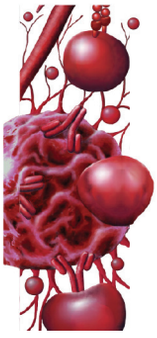
Review

Immunotherapy



Immunotherapy of cancer: targeting cancer during active disease or during dormancy?

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Immunotherapeutic targeting of advanced stage cancers has prolonged the survival of cancer patients, yet its curative efficacy is limited due to tumor immunoediting and escape. On the other hand, human vaccines have been able to eradicate smallpox and control several other infectious diseases. The success has resulted from the administration of vaccines in prophylactic settings, or during latency periods in order to protect an individual during future exposure to the disease rather than curing an established disease. Therefore, administration of immunotherapy at the right time is the key to success. However, instead of focusing on the prevention of cancer, current cancer immunotherapies are often being used in a therapeutic setting with the goal of eliminating tumor cells. The present review of evidence related to cancer immunotherapeutics suggests that immunotherapeutic targeting of tumor dormancy could be more promising than targeting of advanced stage disease to achieve a cure for cancer.

First draft submitted: 21 April 2017; Accepted for publication: 9 August 2017; Published online: 15 September 2017

Current strategies for improving the efficacy of cancer immunotherapy rely on: strengthening antitumor immune responses by modulating tumor cells to become highly immunogenic and/or reprogramming of T cells to increase their affinity and avidity for tumor antigens as well as their sustainability in the host in order to improve humoral and cell-mediated immune responses, overcoming immune suppressive pathways by targeting Tregs and myeloid-derived suppressor cells (MDSCs), and overcoming immune tolerance by the blockade of the immune checkpoint pathways. These strategies, alone or in combination, have shown promising results against established cancers in some, but not all, patients. Very recently, attempts were made to identify and target mutated neoantigens in order to develop personalized immunotherapy, and thus, make it effective for all cancer patients. Here, we provide a review of literature highlighting the challenges that these strategies are facing. This review demonstrates that immunotherapeutic strategies that improve efficacy of tumor-reactive T cells, modulate the tumor-immune cells crosstalk or target some tumor escape mechanisms can at best prolong survival of cancer patients and cannot guarantee cancer cure. Based on recent observations that quiescent dormant tumor cells are not able to undergo immunoediting [1], we suggest that the immunotherapeutic targeting of tumor dormancy with the goal of maintaining tumor dormancy and preventing cancer recurrence, would be an effective strategy in containment or cure of cancer.

Immunotherapeutic targeting of advanced cancer prolongs patient survival but comes short from achieving cancer cure

Studies which demonstrated that the cellular arm of the immune system might be responsible for tissue rejection [2] led investigators to postulate the use of immune cells for the treatment of tumors. The first clinical study in humans demonstrating immune responses generated by tumor infiltrating lymphocytes (TIL) against autologous

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tumors was published in 1987 [3]. TILs have been detected in the stroma of various cancers, and have been harnessed for adoptive cellular therapy (ACT). Conditioning the host environment by a nonmyeloablative (NMA) lymphodepleting regimen (cyclophosphamide and fludarabine) prior to ACT showed increased tumor responses [4]. In order to ascertain the degree of the effect of lymphodepletion, a pivotal follow-up study by Goff *et al.* randomized 51 patients to receive an NMA lymphodepleting regimen (cyclophosphamide and fludarabine) and 50 patients to receive an NMA regimen along with 1200 cGy of total body irradiation (TBI) prior to receiving autologous TIL. The results revealed that even though the objective response (OR) rate was higher in the NMA + 1200 cGy arm (62%) compared with the NMA arm alone (45%), both regimens had almost identical complete response (CR) rates of 24% [5]. In a prior study by the same group, the degree of lymphodepletion (chemotherapy alone) was noted to show increasing CRs of 12, 20 and 40%, respectively [5]. All these patients were previously heavily treated with other regimens for advanced melanoma (high-dose IL-2, anti-CTLA-4, anti-PD-1, a combination of anti-CTLA-4, anti-PD-1, IFN-α, dacarbazine, temizolamide, small molecule inhibitors and biochemotherapy). None of the prior treatment strategies were reflective of any correlation to observed tumor responses in either arm on subgroup analysis [5]. The duration of ongoing CR was 53.4 months as of the date of publication and one patient with CR recurred at 19 months. Even though these studies showed successful ACT and improvement in degree of tumor response with increasing lymphodepletion, this was not sustained in the partial responders and did not reach statistical significance. In a Phase II clinical trial, Chandran *et al.* [6] evaluated the effect of autologous $CD8^+$ T cells clones against MART-1 or gp100 in patients with refractory metastatic melanoma. Fifteen patients treated with these highly avid clones resulted in immune-mediated targeting of skin melanocytes in 11 patients (73%) with minor transient tumor response by Response Evaluation Criteria In Solid Tumors criteria [7] but no OR in spite of successful clonal repopulation and engraftment in the host [6]. Multiple studies in both murine and human models have shown that younger the T cells are the higher the likelihood of antitumor efficacy is [8–10]. In a pilot study, 33 patients were treated with lymphodepleting chemotherapy alone followed by $CD8^+$ enriched young TIL and 23 patients received lymphodepleting chemotherapy and 6Gy TBI followed by $CD8^+$ enriched young TIL (longer telomeres, higher expression of CD27/28). Nineteen of the 33 patients (58%) showed OR by Response Evaluation Criteria In Solid Tumors criteria, including three CR (9%) and 16 partial responders (48%). In the arm receiving additional TBI, 11 out of 23 patients showed an OR (48%) including two patients with CR (9%), with CR similar to previous cohort receiving lymphodepleting chemotherapy alone. It was noticed that in comparison to prior standard TIL therapies, this study cohort that received younger TIL following transfer showed higher level of absolute lymphocyte count on reconstitution suggesting as increased capacity for *in-vivo* expansion for younger TIL compared with selected TIL previously described [11,12]. Analysis among subsets of memory T cells in different studies has indicated that central memory T (T_{CM}) cells are more efficient in antiumor activity in comparison to effector memory T (T_{EM}) cells [13–15]. Among $CD8^+$ memory T cells, T memory stem cells (T_{SCM}) have been identified with even superior antitumor properties compared with other subsets of memory T cells [16].

Modulating the crosstalk between T cells & tumor cells improves the efficacy of cancer immunotherapy but could also induce tumor immunoediting & escape

According to the self-nonself theory of immunity, tumors are often incapable of inducing an effective antitumor immune response because of the expression of self-antigens. Therefore, enhancing immunogenicity of tumor cells and increasing the affinity of T cells for the antigen are expected to modulate the crosstalk between tumor cells and T cells, thereby improving the efficacy of cancer immunotherapy. To test this hypothesis, Yu and colleagues used double-transgenic mice engineered to express both human T-cell receptor chains against gp100 antigenic peptides in T cells and human MHC-I domains in somatic cells. They demonstrated that a mutant gp100 peptide serving as a foreign-like antigen, induced a stronger immune response leading to tumor inhibition compared with a native peptide. However, a complete regression of the tumor was not achieved [17]. In clinical settings, targeting mutant peptides or neopeptides by means of adoptive T-cell therapy resulted in the stabilization of metastatic cholangiocarcinoma for 13 months, and then, disease progression was observed in the lungs [18]. In a separate study, adoptive T-cell therapy using a polyclonal $CD8^+$ TIL recognizing mutant KRAS G12D in a patient with metastatic colorectal cancer resulted in the objective regression of all seven lung metastatic lesions. However, one lesion escaped through loss of heterozygosity of the copy of chromosome 6 that encoded HLA-C*08:02 [19,20]. We also observed complete regression of neu overexpressing mammary carcinoma in wild-type FVB mice in a T-cell-dependent manner recognizing the rat neu protein as a foreign protein. However, a fraction of animals experienced tumor recurrence due to neu antigen loss [21,22]. Similar observations were made in a preclinical

model of breast cancer, and in patients with multiple myeloma when tumor cells were epigenetically modulated by the administration of hypomethylating drugs in order to express cancer testis antigens (CTA) [1,23]. ACT by means of genetically engineered T cell receptor recognizing a cancer testes antigen NY-ESO in patients with either melanoma or synovial cell sarcoma, showed an OR of nine out of 17 patients (52%). Five patients with metastatic melanoma showed OR including two CR (on going at 22, 20 months as of the date of publication), and four out of six patients (66%) with synovial sarcoma showed OR though partial with one lasting 18 months [24]. In the FVBN202 transgenic mouse model of breast carcinoma, adoptive T-cell therapy combined with decitabine prolonged survival of animals bearing lung metastasis, but animals eventually succumbed to metastatic tumors due to tumor immunoediting characterized by the downregulation and loss of tumor antigens as well as upregulation of PD-L1 [1]. In patients with multiple myeloma, use of azacytidine resulted in the expression of CTA in tumor cells and the induction of CTA-reactive immune responses, leading to tumor regression following autologous stem cell transplantation [23]. However, some patients experienced tumor relapse associated with loss of CTA in their tumor cells (Payne *et al.*, Unpublished Data). To this end, modulation of the antigenic profile of tumors improved the efficacy of immunotherapy but was not able to overcome tumor immunoediting and escape from immunotherapy. Similar results were obtained using engineered T cells. Chimeric antigen receptor (CAR) T-cell therapy targeting CD19 resulted in complete remissions in some patients with relapsed/refractory acute lymphocytic leukemia (ALL) [25,26]. This therapy also induced CD19 loss, which is a limiting factor for its therapeutic efficacy. In two patients with refractory $CD19^+$ ALL, CAR T-cell therapy led to a complete remission, which was sustained in one patient during a follow-up period of 9 months, and led to relapse of CD19 negative ALL after 1 month [27]. To overcome tumor escape, T cells were collected from patients whose tumors lost CD19, and modified to target CD22. Again, tumor relapse was evident as a result of CD22 downregulaion or total loss [28]. It appears that IFN-γ produced by T cells is responsible for inducing tumor immunoediting [29,30]. Such tumor immunoediting has not been observed in adults with chronic lymphocytic leukemia [31]. This could be due to the state of dormancy in residual tumor cells since CAR therapy was used after the establishment of stable disease by using bendamustine with rituximab chemotherapies in adults with chronic lymphocytic leukemia. The study did not examine whether stable disease was in the state of cellular dormancy. We have recently reported that quiescent, but not indolent, dormant tumor cells are resistant to immunoediting [1].

Targeting tumor escape mechanisms: MDSCs, Tregs and immune checkpoints

Active solid tumors often induce and recruit MDSCs and/or Tregs, thereby inhibiting the efficacy of antitumor immune responses. A meta-analysis of eight studies that included 442 patients with solid tumors showed that MDSCs were associated with poor overall survival [32]. In patients with advanced non-small-cell lung cancer (NSCLC), multivariate analysis revealed an independent association of MDSCs with decreased progression free-survival and overall survival [33]. A meta-analysis of 18 published studies that included 8562 patients with breast cancer showed an association between Tregs infiltration and poorer prognosis [34]. Similar results were reported from patients with prostate cancer [35]. Analysis of the peripheral blood of 41 patients with prostate cancer and 36 healthy controls showed an increased frequency of MDSCs and Tregs in patients with prostate cancer associated with poor prognosis [35]. In addition, FOXP3 immunohistochemistry analysis of tissue microarray from 2002 prostate cancer patients revealed a higher number of intratumoral $FOXP3^+$ Tregs associated with a more advanced tumor stage [36]. Although, control of MDSCs and Tregs restored antitumor immune responses, it did not produce a curative outcome in cancer patients. In order to target MDSCs and Tregs as well as to increase the efficacy of adoptively transferred TIL, conditioning regimens were used prior to ACT. Murine models and follow-up human studies demonstrated that use of lymphodepletion prior to cell transfer increased the effectiveness of ACT significantly [11]. Lymphodepleting regimens could increase the persistence of transferred T cells [4], deplete endogenous lymphocytes and myeloid cells containing Tregs [37], increase levels of homeostatic cytokines (IL-7 and IL15) as well as remove their sink as seen in both murine and human studies [38]; and finally, they enhance the efficacy of ACT by activating antigen presenting cells via stimulation of toll-like receptors resulting from translocation of commensal microflora across mucosal barriers [39]. Addition of the immune checkpoint inhibitors, however, produced OR in some patients. Use of the phosphodiesterase-5 inhibitor tadalafil has also been associated with depletion in MDSCs [40]. In patients with head and neck squamous cell carcinoma, tadalafil treatment significantly reduced both MDSCs and Tregs, and increased tumor-specific immune responses, though no OR was reported [41]. Therapeutic targeting of immune checkpoints pathways has found to be effective in producing objective clinical responses. The use of neoadjuvant anti-CTLA4, ipilimumab, in patients with regionally advanced melanoma resulted in elevated T-cell responses

against NY-ESO-1, MART-1 and gp100 antigens associated with decreased tumor infiltrating Tregs and MDSCs, and improved progression-free survival for 1 year [42]. Anti-PD1 and anti-PD-L1 immunotherapies have been highly effective for patients with NSCLC, bladder cancer, head and neck cancer and Merckel cell carcinoma. These immune checkpoint inhibitors are the only US FDA approved drugs for bladder cancer in the past 20 years [43].

Immunotherapeutic targeting of tumor dormancy

Four decades ago, Gray & Watkins published a comprehensive review article related to cancer immunotherapy in which they attributed spontaneous regression of neuroblastoma, hypernephroma, choriocarcinoma and melanoma as well as the existence of tumor dormancy to the host-immune system [44]. The notion that tumor dormancy is controlled by the immune system was further supported in six cases of NSCLC exhibiting strong delayed hypersensitivity reactions to the soluble tumor antigens following immunotherapy. These patients ended up with tumor recurrence after an immunosuppressive event or drug treatment [45]. It was also reported that immunization by means of irradiated tumor cells can establish and maintain tumor dormancy in a murine model of B-cell leukemia/lymphoma [46]. Antibody response [47,48] and IFN-γ producing $CD8^+$ T cells [49] were found to be responsible for maintaining the murine B-cell lymphoma in a dormant state. In breast cancer patients, presence of tumor dormancy in the bone marrow was associated with an increase in $CD8^+$ T memory cells that were reactive against HLA-A2/HER-2/neu(p369–377) tumor antigen [50]. Two FDA-approved monoclonal antibodies, trastuzumab and pertuzumab, targeting HER2/neu can also prolong tumor dormancy as evidenced by delaying tumor recurrence and increasing progression free survival and overall survival of patients with invasive breast cancer [51]. Similar observations were made in patients with prostate cancer. Approximately, 70% of patients with prostate cancer have disseminated dormant cells in the bone marrow [52]. Recently, TGF-β was reported to be involved in maintaining prostate cancer dormancy in the bone marrow [53]. It remains to be determined whether TGF-β producing Tregs may contribute to prostate cancer dormancy.

Recent reviews of literature on tumor dormancy and immune response suggest tumor dormancy as the best target for immunotherapeutic prevention of tumor recurrence and advanced disease prophylaxis [54–56]. This is because dormant tumor cells that have been established by chemotherapy or radiation therapy remain susceptible to immunotherapy [1]. A prospective, randomized, multicenter Phase II clinical trial evaluated the efficacy of GP2+GM-CSF vaccine in HLA-A2+, $HER2^+$, node-positive and high-risk node-negative breast cancer patients. The vaccine was administered when patients were found to be disease-free, though might have harbored dormant tumor cells, in other words micrometastatic disease. This vaccination during tumor dormancy resulted in 5-year disease-free survival in 100% of $HER2^+$ patients compared with 89% disease-free in control patients [57]. However, the caveat is that dormant tumor cells could undergo immunoediting and eventually escape and relapse. In particular, indolent dormant cells are susceptible to immunoediting and escape. High grade tumor clones that are susceptible to chemotherapy or radiation therapy could become dormant but low grade tumor clones that do not respond well to these treatments could establish micrometastatic minimal residual disease. While dormant cells contain $Ki67^{low}$ indolent and $Ki67^-$ quiescent tumor cells [1], minimal residual disease is composed of indolent tumor cells, and more susceptible to immunoediting compared with dormant cells. In general, proliferating tumor cells either in the form of active disease or in the form of minimal residual disease or indolent dormancy are prone to immunoediting depending on the selective therapeutic pressure. Cancer therapeutics that could induce G0 cell cycle arrest could establish a quiescent type of tumor dormancy that is incapable of change and escape from therapy. It was reported that IFN-γ producing $CD8^+$ T cells were responsible for establishing and maintaining tumor dormancy, as well as inducing tumor immunoediting and subsequent tumor recurrence [1,21,29]. A very recent report identified IFN-γ as a key cytokine responsible for tumor immunoediting [30]. To this end, we reported that Ki67- quiescent, but not $Ki67^{low}$ indolent, dormant cells were resistant to immunoediting [1]. Therefore, the challenge in immunotherapeutic targeting of tumor dormancy is to dominate a quiescent type of tumor dormancy by means of conditioning regimens prior to immunotherapy in order to overcome tumor immunoediting and escape from immunotherapy. Alternatively, combination of targeted therapies with immunotherapy could inhibit certain immunoediting pathways in indolent dormant tumor cells. For instance, MYC inhibitors could prevent the expression of PD-L1 and CD47, because these immunoediting pathways are regulated by MYC [58]. Also, immunotherapeutic targeting of escape mechanisms such as PD-L1 or CTLA-4 expression could be overcome by immune checkpoint inhibitors [59]. The challenge is that tumor cells utilize numerous escape mechanisms; thus, some tumor clones could still escape from targeted therapies or immune checkpoint inhibitors.

Future perspective

Recently, there have been significant advances in the field of cancer immunotherapy. However, these advances have been limited to increasing patients' survival for a limited period of time when immunotherapeutics are administered in a therapeutic setting against advanced stage disease. For instance, T-cell-based therapies could produce CRs, yet they could not overcome tumor escape and recurrence in some patients. Similar observations were made in other immunotherapeutic approaches when targeting advanced stage diseases. For instance, Sipuleucel-T (Provenge) has extended survival of patients with metastatic prostate cancer by median 4.1 months [60]. The significance of immune checkpoint inhibitors is an increased survival tail in some patients with certain types of cancer, which has not been achieved by standard-of-care chemotherapies. Cumulative response rates for the anti-PD-1 antibody among patients with NSCLC, melanoma and renal cell cancer were 18, 28 and 27%, respectively. Responses were durable such that 20 of 31 responses lasted 1 year or more in patients with 1 year or more of follow-up [61]. To increase the size of survival tails, other checkpoint pathways should be identified and targeted; yet, immune checkpoint inhibitors cannot work for certain types of cancer that are weakly immunogenic to induce antitumor immune responses. To this end, immunogenic chemotherapies or radiation therapies should be considered to render all types of cancer responsive to immune checkpoint inhibitors. Alternatively, administration of immunotherapy including immune checkpoint inhibitors during tumor dormancy as a relapse prophylaxis regimen could be more effective, as prophylactic vaccines have been successful against many infectious diseases, as well as against HPV-associated cervical cancer [62]. In addition, application of stem cell transplantation and donor-derived lymphocyte infusion is successful only against minimal residual disease rather than against active and advanced stage disease. Therefore, it is reasonable to expect that the administration of immunotherapy during minimal residual disease or tumor dormancy could deliver a curative outcome.

Financial & competing interests disclosure

This work was supported by the Office of the Assistant Secretary of Defense for Health Affairs through the Breast Cancer Research Program under Award No. W81XWH-14–1–0087, and partly supported by pilot funding from the VCU Massey Cancer Center supported, in part, with funding from NIH/NCI Cancer Center Support Grant P30 CA016059. Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the US Department of Defense. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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