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COMMENT

Novel immune modulatory vaccines targeting TGFβ

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Immune modulatory vaccines (IMVs) are novel therapies designed to activate anti-regulatory T cells (anti-Tregs) [1]. Anti-Tregs recognize HLA-restricted antigens on the surface of regulatory immune cells derived from tumor microenvironment antigens (TMAs) [2], such as IDO [3], PD-L1 [4], and TGFβ [5, 6]. Having established that specific T cells recognize target cells in a TGFβ-dependent manner, we reported that such TGFβ-specific T cells are associated with clinical benefit and improved survival after combined treatment with radiotherapy (RT) and immune checkpoint inhibitors (ICIs) in patients with pancreatic cancer (PC) [7]. Additionally, we found that a TGFβ-based IMV impacted tumor growth in murine models of PC via immune modulation of the tumor microenvironment (TME) [8]. These data suggest that combining a TGFβ-based IMV with RT/ICI therapy will be beneficial for patients with PC.

Anti-Tregs can kill or modulate regulatory cells such as tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), tolerogenic dendritic cells (DCs), regulatory T cells (Tregs), and cancer-associated fibroblasts (CAFs), in addition to cancer cells [2]. In cancer, the activation of anti-Tregs may thus lead to an attack on tumor cells, but maybe even more importantly, it can also lead to modulation of the TME, creating an immunocompetent and tumor-hostile environment (Fig 1). In contrast to other strategies that target the immunosuppressive TME, IMVs both reprogram and directly kill suppressive cells. This reprogramming includes the conversion of cells such as TAMs into M1-like macrophages but also affects nonimmune cells such as CAFs. TGFβ is a pleiotropic cytokine and is important in tumor immune escape. It is highly expressed in CAFs, among other cells of the TME [9]. Cancer cells are often resistant to the cytostatic effects of TGFβ and instead exploit its role as a promoter of vascularization, tissue invasion, and metastasis. In contrast, tumor-specific T cells are susceptible to the immunosuppressive effects of TGFβ, which critically limit their recruitment, activation, and functionality.

We recently described the existence of T cells in healthy donors and cancer patients that specifically recognized TGFβ-derived epitopes [5, 6]. T cells specifically recognizing an epitope contained in a long TGFβ-derived peptide called TGFβ-15 were able to recognize cancer cells and myeloid cells in a TGFβ-dependent manner. TGFβ-15 was able to stimulate not only specific $CD8^+$ T cells but also $CD4^+$ T cells. Our combined findings on TGFβ-derived epitopes inducing both $CD4^+$ and $CD8^+$ T-cell immunity may be important for the effectiveness of a TGFβ-based IMV because they provide a means to reprogram the TME by both the killing of suppressive cells and the release of proinflammatory cytokines. The importance of combining $CD4^+$ and $CD8^+$ T-cell epitopes in IMVs has been illustrated in vivo in animal models of cancer [8, 10].

In general, PC is associated with a very fibrotic and immunosuppressed TME. One of the key molecules that contributes to this is TGFβ. The important role of TGFβ in resistance to ICIs [9] combined with the description of T cells that specifically recognize a TGFβ-derived epitope [5, 6] led to the investigation of spontaneous TGFβ-specific T-cell immunity in patients with PC treated with ICI combined with RT in a randomized phase 2 study [11]. Interestingly, a strong TGFβ-15-specific T-cell response before treatment initiation was independently associated with both treatment response and prolonged PFS and OS [7]. Additionally, we found that the lack of TGFβ-specific T cells in some patients was not caused by general immune dysfunction, as these patients retained a normal T-cell response to common pathogen-derived epitopes. We further found that TGFβ-15-specific T cells isolated from a patient with a complete clinical response were able to react with and kill autologous regulatory immune cells. Finally, by mimicking vaccination, we showed that repeated antigen stimulation in vitro induced or enhanced TGFβ-15-specific immune responses. Consequently, we believe that therapeutic TGFβ-15-based vaccinations in patients with PC will induce a specific T-cell response in vivo that, in combination with ICI/RT treatment, may lead to a clinical response. We have subsequently shown, in an in vivo model of a "cold" PC tumor, that TGFβ-derived peptide vaccination can control tumor growth [8]. Tumor-draining lymph nodes in vaccinated mice harbored TGFβ-specific T cells, and lymph node enlargement was positively correlated with control of tumor growth. Analysis of immune infiltration and gene expression in the TME of pancreatic tumors revealed that administration of the TGFβ-derived peptide vaccine increased the infiltration of $CD8^+$ T cells and changed the intratumoral M1/M2 macrophage ratio. While the expression of genes involved in lymphoid activation and antigen presentation was increased, the expression of CAF-related genes and genes encoding CAF-derived collagens was reduced. Finally, in functional assays, we confirmed that the TGFβ-derived peptide vaccine modified the TME, as the ability of T cells to proliferate was restored when they were exposed to tumor-conditioned medium from vaccinated mice in contrast to medium from untreated mice. Our data illustrate the ability of a TGFβ-based IMV to control tumor growth in PC and emphasize the potential of TGFβ-derived peptide vaccination as a novel immunotherapeutic approach. We showed that the vaccine combats immunosuppression and fibrosis in the TME by polarizing TME cells toward a more proinflammatory phenotype.

Due to the mechanisms of action of IMVs, combinations of immune-activating IMVs and ICIs are an appealing approach. In a

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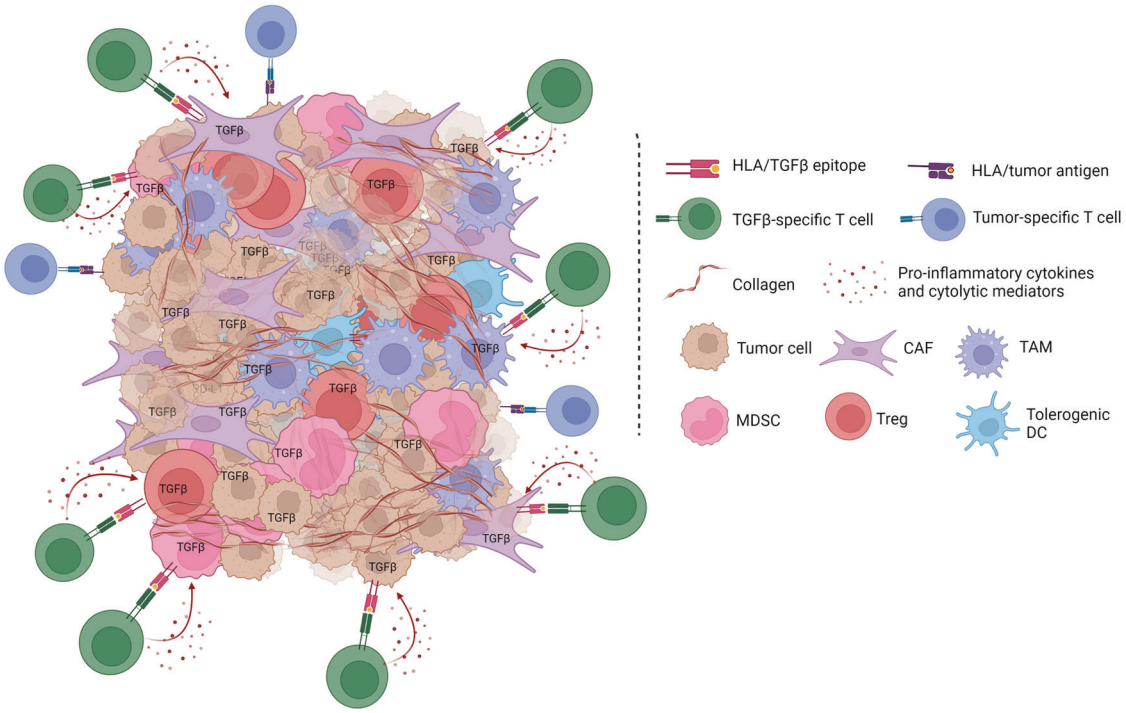

Fig. 1 Cold and fibrotic tumors are characterized by the infiltration of TGFβ-expressing cells such as tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), tolerogenic dendritic cells (DCs), regulatory T cells (Tregs), and cancer-associated fibroblasts (CAFs), in addition to cancer cells. TGFβ-specific T cells may inflame such microenvironments. TGFβ-specific T cells may modulate the TME through both direct killing and reprogramming of immunosuppressive cells (via the release of proinflammatory cytokines). The inflammation of the TME by activated TGFβ-specific T cells may also (re)activate tumor-specific T cells. TGFβ-based immune modulatory vaccines that activate TGFβ-specific T cells are thus attractive treatment modalities for cancers of different origins, including pancreatic cancer. Created with *Biorender.com*

recent phase II trial, the combination of vaccination with the two TMAs (IDO and PD-L1) and an anti-PD1 antibody showed remarkable clinical effects as a first-line treatment in patients with metastatic melanoma [12]. In that study, the objective response rate was 80%, and the complete response rate was 43%. Over the years, many immune-activating therapies have failed to show clinical benefit because we previously lacked understanding of immunosuppression in patients with cancer. Although ICIs can sometimes effectively release the suppression of T-cell activities in the TME, ICI therapy relies on de novo T-cell activation. ICIs are thus known to work best in "hot" (i.e., inflamed) tumors [12]. Thus, for the generally very "cold" PC tumors, ICIs have not been effective as monotherapy, and TGFβ expression in the PC TME is at least partly responsible for this [9]. TGFβ-based IMVs can induce novel T-cell activation and additionally target immunosuppressive cells in the TME. Therefore, the immunomodulatory effects of IMVs in combination with ICIs could increase the number of patients who respond to treatment. We believe that the TGFβ vaccine could enhance the efficacy of ICIs by combating immunosuppression in the TME and that, in turn, ICIs could promote the antitumor activity of the vaccine by reversing the inhibition of vaccine-induced TGFβ-specific T cells and other tumor-reactive cells present in the TME. These ideas will be tested in an upcoming clinical trial at our hospital (EudraCT 2022-002734-13]), approved by the Danish Medicines Agency, with the first patient expected to be enrolled in Q2 2023. Notably, IMVs have been proven to be generally safe with only low-grade toxicity [1]. For instance, the overall safety of an IMV against IDO/PD-L1 combined with nivolumab was comparable to that of nivolumab monotherapy [12]. In line with these findings, we found that the IMV with TGFβ-derived peptides was a safe therapeutic approach in animal models [8].

Thus, TGFβ-based IMVs represent a novel exciting treatment modality that is currently under intense investigation in patients with cancer. Importantly, IMVs should not be pursued only in cancer, as they are just as relevant in infectious diseases [13]. During infections, the immune system protects the host by defeating the pathogen through a balance of stimulating and inhibitory mechanisms to simultaneously prevent a harmful overreaction. Unfortunately, this activation of the regulatory immune system also causes regulatory cells to play a crucial role in the pathogenesis of persistent infections. The activation of the adaptive immune response is an apparent goal of therapeutic interventions for infectious diseases, and IMVs thus represent a novel—and not yet explored—therapeutic option for many different forms of persistent infections. Immune regulatory cells can be induced directly in response to the infection of immune cells with viruses and intracellular pathogens or indirectly through the inflammatory process [14]. For example, exhausted antiviral T cells have been reported in patients with both chronic hepatitis B and C virus infections. IMVs may also be used in common acute infections. Thus, epithelial-derived TGF-β acts as a proviral factor in, e.g. the lungs during flu infection, and increased TGFβ activity has been described during serious COVID-19 infection [15]. Importantly, anti-Tregs have been described to increase T-cell immunity toward viral antigens [1]. Thus, these matters warrant both preclinical and clinical studies to explore the potential of IMVs in many types of infectious diseases.

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COMPETING INTERESTS

MHA is a Founder, shareholder and scientific advisor of IO Biotech ApS.

ADDITIONAL INFORMATION

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