Cancer immunotherapy: moving beyond current vaccines

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Great progress has been made in the field of tumor immunology in the past decade, but optimism about the clinical application of currently available cancer vaccine approaches is based more on surrogate endpoints than on clinical tumor regression. In our cancer vaccine trials of 440 patients, the objective response rate was low (2.6%), and comparable to the results obtained by others. We consider here results in cancer vaccine trials and highlight alternate strategies that mediate cancer regression in preclinical and clinical models.

We now know the molecular identities of many tumor-associated antigens, and this knowledge has provided a major stimulus for the development of new immunotherapies for the treatment of patients with solid cancers[1]. In the field of cancer immunotherapy, most enthusiasm has been directed at the use of cancer vaccines—active immunizations designed to treat growing tumors. A recent review of dendritic cell vaccines mentioned 98 published studies involving over 1,000 patients[2]. A tabulation in 2003 listed 216 ongoing vaccine clinical trials in cancer patients[3]. These studies were conducted, and others are underway, despite the absence of convincing animal data that cancer vaccines used alone can affect invasive, vascularized tumors.

Why this focus on the use of cancer vaccines for solid tumors, especially when other immunotherapeutic approaches currently in preclinical and clinical trials have shown far more positive results[4,5]? The answer to this question is multifold. The widespread success of vaccines for the prevention of viral diseases provided a considerable base of immunologic information as well as a theoretical framework for immunization against cancer antigens, even though antiviral vaccines have not been effective for the treatment of patients with established viral disease. There are also practical reasons for the attractiveness of therapeutic cancer vaccines—they are easily administered to outpatients and generally do not cause significant side effects. Finally, investigators have been enthusiastic about the use of active immunization for patients with solid tumors because of an over-reliance on surrogate and subjective endpoints, such as histologic evidence of tumor necrosis or lymphocyte infiltration, rather than objective cancer regressions. Thus, despite the absence of any significant proportion of

patients who achieved clinical responses, many cancer vaccine trials have been optimistically reported because surrogate or subjective endpoints were achieved. Sensitive techniques such as tetramer or ELISpot assays have been used to demonstrate the generation *in vivo* of antitumor T cells in vaccinated patients, but the scarcity of clinical responses in these patients has made it difficult to validate any of these assays as a useful surrogate of clinical response.

Analysis of trials using standard oncologic criteria

Standard oncologic criteria for evaluating and reporting objective clinical responses to treatment are well established in oncology, and adherence to these guidelines is essential in comparing the results of treatment protocols[6–8]. A set of criteria proposed recently is the Response Evaluation Criteria in Solid Tumors (RECIST): a 30% reduction in the sum of the maximum diameters of lesions to indicate a response, along with the appearance of no new or progressive lesions. The most commonly used definition of objective clinical response, however, is at least a 50% reduction in the sum of the products of the perpendicular diameters of all lesions without the 25% growth of any lesion or the appearance of new lesions. The latter definition has been used in our analysis of our own protocols as well as published studies.

A great deal of effort has been devoted to the preclinical and clinical testing of a variety of cancer vaccines in the Surgery Branch of the National Cancer Institute (NCI). During the past nine years, we, together with our academic and industrial partners, have clinically tested cancer vaccines based on synthetic peptides, 'naked' DNA, dendritic cells, recombinant vaccinia viruses, recombinant fowlpox viruses and recombinant adenoviruses. These efforts include the rigorous testing of many of the commonly used cancer vaccine approaches.

Using conventional oncologic criteria for clinical tumor response, our objective response rate was only 2.6%, which is similar to the overall response rate we determined in a detailed analysis of cancer vaccine trials performed by others. This low clinical effectiveness raises important questions about the appropriate directions for future clinical immunotherapy efforts, especially at a time when alternate approaches such as cell transfer studies confirm the powerful potential of immunotherapy to mediate the regression of large volumes of metastatic disease in experimental models and in humans[4,5,9–11].

Cancer vaccines at the NCI Surgery Branch

Between February 1995 and April 2004, 440 individuals with metastatic cancer were treated with 541 different cancer vaccines at the Surgery Branch, NCI. These individuals signed informed consent

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Table 1 Patient characteristics

		Patients	Percentage
Sex	Male	280	64%
	Female	160	36%
Race	Asian	1	0%
	Black	2	0%
	White	437	99%
Age	11–20	4	1%
	21–30	27	6%
	31–40	74	17%
	41–50	124	28%
	51–60	125	28%
	61–70	65	15%
	Over 70	21	5%
Performance status	0	382	87%
	1	54	12%
	2	4	1%
Disease	Melanoma	422	96%
	Renal cell cancer	10	2%
	Ovarian cancer	4	1%
	Colorectal cancer	3	1%
	Breast cancer	1	0%
Prior treatment	Surgery	440	100%
	Chemotherapy	198	45%
	Radiotherapy	101	23%
	Hormonal	70	16%
	Immunotherapy	310	70%
	Any 2 or more	354	80%
	Any 3 or more	212	48%
Response	CR	4	1%
	PR	9	2%
	NR	428	97%

Total, 440 patients received 541 different vaccines. CR, complete response; PR, partial response; NR, no response. Performance status, Eastern Cooperative Oncology Group performance status.

forms and were entered into clinical trials approved by the NCI Institutional Review Board. The analysis of the 440 participants presented here represents all individuals with metastatic cancer treated with cancer vaccines during this period, with the exception of 13 individuals not able to be evaluated for clinical response and patients who received vaccines along with other agents known to cause cancer regression, such as IL-2 or chemotherapy.

Demographic characteristics of the individuals treated with cancer vaccines are shown in **Table 1**. Of the 440 patients treated, 422 had metastatic melanoma and 18 had other types of cancer. Among these individuals, 65% had visceral disease, 20% had lymph node disease alone or in combination with disease at subcutaneous sites, and 15% had only subcutaneous or cutaneous disease.

The vaccine treatments received by these patients are shown in **Tables 2** and **3**. Peptide vaccines alone (generally at a dose of 1 mg every three weeks) were administered to 323 individuals using peptides derived from one of the following: melanoma-differentiation antigens such as MART-1, gp100, tyrosinase or TRP-2, cancer-testes antigens such as NY-ESO-1 or MAGE-12, or Her2/neu or telomerase proteins (**Table 2**). Fifteen participants received peptides pulsed on dendritic cells. All remaining patients received peptides emulsified in incomplete Freund's adjuvant, except for four who received peptide in saline. Peptide immunization was administered along with IL-12 or GM-CSF to 40 and 18 patients, respectively. One hundred sixty participants received vaccination either with virus (fowlpox, vaccinia or

adenovirus) or with naked DNA encoding tumor antigen (**Table 3**). Results in 244 of these 541 vaccine treatments have been published previously and these reports provide the details of administration of these vaccines[12–21].

Of the participants who received a peptide vaccine, nine showed a partial response and two showed a complete response, for an overall objective response rate of 2.9%. Of participants who received a viral vaccine, two obtained a partial response and one obtained a complete response, for an overall objective response rate of 1.9%. Thus, the overall objective response rate for all vaccine treatments was 2.6%. The 14 individuals who showed objective responses are described in **Table 4**. Of these responders, 11 had disease confined to skin or lymph node sites and only 3 (21%) had visceral disease, compared to 65% of total participants who had visceral disease. This suggests that the vaccine treatments, when successful, were predominantly effective in patients with disease at cutaneous or lymphatic sites.

Separating 'spin' from substance

Hundreds of vaccine clinical trials in patients with metastatic cancer have been published. Some trials do not specify the exact criteria used to determine clinical response; some trials use very 'soft' criteria that make the incidence of cancer regression difficult to evaluate (**Box 1**). Examples include "temporary growth cessation in some individual metastases"[22] or "symptoms disappeared"[23] or "tumor necrosis" or "stable disease" or "unexpectedly long survival." Another analysis included as a partial response "any measurable response in any lesion"[2]. Soft criteria of this sort cause considerable confusion in the analysis of clinical trials because they can occur in the natural course of tumor growth.

Thus we selected 35 reports of vaccine trials that included 765 patients whom we believe are representative of the majority of published trials, including many of the more optimistic trial reports. These studies include patients with multiple cancer types treated with a variety of the most common types of cancer vaccines. Twenty-nine objective responses were reported for an objective response rate of 3.8%. There were 7 (4.0%) responses in 175 patients receiving peptide vaccines, no responses in 206 patients receiving pox viruses, 6 (4.2%) responses in 142 patients receiving native or modified tumor cells and 14 (7.1%) responses in 198 patients receiving dendritic cells. Thus, of the 1,306 vaccine treatments in both the Surgery Branch and the selected trials presented in **Table 5**, a 3.3% overall objective response rate was seen.

In the light of these very large numbers of patients treated with vaccines and the exceedingly low objective response rates reported for the cancer types included in **Table 5**, a reevaluation of future directions for cancer immunotherapy trials would be valuable.

How T cells can destroy large, established tumors

Cellular immune responses have an important role in the immunologic rejection of vascularized tissue in animals and man[24]. In mouse immunotherapy models, transfer of immune T lymphocytes but not antibodies protects mice from tumor challenge; elimination of endogenous $CD8^+$ T cells abrogates both protective and therapeutic antitumor effects; and extensive T cell infiltrates are commonly seen in tumors and allografts undergoing immunologic rejection (reviewed in ref. 24). The induction of $CD8^+$ cells with specific immune reactivity can depend on interactions with other cell types such as $CD4^+$ and antigen-presenting cells, although the final effector in most models is the $CD8^+$ lymphocyte. Thus, the majority of cancer immunotherapy efforts are devoted to stimulat-

ing cellular immune responses against the growing tumor.

Three criteria are required for the immunologic destruction of established tumors: (i) sufficient numbers of immune cells with highly avid recognition of tumor antigens must be generated *in vivo* (ii) these cells must traffic to and infiltrate the tumor stroma, and (iii) the immune cells must be activated at the tumor site to manifest appropriate effector mechanisms such as direct lysis or cytokine secretion capable of causing tumor destruction.

Although immune T cells capable of recognizing tumor antigens can be generated by direct immunization in tumor-bearing mice, there are no cancer vaccine models that reproducibly demonstrate that vascularized tumors can be rejected by this approach. The rapid growth of extensively passaged mouse tumors that often express retroviruses represents an obstacle to the study of cancer vaccines that may require extensive immunizations over a long period of time. Thus, most mouse models of cancer vaccines assess the ability to prevent the outgrowth of tumor injected after vaccination or attempt to treat tumors a few days after transplantation when the tumors are not yet vascularized. The presence of even large numbers of immune T cells capable of recognizing tumor antigens in mice is insufficient to mediate tumor regression[4,25]. T cells must be in the correct state of activation and differentiation in order to mediate antitumor effects. This point is often underappreciated in the analysis of human immunotherapy trials.

In mice transgenic for T cell receptors that recognize tumor antigens, virtually all T lymphocytes can recognize tumor, but tumor growth and lethality are often unaffected. Inadequate numbers or avidity of the immune cells, the inability of the tumor to activate quiescent or precursor lymphocytes, tolerance mechanisms including anergy, and suppressor influences produced by the tumor or the immune system itself are among the mechanisms that can prevent tumor destruction by immune cells[25,26]. These obstacles must be overcome if cancer vaccines are to be effective in mediating cancer regression.

More encouraging, however, are studies that demonstrate the ability of adoptively transferred antitumor immune T cells to mediate the rejection of large vascularized tumors in mice under the appropriate conditions of host immune suppression and antigen stimulation. Large B16 melanomas can be rejected in mice after host lymphodepletion when antitumor T cells are transferred along with antigen-specific vaccination and IL-2 (ref. 4). Cell transfer combined with vaccination, γ_c cytokines and prior host immuno suppression all can maximize tumor destruction[10,11,27].

The success of these cell transfer approaches in mice has its counterpart in recent human clinical trials[5]. In patients with metastatic melanoma refractory to treatment with high dose IL-2 and to chemotherapy, the transfer of *in vitro*–activated and expanded autologous antitumor lymphocytes plus IL-2 into lymphodepleted patients mediated objective cancer regressions in 6 of 13 patients. Persistence of the transferred cells was seen for up to four months after cell administration[5]. Patient entry into this protocol has now been

Table 2 Peptide vaccine immunization of patients with metastatic cancer

Peptide	HLA restriction	Total patients	NR	PR	CR
MART-1$_{27-35}$	A2	23	22	1	0
MART-1$_{27-35}$ + IL-12	A2	12	12	0	0
MART-1$_{26-35}$(27L)	A2	6	6	0	0
TRP-2$_{180-188}$	A2	20	19	1	0
gp100$_{209-217}$	A2	9	8	0	1
gp100$_{209-217}$(210M)[a]	A2	32	32	0	0
gp100$_{209-217}$(210M) + IL-12	A2	28	28	0	0
gp100$_{209-217}$(210M) + GM-CSF	A2	18	18	0	0
gp100$_{280-288}$	A2	9	9	0	0
gp100$_{280-288}$(2889V)[b]	A2	5	5	0	0
gp100$_{154-162}$	A2	10	0	0	0
gp100ES:$_{209-217}$(210)	A2	9	9	0	0
g209-2M + MART-27L	A2	23	23	0	0
g209-2M, g280-9V, MART-27L[c] + tyr3D[d]	A2	16	14	2	0
gp100$_{44-59}$	DR4	4	4	0	0
gp100$_{44-59}$ + g209-2M + MART-27L	A2/DR4	22	21	0	1
Tyrosinase$_{240-251}$	A1	16	15	1	0
gp100$1_{7-25}$	A3	12	12	0	0
Tyrosinase$_{206-214}$	A2	8	8	0	0
TRP-1 ORF1–9	A31	5	5	0	0
Combination peptides	Non-A2	15	15	0	0
MAGE-12$_{170-178}$	Cw7	9	8	1	0
NY-ESO-1$_{157-165}$(165V)	A2	19	19	0	0
NY-ESO-1$_{161-180}$	DP4	6	5	1	0
NY-ESO-1$_{161-180+157-165}$(165V)	A2/DP4	11	11	0	0
Her2/neu$_{369-378}$	A2	6	6	0	0
Telomerase$_{540-548}$	A2	13	13	0	0
Dendritic cells + g209-2M + MART-27L	A2	15	13	2	0
Total		381	370	9	2

Overall objective response rate = 2.9%. HLA, human leukocyte antigen; CR, patients showing complete response; PR, patients showing partial response; NR, patients showing no response. [a]g209-2M. [b]g280-9V. [c]MART-1$_{26-35}$(27L). [d]Tyrosinase$_{368-376}$(370D).

Table 3 Viral vaccine immunization of patients with metastatic cancer

Virus	HLA restriction	Total patients	NR	PR	CR
Fowlpox MART-1	Any	12	12	0	0
Fowlpox gp100	Any	20	20	0	0
Fowlpox gp100(210M, 288V)	A2	15	14	1	0
Fowlpox gp100(ES$_{209-271}$(210M))	A2	46	46	0	0
Vaccinia MART-1	Any	5	5	0	0
Vaccinia gp100	Any	16	16	0	0
Adenovirus MART-1	Any	17	16	0	1
Adenovirus gp100	Any	7	7	0	0
DNA gp100(210M, 288V)	A2	22	21	1	0
Total		160	157	2	1

Overall objective response rate = 1.9%. HLA, human leukocyte antigen; CR, patients showing complete response; PR, patients showing partial response; NR, patients showing no response.

Table 4 Objective responses to vaccine treatment

Patient	Vaccine	Sites	Tumor size		Response duration (months)
			Before	After	
1	MART-1 peptide	Mediastinal lymph node	15.7	5.6	78
2	MAGE-12 peptide	Neck lymph node	6.0	0.4	29+
3	Tyrosinase peptide	Mediastinal lymph node	4.5	1.7	5
4	TRP-2 peptide	Para-aortic lymph node	3.6	0	27+
		lung	0.12	0	
5	gp100 (class I and II) and MART-1 peptide	Inguinal lymph node	1.0	0	19
6	NY-ESO-1 peptide	Mediastinal lymph node	3.8	0.17	12
		subcutaneous	0.73	0	
7	gp100 peptide	Cutaneous/subcutaneous	1.8	0	4
8	Multiple peptides	Cutaneous/subcutaneous	Small multiple		3
9	Multiple peptides	Lung	5.9	0.60	4
		Liver	3.2	0.48	
		Subcutaneous	16.3	2.0	
		Intraperitoneal	15.2	0	
10	Adenovirus MART-1	Mediastinal lymph node	5.6	0	76+
		subcutaneous	4.0	0	
11	Fowlpox-gp100 (210m, 288v)	Cutaneous/subcutaneous (multiple)	55.4	0.1	50+
12	gp100 DNA	Cutaneous	0.1	0	50+
13	Dendritic cells pulsed with peptide	Lung	6.0	1.2	8
		Subcutaneous	5.2	3.2	
14	Dendritic cells pulsed with peptide	Cutaneous	0.58	0.25	2

expanded and we now have observed objective cancer regressions in 18 (51%) of 35 patients, many of whom have bulky disease (data not shown).

The effectiveness of cell transfer immunotherapy also serves to highlight many of the obstacles confronting vaccine therapy approaches and suggests possible means to overcome them. Cancer vaccines often result in low levels of circulating immune cells. Pox virus vaccines have been reported to increase circulating human antitumor antigen-reactive T cells from fewer than 1 in 200,000 to about 1 in 40,000 (refs. 28,29). In some peptide vaccine trials, frequencies of over 1 in 200 antitumor cells can be generated, yet tumor regression is still not seen[30]. The cells generated often have low avidity for tumor recognition. In contrast, antitumor T cells used for cell transfer, generated *in vitro* from tumor infiltrating lymphocytes (TILs) or from peripheral blood lymphocytes, can be obtained in large numbers (up to 1×10^{11}) and can be selected *in vitro* for highly avid recognition of tumor antigens[31]. Transfer of these cells into lymphodepleted hosts can result in 5–75% of circulating CD8+ cells with antitumor activity[5]. Cancer vaccines may need to generate these levels to be clinically effective.

An important reason that T cells generated by cancer vaccines may not destroy solid tumors is the inability of the immune cells to infiltrate and become activated after an encounter with tumor antigen *in vivo*. In contrast to solid tumors, lymphoid tumors allow easier access to the circulation and often express costimulatory molecules that are required in the afferent phase of the immune response, but may also be involved in the activation of memory cells. This may explain why lymphoid tumors have been reported to be more clinically responsive to dendritic cell vaccines[32]. Solid tumors do not express these costimulatory molecules or produce the inflammatory environment necessary to convert quiescent precursor lymphocytes into activated lymphocytes with the effector functions required for tumor eradication. In contrast, immune cells generated and activated *ex vivo* can be infused in a highly activated state, already displaying the necessary lytic and cytokine-secreting activities required to mediate the destruction of even large solid tumor masses. A challenge to the application of cancer vaccines is the development of methods to not only generate long-term

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BOX 1 CRITERIA FOR CLINICAL OBJECTIVE RESPONSE

Conventional

Standard: 50% decrease in the sum of the products of perpendicular diameters of all lesions: no increase in any lesion

RECIST: 30% decrease in the sum of the largest diameters of target lesions; no increase in any lesion

Nonstandard criteria that lead to confusion (each of these 'soft' criteria can occur in the natural course of cancer growth):

"Tumor necrosis"
"Lymphocyte infiltration"
"Antigen loss"
"Stable disease"
"Shrinkage of some lesions"
"Symptom improvement"
"Survive longer than expected"

memory cells but also activate these antitumor cells, possibly by improving methods of stimulating antigen presenting cells with new adjuvants *in vivo* or by creating an inflammatory environment at the tumor site to promote the homing of effector lymphocytes to the tumor.

Recent research has emphasized the importance of active suppressor mechanisms arising both from the tumor and from the immune system itself that can inhibit antitumor immune reactions in vivo[33–35]. Perhaps the most important of these regulatory effects are mediated by $CD4^+CD25^+$ lymphocytes with the ability to suppress both the proliferation and effector functions of immune cells. A major advantage of cell transfer therapies is the ability to deplete host lymphocytes, including these regulatory cells, before cell transfer, and this preparation is critical to the success of many preclinical cell transfer immunotherapies. For cancer vaccines to be effective, it may require the elimination of these regulatory T cells, and although reagents to selectively eliminate these cells *in vivo* are being developed, their clinical efficacy has yet to be established. Chemotherapy- or radiation-induced lymphodepletion can eliminate regulatory cells but cannot be used in conjunction with cancer vaccines because the needed effector cells are also eliminated.

Studies in mouse models have defined additional principles important for human application. Cancer vaccines may be of greatest value when administered as a specific antigenic stimulant to transferred T cells, especially under conditions when host lymphocytes are eliminated that compete with the transferred cells for γ_c homeostatic cytokines such as IL-7, IL-15 and IL-21. Elimination of the 'cellular sinks' for cytokines may enable antitumor T cells to be activated by these endogenous cytokines.

Table 5 Results of clinical vaccine studies in patients with metastatic cancers

Vaccine type	Reference	Cancer type	Vaccine	Total patients	Patients responding
Peptide	43	Melanoma	Tyrosinase + GMCSF	16	0
	44	Melanoma	Peptides in IFA or on DC	26	3
	45	Melanoma	MART-1 + IL-12	28	2
	46	Prostate	Peptides	10	0
	47	Melanoma	Peptides on PBMC + IL-12	20	2
	48	Breast and prostate	Telomerase	7	0
	49	Cervix	HPV16 E7	17	0
	50	Colorectal	Peptides in IFA	10	0
	51	Multiple	NY-ESO-1	12	0
	52	Multiple	Ras in DETOX adjuvant	15	0
	53	Multiple	Peptides in IFA	14	0
Virus	29	Prostate	Vaccinia-PSA	33	0
	54	Prostate	Vaccinia-PSA	42	0
	55	Colorectal	Vaccinia-CEA	20	0
	56	Colorectal	Vaccinia-CEA and B7-1	18	0
	57	Multiple	Avipox-CEA (IGMCSF)	60	0
	58	Multiple	Avipox-CEA	15	0
	59	Multiple	Vaccinia + avipox-CEA	18	0
Tumor cells	60	Melanoma	Transduced with GM-CSF	26	1
	61	Melanoma	Membranes on silicone beads	17	1
	62	Lung	Transduced with GMCSF	26	1
	63	Lung	Transduced with GMCSF	43	3
	64	Breast	Transduced with B7-1	30	0
Dendritic cells	65	Melanoma	Pulsed with peptides	17	0
	66	Melanoma	Pulsed with peptides or lysates	33	3
	67	Melanoma	Pulsed with peptides or lysates	16	5
	68	Melanoma	Pulsed with peptides	24	1
	22	Melanoma	Pulsed with MAGE-3A1 peptide	11	0
	69	Childhood cancers	Pulsed with lysates	15	1
	70	Kidney	Transfected with RNA	15	0
	71	Colorectal	Pulsed with CEA peptides	12	1
	72	Kidney	Pulsed with tumor lysates	35	3
	23	Multiple	Pulsed with tumor lysates	20	0
Heat shock protein	73	Melanoma	Hsp-96	28	2
	74	Multiple	Hsp-96	16	0
			Total	765	29

Objective response rate = 3.8%.

Immunotherapies that cause actual cancer regressions

The description of a wide variety of human cancer antigens that are expressed on multiple cancer types, including many common epithelial cancers, presents new opportunities for the development of cancer immunotherapies. These discoveries have not been successfully exploited to mediate the regression of solid cancers using current cancer vaccine approaches, and changes are required for this approach to bear fruit. The ineffectiveness of cancer vaccine approaches is not commonly appreciated, however, because of the 'spin' often accompanying reports of cancer vaccines. These reports often attribute clinical effectiveness, without standard clinical criteria for tumor regression being achieved. Further confusing the current analysis of cancer vaccines is their application in adjuvant settings, in the absence of measurable disease. Results from this type of use may inappropriately imply effectiveness compared to historical controls[36–38]. Although it is possible that cancer vaccines will be more effective in a minimal disease setting before immunosuppressive chemotherapies have been administered, only randomized, controlled trials can convincingly demonstrate the effectiveness of a therapeutic intervention in the absence of measurable disease or to substantiate claims of 'stable disease.'

The lack of clinical effectiveness of currently available cancer vaccines should not be interpreted to mean that cancer vaccine approaches are at an investigational 'dead end.' Rather, it emphasizes the need for profound changes in the application of this approach. Increased efforts to generate antitumor $CD4^+$ cells that recognize MHC class II–restricted antigens may have impact because of the importance of $CD4^+$ cells in enhancing antitumor reactions and sustaining the activation and survival of $CD8^+$ effector cells. Increased

numbers of T cells with higher avidity are required *in vivo* and exploration of improved adjuvants such as new toll-like[39] receptor agonists to activate innate immunity, the use of agonistic anti-4-1BB antibodies to stimulate CD8^{+} cells[11] or the administration of homeostatic cytokines such as IL-15 require study[40]. Preliminary trials have suggested that immunization with certain peptides[30] or pox viruses[20] can improve response rates to high-dose IL-2, although these observations will require testing in prospective randomized trials. Many current tumor antigens do not derive from molecules essential for cell survival, and thus vaccines that target antigenic molecules critical for cell viability may be more effective. Methods for stimulating an inflammatory environment at the tumor site or introducing costimulatory molecules along with antigen[41] may be required to activate quiescent precursors. Eliminating both tumor and lymphocyte-mediated immune suppressive mechanisms without adversely affecting the desired antitumor effector cells also holds promise. Specifically, the blockade of secreted immunosuppressive molecules such as TGF-β, IL-10, IL-13 or prostaglandins may be required as well as selective means for eliminating CD4^{+}CD25^{+} regulatory cells. Blockade of the negative costimulatory molecule CTLA-4 using a monoclonal antibody can result in regression of established human tumors in limited numbers of patients[42], and further exploration of these manipulations in conjunction with vaccination are needed. Although only small numbers of patients have been treated, cell transfer studies, despite their labor-intensive requirements, are currently very encouraging because they demonstrate that large numbers of adequately activated, tumor-specific T cells in a lymphodepleted host environment can cause the regression of large, vascularized cancers in mice and humans. These cell transfer approaches demonstrate that immunotherapy can be successful in cancer patients and thus increased effort in the development of cancer immunotherapy is needed. Future clinical studies should utilize standard criteria for clinical response and require validation in increased numbers of patients.

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