A Second-Generation Dendritic Cell Cancer Vaccine Preparing to Shine

DCVax-L efficacy may correlate with tumor mutational burden, suggesting mechanism is operable

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Highlights

- DCVax-L is a second-generation autologous dendritic cell vaccine that targets potentially all of the neoantigens on a patient's tumor, a key advantage over first-generation vaccine products that targeted only a single antigen with mixed success.
- DCVax-L newly diagnosed GBM phase III blinded interim results (published May 2018) for the entire cohort and also MGMT-M and MGMT-U subsets compare favorably with historic and contemporary trial results and may improve with additional follow-up.
- The mOS delta between patients with MGMT-M and MGMT-U tumors - an indicator that DCVax-L is working through neoantigens and the proposed vaccination mechanism - compares favorably with historic and contemporary trial results and may improve with additional follow-up.
- Results for patients with partial or complete resection offer the most accurate analysis and compare favorably with Stupp's 2005 and 2017 SOC.
- Safety is impressive, commercial manufacturing is a surmountable challenge, and the regulatory environment for GBM, a deadly disease with severe unmet need, is favorable.
- With imminent phase III results and emerging mechanism evidence, Northwest Biotherapeutics ($NWBO) may be a severely discounted cancer immunotherapy company at a $100M market capitalization.

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Summary

Dendritic cell vaccines are beginning to bear fruit for cancer patients. As they are able to mount broad immune responses to neoantigens, they represent an important tool for cancer immunotherapy strategies. The DCVax-L phase III interim results in newly diagnosed glioblastoma (GBM) patients offer a first look at a second-generation product. Safety is impressive and commercial manufacturing is a surmountable challenge. While we await the formal analysis of efficacy, the interim publication offers fertile data for

debate and speculation. First-principles and Occam's razor suggest there is unrealized value in Northwest Biotherapeutics.

Cancer Immunotherapies and Tumor Mutational Burden

Cancer immunotherapies are designed to evoke an immune response against neoantigens, which are the consequence of genetic alterations accumulated over the life of a tumor.[1–3] Checkpoint inhibitors were initially tested in skin (melanoma) and lung (NSCLC and SCLC) cancers, which have an auspiciously high number of mutations. It has become clear that the tumor mutational burden (TMB) – often expressed as the number of non-synonymous mutations – is positively correlated with clinical outcomes for immunotherapies. In a landmark study by Le et al, the importance of TMB was demonstrated by the strikingly different clinical outcomes for colorectal cancer patients with mismatch-repair deficient (high mutations) vs proficient (low mutations) tumors using immune checkpoint blockade.[4] The correlation between TMB and clinical outcomes for PD-1 blockade extends to a wide range of solid tumors and an equation was derived to predict clinical response rate using mutation number.[5,6] Cancers employ immunoediting and modifications of the tumor microenvironment to avert the innate and adaptive components of the immune system, and while the immunoscore[7] is emerging as a valuable composite prognostic factor because it surveys functional immune response, TMB remains an important biomarker in cancer immunotherapy and provides context for emerging clinical trial results.

Quantitative Antigenicity

The median TMB across various cancer types spans about three orders of magnitude - The number of mutations in MMR-deficient (~1,000s), mutagen-associated (~100s), adult solid (~10s), and liquid and pediatric (few) cancers largely reflects their etiology.[1] However, relatively small differences in TMB have been shown to correlate with clinical outcomes. For the purposes of studying the relationship of immunotherapy response rates and TMB in lung cancer, some clinical trials have binned tumors into tertiles representing low (0 to <143 mutations), medium (143 to 247 mutations), and high (>247 mutations) burden and compared outcomes between these groups.[8,9] For example, nivolumab monotherapy treatment of patients with small cell lung cancer (SCLC) containing low, medium, or high TMB resulted in 3.1, 3.9, and 5.4 months mOS, respectively. Likewise, treatment with nivolumab combined with ipilimumab resulted in 3.4, 3.6, and 22 months mOS, respectively, for SCLC patients with low, medium, and high TMB.[8] Additionally, a substantial mPFS difference of 9.7 vs 4.1 months was shown, respectively, for patients with high vs low-to-medium TMB non-small cell lung cancer (NSCLC) treated with nivolipumab.[9] These studies highlight the importance of TMB as a predictive biomarker for response to immunotherapies and suggest a two-fold difference in number of mutations can be clinically meaningful.

To be clear, TMB is a proxy for the number of productive neoantigens or antigenic neoepitopes. It is estimated that only one tenth of non-synonymous mutations result in useful targets.[10] First, the mutant protein must be expressed, a condition that may eliminate about half of the candidates. And expressed mutant proteins must be either recognized by the immune system on the cell surface in their intact form[11] or become processed into peptides compatible with MHC-I or MHC-II in a patient that has the appropriate HLA-type to present the peptides.[2,12] Finally, the tumor microenvironment - and perhaps the whole patient - must be permissive to mount an immune response.[13]

Each neoantigen provides an opportunity to maximize the efficacy of immunotherapy strategies. Checkpoint inhibitors allow pre-existing or adoptively transferred T cells to perform better against their potentially numerous neoantigen targets. First-generation CAR-T strategies designed against only a single neoantigen were able to generate objective responses in various leukemias and lymphomas leading to drug approvals for Novartis, Kite, and Juno. More recently, complete regression of metastatic breast cancer was achieved in a single patient preconditioned with nonmyeloablative immunodepletion using cyclophosphamide and fludarabine and further treated with a combination of a checkpoint inhibitor and

tumor-infiltrating lymphocytes (TILs) that recognized neoepitopes derived from only four neoantigens.[14] Provenge is a clinically and commercially successful dendritic cell vaccine pulsed against a single antigen for prostate cancer.[15,16] An advantage of second-generation dendritic cell vaccines pulsed against whole tumor lysate is their ability to stimulate an immune response to a variety of neoantigens.

DCVax-L has the opportunity, in theory, to present all the neoantigens that exist in a patient's cancer and represents a second-generation approach. It is widely known that more mutations translate to a higher chance of having "winning" neoantigens and strategies that target more neoantigens are more likely to give better outcomes.[17] Dendritic cells are professional antigen presenting cells well-suited to exploit all the neoantigens – Along with checkpoint inhibitors and adoptive T cell strategies, dendritic cell vaccine therapy development is on the rise and the value is becoming increasingly evident.[18–21]

Epitope Spreading

Immunotherapies have been shown to induce immune responses against additional, non-target neoantigens, including intramolecular or intermolecular spread that may switch between MHC-I or MHC-II restricted responses.[22,23] Epitope spreading can be documented in the blood by an increased diversity of TCRs and antibodies specific to tumor antigens. Cancer immunotherapies designed to target a single antigen (e.g. Provenge, Rindopepimut, and first-generation CAR-Ts) only have one shot at inducing antigen spread. On the other hand, dendritic cell vaccines primed against whole tumor lysate as well as checkpoint inhibitors have many chances of amplifying their assault on cancer cells. Epitope spread takes time and new immune-related response criteria may be required to track this important feature of immunotherapies.[22] Initiating the immune response and epitope spread with more antigens should control tumors faster as the cascade will more rapidly target a sufficient number of good antigens. Provenge worked with a single antigen and epitope spread was documented, but prostate cancers are slow-moving offering patients time to mount an immune response. Since GBMs are notoriously immunosuppressive and progress rapidly,[24] it's possible that the single antigen (EGFRvIII) that failed in the Rindopepimut trial was simply not enough to drive a sufficient spread to other antigens and it's likely that vaccine strategies will require many antigens for successful therapy. An increased abundance and heterogeneity of the anti-cancer adaptive immune response may awaken the force of the innate immune system. Epitope spread may also help to counteract genetic instability and heterogeneity, the hallmarks of cancer.

Unstable Genomes

Cancer genomes are unstable and there is a considerable genetic heterogeneity among cells within and between tumors.[25,1] Intratumoral heterogeneity is associated with reduced overall survival in colorectal cancers.[26] Glioblastoma multiforme are notably heterogeneous and they were historically classified into four distinct tumor types, which were more recently refined to three types.[27,28]

Cancers can escape therapeutic pressure through mutations that have accrued over the life of the tumor,[26,29–31] including those that existed before therapy and those that have been induced by chemotherapies (e.g. alkylating or other DNA damaging agents). For example, treatment with temozolomide (TMZ) damages DNA and increases the genetic heterogeneity in a patient's cancer. In fact, approximately one third of all MGMT-M cancers become MMR-deficient and hypermutable after TMZ treatment.[32] The mutations that allow cancers to escape confer advantages and the tumor mutational landscape is able to evolve under the selective pressure of therapy. Much like the triple-drug cocktails that were able to tame the unstable HIV genome, it appears that therapies targeting at least four good neoantigens may be required for durable cancer outcomes - Four good neoantigens or about 40 non-synonymous mutations.[14]

Adult GBMs overall have a median of 35 non-synonymous mutations,[1] but it was shown more recently that there are 400% more mutations in MGMT-Methylated (MGMT-M) than MGMT-Unmethylated (MGMT-U) GBM tumors prior to chemotherapy and radiotherapy.[33] MGMT converts O^6-methyl-guanine

errors back to guanine, which is an important DNA repair function that helps cells maintain genomic integrity - This function is inactivated in MGMT-M GBM tumors, which results in the accumulation of more mutations (both normally and under DNA-damaging therapy), confers a better prognosis, and improves the response to therapies when compared to patients with MGMT-U tumors.

DCVax-L Phase III Interim and Other Results in GBM

The phase III interim results for DCVax-L in newly diagnosed GBM patients (when adjusted for time-to-randomization) compare favorably with historic and contemporary results for standard-of-care and portend similar or superior results to Optune, which was recently FDA approved (Table 1).[34–43,19] Importantly, censored patients can artificially suppress mOS results, so there is a possibility that the data will improve as follow-up time (mFU) is increased in the DCVax-L trial to achieve parity with historic and contemporary trials (Table 1).[44] Since the DCVax-L study is still blinded, the results report the combined cohort, which includes the placebo (n=45), cross-over (n=54), and DCVax-L-treated (n=232) patients.[19] Based on the vintage (2008), duration (10 years), number of patients (n=331), patient characteristics, and number of international centers (>80) in the DCVax-L trial, it can be reasonably expected that patients treated with SOC (i.e. placebo in this trial) will fall within the tight range found in all of the large (n>150), relevant Phase III trials that also reported intent-to-treat (ITT) data – However, it's difficult to estimate the mOS of the DCVax-L naïve/placebo patients (n=45) when many of the SOC that progressed were crossed-over to vaccine treatment, leaving a potentially healthier population where, in contrast, some may have also died before the tumor detectably grew, leaving a less healthy population (Tables 1 and 2). Better estimates of the mOS for the n=45 SOC subset will be informative. Furthermore, but less well-appreciated, is the fact that the crossed-over patients progressed before they started receiving DCVax-L treatment and, in the Phase II trial, 0% of patients (n= 5) that progressed before vaccination were able to mount a vaccine-induced immune response, nor did they have a pre-existing native immune signature; whereas 100% of patients (n=6) with stable disease at the time of vaccination had measureable CTL responses after vaccination.[19,45] Furthermore, 20 patients that progressed before randomization (an exclusion criteria for the phase III trial) and entered the informational arm to receive DCVax-L treatment were reported to have a mOS of 15.3 months.[46] Patients that made it through the 3.1 month period from surgery to randomization before progressing should fare better than the information arm. This key insight suggests that the crossed-over patients will likely also have a mOS similar to the placebo group. Since patients that progress have a less favorable prognosis, even an intermediate vaccine benefit would be helpful. Because the vaccine may have a much lower immune response rate in patients that progress, comparisons between DCVax-L-treated (n=232) and the combined placebo and crossed-over groups (n=99) may be biologically relevant and offer more statistical power.

While there are serious caveats when using the blinded data to estimate efficacy, the MGMT status can be used as a measure of how well therapies perform in relation to tumor mutational burden. Dendritic cell vaccines primed against a whole tumor lysate and other broad-spectrum immunotherapies (i.e. checkpoint inhibitors) are expected to selectively target tumors with more mutations and, therefore, should be more efficacious in MGMT-M tumors. Radiotherapy is known to induce an immune response against cancers,[47] and temozolomide is known to selectively kill MGMT-M tumors due to their reduced ability to correct DNA damage caused by alkylating agents. Radiotherapy and temozolomide are used in the SOC of newly diagnosed GBM and provide an advantage to patients with MGMT-M compared to MGMT-U tumors – The mOS differences (i.e. M vs U deltas of 10.8, 6.9, 8.9, 6.5 months) published from 2009-2017 for the SOC arms in four clinical trials show how well radiotherapy and temozolomide exploit methylation of MGMT. If an immunotherapy works well, a significant broadening of the mOS delta between MGMT-M and MTMT-U would be expected.

Optune was recently FDA approved for recurrent and newly diagnosed glioblastoma and has achieved impressive results in non-small cell lung cancer, where there is high tumor mutational burden. Optune was originally reported to work by disrupting mitotic processes in cancer cells using radio frequencies.[48]

However, emerging evidence suggests Optune stimulates the immune system by modifying the tumor microenvironment[49,50] and it may be this property that selectively targets MGMT-M tumors, resulting in an improved mOS delta of 14.7 months. The only other published ITT phase III study with such impressive gains for MGMT-M tumors is DCVax-L, which resulted in a mOS delta of 14.9 months; Again, this number includes untreated and crossed-over patients, so the delta for the treated patients is expected to improve when the trial is unblinded, as these 99 patients are expected to give a smaller mOS delta of approximately 6-10 months. Furthermore, since there is a two-fold enrichment of censored patients in the MGMT-M group compared to MGMT-U, it's possible that the delta provided by DCVax-L will expand with additional follow-up as it did for Stupp's SOC-defining 2005 trial when it was refreshed in 2009. It will be useful to carefully consider the delta after refresh of the phase III DCVax-L results.

Table 1: ITT mOS in historical, contemporary, and DCVax-L newly diagnosed GBM Phase III trials

									mOS (months)			delta mOS
Author	Descriptor	Trial ID	Journal	Year	# Patients	Phase	Arm	mFU (months)	All	MGMT-M	MGMT-U	M-U
Stupp	Defining SOC	NCT00006353	NEJM	2005	286	III	RT	28	12.1			
Stupp	Defining SOC	NCT00006353	NEJM	2005	287	III	RT+concurrent and adjuvant TMZ (SOC)	28	14.6			
Hegi	Defining SOC	NCT00006353	NEJM	2005	46M / 54U	III	RT	28	~12.1	15.3	11.8	3.5
Hegi	Defining SOC - 5 yrs	NCT00006353	NEJM	2005	46M / 60U	III	SOC	28	~14.6	21.7	12.7	9
Stupp	Defining SOC - 5 yrs	NCT00006353	Lancet	2009	46M / 54U	III	RT	61	12.1	15.3	11.8	3.5
Stupp	Defining SOC - 5 yrs	NCT00006353	Lancet	2009	46M / 60U	III	SOC	61	14.6	23.4	12.6	10.8
Gilbert	Dose-dense TMZ	NCT00304031	JoCO	2013	411 (122M / 254U)	III	SOC	31.5	18.9	23.5	16.6	6.9
Gilbert	Dose-dense TMZ	NCT00304031	JoCO	2013	422 (123M / 263U)	III	"SOC" using Dose-dense TMZ	31.5	16.8	21.9	15.4	6.5
Chinot	Bevacizumab A	NCT00943826	NEJM	2014	458	III	SOC	15.8	16.7			
Chinot	Bevacizumab A	NCT00943826	NEJM	2014	463	III	SOC + Bev	16.3	16.8			
Gilbert	Bevacizumab B	NCT00884741	NEJM	2014	317	III	SOC	20.5	16.1			
Gilbert	Bevacizumab B	NCT00884741	NEJM	2014	320	III	SOC + Bev	20.5	15.7			
Gilbert	Bevacizumab B	NCT00884741	NEJM	2014	175M / 429U	III	combined SOC and SOC + Bev	~20.5	~Gilbert	23.2	14.3	8.9
Stupp	Cilengitide in MGMT-M	NCT00689221	Lancet	2014	273M	III	SOC	29		26.3		
Stupp	Cilengitide in MGMT-M	NCT00689221	Lancet	2014	272M	III	SOC + Cilengitide	29		26.3		
Weller	Rindopepimut in EGFRvIII	NCT01480479	Lancet	2016	371	III	SOC (ITT)	na	17.4			
Weller	Rindopepimut in EGFRvIII	NCT01480479	Lancet	2016	374	III	SOC + Rindopepimut (ITT)	na	17.4			
Stupp	Optune Maintenance Tx	NCT00916409	JAMA	2015	105	III	SOC	38	15.6			
Stupp	Optune Maintenance Tx	NCT00916409	JAMA	2015	210	III	SOC + Optune (FDA approved here)	38	20.5			
Stupp	Optune refresh	NCT00916409	JAMA	2017	229 (77M / 95U)	III	SOC	40	16	21.2	14.7	6.5
Stupp	Optune refresh	NCT00916409	JAMA	2017	466 (137M / 209U)	III	SOC + Optune (FDA approved)	40	20.9	31.6	16.9	14.7
Liau	DCVax Interim Analysis	NCT00045968	JoTM	2018	331 (131M / 162U)	III	combined SOC and SOC+DCVax	~34	23.1	34.7	19.8	14.9

All the trials in Table I measure mOS from randomization with the exception of the DCVax-L interim analysis which measures from surgery. This difference in methodology adds an absolute, systematic increase to the results for DCVax-L, which can be easily corrected. The median time from surgery to randomization in the DCVax-L trial was reported as 3.1 months[19] and, therefore, the reported mOS numbers for DCVax-L can be reduced by 3.1 months to achieve parity with the trials that started the survival clock later, at randomization. However, this arbitrary clock-start difference does not result in differences in the critical time period from diagnosis to randomization, where different trial strategies may introduce immortal time bias.[51]

Comparisons between trials may be harder to perform if the trials have different degrees of immortal time bias. Immortal time is the period of cohort follow-up in which the outcome can not occur, by design. In practical terms, this means that the time period before randomization may select for healthier patients as patients that die or progress are excluded, introducing a bias. All the trials in Table 1 have some length of immortal time and, therefore, an associated bias. DCVax-L interim results reported a median of 3.1 months from surgery to randomization. The Stupp et. al. 2017 Optune publication notes a median of 3.8 months from diagnosis to randomization. Since GBM progresses quickly and is deadly, the time from diagnosis to surgery is relatively short. For example, Flanigan et. al. describe a median wait time from diagnosis to surgery of 21-26 days, which would put the critical time from diagnosis to randomization for DCVax-L around 3.8 to 4.0 months. Since the Optune and DCVax-L trials have the same time period between diagnosis and randomization and similar exclusion criteria, the immortal time bias should be similar and should not compromise the comparison of the results.

The extent of resection is an important prognostic factor that requires special consideration. In the SOC arm of the Stupp et al 2017 Optune trial, biopsy patients had the worst outcomes followed by partial

resection then complete resection with 11.6, 15.1, and 18.5 mOS results, respectively.[43] A similar trend was found when SOC was defined in Stupp et al 2009.[35] The DCVax-L trial excluded biopsy patients presumably because there was not enough tumor material to reliably create the vaccine. Exclusion of biopsy patients will result in inflated mOS outcomes for the DCVax-L combined cohort, but fortunately Liau et al also published mOS results for patients with partial and complete resections,[19] allowing a more direct comparison to Stupp et al that avoids bias due to biopsy patients. Stupp et al reported 15.1 and 18.5 for SOC in partial and complete resections, respectively, whereas Liau et al reported 21.1 and 25.4 for the DCVax-L combined cohort (Table 2). When adjusted for the clock-start methodology differences, the DCVax-L combined cohort results in 18 and 22.3 months mOS – These results give DCVax-L an estimated therapeutic benefit of 3.9 months for patients with partial resection and 5.1 months for patients with complete resection, which compare favorably with Optune's mOS efficacy (Table 2).

Table 2: Effect of Extent-of-Resection on mOS in Optune compared to DCVax-L

Resection	Stupp 2009 SOC mOS	Stupp 2017 SOC mOS	Stupp 2017 Optune mOS	DCVax-L 2017 Combined mOS	DCVax-L 2017 Combined mOS Clocked	DCVax-L 2017 Estimated mOS (2009 SOC)	DCVax-L 2017 Estimated mOS (2017 SOC)	DCVax-L 2017 Estimated Benefit vs 2009 SOC	DCVax-L 2017 Estimated Benefit vs 2017 SOC	Optune 2017 Actual Benefit vs 2017 SOC
partial	13.5	15.1	21.4	21.1	18.0	19.5	19.0	6.0	3.9	6.3
gross	18.8	18.5	22.6	25.4	22.3	23.5	23.6	4.7	5.1	4.1

Clocked = DCVax-L Combined 2017 mOS - 3.1 months; to adjust for time-to-randomization vs time-to-surgery
Estimated mOS = Clocked + ((Clocked - SOC)/3); Estimated effect on median of DCVax-L treated (n=232) when DCVax-L naïve (n=45) and late-treated (n=54) patients removed
Benefit = mOS treatment - mOS SOC

Because only subsets of patients respond to even the most successful immunotherapies, the most relevant efficacy signal is often demonstrated in the "long tail," which is the period of follow-up beyond the median. Medians can entirely mask the benefit to small but important subsets of patients. For example, if the GBM patient subset representing the most favorable prognosis under SOC were shifted to the right by many years, it would have no effect on the mOS of the entire treatment arm if they were already on the right of the median. If DCVax-L is targeting the 400% higher mutations found in MGMT-M tumors and substantially improving the mOS for this subset, it might not appreciably shift the mOS of the entire cohort since these patients largely reside on the right side of the median. The effect of immunotherapies can be unmasked and fully realized by comparison of survival rates for experimental and control arms at key time points beyond the control arm median. As the understanding of cancer immunotherapy matures, future trials may select subsets of patients that are more likely to respond and the median outcomes will become more sensitive signals for efficacy, but medians will always be confounded and may underestimate or entirely miss important gains for patients.

Results from DCVax-L compassionate use, informational arm, phase I/II and comparator cohorts are instructive although interpretation is limited by significant caveats (Table 3). Newly diagnosed GBM (ndGBM) that progressed rapidly or recurrent GBM (rGBM) patients have poor prognoses which are reflected in the mOS results (Table 3). When treated with DCVax-L, rapid progressors or rGBM patients in various trials had mOS results of 15.1, 14.7, 11.7, and 15.3 months and these numbers were extended to 17.9 months in rGBM with the addition of toll-like receptor (TLR) agonist therapy (shown in red, Table 3). On the other hand, patients with stable disease in Liau et al 2005 had a mOS of 35.8 months (shown in blue, Table 3). Newly diagnosed GMB and the pronueral and mesenchymal subsets also had favorable mOS results of 35.9, 35, and 37.6 months when treated with DCVax-L combined with a TLR agonist. Notably, the mesenchymal subset is known to have a less favorable outcome with standard therapy, but this subset is particularly well-rescued with the mOS shifting from 14.6 to 37.6 months on SOC vs DCVax-L/TLR agonist therapy, respectively. The striking rescue of the mesenchymal subset is thought to be driven by a more pronounced induction of $CD3^+$ and $CD8^+$ T cell response.[52]

Table 3: DCVax-L Compassionate Use, Informational Arm, Phase I/II, and Comparator Results						
Author	**Year**	**Therapy**	**Diagnosis**	**n**	**mOS months (95% CI)**	**Usage Category**
Bosh	2015	DCVax-L	rapid progressor	19	15.1 (10.5-17.2)	Compassionate Use
Brandes	2008	Other	rapid progressor	18	10.2 (na)	comparator
Roldan	2009	Other	rapid progressor	10	9.1 (4.9–19.1)	comparator
Kang	2010	Other	rapid progressor	10	10.8 (na)	comparator
Sanghera	2010	Other	rapid progressor	29	8.3 (na)	comparator
Gunjur	2011	Other	rapid progressor	27	10.4 (na)	comparator
Linhares	2013	Other	rapid progressor	13	9.0 (3.7–14.3)	comparator
Bosh	2015	DCVax-L	recurrent GBM (rGBM)	8	14.7 (na)	Compassionate Use
Friedman	2009	Other	recurrent GBM	?	8.7-9.3	comparator
Liau	2005	DCVax-L	stable GBM (this study contained nd and rGBM)	7	35.8	Phase I
Liau	2005	DCVax-L	progressive GBM (this study contained nd and rGBM)	5	11.7	Phase I
Liau	2005	DCVax-L	all ndGBM and rGBM, stable and progressive	12	23.4	Phase I
Liau	2005	Other	RPA class III, <50 yo, KPS >90, not biopsy	99	18.3	comparator
Prins	2011	DCVax-L + TLR agonists	ndGMB, rGBM	23	31.4	Phase I
Prins	2011	DCVax-L + TLR agonists	ndGMB	15	35.9	Phase I
Prins	2011	DCVax-L + TLR agonists	rGBM	8	17.9	Phase I
Prins	2011	DCVax-L + TLR agonists	Proneural (PN)	5	35.0	Phase I
Prins	2011	DCVax-L + TLR agonists	Mesenchymal (Mes)	9	37.6	Phase I
Lee	2008	Other	PN (>249 days without progression)	59	22.6	comparator
Lee	2008	Other	Mes (>249 days without progression)	63	14.1	comparator
Lee	2008	Other	Mes+ProlferativeMes (>249 days without progression)	82	16.5	comparator
Northwest Bio	2015	DCVax-L	All (entire cohort that progressed too early for Phase III)	51	18.3	Information Arm of Phase III
Northwest Bio	2015	DCVax-L	Rapid progressor	20	15.3	Information Arm of Phase III
Northwest Bio	2015	DCVax-L	Indeterminate status	25	21.5	Information Arm of Phase III
Northwest Bio	2015	DCVax-L	Pseudoprogressor	1	>30	Information Arm of Phase III
Northwest Bio	2015	DCVax-L	Unclassified (MRI did not exist)	5	9.2	Information Arm of Phase III

Other Factors

Prognostic factors for newly diagnosed GBM include age, tumor size, performance status, extent of surgery, radiation dose, and adjuvant chemotherapy use.[53] GBM classification and molecular markers are also important. Mesenchymal have worse outcomes than proneural, for example. MGMT-M and mutIDH-1 are independently associated with better survival and patients with combined mutIDH-1 and MGMT-M seem to survive longer than patients with only one of these markers.[54] IDH-1 mutations conferred a large survival advantage compared to patients with wild-type IDH-1 (mOS 3.8 years vs 1.1 years, respectively, and a hazard ratio for death of 3.6).[55] However, IDH-1 status has opposing effects on survival and response to TMZ, which should be carefully considered in subset analyses.[56] Therapy-induced lymphopenia has also been associated with a reduced survival of up to six months[57] and the statistical package for the DCVax-L trial was adjusted to reflect this variable that emerged contemporaneously.[58]

The DCVax-L interim publication cited other unknown factors for a patient subset (n=100 or about 30% of the entire cohort) with an estimated Kaplan-Meier derived mOS of 40.5 months. There are many emerging prognostic and predictive factors associated with tumor response to immunotherapies that may be contributing to the improved overall survival in this DCVax-L subset population (Table 4). Correlating these immune-factors with mPFS, mOS, survival at various time points, or hazard ratios in various patient subsets may provide early evidence that DCVax-L is working through its presumed immune mechanism.

Race, gender, and age have been shown to effect responses to immunotherapies. African Americans respond significantly better than Whites to Provenge (mOS of 45.3 vs 24.7 months, respectively),[59] presumably because they have more numerous T cells.[60] Males respond better than females to checkpoint inhibitors.[61] Older patients appear to respond relatively better than younger patients, at least partly due to lower regulatory T cells.[62] While age is a negative prognostic indicator in GBM, the Optune trial showed hazard ratios of .51 and .69 for older and younger patients, respectively, suggesting that treated older patients may be more responsive compared to age-matched controls than are younger patients. These examples support the central role of T-cells in the immune response to cancer and suggest that the T-cells induced by dendritic cell vaccines may show similar differences in these populations.

Biomarkers can provide early evidence that DCVax-L is working through the presumed immune mechanism. In ovarian cancer patients, a significant transient increase in serum IFN-gamma and,

conversely, a significant transient decrease in TGF-beta was found after vaccination with an autologous dendritic cell strategy similar to DCVax-L.[43] Response of various solid tumors to DCVax-D (a dendritic cell vaccine that is directly injected into tumors) is inversely correlated with TGF-beta levels in the tumor microenvironment where patients with low tumor-associated TGF-beta had a measurable systemic CTL response and higher overall survival.[45] High TGF-beta in the tumor microenvironment is likely to be a negative predictive factor for response to many immunotherapy strategies. On the other hand, IL8, IL12p40, or TNF-alpha production and CD86 or MHC-II levels on dendritic cells positively correlated with DCVax-D outcomes.[63] Also, activated dendritic, $CD8^+$ T, $CD4^+$ T, and natural killer cells were each associated with improved overall survival, whereas the number of regulatory T cells and MDSCs were associated with reduced overall survival in colorectal cancer.[26] These biomarkers may be useful for interpretation of DCVax-L results.

Neoadjuvant immunotherapy is an emerging idea that aims to take advantage of the more numerous neoantigens that exist prior to surgery.[64–66] Interestingly, in the Optune trial, there was an inverse relationship between extent of resection and improved hazard ratios, where biopsy, partial resection, and total resection gave .50, .56, .70 hazard ratios, respectively, suggesting that immune modulation by radio frequencies may have a relatively larger impact when there is more tumor present. It will be worth following neoadjuvant studies further to see if there is a correlation of tumor burden and response to immunotherapies. In any case, it will be useful to note the hazard ratios for extent-of-resection subsets in the DCVax-L trial. Notably, DCVax-L's companion product, DCVax-D, is a neoadjuvant strategy.

DCVax-L might be expected to differentially modify outcomes in the patients with factors that modulate the immune responses and these differences between patient subsets might be detectable in the interim or unblinded analyses. Correlation of immunoscore, TMB, and MGMT methylation status with DCVax-L efficacy will give insight into the dependence on functional immune responses and the instigating somatic mutations. Other subset analyses may also provide insight into the immune mechanism. For example, did the seven African Americans in the trial fare better than Whites? Does the older population witness a significant improvement to overall survival compared to age-matched historic and contemporary controls? Are males responding better than females? Are these effects visible by hazards ratios? While these are not requirements, they may contribute to the evidence that DCVax-L is a bona-fide immunotherapy.

Table 4: Selected factors that may predict or reflect response to immunotherapies

Factors	Significance	Expected Effect on Survial
tumor mutational burden	high TMB, high neoantigens	Higher responds better
MSI status	high MSI, high neoantigens	Higher responds better
MGMT status	MGMT-M have higher TMB (in addition to the prognotic and predicitve value)	M responds better than U
serum IFNgamma (~24hrs after vaccination)	high IFNgamma, high functional response	Higher responds better
serum TGFbeta (~6hrs after vaccination)	high TGFbeta, high suppression in TME	Lower responds better
tumor-specific native and vaccine-derived CD8+ clone level, heterogeneity, and activity	high CD8 levels and het, better immune response	Higher responds better
tumor-specific native and vaccine-derived CD4+ clone level, heterogeneity, and activity	high CD8 levels and het, better immune response	Higher responds better
age	higher age, lower regulatory T cells	Older responds better than predicted
gender	men seem to respond better than women to checkpoint blockade	Male responds better
race	Afriican Americans (more T cells) respond better to Provenge than Whites	African American responds better than White
extent of surgery (biopsy, partial, total)	Resections with poorer prognosis have more antigens	Biopsy responds better than predicted
mild lymphopenia or non-myeloablative immunodepletion	immunodepletion suppreses suppressor cells	Optimal responds better
ctDNA	Lower ctDNA indicates lower tumor burden	Lower responded better
Delayed-type hypersensitivity (DTH) reaction	Presence of DTH indicates vaccination	Higher responds better
IFN-γ ELISPOT assay to detect cytotoxic T cells targeting tumor lysate	High IFN-γ indicates high functional T cell response	Higher responds better
Natural killer cells	Destroy cancer cells	Higher responds better
Regulatory T cells	Suppress immune response	Lower responds better
MDSC	Suppress immune response	Lower responds better

Discussion

Extensive preclinical and clinical data show exactly how DCVax-L operates as an immunotherapy. Along with favorable mOS data and survival rates at 3, 4, or 5 years, any evidence that DCVax-L is operating through the proposed immune mechanism in the ongoing phase III patients will be highly favorable for regulatory decisions. While the survival benefit is key, it will be useful to monitor the relationship between DCVax-L efficacy and other immune factors in the interim and final analyses for mechanism signals.

DCVax-L efficacy is improved with the addition of PD-1 blockade[67] and further improved with combined PD-1 blockade and suppression of tumor infiltrating myeloid cell (TIM) function.[68] These gains were demonstrated in models with high tumor burden, immunosuppressive tumor microenvironments, and poor prognosis, which – to some degree – emulates patients that have progressed. This triple-combination immunotherapy strategy effectively counters the tumor's attempt to avert the independent effects of each component. These findings provide hope that it will translate to clinical gains for both stable and progressed GBM patients and highlight DCVax-L as an important platform that will allow clinicians to rapidly optimize combination chemo-immunotherapy strategies.[69–71] It will be interesting to compare the relative efficacy of neoadjuvant DCVax-D and post-surgical DCVax-L dendritic cell vaccine strategies in future trials, although these distinct approaches may have different optimal combination strategies.

CancerCommons and the Musella Foundation for Brain Tumor Research are non-profit organizations aiming to more rapidly test therapeutic hypotheses by facilitating small, rigorous clinical trials that seek large efficacy signals. DCVax-L development would benefit from these strategies, perhaps in the right-to-try setting, and the best results could be used to inform larger phase III clinical trials.

By all measures, the safety profile of DCVax-L looks stellar. Importantly, GBM pseudoprogression is correlated with improved survival[72,73] and the DCVax-L results may give early insight into the considerations that may be relevant to developing modified response criteria and safety monitoring. Response criteria and safety monitoring flexibility may be desirable as the immunotherapy field moves into more complex double-, triple-, and chemo-immunotherapy combinations.[69–71]

The FDA, by law, is afforded flexibility when considering potential treatments for diseases with severe unmet need.[74] Likewise, the EMA gives special consideration to areas of unmet need. The DCVax-L trial was originally designed and powered for mPFS as primary and mOS as secondary outcomes. It's not unreasonable to expect regulatory agencies to allow use of mOS in place of mPFS, as survival is the gold-standard and most important outcome. This is particularly likely considering that the FDA approved Bevacizumab for newly diagnosed GBM, which failed for both mOS (primary) and mPFS (secondary) in the Gilbert et al study and also failed for mOS (co-primary) in the Chinot et al study. While Chinot's study suggests there is a mPFS (co-primary) benefit, the toxicity was also increased. While decision-making will rely on standards and the preponderance of evidence, regulatory leniency will not be surprising in the context of GBM, a deadly disease with severe unmet need.

Conclusion

Dendritic cell vaccines are designed to exploit antigens on cancer cells. The expansion of the mOS delta between newly diagnosed GBM patients with high (MGMT-M) and low (MGMT-U) tumor mutational burden suggests DCVax-L may be working through neoantigens and the proposed mechanism. Checkpoint inhibitor, MDSC suppression, Treg suppression, TGF-beta inhibitor, TLR agonist, cytokines and other strategies may further unleash DCVax-L efficacy. The regulatory environment for glioblastoma is highly favorable and the DCVax-L mechanism is tractable, which will allow rapid optimization in follow-on studies as well as an opportunity for agencies to routinely reassess the value for patients. The DCVax-L safety record is impressive and demand under the right-to-try legislation may challenge manufacturing capacity. DCVax-L is primed to be a landmark for dendritic cell vaccines and cancer immunotherapy and will, hopefully, provide a new treatment option for glioblastoma patients.

Contemporaneous to Embargo

Dr. Liau's group recently published an abstract for the upcoming Society for NeuroOncology meeting that shows dendritic cell vaccination in combination with a TLR agonist for GBM patients (WHO Grade III and IV) results in a mOS of 54 months compared to 11 months for the placebo control.[75] Furthermore, the mOS delta between MGMT-M and MGMT-U for the entire cohort was 38 months (57 vs 19 months,

respectively). Improved mOS and, particularly, expansion of the M vs U delta with addition of TLR agonists to dendritic cell vaccination provide further evidence that the mechanism is operable.

Tags
Mutation, Tumor Mutational Burden (TMB), Dendritic Cell Vaccine, DCVax-L, $CD8^+$ Cytotoxic T Cells, $CD4^+$ Helper T Cells, B cells, Natural Killer Cells, Personalized Medicine, Quantitative Immunotherapy, Cancer Immunotherapy, Immuno-oncology, Immuno-editing, Central Tolerance, Peripheral Tolerance, Biomarkers, Neoadjuvant Immunotherapy, MGMT, Methylated, GBM, Glioblastoma, Radiation, Temozolomide, CCNU, Alkylating Agent, Provenge (Sipuleucel-T), First-generation CAR-T, Tumor Infiltrating Lymphocytes (TILs), Pembrolizumab (Keytruda), Checkpoint Inhibitors, CTLA-4, PD-1, PDL-1, Cancer Genome Atlas Research Network (CGARN), Stupp, SOC, Optune, Manufacturing, COGS, Market Size, Pricing, Vogelstein, Diaz, Jaffe, McDonald, Merkel Cell, Future Trials, Rare Pediatric GBM, Priority Review Voucher (PRV), $NWBO, Northwest Biotherapeutics, Open Science Movement.

Disclosures
Carlo Rago, PhD owns Northwest Biotherapeutics (NWBO) Common Stock. In addition, Dr. Rago serves as a Consultant to District 2 Capital and Bigger Capital. Dr. Rago's compensation at District 2 Capital and Bigger Capital depends on the performance of the securities in their portfolios. District 2 Capital and Bigger Capital own both Common Stock and Warrants of NWBO. This review is based solely on publicly available information and does not represent medical or investment advice in any way.

This white paper may contain forward-looking statements, including statements as to anticipated or expected results, beliefs, opinions, and future financial performance. The forward-looking statements are based on current expectations and assumptions and involve risks and uncertainties that may cause the Company's actual experience to differ materially from that anticipated.

The views contained in this white paper represent the opinions of Dr. Rago as of the date hereof. In addition, the efficacy and safety of DCVax-L is only one element in valuing the securities of the Company. Investors and other interested parties of the Company are encouraged to do their own analysis of DCVax-L and the Company. Dr. Rago reserves the right to change any of his opinions expressed herein at any time and for any reason and expressly disclaims any obligation to correct, update or revise the information contained herein. The information contained in the white paper may not contain all of the information required in order to evaluate the value of the Company or its securities. Investors should seek independent scientific or financial advice regarding the efficacy and safety of DCVax-L, the suitability of investing in any securities or of following any investment strategies; Dr. Rago is not offering or providing such services in connection with this white paper or otherwise making a recommendation to buy or sell any of the Company's securities.

About the Author
Dr. Carlo Rago received his PhD in Cellular and Molecular Medicine at the Johns Hopkins School of Medicine and trained as a post-doctoral fellow in the Ludwig Center for Cancer Genetics and Therapeutics at Johns Hopkins where he was the first to demonstrate the dynamics of circulating tumor DNA (now called liquid biopsies)[76] and developed early genome editing techniques[77] to facilitate gene function studies and drug discovery. These and other scientific contributions have found widespread use. Carlo was also instrumental in the formation of a global federation of disease-focused foundations, called the Duchenne Alliance, where he served as their scientific director and initiated their venture philanthropy program. Dr. Rago's software company, OpenOnward, was the first to offer crowd-funding of drug development projects in a secure, online environment and the DuchenneDashboard helped empower the community of foundations to identify and fund the most promising therapies and clinical trials for Duchenne muscular dystrophy. Dr. Rago's privately held biotech company, DMD Therapeutics, was

named on the BioSpace list of top 20 life sciences companies to watch in 2018 for their preclinical development efforts. Carlo has a passion and deep understanding of cancer-immunotherapy and gene therapy strategies and works with the investment community to identify biotechnology opportunities with the most transformative potential.

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