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Vaccines Targeting Helper T Cells for Cancer Immunotherapy

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Abstract

There are compelling arguments for designing cancer vaccines specifically to induce CD4⁺ helper T cell responses. Recent studies highlight the crucial role of proliferating, activated effector memory Th1 CD4⁺ T cells in effective antitumor immunity and reveal that CD4⁺ T cells induce more durable immune-mediated tumor control than CD8⁺ T cells. CD4⁺ T cells promote antitumor immunity by numerous mechanisms including enhancing antigen presentation, co-stimulation, T cell homing, T cell activation, and effector function. These effects are mediated at sites of T cell priming and at the tumor microenvironment. Several cancer vaccine approaches induce durable CD4⁺ T cell responses and have promising clinical activity. Future work should further optimize vaccine adjuvants and combination therapies incorporating helper peptide vaccines.

Graphical Abstract

The goal of cancer therapies is to destroy malignant cells, without damage to healthy tissues. Thus, many immune therapies are designed to take advantage of the specificity and cytotoxic capacity of CD8⁺ T cells (T_{CD8}). However, clinical outcomes with cancer vaccines targeting T_{CD8} has been disappointing [1]. On the other hand, there are compelling arguments for designing vaccines specifically to induce CD4⁺ helper T cell (T_{CD4}) responses instead. This review will summarize preclinical data supporting the critical role of T_{CD4} in antitumor

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immunity, and both preclinical and clinical data for the immunogenicity and clinical activity of cancer vaccines targeting induction of T_{CD4}.

Key roles of T_{CD4} Lymphocytes in Anti-tumor Immune Responses

Murine studies show that T_{CD4} are required for induction of CD8 antitumor T cell responses [2,3]. Recent very comprehensive analysis of cellular subsets in cancer immunity highlight the crucial role of proliferating, activated effector memory Th1 T_{CD4} (CD69⁺ T-bet⁺ CD44⁺ CD62L^{neg} CD27^{low} CD90^{hi}) in effective antitumor immunity [4] and showed that T_{CD4} induce more durable immune-mediated tumor control than T_{CD8}. Depletion of T_{CD4} can abrogate all or part of protective immune responses to cell-based vaccines [5]. Furthermore, adoptive therapy with T_{CD4} has induced tumor protection in some model systems and in humans [6,7]. Thus, protective immunity induced by tumor cell vaccines and other immune therapies appears to depend on T_{CD4}. The mechanisms by which T_{CD4} may promote antitumor immunity include numerous direct and indirect effects of those cells, impacting antigen presentation, co-stimulation, T cell homing, T cell activation, and effector function, both systemically and in the tumor microenvironment (TME), which are detailed below:

Antigen presentation

When Th1 T_{CD4} encounter their cognate antigen, whether expressed by tumor cells or by professional antigen presenting cells (APCs), they can produce IFN γ . Within the TME, effects of IFN γ include the induction of Class I and Class II MHC molecules and upregulation of antigen processing machinery (Figures 1,2). Increased expression of these molecules enhances recognition of tumor-associated antigens by T_{CD8} in a class I-restricted manner, or by T_{CD4} in a class II restricted manner [8–10].

Costimulation

Activated T_{CD4} cells express CD40L [11–13] by which they can activate dendritic cells (DC) through ligation of CD40, for heightened antigen presentation and expression of costimulatory molecules (Figure 1). They also provide help by enabling DC to secrete IL-12 and other cytokines to direct the immune response. Additionally, this interaction triggers release of chemokines CCL3 and CCL4 by the APCs, which guide naïve CD8 T cells to APC within the lymph node, to improve efficacy of T cell priming [14,15]. Mimicking T cell help in the priming phase, with agonistic antibodies to CD40 or CD27, can improve efficacy of vaccines and other immune therapies [16,17]. Furthermore, strong Th1 help produces the proper cytokine milieu to induce immune-mediated tumor destruction [18–20]. Within the TME, T_{CD4} can directly bind to T_{CD8} through co-stimulatory molecules, such as CD27, CD137 and 4-1BB, thus potentiating CD8 proliferation, survival and effector function [21].

T cell homing

As mentioned above, effector Th1 T_{CD4} are a source of IFN γ if they recognize their cognate antigen in the TME (Figure 2). In addition to enhancing antigen presentation, IFN γ also supports homing of T cells to the TME: it induces expression of the IFN-responsive chemokines CXCL9, CXCL10, and CXCL11 [22,23], and also induces expression, by tumor-associated endothelium, of VCAM-1 and other critical ligands for T cell homing

receptors [24]. The receptor for these chemokines is CXCR3, which is expressed by activated T_{CD4} and T_{CD8}, including T cells induced by cancer vaccines [25]. Thus, CXCL9-11 recruit CXCR3+ T cells into the TME [26–28]. Overall, Th1 T_{CD4} have a net effect of enhancing infiltration of tumor by T_{CD8}. [26,29,30]. It is relevant to acknowledge that IFN γ in the TME also induces immunosuppressive and immunoregulatory processes, including indoleamine-2,3-dioxygenase (IDO), regulatory T cells, and PD-L1 expression [31] and may limit effectiveness of cancer vaccines [32]. Interestingly, analysis of TCGA data reveal that increased IFN γ expression in the TME is associated with improved overall survival [33,34] (Figure 3), which supports the favorable association of IFN γ in the TME with melanoma control.

Effector function

T_{CD4} are not classically cytotoxic effector cells; however, some T_{CD4} do have direct antitumor effector function [9,35,36] mediated by expression of granzymes, perforin, TRAIL or FasL [2,37,38] (Figure 2). In addition to direct tumor cell killing, T_{CD4} can also promote antitumor immunity by inhibiting angiogenesis through IFN γ signaling on non-hematopoietic cells [39]. Thus, T_{CD4} cells may support tumor control in some cases by direct lytic effector function, in addition to their more typical role in providing help to T_{CD8} and B cells. Interestingly, they can also provide help to other T_{CD4}; this helper function has supported activation of T_{CD4} to an epitope that otherwise is poorly immunogenic [40].

Memory formation

Classically, CD4 help has been thought to be required for priming T_{CD8} responses only in situations in the absence of a strong primary immune response. A strong danger signal can induce a primary immune response in the presence of toll-like receptor (TLR) ligands or proinflammatory cytokines, which can activate APCs independent of T_{CD4}, thereby circumventing the necessity of T_{CD4} help for proper effector function [41–43]. However, more recent research has shown that, regardless of the strength of the primary T_{CD8} response, CD4 help is always required for optimal CD8 memory cell formation and secondary recall response [44]. Without CD4 help, signaling through receptors important for formation and survival of specific memory T_{CD8} subsets (CD25, CD127 and CD103) is diminished [13,45]. Furthermore, T_{CD8} activated in the absence of CD4 help are more prone to apoptosis when restimulated [46]. Regardless, as the TME is not considered pro-inflammatory, the primary anti-tumor immune response will most likely be weak. Thus, we suggest that to elicit optimal effector as well as memory T_{CD8} responses against tumor antigens, vaccines will require a mechanism to activate T_{CD4}. However, most studies of the role of T_{CD4} in memory have been done in murine models of viral infection. Thus, there is a need for more studies in cancer models and in humans to understand the role of T_{CD4} in optimal immune memory for cancer control.

Summary of preclinical data

Thus, activation of a T_{CD4} response supports cytotoxic T_{CD8} priming, memory formation, recruitment to the tumor, and tumor cell recognition. Additionally, some T_{CD4} have the capacity to contribute directly to tumor cell killing. It is thus not surprising that immune therapies solely focusing on T_{CD8} do not offer optimal results for anti-tumor immunity.

Activation of tumor specific T_{CD4} through helper peptide vaccines or other methods offer promise to enhance anti-tumor T_{CD8} responses and optimal tumor control. Furthermore, the impact of T_{CD4} goes well beyond the effects of cytokines alone or DC licensing alone so that simply mimicking CD4 help through administration of IFNs, IL-2, or anti-CD40 antibodies will not reconstitute the breadth of the beneficial effects of anti-tumor T_{CD4} responses.

Clinical experience with helper peptide vaccines

Several cancer vaccine approaches have targeted T_{CD4} responses and offer promise. Representative examples are summarized briefly here and in Table 1.

Helper peptides from Her2/neu

Patients with breast cancer have decreased Her2-reactive T_{CD4} responses compared to patients without cancer, providing justification for enhancing Th1 responses to Her2 as therapy [47]. Vaccines with these Her2/neu helper peptides have been administered as peptide-pulsed type 1 DC administered intranodally: this regimen has induced durable T_{CD4} responses to those peptides in patients with breast cancer [48] and has induced complete regressions of DCIS [49,50]. The immune responses and clinical activity have been comparable regardless of the route of vaccination (intranodal vs intralesional) [50]. These findings are important because they show evidence for clinical activity of a low toxicity vaccine regimen, at least for early stage disease (DCIS). They are also important because they show activity with a vaccine designed to induce T_{CD4} and that uses an overexpressed antigen, despite presumed pre-existing tolerance.

Telomerase helper peptides

Telomerase (hTERT) is an appealing target for cancer vaccines because it is overexpressed in a wide range of cancers. Several hTERT vaccines have incorporated helper peptides and have induced Th1 T_{CD4} responses [51–53], as well as epitope spreading to Ras peptides [51]. One hTERT vaccine enhanced DC activation in preclinical models, including IL-12 production [54]. A phase III clinical trial of the GV1001 vaccine showed no impact on outcome when added to chemotherapy for pancreatic cancer [55], but others remain in trials. Currently, a clinical trial is testing the safety and effectivity of a vaccine incorporating UCP2 and UCP4 in Incomplete Freund's Adjuvant (IFA) in NSCLC patients (NCT02818426).

Melanoma peptide vaccines administered with dendritic cells

A dendritic cell vaccine includes DC pulsed with multiple peptides including 3 short peptides restricted by HLA-A2, with or without 2 helper peptides restricted by class II MHC. Patients vaccinated with the helper peptides developed helper T cell responses in circulation and in skin infiltrating lymphocytes, and had higher responses to CD8 T cells than those vaccinated only with the T_{CD8} epitopes [56]. There was also a trend to better clinical outcome in those who also received helper peptides [56].

Melanoma peptide vaccines administered in adjuvant emulsion

Our group has evaluated a multi-peptide melanoma vaccine containing 6 peptides from melanocytic proteins and cancer-testis antigens, restricted by HLA-DR molecules (6MHP vaccine) in an emulsion with IFA, with or without GM-CSF [57–59]. This vaccine has induced high rates of Th1 T_{CD4} immune responses [57,60] as well as promising clinical outcomes, with durable clinical responses and durable stable disease lasting up to 7 years in 7–12% of patients, plus stable disease in another 12–29% [57,59]. Overall survival for stage IV melanoma patients who received 6MHP vaccines significantly exceeded that of matched pair controls (5-year survival 57% vs 16%; $p < 0.001$) [61]. Epitope spreading to T_{CD8} was induced in 45% of patients evaluated [62]. Also antibody (IgG) responses to the peptides were induced. Patient survival was significantly associated with T_{CD4} responses and especially to the combination of antibody plus T_{CD4} responses [59,63], supporting the clinical relevance of immune responses induced to the 6MHP vaccine. Ongoing trials are testing the 6MHP vaccine in combination therapy with checkpoint blockade and with BRAF/MEK inhibition.

New directions

Long peptide vaccines to include both T_{CD4} and T_{CD8} specific presented peptides

Preclinical and clinical studies with long (approximately 30-mer) peptides suggest that they may be more effective immunogens than the minimal peptides representing individual CD8 epitopes and that they may also induce T_{CD4} responses. The extra length contributes to a tertiary structure that may protect from peptidases, and they are too long to be presented directly on MHC; so, intracellular processing by professional APCs is required. A vaccine using long peptides for squamous vulvar neoplasia has been associated with high rates of clinical regressions [64], and a vaccine using overlapping long peptides from the cancer-testis antigen NY-ESO-1 has induced T_{CD4}, T_{CD8} and antibody responses [65]. Thus, long peptides offer promise as a form of helper peptide vaccine that may also induce broad integrated immune responses.

Vaccines targeting mutated neoantigens

Melanomas, lung cancers, and other solid tumors have high rates of somatic mutations, and T cells infiltrating melanoma metastases often recognize peptides encompassing these mutations [66]. Furthermore, peptide vaccines have been developed to test whether mutated neoantigens can be predicted and vaccinated against. Personalized vaccines using minimal peptides restricted by HLA-A2-restricted putative neoantigens were successful at inducing T cell responses to 3 peptides per patient in a small study [67]. Ongoing clinical trials are testing long peptide vaccines encompassing cancer-associated somatic point mutations (eg: NCT02950766, NCT02427581, NCT01970358, NCT02287428).

Challenges of vaccines targeting mutated neoantigens

Current efforts to develop neoantigen vaccines are hindered by imprecision of algorithms to predict those epitopes, especially for T_{CD4} cells; the heterogeneity of mutation profiles among different metastases in the same patient [67], plus the time and cost required to

develop vaccines on a per-patient basis. Another critical challenge is that optimal strategies to induce T cell responses to peptides remain unclear. Among the recent and current clinical trials of neoantigen vaccines, the vaccine adjuvants vary widely, reflecting the lack of consensus. Substantial effort is being directed toward addressing these challenges.

Adjuvants for cancer vaccines

The ability of vaccines to induce effective and durable immunity depends on inclusion of local or systemic vaccine adjuvants, to activate DCs and to provide danger signals. Options are numerous and include pulsing DCs with peptide, administering nanoparticles containing peptides plus adjuvant, or administering peptides with incomplete Freund's adjuvant (IFA), TLR agonists, or other agents that may activate innate immunity. Vaccines using DNA, RNA or viral constructs are also options and are in trials. There has been debate about using IFA with a peptide vaccine. In murine studies, vaccines using short peptides in IFA induce chronic inflammation at the site of vaccination that recruits and retains antigen-specific T cells at the vaccine site (rather than supporting T cell homing to tumor) and may deplete antigen-reactive T cells [68]. Similarly, vaccination with short peptides plus TLR agonist and agonistic CD40 antibody has been much more effective than subcutaneous administration in IFA at inducing strong circulating T_{CD8} responses in murine models [69]: that approach holds promise but has not yet been explored in humans, but deserves study when CD40 antibodies are available for that purpose. For induction of T_{CD4} in mice, antigen-reactive T_{CD4} cells may also accumulate preferentially at sites of vaccination with IFA [70]; however, use of IFA with a long peptide (20-mer) did not have the same negative effects that were observed with a short 9-mer peptide [68]. In humans, on the other hand, vaccines incorporating IFA, with or without a TLR agonist, can induce strong and durable Th1 dominant T cell responses, especially with intermediate length peptides (14–23 mers) or long peptides (30 mers) [60,71], and addition of a TLR agonist further enhances T_{CD4} responses, as well as antibody and CD8 T cell responses [71]. There is no consensus about the optimal strategy to induce durable responses. There is a need to optimize adjuvants further and to reach some consensus on optimal adjuvant. Mutated neoantigen vaccines present a challenge for testing adjuvants: because each vaccine is different, it is not possible to interpret differences in outcome related to choice of adjuvant when the peptides are not controlled. Thus, there is rationale for using defined antigen vaccines to identify optimal adjuvants, which subsequently may be selected for use with neoantigen vaccines.

Summary

Immune therapy with checkpoint blockade antibodies can induce dramatic and durable cancer control if there is already an immune response to the cancer. However, active induction of immune responses may be required to enable tumor control by these immune therapies in tumor without an active immune response present. T_{CD4} have myriad roles in enhancing tumor control both during priming of effector T cells as well as in the tumor microenvironment. More importantly, in a suppressive environment such as the tumor, effector T cell responses are often suboptimal without T_{CD4} help. Despite this, the role of CD4 T cells has not been addressed sufficiently in current approaches to cancer immunotherapy. Vaccines designed to induce Th1 T_{CD4} responses are showing significant promise both for induction of durable T cell responses and also for induction of clinical

activity in a subset of patients with melanoma and breast cancer. Helper peptide vaccines can induce circulating T_{CD4} I responses that enable recognition of tumor antigen in the tumor microenvironment, and should be able to enhance homing and expansion of T cells to the tumor. Helper peptide vaccines have induced epitope spreading to epitopes in the same or different proteins, and presented by Class I or II MHC. Thus, it is possible that helper peptides, even without incorporating somatic mutations, may also enhance reactivity to mutated neoantigens by epitope spreading or by enhancing responses that are otherwise suboptimal. Definitive testing of these hypotheses is warranted. Other questions remain about how to optimize vaccine adjuvants and combination strategies. Many promising trials are underway with helper peptide vaccines and combinations, and these should advance this field over the next few years.

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Highlights

- Activated CD4+ helper T cells support DC activation by expression of CD40L.
- CD4+ helper T cells enhance antigen presentation and MHC expression.
- Vaccines incorporating epitopes for helper T cells induce epitope spreading.
- CD4+ T cells activated by vaccines support expansion of CD8 T cells in the tumor.
- Helper peptide vaccines have clinical activity for breast cancer and melanoma.

Figure 1. Role of helper T cells in priming of tumor specific effector T cells

Step 1. Vaccination with helper peptides allows antigen presenting cells (APCs) to take up tumor specific helper peptides in addition to tumor antigen directly from dying tumor cells. Step 2). APCs migrate to the lymph node where they interact with CD4 T cells through MHC class II molecules. Step 3). CD40 on the CD4 T cells ligates CD40L to mature the APC, which leads to enhanced MHC class I expression as well as costimulatory molecules CD70 and CCL3 and CCL4. Step 4) The chemokines recruit CD8 T cells to the complex, and binding the MHC molecules with costimulation induces optimal effector priming. The primed effectors proliferate and are capable of trafficking to the tumor site.

Figure 2. Role of CD4 T cells in the tumor microenvironment

Step 1). CD4 T cells can home to the tumor where they can interact with tumor cells when they express MHC class II. When activated Th1 CD4⁺ cells produce IFN γ . Step 2). IFN γ enhances MHC expression by tumor cells. Additionally, it induces CXCL9 and CXCL10 expression by the vasculature, to optimally recruit CD8 effector T cells to the tumor site. Step 3). Some CD4 T cells also are capable of direct tumor cell killing through FasL and TRAIL interactions as well as T cell receptor mediated cytotoxicity. Additionally, the T cells produce IL-2 which supports CD8 effector T cells in survival, proliferation and cytotoxic activity.

Figure 3. Association of IFN γ expression with survival in melanoma

Data from the Cancer Genome Atlas (TCGA) were interrogated through cbiportal.org for 459 patients.

Overexpression of IFN γ in the tumor microenvironment ($z > 0.5$, 11% of patients), was associated with enhanced overall survival (148 vs 69 months median survival, $p = 0.011$). Similar significant differences were evident with z scores of 0.8, 1, 1.2, 1.5 ($p < 0.006$ to $p = 0.02$, data not shown).

Table 1

Helper peptide vaccines with favorable immunologic and/or clinical outcomes

Cancer target	Source proteins: Peptides in vaccines	Adjuvant/delivery	Immunologic outcome	Clinical outcomes
Invasive breast cancer, DCIS	[Her2/neu]: Her2 (42-56), (98-114), (328-345), (776-790), (927-941), (1166-1180) +/- HLA-A2 peptides Her2 (369-377), (689-697)	Peptide-pulsed type 1 DC (matured in IFN γ and LPS), intranodal. Also evaluated intralesional vaccines.	Durable T _{CD4} responses in about 80% of patients [48].	Complete regressions of DCIS in 19%–29%; decreased Her2 expression [49] [50].
NSCLC and pancreatic cancer	[hTERT]: GV1001 vaccine : hTERT (611-626)	Administered to patients with NSCLC and also with gemcitabine for patients with pancreatic cancer.	Polyfunctional Th1 cells induced to hTERT (59%); epitope spreading to Ras (29%), but transient and weak responses.[51]	Survival for NSCLC enhanced in immune responders (54 vs. 13 mos, p<0.001) [52]. Failed to impact survival in randomized phase III trial [55].
Prostate cancer, renal cell cancer	[hTERT]: GX301 vaccine : hTERT (611-626), (672-686), (766-780); plus HLA-A2 restricted hTERT (540-548)	Each peptide administered with IFA and topical imiquimod in the skin of the abdomen: each peptide in a different site. Each vaccine administration in the same sites.	Immune response in all patients.	SD in 4 patients
Melanoma	[gp100, tyrosinase]: HLA-A2 restricted: gp100 (154–162); tyros (369–377); +/- MHC-II–restricted gp100 (44–59); tyros (448–462).	Monocyte-derived DC prepared in IL4, GM-CSF and matured PGE2 and TNF α . Also pulsed with KLH. Intranodal injection.	IFN γ response of SKIL to melanoma cells 29% vs 7%; Tetramer+ in blood 17% vs 0% for T _{CD8} , 23% for T _{CD4} .	PFS enhanced for addition of helper peptides (5 vs 2.8 mos, p < 0.01)
Melanoma	[gp100, tyrosinase, MelanA, MAGE-A] 6MHP vaccine : gp100 (44-59); tyros (56-70); tyros (386-406); Melan-A (51-73); MAGE-A3 (281-295); MAGE-A1-3,6 (121-134);	Peptides + IFA +/- GM-CSF, administered half SC, half ID.	T _{CD4} response in 81% (blood or node); epitope spreading to T _{CD8} responses (different antigens) in 45% tested; induction of Ab responses.	ORR 7–12% (duration up to 7 years). Survival associated with T _{CD4} response. Survival exceeds matched controls. 5 year survival 74% for resected stage IV.

Abbreviations: AdenoCA = adenocarcinoma; tyros = tyrosinase; SKIL = skin test infiltrating lymphocytes; LPS = lipopolysaccharide; IN = intranodal; SC = subcutaneous; ID = intradermally; ORR = objective response rate (PR + CR); NSCLC: non small cell lung cancer.

Amino acid sequences of selected peptides are: gp100_{154–162}(KTWGQYWQV); gp100_{280–288}(YLEPGPVTA); tyros_{369–377}(YMDGTMSQV); gp100_{44–59}(WNRQLYPEWTEAQRLD); tyros_{448–462}(DYSYLQSDPDSFQD); tyros_{56–70}(AQNILLSNAPLGPQFP); tyros_{386–406}(FLLHHAFVDSIFEQWLQRHRP); Melan-A_{51–73}(RNGYRALMDKSLHVGTCALTRR); MAGE-_{3281–295}(TSYVKVLHMHVKISG); MAGE-1,2,3,6_{121–134}(LLKYRAREPVTKAE); hTERT_{611–626}(EARPALLTSRLRFIPK)