Overcoming barriers to T cell activation, infiltration and function in tumors

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Chapter 2

Vaccines targeting helper T cells for cancer immunotherapy

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Curr Opin Immunol. 2017 Aug;47:85-92.

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.

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 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 IFNγ. Within the TME, effects of IFNγ include the induction of Class I and Class II MHC molecules and upregulation of antigen processing machinery (Figures 1 and 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].

Co-stimulation

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 T_{CD8} proliferation, survival and effector function [21].

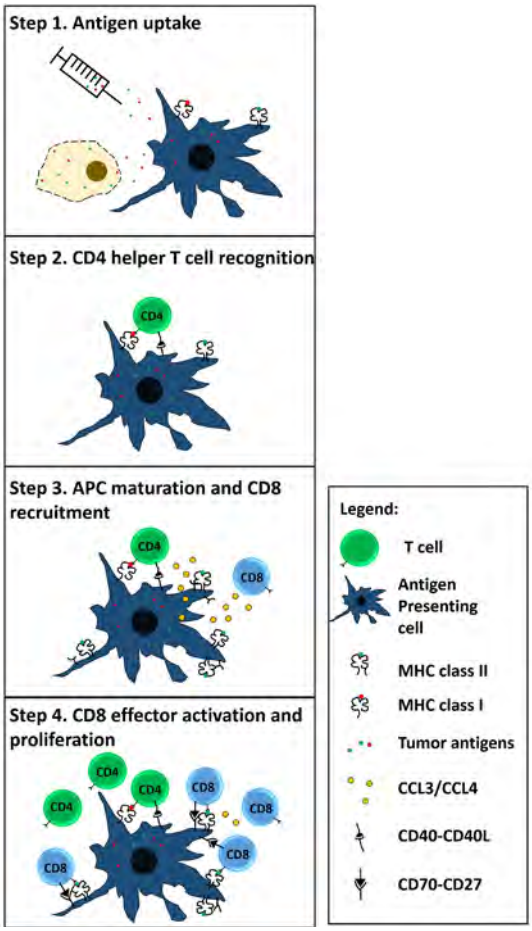

Figure 1. Role of helper T cells in priming of tumor specific effector T cells. Step 1. Vaccination with helper peptides allows antigen presenting cells (APCs) to take up tumor specific helper peptides in addition to tumor antigen directly from dying tumor cells. Step 2. APCs migrate to the lymph node 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.

T cell homing

As mentioned above, effector Th1 T_{CD4} are a source of IFNγ if they recognize their cognate antigen in the TME (Figure 2). In addition to enhancing antigen presentation, IFNγ 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 IFNγ 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 IFNγ expression in the TME is associated with improved overall survival [33,34] (Figure 3), which supports the favorable association of IFNγ in the TME with melanoma control.

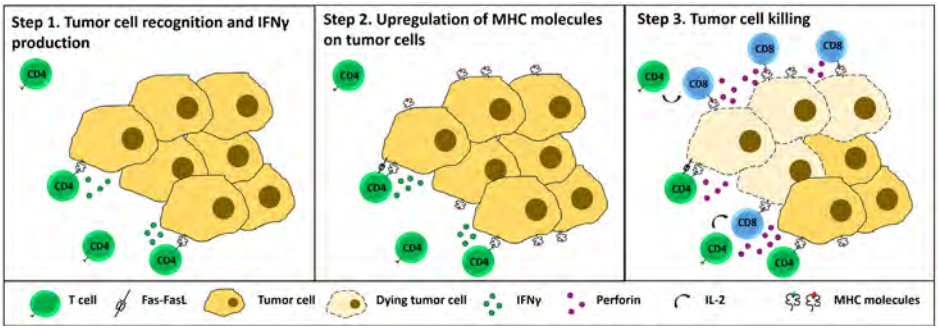

Figure 2. Role of CD4 T cells in the tumor microenvironment. Step 1. CD4 T cells can home to the tumor where they can interact with tumor cells when they express MHC class II. When activated Th1 CD4+ cells produce IFNg. Step 2. IFNg enhances MHC expression by tumor cells. Additionally, it induces CXCL9 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

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 IFNγ 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].

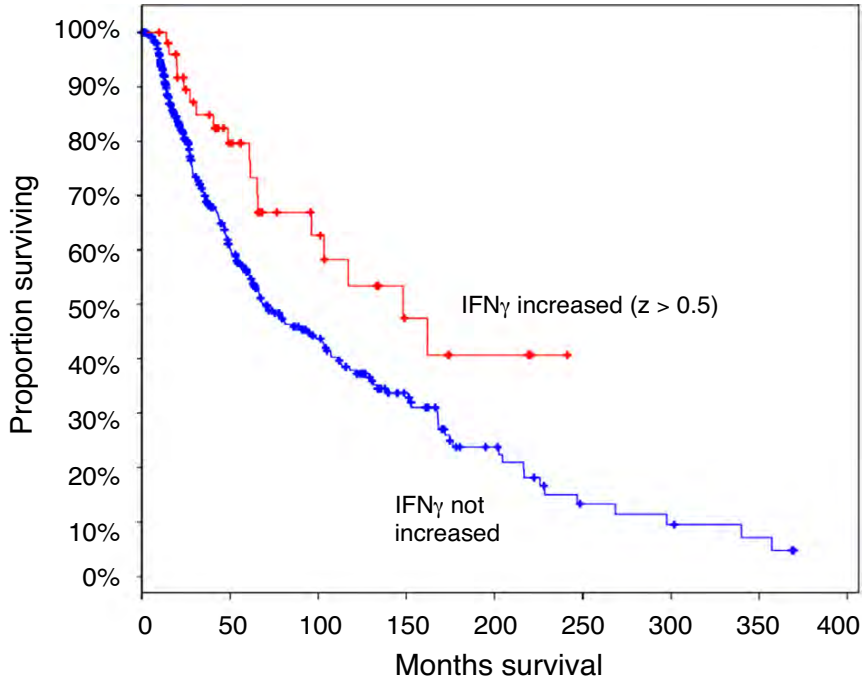

Figure 3. Association of IFNγ expression with survival in melanoma. Data from the Cancer Genome Atlas (TCGA) were interrogated through cbioportal.org for 459 patients. Overexpression of IFNγ in the tumor microenvironment (z > 0.5, 11% of patients), was associated with enhanced overall survival (148 versus 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).

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 versus 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].

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 IFNγ 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

table continues

Cancer target	Source proteins: Peptides in vaccines	Adjuvant/ delivery	Immunologic outcome	Clinical outcomes
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 TNFa. Also pulsed with KLH. Intranodal injection.	IFNγ 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); $tyros_{448–462}$(DYSYLQDSDPDSFQD); $tyros_{56-70}$(AQNILLSNAPLGPQFP); $tyros_{386-406}$(FLLHHAFVDSIFEQWLQRHRP); Melan-A_{51-73}(RNGYRALMDKSLHVGTQCALTRR); MAGE-$3_{281-295}$(TSYVKVLHHMVKISG); MAGE-1,2,3,$6_{121-134}$(LLKYRAREPVTKAE); hTERT$_{611-626}$(EARPALLTSRLRFIPK)

Melanoma peptide vaccines administered in adjuvant emulsion

Our group has evaluated a multipeptide 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% versus 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 (e.g.: 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 T_{CD8} 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 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} 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.

ACKNOWLEDGEMENTS

This work was supported by the National Institutes of Health [grant numbers R01 CA178846, P30 CA044579] and a Cancer Research Institute Clinical Laboratory Integration Project (CLIP) award.

REFERENCES

1. Rosenberg SA, Yang JC, Restifo NP: Cancer immunotherapy: moving beyond current vaccines. Nat Med 2004, 10:909-915.
2. Kim HJ, Cantor H: CD4 T-cell subsets and tumor immunity: the helpful and the not-so-helpful. Cancer Immunol Res 2014, 2:91- 98.
3. Kumai T, Lee S, Cho HI, Sultan H, Kobayashi H, Harabuchi Y, Celis E: Optimization of peptide vaccines to induce robust antitumor CD4 T-cell responses. Cancer Immunol Res 2017, 5:72-83.
4. Spitzer MH, Carmi Y, Reticker-Flynn NE, Kwek SS, Madhireddy D, Martins MM, Gherardini PF, Prestwood TR, Chabon J, Bendall SC et al.: Systemic immunity is required for effective cancer immunotherapy. Cell 2017.
5. Kayaga J, Souberbielle BE, Sheikh N, Morrow WJ, Scott-Taylor T, Vile R, Chong H, Dalgleish AG: Anti-tumour activity against B16- F10 melanoma with a GM-CSF secreting allogeneic tumour cell vaccine. Gene Therapy 1999, 6:1475-1481.
6. Hunder NN, Wallen H, Cao J, Hendricks DW, Reilly JZ, Rodmyre R, Jungbluth A, Gnjatic S, Thompson JA, Yee C: Treatment of metastatic melanoma with autologous CD4+ T cells against NY-ESO-1. N Engl J Med 2008, 358:2698-2703.
7. Kahn M, Sugawara H, McGowan P, Okuno K, Nagoya S, Hellstrom KE, Greenberg P: CD4+ T cell clones specific for the human p97 melanoma-associated antigen can eradicate pulmonary metastases from a murine tumor expressing the p97 antigen. J Immunol 1991, 146:3235-3241.
8. Xie Y, Akpinarli A, Maris C, Hipkiss EL, Lane M, Kwon EK, Muranski P, Restifo NP, Antony PA: Naive tumor-specific CD4(+) T cells differentiated in vivo eradicate established melanoma. J Exp Med 2010, 207:651-667.
9. Quezada SA, Simpson TR, Peggs KS, Merghoub T, Vider J, Fan X, Blasberg R, Yagita H, Muranski P, Antony PA et al.: Tumorreactive CD4(+) T cells develop cytotoxic activity and eradicate large established melanoma after transfer into lymphopenic hosts. J Exp Med 2010, 207:637-650.
10. Redondo M, Ruiz-Cabello F, Concha A, Hortas ML, Serrano A, Morell M, Garrido F: Differential expression of MHC class II genes in lung tumour cell lines. Eur J Immunogenet 1998, 25:385-391.
11. Ridge JP, Di RF, Matzinger P: A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell. Nature 1998, 393:474-478.
12. Bennett SR, Carbone FR, Karamalis F, Flavell RA, Miller JF, Heath WR: Help for cytotoxic-T-cell responses is mediated by CD40 signalling. Nature 1998, 393:478-480.
13. Smith CM, Wilson NS, Waithman J, Villadangos JA, Carbone FR, Heath WR, Belz GT: Cognate CD4(+) T cell licensing of dendritic cells in CD8(+) T cell immunity. Nat Immunol 2004, 5:1143-1148.
14. Castellino F, Huang AY, Altan-Bonnet G, Stoll S, Scheinecker C, Germain RN: Chemokines enhance immunity by guiding naive CD8+ T cells to sites of CD4+ T cell-dendritic cell interaction. Nature 2006, 440:890-895.
15. Kumamoto Y, Mattei LM, Sellers S, Payne GW, Iwasaki A: CD4+ T cells support cytotoxic T lymphocyte priming by controlling lymph node input. Proc Natl Acad Sci U S A 2011, 108:8749- 8754.

16. Ahrends T, Babala N, Xiao Y, Yagita H, van Eenennaam H, Borst J: CD27 agonism plus PD-1 blockade recapitulates CD4+ T-cell help in therapeutic anticancer vaccination. Cancer Res 2016, 76:2921-2931.
17. Hassan SB, Sorensen JF, Olsen BN, Pedersen AE: Anti-CD40- mediated cancer immunotherapy: an update of recent and ongoing clinical trials. Immunopharmacol Immunotoxicol 2014, 36:96-104.
18. Hung K, Hayashi R, Lafond-Walker A, Lowenstein C, Pardoll D, Levitsky H: The central role of CD4+ T-cells in the antitumor immune response. J Exp Med 1998, 188:2357-2368.
19. Matsui S, Ahlers JD, Vortmeyer AO, Terabe M, Tsukui T, Carbone DP, Liotta LA, Berzofsky JA: A model for CD8+ CTL tumor immunosurveillance and regulation of tumor escape by CD4 T cells through an effect on quality of CTL. J Immunol 1999, 163:184-193.
20. Schoenberger SP, Toes RE, van der Voort EI, Offringa R, Melief CJ: T-cell help for cytotoxic T lymphocytes is mediated by CD40– CD40L interactions. Nature 1998, 393:480-483.
21. Giuntoli RL 2nd, Lu J, Kobayashi H, Kennedy R, Celis E: Direct costimulation of tumor-reactive CTL by helper T cells potentiate their proliferation, survival, and effector function. Clin Cancer Res 2002, 8:922-931.
22. Dengel LT, Norrod AG, Gregory BL, Clancy-Thompson E, Burdick MD, Strieter RM, Slingluff CL Jr, Mullins DW: Interferons induce CXCR3-cognate chemokine production by human metastatic melanoma. J Immunother 2010, 33:965-974.
23. Peng W, Liu C, Xu C, Lou Y, Chen J, Yang Y, Yagita H, Overwijk WW, Lizee G, Radvanyi L et al.: PD-1 blockade enhances T-cell migration to tumors by elevating IFN-gamma inducible chemokines. Cancer Res 2012, 72:5209-5218.
24. Wang X, Michie SA, Xu B, Suzuki Y: Importance of IFN-gammamediated expression of endothelial VCAM-1 on recruitment of CD8+ T cells into the brain during chronic infection with Toxoplasma gondii. J Interferon Cytokine Res 2007, 27:329-338.
25. Clancy-Thompson E, King LK, Nunnley LD, Mullins IM, Slingluff CL Jr, Mullins DW: Peptide vaccination in Montanide adjuvant induces and GM-CSF increases CXCR3 and cutaneous lymphocyte antigen expression by tumor antigen-specific CD8 T cells. Cancer Immunol Res 2013, 1:332-339.
26. Bos R, Sherman LA: CD4+ T-cell help in the tumor milieu is required for recruitment and cytolytic function of CD8+ T lymphocytes. Cancer Res 2010, 70:8368-8377.
27. Nakanishi Y, Lu B, Gerard C, Iwasaki A: CD8(+) T lymphocyte mobilization to virus-infected tissue requires CD4(+) T-cell help. Nature 2009, 462:510-513.
28. Harlin H, Meng Y, Peterson AC, Zha Y, Tretiakova M, Slingluff C, McKee M, Gajewski TF: Chemokine expression in melanoma metastases associated with CD8+ T-cell recruitment. Cancer Res 2009, 69:3077-3085.
29. Dosset M, Vauchy C, Beziaud L, Adotevi O, Godet Y: Universal tumor-reactive helper peptides from telomerase as new tools for anticancer vaccination. Oncoimmunology 2013, 2:e23430.
30. Wong SB, Bos R, Sherman LA: Tumor-specific CD4+ T cells render the tumor environment permissive for infiltration by low-avidity CD8+ T cells. J Immunol 2008, 180:3122-3131.
31. Spranger S, Spaapen RM, Zha Y, Williams J, Meng Y, Ha TT, Gajewski TF: Up-regulation

of PD-L1, IDO, and T(regs) in the melanoma tumor microenvironment is driven by CD8(+) T cells. Sci Transl Med 2013, 5:200ra116.
32. Cho HI, Lee YR, Celis E: Interferon gamma limits the effectiveness of melanoma peptide vaccines. Blood 2011, 117:135-144.
33. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E et al.: Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013, 6:pl1.
34. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E et al.: The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012, 2:401-404.
35. Matsuzaki J, Tsuji T, Luescher I, Old LJ, Shrikant P, Gnjatic S, Odunsi K: Nonclassical antigen-processing pathways are required for MHC class II-restricted direct tumor recognition by NY-ESO-1-specific CD4(+) T cells. Cancer Immunol Res 2014, 2:341-350.
36. Matsuzaki J, Tsuji T, Luescher IF, Shiku H, Mineno J, Okamoto S, Old LJ, Shrikant P, Gnjatic S, Odunsi K: Direct tumor recognition by a human CD4(+) T-cell subset potently mediates tumor growth inhibition and orchestrates anti-tumor immune responses. Sci Rep 2015, 5:14896.
37. Kennedy R, Celis E: Multiple roles for CD4+ T cells in anti-tumor immune responses. Immunol Rev 2008, 222:129-144.
38. Tateyama M, Oyaizu N, McCloskey TW, Than S, Pahwa S: CD4 T lymphocytes are primed to express Fas ligand by CD4 crosslinking and to contribute to CD8 T-cell apoptosis via Fas/FasL death signaling pathway. Blood 2000, 96:195-202.
39. Qin Z, Blankenstein T: CD4+ T cell – mediated tumor rejection involves inhibition of angiogenesis that is dependent on IFN gamma receptor expression by nonhematopoietic cells. Immunity 2000, 12:677-686.
40. Zanetti M: Tapping CD4 T cells for cancer immunotherapy: the choice of personalized genomics. J Immunol 2015, 194:2049- 2056.
41. Buller RM, Holmes KL, Hugin A, Frederickson TN, Morse HC 3rd: Induction of cytotoxic T-cell responses in vivo in the absence of CD4 helper cells. Nature 1987, 328:77-79.
42. Wu Y, Liu Y: Viral induction of co-stimulatory activity on antigen-presenting cells bypasses the need for CD4+ T-cell help in CD8+ T-cell responses. Curr Biol 1994, 4:499-505.
43. Bevan MJ: Helping the CD8(+) T-cell response. Nat Rev Immunol 2004, 4:595-602.
44. Sun JC, Bevan MJ: Cutting edge: long-lived CD8 memory and protective immunity in the absence of CD40 expression on CD8 T cells. J Immunol 2004, 172:3385-3389.
45. Laidlaw BJ, Zhang N, Marshall HD, Staron MM, Guan T, Hu Y, Cauley LS, Craft J, Kaech SM: CD4+ T cell help guides formation of CD103+ lung-resident memory CD8+ T cells during influenza viral infection. Immunity 2014, 41:633-645.
46. Janssen EM, Droin NM, Lemmens EE, Pinkoski MJ, Bensinger SJ, Ehst BD, Griffith TS, Green DR, Schoenberger SP: CD4+ T-cell help controls CD8+ T-cell memory via TRAIL-mediated activation-induced cell death. Nature 2005, 434:88-93.
47. Datta J, Fracol M, McMillan MT, Berk E, Xu S, Goodman N, Lewis DA, DeMichele A, Czerniecki BJ: Association of depressed anti-HER2 T-helper type 1 response with recurrence in patients with completely treated HER2-positive breast cancer: role for

immune monitoring. JAMA Oncol 2016, 2:242-246.
48. Koski GK, Koldovsky U, Xu S, Mick R, Sharma A, Fitzpatrick E, Weinstein S, Nisenbaum H, Levine BL, Fox K et al.: A novel dendritic cell-based immunization approach for the induction of durable Th1-polarized anti-HER-2/neu responses in women with early breast cancer. J Immunother 2012, 35:54-65.
49. Sharma A, Koldovsky U, Xu S, Mick R, Roses R, Fitzpatrick E, Weinstein S, Nisenbaum H, Levine BL, Fox K et al.: HER-2 pulsed dendritic cell vaccine can eliminate HER-2 expression and impact ductal carcinoma in situ. Cancer 2012, 118:4354-4362.
50. Lowenfeld L, Mick R, Datta J, Xu S, Fitzpatrick E, Fisher CS, Fox KR, DeMichele A, Zhang P, Weinstein S et al.: Dendritic cell vaccination enhances immune responses and induces regression of HER2pos DCIS independent of route: results of randomized selection design trial. Clin Cancer Res 2016.
51. Staff C, Mozaffari F, Frodin JE, Mellstedt H, Liljefors M: Telomerase (GV1001) vaccination together with gemcitabine in advanced pancreatic cancer patients. Int J Oncol 2014, 45:1293-1303.
52. Hansen GL, Gaudernack G, Brunsvig PF, Cvancarova M, Kyte JA: Immunological factors influencing clinical outcome in lung cancer patients after telomerase peptide vaccination. Cancer Immunol Immunother 2015, 64:1609-1621.
53. Fenoglio D, Traverso P, Parodi A, Tomasello L, Negrini S, Kalli F, Battaglia F, Ferrera F, Sciallero S, Murdaca G et al.: A multipeptide, dual-adjuvant telomerase vaccine (GX301) is highly immunogenic in patients with prostate and renal cancer. Cancer Immunol Immunother 2013, 62:1041-1052.
54. Dosset M, Godet Y, Vauchy C, Beziaud L, Lone YC, Sedlik C, Liard C, Levionnois E, Clerc B, Sandoval F et al.: Universal cancer peptide-based therapeutic vaccine breaks tolerance against telomerase and eradicates established tumor. Clin Cancer Res 2012, 18:6284-6295.
55. Middleton G, Silcocks P, Cox T, Valle J, Wadsley J, Propper D, Coxon F, Ross P, Madhusudan S, Roques T et al.: Gemcitabine and capecitabine with or without telomerase peptide vaccine GV1001 in patients with locally advanced or metastatic pancreatic cancer (TeloVac): an open-label, randomised, phase 3 trial. Lancet Oncol 2014, 15:829-840.
56. Aarntzen EH, De Vries IJ, Lesterhuis WJ, Schuurhuis D, Jacobs JF, Bol K, Schreibelt G, Mus R, De Wilt JH, Haanen JB et al.: Targeting CD4(+) T-helper cells improves the induction of antitumor responses in dendritic cell-based vaccination. Cancer Res 2013, 73:19-29.
57. Slingluff CL Jr, Petroni GR, Olson W, Czarkowski AR, Grosh WW, Smolkin M, Chianese-Bullock KA, Neese PY, Deacon DH, Nail CJ et al.: Helper T cell responses and clinical activity of a melanoma vaccine with multiple peptides from MAGE and melanocytic differentiation antigens. J Clin Oncol 2008, 26:4973-4980.
58. Slingluff CL Jr, Petroni GR, Chianese-Bullock KA, Smolkin ME, Ross MI, Haas NB, von Mehren M, Grosh WW: Randomized multicenter trial of the effects of melanoma-associated helper peptides and cyclophosphamide on the immunogenicity of a multipeptide melanoma vaccine. J Clin Oncol 2011, 29:2924- 2932.
59. Slingluff CL Jr, Lee S, Zhao F, Chianese-Bullock KA, Olson WC, Butterfield LH, Whiteside TL, Leming PD, Kirkwood JM: A randomized phase II trial of multiepitope vaccination with melanoma peptides for cytotoxic T cells and helper T cells for

patients with metastatic melanoma (E1602). Clin Cancer Res 2013, 19:4228-4238.
60. Dillon PM, Olson WC, Czarkowski A, Petroni GR, Smolkin M, Grosh WW, Chianese-Bullock KA, Deacon DH, Slingluff CL Jr: A melanoma helper peptide vaccine increases Th1 cytokine production by leukocytes in peripheral blood and immunized lymph nodes. J Immunother Cancer 2014, 2:23.
61. Hu Y, Kim H, Blackwell CM, Slingluff CL Jr: Long-term outcomes of helper peptide vaccination for metastatic melanoma. Ann Surg 2015, 262:456-464 (discussion 462-454).
62. Hu Y, Petroni GR, Olson WC, Czarkowski A, Smolkin ME, Grosh WW, Chianese-Bullock KA, Slingluff CL Jr: Immunologic hierarchy, class II MHC promiscuity, and epitope spreading of a melanoma helper peptide vaccine. Cancer Immunol Immunother 2014, 63:779-786.
63. Reed CM, Cresce ND, Mauldin IS, Slingluff CL Jr, Olson WC: Vaccination with melanoma helper peptides induces antibody responses associated with improved overall survival. Clin Cancer Res 2015, 21:3879-3887.
64. Kenter GG, Welters MJ, Valentijn AR, Lowik MJ, B-vdM DM, Vloon AP, Essahsah F, Fathers LM, Offringa R, Drijfhout JW et al.: Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. N Engl J Med 2009, 361:1838-1847.
65. Tsuji T, Sabbatini P, Jungbluth AA, Ritter E, Pan L, Ritter G, Ferran L, Spriggs D, Salazar AM, Gnjatic S: Effect of Montanide and poly-ICLC adjuvant on human self/tumor antigen-specific CD4+ T cells in phase I overlapping long peptide vaccine trial. Cancer Immunol Res 2013, 1:340-350.
66. Cohen CJ, Gartner JJ, Horovitz-Fried M, Shamalov K, TrebskaMcGowan K, Bliskovsky VV, Parkhurst MR, Ankri C, Prickett TD, Crystal JS et al.: Isolation of neoantigen-specific T cells from tumor and peripheral lymphocytes. J Clin Invest 2015, 125:3981-3991.
67. Carreno BM, Magrini V, Becker-Hapak M, Kaabinejadian S, Hundal J, Petti AA, Ly A, Lie WR, Hildebrand WH, Mardis ER et al.: Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. Science 2015, 348:803-808.
68. Hailemichael Y, Dai Z, Jaffarzad N, Ye Y, Medina MA, Huang XF, Dorta-Estremera SM, Greeley NR, Nitti G, Peng W et al.: Persistent antigen at vaccination sites induces tumor-specific CD8(+) T cell sequestration, dysfunction and deletion. Nat Med 2013, 19:465-472.
69. Cho HI, Celis E: Optimized peptide vaccines eliciting extensive CD8 T-cell responses with therapeutic antitumor effects. Cancer Res 2009, 69:9012-9019.
70. Reinhardt RL, Bullard DC, Weaver CT, Jenkins MK: Preferential accumulation of antigen-specific effector CD4 T cells at an antigen injection site involves CD62E-dependent migration but not local proliferation. J Exp Med 2003, 197:751-762.
71. Diefenbach CS, Gnjatic S, Sabbatini P, Aghajanian C, Hensley ML, Spriggs DR, Iasonos A, Lee H, Dupont B, Pezzulli S et al.: Safety and immunogenicity study of NY-ESO-1b peptide and montanide ISA-51 vaccination of patients with epithelial ovarian cancer in high-risk first remission. Clin Cancer Res 2008, 14:2740-2748.