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SPOTLIGHT

COMMENTARY

How biomarkers can be used to optimize the clinical development of dendritic cell vaccines in immuno-oncology

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Numerous clinical studies with dendritic cell (DC) vaccines to treat cancer have been conducted in the past two decades. While DC-based therapies have been shown to induce immune responses and to be safe, clinical outcomes have been disappointing. Nonetheless, emerging research suggests DC-based treatments might improve survival and there is renewed interest in next generation DC-based vaccine approaches, particularly in combination with other emerging immunotherapies such as checkpoint inhibitors. This article explores how predictive or prognostic biomarkers, either to select patients or to guide treatment, could be applied to improve outcomes of this novel therapeutic approach. Specifically, we discuss two main approaches: establishment of eligibility criteria based on confirmation of expression of the tumor-associated antigens used in the vaccine, and implementation of a delayed type hypersensitivity test to screen responders so as to extend treatment.

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A NEED FOR IMPROVED ENDPOINTS

For over two decades, dendritic cell (DC) vaccines have been used in clinical trials for a range of cancers. As summarized by Garg *et al.*, DC vaccines have been applied against various malignancies in over 200 clinical trials with the four most targeted cancer types being melanoma (>1000 patients), prostate cancer (>750 patients), glioblastoma (GBM; >500 patients), and renal cell carcinoma (RCC; >250 patients) [1]. This extensive body of clinical trials has shown that DC-based immunotherapy is safe and can induce anti-tumor immunity, even in patients with advanced disease. However, clinical responses have been disappointing, with objective tumor response rates rarely exceeding 15% [2]. As other emerging immunotherapies such as immune checkpoint inhibitors and CAR T cells started delivering breakthrough results, the interest in DC therapies waned.

Some recent reviews and new clinical data, however, have shed new insights that are putting the field back into the spotlight. The review by Anguille *et al.* for example proposes that the assessment criteria used as the primary endpoint in most of these early trials was simply not appropriate. Typically, the primary endpoint used in this extensive body of trials was the classic response assessment criteria such as RECIST, which is a measure of tumor 'burden'. Anguille *et al.* were able to demonstrate that an increasing number of trials that had survival secondary endpoints indicate that DC therapy could confer a survival benefit. Specifically, an increase in median overall survival of at least 20% has been documented in most studies that had a secondary survival endpoint. Although many of these trials were early phase and not designed primarily to measure survival, the results obtained are nevertheless noteworthy, especially in view of the fact that the bar for establishment of a clinically meaningful improvement in median overall survival is generally set at 20% [2]. Thus, the authors concluded that new clinical trials should either use overall survival as the primary endpoint or surrogate endpoints for clinical effectiveness. This absence of association between objective response and overall survival has been also reported with the use of other immunotherapies and as a result, RECIST criteria and improved endpoints for cancer immunotherapy have received significant attention [3].

But using survival as the main endpoint does nothing to help select patients that could respond best to therapy nor does it helps guide clinical treatment. What could be biomarkers or surrogate endpoints to guide DC therapy? As the investment in the field has dwindled after these initial set of clinical trials there are only a handful of recent publications exploring the subject of how to use biomarkers to achieve better outcomes with DC therapies. Nonetheless, there are some emerging directions that will be discussed below.

LOOKING FOR CLUES IN THE IMMUNE RESPONSE

The first port of call is the immune response itself. The mode of action of a DC vaccine is to induce an immune response in the form of clonal

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expansion of antigen-specific T cells that then need to infiltrate the tumor and exert a cytotoxic action. Therefore, as discussed by Lesterhuis *et al.*, as clinical responses were not obvious or did not occur in the majority of patients, researchers have looked for validated assays that can monitor immunological outcome. Most studies have focused on monitoring of antigen-specific T-cell responses in peripheral blood, which proved difficult as often it required in vitro re-stimulation due to very low precursor frequencies. Tumor tissue and lymph nodes would be more interesting compartments to monitor these responses but unfortunately, lymph nodes and the tumor site itself are not always readily accessible for monitoring purposes [4] and the early technologies to detect antigen-specific T cells were based on MHC class I tetramer staining which is limited by the sensitivity required to detect low frequency events [5]. Therefore, monitoring of antigen-specific T-cell responses was not adopted as a practical biomarker of response to treatment.

Several groups have explored other immune related measures or events as predictive biomarkers of an ongoing response. For example, Boudewinjs *et al.* evaluated the correlation between side effects and immunologic and clinical outcomes in stage III and IV melanoma patients. For this, a retrospective analysis of 82 stage III patients and 137 stage IV patients vaccinated with an autologous DC vaccine loaded with gp100 and tyrosinase tumor-associated antigens was conducted. Treatment-related adverse events occurred in 84% of patients of which flu-like symptoms (74%) and injection site reactions (50%) were the most common and both correlated with the presence of tetramer positive $CD8^+$ T cells. In stage III melanoma patients experiencing flu-like symptoms overall survival (OS) was not reached (median follow up time was 54.3 months) versus 32.3 months for patients not experiencing flu-like symptoms. Median OS in patients with an injection site reaction was not reached versus 53.7 months in patients without an injection site reaction. Superior clinical outcomes were also observed for stage IV melanoma patients [6].

Others have also established this correlation. Teramoto *et al.* have explored immune-related adverse events and presence of peripheral lymphocytes as possible predictive biomarkers. Specifically, the research group evaluated the effectiveness of a MUC1-targeted DC vaccine in patients with refractory non-small cell lung cancer. For this, forty patients were treated during a period of 10 years between August 2005 and May 2015. The median survival time (MST) after the initial vaccination was 7.4 months while the 1-year survival rate was 39.3%. Given that following vaccination it may take several months for activation of an anti-tumor response [3], Teramoto *et al.* explored the relationship between the number of vaccinations that patients received with survival outcome and established that the group that received six or more vaccinations achieved significantly higher MST and 1-year survival rate than those that received fewer vaccinations. It is noteworthy that the authors also evaluated the anti-tumor response via conventional RECIST criteria which showed no response, confirming the analysis of Anguille *et al.* and suggesting that new endpoints are required to

assess the clinical response to DC vaccines. Predictive biomarkers of a clinical response were then explored in the patients that received more vaccinations. In this cohort, patients who experienced immune-related adverse events, including skin reactions at the vaccination site and fever, had significantly longer survival times compared with patients without such immune-related adverse events (12.6 vs 6.7 months; p = 0.042). Longest survival times were also noticed in patients whose peripheral white cells contained over 20% lymphocytes (12.6 vs 4.5 months; p = 0.014). Importantly, MUC1-specific cytotoxic responses were achieved in all seven patients analyzed who received at least six vaccinations. Based on this, the authors concluded that immune-related adverse events and a higher percentage of peripheral lymphocytes prior to vaccination are useful to predict clinical responses [7]. It is important to note also that Teramoto *et al.* (and others) established that the robustness of the patient's immune system correlates with clinical response. In other words, patients that have a healthy presence of peripheral lymphocytes have better treatment prognosis. This has been interpreted to suggest that DC vaccines are best used early as advanced stage cancer patients frequently have weak immune systems showing low percentages of lymphocytes in the peripheral blood [7]. This has been noted before, for example, Aartzen *et al.* observed that an intact and proper functioning immune system seems to have a higher potential to react to immune therapy and concluded that "we might take better advantage of the unique capacity of DC to direct the immune response by exploiting DC-based cellular therapy earlier in the disease course" [8].

Using immune-related adverse events as a biomarker is of limited use, however. Typical adverse events are skin redness/swelling/rubor and fever. These measures are highly variable and subject to other confounding factors. Fever in particular may be affected if the patient is taking analgesic/antipyretic medicines such as ibuprofen.

GOING A STEP FURTHER: THE DTH TEST

A number of other groups have explored immune-related skin irritation further: specifically, the association between a positive reaction for the skin-delayed type hypersensitivity (DTH) test and clinical outcome. Escobar *et al.* claim to be the first to report a significant correlation between DTH positive reaction against tumor antigens and an increase of short-term progression free survival. In this study, 20 patients with malignant melanoma in stages III or IV were vaccinated with autologous DCs pulsed with a melanoma cell lysate, alone (n = 13) or in combination with low doses of subcutaneous IL-2 injections (n = 7), to assess toxicity, immunological and clinical responses [9].

To analyze the tumor cell lysate-specific reactivity, patients were evaluated using 400 µg/ml of tumor cell lysate in 200 µl aqueous solution, injected intradermally at a separate site in a volume of 100 µl. Saline solution was used as a negative control. At least 5 mm of induration or erythema, read 48 h after intradermal injection, were required to score a skin test as positive. This evaluation was made 1

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month after the end of therapy. The group found significant correlation between DTH positive responder patients and a longer stability of disease, and also a longer post-vaccination patient survival. In the study, 8 stage IV patients who showed a positive reaction showed a median TTP of 13.4 months while the group of 8 stage IV patients who did not show a DTH reaction to tumor cell lysate had a median TTP of 2.4 months. The post vaccination survival was also significantly longer in DTH responder patients (17.3 months) than in non-responders (8.6 months) [9]. Subsequently several groups have also established this correlation. Okamoto *et al.*, for example, conducted a retrospective analysis of 255 patients with inoperable pancreatic cancer who received standard chemotherapy combined with peptide-pulsed DC vaccines. The median OS from diagnosis was 16.5 months and that from the 1st vaccination was 9.9 months. The authors report that survival time of the patients with positive DTH was significantly prolonged as compared to that with negative DTH [10].

The DTH test clearly provides for a controlled assessment of the skin reaction which has advantages versus using skin-related adverse events to identify responders. Subsequent to the study discussed above, the same research group has continued to use DTH to establish response to their proprietary tumor cell lysate pool derived from metastatic melanoma cell line, TRIMEL, used in their DC vaccine TAPCells product [11]. This group also established that positive DTH and prolonged patient survival correlates with increased proinflammatory cytokine profiles. Specifically, Duran-Anioz *et al.* determined that peripheral blood lymphocytes from melanoma patients have an increased proportion of Th3 ($CD4^+$ $TGF\text{-}\beta^+$) regulatory T lymphocytes compared with healthy donors and that DTH positive patients showed a threefold reduction of Th3 cells compared with DTH negative patients after DC vaccine treatment. Furthermore, in this study it was also observed that DC vaccination resulted in a threefold increase of the proportion of IFN-γ releasing Th1 cells and in a twofold increase of the IL-17-producing Th17 population in DTH-positive compared to DTH-negative patients. The authors concluded that increased Th1 and Th17 cell populations in both blood and DTH-derived tissues may be related to a more effective anti-melanoma response [12].

The DC vaccine TAPCells is now being used commercially and a publication by Lopez *et al.* describes the use of DTH testing to assess response criteria ("patients were defined as immunologic responders if they displayed activity against TRIMEL in DTH assays"). The authors report that more than 60% of patients showed a positive DTH reaction to TRIMEL and that stage IV DTH-positive patients had a median survival of 33 months compared with 11 months observed for DTH-negative patients [13]. This approach has limitations, however. A study conducted by Dillman *et al.* concluded that DTH to autologous tumor cells (irradiated tumor cells) was neither prognostic for survival nor predictive of benefit in their MACVAC trial. This was a 5-year follow-up of a randomized Phase 2 trial of autologous DC vaccines versus autologous tumor cell vaccines in metastatic melanoma [14]. The main difference versus other

approaches appears to be that the DTH test was conducted before and shortly (1 week) after completing treatment while Escobar *et al.* specifically reported that the evaluation was conducted 1 month after the end of therapy, suggesting there is a time gap for response to the DC vaccine. It could also be that irradiated tumor cells are altogether different from the tumor cell lysates used by the TAPCells group or single peptide antigen as used by Okamoto *et al.*

USING THE DTH TEST TO MONITOR THE T-CELL RESPONSE

Coming back full circle, some research groups have gone beyond the DTH test to specifically investigate infiltrating T cells in biopsies. Lesterhuis *et al.*, actually explored this approach with success as early as 2005 and concluded that biopsies from DTH sites after DC vaccination of melanoma patients represent a convenient approach to detecting antigen-specific T-cell responses that highly correlate with clinical outcomes in stage IV melanoma patients [15]. In a subsequent study with colorectal cancer patients, Lesterhuis *et al.* reported that DTH testing provided superior results in the monitoring of antigen-specific T-cell responses compared with peripheral blood. Specifically, in none of the patients could they detect an increase of CEA-specific T cells in unstimulated peripheral blood by direct tetramer analysis, while in 7/10 patients CEA-specific infiltrated T cells were detected by tetramer analysis in DTH biopsies. These T cells were also able to be evaluated for functionality. Unfortunately, given small patient numbers and short duration of the study (the trial had to be stopped due to lack of funding) the authors could not establish correlation with clinical outcomes [4]. The conclusion of the authors was that skin testing provided superior results in the monitoring of antigen-specific T-cell responses compared to peripheral blood, lymph nodes and tumor tissue. It appears, however, that this line of reasoning has not been pursued by other groups that progressed with the translation of DC vaccines.

Clearly, being able to identify responders early during treatment can be a tool that helps clinicians improve outcomes. The ability to detect and assess the functionality of infiltrating T cells in DTH test biopsies would justify the continuation of treatment for responders and possibly improve outcomes. Thus, I would favor further exploring and validating the approach introduced by Lesterhuis *et al.*

GOING BEYOND DTH TESTING: LOOKING FOR CLUES IN MOLECULAR SIGNATURES

There is now a vast literature of reported 'molecular signatures' of disease progression due to the advent of new 'omics' technologies including gene sequencing, high throughput technologies, etc. However, there appear to be very few studies trying to identify molecular signatures in response to DC vaccination.

The exception appears to be the group responsible for developing TAPCells. This group is routinely treating patients and has established the use of DTH testing to identify responders to treatment. In a recent

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publication they reported that the DTH reaction was associated with the presence of distinct cell subpopulations in peripheral blood and have conducted molecular studies to identify gene expression markers that might serve as potential molecular biomarkers. Specifically, Garcia-Salum *et al.* used microarray analysis to profile the transcriptome of patients during treatment. Researchers identified 17 genes over-expressed in responder patients after vaccination relative to non-responders, from which ten were linked to immune responses and five were linked to cell cycle control and signal transduction. In immunological responder patients, increased protein levels of CXCR4 and CD32 were observed on the surface of $CD8^+$ T cells and B cells and the monocyte population confirming gene expression results. The clinical use of these findings as biomarkers, however, requires further investigation [16].

PATIENT STRATIFICATION TO IMPROVE CLINICAL OUTCOMES

Can patients most likely to respond to DC therapy be selected at the very start of the trial so as to maximize clinical benefit? The approach to 'arming' the vaccine may be a good place to start.

Most DC vaccines tested have been loaded with single or simple recombinant/synthetic antigenic peptide cocktails, usually targeting well-established tumor-associated antigens (TAAs) such as CEA, MUC1, gp100 or with tumor cell lysates prepared via various treatments ensuring 100% cancer cell death [17].

For DC vaccines that are 'armed' with defined TAAs, the obvious first port of call for a stratification strategy should be based, where viable, on confirmation of expression of the TAA in question. While this seems obvious, it was not routine practice in most of the early trials and perhaps partly explains why outcomes have fallen short of expectations. Teramoto *et al.* consider that selection of patients with high expression of target antigens on cancer cells is critical [7]. In the specific case of their MUC1-loaded DC vaccine, Teramoto *et al.* report that their immunohistochemistry data demonstrate that the expression of MUC1 on more than 60% of adenocarcinoma cells occurs in only about 40% of patients. Expression of MUC1 on more than 60% of adenocarcinoma was, in fact, a key eligibility criterion in their trial.

For DC vaccines that are 'armed' with tumor cell lysate, the picture is more complicated. In the case of TRIMEL, the allogeneic proprietary tumor cell lysate pool derived from metastatic melanoma cell line used in TAPCells, main antigens can be characterized and eligibility criteria can be established based on threshold levels of these in the patient's tumor tissue, if that is available. That is definitely applicable in certain clinical scenarios where tumor biopsies are available, but not all. There is, however, a need to identify universal biomarkers that could be used to identify responders at the very beginning.

TRANSLATIONAL INSIGHT

Putting it all together, there are clear learnings that can be implemented at patient selection and during

treatment to maximize clinical outcomes with DC vaccines:

- Recruit patients with robust immune systems, i.e., in early stages of cancer progression and by means of eligibility criteria based on % of peripheral lymphocytes, i.e., 20%.

- In DC treatments where tumor biopsies are available and where the antigen source is either a single defined TAA or a cocktail of defined TAAs or an allogeneic tumor cell lysate source, establish eligibility criteria based on confirmation of expression of the TAA in question. There is an argument that DC treatment may not be the best approach in clinical settings where this cannot be established.

- Implement a DTH test including analysis of biopsies to detect antigen-specific T-cell responses and use this to screen responders so as to extend treatment. The proven correlation between DTH-positive testing and improved outcomes [12,13] and the ability to detect and assess the functionality of infiltrating T cells in DTH test biopsies [15] would justify the continuation of treatment for responders which is desired as Teramoto *et al.* established that receiving more vaccinations improves outcomes.

Clearly there are many open questions:

- What should be thresholds to establish eligibility criteria either in terms of disease stage or in terms of % of peripheral lymphocytes?

- For specific TAAs or main antigens in a tumor lysate pool used to arm in a vaccine, what should be the appropriate thresholds of expression to establish eligibility criteria?

- What should be specific criteria in infiltrating T-cell composition and functionality that would warrant continuation or adjustment of treatment?

- For how long and with what frequency should vaccination continue and what should the clinician look for in the analysis of DTH biopsies to guide this?

It is noteworthy that, while the median number of vaccinations for the patients that received more than six vaccinations in the Teramoto *et al.* study was 10, the range was very wide, 6 to 42 vaccinations in total [7]. These were given bi-weekly, so patients had treatment that ranged from three months to 24 months. The correlation of presence and functionality of antigen-specific T cells in DTH biopsies with outcomes could be used to provide guidance to treatment duration. In other words, this biomarker(s) might be used to establish how much time it takes for a DC therapy to mount an effective anti-tumor immune response and establish T-cell memory.

The answers to these questions can only be explored in the clinic. The ability to conduct retrospective or meta analyses in this field is limited so shedding light on these questions will require prospective clinical work, most likely in the form of Phase 2 trials. A good example of this is the Phase 2 trial reported by Lopez *et al.* and Escobar

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et al. Specifically a Phase 2 trial with survival primary endpoints, eligibility criteria based on disease state and immune state, and ongoing monitoring of target T-cell responses via the DTH test with analysis of biopsies. A complexity to be considered in trial design, and outside the scope of this article, is that future DC trials will most likely be in the context of combination with other immune therapies, i.e., checkpoint inhibitors. This will certainly provide for more complex trial designs so as to read the effect of each therapy alone before assessing the effect of the combination therapy.

While there is still an unmet need to have robust validated assays to monitor the immunological outcome of DC vaccination in order to predict response and guide treatment, there are some basic approaches to implement and further explore in future trials which can help improve outcomes, even at an exploratory stage.

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REFERENCES

1. Garg DA, Coulie PG, Van den Eynde BJ, Agostinis P. Integrating Next-Generation Dendritic Cell Vaccines into the Current Cancer Immunotherapy Landscape. *Trends Immunol.* 2017; 38(8): 577–93

2. Anguille S, Smits EL, Lion E, Van Tendelo VFo, Berneman ZN. Clinical use dendritic cells for cancer therapy. *Lancet Oncol.* 2014; 15: e257–67

3. Hoos A, Eggermont AMM, Janetzki S *et al.* Improved Endpoints for Cancer Immunotherapy Trials. *J. Natl Cancer Inst.* 2010; 102(18): 1388–97.

4. Lesterhuis WH, De Vries IJ, Schuurhuis DH *et al.* Vaccination of Colorectal Cancer Patients with CEA-Loaded Dendritic Cells: Antigen-Specific T Cell Responses in DTH Skin Tests. *Ann. Oncol.* 2006; 17(6): 974–80.

5. Hedge PS, Karanikas V, Evers S. The Where, the When, and the How of Immune Monitoring for Cancer Immunotherapies in the Era of Checkpoint Inhibition. *Clin. Cancer Res.* 2016; 22(8): 1865–74.

6. Boudewijns S, Westdorp H, Koornstra *et al.* Immune-related Adverse Events of Dendritic Cell Vaccination Correlate With Immunologic and Clinical Outcome in Stage III and IV Melanoma Patients. *J. Immunother.* 2016; 39(6): 241–8.

7. Teramoto K, Ozaki Y, Hanaoka J *et al.* Predictive biomarkers and effectiveness of MUC1-targeted dendritic cell based vaccine in patients with refractory non-small cell lung cancer. *Ther. Adv. Med. Oncol.* 2017; 9(3): 147–57.

8. Aartntzen EHJG, Figdor CG, Adema GJ, Punt CJA, De Vries IJM. Dendritic cell vaccination and immune monitoring. *Cancer Immunol. Immunother.* 2008; 57: 1559–68.

9. Escobar A, Lopez M, Serrano A *et al.* Dendritic Cell Immunizations Alone or Combined with Low Doses of Interleukin-2 Induce Specific Immune Responses in Melanoma Patients. *Clin. Exp. Immunol.* 2005; 142(3): 555–68.

10. Okamoto M, Kobayashi M, Yonemitsu Y, Koido S, Homma S. Dendritic cell-based vaccine for pancreatic cancer in Japan. *World J. Gatrointest. Pharmacol. Ther.* 2016; 7(1): 133–8.

11. Salazar-Onfray F, Perda C, reyes D, Lopez M. TAPCells, the Chilean Dendritic Cell Vaccine against Melanoma and Prostate Cancer. *Biol. Res.* 2013; 46(4): 431–40.

12. Durán-Aniotz C, Segal G, Salazar L *et al.* The Immunological Response and Post-Treatment Survival of DC-Vaccinated Melanoma Patients Are Associated with Increased Th1/Th17 and Reduced Th3 Cytokine Responses. *Cancer Immunol. Immunother.* 2013; 62(4): 761–72.

13. López M, Pereda C, Segal G *et al.* Prolonged Survival of Dendritic Cell–Vaccinated Melanoma Patients Correlates With Tumor-Specific Delayed Type IV Hypersensitivity Response and Reduction of Tumor Growth Factor β-Expressing T Cells. *J. Clin. Oncol.* 2009; 27(6): 945–52.

14. Dillman R, Cornforth AN, Nistor G, McClay EF, Amatruda TT, Depriest C. Randomized Phase II Trial of Autologous Dendritic Cell Vaccines versus Autologous Tumor Cell Vaccines in Metastatic Melanoma: 5-Year Follow up and Additional Analyses. *J. Immunother. Cancer* 2018; 6 (1): 19.

15. Lesterhuis WJ, de Vries IJ, Shuurhuis D *et al.* Vaccination of Colorectal Cancer Patients with Carcinoembryonic Antigen Peptide-Loaded Dendritic Cells. *J. Clin. Oncol.* 2005; 23(16 Suppl.): 2544.

16. Garcia-Salum T, Villablanca A, Matthaus F *et al.* Molecular signatures associated with tumor-specific immune response in melanoma patients treated with dendritic cell-based immunotherapy. *Oncotarget* 2018; 9(24): 17014–27.

17. Banchereau J, Palucka K. Cancer Vaccines on the Move. *Nat. Rev. Clin. Oncol.* doi:10.1038/nrclinonc.2017.149 (Epub ahead of print).

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