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Cancer immunotherapy: Progress and challenges in the clinical setting

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Cancer immunologists have made enormous efforts to prove that innate and adaptive immune cells recognize tumor cells and induce tumor rejection in experimental animal models. These observations provided the rationale to study the role of the immune system in the control of tumor growth in cancer patients and to develop immunotherapeutic strategies to treat patients with cancers. Several lines of evidence suggest that anti-tumor immune responses may correlate with better clinical outcome in patients with cancers. Among them is the correlation between the presence of tumor-infiltrating T cells and the improved clinical outcome for patients with solid tumors [1–3]. In addition, a number of immunotherapies, such as high-dose IL-2 [4] and TA-specific monoclonal antibodies (mAb) [5], have provided long-term clinical benefits to a minority of cancer patients.

Most importantly, the recent approval by the US FDA of the mAb ipilimumab directed against the co-inhibitory molecule CTLA-4 for patients with unresectable or metastatic melanoma represents a major breakthrough for mAb-based therapies in oncology practice [6]. The successful outcome of the randomized phase III clinical trial with ipilumumab has provided the much-needed incontrovertible clinical evidence that in humans, as in experimental animal models, the host's immune system can control tumor growth. Furthermore, it has infused a considerable amount of optimism among tumor immunologists and clinical oncologists about the clinical potential of immunotherapy for the treatment of advanced cancers. However, there are also many examples of spontaneous or vaccine-induced TA-specific T- and B-cell immune responses that do not correlate with improved clinical status [7–9].

This discrepancy between immune and clinical responses underlines the need to better dissect the molecular and cellular events leading to tumor rejection in humans. Such an endeavor has greatly benefited from the molecular identification of TA expressed by human tumor cells, which are recognized by T cells and antibodies [5, 10, 11]. As a result, TA-specific immunotherapies have been implemented in clinical trials with molecularly defined cancer vaccines, TA-specific mAb and adoptive transfer of TA-specific T cells. Novel generations of cancer vaccines with molecularly defined TAs and potent adjuvants like toll-like receptor ligands appear to stimulate strong TA-specific T-cell responses but have shown evidence of clinical benefits in only a minority of patients with advanced cancer [7, 8, 12]. The adoptive transfer of TA-specific T cells remains technically challenging and the promising data obtained in terms of objective clinical responses and durability of responses from small monocentric clinical trials will need to be further confirmed in large multicenter clinical trials [13]. TA-specific mAb are clinically effective in a number of hematological malignancies and solid tumors and are routinely used in the clinic [5].

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We now have a better understanding of the multiple mechanisms of tumor-induced immune escape, which are likely to cause the failure of the spontaneous or vaccine-induced immune responses to promote tumor regression in humans. In the tumor microenvironment, a number of negative regulators dampens anti-tumor immune responses and/or their therapeutic efficacy, including the production of cytokines (like TGF-β or IL-10), suppressive cells (regulatory T cells, myelosuppressive dendritic cells), defective antigen presentation by tumor cells (HLA or tumor antigen loss, antigen processing machinery defects), amino-acid catabolizing enzymes (indoleamine-2-3dioxygenase, arginase) and co-inhibitory pathways (like CTLA-4/CD28, PD-1/PD-L1) [14–17]. As a consequence, a number of therapies to specifically target these pathways are being developed to enhance TA-specific immune responses and to increase the likelihood of clinical benefits.

In this article, commissioned to recognize National Cancer Survivors Day (the first Sunday in June each year, 5 June in 2011, see www.ncsdf.org), we will comment on the successes of immunotherapy of cancer in the clinical setting. In addition, we will discuss the challenges to optimize the use of cancer immunotherapies in the clinic.

Targeting tumor cells

The enthusiastic application of the hybridoma methodology by a number of tumor immunologists in the late 1970s led to the development of mouse mAb to many human TA. Some of them, such as the carcinoembryonic antigen (CEA) [18] were known and extensively characterized TAs, while others, such as the chondroitin sulphate protidoglycan 4 (CSPG4) [19], were newly identified TAs. Given their high degree of specificity and their availability in large amounts in a purified and well-standardized form, TA-specific mAbs overcame most, if not all, of the obstacles that had until then hindered the clinical application of immunotherapy of malignant diseases with TA-specific antisera. As a result many TA-specific mAbs were utilized in clinical trials at various centers and a large number of patients were treated. However, contrary to expectations, the clinical results were quite disappointing, most likely because the mouse mAbs were quite immunogenic in patients and also failed to successfully recruit human effector mechanisms [5].

These problems have been overcome by the development of chimeric, humanized and human mAbs, which are either not immunogenic or only poorly immunogenic in patients and are more effective in recruiting human anti-tumor effector mechanisms. Owing to their therapeutic efficacy, some TA-specific mAbs have become part of the armamentarium used for the treatment of some hematological malignancies and solid tumors [5]. Such mAbs include the CD20-specific mAb rituximab for lymphoma, the human epidermal growth factor receptor 2 (HER2/*neu*)-specific mAb trastuzumab for breast cancer and the epidermal growth factor receptor (EGFR/HER1)-specific mAbs cetuximab and panitumumab for head and neck cancer and colorectal carcinoma. The results obtained with a large number of patients with different types of cancer have shown that TA-specific mAb-based immunotherapy yields response rates (including objective clinical responses, increased relapse free and overall survival) of 8–10% when mAb are used as single agents and up to 30% when they are used in combination with chemotherapy and/or radiotherapy.

In general hematologic malignancies have been found to be easier than solid tumors to target with TA-specific mAb because the dose required to achieve therapeutic efficacy is lower and the tumors can be more easily penetrated. Furthermore, hematologic malignancies such as non-Hodgkin lymphoma are more sensitive than solid tumors to radio-immunotherapy. However, the clinical use of radiolabelled TA-specific mAb is hampered by the complexities in manufacturing them, by safety concerns and by poor specificity because of slow pharmacokinetics and tumor perfusion [20].

Adverse events caused by the administration of nonconjugated TA-specific mAbs are in general mild. They reflect allergic or hypersensitivity reactions to a protein containing xenogeneic sequences and occur during or immediately after the first mAb administration. Despite the expression in normal tissues of the TA that are currently used as targets of antibody-based immunotherapy, the side effects caused by the binding of the injected mAb to normal tissues are rare. A few examples include the transitory B-lymphocyte depletion in patients treated with rituximab, cardiac dysfunction in those treated with trastuzumab, and seborrheic dermatitis and acneiform eruptions in those treated with cetuximab. Whether the resistance of normal cells to mAb-based immunotherapy in comparison with their malignant counterparts reflects differences in the expression level of the targeted TA and/or in the activation of signaling pathways associated with survival and apoptosis remains unknown. The low or absent sensitivity of normal cells, which express the targeted TA, is not unique to TA-specific mAb-based immunotherapy, since it has been also observed in patients treated with T-cell-based immunotherapy. Unraveling the mechanism(s) underlying the low or absent sensitivity of normal cells to the detrimental effects of immunotherapy may teach us how to overcome tumor cell resistance to antibody-based immunotherapy.

Noteworthy, not all patients with a given type of cancer respond clinically to mAb-based immunotherapy in spite of the expression of the targeted TA in their malignant lesions. In addition, not all the malignancies expressing a TA targeted by a clinically effective mAb are sensitive to mAb-based immunotherapy. Together, these results indicate that expression of the targeted TA in the malignant lesions is not sufficient for a clinical response to mAb-based immunotherapy to occur, and that a number of other variables play a role. The identification of these variables represents a challenge that tumor immunologists and clinical oncologists are currently facing. Obtaining this information may contribute to define the mechanism(s) underlying the anti-tumor activity of TA-specific mAb as well as patients' differential clinical responses to TA-specific mAb-based immunotherapy.

In vitro experiments and studies in animal models have shown that the clinically used TA-specific mAb can utilize both immunological effector mechanisms and inhibition of the activation signals needed for continued malignant cell growth and/or viability to effect their anti-tumor activity. To mention a few examples, the CD20-specific mAb rituximab mediates complement- and cell-dependent cytotoxicity (ADCC) of target cells and inhibits cell survival pathways [21]. The EGFR-specific mAb cetuximab mediates cell-dependent lysis of target cells and inhibits multiple signaling pathways associated with cell survival and proliferation such as the PI3K/AKT and the Ras/MAPK pathways [22–25].

Clinical results support the role of immunological mechanisms and of signal transduction pathway blockade in the therapeutic efficacy of TA-specific mAb-based immunotherapy. For instance, the statistically significant association between the clinical course of the disease and polymorphism of the Fcγ receptors expressed on NK cells and monocytes, the major effector cells in ADCC, argues in favor of this immunological mechanism as a major player in the clinical response to TA-specific mAb-based immunotherapy [22, 26]. A similar conclusion could be drawn for inhibition of signaling pathways, since inhibition of EGFR activation has been reported to be associated with major clinical responses in patients with head and neck cancer treated with the EGFR-specific mAb cetuximab [27, 28] and inhibition of AKT activation has been reported to be associated with tumor shrinkage in patients with breast cancer treated with HER2/*neu*-specific mAb trastuzumab [29]. However malignant lesions do not regress in patients in a matter of a few hours or days following the administration of TA-specific mAb as one would expect, should the lysis mediated by the innate cells or the inhibition of signaling pathways contribute significantly to the anti-tumor activity of the TA-specific mAbs. The length of time, at least 1 wk, required for a clinical response to occur following the administration of TA-specific mAb to patients who respond

to TA-specific mAb-based immunotherapy argues against inhibition of signaling pathways and complement- and cell-dependent lysis of tumor cells as major mechanisms underlying patients' clinical responses to mAb-based immunotherapy.

The kinetics of clinical responses following the administration of TA-specific mAb has been taken as evidence that TA-specific mAb can enhance the immunogenicity of TA and induce TA-specific T cellular immunity. This potential mechanism is supported by several lines of evidence generated by in vitro experiments, by studies in animal model systems and by clinical investigations. As we have recently reviewed in two papers [20, 22] to which we refer the interested reader for a more extensive discussion of the topic, TA-specific mAb may induce or augment TA-specific T cellular immunity by enhancing TA uptake, internalization and presentation to $CD8^+$ T cells by dendritic cells and cross-presentation. Should induction of TA-specific cellular immunity by TA-specific mAb be the major mechanism underlying the therapeutic efficacy of TA-specific mAb-based immunotherapy, one might wonder why TA-specific T cellular immunity induced or enhanced by TA-specific mAb is therapeutically more effective than that elicited by the various types of vaccines which have been used over the years. Does this difference reflect the different types of TA-recognized by the T cells elicited by TA-specific mAb and by the other types of vaccines used? Can combining the administration of TA-specific mAb with that of vaccines, adjuvants and/or check point-specific mAb enhance the therapeutic efficacy of TA-specific mAb-based immunotherapy? In addition, if HLA class I antigen restricted, TA-specific T cells are the major players in patients' clinical responses to TA-specific mAb-based immunotherapy, this type of immunotherapy will be affected by the multiple escape mechanisms which have been shown to be a major obstacle to the successful clinical application of T-cell-based immunotherapy [14–17, 30].

Significant improvements in gene transfer and in the understanding of immunological pathways have led to the clinical development of chimeric antigen receptor (CAR)-transduced T cells (CAR-T cells). CARs result from combining the antigen site of an antibody with the signal-activating domain of immune receptors responsible for initiating signal transduction that leads to lymphocyte activation. Like mAb and HLA class I antigen-restricted, TA-specific CTLs and T cells transduced with T-cell receptors, CAR-T cells are highly specific [31–33]. In comparison with mAbs, CAR-T cells offer the advantage to traffic to the tumor site, expand in vivo and persist for a long time. CAR-T cells recognize a broad range of TAs, which include both glycoproteins and glycolipids. They can be used in patients independently of the expression of certain HLA class I antigens and are not affected by defects in the expression and/or function of the HLA class I antigen processing machinery. These defects, which are present with different frequency in malignant cells, have a negative impact on the generation and/or expression of HLA class I antigen-TA-derived peptide complexes recognized by T cells [34].

Like antibodies, CAR-T cells can recognize only TA expressed on tumor cell membranes. The TAs used as targets of CAR-transduced T cells include CEA, CSPG4, folate-binding protein, GD2 ganglioside, GD3 ganglioside, and HER2/*neu*. The cells used as effector cells include $CD8^+$ and $CD4^+$ T cells and NK cells. CAR-T cells have been shown to lyse tumor cells in vitro and in animal model systems. To date, the clinical efficacy of this strategy has been modest, emphasizing the need to improve the lytic activity of CAR-T cells. To this end, second-generation CAR-T cells include co-stimulatory molecules such as CD28, OX40 and 4-1BB. Furthermore, CARs have been grafted into virus-specific CTL, which can be stimulated with the cognate antigen in order to expand them. Along the same lines, administration of T-cell growth factors such as IL-2, IL-7 or IL-15 may improve the survival of CAR-T cells [35–37]. Finally, upregulation of TA expression by hypomethylating agents

and histone deacetylase inhibitors can also enhance the sensitivity of target cells to cognate CAR-transduced T cells [38].

Targeting co-inhibitory pathways

A number of co-inhibitory molecular pathways play a role in decreasing TA-specific immune responses. Two of these co-inhibitory pathways, i.e. CTLA-4 and PD-1, have already been targeted in the clinic with mAbs.

CTLA-4 is a co-inhibitory receptor expressed by activated T cells and Tregs. It acts as a negative regulator of T-cell activation, serving as a checkpoint blockade to prevent excessive T-cell proliferation and immune-mediated damage to normal tissues [39]. CTLA-4 binds to B7 molecules expressed by antigen presenting cells with a higher affinity than CD28, also a ligand for B7 molecules. Treatment with an anti-CTLA-4 mAb has been shown to contribute to tumor rejection in experimental animal models [40]. These preclinical results led to the implementation of clinical trials with anti-CTLA-4 humanized mAbs. A dose response phase clinical trial with one such mAb ipilumumab at three dose levels (0.3, 3, and 10 mg/kg) in 217 patients with unresectable melanoma has shown evidence of clinical responses [41]. The higher response of 11% was observed in the 10 mg/kg cohort with a median overall survival of 14 months. A large phase III randomized trial of ipilimumab (3 mg/kg) in combination with or without a gp100 peptide vaccine versus the peptide vaccine alone in stage IV melanoma patients demonstrated that ipilimumab improved overall survival with evidence of durable clinical responses among the responders [6].

This type of immunotherapy presents major challenges in oncology practice, however. First, such therapy goes along with a significant number of grade 3–4 adverse effects including severe colitis, which are most often immune related (10–15% of patients) and which need specific clinical care. Second, because a subset of patients with advanced melanoma appears to benefit from such therapy, it is critical to identify who will respond clinically, in order to avoid exposing the rest of the patients to serious side effects without any clinical benefits. In this regard, it is important to acknowledge that we still need to precisely determine the anti-CTLA-4-mA-binduced immune mechanisms directly responsible for the improved clinical outcome. Third, anti-CTLA-4 mAb treatment has provided many examples of patients who did not respond immediately to therapy but exhibit either late or slow responses over time, suggesting that the evaluation of objective clinical responses over a short term may not correctly predict the response to this therapy. These observations have supported the proposition of novel, immune-related response criteria (irRC) to avoid the premature exclusion of patients who may initially progress before responding to immunotherapy [42].

A number of experimental studies in animals [43, 44] and in vitro [45] have suggested the role of PD-1/ PD-L1 interactions in inhibiting the effector functions of TA-specific $CD8^+$ T cells [43, 44]. PD-1 is a co-inhibitory receptor expressed by activated T and B cells [46–49]; it binds to two known ligands: PD-L1 (B7-H1) [47, 50] and PD-L2 (B7-DC) [51, 52]. PD-1 negatively regulates T-cell functions through the engagement of PD-L1, which is expressed by a wide variety of tissues [47, 49, 50]. PD-L1 is also expressed by human tumors, either constitutively or after treatment with IFN-γ [43, 44]. Dysfunctional (“exhausted”) T cells upon exposure to high antigen load have been shown to upregulate PD-1, and blockade of the PD-1/PD-L1 pathway has led to increased cytokine production and proliferation, resulting in a significant reduction of the viral load [53].

In cancer patients, TA-specific CTLs present in PBLs or at tumor sites have been shown to upregulate PD-1 expression and PD-1 appears to play a critical role in regulating the expansion of TA-specific $CD8^+$ T cells [54]. Blocking anti-PD-1 and anti-PD-L1 mAbs

have been implemented in pilot trials in patients with cancers. MDX-1106 (Bristol-Myers) is a fully humanized anti-PD-1 IgG4 antibody, which has been tested in phase I dose escalation trial of 39 patients with solid tumors. No major adverse event was observed even at the highest dose tested (10 mg/kg) and there was some evidence of objective clinical responses (1 complete, 2 partial and 2 mixed responses). An additional trial with multiple doses of anti-PD-1 mAbs has shown evidence of clinical activity and durable clinical responses in patients with advanced solid tumors (renal cancers, melanoma) [55]. The absence of any major autoimmune side effects observed to date, was unexpected because of the role of the PD-1 pathway in immune tolerance. At any rate, the clinical effect of anti-PD-1mAb therapy will need to be confirmed in larger randomized trials.

It is now clearly established that "exhausted" T cells upon chronic antigen stimulation co-express multiple co-inhibitory receptors, supporting the implementation of combined co-inhibitory blockades to enhance TA-specific immune responses and reverse tumor-induced T cell dysfunction [56]. As a result, the combination of anti-CTLA-4 and anti-PD-1 mAbs, which appears promising in experimental animal models [57], is being pursued in the clinic. Most recently, a subset of highly dysfunctional TA-specific $CD8^+$ T cells have been identified in patients with advanced melanoma and shown to upregulate both PD-1 and Tim-3 [58]. Tim-3 is a co-inhibitory receptor, which upon interaction with its ligand galectin-9 induces death in Th1 cells [59]. PD-1 and Tim-3 blockade strongly enhanced TA-specific $CD8^+$ T cell expansion and function in patients with advanced cancers. In addition, targeting PD-1 and Tim-3 in vivo induced tumor regression in experimental animal models. Therefore, the combination of PD-1 and Tim-3 blockade either alone or in combination with cancer vaccines appears to be the next logical step to further reverse tumor-induced T cell dysfunction.

Targeting immunostimulatory pathways

CD40 is a member of the TNF receptor superfamily and is expressed by APCs including monocytes, macrophages and dendritic cells, B cells and some tumors. Therefore, CD40 engagement is a promising approach to activate APCs and enhance TA-specific immune responses. A fully humanized anti-CD40 mAb (CP 870,893, Pfizer, New-York, USA) was recently evaluated as a single agent in a phase I trial or in combination with gemcitabine chemotherapy in a small cohort of incurable pancreatic adenocarcinoma [60]. There was evidence of clinical responses. Tumor regression efficacy appeared to require macrophages but not T cells. CD40-activated macrophages were shown to rapidly infiltrate tumors, exhibit anti-tumor activities and destroy tumor stroma.

Future developments of immunotherapies for cancer patients

The data we have reviewed clearly indicate that both antibody- and T-cell-based immunotherapies, can improve clinical outcome in cancer patients. They also suggest that multiple challenges lay before us to improve the efficacy of cancer immunotherapies in the clinic. First, it is important to define the mechanism(s) underlying the anti-tumor activity of the used immunotherapies. This information will contribute to design combinatorial immunotherapeutic strategies to target tumor cells and tumor microenvironment and counteract the multiple immune escape mechanisms utilized by tumor cells.

To this end, the possibilities are many and will need to be carefully investigated in pilot clinical trials to evaluate their safety, toxicity and efficacy. Innate immune cells including NK, NKT and dendritic cells can be activated by a number of clinically available TLR ligands, glycolipids and a number of cytokines (IL-15, IL-21). $CD8^+$ and $CD4^+$ T cells can be successfully expanded with molecularly defined cancer vaccines and potent adjuvants like TLR ligands. Alternatively, PBLs genetically engineered to express TCRs can be

adoptively transferred into patients. Multiple negative regulators of immune responses can be inhibited with a number of novel reagents including mAbs to checkpoint molecules, such anti-CTLA-4 and anti-PD-1 mAbs, and small molecules, such as IDO inhibitors. Chemotherapeutic agents can be used to induce immunogenic cell death-releasing tumor derived proteins (like calcireticulin or HMGB1) to activate APCs and promote anti-tumor immune responses [61]. An area, which has been poorly investigated, is the potential clinical significance of anti-idiotypic (anti-Id) responses elicited by TA-specific mAbs. In this regard, anti-Id antibodies may potentially inhibit the binding of TA-specific mAbs to the targeted TAs and, therefore, reduce their therapeutic efficacy. Alternatively, anti-Id, which mimic the TAs may also induce TA-specific immune responses in patients treated with TA-specific mAbs.

The cancer immunology community has now to face the difficult choice to define what are the most promising approaches to be actively explored in the clinical setting. Such necessity has led to multiple clinical initiatives like the Cancer Immunotherapy Trial Network (CITN) under the auspices of the NCI, and the Cancer Vaccine Collaborative from the Cancer Research Institute, which regroup many Cancer Immunologists in the USA and abroad.

One additional challenge lies in the availability of clinical-grade reagents most often produced by pharmaceutical industries whose objectives may not always match the ones of cancer immunologists interested in combinatorial approaches instead of monotherapies. Because they fail to show strong efficacy in early phase trials or because of internal priorities, too many promising reagents are not further developed for additional clinical trials although there may be a strong rationale to support their activity in combinatorial approaches. Pharmaceutical industries, national agencies and cancer immunologists will need to find a common ground to retrieve these lost “arks” from their wooden crate!

Although, immunotherapies have been most often tested in patients with advanced cancers, one major challenge will be to evaluate the role of immunotherapy in the prevention (i.e. patients who will develop tumors) or the adjuvant setting (i.e. patients who became disease-free after tumor resection and who are at high risk of relapse). Such strategies are very appealing because it is likely that the development of potent TA-specific immune responses will be easier in patients with low tumor burden and whose tumors may not have developed resistance to immune attack. Few immunotherapies have been evaluated in the adjuvant setting. High dose interferon has shown evidence of modest clinical benefit in patients with resected melanoma [62]. Ipilimumab and vaccines with the MAGE-A3 protein in combination with adjuvants are now being investigated in large trials in patients with resected melanoma.

The successful development of preventive and adjuvant immunotherapies faces multiple hurdles. First, testing immunotherapies in the prevention or adjuvant setting requires costly and large randomized trials with control arms and long-term follow-up. Second, it will be important to define biomarkers that predict the patients at high risk of cancer or relapse to focus on this population who may benefit from treatment. Finally, the recent evidence that a gene signature from the tumor microenvironment may identify a subset of patients who respond to cancer vaccines has raised the hypothesis that a limited number of cancer patients may benefit from immune interventions [63, 64]. It will therefore be important to investigate such gene signatures in the context of the multiple immunotherapies implemented in the clinic to further determine whether or not we may identify a subset of patients who may electively benefit from immune interventions.

In summary, it is hoped that pursuing the above approaches, noting and addressing their limitations, will lead to more cancer survivors and greater cause for celebration on the next National Cancer Survivors Day.

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