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Twelve immunotherapy drugs that could cure cancers

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Summary: Immune T cells can kill cancer cells. Cancer vaccines function by increasing the number of immune T cells. There are exceedingly strict biologic limits imposed on the immune system to prevent excessive T-cell activation and expansion. The same biological restrictions limit cancer vaccines. Immunotherapeutic agents that circumvent the biological restrictions have been invented and formulated, including (i) dendritic cell activators and growth factors, (ii) vaccine adjuvants, (iii) T-cell stimulators and growth factors, (iv) immune checkpoint inhibitors, and (v) agents to neutralize or inhibit suppressive cells, cytokines, and enzymes. Few of these agents are broadly available for the development of effective multiple component regimens. The major problem facing immunotherapy today is a lack of broad availability of agents already in existence. The National Cancer Institute has developed a well-vetted ranked list of agents with high potential to serve as immunotherapeutic drugs. This review focuses on 12 of the agents, all with proven ability to augment T-cell responses. Alone, each has little chance of making substantial inroads into cancer therapy. In combinations dictated by biology, the agents are overwhelmingly likely to have an impact. Future availability of these agents for development of innovative combination cancer therapy regimens will provide a benchmark for the resolve of the national cancer therapy translational research enterprise.

Keywords: *T cell, vaccine, adjuvant, cancer, immunotherapy, translational*

Introduction

It is well known that cancer can be cured by immune T cells. Hematologic malignancy can be cured by allogeneic T cells. Probability of relapse after bone marrow transplantation (BMT) for leukemia varies according to type of marrow graft and subsequent development of graft versus host disease (1). 'Mini-transplants' using non-myeloablative regimens with high dose immunosuppression to prevent rejection of the transplanted allogeneic bone marrow can eliminate leukemia and lymphoma exclusively by the donor T-cell-mediated allogeneic graft versus leukemia effect (2).

More common epithelial tumors can also be treated with allogeneic T cells, but less effectively. Mini-transplants for renal cell cancer (3) and breast cancer (4) have induced responses including complete remissions. The approach is limited by inordinate toxicity but establishes the proof of concept that T cells can eliminate epithelial tumors.

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A major endeavor for tumor immunotherapists has been trying to figure out how to invoke the therapeutic potential of autologous immune T cells to reproduce the therapeutic efficacy of allogeneic T cells without the inordinate toxicity. It is eminently clear that cancer cells are immunogenic and can be recognized and killed by autologous T cells. Cancer is the result of multiple genetic alterations occurring over many years. The cumulative effect of genetic alterations results in loss of control of cell growth and differentiation. Each change in protein structure, resulting from genetic alterations, represents a potential cancer antigen and target for T-cell attack. Cancer cells commonly posses many tumor-specific and tumor-associated antigens that can be shared or are unique (5). Ongoing and nascent immune responses to autochthonous cancer cells are commonly observed with both circulating and tumor-infiltrating immune T cells with the potential to recognize and kill cancer cells.

It is axiomatic that if large number of immune T cells capable of recognizing and killing cancer cells could be generated *in vivo* with cancer vaccine regimens or *ex vivo* for adoptive transfer many patients could benefit. The prognosis of cancer patients in many instances correlates with the number and character of immune T cells and tumor infiltrating T cells (6, 7). Autologous immune T cells have been used effectively in therapy in small numbers of cancer patients (8). Vaccines that induce immune T-cell responses have proven to be effective, but less so (9).

Immune T-cell number and function are both important components of vaccine efficacy (10). However, the fundamental role of cancer vaccines is to increase the number of cancer antigen-specific T cells *in vivo*. At the simplest level, the therapeutic effect of immune T cells, and thus vaccines, is quantitative and related to the number of T cells present. As the number of immune T cells increases, the effect of the vaccine should increase, all else being equal. The efficacy is related not only to the quantity of T cells but also to the length of time the T cells persist, i.e. in pharmacologic terms, the 'area under the curve.'

Cancer vaccines present antigen to the small number of T cells capable of 'recognizing and responding' to the antigen, which are thereby stimulated to proliferate and increase in number. There are exceedingly strict biologic limits imposed on the immune system to prevent untoward and excessive T-cell expansion. Many of the components and factors that allow T-cell activation and expansion in number have been identified and constructed as immunotherapeutic agents. Similarly, many of the components and factors that disallow activation, expansion, or persistence of immune effector cells have been identified, and agents that interfere with the disallowment have also been constructed as immunotherapeutic agents (11).

Taken together, the agents needed to greatly augment immune T-cell responses have been discovered. Many new biological agents are available as potential immunotherapeutic drugs to substantially increase the number and therapeutic function of immune T cells *in vivo*.

Agents that could take cancer vaccines from interesting biological phenomena to effective therapeutic agents include:

- T-cell growth factors to increase number and repertoire of naive T cells
- Growth factors to increase the number of dendritic cells (DCs)
- Agonists to activate DCs and other antigen-presenting cells (APCs)
- Adjuvants to allow and augment cancer vaccines
- Agonists to activate and stimulate T cells
- Inhibitors of T-cell checkpoint blockade
- T-cell growth factors to increase the growth and survival of immune T cells
- Agents to inhibit, block, or neutralize cancer cell and immune cell-derived immunosuppressive cytokines

Agents in each category have been discovered, invented, constructed, and proven to have immunologic function in animal models and in human clinical trials. However, the majority of agents needed to improve cancer vaccines are not broadly available for testing in patients, especially for use in innovative multi-component regimens.

Lack of broad availability of immunotherapeutic agents: adjuvant example

The major problem facing immunotherapy today is no longer a lack of knowledge or a lack of agents with proven immunologic effects, rather a lack of broad availability of agents with already proven immunologic function. The example of adjuvants exemplifies that problem. There is one 'Universal Truth' about vaccines: adjuvants are needed to achieve highest levels of immune response. Despite this Universal Truth, few vaccine adjuvants are commonly available for broad testing. The question is 'Why aren't adjuvants available?' The National Cancer Institute (NCI), Food and Drug Administration (FDA), and industry all play a role.

The NCI and other funding agencies have spent literally hundreds of millions, if not billions, for cancer vaccine development. Very little has been spent for essential vaccine components. It is commonly accepted that adjuvants are needed to achieve highest level of immune response, in yet

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there is no coordinated effort to assure that the appropriate adjuvants are available, even for ongoing NCI-funded cancer vaccine trials.

By FDA policy and custom, adjuvants are approved only as components of vaccines. Accordingly, the commonest adjuvants used by academics are agents approved for non-adjuvant purposes. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is commonly used in academic vaccine trials, in part because it is readily available. GM-CSF was approved by the FDA as a hematopoietic growth factor, not as a vaccine adjuvant. If GM-CSF had activity only as an adjuvant, it would not be available for testing in cancer vaccines. In a similar vein, imiquimod, a drug approved for therapy of genital warts and superficial basal cell cancer, not as an adjuvant, is beginning to be used as an adjuvant in NCI-funded cancer vaccine trials. The 'Catch 22' is that adjuvants approved for non-adjuvant purposes are broadly available, whereas, adjuvants that function only as adjuvants are not broadly available, regardless of potency. The 'majority of cancer drug development takes place post-approval,' an observation of Robert Capizzi. Adjuvants are not approved as adjuvants and thus not available for cancer drug development. The FDA has given no clear path forward for broad testing and approval of adjuvants that are not effective as monotherapy.

Industry decisions are driven by the 'Invisible hand of the market.' Rational decisions are made based on regulatory and commercial concerns. Companies with great adjuvants have tended to develop them as monotherapy, e.g. CpG and interleukin-12 (IL-12). Agents with adjuvant potential that fail as monotherapy are often 'left on the shelf,' as companies do not see a clear and rational regulatory and commercial path forward.

NCI immunotherapy agent workshop to rank agents with high potential to serve as immunotherapeutic drugs

What is the solution to the lack of availability vaccine adjuvants and other previously invented critical immunotherapeutic agents? The NCI has taken one small step by developing a ranked list of agents with high potential to serve as immunotherapeutic drugs (12). The ranking was exceedingly well vetted, with open and substantial input. The list represents a 'consensus' of what is most needed. There was a range in participant representation from academia, industry, the NCI, and the FDA. Participants were selected from suggestions by the American Association for Cancer Research, American Association of Immunologists, American Society of Oncology, American Society of Hematology, the Cancer Vaccine Consortium, and the International Society of Biological Therapy of Cancer, and NCI intramural and extramural program heads.

These agents have wide appeal to the immunotherapy community and, by consensus, hold particular promise for use in cancer therapy. The exceedingly broad nature of the consensus behind this list should facilitate NCI discussions on the availability of clinical grade immunotherapeutic drugs for human trials. The agents were not selected specifically with cancer vaccines in mind but are essentially the agents needed to improve cancer vaccines.

The process of developing a ranked list of immunotherapy agents began with the NCI opening a web site to ask for suggestions. The web site was advertised and promoted broadly. A total of 124 agents were suggested. Most had demonstrated immunological or physiological function. This response is substantial evidence of the vigor of the immunology and immunotherapy fields. There was a broad desire for vaccine adjuvants, T-cell growth factors, agents to inhibit immune checkpoint blockade, functional antibodies, cytokines, ligands and receptors to activate or augment T-cell responses and/or to inhibit suppressor circuits, and agents left on the shelf by drug companies.

A workshop was held at the NCI July 12, 2007 to develop a ranked list of agents with high potential for use in cancer therapy (12). The organizing committee selected the top 30 agents winnowed from the 124 suggested agents. The committee focused on agents with the greatest potential for broad usage by multiple investigators in multiple different regimens and excluded specific antigens for vaccines and antigen-specific antibodies regardless of attractiveness or potential utility. The ranked list of 20 agents (*Table 1*) derives value from the broad and substantial input. The process, participants, and full report including PowerPoint® presentations can be viewed and downloaded from the NCI web site (http://web.ncifcrf.gov/research/brb/workshops.asp) (12).

The criteria used for inclusion on ranked list included (i) potential for use in cancer therapy, (ii) perceived need by multiple, independent clinical investigators, (iii) potential use in more than one clinical setting, i.e., against different tumor types or as part of multiple therapy regimens, and (iv) not broadly available for testing in patients. Criteria not used included (i) prior failed attempts to commercialize and (ii) intellectual property.

The final ranked list derived from discussions of each agent. Agents at the top of the list were considered the most desirable based on current evidence. It was well recognized by the participants that many agents with fewer data, including

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Table 1. **Rankings of agents with high potential for use in treating cancer**

Rank*	Agent	Agent category
1	IL-15	T-cell growth factor
2	Anti-PD1 and/or anti-B7-H1 (PD-1L)	*T-cell checkpoint blockade inhibitor
3	IL-12	Vaccine adjuvant
4	Anti-CD40 and/or CD40L	Antigen presenting cell stimulator
5	IL-7	T-cell growth factor
6	CpG	Vaccine adjuvant
7	1MT	Enzyme inhibitor
8	Anti-CD137 (anti-4-1BB)	T-cell stimulator
9	Anti-TGF-β	Signaling inhibitor
10	Anti-IL-10 Receptor or Anti-IL-10	Suppression inhibitor
11	Flt3L	Dendritic cell growth factor/ vaccine adjuvant
12	Anti-glucocorticoid-induced TNF receptor (GITR)	T-cell stimulator
13	CCL21 adenovirus	T-cell attracting chemokine
14	MPL	Vaccine adjuvant
15	Poly I:C and/or PolyICLC	Vaccine adjuvant
16	Anti-OX40	T-cell stimulator
17	Anti-B7-H4	T-cell checkpoint blockade inhibitor
18	Resiquimod and/or 852A	Vaccine adjuvant
19	LIGHT and/or LIGHT vector	T-cell stimulator
20	Anti-lymphocyte activation gene-3 (LAG-3)	T-cell checkpoint blockade inhibitor

*Anti-CTLA-4, a T-cell checkpoint blockade inhibitor, was considered of exceedingly high value but was not included on the list, as it is being produced by two companies and is likely to be approved by the FDA within the foreseeable future.

agents not currently on the list, will ultimately prove to be more important than those at the top of the list. Although the ranking is well vetted and based on the cumulative knowledge of the broad immunotherapy and cancer research communities, the choice and desirability of individual agents will undoubtedly change with new knowledge. Because the priorities are based on incomplete knowledge, the process should be a dynamic one that can be revised as more data appear. A common suggestion was that a mechanism should be developed to continually update the list.

Possible positive outcomes of having a well-vetted ranked list based on a broad consensus of the immunology and immunotherapy community could include encouragement of (i) Rapid Access to Interventional Development (RAID) applications for the manufacture, (ii) distribution of company-manufactured agents through RAID or the Cancer Therapy Evaluation Program, and (iii) reinvigoration of pharma/biotech efforts to develop them. This rank-setting exercise should also serve as a report card. Future availability of these agents for broad testing and development will provide a benchmark for the strength and resolve of the national cancer therapy development enterprise.

This review focuses on 12 agents on the list that have proven immunologic efficacy and have been in clinical trials or will shortly be taken into clinical trials (*Table* 2). If tested creatively and intelligently in multiple combinations and regimens with cancer vaccines, regimens will surely be derived that will be able to benefit many patients with cancer. The major message is that the drugs needed to treat many patients with cancer have already been invented.

T-cell growth factor: IL-15

IL-15 is a T-cell growth factor, similar to IL-2 (13–22). Preclinical data overwhelmingly suggest that IL-15 could have an exceedingly important role in cancer vaccine and adoptive T-cell regimens. In contrast to IL-2, IL-15 inhibits antigen-induced cell death of T cells, whereas IL-2 promotes antigen-induced cell death. IL-15 is made by DCs, macrophages, and stromal cells, not T cells. It acts on $CD8^+$ T cells, $CD4^+$ T cells, natural killer (NK) cells, and mast cells. It uses a unique IL-15Rα chain, but shares the β and γ chains of the IL-2R.

IL-15 is necessary for maintenance of $CD8^+$ memory cells and NK cell development. In vaccines, it promotes induction of longer-lived and higher-avidity $CD8^+$ T cells that can kill tumor cells very effectively. Greenberg's group (22) showed that IL-15 can reverse T-cell anergy. Palucka's group (14) demonstrated that IL-15 promotes *in vitro* differentiation of

Table 2. **Twelve immunotherapy drugs that could cure cancer**

Agent category	Agent
T-cell growth factors	IL-15
	IL-7
T-cell checkpoint blockade inhibitor	Anti-PD1 and/or anti-B7-H1 (PD-1L)
Antigen-presenting cell stimulator	Anti-CD40 and/or CD40L
Enzyme inhibitor	1MT
T-cell stimulator	Anti-CD137 (anti-4-1BB)
DC growth factor/ vaccine adjuvant	Flt3L
Vaccine adjuvants	IL-12
	CpG
	MPL
	Poly I:C and/or PolyICLC
	Resiquimod and/or 852A

Eight agents on the NCI ranked list (*Table 1*) are excluded from this list, only as less is known about their effects on T-cell vaccine responses. The agents not included as well as many other might ultimately prove to have greater immunotherapy efficacy than the agents chosen for this review.

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monocyte-derived DCs that are potent inducers of $CD8^+$ T cells. Unpublished data from Berzofsky's laboratory indicate that IL-15 can overcome lack of $CD4^+$ T-cell help in cytotoxic T-lymphocyte (CTL) induction. Unpublished data from Khleif and Pavlakis and Felber show that IL-15-expressing plasmids can induce tumor regression in mice via intratumoral injection or hydrodynamic delivery. No clinical data are available.

The information on IL-15 in this section is taken, largely verbatim, from a presentation by Jay Berzofsky at the NCI Immunotherapy Agent Workshop (12). A full report of the presentation with slides and comments, as well as presentations on the subsequent agents to be discussed in this review, can be downloaded from the NCI web site (http://web.ncifcrf.gov/research/brb/workshops.asp).

The contemplated uses for IL-15 include as (i) a vaccine adjuvant to induce longer-lived, higher avidity, more efficacious $CD8^+$ T cells, single agent to overcome T-cell anergy, (ii) as a T-cell growth factor to be used in conjunction with adoptive T-cell immunotherapy, in place of IL-2, (iii) induction of DC precursors into Langerhans-like DCs for DC-based vaccines, and (iv) intratumoral or systemic cytokine therapy for cancer.

T-cell growth factor: IL-7

IL-7 is a T-cell growth factor responsible for homeostatic expansion of naive cells during lymphopenia (23–31). In mice and primates, IL-7 treatment (24, 26) induces dramatic expansions in peripheral T-cell numbers without obvious toxicity. In mice and primates, following BMT, IL-7 increases the rate and degree of immune reconstitution (27). In humans, IL-7 is required for T-cell development and for naive T-cell survival in the periphery. During lymphopenia, IL-7 accumulates as a result of diminished utilization.

Two-phase I/II clinical trials have been completed in patients with cancer (31, Sportes *et al.*, manuscript submitted). The trials established proof of principle. IL-7 induced dramatic increases in total body $CD4^+$ and $CD8^+$ T cells with modest increases in NK cells and $\delta\gamma$ T cells with no change in mature B cells or selective increase in T-regulatory cells (Tregs). There appeared to be a preferential expansion of naive T cells with an increased T-cell repertoire diversity, likely related to cycling of recent thymic emigrants. In mice, IL-7 is also a vaccine adjuvant (30) with its most dramatic effects on subdominant responses.

IL-7 and IL-15 are both T-cell growth factors and vaccine adjuvants, but they work at different phases of T-cell activation. Both alter T-cell homeostasis but work on different populations. IL-7 signals resting T cells and preferentially expands naive T cells, leading to increased repertoire diversity. IL-15 preferentially signals activated T cells and expands effector/memory populations. The information on IL-7 is taken, largely verbatim, from a presentation by Crystal L. Mackall (12).

The contemplated uses of IL-7 are (i) immunorestorative T-cell growth factor for naive T cells, especially in elderly patients and patients with prior lympholytic chemotherapy, and (ii) vaccine adjuvant to enhance the effectiveness of tumor vaccines targeting weak self-antigens.

T-cell checkpoint blockade inhibitor: anti-programmed death-1

Programmed death-1 (PD-1) is a negative regulator of T-cell function (32–38). Blocking PD-1 can increase anti-tumor T-cell immunity. PD-1, a receptor member of the immunoglobulin superfamily, structurally related to cytotoxic T-lymphocyte antigen-4 (CTLA-4) and CD28, is upregulated on activated T and B cells and monocytes. Binding to PD-1 ligand (PD-1L) results in inhibition of T-cell proliferation. PD-1L is expressed on T and B cells, macrophages, and DCs, as well as parenchymal and tumor cells. In mice, PD-1 is implicated in tolerance induction and appears to 'protect' them from immune attack by CTLs. Blockade of PD-1 or PD-L1 results in long-lasting tumor regression. In humans, abrogation of PD-1 can increase the number of functional cytokine-secreting CTLs, presumably through increased proliferation. PD-L1, expressed on many human tumors, is associated with a reduced T-cell infiltrate and a poorer prognosis. Hamanishi *et al.* (38) showed that ovarian cancer patients who had greater levels of PD-1L on tumor cells had less T-cell infiltration and lower survival rates than those who expressed little or no PD ligand.

PD-1L is expressed by esophageal, colon, lung, and ovarian cancer and melanoma by immunohistochemistry. Thus, these tumors might be appropriate candidates for initial testing of anti-PD-1, especially for those tumors with T-cell infiltration. Use of antibody to block PD-L1 is an alternative approach that might also be useful. However, targeting the tumor may be more difficult than targeting T cells, if saturation of all tumor tissue is needed. The information on anti-PD-1 is taken, largely verbatim, from a presentation by Jeffrey Weber (12).

The contemplated uses of anti-PD-1 are (i) combined with vaccines to increase the number of activated T cells, (ii) as monotherapy for cancers that have infiltrating T cells or a nascent or ongoing immune response, and (iii) combined with anti-CTLA-4 therapy.

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APC stimulator: CD40 agonists

Anti-CD40 and CD40L are potent agonists for increasing APC function (39–50). CD40, a member of the tumor necrosis factor (TNF) receptor superfamily, is a costimulatory protein found on APCs. Ligation of CD40 on APCs by CD40L expressed by T cells enhances the expression of major histocompatibility complex and costimulatory molecules on APCs, stimulates the production of pro-inflammatory cytokines such as IL-12, and leads to T-cell activation and cell-mediated immunity (44). In mice, agonist CD40 monoclonal antibody (mAb) can mimic the signal of CD40L and substitute for the function of $CD4^+$ lymphocytes (39). Agonist CD40 mAb can also overcome T-cell tolerance in tumor-bearing mice, evoke effective CTL responses, and enhance the efficacy of anti-tumor vaccines (42).

In addition to APC activation and augmentation of T-cell immunity, CD40 agonists have other potentially therapeutic effects including direct tumor inhibition, anti-angiogenesis (41), and activation of NK cells (45) and macrophages (40). Engagement of CD40 on tumor cells *in vitro* has been shown to inhibit the growth of solid tumor cells and high-grade B-cell lymphoma lines, a direct cytotoxic effect resulting in tumor regression through apoptosis and necrosis (43).

Two categories of agents have proven efficacy as CD40 agonists, recombinant trimeric ligand, and mAb. Dr Sondel described available unpublished and published data on clinical experience (47–50). One phase I trial enrolled 29 patients with melanoma or other solid tumors. Four subjects had measurable objective responses by RECIST criteria. Most showed upregulation of the CD86 costimulatory molecule. Another phase I trial with recombinant human trimeric CD40 ligand showed two partial responses out of 32 solid tumors or non-Hodgkin's lymphoma (49, R. H. Vonderheide, personal communication regarding unpublished observations, July 2007).

CD40 agonists have great potential for use in combination with other agents. Preclinical models suggest efficacy with chemotherapy, radiation therapy, Toll-like receptor (TLR) agonists, and cytokines. The information on anti-CD40 is taken, largely verbatim, from a presentation by Paul M. Sondel (12).

The contemplated uses for anti-CD40 are (i) for systemic or local activation of APCs as a component of a regimen to induce or augment T-cell responses, (ii) to direct inhibition of $CD40^+$ tumors, and (iii) in combination with other cancer therapies.

Inhibitor of an immunosuppressive enzyme inhibitor: 1-methyl tryptophan

1-Methyl tryptophan (1MT) is a potent small molecule inhibitor of the immunosuppressive enzyme indoleamine 2,3-dioxygenase (IDO) (51–59). IDO is highly expressed in tumor cells and plasmacytoid DCs in tumor-draining lymph nodes. IDO catalyzes tryptophan degradation, leading to insufficient tryptophan and thereby limiting antigen-induced T-cell activation and mediating immunosuppression in cancer. 1MT inhibits IDO, reversing the immunosuppressive effects of IDO. IDO knockout mice are resistant to inflammatory skin carcinogenesis. 1MT limits growth of grafted or transgenic tumors in combination with chemotherapy in models dependent on $CD4^+$ T-cell responses. In humans, clinical trials are being planned. The information on 1MT is taken, largely verbatim, from a presentation by George Prendergast (12).

The contemplated uses of 1MT are (i) as a general reversal of a mechanism of tumoral immune suppression alone or in combination with cytotoxic chemotherapy, tumor vaccines, TLR agonists (e.g. CpG), radiotherapy, mAbs, or drugs that target other mechanisms of immune suppression (e.g. OX40, PDL-1), and (ii) for cancer prevention in clinical states of chronic inflammation.

T-cell stimulator: anti-CD137 (anti-4-1BB)

Anti-CD137 is a T-cell stimulator with potent costimulatory, anti-apoptotic, and proliferative activity (60–69). The target, CD137, as a member of the TNF superfamily of receptors and is present on activated T cells, NK cells, and NKT cells. The receptor is not present on tumors. In preclinical studies, anti-CD137 could costimulate T cells that are suboptimally stimulated with anti-CD3, enhance T-cell responses to simian immunodeficiency virus gag DNA vaccines, and treat immunogenic murine tumors as monotherapy or in conjunction with vaccination. Anti-tumor effects have been observed with murine anti-4-1BB in various *in vivo* tumor models. Preclinical toxicity studies in mice suggested a predominance of NKT cells in the liver (e.g. hepatic necrosis, elevated transaminases). In monkey models, occasional mild colitis was observed, possibly related to the high number of activated lymphocytes in intestinal mucosa.

A phase I, first-in-human study is ongoing and is being expanded to a phase II trial. The subjects are advanced cancer patients with a variety of tumors. Toxicities have consisted of faint skin rash, mild neutropenia, and hepatoxicity (likely to be dose limiting). A phase I trial of anti-4-1BB in combination with paclitaxel and carboplatin is accruing, and another one involving radiotherapy or chemoradiotherapy is being planned. The information on 1MT is taken, largely verbatim, from a presentation by Kim Margolin (12).

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The contemplated uses of 1MT as part of multiple different antigen-specific strategies involving suboptimal T-cell responses include (i) vaccine and cytokine therapy, (ii) augmentation of nascent anti-tumor responses, and (iii) in combination with checkpoint blockade (e.g. anti-CTLA-4A).

DC growth factor/vaccine adjuvant agent: Flt3 ligand

Flt3 ligand (Flt3L) is a hematopoietic growth factor that induces expansion and differentiation of all DC progenitors, especially interferon-producing killer and plasmacytoid DCs (70–76). Systemic administration of Flt3L increases DC numbers circulating in blood and in secondary lymphoid tissues as well as tumors. Some preclinical models report anti-tumor activity with systemic Flt3L protein alone, though effects are very variable. There are a number of reports demonstrating synergy between Flt3L and DC activators such as CpG and anti-CD40 in generating anti-tumor activity.

Phase I/II studies have examined Flt3L alone, with peptide vaccines, as DC stimulators, and after BMT. Increases in circulating DCs are consistently reported. Increased T-cell responses are commonly reported. CEA-loaded Flt3L-mobilized DC vaccine for colon cancer reported increased $CD8^+$ T-cell priming and clinical responses. MAGE-loaded Flt3L-mobilized DC vaccine (with CD40L *in vitro* DC activation for melanoma) reported increased $CD8^+$ T-cell responses. Studies were generally too small and variable to make significant statements about clinical efficacy. Immunex terminated trials after lack of obvious efficacy as single agent or with sCD40L. The information on Flt3L is taken, largely verbatim, from a presentation by Drew Pardoll (12).

The contemplated uses of Flt3L are (i) in combination with vaccines, BMT, BMT+vaccine, adoptive T-cell therapy, TLR agonists, and other immune stimulants, (ii) local administration (topical, admixture with vaccine preparations, intratumoral injection) to enhance DCs, (iii) transduction of Flt3L gene into engineered tumor vaccines or *in vivo* delivery (i.e. plasmid vaccination or in viral carriers), and (iv) for DC mobilization for *ex vivo* DC vaccines.

Vaccine adjuvant: IL-12

IL-12 is an exceedingly potent immune adjuvant (77–87). IL-12 promotes interferon-γ release by IL-12R-expressing T and NK cells and induces T-helper 1 cell (Th1) polarization, as well as proliferation of interferon-γ-expressing T cells. IL-12 plays a central role in resistance to mycobacterial and intracellular pathogens (e.g. parasites). It also plays an important part in anti-cancer development and immunity in animal systems. Phase I and II clinical trials showed very modest efficacy alone with a handful of melanoma and renal cell cancer responses. It did not demonstrated sufficient clinical activity as a stand-alone drug to warrant further development, according to the standard oncology paradigm. It was originally developed as a systemic cytokine, but it proved challenging to administer safely. However, studies in mice have shown IL-12 to be exceedingly effective as a vaccine adjuvant. Owing to systemic toxicity, delivery might best be by local injection including use of viral or plasmid vectors. The information on IL-12 is taken, largely verbatim, from a presentation by Jeffrey Weber (12).

The contemplated uses for IL-12 are (i) as vaccine adjuvant, and (ii) as an adjuvant to cytokine therapy to polarize Th1 responses and augment $CD8^+$ T-cell responses in any antigen-specific strategy.

Vaccine adjuvant: CpG

CpG is a potent TLR9 agonist (88–97). Unmethylated CpG is prevalent in bacterial and viral DNA but not in vertebrate genomes. CpGs target TLR9 with activity as a vaccine adjuvant and immune modulator. In humans, TLR9 is expressed on B cells and plasmacytoid DCs. In the mouse, it is expressed on B cells, monocytes, and all DCs. These species-based differences make it a bit difficult to extrapolate preclinical data. Targeting TLR9 with CpG leads to B-cell proliferation and differentiation to plasma cells, immunoglobulin G isotype switching and antibody secretion, maturation of plasmacytoid DCs with type I interferon secretion, activation of NK cells with enhancement of antibody-dependent cell-mediated cytotoxic activity, stimulation of Th1 cell response with CTL activation, but also has opposing effects, e.g. Treg generation, IDO expression, and anti-inflammatory (IL-10) cytokine secretion.

CpG has been studied in phase I and II trials as monotherapy, in combinations, and as a vaccine adjuvant. CpG is a category of agent with many different nucleotide sequences producing similar but not identical results. Two randomized trials of CpG in combination with chemotherapy in patients with non-small cell lung cancer failed to show benefit.

CpG has shown substantial activity as a vaccine adjuvant for peptide, protein, DC, and autologous tumor cell vaccines. It induces strong Th-1-promoting effects and CTL responses, compared with other TLR ligands, and appears to be synergistic with GM-CSF and other adjuvants. As an immune modulator, it has activity in combination with many other types of therapy including anti-CD40, anti-CTLA-4, IL-13 exotoxin, chemotherapy, radiation therapy, and cryoablation.

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The information on CpG is taken, largely verbatim, from a presentation by Ellis Reinherz (12).

The contemplated uses of CpG are (i) as a vaccine adjuvant and (ii) in combination with multiple other types of cancer therapeutic interventions.

Vaccine adjuvant: monophosphoryl lipid A

Monophosphoryl lipid A (MPL or MPLA) is an effective and exceedingly safe and non-toxic adjuvant vaccine adjuvant (98–107). It is a highly specific TLR4 agonist. MPL is a component of endotoxin, i.e. lipopolysaccharide (LPS). LPS functions as a vaccine adjuvant but is considered too toxic for clinical use. However, purifying MPL from *Salmonella minnesota* endotoxin yields an excellent, low-toxicity adjuvant capable of activating macrophages and especially DCs. MPL has been shown in animal models to elicit responses to antigens of low immunogenic potential and can be administered by various routes and used in multiple formulations, including combinations with other adjuvants. It is a safe adjuvant with a side effect profile equivalent to that of alum. More than 120 000 doses have been administered to more than 50 000 human subjects. It has been approved as a component of a hepatitis B virus vaccine in the European Union. MPL is being used in combination with QS-21 and CpG in a proposed pivotal trial MAGE-A2 protein for non-small cell lung cancer by GlaxoSmithKline. Synthetic TLR4 agonists are being developed with similar potential as MPL. The information on MPL is taken, largely verbatim, from a presentation by Martin Cheever (12).

The contemplated uses for MPL are (i) as an adjuvant for vaccines as a single adjuvant or combined with other adjuvants with multiple different types of antigens, different formulations, different routes of administration and adjuvant combinations, and (ii) for activation of innate immune responses.

Vaccine adjuvants: polyI:C and polyICLC

PolyI:C and polyICLC are potent TLR3 agonists with vaccine adjuvant potential (108–117). PolyI:C is double-stranded polyinosinic:polycytidylic acid. When stabilized with poly-L-lysine and carboxymethylcellulose, it is known as polyICLC, and is more stable and, in that regard has greater activity. Preclinical studies have demonstrated that these TLR3 agonists activate human DCs, improve antigen presentation, and enhance Th1 polarization and adjuvant vaccines. In humans, they are strong activators of Th1 responses, $CD8^+$ T cells, and NK cells. PolyI:C and related compounds are being tested broadly in humans in clinical trials in viral infections. In cancer, an ongoing phase I/II trial of polyICLC involves patients with malignant gliomas. The agent is also being tested in prostate cancer patients for adjuvant effect with a MUC1 100-mer peptide vaccine. TLR3 agonists have uncertain activity as monotherapy but substantial potential activity as vaccine adjuvants. The information on polyI:C and polyICLC is taken, largely verbatim from a presentation by Anna Karolina Palucka (12).

The contemplated uses for polyI:C and polyICLC are (i) as adjuvant for cancer vaccines for use alone and in combination with other categories of adjuvants and (ii) for prevention or mitigation of viral infections.

Vaccine adjuvants: resiquimod and 852A

The imidazoquinolinamines resiquimod and 852A are TLR7/8 agonists, which induce innate and adaptive immune responses (118–125). Their biology is similar to that of imiquimod, aTLR7 agonist, which is currently FDA approved as a topical medication for genital warts and basal cell skin cancer. Anecdotal reports have indicated that imiquimod is useful for managing some cases of melanoma with cutaneous metastases. Resiquimod induces production of interferon-α, IL-6, IL-8, IL-12, and TNF-α from DCs, monocytes, and macrophages. Activation stimulates the innate immune response and leads to subsequent Th1 cell-mediated immune responses.

Another TLR7 agonist is 852A, which stimulates plasmacytoid DCs and can be administered as an intravenous solution. Scant data are available on 852A, although indications are that it may be more potent than resiquimod. Clinical responses have been reported for carcinoid tumor, melanoma, and breast cancer (124). There is a dearth of publications on 852A. However, mixed TLR 7/8 agonists would be very interesting for local use. Having TLR7 agonists available would add to vaccine adjuvant options. Having topical and systemic formulations could also be useful. The information on resiquimod and 852A is taken, largely verbatim, from a presentation by Louis M. Weiner (12).

The contemplated uses for resiquimod and 852A are (i) as vaccine adjuvant, (ii) as monotherapy for immune activation especially for topical administration, (iii) in combination with chemotherapy agents, and (iv) in combination with antibodies.

Conclusion

The tools needed to raise T-cell levels to extraordinary levels *in vivo* are at hand. The 12 agents described all have proven ability to substantially increase specific T-cell responses. Alone the

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agents have very little chance of making substantial inroads into cancer therapy. Together, in innovative and intelligent combinations, the agents are overwhelmingly likely to have an impact.

Imagine sequential treatment of cancer patients with IL-7 to increase the number and repertoire of naive T cells, Flt3 to increase the total body burden of DCs, anti-CD40 to activate the DCs, a polyvalent cancer vaccine with a combination of IL-12, CpG, MPL, polyI:C and resiquimod adjuvants to activate and induce the proliferation of cancer immune T cells, 4-1-BB to further stimulate activation and proliferation of the immune T cells, anti-PD1 to inhibit T-cell checkpoint blockade, IL-15 to maintain proliferation and survival of the immune T cells, and 1MT to inhibit immunosuppression by IDO. The number of immune T cells achievable should make the *in vivo* environment exceedingly inhospitable for cancer cells. If that were not enough, it would be straightforward to expand the tumor immune T cells *ex vivo* and transfer them *in vivo* to achieve even larger numbers.

The above scenario is fraught with difficulties, but development of biologically directed multi-component immunotherapeutic regimens including the use of standard cancer therapies are eminently doable with today's technology. The scientific discoveries are at hand. Applying the immunotherapy agents already discovered is essentially an engineering feat. The immunology scientific community has done an extraordinary job of developing the principles of T-cell immune responses. Preclinical scientists in academia and industry have combined to develop the immunotherapy agents needed to induce and greatly augment immune T-cell responses. The time is ripe to apply all of the principles of immunology to the therapy of cancer using the immunotherapy agents close at hand. The priorities have been set by the broad immunotherapy community (12).

The list of agents (*Table 1*) with high potential to serve as immunotherapeutic drugs contained eight additional agents, each with great potential: a signaling inhibitor, anti-transforming growth factor-β; an immune suppression inhibitor, anti-IL-10 receptor; three T-cell stimulators, anti-glucocorticoid-induced TNF receptor, anti-OX40 and LIGHT; a T-cell attracting chemokine, CCL21; and two T-cell checkpoint blockade inhibitors, anti-B7-H4 and anti-lymphocyte activation gene-3. There is every reason to think that these agents as well as other agents in development will prove to be as effective as the 12 described in this review. The 12 described are just the start.

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