EDITORIAL

Is immunotherapy a viable option in treating mesothelioma?
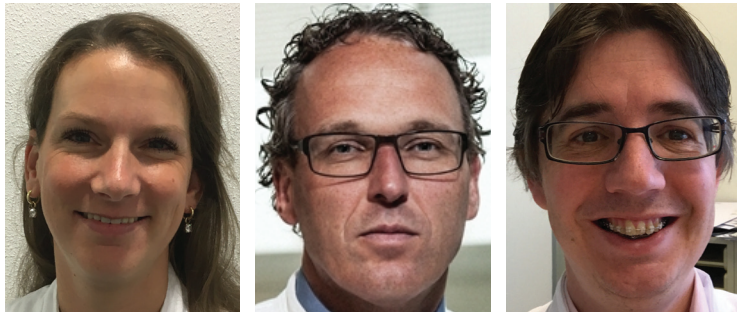
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Over the last years, immunotherapy has been on the rise as an effective treatment option in cancer. For example, immunotherapy targeting PD(L)-1 was found to be effective in non-small-cell lung cancer (NSCLC) [1], with response rates of approximately 20% in an unselected population and 45% in a selected population of patients with high PD-L1 expressing tumors [2]. Therefore, anti-PD-(L)1 has become a standard care for treating NSCLC in second line and in patients with PD-L1 expression ≥50% in first-line therapy.

In malignant pleural mesothelioma, treatment with cisplatinum/pemetrexed resulted in a survival benefit of 3 months [3]. Recently, the addition of bevacizumab resulted in an additional survival benefit of nearly 3 months [4]. Currently, no other therapy has shown activity in a randomized controlled Phase III trial. Therefore, new treatment options are more than desirable. Similar to NSCLC, immunotherapy might have the potency to provide a tumor response with durable survival in mesothelioma [1]. Although, to date no randomized Phase III study using immunotherapy has proven effective in mesothelioma, the research continues extensively. This Editorial aims to answer the question if immunotherapy could be a viable option in mesothelioma by analyzing the immune response needed for an effective immune-mediated tumor killing.

Opportunities to make immunotherapy viable in mesothelioma

Anticancer immunity is a process consisting of consecutive steps [5]. First, tumor-associated antigens are released by the tumor while it develops. Immature dendritic cells (DCs) can take up these tumor-associated antigens. Afterward, DCs mature, followed by migration to the lymphatic system. There, they can present the captured antigens in presence of major histocompatibility complex-I molecules on the cell membrane to T cells. In turn, these CD8 T cells need to travel to the tumor site, recognize the tumor and invade the tumor and initiate tumor killing.

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Future
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• Antigen recognitions & dendritic cell maturation

Mesothelioma is a tumor with evident less mutational load than NSCLC, therefore, less tumor antigens are expected, resulting in a lower chance of tumor recognition by DCs [6]. In addition, the tumor microenvironment in mesothelioma has shown to be immune-suppressive with the presence of, for example, M2 macrophages and regulatory T cells (Tregs), as well as the presence of IL-10, M-CSF, VEGF, prostaglandins and TGF-β. This also impedes DCs from their potency to act as antigen-presenting cell to T cells [7]. In addition, tumors can inactivate damage-associated molecular pattern, which is needed for DC maturation.

For immunotherapy to be effective, tumor recognition is mandatory. *Ex vivo* generated activated DCs that have been 'trained' to present tumor antigen could provide a possibility to overcome the problems stated above [8,9].

In 2010, the first clinical trial was conducted with ten mesothelioma patients using autologous tumor lysate-pulsed DCs. After completion of chemotherapy, these mature DCs were injected intradermally and intravenously. Nine patients showed a significant increase in peripheral blood mononuclear cells. Four patients showed an increased cytotoxic activity against autologous tumor cells. This vaccination with DCs was well tolerated [8].

A follow-up trial using cyclophosphamide in addition to DCs resulted in a significant immunoactivity against the tumor [9]. The mechanism of cyclophosphamide will be explained further below. To circumvent the need for autologous tumor material, in the follow-up trial, an allogeneic tumor lysate was used, which resulted in similar antitumor response [10]. Currently, a randomized Phase III study named DENIM with a planned enrollment of 230 patients is about to start.

Another option that is currently tested to activate DCs is vaccination with *Listeria* [11]. Uptake of the *Listeria*-based vaccine results in activation of both adapted and innate effector cells, by directly targeting and activating DCs *in vivo*. Engineered *Listeria*-based vaccine can express human mesothelin antigen, which is highly expressed in mesothelioma. Uptake of this engineered *Listeria* by DCs triggers a mesothelin targeted T-cell response.

• Antigen presentation to T cell

The next step in anticancer immunity is the antigen presenting of DCs to the T cells. In the case that DC maturation is progressing well,

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optimal T-cell activation may be insufficient due to lower levels of costimulatory ligands and MHC molecules [12]. Activation of T-cell receptor in absence of appropriate costimulation leads to dysfunctional T cells. By using stimulatory antibodies (such as anti-OX40) [5], anticancer immunity could be increased.

The protein CTLA-4 (cytotoxic T lymphocyte antigen-4) is mainly found on T cells and has an immune inhibitory effect. T-cell activation decreases when CTLA-4 is bound to CD-80 and CD-86. Blocking CTLA-4 could therefore be an effective antitumor treatment [13].

CTLA-4 blocking IgG2 antibody tremelimumab has been investigated in mesothelioma in the DETERMINE trial: a Phase IIB placebo-controlled study of tremelimumab in 571 advanced mesothelioma patients as second- and third-line treatment. In terms of patient number, it was the largest immunotherapy study in mesothelioma to date. Unfortunately, no survival benefit was seen in patients using tremelimumab, which might be explained by the treatment in second- and third-line setting. Of note, the authors of this Editorial have seen a response in a patient participating in this trial, showing the need for more insight into the immune response present in a patient. One treatment probably will not fit all patients [14].

Another option to overcome tumor recognition by DCs and also T-cell presentation is to produce *ex vivo* generated T cells. Chimeric antigen receptors (CARs) T-cell therapy is a form of immunotherapy whereby genetic engineered receptors expressed on T cells bind to a specific tumor-associated antigen and activate T-cell cytotoxicity. These antigen-specific CARs bound T cells are reinfused to the patient in an effort to eradicate tumors. A murine model showed that administration of CAR T cells intrapleurally needs a 30-fold lower dose to eradicate pleural tumors than intravenously administered CAR T cells, which could be an interesting strategy in mesothelioma patients. The main disadvantage is that CAR T cells only target a specific tumor antigen, allowing for tumor escape mechanism avoiding that single antigen [15]. Another hurdle encountered in the use of CAR T cells in solid tumors is the difficulty in migrating to and adequately penetrating the tumor to unleash their cytotoxic function [16].

• T-cell trafficking & infiltration

The next step in anticancer immunity is infiltration of activated T cells into the tumor. T-cell adhesion molecules are needed for T-cell

extravasation. These adhesion molecules are downregulated by, for example, VEGF, which is produced in the tumor microenvironment. By targeting VEGF by, for example bevacizumab, several tumor mechanisms are targeted, among which is ineffective T-cell trafficking. The positive result using bevacizumab in the MAPS study might be partially due to more effective T-cell trafficking [4].

• **Tumor cell killing**
If the previous stages in the immunity cycle were passed effectively in a patient, it should result in an influx of CD8+ cells in the tumor. This is also called the 'inflamed tumor microenvironment'. However, recognition of MHC complexes on tumor cells by CD8+ T cells is needed for tumor cell killing. T cells are releasing IFN-γ, which results in releasing of additional cancer antigens but also in expression of multiple coinhibitory receptors such as PD-1, LAG-3, TIM-3 and TIGIT, which together with chronic T-cell receptor signaling induces T-cell dysfunction [17]. An effective tumor treatment could be induced by blocking this negative feedback signal. Several trials have investigated PD-1/PD-L1 inhibitors in mesothelioma. The Keynote 28 is a Phase IB trial in which mesothelioma patients with a PD-L1 ≥1% were treated with pembrolizumab. This trial showed a disease control rate of 72% and an objective response rate of 20%. The median overall survival was 18 months [18].

The Phase II NivoMes study (nivolumab) and the Phase IB JAVELIN trial (avelumab) showed a disease control rate of approximately 50% by treating mesothelioma without a PD-L1 expression selection criterion. The overall response rate was 28% in NivoMes study and 9.4% in JAVELIN trial [19,20].

Local immune suppression due to immune cells in the tumor microenvironment also impedes effective tumor killing. Although M1 macrophages protect the host by producing IL-12 which stimulates the activation of NK and Th1 cells, M2 macrophages downregulate these functions and produce IL-10, which stimulates Tregs and Th2 cells and so suppresses CD8+ T-cell response [21,22].

Inhibition of M2 macrophages in mice resulted in reduced monocytes and neoangiogenesis, but without improving survival. However, combination with DC therapy resulted in improved survival and enhanced CD8+ T cells, which induces durable antitumor immunity [23].

In addition, Tregs can promote tumor progression by inhibiting CD8+ T-cell response [22]. Low-dose cyclophosphamide can reduce the number of Tregs. This mechanism was demonstrated in combination with DC immunotherapy. This trial was not powered for efficacy of cyclophosphamide alone, however [8].

Conclusion
As stated above, mesothelioma tumors have several features that can result in inferior outcomes in trials using the current checkpoint inhibitors that have resulted in positive trials in NSCLC. Most mesothelioma tumors are indeed noninflamed, with low T-cell infiltration. In those patients, treatment must focus on initiating this T-cell response. If the tumor is already inflamed or this has been archived by using a form of immunotherapy and no response is seen then treatment must focus on checkpoint inhibition or the tumor microenvironment. Treatment must be tuned to the missing step(s) in the cancer immunity cycle, and therefore combination treatment can be of added value. By using these tools, immunotherapy may prove to be a viable option for mesothelioma patients.

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