Retrospective Review of Adult Patients with Leptomeningeal Disease Secondary to Melanoma at H. Lee Moffitt Cancer Center & Research Institute: Diagnosis, Treatment, and Outcomes.

Yolanda Piña, M.D., Brittany R. Evernden, P.A., Nam D. Tran, M.D., Inna Smalley, Ph.D., Nikhil I. Khushalani, M.D., Emily P. Sparr, B.S., Vincent W. Law, M.S., John A. Puskas, Ph.D., Keiran Smalley, Ph.D., Peter Forsyth, M.D.

H. LEE MOFFITT CANCER CENTER & RESEARCH INSTITUTE,
AN NCI COMPREHENSIVE CANCER CENTER – Tampa, FL
1-888-MOFFITT (1-888-663-3488) | MOFFITT.org

BACKGROUND

Leptomeningeal disease (**LMD**), the metastases of tumor cells to the cerebrospinal fluid (**CSF**) and leptomeninges, is a devastating disease with a median survival **(MS)** of only 8-10 weeks. Its incidence is unknown, and diagnosis is typically performed at late stages of the disease process. Nearly 5-8% of solid cancers present with LMD, most commonly involved melanoma, breast, and lung cancers.[Raizer 2008, Harstad 2008]. It affects approximately 5% to 25% of melanoma patients,(Fedorenko, Evernden et al. 2016, Smalley, Fedorenko et al. 2016).

The pathophysiology of LMD remains unknown except for a single study (Boire 2017) implicating C3 expression on CSF circulating tumor cells (**CTCs**) that activates the C3a receptor on the choroid plexus to allow serum growth factors to enter the CSF. Patients with LMD have a very poor prognosis, and effective treatments are virtually non-existent. The standard of care is predominantly the use of whole brain radiation therapy (**WBRT**) +/- local spinal radiation therapy (**RT**) and intrathecal (**IT**) chemotherapy using various agents. Treatment response rates for patients with solid tumors are <20% and difficult to evaluate since the standard technology with cytology is based on morphology. Most patients with LMD from melanoma, lung and other solid tumors decline very rapidly regardless of treatment.(Fedorenko, Evernden et al. 2016)

In an attempt to better understand this patient population, we performed a retrospective chart review of adult patients with suspected LMD secondary to melanoma who were enrolled in the MCC 19332 or 19648 study from September 2013 to February 2019 at H. Lee Moffitt Cancer Center (**MCC**) & Research Institute. The aim of this study was to evaluate the demographics, validity of different diagnosis methods (i.e., the Veridex CellSearch® System adapted to enumerate CTCs from cerebrospinal fluid analysis [CSF], CSF analysis, treatment, and prognosis, in order to understand and improve these approaches.

METHODS

Patients who were enrolled in the studies underwent Ommaya reservoir placement and standard of care with different treatments as deemed appropriate by the physician(s). Samples of CSF were obtained from lumbar puncture **(LP)**, surgery for Ommaya reservoir placement, and/or Ommaya reservoir, and were sent for analysis.
Peripheral blood (**PB**) and CSF were evaluated for detection and quantification of CSF circulating tumor cells (**CTCs**) with the Veridex CellSearch® System (Le Rhun 2013) **(Fig. 1).**

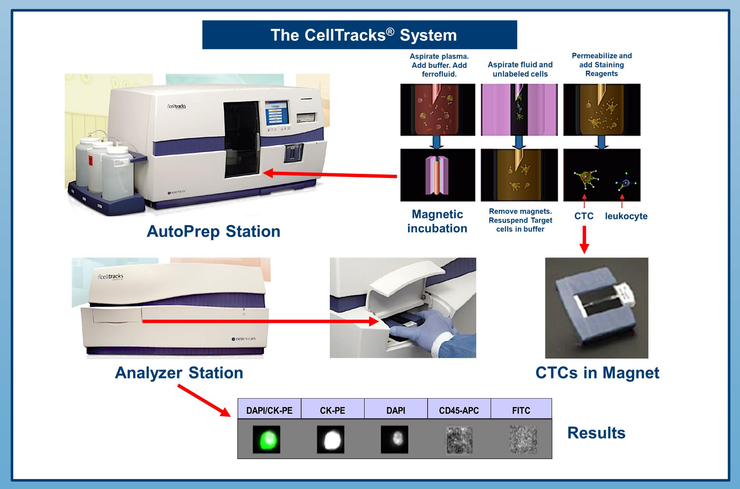

Figure 1. Peripheral blood (PB)/CSF-CTCs were detected by Veridex CellSearch® System and the circulating melanoma cell kit. Melanoma cell enrichment and detection was based on anti-CD146 and anti-high molecular weight melanoma associated antigen (HMW-MAA-PE [MEL-PE]). Anti-CD34-APC was used for ruling out endothelial cells, anti-CD45-APC was used for ruling out leukocytes and DAPI was used for nuclear staining. Both cfDNA and caDNA were extracted and sequenced with TruSight Tumor 26 panel, Illumina Inc and then profiled in Mela Carta MassArray System.

RESULTS

More than 100 patient charts were reviewed of patients with suspected LMD secondary to melanoma who were enrolled in the MCC 19332 or 19648 study from September 2013 to February 2019 at **MCC**. Only patients with melanoma as primary tumor (N=48) were included in the analysis. Forty-eight patients were evaluated with ages ranging between 29 and 80 years of age. Criteria for LMD includes positive CSF cytology (i.e., diagnostic or suspicious for malignancy), MRI brain or spine findings of LMD, or known intraventricular tumor. n=28 (58%) met criteria for LMD, with a median age of 59 (ranging between 31 and 76 years of age), and a male predominance of 21 (ratio of male to female 3:1). n=6 (12.5%) did not meet diagnostic criteria for LMD but had positive CSF CTC's by CellSearch analysis, and n=15 (31%) did not meet criteria for LMD and had negative CSF CTC's.

Out of the 28 patients with LMD, n=26 patients had cutaneous melanoma as primary tumor; BRAF mutant n=17 (V600E n=14; G469E n=1; unknown subtype n=2); BRAF wild type n=8; unknown mutation n=1. n=2 patients had ocular melanoma as primary tumor with GNAQ mutation. n=19 (68% of those with LMD) had known brain metastatic lesions and n=10 (36%) had undergone craniotomy for tumor resection prior to the diagnosis of LMD. Patients with LMD were diagnosed with a median of 4 years (0-38) following their diagnosis of melanoma and 0.5 years (0-4) after their diagnosis of MBM if existent. At the time of the LMD diagnosis, KPS score had median of 70 (30-100), with most prevalent symptoms of headaches, altered mentation, focal weakness, and nausea/vomiting **(Table 1).**

Table 1. Most prominent symptoms in patients diagnosed with LMD at the time of diagnosis.

Symptoms at presentation	n (%)
headaches	14 (50%)
altered mental status	7 (25%)
focal weakness	6 (21%)
nausea and vomiting	6 (21%)
balance problems, unsteady gait	4 (14%)
vision changes	2 (7%)
back pain	2 (7%)
back pain with radicular symptoms	1 (3%)
speech changes/aphasia	2 (7%)
facial pain	1 (3%)
focal seizures	1 (3%)
hallucinations	1 (3%)
irritability	1 (3%)

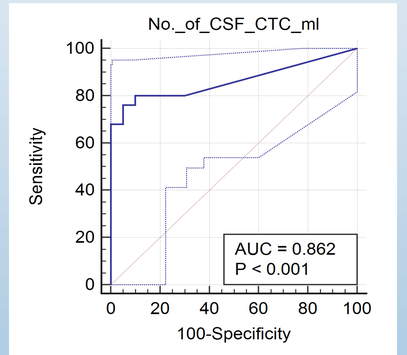

Figure 2. ROC curve with sensitivity and specificity of the Veridex CellSearch® System, detecting and quantifying CSF CTCs. Significance level of p<0.0001.

Area under the ROC curve (AUC)	
Area under the ROC curve (AUC)	0.862
Standard Error [a]	0.0544
95% Confidence interval [b]	0.726 to 0.947
z statistic	6.653
Significance level P (Area=0.5)	<0.0001

[a] DeLong et al., 1988
[b] Binomial exact

From the patients with LMD, n=20 (71%) patients were diagnosed with LMD by MRI brain or spine (n=2 by MRI spine) and n=5 (18%) by CSF cytology. Out of 27 patients who had a lumbar puncture (LP) performed, n=14 (52%) had positive cytology on first LP, n=18 (67%) on first two LPs or on all attempts, and n=9 (33%) had negative CSF cytology despite multiple LPs attempted.

CSF was sent for analysis with the Veridex CellSearch® System to detect and quantify CTCs (**Fig. 1**) from n=25 patients with LMD and n=20 patients who did not meet criteria for LMD diagnosis. From 25 patients with LMD, n=22 (88%) patients had positive CSF CTCs; n=18 (72%) on first sample sent for analysis, n=20 (80%) on first two samples, and n=3 (12%) were negative for CSF CTCs. From the 20 patients who did not meet criteria for LMD diagnosis, n=6 (30%) of patients had positive CSF CTCs and n=14 (70%) had negative CSF CTCs. The Veridex CellSearch® System had a sensitivity of 88% (22[22+3]) and specificity of 70% (14[14+6]), with positive and negative predictive values (PPV/NPV) of 79% (22[22+6]) and 82% (14[14+3]), respectively. The false positive and false negative rates were 30% and 12%, respectively, with a positive and negative likelihood ratios of 2.93 and 0.17, respectively. The false discovery and false omission rates were 21% and 18%, respectively **(Fig. 2).** Survival in months plotted against the percentage of CSF-CTCs as measured by CellSearch showed a significant value of p=0.0377 **(Fig. 3).**

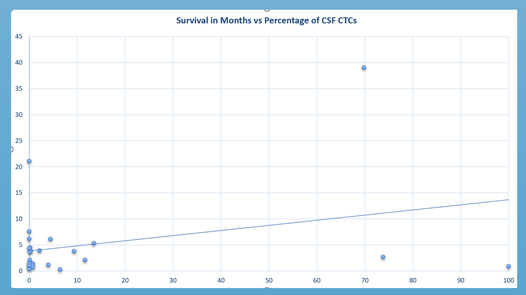

Figure 3. Survival in months vs. percentage of CSF CTCs as measured by CellSearch system. Significance level of p<0.0377.

Paired samples t-test	
Mean difference	-359.3200
Standard deviation of differences	816.5590
Standard error of mean difference	163.3118
95% CI of difference	-696.3790 to -22.2610
Test statistic t	-2.200
Degrees of Freedom (DF)	24
Two-tailed probability	P = 0.0377

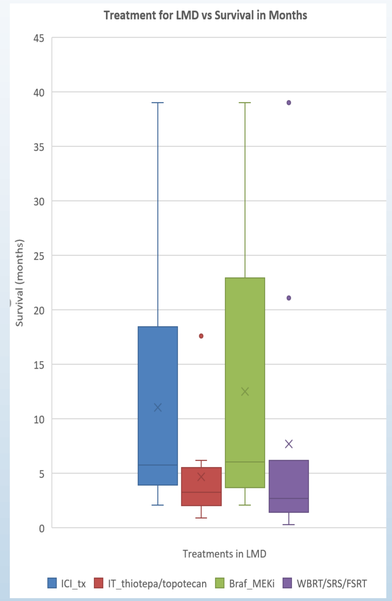

Figure 4. Treatments in patients with LMD and survival in months.

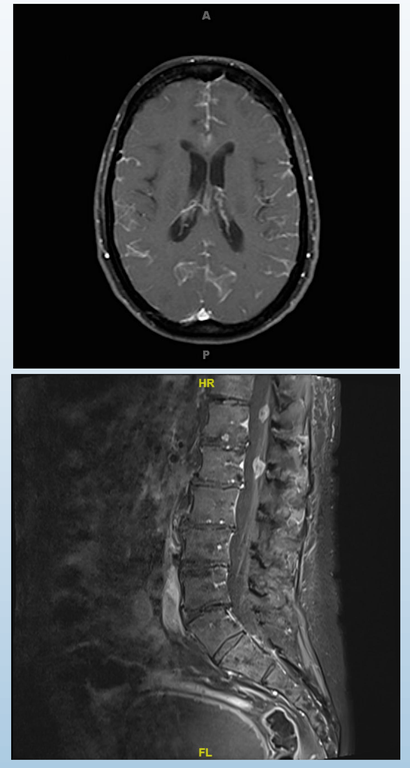

Figure 5. MRI brain (top) and lumbar spine (bottom) with and without contrast of patients with leptomeningeal carcinomatosis secondary to melanoma malignancy.

Initial LP on patients with LMD showed a median OP of 23.5 cmH2O (range of 9 - 65), with CSF analysis on initial LP revealing median (range) WBC 10/cumm (0-313) with a predominant lymphocytic pleocytosis 70.5 % (8-85), RBC 106/cumm (0-34325), total protein 95 mg/dL (12-600), glucose 54 mg/dL (9-101), and atypical cells of 4% (0-83). Inflammatory markers were measured in 6 patients with LMD, which showed an IgG index median of 0.6 (0.5-0.9), IgG synthesis rate in CSF of 35 (0-62.6), oligoclonal bands positive in 4 patients (33%), with median no. of 0.5 (0-5), and myelin basic protein median of 5.2 (4.8-5.5), indicative of a chronic inflammatory process affecting myelin.

In patients with LMD, prior to LMD diagnosis, patients were treated with either immune checkpoint inhibitors (ICI's) n=14 (50%), BRAF +/- MEK inhibitors n=8 (29%), craniotomy for tumor resection n=9 (32%), spinal surgery n=1 (4%), and/or RT n=11 (39%; WBRT n=3 [11%], SRS n=4 [14%], and/or FSRT n=7 [25%]). Within 1 month preceding the diagnosis of MLD, they were treated with craniotomy for tumor resection n=5 (18%), FSRT n=3 (11%), BRAF +/- MEK inhibitors n=2 (7%), or vemurafenib/XL888 n=1 (4%), ICIs n=3 (11%). Once LMD was diagnosed or suspected, they were treated with Ommaya reservoir placement n=19 (69%), VP shunt placement n=3 (11%), WBRT n=9 (32%), SRS n=2 (7%), HFSRT n=1 (4%), RT to spine n=1 (4%), ICIs n=9 (32%; nivo+ipi n3 [11%], Ipi n=3 [11%], Nivo n=4 [14%], pembrolizumab n=2 [7%]), BRAF +/- MEK inhibitors n=7 (25%), IT thiotepa n=10 (36%), or IT topotecan n=1 (4%) **(Fig. 4**).

Most patients with LMD died n=24 (86%), with a median overall survival **(OS)** of 3.15 months after diagnosis (0.30-39; 6.85 months [4.33-21] in those still alive), with two patients who outlived their counterparts by 21.1 and 39.0 months after their diagnosis, one of them is still alive but with a very poor functional status. These two long-term survivors were both treated with WBRT and either pembrolizumab or ipilimumab+nivolumab.

CONCLUSIONS

These results indicate the potential value of the Veridex CellSearch® System to detect and quantify CTCs in CSF, as an additional tool to the gold standard in the diagnosis of LMD in patients with high suspicion of the disease. Future directions involve doing prospective studies to further validate this method.