Four patients (9%) had LMD but no history of systemic melanoma. Among the remaining patients, eight (19%) had no evidence of active systemic disease at the time of IT IL-2 induction; 20 (47%) had stable systemic disease; and 11 (26%) had progressive systemic disease. Previously treated or concomitant parenchymal brain metastases were present in 34 (79%) patients, including 17 with progressing parenchymal metastases.

The majority (74%) of patients had received prior systemic therapy, which included BRAF and MEK inhibitors, temozolomide, and other chemotherapy agents. Twenty patients (47%) had received prior systemic immunotherapy, including ipilimumab, anti- PD-1 antibodies, adoptive cell therapy with tumor infiltrating lymphocytes (ACT TIL), and biochemotherapy.

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Table 3: Demographics of LMD patients

Variable	Category	N (%)
Sex	Male	32 (74%)
	Female	11 (26%)
Mutation Status	BRAF	21 (58%)
	NRAS	9 (25%)
	None	4 (25%)
	Other	2 (6%)
	Unknown	7
Diagnosis Basis	CSF Positive Only	3 (7%)
	Radiology Positive Only	11 (26%)
	Surgery Only	1 (2%)
	CSF And Radiology Positive	28 (65%)
Prior Systemic Therapy	No	11 (26%)
	Yes	32 (74%)
Previous Temodar Therapy	No	29 (67%)
	Yes	14 (33%)
Prior Immunotherapy	No	23 (53%)

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	Yes	20 (47%)
Prior BRAF, BRAF/MEK Inhibitors	No	33 (77%)
	Yes	10 (23%)
Prior Radiation Therapy	No	16 (37%)
	Yes	27 (63%)
Steroids	No	26 (60%)
	Yes	17 (40%)
Previous Parenchymal Brain Mets	No	9 (21%)
	Yes	34 (79%)
LDH > ULN	No	15 (35%)
	Yes	28 (65%)
Neuro Symptoms	No	22 (51%)
	Yes	21 (49%)
Extracranial Disease	None	8 (19%)
	LMD Only	4 (9%)
	Systemic Controlled	20 (47%)
	Systemic Uncontrolled	11 (26%)

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Concomitant Medications	None	27 (63%)
	Targeted	7 (16%)
	Other	9 (21%)

IT IL-2 Treatment and Concomitant Therapies

Patients received a median of nine IT doses of IL-2 during the induction period (range, 3-14). All patients developed toxicities from IT IL-2, including fever, chills and/or symptoms of elevated ICP, which included nausea and headache. Some patients experienced vomiting and temporary changes in mental status as well as deterioration of their performance status. All patients required additional CSF drainage for symptom control and ICP relief during the induction period and received supportive medications as needed. In eight patients, CSF drainage had to be performed thrice on the same day as IT IL-2 administration. Almost half the patients (44%) required IT IL-2 dose reduction to either 1.0 mIU or 0.6 mIU during the induction phase. Five patients had shunts in place due to increased ICP or hydrocephalus, and four patients were converted to a shunt either during induction (n=3) or while on maintenance therapy. Despite this intensive therapy, no patients died of toxicity attributed to IT IL-2.

Seventeen patients (40%) received concomitant corticosteroids with IT IL-2, with a median daily dexamethasone dose of 8 mg (range 1-24 mg). Twenty-seven patients did not receive any concomitant anti-cancer therapy during IT IL-2 induction. Six patients received radiation therapy during the induction period, including stereotactic radiosurgery (SRS, n=1), focal sinonasal radiation (n=1), and whole brain radiotherapy (WBXRT, n=4). Other patients continued systemic therapy that had been initiated prior to the first dose of IT IL-2, including ipilimumab (n=2); BRAF inhibitor (BRAFi) alone or in combination with MEK inhibitor (MEKi) or

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temozolomide (n=7); temozolomide in combination with WBXRT (n=3); and biochemotherapy (n=1).

Overall survival

The median overall survival (OS) for all patients (n=43) treated with IT IL-2 from the start of treatment was 7.8 months (range, 4.7-16.3 months), with 1-, 2-, and 5-year OS rates of 36%, 26%, and 13%, respectively (Figure 3).

Patients with prior but no systemic disease or LMD with no prior or concurrent systemic disease (n=12) had a median OS of 20.2 months from the start of IL-2 treatment (range 1.7-90.8 months) (Figure 3). Patients with concurrent but controlled systemic disease (n=20) had a median OS of 10.6 months (range, 0.5-47.0 months), while patients with progressive systemic disease (n=11) had a median OS of 4.3 months (range, 0.4-55.9 months, Fig. 4). No significant difference in OS (p=0.21) was observed in patients with elevated LDH (Fig. 5), requiring steroids (Fig 6) or in patients with or without parenchymal brain metastases (controlled or uncontrolled, Fig 7). The presence of neurological symptoms (HR 2.1, p=0.03), positive CSF cytology prior to the start of treatment (HR 4.1, p=0.001), and concomitant use of targeted therapy (HR 3.0, p=0.02) was associated with shorter OS on univariate analysis (Table 4, 5).

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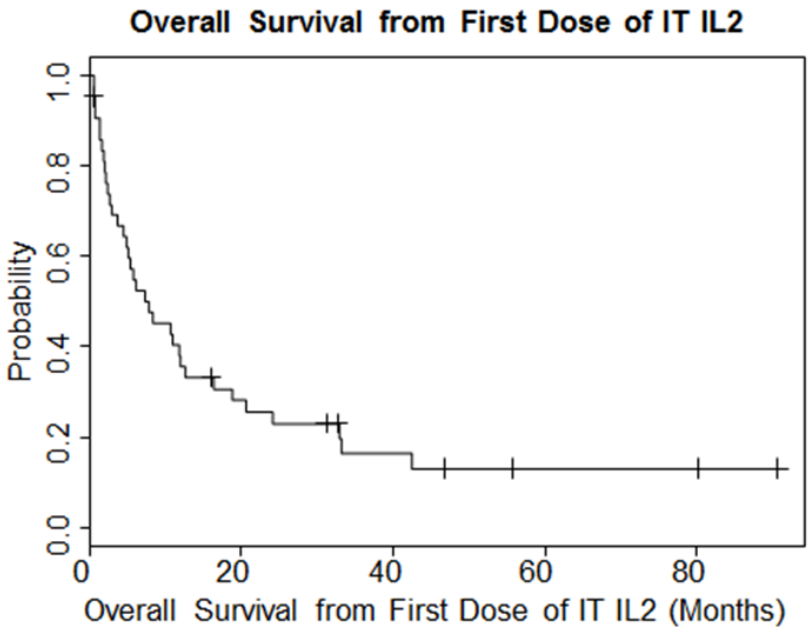

Fig. 3: The overall survival (OS) for all patients (n=43) treated with IT IL-2 from the start of treatment.

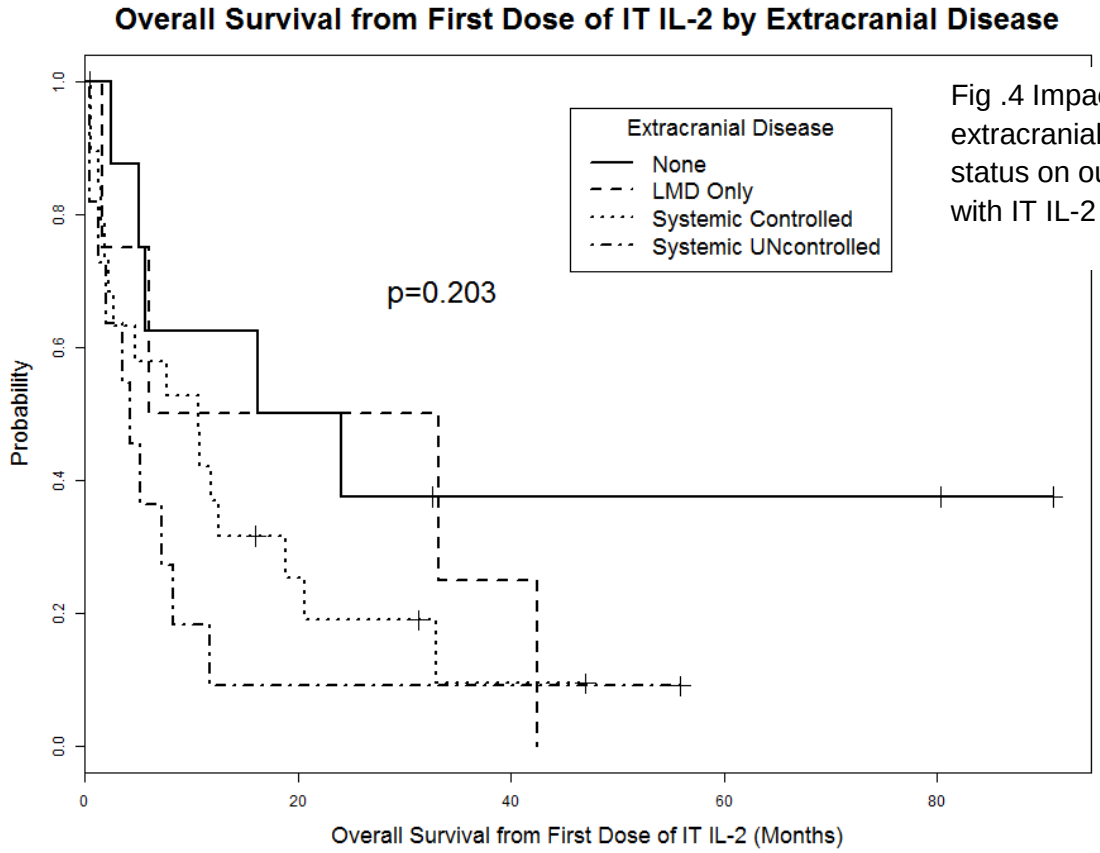

Fig .4 Impact of extracranial disease status on outcomes with IT IL-2

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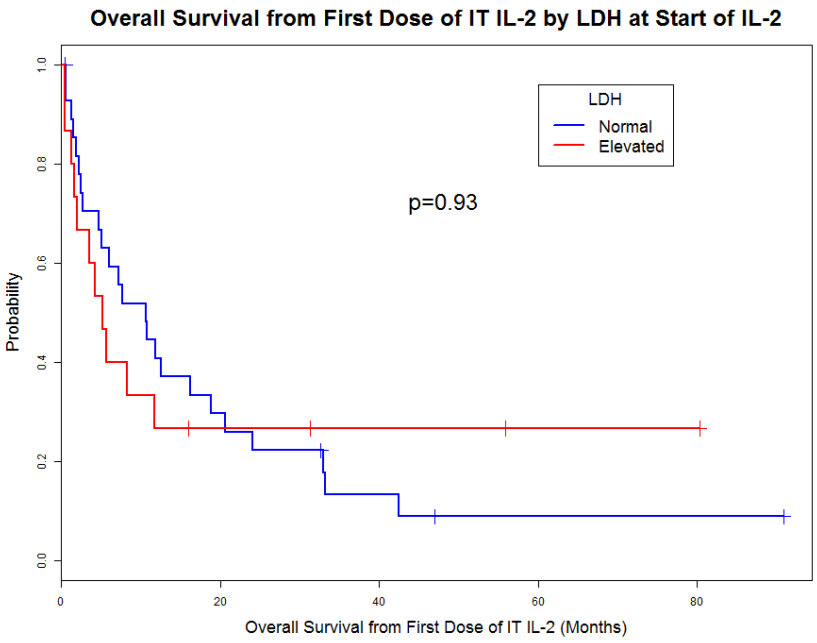

Fig. 5 Impact of serum LDH status on outcomes with IT IL-2

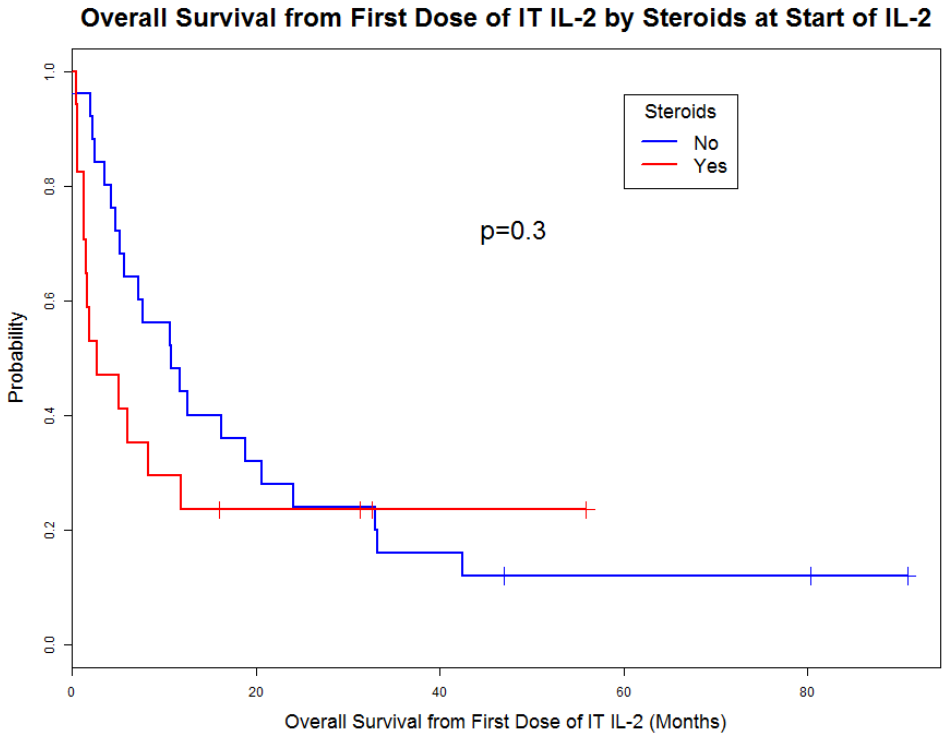

Fig. 6 Impact of concurrent steroids use on outcomes with IT IL-2

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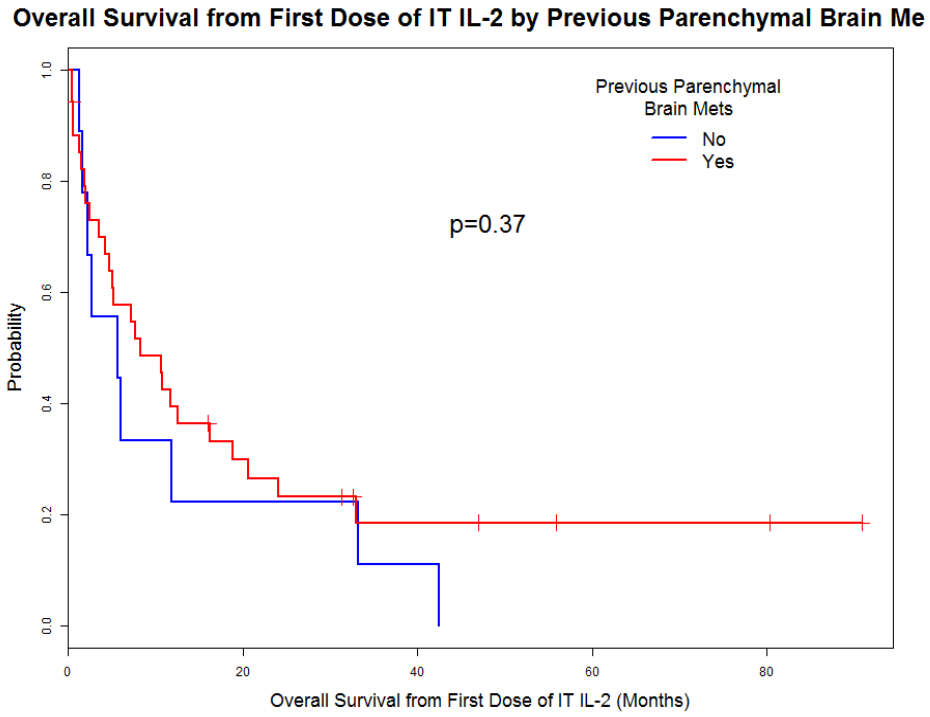

Fig. 7 Impact of parenchymal brain metastasis status on outcomes with IT IL-2

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Table 4: Associations of clinical factors with overall survival with IT IL-2

Variable	Level	Total	Total Deaths	Median OS	Hazard Ratio	95% CI	P-Value
Age	Per Year	43	35	7.8	1.001	0.97, 1.03	0.92
Sex	Male	32	26	5.9	-----	-----	-----
	Female	11	9	9.6	0.93	0.43, 1.99	0.85
LDH	Normal	28	24	10.6	-----	-----	-----
	Elevated	15	11	5.2	1.03	0.51,	0.93

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						2.12	
BRAF Mutation	No	15	11	16.3	-----	-----	-----
	Yes	21	17	10.6	1.42	0.66, 3.04	0.37
	Missing	7	7	*	*	*	*
Neuro Symptoms**	No	22	16	11.7	-----	-----	-----
	Yes	21	19	2.8	2.14	1.09, 4.19	0.03
Dose Reduction	No	24	18	11.8	-----	-----	-----
	Yes	19	17	5.1	1.73	0.88, 3.41	0.11
Total IL-2 doses received during induction	Per Dose	43	35	7.8	0.89	0.77, 1.01	0.08
Extracranial Disease	None/LMD Only	12	9	20.1	-----	-----	-----
	Systemic Controlled	20	16	10.6	1.68	0.73, 3.85	0.22

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	Systemic Uncontrolled	11	10	4.3	2.57	1.03, 6.45	0.04
Concomitant Medications	None	27	19	11.8	-----	-----	-----
	Targeted	7	7	4.7	3.02	1.18, 7.74	0.02
	Other	9	9	8.3	1.88	0.82, 4.34	0.14

Abbreviations used: IL-2, interleukin-2; LDH, lactate dehydrogenase; LMD, leptomeningeal disease; *: NA; **: at initiation of therapy

Review of the death notifications and last visit/contact notes in the electronic medical record system was performed to determine whether LMD contributed directly to patient demise among those who died (n=35, and one lost to follow-up). Death was attributable to LMD in 16 patients, and to overwhelming systemic disease or from complications arising from parenchymal brain metastases in 10 patients. The cause of death was not specified for eight patients, but presumed due to melanoma.

CSF clearance

Thirty patients had positive CSF cytology prior to starting IT IL-2 treatment. Nine of these patients had CSF that became negative for melanoma cells during the induction period, and remained negative subsequently. Conversion to a negative CSF cytology was not associated with significantly improved OS (HR 1.27, p=0.58) (Table 4).

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Table 5: Association of CSF features with overall survival with IT IL-2

Variable	Level	Total	Total Deaths	Median OS	Hazard Ratio	95% CI	P-Value
Cytology at Diagnosis	Negative	13	7	33.2	-----	-----	-----
	Positive	30	28	5.1	4.09	1.72, 9.74	0.001
CSF Clearing	No	20	19	4.5	-----	-----	-----
	Yes	9	8	9.3	0.98	0.42, 2.29	0.96
	Prev. Negative	13	7	33.2	0.25	0.10, 0.61	0.002
	Missing	1	1	*	*	*	*
CSF Turning Positive During Induction	No	7	3		-----	-----	-----
	Yes	6	4	16.2	2.26	0.49, 10.40	0.29
	Missing	30	28	*	*	*	*

Abbreviations used: CSF, cerebrospinal fluid; *: NA

Thirteen patients did not have melanoma cells on CSF examination at initiation of the IT IL-2. Twelve of these patients had MRI findings supporting the diagnosis of LMD, and one patient was found to have LMD during a craniotomy. During the IT IL-2 induction, six (46%) of these patients were detected to have developed positive CSF cytology. All but one of these patients eventually converted to negative CSF cytology status during their maintenance phase. One additional patient with negative CSF cytology at baseline had melanin pigment detected in the CSF but no melanoma cells. Among the other six patients with negative baseline CSF cytology,

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only atypical cells were repeatedly detected in the CSF (examined volume ranging from 2-10 ml), but staining and morphology did not confirm melanoma.

Opening pressure, and CSF analysis

Twelve patients (28%) had elevated ICP at initiation of IT IL-2, with recorded levels of up to 40 cm H_2O (normal ICP < 15 cm H_20). Opening pressures ranged between 0-40 cmH_2O. The median opening pressure was 11.5 cmH_2O. Opening pressure increased in all patients during the induction treatment and on day 21, in patients whose opening pressures were recorded, the values ranged from 5- 60 cmH_2O, with a median of 20 cmH_2O. The median change between day 1 and day 21 opening pressure was 7 cmH_2O. Baseline opening pressures (HR=1.03 95% CI= (0.99, 1.07), P=0.22), opening pressure on day 21 (HR=0.99 95% CI= (0.97, 1.02), P=0.59) and change in opening pressure while on treatment (HR=0.98 95% CI= (0.96, 1.01), P=0.19) were not associated with survival.

While the total white blood cell count (WBC) in the CSF at baseline was not significantly associated with survival (p=0.59), the day 21 value of CSF WBC count was of marginal significance (HR=0.95 per 10 units, p=0.07), and the observed difference between baseline and day 21 levels was significant (HR= 0.97 per 10 units, p=0.03) (Table 6).

Table 6: Association of CSF WBC with Survival

Variable	Level	Total	Total Deaths	Median OS	Hazard Ratio	95% CI	P-Value
WBC: Baseline	Per 10 Units	43	35	7.8	1.01	0.97, 1.05	0.59

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WBC: Day 21 **	Per 10 Units	38	31	8.5 ***	0.95	0.90, 1.004	0.07
WBC: Day 21-Baseline **	Per 10 Units	38	31	8.5 ***	0.97	0.95, 0.997	0.03

Median level of protein in the CSF was 23 (range -1132) and median glucose level was 67 (range 28-99). An elevated protein level in the CSF was considered to be > 55 mg/dL and was not associated with worse outcome (HR=1.75, 95% CI= (0.76, 4.04); P=0.18) in the 7 patients who had elevated baseline CSF protein levels.

Transient Hyponatremia

Transient hyponatremia during the induction phase was observed in most patients, and represented a toxicity that rarely required further intervention. One patient continued to have transient hyponatremia with each maintenance dose, requiring 3-4 days of hospitalization after each maintenance dose until the neurological symptoms associated with his hyponatremia (gait instability, confusion and decreased mentation) resolved. His sodium levels are shown below in Figure 8.

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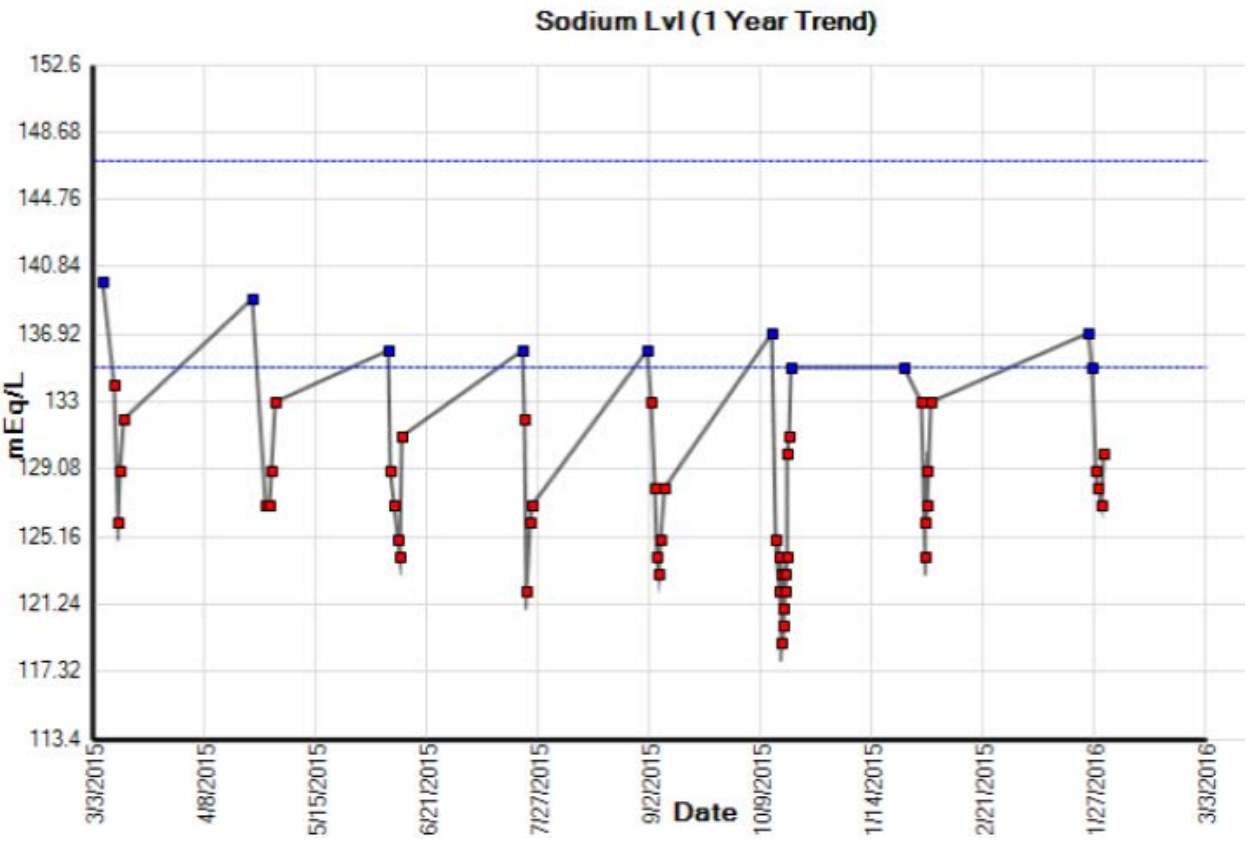

Fig. 8 Transient hyponatremia as one of the adverse events observed with IT IL-2

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CHAPTER IV

DISCUSSION

LMD is associated with an extremely poor prognosis in virtually all tumor types, including melanoma. The results reported here represent the largest known cohort of patients with LMD in which the safety and efficacy of IT immunotherapy have been evaluated to date in any type of cancer. These results provide evidence that IT IL-2 therapy can produce long-term survival in selected metastatic melanoma patients with LMD, and provides proof-of-concept that IT immunotherapy may be an effective strategy for these patients.

This study of IT IL-2 represents the largest cohort of melanoma patients with LMD evaluated for outcomes with a specific therapy reported to date, and with the longest follow-up for overall survival. The demonstration of prolonged survival in a subset of these patients is a unique and important result, and one needs to be aware that this long-term survival observed is similar to the prolonged survival now repeatedly seen with immunotherapies. [65-67] While retrospective studies by our group and others support that the median survival of melanoma patients with LMD is generally < 2 months, the median OS in this cohort was 7.8 months. Particularly impressive results were observed in patients with no prior history of non-LMD disease (median OS 19.6 months), and in patients with prior but no concurrent systemic disease (median OS 20.2 months), who together comprised 28% of the patients in the study. Relatively favorable results were also observed in patients with concurrent but controlled systemic disease (46% of patients, median OS 10.6 months). In contrast, patients with uncontrolled systemic disease had a median OS of only 4.3 months. Importantly, a subset of the patients treated with IT IL-2 achieved durable OS, with 26% and 13% of the patients alive 2 and 5 years after the start of treatment, respectively. We also recently reported the outcomes of 178 patients with metastatic melanoma and LMD, diagnosed at our institution between 2000 and 2015. The overall survival

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for all patients was only 3 months. Such outcomes are essentially unprecedented in melanoma patients with LMD, and add to the expanding number of reports documenting durable OS in metastatic melanoma patients treated with immunotherapy. [68-69]

The presence of neurological symptoms has previously been identified as a negative prognostic factor in a number of outcome analyses of melanoma patients with CNS involvement. [70] Thus, neurological symptoms are likely prognostic and not predictive in patients with LMD receiving IT IL-2. In this study, patients could be enrolled based on radiographic, CSF cytology, or surgical findings of LMD. The observed improved outcomes in patients with negative CSF cytology raises questions as to the certainty of LMD diagnosis by MRI imaging alone. However, 50% of the patients with negative cytology at baseline were eventually determined to have a positive cytology during the induction period, and several others had melanin and/or atypical cells identified. The observed frequent conversion of CSF from negative to positive with treatment could be due to the serial sampling performed in this trial, as it is known that repeated cytological analysis increases the sensitivity of detection of malignant cells in CSF.[4] Alternatively, it is possible that the IT IL-2 treatment could cause cells to detach from the leptomeninges, allowing improved detection. Notably, in patients with positive CSF cytology at baseline, there was no significant correlation between CSF clearance and OS. Thus, along with the challenging nature of assessing radiographic responses in LMD [4], we believe that these findings support the use of OS as a primary endpoint in clinical studies in melanoma patients with LMD, and support the significance of the findings observed in this cohort of patients treated with IT IL-2. The findings in this trial also support the need for the development of additional diagnostic methods on CSF to improve the ability to diagnose and to evaluate treatment responses in LMD patients in the future. [11]

Furthermore, our own review of all melanoma LMD patients seen at MD Anderson showed a similar association with above discussed factors and poor overall survival. Importantly, in both

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studies, neither a history nor presence of concurrent parenchymal brain metastasis affected overall survival.

Unfortunately, the performance status of patients receiving intrathecal IL-2 was not consistently evaluated, and therefore univariate analysis not possible. It should be mentioned thought that patients in general would have not been treated if they had an ECOG >2, because the anticipated significant toxicity from treatment required good baseline performance status. Neurological symptoms could be potentially a surrogate marker for LMD disease burden, and depending on type and location of CNS involvement, leading to further morbidity and worsening in the performance status. Multiple studies, including our own, have shown that there is better outcome associated with better performance status, which ultimately might lead to more aggressive multidisciplinary treatment. Fifteen of our patients had elevated LDH at treatment initiation, but unlike other reports, this had no impact on outcome.

In contrast to other studies, elevated intracranial pressure and elevated CSF protein, which is often observed together in patients with LMD, did have no significant impact on survival. This might be due to overall still small number, with only 7 patients having elevated CSF protein levels.

While changes in the CSF have been observed in the initial intrathecal IL-2 and interferon case reports, virtually nothing is known about the cell composition of the CSF at baseline and the changes of this “microenvironment” as response to intrathecal immunotherapy. This is the first study to report a change within the CSF leukocyte count to be associated with marginally improved survival. While this is not completely surprising based on the mechanism of action of IL-2, many questions remain open and will require further assessment. It remains unclear if the total cell count or the type of cells has a higher impact on survival. Initial flow cytometry of CSF leukocytes of a patient deriving benefit (responder) and a non-responder patient showed that

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the cell population in the responder mainly consisted of T-cell (85%), the majority of which were CD8+ (68%) cells. In contrast, the nonresponder had a higher proportion of NKD cells (52%), while the T cells were predominantly CD4+ (73%) (unpublished data, TIL lab UT MDA). These findings are not surprising in light of recent publications uncovering differences in the tumor microenvironment, CD8+ infiltration and upregulation of genes leading to an inflamed microenvironment and responders to immunotherapy with either anti-CTLA4 or anti-PD1. While paucity of CSF cells and absence of true tumor tissue will make monitoring the CSF more challenging, further analysis is warranted. These "liquid biopsies" could identify differences in the immune cell repertoire of patients with LMD and their response to intrathecal immunotherapy, and in exchange leading to strategies overcoming treatment resistance.

Lack of treatment response could be due to just simply paucity of cells, and we attempted to overcome this by direct injection of tumor infiltrating lymphocytes into the CSF. [71] This is, to our knowledge, the first case report that described this approach, while another case report described the safe use of IT cytotoxic T cells. [71-72] Both of these reports are based on clinical experience with adoptive cell therapy using autologous TIL in conjunction with high-dose IL-2. [73-74] Our patient, who initial diagnosis of cutaneous melanoma was 3 years prior, was diagnosed with stage IV disease in January 2010. After multiple lines of treatments, he developed lower-extremity weakness and numbness of the lower extremities after requiring surgery for splenectomy and bowel resection. At this point, CNS imaging revealed LMD in the distal cord and nerve roots at the lumbar level. As the standard of care, he was started on IT IL-2, but unfortunately experienced progression of LMD both clinically and radiologically. Despite palliative radiation to the spine, he still experienced progression of LMD, but his systemic disease remained grossly stable.

He then received IT tumor-infiltrating lymphocytes (TIL) under a compassionate-use investigational new drug application (CIND 10-0060) at the beginning of January 2011. He

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received a total of three IT TIL injections, with doses of 0.3 x 10^9, 1 x 10^9 and 3 x 10^9 IT TIL. Each IT TIL dose was supported by IT IL-2, which was given twice weekly.

While the patient eventually died 5 months later from systemic and parenchymal brain disease progression, it should be noted that his LMD remained stable in imaging and on clinical exam.

CSF was banked during this treatment and later retrospectively analyzed. One of the concerns related to administration of IT TIL was the development of a cytokine storm; while elevated levels of cytokines were found in the CSF, clinically the patient tolerated all three dose levels of IT TIL very well. [71] Based on this experience, I hypothesize that the intrathecal administration of TIL can be safe and will improve the efficacy of IT IL-2, particularly as this approach could also overcome resistance to IT IL-2 caused by decreased trafficking of anti-tumor T cells into the CSF. A clinical trial based on this concept is currently ongoing (NCI NCT00338377), and was developed based on the results of this thesis work.

Despite these promising results, we acknowledge that the retrospective nature of this non-randomized study imposes some inherent limitations on the interpretation of the results. All patients were treated under an institutional CIND, and additional treatments were administered at the discretion of the treating physician. None of these concomitant treatment approaches has been shown to prolong OS in patients with LMD, but a synergism cannot be excluded. While systemically administered BRAF inhibitors (BRAFi) have been shown to achieve clinical responses in a significant subset of melanoma patients with parenchymal brain metastases, concurrent targeted therapy treatment was associated with shorter OS in our cohort of patients with LMD.[30] This finding was in contrast to a recently published retrospective review of 39 melanoma patients with LMD. While the full cohort had a median OS of only 1.7 months, patients who received treatment with either systemic therapy with targeted and/or immunotherapy had a median OS of 5.4 months. [12] While that study did not specify how many

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patients had received therapy prior to the diagnosis of LMD, all 7 patients in our cohort that received concomitant BRAFi and IT IL-2 therapy developed (progressed with) LMD while taking BRAFi prior to the start of IT IL-2, supporting resistance to the targeted therapy at baseline. Two of these patients died within 3 weeks of IT IL-2 initiation from LMD progression. One of these patients had a significantly elevated LDH level (1826 IU/L, range 265-1826) at time of IT IL-2 treatment initiation; both patients had markedly elevated opening pressures prior to the first dose of IT IL-2 (27 and 33 cm H_20 respectively); and both patients had significant LMD burden. Thus, we postulate that the observed association of concurrent targeted therapy during the induction phase of treatment with shorter OS is most likely due to confounding factors, as opposed to a detrimental effect on the IT IL-2 treatment. However, evaluation of additional patients, including patients without prior progression on targeted therapy, will be needed to confirm this.

Another limitation of this study of IT IL-2 is that the retrospective nature of this cohort of patients treated on the basis of individual INDs also precludes detailed toxicity reporting and analysis. All patients developed toxicity during the induction phase and were hospitalized throughout that time for treatment and toxicity management. While unable to establish retrospective grading for the observed symptoms, they were likely similar in incidence and severity as previously presented by Papadopoulos et al in the initial description of the effects of IT IL-2. [39] That study reported chills (100% all grades, no grade 3), fever (98% all grades, 11% grade 3), nausea (95% all grades, 30% grade 3) and headache (99% all grades, 57% grade 3) as the most common clinical toxicities of IT IL-2. The frequent and significant toxicity incurred by this regimen will likely limit its broad dissemination to cancer centers, similar to the experience with systemic IL-2 therapy. Despite these challenges, our highly trained and motivated clinical team did not observe any treatment-related deaths with IT IL-2. In addition, we generally observed a significant decrease in symptom burden in patients receiving intermittent maintenance therapy,

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with only overnight observation required. While the observation of prolonged survival in a subset of patients is very encouraging, the use of this therapeutic approach will be enhanced by the development of more robust predictors of clinical benefit. Thus, further interrogation of clinical, molecular and immunological correlates of response and long-term OS is warranted. In addition, our long-term outcomes also support the rationale to evaluate recently approved and experimental immunotherapies in patients with LMD, including those with IT administration.

In conclusion, this study reports the outcomes of the largest cohort of metastatic melanoma patients with LMD receiving a specific therapeutic intervention to date. Our results demonstrate that despite their historically dismal prognosis, a subset of metastatic melanoma patients with LMD treated with IT IL-2 achieve long-term survival. Additional studies are needed to identify biomarkers that predict the clinical benefit of IT IL-2 so that patients who have the greatest chance of responding to this treatment can be identified and selected for this high-risk therapy in the future. In addition to supporting the potential clinical benefit of this treatment, these results strongly support the rationale for additional clinical trials in this patient population. Despite the tremendous advances that have been made in the field of melanoma in recent years, effective treatments and clinical trials for patients with LMD remain critical unmet needs. Our results show that long-term survival can be achieved with IT immunotherapy, but additional clinical trials and translational research efforts are needed to further improve the outcomes of these patients.

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Chapter V

LIMITATIONS

As outlined, the retrospective nature of this work prevents complete and graded toxicity reporting. While we used a descriptive approach, this does not enable us to further analyze toxicity data in association with survival. We therefore were obliged to use dose reduction and total number of IT IL-2 doses received as a surrogate for significant toxicity.

Furthermore, only a few patients had CSF banked in our institutional melanoma tissue bank, thereby preventing retrospective analysis of CSF samples. This would have allowed us to better describe the changes in the immune cell population observed while patients were receiving IT IL-2, as well as potentially uncover the mechanism of resistance to this therapy.

Finally, the diagnosis and response evaluation of LMD can be very challenging. While the evaluation of CSF by cytology has remained the gold standard for LMD diagnosis, this approach lacks sensitivity, and repeated CSF evaluation is often required. [3-4, 75-76](3, 4, 75, 76)(Le Rhun, Taillibert, et al., 2013; Le Rhun, Tu, et al., 2013; Le Rhun et al., 2014; Tu et al., 2015)[3,4,75,763,4,74,753,4,74,753,4,74,753,4,74,75] As a consequence, often patients are diagnosed based on CNS imaging by MRI alone, and it is possible that the initial "clearing" of the CSF was due to lack of sensitivity. Thus, the development and validation of methods that can accurately diagnose and assess treatment responses and clinical benefit in patients with LMD will facilitate future clinical trials in these patients. Therefore, our upcoming clinical trial (described below in "Future Direction") will prospectively use the RANO- LM criteria, which incorporate neurological examination, CSF cytology, and radiographic evaluation, although it has yet to be validated in a prospective clinical trial. Further, there is evidence in melanoma and other disease types that serial evaluation of mutations detected in tumor-derived cell-free DNA in the blood may serve as an alternative "liquid biopsy" to evaluate treatment responses, and to detect resistance (i.e.

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circulating $BRAF^{V600E}$ mutations with BRAF inhibitor therapy). [11, 77-78] (11, 77, 78) (Li et al., 2016; Pan, Gu, Nagpal, Gephart, & Quake, 2015; Wang et al., 2015) [11,77,78] [11,76,77] [11,76,77] Recent data also support that mutations can be detected in cfDNA isolated from the CSF, but to date this approach has not been evaluated prospectively in patients with LMD receiving therapy. Therefore, any trial enrolling LMD patients should strive to incorporate these novel response and assessment tools.

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Chapter VI

FUTURE DIRECTIONS

While these results provide the proof-of-concept that IT immunotherapy with IL-2 is feasible and may achieve long-term survival in patients with LMD, there is a strong rationale to explore other treatments that may have greater efficacy and less toxicity. The results are very encouraging, but much remains unknown, and as part of this project, additional new collaborations started. For example, we have increased our CSF banking efforts significantly, and most melanoma patients who are receiving work-up for LMD will have part of their initially obtained CSF stored in our MelCore, and therefore readily available for future testing. Furthermore, I have built the largest melanoma LMD database in the world, which will be invaluable to learn even more prognostic and predictive factors in the treatment of patients with LMD.

Given that the CSF represents a microenvironment, with details about immune cell populations and their changes in regards to systemic and intrathecal therapy still largely unknown, efforts are undergoing to evaluate baseline CSF samples by CyTOF. Finally, I recognize the challenge of diagnosing LMD. While the evaluation of CSF by cytology has remained the gold standard for LMD diagnosis, this approach lacks sensitivity and repeated CSF evaluation is often required. In addition, there is a critical need to improve our understanding of the molecular features of LMD from melanoma. There is growing evidence in melanoma and other cancers that serial evaluation of mutations detected in tumor-derived cell-free DNA may serve as an alternative "liquid biopsy" to evaluate treatment responses (i.e. circulating $BRAF^{V600E}$ mutations with BRAF inhibitor therapy) and molecular features.[11] While most studies to date have evaluated cfDNA in blood, recent data supports the feasibility of this approach for CSF as well, including in samples in which malignant cells are not detected by traditional cytology (Fig.5). Thus, in this clinical trial, and in our parallel treatment of patients with IT IL2 and IT TIL, there is an opportunity to

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evaluate if mutational analysis of cfDNA can be a surrogate for clinical activity in patients with LMD. Comparison of the mutations detected in cfDNA from the CSF to mutations detected in other disease sites, including the blood, in individual patients may also provide new insights into the molecular basis and pathogenesis of LMD.

Nivolumab (Nivo) is a fully humanized blocking antibody against the PD-1 receptor, which is a critical checkpoint regulator on T cells. Previous clinical trials in patients without CNS metastases have demonstrated that systemic Nivo achieves clinical responses in ~40% of metastatic melanoma patients, and that <10% of patients have to discontinue treatment for toxicity. As the systemic activity and toxicity of Nivo is superior to HD IL2, *we hypothesize that IT Nivo treatment will be safe, immunogenic, and clinically beneficial in MM patients with LMD*. We therefore developed a clinical protocol which has now received full IRB approval.

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VITA

Isabella Claudia Glitza Oliva was born on June 3rd 1977 in Schwetzingen, Germany, the daughter of Siegfried and Barbara Glitza. After completing high school in Hockenheim/Germany in 1996, she worked as an au pair in Cleveland, Ohio, with the main goal of mastering the English language. In 1997, she enrolled as a medical student at the Ruprecht-Karls University in Heidelberg, Germany. She graduated in 2004 with her Doctorate of Medicine, and received her Ph.D. with Magna Cum Laude the following year. From 2005 to 2007 she worked as a medical advisor for Janssen-Cilag, a Johnson & Johnson company in Neuss, Germany.

In the summer of 2007 she started her Internal Medicine residency at Albert Einstein Medical Center, where she stayed for an additional year as a hospitalist after completion of her residency in 2010. She then completed her fellowship in Hematology/Oncology at The University of Texas MD Anderson Cancer Center in June 2014. Since that time, she has been a faculty member at MD Anderson in the Department of Melanoma Medical Oncology, where she is currently an Assistant Professor.

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APPENDIX Tables:

Table 7: Patient demographics, basis for LMD diagnosis, previous therapies received, LDH levels, steroids use and presence of neurological deficits at time of LMD diagnosis

Patient #	Gender 1=male, 2=female	Mutation 1=BRAF 2=NRAS 3=neg 4=unknown or not done 5=other	CSF positive 0=no, 1=yes	CNS MRI imaging positive for LMD 0=no, 1=yes	Op report 1=pos 2=neg 6=not done or no info	Previous Systemic Therapy 0=no, 1=yes	Previous Radiation Spine/ Brain therapy 0=no, 1=yes	Previous Systemic Therapy with Temodar 0=no, 1=yes	Previous Systemic Therapy with BRAF and/or MEK inhibitor 0=no, 1=yes	Previous Systemic Therapy with Immunotherapy (Ipilimumab, IL-2, anti- PD1, Immunotherapy as part of Biochemo) 0 = no, 1 = yes	LDH above institutional limit 0=no, 1=yes	Neurological Deficits from LMD 0=no, 1=yes	Steroids at time of first IT IL-2, and dose 0=no, 1=yes
1	1	1	1	1	6	1	1	0	1	0	0	1	0
2	1	1	1	1	6	1	1	1	1	1	1	1	1
3	1	3	0	1	2	1	1	0	0	0	0	0	0
4	1	2	1	1	6	1	1	0	0	0	0	0	0
5	1	2	1	1	6	0	0	0	0	0	1	0	1
6	1	1	1	1	6	1	0	0	1	1	0	0	0
7	1	5	0	1	2	1	1	0	0	1	0	0	1
8	2	1	1	1	6	1	1	1	1	0	0	0	0
9	2	1	1	1	6	1	0	0	1	1	0	0	1
10	2	2	0	1	1	0	1	0	0	0	0	0	0
11	1	1	1	1	6	1	1	0	0	0	1	0	0
12	1	1	1	0	6	1	1	1	0	1	1	1	0
13	1	1	1	1	6	1	1	1	0	0	1	1	0
14	1	4	0	1	6	1	1	1	0	0	1	0	0
15	1	1	0	1	2	1	1	1	1	0	0	0	0
16	2	4	1	1	6	0	1	0	0	0	0	1	0
17	1	4	1	1	6	1	0	1	0	0	0	1	1

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18	1	2	1	1	6	1	1	0	0	1	0	1	0
19	1	4	1	1	1	1	1	1	0	0	0	1	0
20	1	1	1	1	6	1	1	0	1	1	1	1	0
21	1	2	1	1	6	1	1	0	0	1	1	1	1
22	1	1	1	1	6	1	1	0	0	1	0	1	0
23	1	3	1	0	6	0	0	0	0	0	0	1	0
24	1	1	1	1	6	1	0	1	0	1	0	0	1
25	1	2	0	1	6	0	1	0	0	0	0	0	1
26	1	4	1	1	6	1	1	1	0	0	1	1	1
27	1	3	1	1	6	1	1	1	0	1	1	1	1
28	2	1	1	1	6	0	0	0	0	0	0	0	0
29	1	1	1	1	6	0	0	0	0	0	0	0	1
30	2	2	1	0	6	1	0	0	0	1	0	1	0
31	2	2	0	1	6	1	1	1	0	1	1	0	1
32	1	3	0	1	1	0	0	0	0	0	1	0	0
33	2	1	1	1	6	1	0	0	0	1	0	1	1
34	1	4	1	1	6	0	0	0	0	0	0	1	1
35	1	1	0	1	6	1	1	0	0	1	0	0	0
36	1	5	1	1	6	1	1	0	0	1	0	1	1
37	1	2	0	1	6	1	1	1	0	1	1	1	0
38	2	1	0	1	6	0	0	0	0	0	1	1	1
39	2	1	1	1	6	1	0	0	1	0	0	0	0
40	1	1	0	0	1	0	0	0	0	0	0	0	0
41	1	1	1	1	6	1	1	0	1	1	0	0	0
42	2	1	1	1	6	1	0	0	1	1	0	1	1
43	1	4	1	1	6	1	1	1	0	1	1	0	0

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Table 8: CSF opening pressures, protein and glucose in the individual patient

	Opening Pressures		CSF Cell composition, protein and Glucose									
Patient #	Day 1	Day 21	WBC	RBC	Histiocytes	Lymphocytes	Eosinophils	Basophils	Other cells	Protein	Protein Day 1 abnormal 1=yes, 0=no (cutoff 55)	Glucose
1	26	23	1	675	9	71	NR	NR	1	41	0	71
2	27	27	8	3	27	67	NR	NR	3	68	1	43
3	15	37	13	600	13	56	21	NR	NR	48	0	65
4	11	55	4	48	6	73	NR	NR	3	115	1	76
5	14	16	0	40	25	75	NR	NR	NR	12	0	71
6	11.5	32.5	3	18	6	20	2	NR	2	15	0	74
7	5	5	2	40	10	81	4	NR	NR	12	0	64
8	8	8	0	193	45	55	NR	NR	NR	12	0	49
9	3	28	0	0	13	36	NR	NR	48	20	0	67
10	7	52	0	10	45	45	NR	NR	NR	12	0	57
11	13	5	13	12	33	50	NR	NR	8	42	0	67
12	11	17	0	18	6	25	NR	NR	64	15	0	66
13	3	7.5	2	330	1	14	NR	NR	80	18	0	45
14	11	23	1	573	56	33	NR	NR	NR	17	0	75
15	8	13	2	265	24	73	NR	NR	1	27	0	70
16	13	18	0	0	22	71	3	NR	3	50	0	47
17	14	20	1	184	32	61	NR	NR	4	18	0	74
18	12	35	3	26	10	26	NR	NR	2	33	0	71
19	14	27	15	67	50	39	NR	NR	5 + 6% TC	120	1	54
20	4	16	0	12	5	73	NR	NR	9 + 10% TC	23	0	73
21	13	6	5	975	16	12	0	0	1 + 4% TC	56	1	57
22	19	45	12	180	1	1	NR	NR	1	73	1	77

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23	30	45	14	31	19	79	NR	NR	1	22	0	55
24	17	20	1	27	48	37	NR	NR	11	27	0	73
25	5	8	0	4	57	43	NR	NR	NR	12	0	55
26	5	7	0	0	22	40	1	NR	37	462	1	54
27	8	12	5	4	12	74	NR	NR	8	33	0	58
28	6	20	1	80	10	17	NR	NR	4	20	0	71
29	15	16	0	39	75	25	NR	NR	NR	16	0	61
30	16	25	5	19	10	86	NR	NR	1 + 2% TC	12	0	64
31	6	5	0	0	33	66	NR	NR	NR	12	0	79
32	17	45	1	44	9	87	NR	NR	2	24	0	77
33	35	37	12	1550	2	7	1	NR	90	55	0	50
34	1	17	506	69000	8	43	NR	NR	5	1142	1	28
35	9	16	2	18	14	85	1	NR	NR	38	0	73
36	15	15	0	1	29	68	NR	NR	1	55	0	99
37	11	45	0	65	47	44	NR	NR	6	18	0	70
38	0	16	0	7	43	57	NR	NR	NR	3	0	87
39	7	30	93	93	14	39	NR	NR	3	12	0	59
40	4	20	6	1980	17	30	NR	NR	1	54	0	94
41	30	40	2	120	6	59	NR	NR	5	19	0	55
42	18	22	2	24	39	31	NR	NR	28	47	0	39
43	22	60	8	50	17	15	NR	NR	5	23	0	68

Table 8: Ongoing clinical trial for brain metastasis

NCT Number	Name of Study	Treatment	Phase	Estimated Accrual	Cancer type	Patients with LMD allowed?
Immunotherapy						
NCT02681549	Pembrolizumab Plus Bevacizumab for Treatment of Brain Metastases in	Pembrolizumab plus Bevacizumab	II	53	NSCLC, melanoma	no

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	Metastatic Melanoma or Non-small Cell Lung Cancer					
NCT02886585	Pembrolizumab In Central Nervous System Metastases	Pembrolizumab	II	102	Solid tumors	yes
NCT02085070	MK-3475 in Melanoma and NSCLC Patients With Brain Metastases	Pembrolizumab	II	64	NSCLC, melanoma	no
NCT02621515	Nivolumab in Symptomatic Brain Metastases (CA209-322)	Nivolumab	II	70		yes
NCT02460068	A Study of Fotemustine (FTM) Vs FTM and Ipilimumab (IPI) or IPI and Nivolumab in Melanoma Brain Metastasis (NIBIT-M2)	Fotemustine; Fotemustine and Ipilimumab; Ipilimumab and Nivolumab	III	168	melanoma	not mentioned
NCT02374242	Anti-PD 1 Brain Collaboration for Patients With Melanoma Brain Metastases (ABC)	Nivolumab vs. Nivolumab with Iipilimumab	II	76	melanoma	concurrently with measurable brain metastases
NCT02320058	A Study to Evaluate Safety and Effectiveness in Patients With Melanoma That Has Spread to the Brain Treated With Nivolumab in Combination With Ipilimumab Followed by Nivolumab by Itself (CheckMate204)	Nivolumab plus Ipilimumab followed by Nivolumab monotherapy	II	110	melanoma	no
Targeted Therapy						
NCT01978236	Dabrafenib/Trametinib, BRAF or BRAF AND MEK Pre-op With BRAF and MEK Post-op, Phase IIB, Melanoma With Brain Mets,Biomarkers and Metabolites	Dabrafenib plus Trametinib	II	30	melanoma	no
NCT02452294	Buparlisib in Melanoma Patients Suffering From Brain Metastases (BUMPER)	Buparlisib	II	22	melanoma	no
NCT02308020	A Phase 2 Study of Abemaciclib in Patients With Brain Metastases Secondary to Hormone Receptor Positive Breast Cancer, Non-small Cell	Abemaciclib	II	247	breast, NSCLC, melanoma	yes

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	Lung Cancer, or Melanoma					
NCT01904123	A Phase I Trial of WP1066 in Patients With Recurrent Malignant Glioma and Brain Metastasis From Melanoma	WP1066	I	33	melanoma, recurrent glioma	not mentioned
NCT02039947	Study to Evaluate Treatment of Dabrafenib Plus Trametinib in Subjects With BRAF Mutation-Positive Melanoma That Has Metastasized to the Brain	Dabrafenib plus Trametinib	II	120	melanoma	no
NCT02537600	Vemurafenib and Cobimetinib Combination in BRAF Mutated Melanoma With Brain Metastasis (CONVERCE)	Vemurafenib plus Cobimetinib	II	137	melanoma	no
Radiation plus systemic therapy						
NCT02716948	Stereotactic Radiosurgery and Nivolumab in Treating Patients With Newly Diagnosed Melanoma Metastases in the Brain or Spine	Nivolumab plus SRS	Pilot	90	melanoma	not mentioned
NCT02097732	Ipilimumab Induction in Patients With Melanoma Brain Metastases Receiving Stereotactic Radiosurgery	Ipililumab plus SRS	II	40	melanoma	only if SRS is considered for LMD
NCT01703507	Phase I Study of Ipilimumab Combined With Whole Brain Radiation Therapy or Radiosurgery for Melanoma	Ipilimumab plus WBRT	II	24	melanoma	not mentioned
NCT02115139	GEM STUDY: Radiation And Yervoy in Patients With Melanoma and Brain Metastases (GRAY-B)	Ipilimumab plus WBRT	II	66	melanoma	not mentioned
NCT02858869	Pembrolizumab and Stereotactic Radiosurgery for Melanoma or Non-Small Cell Lung Cancer Brain Metastases	Pembrolizumab plus SRS	Pilot	43	NSCLC, melanoma	no
NCT01721603	A Phase 2 Prospective Trial of Dabrafenib With Stereotactic Radiosurgery in BRAFV600E Melanoma Brain Metastases	Dabrafenib plus SRS	II	39	melanoma	not mentioned

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