**Betting on improved cancer immunotherapy by doubling down on CD134 and CD137 co-stimulation**

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The ability of T cells to recognize a vast array of antigens enables them to destroy tumor cells while inflicting minimal collateral damage. Nevertheless, tumor antigens often are a form of self-antigen, and thus tumor immunity can be dampened by tolerance mechanisms that evolved to prevent autoimmunity. Since tolerance can be induced by steady-state antigen-presenting cells that provide insufficient co-stimulation, the exogenous administration of co-stimulatory agonists can favor the expansion and tumoricidal functions of tumor-specific T cells. Agonists of the co-stimulatory tumor necrosis factor receptor (TNFR) family members CD134 and CD137 exert antitumor activity in mice, and as monotherapies have exhibited encouraging results in clinical trials. This review focuses on how the dual administration of CD134 and CD137 agonists synergistically boosts T-cell priming and elaborates a multi-pronged antitumor immune response, as well as how such dual co-stimulation might be translated into effective anticancer therapies.

Introduction

The ultimate goal of cancer research is to develop therapeutic strategies that specifically target tumor cells with minimal collateral damage to healthy tissue. The discovery of common mutations that drive tumorigenesis has enabled the development of drugs that produce dramatic clinical responses—at least in specific clinical settings—along with minimal side effects. Examples of this approach include the tyrosine kinase inhibitor imatinib (Gleevec®), which targets the chronic myelogenous leukemia (CML)-specific kinase BCR-ABL, and PLX4032, which inhibits mutated BRAF in a subset of metastatic melanoma patients. Despite these successes, non-specific approaches to cancer such as chemo- and radiotherapy, which impart substantial toxic side effects, remain the standard of care for the vast majority of cancer patients.

One of the hallmarks of cancer is genetic instability, resulting from chromosomal rearrangements and defects in DNA repair mechanisms that normally operate during DNA replication. Genetic mutations can give rise to new antigenic determinants that are selectively expressed by tumor, but not by healthy, cells, the number of which likely increases with disease progression. The random recombination of genes encoding T-cell receptor (TCR) chains endows the T-cell compartment of the adaptive immune system with a diverse repertoire of specificities. These can recognize short peptides derived from virtually any microbe when complexed with major histocompatibility (MHC) molecules expressed on the surface of infected cells or specialized antigen-presenting cells (APCs). This diversity also raises the potential for T cells to recognize tumor-specific peptides, which has fueled the development of therapeutic strategies to induce antitumor T-cell immunity.

Immunosuppressive Nature of the Tumor Microenvironment

Although the immune system (and T cells in particular) can recognize tumor antigens, the high prevalence of cancer indicates that tumors must activate immunosuppressive mechanisms that thwart naturally arising antitumor T-cell responses. CD4+ T cells classically function as “helpers” to facilitate the function of cytotoxic CD8+ T cells (CTLs), natural killer (NK) cells, B cells and macrophages. It thus seemed paradoxical when North and colleagues found that depleting CD4+ T cells from tumor-bearing mice could promote the regression of advanced neoplastic lesions. It was subsequently found that these tumor-induced immunosuppressive T cells (termed regulatory T cells or Tregs) are defined by the constitutive expression of CD25, the α chain of the interleukin (IL)-2 receptor (which confers high affinity for IL-2), as well as the forkhead transcription factor FOXP3. CD25+FOXP3+ Tregs can suppress autoreactive T cells and control the magnitude of pathogen-specific T-cell responses. Tregs can be recruited into the tumor microenvironment, where their presence correlates with unfavorable prognosis, by factors such as the chemokine CCL22. Tregs appear to impede the immune control of tumor growth through multiple mechanisms. For instance, they can promote the expression of anti-inflammatory cytokines within the tumor microenvironment and suppress
the ability of tumor-infiltrating CD8+ T cells to mediate antitumor effector functions.\textsuperscript{21,22}

In addition to Tregs, there are several other immunosuppressive cell types that can localize within the tumor microenvironment. For instance, tumor infiltrating myeloid-derived suppressor cells (MDSCs) can desensitize tumor-specific CD8+ CTLs to cognate antigens by inducing covalent modifications of their TCR through the release of reactive oxygen species and peroxynitrite.\textsuperscript{23} In addition, tumor-associated macrophages (TAMs) can facilitate tumor angiogenesis, invasiveness and metastasis.\textsuperscript{24} Tregs, MDSCs, TAMs and other myeloid-derived cells function within an intricate, but not yet fully understood, immunosuppressive network within the tumor microenvironment.\textsuperscript{25}

**Tumor-Reactive T cells and Tolerance**

To prevent autoimmunity, the bulk of developing T cells expressing self-reactive TCRs undergo negative selection in the thymus.\textsuperscript{26,27} A variety of peripheral tolerance mechanisms such as deletion,\textsuperscript{28} functional inactivation (i.e., anergy\textsuperscript{29}) and suppression by Tregs\textsuperscript{30} control the activity of T cells recognizing self antigens that are not presented in the thymus. These peripheral tolerance mechanisms are not necessarily mutually exclusive, and in fact are likely to be deeply interrelated. For instance, self-reactive T cells often become anergic prior to undergoing deletion\textsuperscript{30,31} and under certain conditions anergic T cells express regulatory functions.\textsuperscript{32}

Dendritic cells (DCs) are a central player in programming peripheral T-cell tolerance. Paradoxically, these bone marrow-derived APCs were originally defined by their potent ability to prime pathogen-specific T cells to develop effector and memory functions. Thus, in addition to their ability to efficiently acquire, process and present peptide epitopes from microbe-infected cells, DCs are induced by pathogen-associated molecular patterns (PAMPs) to express co-stimulatory ligands and cytokines that provide critical signals to enable antigen-stimulated naïve T cells to proliferate and develop effector functions.\textsuperscript{33–35} Co-stimulatory ligands principally belonging to the Ig superfamily such as CD80 (B7.1) and CD86 (B7.2) bind the co-stimulatory receptor CD28 on antigen-stimulated T cells to induce the initial phase of T-cell activation.\textsuperscript{36–38} DCs can also supply a second wave of co-stimulatory signals, mainly via members of the tumor necrosis factor receptor (TNFR) superfamily, which provide antigen-stimulated T cells with anti-apoptotic signals as well as with signals that program effector function and the capacity to form memory cells.\textsuperscript{39,40} Importantly, in steady-state conditions, DCs only express low levels of CD80 and CD86 and little-to-no levels of TNFR family members or cytokines such as IL-12. Thus, when steady-state DCs acquire and present self-antigens, their low expression of co-stimulatory ligands and cytokines causes cognate self-reactive T cells to undergo an abortive proliferative response that culminates in anergy, deletion or in the development of regulatory functions.\textsuperscript{41–44}

Although the immune system can—at least theoretically—respond to tumor antigens, there is a fundamental difference between the targets of tumor-specific, as opposed to pathogen-specific, responses. Thus, pathogen-derived antigens are typically associated with PAMPs that potently activate DCs to prime cognate T cells, while tumor antigens are a form of self antigen, and thus are typically presented under steady-state conditions that are associated with the induction of tolerance. An exception to this rule are antigens that derive from oncogenic viruses such as the human papillomavirus\textsuperscript{45} and Epstein-Barr virus,\textsuperscript{46} which promote cervical tumors and B-cell lymphomas, respectively. Many tumor-associated antigens are non-mutated self antigens that are expressed on both tumors as well as healthy cells. Prototypical examples of such tumor differentiation antigens are the melanocytic antigens tyrosinase,\textsuperscript{47,48} TRP-2\textsuperscript{49,50} and Pmel-17/gp100.\textsuperscript{51,52} Vaccination strategies against these antigens can elicit CD8+ CTLs with the potential to destroy both melanoma cells as well as healthy melanocytes (i.e., resulting in autoimmune vitiligo).\textsuperscript{53,54} Nevertheless, the quality and magnitude of CTL responses to tumor differentiation antigens can be limited by peripheral tolerance, as induced by the same antigens derived from healthy tissue.\textsuperscript{55–57} Taken together, T-cell tolerance mechanisms can limit the magnitude and effectiveness of antitumor immunity directed toward tumor differentiation antigens. In this scenario, when tolerance is overcome, autoimmunity may be a side effect of tumor immunity.

Tumor differentiation antigens most often induce T-cell tolerance prior to the onset of tumorigenesis, because they are expressed on healthy tissues.\textsuperscript{58} Thus, it might seem reasonable that T cells would be less tolerant of tumor-specific antigens that derive from mutated self proteins that are not encoded in the genome of healthy cells. Nevertheless, peripheral T-cell tolerance to tumor-specific antigens occurs in both transplantable and autochthonous tumor models.\textsuperscript{59–62} Specifically, steady-state DCs can acquire tumor antigens and present them in the same tolerogenic manner as self antigens deriving from healthy tissues.\textsuperscript{63,64} ‘This said, tolerance does not always occur,\textsuperscript{65} and in some situations cognate T cells can develop tumoricidal effector functions.\textsuperscript{66–68} The ability of tumors to prime rather than tolerize cognate T cells may depend on whether they release inflammation-inducing endogenous danger-associated molecular patterns (DAMPs) such as heat shock proteins,\textsuperscript{69} uric acid,\textsuperscript{70} or HMGB1\textsuperscript{71} when they metastasize or invade across basement membranes.\textsuperscript{72} Tumors that prime cognate T cells typically engage immunosuppressive mechanisms to dampen the activity of infiltrating tumor-specific effector T cells (see above). Additionally, tumors can undergo “immunoediting,” a process whereby some cells within a heterogeneous tumor mass are eliminated by effector T cells and innate immune cells while another cell population that has downregulated cognate T-cell epitopes and ligands for innate immune cells expands to form a non-immunogenic tumor.\textsuperscript{73} Notably, tumor-specific T cells can mediate immunoediting while simultaneously undergoing tolerance,\textsuperscript{74} which may help to explain the early paradoxical observation that human cancer patients often harbor clonally expanded populations of anergic tumor-specific T cells.\textsuperscript{75} Hence, the initial phase of the antitumor immune response appears to promote the outgrowth of non-immunogenic tumors.
Co-stimulatory agonists enable T cells responding to tolerogenic tumor antigens to undergo expansion and effector differentiation. An example is the TNFR family member CD134 (OX40), which is expressed on T cells following TCR stimulation and normally bound by CD252 (OX40L) on activated, but not steady-state, DCs. When naïve T cells are primed by cognate peptide-presenting activated DCs, the CD252-CD134 interaction initiates signals that program T-cell survival and effector differentiation. CD134 agonists thus enable T cells stimulated by steady-state DCs that lack CD252 to avoid tolerance and undergo expansion and effector differentiation. Importantly, agonists to several TNFR co-stimulatory family members such as CD40, CD134, CD137, and GITR can elicit antitumor immunity in mice. Further, humanized agonists to CD134 and CD137 are being tested in clinical trials to treat human neoplasms.

Because tumor-reactive T cells can undergo tolerization when tumors elicit insufficient inflammation to induce co-stimulatory activity on DCs (Fig. 1A and B), agonistic monoclonal antibodies to co-stimulatory ligands and receptors have been used to program tumor-reactive T cells to expand and acquire tumoricidal effector functions (Fig. 1C and D). For instance, CD40 is a TNFR family member expressed on DCs that—when bound by the its ligand (CD40L or CD154) expressed on activated CD4+ helper T cells—upregulates MHC molecules, co-stimulatory ligands such as CD80 and CD86, as well as cytokines such as IL-12, and hence has the potential to effectively prime CD8+ CTLs. CD40 agonists thus can compensate for the absence of activated CD4+ helper T cells, and—similar to classical adjuvants (e.g., PAMPs that bind Toll-like receptors)—can induce DCs to express co-stimulatory ligands and release cytokines enabling effective T-cell priming (Fig. 1C). A reciprocal approach is to employ agonists to co-stimulatory receptors expressed on T cells (Fig. 1D). An example is the TNFR family member CD134 (OX40), which is expressed on T cells following TCR stimulation and normally bound by CD252 (OX40L) on activated, but not steady-state, DCs. When naïve T cells are primed by cognate peptide-presenting activated DCs, the CD252-CD134 interaction initiates signals that program T-cell survival and effector differentiation. CD134 agonists thus enable T cells primed by steady-state DCs that lack CD252 to avoid tolerance and undergo expansion and effector differentiation. Importantly, agonists to several TNFR co-stimulatory family members such as CD40, CD134, CD137, and GITR can elicit antitumor immunity in mice. Further, humanized agonists to CD134 and CD137 are being tested in clinical trials to treat human neoplasms.

Co-stimulatory Agonists can be Applied in Synergistic Therapeutic Combinations

The TNFR superfamily contains at least 29 receptors (and 19 ligands), several pairs of which play a role in T-cell co-stimulation: CD134-CD252, CD27-CD70, CD30-CD30L, CD137-4-1BBL,
LIGHT-HVEM, CD40-CD40L and GITR-GITRL. This plethora of co-stimulatory mechanisms likely reflects the fact that individual pathways program unique facets of T-cell functions. For instance, although CD134 and CD137 are both expressed on activated CD4+ and CD8+ T cells, CD134 co-stimulation generally has a greater impact on CD4+ T-cell function while CD137 more significantly impacts CD8+ T cells. Further, innate immune cell types such as DCs and NK cells are more responsive to CD137 than CD134. The particular combination of co-stimulatory signals engaged during an immune response is therefore likely to influence the overall functional outcome of T-cell priming. Given that the most effective antitumor immune responses may involve the recruitment of multiple immune effector arms, the administration of co-stimulatory agonists in combination may elicit the most potent therapeutic responses.

The potential to elicit synergistic effects by multiple co-stimulatory pathways may depend (at least in part) on the ability of each pathway to trigger unique downstream signaling events. Thus, although all TNFR family members initiate cytoplasmic signaling through one or more of the six TNFR-associated factors (TRAF1-6) that interact with their intracellular domains and engage several downstream signal transduction pathways such as ERK-, JNK-, p38- and NFκB-dependent pathways, the distinct TNFR family members engage different combinations of TRAFs. Furthermore, TNFR family members differ in their expression patterns. For instance, CD40 is principally expressed on APCS, while CD134 is transiently expressed on TCR-stimulated conventional T cells but constitutively present on the surface of FOXP3+CD25+ Tregs, while CD137 can be expressed on T cells, NK cells, DCs and other innate cells. Thus, the potential for individual TNFR agonists to exert distinct effects in shaping T-cell responsiveness may be determined by a combined effect of triggering unique combinations of intracellular signaling pathways in distinct cell subsets.

One example of an effective combination therapy involves the co-administration of CD40, CD137 and DR5 (apoptosis-inducing receptor for TNF-related apoptosis-inducing ligand, TRAIL) agonists, eliciting a CD8+ T cell-dependent eradication of pre-established tumors in mice. In analyzing the potential of various combinations of CD40, CD134 and CD137 agonists to program CD8+ T cell responsiveness, multiple mouse studies have revealed that the combination of CD134 plus CD137 agonists was particularly effective in boosting CD8+ T-cell expansion, effector function and antitumor immunity. The fact that the effects of CD134 plus CD137 co-stimulation were not simply additive was demonstrated both by their synergistic effect in boosting CD8+ T-cell clonal expansion as well as by the necessity for CD137 co-stimulation to enable CD134 agonist to program CD8+ T cells to differentiate into interferon γ (IFNγ) superproducers.

### CD134 plus CD137 Co-Stimulation Programs a Multi-Pronged Antitumor Immune Response

A major advantage of CD134 plus CD137 dual co-stimulation therapy is its potential to elicit a multi-pronged antitumor response. A first prong of this program is a robust CD8+ CTL response. A second one stems from ability of CD137 alone to activate innate immune cells such as DCs and NK cells. A third, and unexpected, prong is the ability of dual co-stimulation to program CD4+ T cells to differentiate into cytotoxic T\(_{\text{eff}}\) effects that not only produce IFNγ, but also kill target cells presenting cognate MHC Class II-restricted peptides. Cytotoxic functions are classically associated with CD8+ CTLs and NK cells, and although it was known that cultured CD4+ T cells can develop cytotoxic potential in vitro, it has only recently become clear that these cells can be induced in vivo in response to certain infections. Cytotoxic CD4+ T cells might be useful in targeting MHC Class II+ tumors and notably melanomas, which can express MHC Class II molecules but have a propensity to downregulate their Class I counterparts. Indeed, cytotoxic CD4+ T\(_{\text{H1}}\) cells can effectively target murine melanoma. Moreover, CD134 plus CD137 co-stimulated CD4+ T cells exert antitumor activity against murine melanoma.

Given that humanized CD134 and CD137 agonists are being tested in human cancer patients, it will be important to understand how CD134 plus CD137 co-stimulation induces cytotoxic CD4+ T\(_{\text{H1}}\) cells and fully explore their therapeutic potential. Consistent with the notion that CD4+ T cells are typically more responsive to CD134 co-stimulation, a CD134 agonist, but not 4-IBB, is sufficient to program the cytotoxic CD4+ T\(_{\text{H1}}\) functional profile (i.e., the ability to express IFNγ and the apoptosis-inducing serine protease granzyme B, GzmB). Nevertheless, the addition of CD137 co-stimulation maximizes the clonal expansion of cytotoxic CD4+ T\(_{\text{H1}}\) cells, an effect that might promote their therapeutic potential. Mechanistically, cytotoxic T\(_{\text{H1}}\) differentiation depends upon the cytokine IL-2 and the T-box transcription factor Eomesodermin (Eomes). Eomes was initially characterized as a CD8+ T cell-specific factor that drives the expression of GzmB, perforin and IFNγ, indicating that CD134 plus CD137 co-stimulation programs a sort of “CD8-like” CD4+ T cells by inducing a transcription factor normally expressed by CD8+ T cells in a restricted fashion. An intriguing facet of this dual co-stimulation response is that while antigen-responding CD4+ T cells undergo cytotoxic T\(_{\text{H1}}\) differentiation, antigen-non-responding (bystander) T cells are also induced to express GzmB. A common reason for the failure of T cell-based antitumor therapies is the outgrowth of antigen-loss variant tumor cells that lack expression of the targeted epitopes. Given that dual co-stimulation-programmed GzmB bystander T cells have a diverse polyclonal TCR repertoire, they may have the potential to target such antigen-loss variant tumor cells.

A fourth prong derives from the fact that CD134 plus CD137 co-stimulation can program effector T cells to elaborate TCR-independent effector functions. Recently, it has been shown that dual-co-stimulated CD8+ T cells produce prodigious amounts of IFNγ when exposed to IL-33 in the context of IL-12. Unlike IL-12, active IL-33 is typically released by necrotic cells to alert the immune system of danger. This new finding has yet to be exploited in tumor models. Thus, the potential of IL-33 plus...
IL-12 administered directly into tumors to trigger dual-co-stimulated CD8+ effector T cells to secrete IFNγ may bypass the consistent problem of MHC downregulation by malignant cells, which theoretically precludes the TCR-triggered elaboration of effector functions. This concept provides a novel approach that may avoid toxic side effects associated with systemic high dose IL-12. Thus, dual co-stimulation may lower the overall threshold for effector cell activation by programming both TCR-dependent and -independent effector functions.

**Potential Therapeutic Advantages and Disadvantages of Dual Co-Stimulation Therapy**

Like any experimental therapy, CD134 plus CD137 co-stimulation has both potential advantages and disadvantages. As described above, the strong therapeutic potential of this approach stems from a multi-pronged immune response that involves cells from the innate immune system, antigen-specific CD8+ CTLs, cytotoxic T,1 CD4+ cells, and bystander CTLs. This broad attack that engages both innate and adaptive immune components should help to minimize outgrowth of tumors resistant to individual (and even multiple) immune effector arms.

A common toxicity associated with T-cell-based cancer therapies that target tumor differentiation antigens is autoimmune reactions directed against the healthy tissues from which the tumors develop. Notably, an antagonist to the T-cell immune checkpoint protein CTLA-4 (ipilimumab) that has recently received FDA approval for the treatment of advanced melanoma patients can elicit autoimmune side effects. Co-stimulatory modulators can also elicit antigen-unrelated immune toxicities. Thus, in a clinical trial testing the tolerability of a CD28 superagonist (that was expected to only activate Tregs) six out of six healthy volunteers experienced multiorgan failure in association with a storm of pro-inflammatory cytokines.

Thus far, in a Phase I clinical trial, CD134 agonist monotherapy has not produced any toxicities while in Phase II trials CD137 monotherapy has exhibited both clinical efficacy as well as some degrees of liver toxicity. It has previously been shown that the careful titration of CD134 and CD137 agonists in mice significantly lowers the effective dose required to achieve optimal CD8+ T-cell responses. This approach may thus preserve beneficial antitumor activity while limiting adverse side effects. The use of lower doses of agonistic antibodies might also limit the development of human anti-chimeric antibodies (HACA), hence allowing for multiple dosing. This strategy seems less feasible with single co-stimulators, and thus represents a potential in-built clinical advantage of dual co-stimulation-based therapeutic approaches over monotherapies.

**Translating Dual Co-Stimulation into Effective Anticancer Therapies**

The successful translation of CD134 plus CD137 co-stimulation into an effective therapy for human cancer will be facilitated by efforts in several areas. First, an increased understanding of how such agonists regulate human immune responses is required. The bulk of our understanding of how these co-stimulatory pathways stimulate immunity derive from mouse models. Although it is known that CD134 and CD137 can co-stimulate human T cells and results from clinical trials so far support the anti-neoplastic potential of individual humanized agonists, it will be critical to gain deeper insight into how dual co-stimulation impacts human immune responses. Preliminary studies demonstrating that CD134 plus CD137 co-stimulation boosts the priming of human T cells in vitro beyond the effects of either co-stimulator alone support a potential of dual co-stimulation therapy to elicit therapeutic responses in cancer patients.

It will also be important to further dissect how CD134 and CD137 mechanistically synergize to program robust effector T-cell responses. For instance, CD134 co-stimulation (occurring on either CD8+ T cells or innate immune cells) enables CD134 agonist to elicit supereffector CD8+ T cells that express high levels of both IFNγ and TNF. However, it is not clear how CD137 engages the CD134 pathway. In addition, CD134, but not CD137, induces robust IFNγ and GzmB expression in CD4+ T cells, while the addition of CD137 co-stimulation maximizes the clonal expansion of cytotoxic CD4+ T cells. Given the overlap in the intracellular signaling pathways initiated by the these two TNFR family members (both involving TRAFs), it is surprising that they play distinct (rather than simply additive) roles in programming both CD4+ and CD8+ T-cell responses. One possibility is that subtle differences in the respective downstream signaling pathways confer distinct effects in programming T-cell responsiveness. Also, critical signaling events might occur on different cells. Specifically, the mostly T-cell-restricted expression pattern of CD134 suggest that CD134 co-stimulation presumably needs to occur on antigen-stimulated T cells. In contrast, numerous innate cell types express CD137 and CD137 co-stimulation in innate cells can impact specific T-cell responses. Thus, the synergetic effect of CD134 and CD137 co-stimulation may occur through both cell-extrinsic and cell-intrinsic mechanisms.

Advanced cancer is inherently difficult to treat in part due to its high degree of genetic instability. This can lead to the outgrowth of tumor clones that are resistant not only to a particular immunotherapy, for instance owing to variants that have downregulated MHC Class I molecules or specific CTL epitopes, but also to non-immunological therapies such as oncoprotein-targeted small molecules like imatinib and BRAF inhibitor. In some cases, it has been possible to control chemoresistant tumors using drug combinations that differentially target the same oncogenic pathway (e.g., using dasatinib to treat imatinib-resistant tumors). As discussed above, dual co-stimulation therapy may be effective in limiting the outgrowth of antigen-loss variants, given its potential to engage multiple tumoricidal immune effector arms. Nevertheless, dual co-stimulation might become more effective if combined with antagonists to immune checkpoint molecules such as CTLA-4 and PD-1, which themselves possess therapeutic potential but elicit more potent therapeutic responses when combined with immune stimulators. Another potential area to exploit is the use of alarmins (e.g., IL-33) or cytokines that dual-co-stimulated effector T cells have become able to respond to.
Combining dual co-stimulation with standard therapies might produce the most beneficial clinical responses, as the genetic alterations conferring resistance to these different treatment modalities are very unlikely to overlap. Support for this idea come from studies in which tumor vaccines elicited more durable therapeutic responses against lymphoma when given following bone marrow transplantation, and against solid tumors when given following the administration of chemotherapeutic drugs. Importantly, besides establishing states of minimal residual disease (and hence minimize the tumor burden for the immune response to eliminate), these standard of care modalities may also promote antitumor immune responses. Anti-lymphoma vaccines administered following bone marrow transplantation may elicit stronger antitumor T-cell responses due to lymphopenia resulting from the pre-conditioning regimen. Chemotherapeutic drugs may augment antitumor immunity by eliciting the production of Type I interferons or by inducing the release of DAMPs from dying tumor cells, which facilitate tumor antigen uptake by DCs and their activation. The fact that a CD134 agonist and the chemotherapeutic drug cyclophosphamide synergize in controlling melanoma growth by inducing Treg apoptosis within tumors as well as by priming tumoricidal CD4+ effector T cells suggests that it may be worthwhile to examine the therapeutic efficacy of combining CD134 plus CD137 co-stimulation with chemotherapy.

Prostate cancer, the most common malignancy in American men, is an attractive target for T cell-based therapies in part because potential autoimmune side effects directed against healthy prostatic tissue should be tolerable, given the non-vital nature of the prostate gland. Further, T cell-based therapies might be particularly effective when given in conjunction with the standard of care treatment for advanced prostate cancer (Fig. 2). Thus androgen ablation/ deprivation is used for advanced disease, which cannot be treated by surgery or radiation. Although androgen ablation is typically effective in initially reducing tumor burden, because most prostate tumor cells depend upon androgens to grow and survive, disease inevitably recurs due to the outgrowth of tumor clones in which the androgen receptor signaling axis functions even in the absence of normal androgen levels or in the presence of anti-androgens. The development of autochthonous prostate tumors in mice induces T-cell tolerance to prostate-specific antigens, but—importantly—androgen ablation diminishes this effect, apparently by reducing tumor mass and hence the quantity of tumor antigens available for presentation by steady-state tolerogenic DCs. Clinical trials are currently testing the idea that T cell-based prostate cancer therapies may be most effective when administered soon after androgen ablation. Androgen ablation may improve the clinical outcome of immune therapy (at least in part) by reducing the number of tumor cells to be eliminated by the immune system. Additionally, androgen ablation reverses age-related thymic atrophy, and is therefore likely to increase the number of newly generated naïve prostate-specific T cells available for priming. Furthermore, CD134 agonists can reverse pre-existing T cell anergy, and thus dual co-stimulation may also be able to engage previously tolerized prostate-specific T cells.

Concluding Remarks

As discussed above, translating dual co-stimulation into an effective anticancer therapy will require the resolution of several outstanding questions: (1) how CD134 and CD137 synergize at the genetic and biochemical level to program TCR-dependent effector functions beyond those elicited by single co-stimulators;
(2) whether dual co-stimulation-programmed TCR-independent effector functions (e.g., the release of IL-33) can be exploited for therapeutic use; and (3) whether potential synergies between dual co-stimulation and other immune modulators (e.g., CTLA-4 antagonist) as well as non-immune-based therapeutic modalities may result in superior antineoplastic effects.

It is commonly known that “two heads are better than one” and we propose that dual co-stimulation is better than mono co-stimulation. The capacity to administer lower doses of agonists while achieving greater clinical benefit is a critical goal that can be achieved by triggering specific combinations of co-stimulatory pathways. As not all co-stimulatory pathways fit into this category, a goal for the field is to uncover why some agonistic combinations work better than others. Understanding the basis of this synergism will allow for the rational design of small molecules that operate similarly to humanized reagents and potentially spawn a more personalized approach to cancer treatment. In sum, many co-stimulatory pathways are known, but finding the right combination of co-stimulation or cytokines for selected clinical circumstances may be the holy grail for the efficient treatment against different forms of cancer that strike humans.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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