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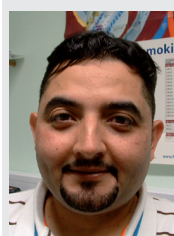
5T4 as a target for immunotherapy in renal cell carcinoma

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“5T4 restricted expression on tumor tissues and its association with tumor progression and poor prognosis has driven the development of 5T4 vaccine and antibody-targeted immunotherapies.”

Renal cell carcinoma (RCC) accounts for 2% of all new cancer cases worldwide [1]. The management of metastatic RCC (MRCC) represents a therapeutic challenge. Radical nephrectomy can be curative for early-stage disease; however, approximately a third of patients present with metastatic disease, and a further third will subsequently relapse after initial surgery [2,3]. MRCC is resistant to cytotoxic chemo- [4], hormone- and radio-therapies [5] and responds modestly to immunotherapies, including cytokines, monoclonal antibodies and tumor vaccines [6]. Recently developed targeted agents, such as the VEGF receptor and mTOR inhibitors, are currently the standard of care for most MRCC patients. Although they provide good rates of disease control and have relatively manageable toxicities for most patients, the benefit is relatively limited and there are few if any durable remissions once treatment is stopped [7]. In view of the occasional spontaneous regression of metastasis after nephrectomy [8] and the durable objective response observed following administration of immunomodulatory therapies, such as IL-2 [9], RCC is considered an immune-sensitive tumor and attempts to increase the rate of sustained responses using immunotherapeutic modalities are justified.

5T4 oncofetal antigen

A useful target tumor-associated antigen (TAA) for RCC would be expressed by most RCCs but have no or only limited expression in normal tissues [10,11]. 5T4 oncofetal antigen, a 72-kDa membrane glycoprotein, was discovered while searching for molecules with invasive

properties likely to be shared by trophoblast and cancer cells [12]. It is expressed by many different carcinomas while showing only low levels in some normal tissues [13]. Our immunohistochemistry studies have shown that the majority of RCCs strongly express 5T4 [14], making this tumor an attractive target for 5T4-directed immunotherapy. 5T4 expression has been shown to influence adhesion, cytoskeletal organization and motility properties [15–17], which might account for its association with a poorer clinical outcome in some cancers. Recent studies have shown that upregulation of 5T4 expression is a marker of loss of pluripotency in the early differentiation of human and murine embryonic stem cells [18,19] and forms an integrated component of an epithelial mesenchymal transition [20,21]. Epithelial mesenchymal transition occurs during embryonic development and is believed to be important for the metastatic spread of epithelial tumors [22]. 5T4 restricted expression on tumor tissues and its association with tumor progression and poor prognosis has driven the development of the 5T4 vaccine and antibody-targeted immunotherapies.

5T4 vaccine (TroVax®)

TroVax® consists of a highly attenuated and efficacious modified vaccinia virus Ankara (MVA) strain, expressing human 5T4 (h5T4). Preclinical studies in mice have demonstrated, in active treatment studies, that inoculation with MVA-h5T4 was able to treat established CT26-h5T4 lung tumors and B16-h5T4 subcutaneous tumors. Furthermore, vaccination with MVA-mouse 5T4 (m5T4)

was immunogenic (breaking tolerance) in mice but had no signs of autoimmune toxicity [23]. In humans, CD8⁺ and CD4⁺ T cells recognizing h5T4 can be generated from peripheral blood lymphocytes of healthy individuals [24,25]. Importantly, h5T4-specific CD4⁺ T cells are more readily generated with prior depletion of CD25⁺ Tregs. These observations imply that the availability of the h5T4 repertoire may only be optimally accessed in the absence of Treg activity, and these cells play an important role in controlling their activation/expansion.

Overall these data have supported the use of TroVax in several Phase I and II studies in cancer patients that have provided evidence of immunogenicity and safety [26–28]. In a recent Phase II trial, two TroVax vaccinations were given to patients both pre- and post-surgical resection of liver metastases secondary to colorectal cancer. 5T4-specific cellular responses were assessed at entry and 2 weeks after each vaccination by proliferation of fresh lymphocytes and ELISA for antibody responses, and 95% of patients showed a 5T4-specific cellular and/or humoral response [29]. In patients who received at least four vaccinations and potentially curative surgery, those with above median 5T4-specific proliferative responses or T-cell infiltration into the resected tumor showed significantly longer survival compared with those with below median responses [29]. These data suggest that the magnitude of 5T4 proliferative responses and the density of CD3 cells in colorectal cancer liver metastases are associated with longer survival. We also assessed the levels of systemic Tregs, plasma cytokine levels, phenotype of tumor-infiltrating lymphocytes (TILs), including Tregs, and tumor HLA class I loss of expression. More than half of the patients showed phenotypes consistent with relative immunosuppression and/or escape, making it difficult to define any simple correlation with clinical outcome [30]. Tregs are important regulators for immune homeostasis but they are also able to suppress anti-tumor immunity and consequently impair immunotherapy. Another study has shown that Tregs inhibiting h5T4-specific immune responses are increased in colorectal cancer patients, compared with healthy donors [31].

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Phase II trials of TroVax combined with IL-2 or IFN- α in MRCC have also established immunogenicity and safety [32–35]. Administration of TroVax with IFN- α was shown to be safe with evidence of 5T4-specific immune response and encouraging survival [32,34]. A randomized, placebo-controlled Phase III study (TroVax Renal Immunotherapy Survival Trial [TRIST]), comparing TroVax or placebo in combination with standard treatment has recently been completed. This study investigated whether TroVax added to first-line standard of care (IFN- α , IL-2 or sunitinib were allowed as standard care according to local preference) prolonged the survival of patients with locally advanced or metastatic clear cell renal adenocarcinoma. TroVax was well tolerated when administered alongside IL-2, IFN- α

or sunitinib. While the primary end point was not met, a significant survival advantage was seen in good-prognosis patients treated with IL-2 plus TroVax compared with patients treated with IL-2 alone [36]. This combination of IL-2 and TroVax appears promising and may be worthy of exploring further in this subgroup of patients. Antibody responses against 5T4 were induced in most TroVax-treated patients and were associated with enhanced survival irrespective of the health status of the patient [36].

“The group of patients receiving the highest drug [5T4Fab–SEA] exposure lived almost twice as long as expected, while the low-dose group lived as expected from their Motzer scores.”

The lack of benefit seen in other subgroups may reflect difficulties in optimizing immune responses, and obtaining the optimal type of immune response especially when combinations of therapies are used. For example, it has been shown that a significant increased frequency of systemic Treg levels in RCC patients compared with normal donors is observed and this was associated with adverse overall survival [37]. Although CD4⁺ T cells spontaneously recognizing a 5T4 epitope restricted by HLA-DR have been identified in TILs from a RCC lung metastasis [24], such h5T4-specific CD4⁺ T cells that may be boosted or induced by vaccination could deliver both positive and negative modulation of anti-tumor responses. Importantly, in the latter work, these cells secreted both IFN- γ and IL-10, which could indicate a Treg phenotype. Approaches to modulating Treg activity and boosting 5T4 immunity in patients are in progress. A Phase I trial to assess the feasibility and safety of adoptive transfer of Treg-depleted autologous T cells following conditioning chemotherapy in six patients with advanced RCC reported one patient with increased specific response to 5T4 coinciding with the nadir of Treg [38]. However, for routine clinical practice, simplified methods of Treg depletion are required.

5T4 antibody-directed superantigen therapy

The exploitation of active immunization may be particularly favorable for TAA where tolerance is incomplete but passive immunization may offer an additional strategy where the immune repertoire is affected by either tolerance, immunosuppression or tumor escape. Tumor-targeted superantigens treat tumors via the local activation of the patients' cytotoxic T cells through a process termed superantigen antibody-dependent cellular cytotoxicity. Using this approach, 5T4 was targeted by genetically fusing a murine Fab fragment of the monoclonal antibody 5T4 to a mutated superantigen staphylococcal enterotoxin A (SEA) and the maximum tolerated dose and safety was established in a trial in non-small-cell lung carcinoma patients [39]. A Phase II study of RCC patients receiving at least two 4-day cycles of individualized doses of 5T4Fab–SEA (ABR-214936) treatment approximately 1 month apart has been completed. When stratified by the Motzer prognostic criteria, there is a prolonged survival compared with published

expectation. The group of patients receiving the highest drug exposure lived almost twice as long as expected, while the low-dose group lived as expected from their Motzer scores [40]. The increase in circulating IL-2 levels after treatment provides a useful biomarker for clinical effect since patients with the highest increase in IL-2 at the second treatment day lived significantly longer [40]. The next generation of drug, known as ABR-217620 or ANYARA, has been developed. The principle advantages are reduced binding to preformed superantigen-specific antibodies, lower toxicity, higher affinity for 5T4 and improved tumor cell killing [41]. ABR-217620 was shown to be safe for the treatment of RCC and non-small-cell lung carcinoma in a Phase I trial and demonstrated evidence of immunological and anti-tumor activities [42]. A randomized Phase II/III trial of ABR-217620 combined with IFN- α versus IFN- α alone in patients with advanced RCC has been completed, and clinical and immunological results are awaited.

T-cell-based immunotherapy

In adoptive cell therapy, tumor-specific T cells isolated from peripheral blood mononuclear cells or TILs could be expanded *ex vivo* prior to infusion into patients. T-cell-based immunotherapy using natural or gene-modified T cells has shown success for treating metastatic melanoma. However, similar attempts in MRCC have not been encouraging and we have recently reviewed the potential strategies for improving the efficacy of T-cell-based immunotherapy in MRCC [43].

“5T4 oncofetal antigen is a suitable tumor-associated antigen target for immunotherapeutic approaches in renal cell carcinoma.”

Another way to harness T cells with the specificity of antibody is via their transduction to express chimeric immune receptors (CIRs) consisting of a scFv fused to an extracellular spacer domain, coupled to a transmembrane and cytosolic region of a key signaling molecule (CD3 ζ). Although 5T4 has not yet been used as a target for T-cell therapy in clinical trials, a CIR that targets 5T4 has been tested *in vitro* [14]. T cells from RCC patients can be used to generate CIR effectors able to kill 5T4-expressing RCC cells *in vitro* [14]. This could be a powerful strategy that bypasses a number of mechanisms that allow tumors to escape T-cell killing and it can be readily scaled up for

clinical use [44]. Other preclinical studies have generated murine T cells expressing CIR against h5T4 and evaluated their tumor therapeutic efficacy alone and in combination with immunization using a replication defective adenoviral h5T4 vaccine and bone marrow-derived dendritic cells (DCs). In the B16-h5T4 melanoma model, early local subcutaneous, but not systemic administration of syngeneic 5T4-specific CIR T cells resulted in significantly improved survival [45]. This anti-tumor activity was enhanced when combined with a post-tumor challenge immunization with vaccine followed by post-CIR T-cell treatment with DCs. This synergistic effect was lost without delivery of the DCs. These findings suggest that combining engineered T cells with specific vaccination strategies can improve the active tumor therapy [45].

Conclusion

Renal cell carcinoma remains a difficult malignancy to treat, with immunotherapy being an option that can achieve durable objective responses, albeit sporadically. 5T4 oncofetal antigen is a suitable TAA target for immunotherapeutic approaches in RCC. Large-scale trials of both vaccines and antibody-based therapies have been completed and the full results are eagerly awaited. T-cell-based immunotherapy targeting h5T4 warrants further investigation using either natural or gene modified 5T4-specific T cells. Further evaluation and development of 5T4 immune targeting by combining with other immunotherapeutic modalities (e.g., cytokines, Treg depletion/inhibition or anti-CTLA4) in selected cancer patients may provide synergy and improved clinical outcomes. Identifying biomarkers prognostic of patient clinical response and/or immune parameters that correlate with efficacy is of prime importance. With respect to TroVax, candidate biomarkers for further investigation include pre-existing lymphocyte proliferative response, vaccine-induced 5T4-specific antibody and/or lymphoproliferative responses, as well as the balance of TILs phenotype.

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