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Overview

Recent advances in clinical anti-cancer immunotherapy

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Abstract

Recent successful results with the relatively novel immunotherapeutic anti-cancer strategies such as adoptive T cell transfer (ACT), engineered T cells with chimeric antigen receptors (CARs), therapeutic Sipuleucil-T vaccine and checkpoint blockade inhibitors, do indicate that patient's immune system can be effectively used against autologous tumor cells. Interactions between the immune system and the malignancy are complex but the results obtained using the above mentioned therapeutic approaches indicate acceptable clinical utility, efficacy and safety against several types of cancer. Much work still lies ahead but the success achieved with these modern immunotherapies is undeniable. This paper aims to present a short basic overview of these recent advances in cancer immunotherapy, but one should keep in mind that this field is in a dynamic stage given its success and that many immunotherapeutic agents, not all of them mentioned, are undergoing active clinical testing.

INTRODUCTION

The established clinical therapeutic modalities for cancer treatment are surgery, cytotoxic chemotherapy and radiotherapy applied either alone or in combinations depending on the tumor type, tumor stage, patient's general condition and functional organ reserves. The treatment intention may be curative or the palliative. Newer molecularly targeted drugs and biologic agents have shown activity in some cancers refractory to traditional chemotherapeutics, but in some tumor types they too do not appear to be as effective as initially thought. Besides the primary goal of having an effective anti-cancer therapy, the side effects ranging from tolerable ones to sometimes even fatal ones should also always be taken into account in therapy planning. Moreover, the price of such newer drugs and treatments compared to their effectiveness is becoming more and more relevant, especially where the cure or long term tumor control is not predictable or expected. The monthly prices of newer drugs can be up to several tens of thousands of dollars or euros, raising the question of cost-effectiveness *(1–3)*.

The idea to use the immune system effector mechanisms against autologous tumor cells is an attractive idea, partly based on the proven effectiveness (body protection), specificity and tolerable side effects obtained with vaccines against a variety of infectious pathogens. This idea that an immune system can also be used against autologous tumor cells,

i.e. to recognize autologous tumor cells as body-foreign cells can be traced back more than 100 years to the past. For example, in works and publications by William B. Coley ("Coley's Toxin") and by Paul Ehrlich. Later, for example, an idea of cancer "immunosurveillance" was proposed by Macfarlane Burmet and Lewis Thomas more than 50 years ago, where the immune system should have a homeostatic role in controlling cancer. When oncogenic or other mutations occurred, the immune system was, in theory, thought to recognize the encoded mutated proteins and respond, thereby preventing the development of a tumor, at least when the system was operating normally *(1, 4–9)*.

Later experimental evidence, mainly from murine tumor models in the second half of the 20th century, supported this idea of the immune system-based possibility of cancer cell elimination. It was demonstrated that the immune response against cancer involves different effector mechanisms of adaptive and innate immunity, with the predominant role of cytotoxic CD8+ T cells (CTLs), but it may also include antibodies, natural killer (NK) cells and granulocytes and macrophages. Despite very effective and curative anti-tumor responses obtained in various experimental animal models, in daily clinical practice these various immune-based approaches were until recently of limited effectives and consequently of limited clinical applicability. Various mechanisms ranging, for example, from low immunogenicity and low expression of potential tumor antigens on tumor cells, their downregulation, presence of various soluble immunosuppressive factors and/or cell-bound molecules on regulatory (suppressor) immune cells or on tumor cells within the tumor microenvironment may have an adverse effect on the afferent and the efferent phases of the immune anti-tumor response. Due to their similarity to normal cells, cancer cells have in fact many ways of evading and thus escaping an otherwise possible effective immune response against microorganisms and against allogeneic cell transplants. It seems that tumor-related antigens may not be properly presented, antigens may be recognized as "self" and may induce anergy, T-lymphocytes may not be appropriately activated, or T-lymphocytes may be excessively inhibited *(1, 2, 9–16)*.

In the past 10 to 15 years, several immunotherapeutic approaches have been shown to have promising clinical impact. In addition to the relatively high price, for some of these approaches, although with promising results, there may be a practical problem in the routine applicability in a daily busy clinical setting. These newer immunotherapeutic approaches include several variations of the **adoptive T cell transfer therapy** *(17–22)*; **vaccines** *(2, 23, 24)* such as the first therapeutic cellular vaccine approved by the United States Food and Drug Administration (FDA), Sipuleucel-T *(25)*; and **checkpoint blockade inhibitors**, such as the FDA-approved anti-CTLA4 antibody (mab) (ipilimumab) *(26, 27)* and the anti-PD-1:PD-1 ligand mabs *(28–32)*.

The antitumor responses obtained with the vaccine and checkpoint immune modulators may be time-delayed and mixed (lesions may enlarge before shrinking, lesions may remain stable or slowly regress over time). These type of tumor response dynamics can be explained by the time required for the T-cell activation *in vivo*, tumor infiltration, antigen modulations on target (tumor) cells, as well as by intra-patient heterogeneity of tumor–host interactions. Also, since an anti-tumor response is usually obtained in only a subset of patients, this also results in ongoing studies aiming to identify the response predictors (biomarkers) *(33–36)*.

ADOPTIVE T-CELL TRANSFER

Adoptive T cell transfer (**ACT**) immunotherapy employs the reinfusion of large numbers of autologous T cells previously expanded and activated *in vitro* (*ex vivo*) with high avidity for tumor antigens or cells. The source of tumor-specific T cells is either naturally-occurring autologous T cells from the tumor microenvironment (e.g., tumor-infiltrating lymphocytes, **TILs**) or blood or genetically engineered T cells expressing high affinity tumor-specific T cell receptors (**TCRs**). These cells are expanded *in vitro* in the presence of various growth factors and infused to patients after they normally receive a preparative lymphodepleting regimen. To enhance T-cell activation, interleukin-2 (IL-2) might be used. Using these approaches, objective responses reaching 50% with lasting remissions have been achieved in patients with metastatic melanoma treated with autologous TILs *(17–22)*.

The main problems or limitations in the clinical development and application of ACT pertain to the time required together with the labor-intensive methods and the use of sophisticated technologies to develop and grow specific T cell clones or T cell lines *in vitro* in a sufficient number, their short half-life after transfer into the patient and the need for an individual development of T cells due to HLA-restriction. The *in vivo* half-life of the transferred T cells could be increased after lymphodepletion of recipients before adoptive transfer.

Advances in T cell engineering using lentiviral and retroviral vectors carrying genetically engineered TCRs expanded the opportunities for ACT. Recombinant viruses encoding either conventional αβT−cell receptors (TCR) or **chimeric antigen receptors** (**CARs**) are capable of inserting genes into the genome of human lymphocytes with efficiencies exceeding 80%. CARs are chimeric single-chain constructs composed of antibody-derived complementarity-determining region fused to a T-cell receptor (TCR) signaling domain. Genetic transfer of CAR genes to autologous T cells results in T cells that are activated and proliferated *in vivo* upon contact with

their antigen. The advent of CARs bypasses the need for tumor cells to possess functional antigen-processing machinery and express antigen through MHC class I or II molecules; transduced T cells are able to recognize the intact surface protein through the artificial CAR. Clinically, this may lead to both lysis of a large tumor burden and development of immunologic memory for that specific target antigen. Preclinical and clinical evaluations have resulted in stepwise improvements in the constructs used to produce CARs. Genetically engineered T cells were shown to recognize and destroy hematopoietic tumor cells, particularly those involving anti-CD19-based CARs for the treatment of B-cell malignancies or some solid tumors expressing the cognate antigens. The CARs approach combines elements of genetic engineering and molecular biology to create new biological structures with enhanced functionalities. CAR therapy, as it currently exists, requires consequently a multidisciplinary team composed of devoted molecular biologists, immunologists and clinicians within a hospital environment, so at present time it is in practice in several high-quality and high-ranking academic institutions, predominantly in the USA. Clinical trials have already shown clinically significant antitumor activity in chronic lymphocytic leukemia, B cell lymphoma, neuroblastoma. Trials targeting a variety of other adult and pediatric malignancies are also under way. The clinical responses to the ACT transfer are most often observed in days to weeks, in contrast to usually much slower time-response to tumor vaccines and checkpoint blockade. Reported toxicities such as fever, hypotension and metabolic complications can be related to the elevated proinflamatory serum cytokine levels and to the systemic release of various intracellular molecules due to the tumor cells lysis and in depletion of normal B cells in case of anti-CD19-based CARs application *(17–22)*.

VACCINATION

Sipuleucel-T (Provenge) is to date the only **therapeutic anti-cancer vaccine** that has been licensed for use in clinical practice. It was first licensed by the U.S. Food and Drug Administration (FDA) in 2010 for use in the treatment of asymptomatic/minimally symptomatic metastatic castration-resistant prostate cancer (mCRPC) *(25)*. It involves an autologous cell transplant, whereby peripheral blood mononuclear cells (PBMCs) in a sufficient number are taken from the patient using the leukapheresis procedure and incubated with a fusion protein consisting of recombinant prostate acid phosphatase (PAP; a tumor associated antigen expressed in prostate tumor cells) and granulocyte-macrophage colony stimulating factor (GM-CSF). Dendritic cells in the PBMC sample should take up PAP and express it as part of a major histocompatibility complex (MHC) on their cell surface. GM-CSF is used as an adjuvant co-stimulant for functional maturation of the dendritic cells in order for them to activate specific CTLs. These specific CTLs are then activated themselves and can replicate to form a reservoir of CTLs against PAP. They CTLs are then used to form the sipuleucel-T vaccine, which is administered to the patient. Each vaccine preparation is patient-individualized, i.e. each vaccine is autologous to the patient and thus avoids human leukocyte antigen (HLA) mismatching. A phase III trial (IMPACT) randomized 512 patients with asymptomatic or minimally symptomatic metastatic castrate-resistant prostate cancer (CRPC) in a 2:1 fashion, to receive either sipuleucel-T or placebo. The IMPACT trial was successful in demonstrating prolonged overall survival rates for patients with mCRPC vaccinated with sipuleucel-T compared to a placebo/control group, although there were no significant differences between the two groups in time to cancer progression. A 4.1 month improvement in median survival was achieved: 25.8 months in patients treated with sipuleucel-T versus 21.7 months in the control patients arm (a 22% relative reduction in risk of death, hazard ratio 0.78, 95% confidence interval 0.61–0.98 P=0.03). Adverse events were more prevalent in the sipuleucel-T–treated group, but they were generally mild and flulike in nature *(24, 25)*.

In comparison with the above mentioned ACT and CARs immunotherapy approach, the technology to generate *in vitro* autologous activated dendritic cells and activated CTLs is relatively less complex so this approach may have a wider applicability for other tumor types. Regarding the clinical indication for the Sipuleucel-T application in practice, it is in the meantime becoming an indication more "squeezed", because there are now new options for additional prostate hormonal manipulations. For example, with the abiraterone acetate or enzalutamide, both in the form of tablets in chemotherapy-naive patients having no or minimal symptoms *(24, 25, 36, 37)*.

CHECKPOINT BLOCKADE

Another novel and perspective immunotherapeutic approach is **immune checkpoint blockade**. It has emerged as one of the most clinically promising strategies. This strategy is based on the **nonspecific immune activation of T lymphocytes** in cancer patients which can be achieved by monoclonal antibodies that inhibit co-inhibitory signaling pathways. Physiologically, the amplitude and duration of T-cell response is regulated by a balance between co-stimulatory and co-inhibitory signals (that is, **immune checkpoints**). Through these functionally opposite signaling pathways, immune homeostasis and avoidance of immune over-activation (autoimmunity) is maintained. The antibodies that block immune checkpoints do not target tumor cells directly, but instead target lymphocyte receptors for inhibitory signals or their ligands. By blocking the T cell receptors for the inhibitory signals or inhibitory ligand molecules for these receptors on other cells (for example, on antigen presenting cells or on normal

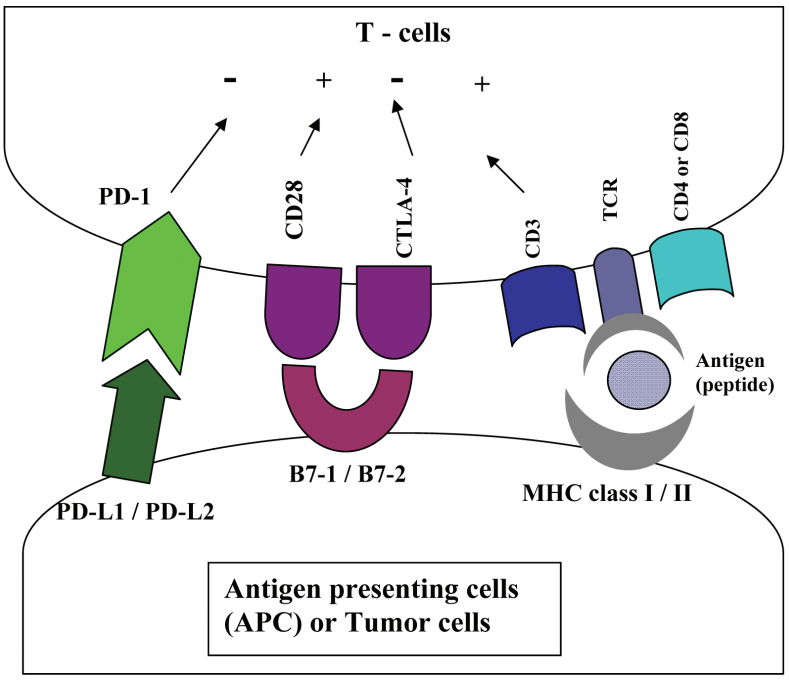

Figure 1. *Simplified schematic representation of CTLA-4 and PD-1 immune checkpoints. In the priming phase, antigen-presenting cells (APC) present antigens to the T-cells. Antigen (short peptide) is presented in the context of the major histocompatibility complex (MHC) class I or class II molecules. It is recognized by the T-cell receptor complex composed of (TCR) heterodimer protein chains, cluster of differentiain 3 (CD3) molecules nad T-cell co-receptor molecules CD8 or CD4, respectively. For T-cells to become fully activated second signal is required. This second signal, the „co-stimulatory" signal, is antigen nonspecific and is provided by the interaction between co-stimulatory molecules expressed on the membrane of APC and the T-cell. Co-stimulatory signals are provided by binding of the CD28 receptor on the T-cell surface to its ligand B7-1 or B7-2 on the APC. These interactions enhance stimulation of the T-cell, whereas failure of this event results in T-cell anergy (non-activating event). As a regulatory mechanism, cytotoxic T-lymphocyte antigen-4 (CTLA-4) is upregulated after T-cell activation and inhibits the T-cell response. Binding of CTLA-4 to B7-1 or B7-2 acts as a suppressor response. This step acts as a checkpoint in the immune response cascade and prevents adverse and harmful immune activities of T-cells. Anti-CTLA-4 antibodies bind to CTLA-4, turning off the „inhibitory signal", thus resulting in an enhancement of T-cell function. In the effector phase, the programmed cell death-1 (PD-1) inhibitory receptor is expressed by the T-cell and, when it is engaged by its ligands PD-L1 and PD-L2, it serves to inhibit the T-cell response. Anti-PD-1 or anti-PD-L1 antibodies bind to PD-1 or PD-L1, respectively, turning off the „inhibitory signal" in the peripheral tissues and enhancing T-cell function. PD-1/PD-L1 interactions are complex, and this interaction is also involved in the priming phase.*

and tumor cells), nonspecific (over)activation of T-lymphocytes can be obtained, which may also evoke the endogenous antitumor activity. In cancer, inhibitory pathways seems to be important in the tumor microenvironment and draining lymph nodes and might lead to a state of T-cell anergy, thereby allowing tumor to escape from immune surveillance, and unchecked tumor growth *(34–39)*.

Cytotoxic T–lymphocyte associated antigen 4 (CTLA-4; also known as CD152) and programmed death-1 antigen (PD-1; also known as CD279) were the first two immune checkpoints to be evaluated extensively in the setting of clinical cancer immunotherapy. They differ in the manner and level at which they negatively regulate the immune system. Inhibitory pathway CTLA-4 regulates T-lymphocytes at the level of initial activation, while the PD-1 regulates immunity at multiple phases of the immune response, including exerting its effect on effector T-lymphocyte activity in the peripheral tissues. In the priming phase, antigen-presenting cells present antigens to the T-cell. Two signals are required to initiate a T-cell response. CTLA-4 is upregulated after T-cell activation and inhibits the T-cell response set in motion. Anti-CTLA-4 antibodies bind to CTLA-4, turning off the "inhibitory signal", thus resulting in an enhancement of T-cell function. In the effector phase, the PD-1 inhibitory receptor is expressed by the T-cell and, when it is engaged by its ligands PD-L1 and PD-L2, it serves to inhibit the T-cell response. Anti-PD-1 antibodies bind to PD-1, turning off the "inhibitory signal" in the peripheral tissues and enhancing T-cell function. PD-1/PD-L1 interactions are complex, and this interaction is also involved in the priming phase. In addition to its activity in cancer immunotherapy, PD-1 has been shown to play a role in allergy, autoimmunity, infectious disease, and transplantation immunity. PD-1 is highly expressed on tumor-infiltrating lymphocytes (TILs) in the effector phase and serves to inhibit T-lymphocyte activity during chronic antigen ex-

posure when it is engaged by its ligands. In peripheral tissues, tumor cells and other cells in the tumor microenvironment may express PD-1 ligands, which may protect the tumor cells from immune destruction. Two PD-1 ligands are known: PD-L1 (also known as B7-H1 or CD274) and PD-L2 (also known as B7-DC or CD273). PD-1 ligands are also expressed on different types of tumors. PD-L1 is most commonly expressed on solid tumors, including melanoma, ovarian, lung, and renal carcinomas. PD-L2 has been reported to be upregulated in different types of lymphoma *(34–39).*

Several immune checkpoint antibodies are currently in clinical trials, with promising results including high objective durable response rates (ORR) and a favorable side effect profile. These immune checkpoint antibodies seems to be clinically active in a variety of malignancies, including those not traditionally classified as immunogenic, such as non-small-cell lung cancer (NSCLC). Encouraging clinical results, even with improved survival, such as in patients with metastatic melanoma, have been obtained. In addition to ipilimumab (Yervoy), antibodies against PD-1 such as nivolumab (Obdivo) and pembrolizumab (Keytruda) are also FDA approved for the treatment of advanced melanoma. An ongoing challenge is learning how to use these agents to optimize tumor regression while avoiding unacceptable toxicity due to enhanced autoreactivity of T cells to benign cells. For example, the most common adverse events (immune related adverse events, irAE's) following ipilimumab include pruritus, rash, diarrhea, hepatotoxicity, endocrinopathies and uveitis. Similar irAE's have been described with anti-PD-1 agents including also fatigue and pneumonitis (several cases of unfortunately fatal pneumonitis were reported). Toxicities may vary across tumor types, depending probably on antigen expression and recognition and on intensity of the autoreactivity. Treatment algorithms of irAE's include measures such as early treatment with supportive care and immunosuppressive medications (for example, diet and hydrations recommendations, loperamide, corticosteroids) *(34–40).*

CONCLUSION

In conclusion, it seems that the recent ongoing clinical studies with the ACT, vaccine Sipuleucel-T and with the immune check-point activation modulation are demonstrating, after decades of disappointing immunotherapy trials, that the patient's immune system can be used effectively in a clinical setting against autologous tumor cells. The field of cancer immunotherapy has in fact a long history of development and of various experimental approaches and strategies with mixed results. Because of these mixed results, a doubt existed for many years as to whether the immune system is capable of eliminating autologous cancer. All this, indirectly, indicates that the interplay between immunity and cancer is complex. It has also become obvious over these many years of research that the immune system is able not only to suppress tumor growth by destroying cancer cells or inhibiting their outgrowth but also under certain conditions to promote tumor progression either by selecting tumor cells that are more fit to survive in an immunocompetent host or by establishing conditions within the tumor microenvironment that facilitate tumor outgrowth ("cancer immunoediting"). Moreover, a chronic inflammatory microenvironment composed of various immune cells (macrophages, myeloid-derived suppressor cell, T-regulatory cells) within the tumor, and also some cytokines and metabolic product can cause T cell dysfunction. Over the past two to three decades, however, cancer immunotherapy has been undergoing a remarkable transition in effectiveness due to the advances in our understanding of the immune system, in the translation of this into the clinical practice and also due to the development of novel technologies and immunotherapeutic agents. Still, many key questions remain. It is to be expected that more relevant and clinically applicable strategies for the identification of possibly responding patients and assay for the immune monitoring of patients undergoing cancer immunotherapy will also be tested and developed concomitantly *(1, 2, 20, 22, 23, 34, 36, 40).*

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