Autologous Tumor Lysate-Loaded Dendritic Cell Vaccination (DCVax-L) in glioblastoma: breakthrough or fata morgana?

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Glioblastoma is the most common malignant primary brain tumor of adults and associated with high morbidity and mortality. Increased biological insights have so far not translated into new treatments and the current standard of care therapy for newly diagnosed glioblastoma comprises maximal safe resection and radiochemotherapy with temozolomide, while no treatment standard exists for recurrent disease.[1] Molecularly directed treatments such as EGFR inhibition, integrin inhibition, VEGF inhibition, immune checkpoint inhibitors and others have failed to show clinical benefit in prospective randomized clinical trials. Recently, Liau et al. reported data on autologous tumor lysate-loaded dendritic cell vaccination (DCVax-L) in *JAMA Oncology* on November 17th 2022 and at the 27th Society of Neuro-Oncology (SNO) Annual meeting on November 20th 2022.[2] While the efforts of all contributors to the study need to be acknowledged, there are significant concerns about the study report and conclusions.

The study (NCT00045968) was initially designed in 2007 as a prospective multicentric placebo-controlled randomized phase III trial. Patients with newly diagnosed glioblastoma were to be randomized in a 2:1 ratio to standard radiochemotherapy with either placebo or DCVax-L. With cross-over to the active treatment for the patients in the control arm at the time of progression being part of the study protocol, the primary endpoint had to be progression-free survival (PFS). The trial was conducted at 94 sites in 4 countries (US, Canada, UK, and Germany) and enrolled a total of 331 patients (232 patients randomized to the DCVax-L group, and 99 patients randomized to the placebo group) over a period of eight years (August 2007 until November 2015), with the vast majority of patients (n=303, 91.5%) accrued between 2012 and 2015. An initial report on the trial in 2018 reported only on the combined overall survival (OS) data of both study arms and failed to report on PFS, the primary study endpoint.[3] The argument for not publishing the primary endpoint was an ambiguous statement about an expert panel being required because of the complex determination of progression. Nonetheless, the authors concluded that the patients in this trial were living longer than expected. Now, more than four years later, a second report is available, which is surprisingly named “A Phase 3 Prospective Externally Controlled Cohort Trial”. This is a remarkable title, as the investigators have re-analyzed the OS data of the study against published external controls and present this as a prospective trial. It is obvious, however, that this is not a prospective analysis but a *post-hoc* retrospective analysis: the investigators had seen the data, both of their own study and of the cohorts taken for comparison and then decided to go ahead with cross-trial comparisons. The authors state that the PFS endpoint became infeasible because of pseudo-progression issues, however, to the best of our knowledge in no other study that issue has resulted

in abandoning the primary endpoint. Fortunately, the numerical PFS data are now presented: the median PFS was 6.2 (95% confidence interval CI, 5.7-7.4) months for patients receiving DCVax-L and 7.6 (95% CI, 5.6-10.9) months for the placebo group and not statistically significantly different (p= 0.47). Thus, the clinical trial did not reach its prospectively defined primary endpoint and with that, the investigators *de facto* declare the randomized trial in its original and prospective design to be negative.

The report presented now is an attempt to save the trial from this negative conclusion. This is done by building further on the OS observation already made in the 2018 paper, leading the authors to the conclusions that the 'survival data suggest that patients in this phase 3 trial are living longer than expected'.[3] Thus, the primary endpoint was changed into OS, based on the argument that pseudo-progression in the active treatment arm might have interfered with the assessment of PFS. Then, to assess the *post-hoc* defined primary outcome measure an external control population (ECP) of patients obtained from selected published randomized clinical trials is introduced. The ECP was compiled by a company providing „consultancy & research in health economics" and comprised a total of 1366 patients with newly diagnosed glioblastoma treated with standard of care within 5 completed and published trials, which met „fit for purpose" set of 14 criteria. It is however not a meta-analysis on patient level data, but a comparison at the trial level with survival data reconstructed by digitizing the published Kaplan-Meier curves using an algorithm. Because of the high cross over rate (approximately 90%) the two arms (investigational and control) from the original DC-Vax study were combined in the analyses. An analysis of patients receiving DCVax-L at progression is presented as a separate analysis. The endpoint is here defined as OS of patients with recurrent glioblastoma measured from first recurrence to death of any cause. The ECP for a secondary end point analyses was selected through a similar methodology as the primary endpoint ECP and comprised of a total of 640 patients with first recurrence of glioblastoma treated with either standard of care therapies (lomustine, bevacizumab, or best supportive care) or a placebo in the control groups of 10 comparator RCTs.

The investigators performed further analyses to validate their methodology. The ECP was compared to the treatment groups of the individual external trials. The analysis of each of the 15 comparator trials, substituting the ECP for the original control groups, confirmed that the outcomes were the same as originally reported (primary end point met or not met). Furthermore, sensitivity analyses to

check for biases and a matching-adjusted indirect comparison (MAIC) to adjust for imbalances in individual patient characteristics were applied. In summary, the results for the adapted primary endpoint of this study showed a median OS of 19.3 (95% CI, 17.5-21.3) months for the 232 patients with newly diagnosed glioblastoma receiving DCVax-L and 16.5 (95% CI, 16.0-17.5) months for the ECP patients (HR = 0.80; 98%CI, 0.00-0.94; p= 0.002). The survival rate at 48 months from randomization was 15.7% in patients receiving DCVax-L and 9.9% in ECP patients, while at 60 months it was 13.0% and 5.7%, respectively. For the 64 patients with recurrent glioblastoma receiving DCVax-L analyzed for the adapted secondary endpoint, median OS was 13.2 (95%CI, 9.7-16.8) months, while it was 7.8 (95% CI, 7.2-8.2) months among ECP patients (HR, 0.58; 98% CI, 0.00-0.76; p< 0.001).

So, do these results prove activity of DCVax-L in glioblastoma? The answer to this question is clearly: no, they do not. Indeed, there is a trend towards the recognition of the validity of external controls for comparison, but it really requires a near total similarity between trials or datasets – from the way diagnoses are made, the trial design, inclusion criteria, endpoints and the centers participating.[4,5] And here, major differences are present.

The DCVax-L trial was special in its design in that it randomized the patients after the end of radiotherapy. The five trials that were selected for comparison of the newly diagnosed patients also randomized the patients after radiotherapy, and for that aspect they are well selected. But even looking at the 5 trials on newly diagnosed glioblastoma, there are striking differences to the DC-Vax trial, which present a major limitation. The DCVax-L trial was limited to patients with tumors in one hemisphere, the surgical resection needed to have the intent for a gross or near total resection of the contrast-enhancing tumor mass and biopsy only patients were excluded. The leukapheresis required for the harvesting of antigen presenting cells was done 3 weeks after surgery and steroids needed to have been discontinued ten days prior to that. Patients with progression at the completion of radiotherapy were excluded. These are high level inclusion criteria, aiming at enrolling a select group of patients with very favorable clinical characteristics assumed necessary to show efficacy of the vaccine strategy – but also likely to be associated with improved OS. While selecting such favorable prognosis patient selection may be an appropriate strategy for the conduct of a vaccine trial, they severely limit the comparison of the data with the data from other trials not using such stringent criteria.

Indeed, two of the comparator studies were reflecting community trials that did allow radiological progression at the time of randomization and for the extent of resection only required that at least a tumor block of 1 cm^3 was available for translational research.[6,7] The trial on tumor treating fields (TTF) did not restrict inclusion because of steroid use or extent of resection.[8] Two other used trials are also vaccine approaches, with similar precautions on the extent of resection and steroid use. This resulted in important differences between these trials in exclusion rates for progression during the radio-chemotherapy phase: the two trials that reflected a community approach and the TTF trial excluded between 2% and 8% of patients registered into the trial for progression during concomitant radio-chemotherapy, whereas no less than 18% of patients registered into the DCVax-L trial were excluded for this reason.

Interestingly, the rindopepimut study had two groups, centrally determined: a 'minimal residual disease' group (MRD) subject to the primary analysis, and a 'significant residual disease' (SRD) group, in which tumor load on the post radiotherapy scans was centrally determined and based on < or ≥ 2 cm residual disease. For this discussion, the observed median overall survival in the control arms of these subgroups is highly relevant: it was 20.0 months in the MRD group, and 14.1 months in the SRD group.[9] These data show the huge impact of residual enhancement at the time of progression, which is also present in the DCVax-L trial resulting in a 3 months difference in OS between minimal and significant residual disease using a similar definition. The potential for bias is clear, especially when considering that in three of the 5 newly diagnosed glioblastoma trials used for comparison no attempt was made to control for residual tumor volume.

So yes, all these trials were on glioblastoma and all randomized patients after radiotherapy but major differences in trial design and enrolled patients exist which correlate to well-established prognostic factors. Although the authors state that patient demographic characteristics and prognostic factors of the DCVax-L cohorts were well matched with the ECPs for both the primary and secondary end points based on the criteria prespecified in the statistical analysis plan of this retrospective study, major prognostic patient and tumor related factors such as age, steroid use, performance status, and extent of resection were not used for matching. The authors themselves note that "this method relies on the unverifiable assumption that outcomes can be fully predicted from the treatment and that all important prognostic factors and effect modifiers are included in the model". That also assumes that the same methodological approach is used in all trials with the same granularity of the data presented in the trial reports.[5] However, this is not the case for the studies used here: while some of the data were extracted from the publications on the ECP trials, they were

available only as grouped categories for age (<50 versus >=50) and Karnofsky performance group (<90 versus >=90). Moreover, the gross description of extent of resection does not allow a meaningful comparison between trials. The efforts by the investigators to minimize error due to confounding factors in their adapted study design need to be acknowledged, however they do neither exclude bias nor provide controls equivalent to that generated by prospective randomization.

And what about that presumed 'noteworthy tails of long-term survival curves'? In the DCVax-L group 15.7% of patients were alive 48 months after randomization, whereas in the pivotal EORTC trial on chemo-irradiation with temozolomide without the stringent favorable prognosis patient selection and patients randomized prior to the start of radiotherapy, this was 12.1%.[10] So here also, assumptions remain to be proven and molecular analyses (like IDH status) are needed.

Trial designs that leverage external data have recently been discussed as potentially valuable inferences in settings in which single-arm trials are suboptimal and RCTs are infeasible.[4] The availability of high quality patient-level patient data, similar patient selection and consistent definition of covariates and endpoints have been emphasized as major prerequisites for meaningful externally augmented clinical trial analyses.[5] The lack of such individual patient-level data in the present study precluded well established statistical methods such as propensity score matching or inverse probability weighting, which have specifically been developed for estimation of treatment effects in non-randomized studies.

In summary, despite this report on a research program spanning 15 years, clear conclusions on the activity of DCVax-L in glioblastoma cannot be drawn - the major methodological limitations of the recent publication with clear confounding factors simply do not allow that. Moreover, while the investigators describe their approach as innovative with wider general applicability, the mutation of a negative randomized clinical trial with replacement of primary and secondary endpoints as well *post-hoc* introduction of 'control patients' by pooled and inferred external control data requires validation by prospective studies before it is considered for implementation as high-level evidence. Importantly, overly positive interpretation of the study data is misleading to patients and must be avoided, as the current data simply do not warrant application of DCVax-L in patients with newly diagnosed or recurrent glioblastoma. Our mission is not to give more treatments to patients but to deliver treatments that improve outcome, based on solid evidence and not speculation.

An issue that is worthy of further discussion with a broader perspective with regard to general codes of conduct for academic publishing is the fact that the corresponding author is an employee of the company, with patents pending. In our view, this should be specifically addressed and discouraged in journal policies as relevant conflicts of interest. Here, it should be remembered that the primary study outcome was not published in 2018 and is now only presented as some secondary measure.

What can be learned from this? The trial design allowing 'cross-over at progression' prevented the use of OS as the primary endpoint. This is still the 'gold standard' endpoint in oncological phase III studies, and PFS is indeed a problematic endpoint in trials on newly diagnosed glioblastoma. Despite the argument used to allow cross-over at the time of the study design, a more general question is whether cross-over designs may actually obscure the signal of activity of the investigational agent by interfering with endpoint analyses. If so, not allowing cross over will result in a more robust trial design and the more robust the trial design, the larger the likelihood results will translate quickly into a modified and superior standard of care if the trial is positive. Then, the investigators can be criticized for not taking an obvious step: the data presented in 2018 are basically the same and if that signal was conceived as positive, a dedicated well-designed 2nd trial would have been the real answer instead of a round of intrinsically flawed data constructions. *Post hoc* analyses are hypothesis generating and nothing else.

To conclude, stringently designed and executed prospective randomized clinical trials remain the gold standard for evaluation of efficacy of novel treatments. Control of known, but also unknown, confounders are crucial in clinical trials, and the present analyses of the DCVax-L trial offer neither. Further methodological research and definition of standards that ensure adequate scientific rigor is needed to define the possible role of externally controlled clinical trial data for retrospective analyses or within prospective clinical trials. This concerns both healthcare and regulatory decision making in (neuro-)oncology.

Declaration of interests:

MP has received honoraria for lectures, consultation or advisory board participation from the following for-profit companies: Bayer, Bristol-Myers Squibb, Novartis, Gerson Lehrman Group (GLG), CMC Contrast, GlaxoSmithKline, Mundipharma, Roche, BMJ Journals, MedMedia, Astra Zeneca, AbbVie, Lilly, Medahead, Daiichi Sankyo, Sanofi, Merck Sharp & Dome, Tocagen, Adastra, Gan & Lee Pharmaceuticals.

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