Productive adaptive immune responses are the end product of a complex set of cellular and molecular interactions fine-tuned by various stimulators and inhibitors, also referred as immune checkpoint blockers, of T cells to ensure optimal immune response for elimination of external and internal threats followed by resolution of the effector T cells without collateral damage and autoimmune consequences. In this context, the CD28 and tumor necrosis factor receptor (TNFR) super family costimulatory and coinhibitory molecules are central to immune modulation and immune homeostasis. These signals bridge innate, adaptive, and regulatory immunity through a coordinated communication network that ensures the generation of productive immune responses against pathogens without collateral damage to the host. Costimulatory receptor ligand pairs, such as CD28/CTLA-4 and PD-1, are evolved to contribute to peripheral tolerance to self-antigens as well as to ensure homeostatic control of unregulated T cell activation/proliferation ensuing immune responses to pathogens [2]. Therefore, costimulatory and co-inhibitory signaling pathways work in balance to ensure physiological immune responses to infection and cancer in the absence of collateral damage and autoimmunity.

The discovery of tumor specific and/or associated antigens (TAAs) expressed by cancerous cells taken together with numerous preclinical and clinical studies providing evidence for the importance of immunosurveillance in controlling cancer revitalized enthusiasm to tumor immunologists to formulate approaches where intrinsic TAA specific antitumor responses can be potentiated for durable therapeutic responses against cancer. Varieties of cancer vaccine antigens as well as to ensure homeostatic control of unrestrained T cell responses can be viewed as golden era for cancer immunotherapies in preclinical models and translation to the clinic.

While these important discoveries led to a boost of enthusiasm in the field for the development of various cancer vaccines and immunotherapies in preclinical models and translation to the clinic, a new class of immunotherapeutic approach is focused on unleashing the breaks of immune system by inhibiting the signaling of immune check point blockers, such as CTLA-4 and PD-1. Two immune checkpoint receptors have been most extensively studied. The importance of CTLA-4 signaling in controlling adaptive immune responses was hinted by observations that mice deficient for this receptor displayed massive generalized lethal autoimmune/hyper immune like syndrome due to their inability to limit the amplitude of naïve and memory T cells systemically [4]. Decades of studies by Allison and colleagues and others first in preclinical models and then in the clinic led to FDA approval in 2011 of an antibody to CTLA-4 (ipilimumab) after its historic success in phase III clinical trial demonstrating survival benefit in melanoma patients [5]. HLA-A*0201-positive patients (n=676) with stage III or IV un-resectable metastatic melanoma which have gone through standard of care therapy, were arbitrarily assigned, in 3:1:1 ratio, and received ipilimumab with gp100 (403 patients), ipilimumab only (137), or gp100 alone (136). The primary end point for the study was overall survival, ipilimumab, with or without gp100 peptide, improved overall survival in these patients as compared to gp100 peptide alone group. Grade 3 or 4 immune-associated adverse effects occurred in a small fraction of patients that received ipilimumab. These adverse side effects can be severe but most can be mitigated with appropriate treatments.

The success of anti-CTLA-4 Ab in the clinic diverted attention to other co-inhibitory molecules, in particular PD-1. PD-1 is expressed on activated T cells and has two well characterized ligands; PD-L1 (also known as B7-H1) and PD-L2 (B7-DC) [6,7]. The ligands of PD-1 are upregulated in response to inflammation. PD-L1 is upregulated on hematopoietic, endothelial, and epithelial cells in response to pro-inflammatory cytokines, particularly IFN-γ, whereas PD-L2 upregulation is restricted to dendritic cells (DCs) and macrophages in response to IL-4 [8,9]. Signaling through PD-1 was shown to limit T cell responses in peripheral organs during inflammatory responses to infections, and as such limit organ specific autoimmunity [10,11]. For example, PD-1 deficient mice on C57BL/6 background developed lupus like syndrome involving joints and kidneys [10], whereas PD-1 deficient mice on BALB/c background developed myocarditis [11]. Initial disappointment due to delayed (6-9 months) manifestation of mild deleterious phenotype of PD-1 knockout mice seems advantageous for targeting cancer immunotherapy with this pathway. These findings established PD-1 as an inhibitory receptor for control of organ specific autoimmunity. Phenotype of PD-1 KO mice became more clear by further studies demonstrating that acceleration of organ specific autoimmunity in autoimmune prone mice [12].
Further studies demonstrated that signaling through PD-1 receptor
indeed have crucial role in the inhibition of T cell responses [6,7]. The
discovery that PD-L1 can bind to B7-1 in addition to PD-1 to inhibit
the co-stimulatory signaling as well as inhibit T cell activation in PD-1
independent manner [13] introduced another complexity with added
opportunity for targeting this pathway for immunotherapy.

The importance of PD-1 signaling in the control of peripheral
immune response to cancer was demonstrated by forced expression of
PD-L1 in mouse tumor cells, which conferred resistance to immune
attack [14,15]. Subsequent studies demonstrated selective upregulation
of PD-L1 in many human cancers [16]. PD-1 is expressed on majority
of tumor infiltrating lymphocytes (TILs) and enhanced expression of
PD-1 on CD8+ T cells against tumors and chronic viral infections
correlated with their nonresponsive/anergic state [17,18]. In a chronic
viral infection model, blockade of PD/ PD-L1 signaling restored the
function of anergic virus specific CTLs [18]. Like CD8+ T cells,
expression of PD-1 was also observed on antigen-non-responsive
CD4+ T cells in chronically infected patients with hepatitis C virus.
The blockade of PD-1 partially restored the CD4+ T cell function [19].
In addition to inducing anergy in T effector cells, PD-1 expression on
CD4+ T effector cells in the PD-L1 enriched tumor microenvironment
actively converts these cells into induced CD4+CD25+FoxP3+ T
regulatory (Treg) cells, which play critical roles in tumor immune
evasion mechanisms [20]. Importantly, PD-1 signaling on B cells
was shown to mediated depletion of activated memory B cells during
simian immunodeficiency virus infection in a primate model and PD-1
blockade reversed this effect, restoring antibody titers [21]. Therefore,
the pleiotropic effects of PD-1 signaling on multiple immune cell
types, such as CD4+ T cell, CD8+ T cells, Treg cells, and B cells, and
its demonstrated role in immune evasion mechanisms presents this
receptor as an important target for cancer immunotherapy.

These insights into the biology of PD-1 and its ligand reinforce the
notion that PD-1 and PD-L1 blockade may synergize multiple arms of
adaptive immunity within tumor microenvironment for a productive
and durable antitumor response required for clinical efficacy. There are
currently four agents, MDX-1106, CT-011, MK-3475 and AMP-224, in
clinical trials targeting the PD-1 pathway for cancer immunotherapy.
The first three are anti-PD-1 monoclonal antibodies (mAbs) while the
last is a B7-DC/IgG1 protein. MDX-1106 is a fully human IgG4mAb
that was first tested in humans with various metastatic solid tumors,
such as melanoma, renal cell carcinoma (RCC), colorectal cancer (CRC),
and non-small cell lung cancer (NSCLC). The trials culminated in
demonstration of impressive clinical efficacy in terms of objective
and durable responses in advanced melanoma (~35%), RCC (~50%)
and NSCLC (~25%) patients. The overall efficacy of the partial or
complete responses of durable nature collectively in all of these cancer
types was over 33%, which far exceeds the responses observed by
standard chemotherapy in these cancer settings [22].

The humanized second mAb, CT-011, was first tested in a single
and well-tolerated dose regimen of advance hematologic malignancies
[23]. Encouraging results from this trial led to its testing in additional
clinical trials for patients with advanced hematologic malignancies
and solid tumors. This Ab in vitro enhanced human NK cell function against
autologous, primary multiple myeloma (MM) cells, seemingly through
effects on NK cell trafficking, immune complex formation with MM
cells, and cytotoxicity specifically toward PD-L1 expressing MM tumor
cells, but not normal cells. These observations demonstrate the function
of CT-011 on the innate immune system [24], and as such indicate the
potential utility of this agent for elimination of MHC class I negative
tumor cells. The third humanized MK-3475 mAb being tested in a
phase I clinical trial. Finally AMP-224, a recombinant protein made by
fusing the extracellular domain of PD-L2 to IgG1, is presently being
tested in a phase I clinical trial against refractory metastatic cancer
cells. The clinical performance of this agent is of significant interest due
to the use of a natural ligand, rather than an Ab, given the toxicity and
efficacy differences observed between natural ligands and agonistic Ab
to costimulatory molecules, such as 4-1BB [25,26].

Moving forward, it is anticipated that one or more anti-PD-1
mAbs will be approved by 2015 based on the impressive early clinical
responses in various tumor models. The anti-PD-1 mAbs may show
even more impressive clinical benefits when used in combination with
other checkpoint blockers, such as CTLA-4, or immune costimulatory
molecules, such as agonists of 4-1BB, OX-40, HVEM, CD40 receptors.
These costimulatory molecules may work with checkpoint blockers
in true synergy, which provide strong rationale for the development of
costimulatory agonists for cancer immunotherapy. Another level
of synergy can be achieved by using the agonists of costimulatory
molecules and antagonist of coinhibitory molecules in combination
with TAAs as novel vaccine candidates. In this configuration, TAAs may
prime tumor-specific T effector cells while the costimulatory agonists
and coinhibitory antagonist augment the ongoing T cell response for a
robust and durable anti-tumor response with therapeutic efficacy.

The incorporation of agents that target innate immunity
and antigen presentation, such as DCs, into the costimulatory/
coinhibitory immunotherapeutic configuration may further improve
immunotherapies, particularly cancer vaccines. In this context,
the use of toll like receptor agonists, such as FDA approved agent
monophosphoryl lipid A (MPL-A), CpG-ODNs, or flagellins, is a
logical choice. These agonists are expected to jump start the adaptive
immunity by first activating DCs for antigen uptake, processing,
and presentation to T cells in addition to the elaboration of various
inflammatory cytokines, such as IL-12, required for up regulation of
costimulatory receptors and initiation/expansion of T cell responses
with therapeutic efficacy and long-term immune memory controlling
micrometastases. However, given the potent nature and pleiotropic
effects of these immune modulators, the combinatorial use of these
molecules for cancer immunotherapy will require homework well done
for achieving clinical benefit in the absence or with tolerable toxicity.
Regardless, the field of tumor immunotherapy is finally ripe to deliver
the long-anticipated promise of controlling/curing cancer and the
years ahead will assess the feasibility of this promise.

Acknowledgements

Funded in parts by grants from Kentucky Lung Cancer Research Program,
W. M. Keck Foundation, and the Commonwealth of Kentucky Research Challenge
Trust Fund.

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