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Harnessing neoantigen specific CD4 T cells for cancer immunotherapy

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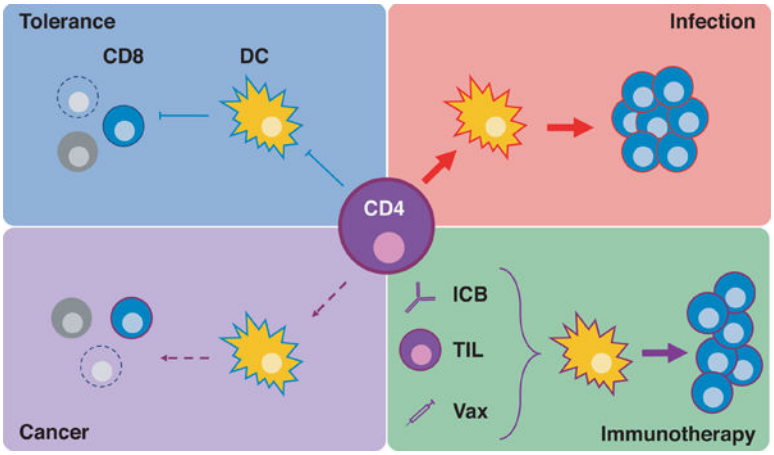
Graphical Abstract:



CD4$^+$ T cells can either bolster or constrain CD8$^+$ T cell responses by conditioning dendritic cells. In the context of anti-tumor immunity, the capacity of CD4$^+$ T cells to do so is greatly diminished. Thus, in this review we will discuss how augmenting neoantigen-specific CD4$^+$ T cells via immune checkpoint blockade (ICB), adoptive cellular therapy (ACT), and tumor-specific vaccines (Vax) could lead to robust CD8$^+$ T cell responses.

Introduction

The idea that cells of the adaptive immune system—specifically of the T lineage—surveil, recognize, and eliminate cells expressing *mutated-self* antigens (neoantigens, NeoAg) lies at the heart of the current immunotherapy revolution in oncology. While these ideas are hardly new (1), our ability to clinically manipulate the immune system to elicit such potent antitumor response has only recently matured. Somatic mutations in the functional domains of key genes not only set in motion the transformation of normal cells into cancer but also serve as potential targets for T cells (2). The major avenues of cancer immunotherapy–immune checkpoint blockade (ICB), cancer vaccines, and adoptive cell therapy (ACT)—

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each focus on directing a cytotoxic T lymphocyte (CTL) response against the tumor; however, the role of helper $CD4^+$ T cells in enhancing CTL function is often overlooked. In this review, we aim to highlight our current understanding and therapeutic value of $CD4^+$ T cell help in cancer immunotherapy.

T helper immunity in the context of cancer immunotherapy

Helper T cells shape and orchestrate immune responses through direct cellular interactions and soluble factors. For example, direct TCR:MHCII interactions result in the selection of high affinity B cell clones in germinal centers via CD40-CD40L interactions (3). As antigen-presenting cells (APC), B cells can engage in this direct communication with $CD4^+$ T cells. Similarly, $CD4^+$ T cells help CTLs but through an APC intermediate. Older models suggested that $CD4^+$ T cell cytokine production (particularly IL-2) in proximity to $CD8^+$ T cells interacting with the same dendritic cell (DC) imparts the coveted help signal (4,5). However, numerous subsequent studies have upheld a dynamic, stepwise model involving coordinated cellular interactions. Following initial TCR:MHCII interactions, $CD4^+$ T cells condition an APC via CD40-CD40L to provide proper costimulation to cognate $CD8^+$ T cells reacting to a cross-presented antigen on the same APC (6–9). Recently, key studies have refined previous models and identified cellular interactions between different DC subsets and T cells that are spatiotemporally distinct (10). Specifically, incoming, antigen-loaded migratory DCs prime $CD4^+$ T cells and transfer antigen to lymph node (LN)-resident DCs capable of efficient cross-presentation and CTL priming (11–13). While these studies primarily used viral infection models, the question of whether these same rules apply, differ, or are rendered defunct in the context of cancer immunity is an active area of research (14). Importantly, this inter-DC antigen transfer phenomenon was shown to be highly efficient and maintains peripheral tolerance (15–17). Thus, we propose that the paucity of presented NeoAgs relative to autoantigens and lack of pattern recognition receptor (PRR) and, thereby, innate immune system engagement, both contribute to the impaired initiation of a proper CTL response (Figure 1).

Without this highly choreographed dance between T cell and DC subsets, the consequences of a “helpless” CTL response include poor memory formation, secondary expansion, effector function, and survival (18–22). There exist a number of virulent infections which apparently do not require T help to generate a sufficient cytotoxic response, and in these cases, overzealous PRR activation has been thought to circumvent the requirements for help (9,23). However, in the context of a relatively non-inflammatory tumor, helpless cytotoxic responses are likely to be inadequate to control or eradicate the malignancy (Figure 1C). Strong evidence for the help requirement was demonstrated by seminal experiments in *Rag2*$^{-/-}$ mice: a significantly higher frequency of these mice developed sarcomas when exposed to the mutagen 3’-methylcholanthrene (MCA) than did their wildtype counterparts. Importantly, when tumors from *Rag2*$^{-/-}$ mice were transplanted into wildtype mice, many were spontaneously rejected in a $CD4^+$ or $CD8^+$ T cell dependent manner (24). This line of investigation established two key observations: 1) Both $CD4^+$ and $CD8^+$ T cells react and respond to cancer antigens and 2) the cytotoxic response alone is insufficient to control tumor progression. Thus, ICB, cancer vaccines, and ACT should aim to exploit the rules of proper T cell activation.

Which antigens do T cells recognize in cancer?

Tumor antigens can be broadly divided into two classes: self-antigens derive from proteins selectively expressed or overexpressed by tumor cells, and non-self-antigens deriving from mutated proteins, or NeoAg. Self-antigens include the tissue-specific cancer-testis antigens, such as MAGE-A, NY-ESO-1, and SSX-2, and lineage-specific proteins, such as MART-1, gp100, and tyrosinase (25). Given that these antigens arise from wildtype proteins, they are more likely to be shared between patients than NeoAg. Several clinical trials have been conducted investigating vaccines and cellular therapies targeting these shared tumor antigens. In one such trial, melanoma patients immunized with NY-ESO-1 peptides and CpG adjuvant mounted $CD8^+$ and $CD4^+$ T cell responses (26). Despite apparent antigen-specific T cell responses, clinical efficacy has been limited in patients with established tumors receiving these and similar vaccines. This lack of efficacy may be due to either the immunosuppressive tumor microenvironment or an inherent defect in the T cell repertoire recognizing these antigens. One preclinical study has demonstrated that the avidity of TCRs recognizing tumor self-antigens TRP-2 and gp100 are lower than those of TCRs recognizing a non-self, viral tumor antigen, resulting in worse tumor control after vaccination (27). This may be due to elimination of highly avid T cells recognizing tumor self-antigens during thymic selection. Indeed, another group has demonstrated that the tumor self-antigen carcinoembryonic antigen (CEA) is expressed by medullary thymic epithelial cells in CEA-transgenic mice, thus limiting the $CD4^+$ T cell repertoire responding to CEA vaccination (28). In addition to concerns regarding efficacy, safety concerns abound with immunotherapies targeting wildtype antigens (29,30).

NeoAg are ideal targets for immunotherapy because they are exclusively expressed by malignant tissue, and because NeoAg-specific clones are likely to survive thymic selection or peripheral tolerance. Indeed, ACT with tumor infiltrating lymphocytes (TIL) harboring NeoAg specificities has induced tumor regression in patients with metastatic breast cancer and melanoma (31,32). Furthermore, patients with higher mutational burden malignancies (and therefore a greater diversity of NeoAg targets) experience a greater response rate to both TIL ACT and ICB (33–35). NeoAg-specific T cells have also been identified as effectors of antitumor immunity in mouse models (36–38). Specifically, NeoAg-specific T cell populations increased in frequency and a greater percentage produced effector cytokines in mice bearing MCA-induced sarcomas receiving ICB (37). This phenomenon has also been observed in patient responders to ICB (34).

Methods of NeoAg identification

In order to test immune recognition of putative NeoAg, tumor mutations within the protein coding region of the genome must first be identified. The advent of next generation sequencing (NGS) technology permits high-throughput sequencing of multiple tumor specimens and provides a comprehensive map of somatic mutations across a range of human malignancies (39). These data reveal that 10–1000s of nonsynonymous mutations exist in tumor tissue and could serve as targets for immune therapies. Accordingly, numerous strategies have been developed to identify NeoAg and test them for immunogenicity. To date, *in silico* prediction algorithms have been utilized to identify putative NeoAg peptides

based on their calculated ability to bind MHC-I and II (40,41). These prediction algorithms have been a necessary first step for NeoAg identification for high mutation rate malignancies; however, this approach carries the risk that *de facto* NeoAg will be missed and therefore remain untested (42,43). Indeed, many predicted NeoAg are unable to generate detectable T cell responses *in vivo* after therapeutic vaccination (44,45). While additional bioinformatics packages exist that may be used to augment the performance of MHC prediction algorithms, a recent study from our colleagues in the Peters lab found that the addition of predictions for proteasomal cleavage, TAP transport, and MHC-peptide complex stability did not improve the predictive power of NetMHCpan—a widely used prediction algorithm for MHC I binding (46). Lastly, in a study from the Riddell group, mutations were selected for screening in an HLA agnostic manner (47). Instead, mutations were ranked based on their mean expression levels in The Cancer Genome Atlas for the given cancer type or expression as determined by RNA sequencing of a patient derived xenograft. The expression of mutated genes within a tumor is an important metric to consider given the influence of the transcriptome on antigen presentation (48). In a retrospective analysis they found that only one of three validated $CD4^+$ T cell epitopes was predicted to bind its restricting MHC allele using NetMHCIIpan.

Alongside the development of algorithms for the prediction of NeoAg, experimental approaches have been investigated to verify presentation of putative NeoAg. Some groups have been able to identify NeoAg by eluting peptides from MHC molecules on the cell surface and subsequently using mass spectrometry to verify the presentation of mutant epitopes (43). However, such an approach will likely not be feasible for widespread clinical applications, at least not on a per-patient basis. Moreover, given that most tumors do not express MHC class II, NeoAg identified in this manner would be highly if not entirely biased towards class I epitopes. Rosenberg and colleagues have employed traditional functional assays such as ELISpot to identify NeoAg-specific responses among TIL and circulating T cells. In these assays, autologous APCs are either pulsed with 8–11mer peptides corresponding to mutant epitopes or transfected with tandem minigene thus allowing to screen both MHC class I and II, NeoAg-specific T cell responses (49). Given that $CD4^+$ T cells predominantly recognize antigen derived from endocytosed dying cells or cell debris *in vivo*, the degree to which either peptide pulsing or transfection with tandem minigenes accurately reflects this process remains in question. So called “type B” $CD4^+$ T cells have been identified in the context of autoimmune disease and recognize APCs directly pulsed with soluble peptides but are unable to recognize APCs incubated with the larger parent protein (50,51). To rule out such responses, which may be unproductive *in vivo*, surrogate assays for class II presentation should be employed. This can be accomplished by either feeding HLA-matched DCs irradiated or otherwise killed cell lines expressing the mutant proteins or employing *in vitro* transcribed RNA constructs linking the target antigen to a membrane trafficking sequence and transmembrane domain (52). *In toto*, future approaches should couple bioinformatic identification of putative NeoAgs in an HLA-agnostic manner followed by rigorous testing using standard immunoassays.

Help in Therapeutic Context

Immune Checkpoint Blockade

Currently, the most widely used immunotherapeutic strategy is ICB. Blockade of the inhibitory molecules CTLA4 and/or PD1/PDL1 results in the activation of a preexisting pool of NeoAg or tumor-specific T cells within the patient (53). Tumor regression in the context of PD1:PDL1 blockade presumably works via the transient reversal of $CD8^+$ T cell exhaustion (54); however, the role of help in PD1 blockade efficacy is yet to be ascertained. Importantly, a new report reveals a *de novo* tumor-specific cytotoxic response following PD1 blockade in humans with basal or squamous cell carcinoma rather than the expansion of preexisting clonotypes (55). This phenomenon supports other recent findings indicating that T cells within the tumor microenvironment enter a state of epigenetically programmed, irreversible exhaustion (56,57). Thus, whether the recruitment of these novel $CD8^+$ NeoAg specificities is dependent on help remains an open question.

In contrast to PD1, CTLA4 outcompetes CD28 for B7–1 and 2 (58,59), and thus imparts a strong inhibitory effect on T cell activation. Consequently, CTLA4 deficient mice succumb to a lymphoproliferative disease similar to FOXP3-deficient mice, indicating that CTLA4 is a key mediator of peripheral tolerance (60,61). In Nobel-worthy mouse studies, CTLA4 blockade lead to potent anti-tumor responses (62). Importantly, the antitumor response in certain tumor models sensitive to CTLA4 blockade was found to be CD4 dependent (63–65). Whether this dependence was due to help provided or Treg depletion remains controversial (66,67); however, recent studies have highlighted that in humans, CTLA4 therapy does not deplete Tregs but results in T cell activation (68,69). Despite the lack of clarity surrounding its effects on the natural course of a *bona fide* cytotoxic response, ICB's clinical results are impressive especially in highly mutated tumors such as smoking induced lung cancer and melanoma (70,71).

The moniker of "exhaustion" was originally applied to CTLs, but the dysfunction that ICB aims to reverse also applies to helper T cells. Indeed, chronic antigen exposure drives $CD4^+$ T cells into a dysfunctional state similar to the exhaustion phenotype seen in $CD8^+$ T cells (72). Exhausted $CD4^+$ and $CD8^+$ T cells express many of the same coinhibitory receptors, but there is evidence in chronic viral infection models of biased expression (72). Specifically, dysfunctional $CD4^+$ T cells appear to express higher levels of CTLA4 at late time points in their activation cycle than their $CD8^+$ counterparts. This may partially explain why, in many murine cancer models, ICB with anti-CTLA4 alone or in combination with anti-PD1 induces the expansion of a T_H1-like subset of $CD4^+$ T cells (73,74). Whether this expansion is the result of a "rescue" of dysfunctional $CD4^+$ T cells or the priming of novel clonotypes requires further investigation. Combination treatments employing NeoAg vaccines and ICB will likely yield optimal responses by overcoming exhaustion of newly primed clones. Such combinations may also lead to responses in patients with a lower mutational burden that would have previously been unresponsive to ICB alone.

Cancer Vaccines

Studies of NeoAg vaccines to date have identified a peculiar phenomenon: peptides selected *in silico* for their ability to bind MHC class I largely yield $CD4^+$ T cell responses *in vivo* (45). Indeed, NeoAg-specific $CD4^+$ T cells arise spontaneously in various human malignancies and are readily identifiable (32,75). The role of these NeoAg-specific $CD4^+$ T cells, however, is incompletely understood. We propose that these cells have a dominant function as "helpers" for cytotoxic $CD8^+$ T cells through the licensing of dendritic cells via CD40/CD40L interactions (7,76). $CD8^+$ T cells primed in the absence of $CD4^+$ T cell help are unable to undergo secondary expansion and instead are subject to activation-induced cell death (AICD) (18). It therefore stands to reason that $CD4^+$ T cell epitopes are a crucial component of effective NeoAg vaccination (Figure 2A). It has been demonstrated that the inclusion of "helper" epitopes in therapeutic cancer vaccines improves the antitumor response by increasing the expansion of $CD8^+$ T cells, as well as reducing their expression of coinhibitory receptors and increasing their migratory potential (77).

In addition to aiding in the productive priming of $CD8^+$ T cells in secondary lymphoid organs, effector roles of $CD4^+$ T cells in the tumor microenvironment have been proposed. These include the activation of local NK cells via secretion of effector cytokines, recruitment of $CD8^+$ T cells by IFNγ-inducible chemokines such as CXCL-10, and even class-II dependent killing of tumor cells (78–80). In a recent publication from the Schreiber laboratory, KPC (*LSL-Kras$^{G12D/+}$; LSL-Trp53$^{R172H/+}$; Pdx-1-Cre*) tumors lacking natural NeoAg were transduced to express a single MHC-I-restricted NeoAg with or without an additional MHC-II-restricted NeoAg (81). Contralateral injection resulted in a significant increase in the numbers of antigen-specific $CD8^+$ T cells, total $CD8^+$ and $CD4^+$ T cells, and $iNOS^+$ macrophages infiltrating the MHC-II NeoAg-expressing tumor. Importantly, KPC tumors *not* expressing MHC-II NeoAg continued to grow indicating that $CD4^+$ T cell effectors must recognize antigen locally to mediate their antitumor effects. Furthermore, another recent study has demonstrated that across a range of solid tumor types MHC-II expression is rare among tumor cells, which implies that the local effects of $CD4^+$ T cells previously mentioned are likely dependent on antigen recognition in the context of APCs and stromal cells (82). Overall, these and other effector roles of $CD4^+$ T cells require further exploration. If the dominant role of $CD4^+$ T cells in the antitumor immune response is the provision of help, NeoAg vaccines can simply include promiscuous helper epitopes such as the pan-DR binding epitope (83). If, however, there is an additional role for tumor-specific $CD4^+$ within the tumor, class II NeoAg must be identified and targeted specifically.

Adoptive Cellular Therapy

The promise of ACT is that it provides anti-tumor specificities that were never present or are exceeding rare to be beneficial. Broadly, ACT can be divided into 3 categories based on the cellular product delivered: Chimeric antigen receptor (CAR) T cells, TIL, and TCR engineered T cells. While TIL and TCR engineered T cells recognize antigen by conventional peptide-MHC interactions, CAR T cells target cell lineage determining antigens such as CD19 via the introduction of antibody specificity linked to intracellular T cell activating signal motifs into autologous T cells (84–86).

CART-19 therapy has vastly improved overall survival in certain B cell lymphomas relative to past standard of care (87,88), but both normal and malignant B cells are eliminated. While CAR T cells to date target cell surface proteins, TCR "mimic" (TCRm) antibodies have been described that bind tumor associated antigens in the context of HLA (89). Thus, NeoAg-reactive TCRm antibodies could be used with current CAR technology to provide specificity against NeoAgs in the near future. Surprisingly, recent mouse studies have highlighted that $CD4^+$ CAR T cells *alone* mediate superior antitumor activity relative to their $CD8^+$ counterparts (90–93). While the cytotoxic capacity of $CD4^+$ T cells in tumor models is hardly novel (80,94–96), in the context of CAR T therapy, $CD4^+$ T cells also mediate tumor cytotoxicity directly, resist AICD, do not help CTLs resist exhaustion, and are in fact impeded by their $CD8^+$ compatriots (90). Future CAR trials should test whether enriched $CD4^+$ T cells are similarly superior in humans as they are in mice.

TIL therapy expands a preexisting pool of T cells isolated from the patient's tumor. Tumor tissue is cultured with high concentrations of IL-2 to allow for TIL extravasation and growth and expanded TIL cultures are then infused back into the patient (97). While NeoAg-specific T cells are found within TIL, so too are T cells with irrelevant specificities (98). We propose that selection and expansion of TIL cultures with a high frequency of NeoAg-specific T cells as assessed by the methods previously described holds the most promise for effective cancer therapy (99) (Figure 2B). Apart from tumor specificity, several studies have correlated higher objective responses with a higher proportion of $CD8^+$ T cells within TIL products in melanoma patients (100,101); however, a randomized control trial failed to prove this hypothesis with a $CD8^+$ T cell enrichment step (102). Thus, understanding the relative contributions of $CD4^+$ or $CD8^+$ T cells with TIL studies is incomplete.

Specific anticancer responses may be achieved by engineering patient autologous T cells from blood with NeoAg-specific TCRs. Indeed, T cells have been engineered to express TCRs targeting tumor self-antigens using retroviral and more recently CRISPR based methodologies capable of simultaneously disrupting the endogenous TCR (103,104). This approach may require lower total numbers of T cells for ACT given that the frequency of engineered tumor-specific cells will be much higher than in TIL. In addition, T cell clones within TIL may be dysfunctional as a consequence of the immunosuppressive tumor microenvironment and chronic antigen stimulation (56,57). Therefore, engineered "fresh" T cells may have enhanced antitumor function on a per cell basis. Moreover, some tumor-specific TCRs are of such high affinity that they can recognize antigen in a co-receptor independent manner (105). Since $CD4^+$ T cells can now recognize NeoAg directly on tumors via MHC class I, the consequences of these interactions will be an important avenue of investigation. A clinical trial investigating TCR engineering with NeoAg-specific TCRs targeting metastatic disease in a range of malignancies is currently underway at the National Cancer Institute (NCT03412877).

Although efficacy endpoints for $CD4^+$ T cells in ACT thus far focus on direct tumor cytotoxicity, assessing how ACT functions through the help paradigm will be informative (Figure 2B). The value of tumor-specific $CD4^+$ T cells in the context of ACT has been demonstrated in numerous mouse models implicating a downstream role for macrophages, NK cells, and CTLs (95,96). Compelling clinical evidence exists in which a patient

receiving TIL therapy containing a BRAFV600E-specific $CD4^+$ clone experienced complete remission (32). In this case, the researchers identified the subsequent expansion of $CD8^+$ T cells recognizing several tumor-associated antigens, which may not have been primed in the absence of this expanded and activated $CD4^+$ clone (Figure 2B). Whether similar results can be obtained using NeoAg-specific $CD4^+$ TCR engineering remains unknown.

Lastly, many studies have indicated that a T_H1 phenotype is preferable for ACT; however, recent work indicates that T_H17 polarized $CD4^+$ T cells are able to exert superior antitumor immunity over T_H1 cells by resisting apoptosis and senescence (106). Importantly, tumor destruction still depends on the T_H1 cytokine IFNγ and $CD8^+$ T cells. Adoptively transferred T_H17 cells also may have a greater capacity to recruit DC subsets, promote $CD8^+$ T cell differentiation directly via IL-17, and ultimately control large tumors (107,108). Given that other studies have identified IL-17 and related gene signatures as negative prognostic markers in non-small cell lung and colorectal cancers, therapeutic engagement of T_H17 $CD4^+$ T cells requires further investigation (109,110).

Considerations for NeoAg-specific therapies

Personalized NeoAg vaccines tested thus far have demonstrated cautiously encouraging clinical responses that can be improved to provide a less costly alternative to ACT with either TIL or engineered T cells. RNA- and peptide-based NeoAg vaccines in melanoma and glioblastoma have induced the expansion of T cells recognizing targeted epitopes (44,111,112). Patients receiving RNA-based multiepitope vaccines experienced significantly reduced incidence of metastatic events compared to pretreatment (112). We echo what many have already shown and proposed: in order to achieve optimal $CD8^+$ T cell priming, NeoAg vaccines must include $CD4^+$ helper epitopes (111,113,114) (Figure 2A). We also speculate that these epitopes should be physically linked to ensure their delivery to the same APC, given that cross presentation is dependent on $CD4^+$ T cell help (115). In the case of multimer nucleic acid vaccines, a single vector encoding both $CD8^+$ and $CD4^+$ epitopes would ensure their expression within the same cell. For peptide vaccines, a flexible linking moiety joining these epitopes or nanoparticles should be considered for efficient codelivery. This “linker” strategy ensures that both helper and CTL neoepitopes will be deposited and transferred to appropriate DC subsets within the draining LNs thus maximizing the cytotoxic response (10–13). Lastly, attention must be given to proper adjuvant selection in order to drive a potent CTL response. Failure to induce a T_H1 and/or T_H17 response can result not only in poor anti-tumor cytotoxicity but also tolerize the immune response to the malignancy (116–118). The nuances of proper cancer adjuvant selection are beyond the scope of this review but are well summarized elsewhere (119).

In addition to including epitopes recognized by both $CD8^+$ and $CD4^+$ T cells, epitopes across multiple HLA alleles should be targeted to reduce the chance of immune escape by loss of heterozygosity (LOH), which is now a well-documented phenomenon in patients experiencing relapse after immunotherapy (111,120,121). We speculate that this may be an inherent benefit of $CD4^+$ T cell-directed therapies because help functions through antigen presented by APCs and not the tumor cells themselves (122). Moreover, $CD4^+$ T cells can direct a diverse CTL response through a variety of MHCI alleles rather than one in addition

to recruiting NK cells and macrophages, thus trapping the tumor from mutating further. Indeed, the same considerations apply for TCR engineering. Whereas the majority of early studies have prioritized single TCRs recognizing targets in the context of the common HLA-A*02:01 allele (of North American and European Caucasian populations), only recently have investigators searched for TCRs restricted to other common alleles (123). Priority targeting of known driver mutations, which are more likely to be clonal "trunk" mutations, will be advantageous so as to limit immune escape from antigen loss or incomplete clearance due to preexisting intratumor heterogeneity (124–126). A final consideration for effective NeoAg-specific therapies is that of optimal TCR avidity. Studies employing minimally altered tumor epitopes to model varying TCR-MHC interaction strengths have demonstrated that vaccination with intermediate affinity epitopes provides optimal tumor control, while the highest affinity epitope vaccinations result in a dysfunctional T cell response (127). Few published studies exist defining the rules of $CD4^+$ TCR affinity in the context of cancer. We would speculate that, similar to $CD8^+$ T cells, higher affinity TCR-MHC interactions in $CD4^+$ T cells may lead to dysfunctional responses, but this hypothesis must be experimentally tested.

Concluding Remarks

NeoAg-specific therapies represent perhaps the most advanced foray into personalized medicine for cancer. While NeoAg prediction methods require further investigation in a broader range of patient cohorts, we are beginning to learn which approaches are optimal for which contexts. Additionally, we are uncovering roles for $CD4^+$ T cells that may inform the next wave of therapies specifically designed to target this cell type. By employing appropriate methods for detection and validation along with rational design of both vaccination and cellular therapies, we can improve upon the already impressive recent track record of immunotherapies against cancer.

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Abbreviations:

NeoAg	Neoantigen
TCR	T Cell Receptor
CTL	Cytotoxic T Lymphocyte
MHC	Major Histocompatability Complex
HLA	Human Leukocyte Antigen
APC	Antigen Presenting Cell
DC	Dendritic Cell
ICB	Immune Checkpoint Blockade

ACT	Adoptive Cellular Therapy
AICD	Activation-Induced Cell Death
PRR	Pattern Recognition Receptor

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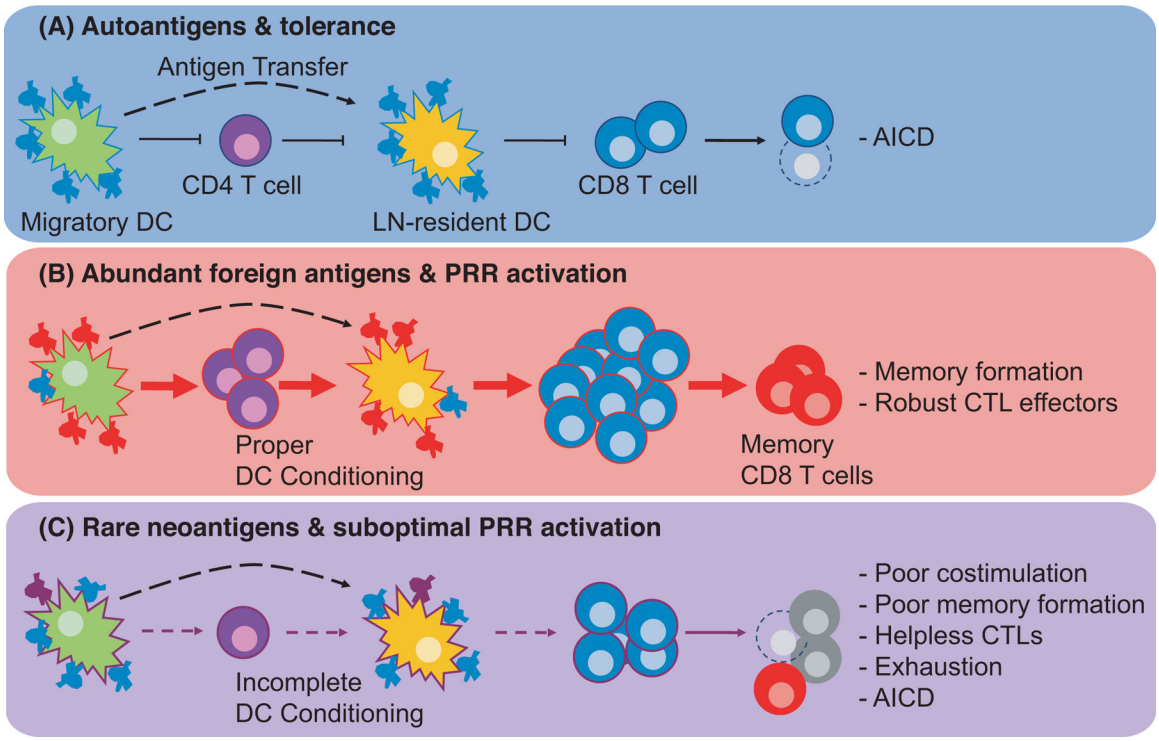

Figure 1. Context dependent CTL activation.

Migratory DCs capture antigen and traffic to the LN where they can present to $CD4^+$ T cells and transfer antigen to LN-resident DCs. (**A**) In the case of self-antigens (blue), $CD4^+$ T cells are not activated and thus LN-resident DCs capable of cross presentation are not licensed or conditioned to provide proper costimulation to potentially autoreactive CTLs, leading to no activation or AICD. (**B**) In the context of an acute pathogenic insult, abundant foreign antigen (red) and PRR engagement leads to $CD4^+$ activation and proper conditioning of LN-resident DCs via CD40:CD40L interactions. Ultimately, cognate $CD8^+$ T cells undergo robust expansion and memory formation due to optimal costimulation. (**C**) Rare tumor antigens (purple) relative to autoantigens (blue) and lack of PRR activation leads to incomplete costimulation. The resulting helpless or exhausted CTLs may be insufficient to control the tumor. Some CTLs might receive all necessary cues and form proper memory; however, the clonal diversity of the effective CTL response is dramatically decreased and may lead to tumor escape.

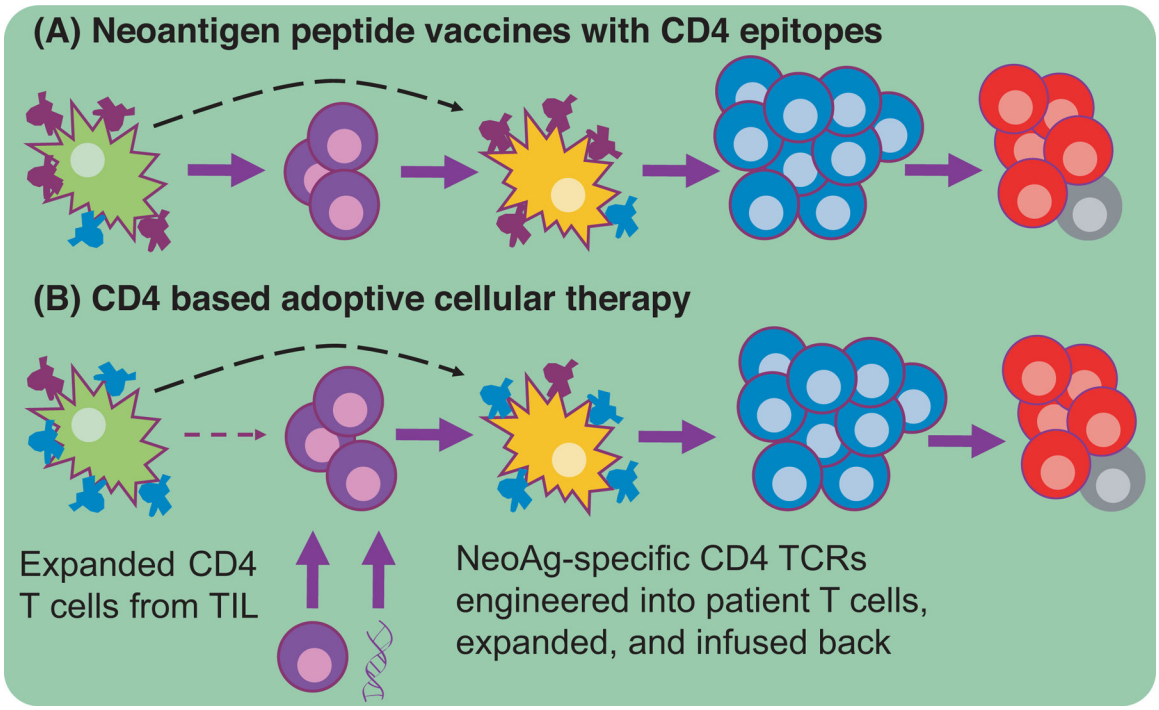

Figure 2. Help-centric therapeutic avenues.

A major issue with anti-tumor immunity is the paucity of NeoAgs available for cross-presentation. (A) Peptide vaccines containing CD4-NeoAg epitopes (purple) increase the amount of NeoAg migratory DCs carry to LNs where activated $CD4^+$ T cells will subsequently result in LN-resident DC conditioning, proper CTL priming, robust CTL expansion, and memory formation. (B) An alternative therapeutic modality functions via the expansion of $CD4^+$ T cells found in the TIL of a patient or the genetic introduction of NeoAg-specificity. Both ACT strategies circumscribe the need for migratory DC to carry NeoAgs to the LN. The expanded helper cells will presumably condition the resident DC and ultimately lead to polyfunctional CTLs.