Differentiation and Regulation of TH Cells: A Balancing Act for Cancer Immunotherapy

Amrita Basu1, Ganesan Ramamoorthi1, Gabriella Albert1, Corey Gallen1, Amber Beyer1, Colin Snyder1, Gary Koski2, Mary L. Disis3, Brian J. Czerniecki1,4,5 and Krithika Kodumudi1,2*

1 Clinical Science Division, Moffitt Cancer Center, Tampa, FL, United States, 2 Department of Biological Sciences, Kent State University, Kent, OH, United States, 3 UW Medicine Cancer Vaccine Institute, University of Washington, Seattle, WA, United States, 4 Department of Oncological Sciences, University of South Florida, Tampa, FL, United States, 5 Department of Breast Cancer Program, Moffitt Cancer Center, Tampa, FL, United States

Current success of immunotherapy in cancer has drawn attention to the subsets of TH cells in the tumor which are critical for activation of anti-tumor response either directly by themselves or by stimulating cytotoxic T cell activity. However, presence of immunosuppressive pro-tumorigenic TH subsets in the tumor milieu further contributes to the complexity of regulation of TH cell-mediated immune response. In this review, we present an overview of the multifaceted positive and negative effects of TH cells, with an emphasis on regulation of different TH cell subtypes by various immune cells, and how a delicate balance of contradictory signals can influence overall success of cancer immunotherapy. We focus on the regulatory network that encompasses dendritic cell-induced activation of CD4+ TH1 cells and subsequent priming of CD8+ cytotoxic T cells, along with intersecting anti-inflammatory and pro-tumorigenic TH2 cell activity. We further discuss how other tumor infiltrating immune cells such as immunostimulatory TH9 and Tfh cells, immunosuppressive Treg cells, and the duality of TH17 function contribute to tip the balance of anti- vs pro-tumorigenic TH responses in the tumor. We highlight the developing knowledge of CD4+ TH1 immune response against neoantigens/oncodrivers, impact of current immunotherapy strategies on CD4+ TH1 immunity, and how opposing action of TH cell subtypes can be explored further to amplify immunotherapy success in patients. Understanding the nuances of CD4+ TH cells regulation and the molecular framework undergirding the balancing act between anti-vs pro-tumorigenic TH subtypes is critical for rational designing of immunotherapies that can bypass therapeutic escape to maximize the potential of immunotherapy.

Keywords: T helper, CD4, neoantigen, tumor associated antigen, immunotherapy
**CD4⁺ T CELLS CLASSIFICATION**

As immunotherapy emerges as an effective therapeutic strategy in cancer, T helper (TH) cells have received widespread interest owing to their integral role in anti-tumor immune responses as has been demonstrated by diverse pre-clinical and clinical models (1, 2). While CD8⁺ cytotoxic T lymphocyte (CTL) function has been explored extensively in recent years in the context of immunotherapy (3), research shows the crucial role of CD4⁺ TH cells and its interaction with dendritic cells (DC) to transmit necessary molecular help that stimulates CTL function (4). TH1 and TH2 subclasses of helper T cells engage in molecular crosstalk with multiple immune signaling pathways and have been investigated for their immunotherapeutic relevance in cancer. Considering the multidimensional role of CD4⁺ TH cells, it is of utmost importance to understand the biology of these cells and how they contribute to tumor immune responses. Non-naïve CD4⁺ T cells are categorized as either effector or memory T cells. CD4⁺ memory T (TM) cells constitute a subpopulation of CD4⁺ T cells crucial in the immune system response against infections and non-infectious antigen exposure. Detailed mechanisms of differentiation and function of each TM cell subtype is not discussed in this review, since it has been extensively reviewed elsewhere (5, 6). TM cells are broadly subclassified into TRM (tissue-resident memory cells) which are thought to reside specifically in the area of previously infected tissue, while TCM (T central memory) and TEM (T effector memory) cells are found circulating in the blood (both subtypes), lymphoid organs (TCM cells) and peripheral organs (TEM cells) (7), and overall, has been shown to be critical for eliciting anti-tumor immune response (8).

**CD4⁺ T Cells in Cancer**

While distinct surface marker and functional profiles set TM cell subtypes apart, TRM cells have been crucial in anti-tumor immunity since a TRM cell signature in the tumor has been associated with favorable prognosis in terms of disease-free survival and overall survival in breast cancer, ovarian cancer, cervical cancer, melanoma, lung cancer, head and neck squamous cell carcinoma, gastric cancer, bladder cancer and pancreatic cancer (5). Along with the expression of CD103 (integrin-αE) and CD69 surface markers, expression of immune checkpoint regulator genes such as PD-1, CTLA-4, TIM-3 and LAG-1 on TRM cells obtained from solid tumors, clonal expansion of PD-1⁺TIM-3⁺ TRM cells with high expression of proliferation and cytotoxicity markers, and enrichment of this specific cell type in lung cancer patients responding to PD-1 antibody therapy (9) suggest TRM cells are a promising target for checkpoint inhibitor antibodies to offer therapeutic benefit in a myriad of solid tumor types.

In >300 patients with early stage triple negative breast cancer (TNBC), Savas et al. identified a gene signature of TRM cells (high expression of the integrin αEβ7 αE chain (CD103) and significantly lower expression of SELL, KLRG1, KLF2, S1PR1 and S1PR3 genes) by single cell sequencing that shows significant positive association with reduced risk of recurrence and overall survival (10). In TNBC patients receiving combination therapy of chemotherapy with immunotherapy, specifically pembrolizumab, and/or targeted therapy, TRM cell gene signature was associated with higher pathological complete response rate (pCR) in the I-SPY 2 neoadjuvant trials with 989 patients (11) and in the KEYNOTE-086 trial, in 200 patients with advanced-stage TNBC receiving pembrolizumab monotherapy (12–14). Compared to their CD8⁺ counterparts, CD4⁺ TM cells appear to be persistent and regulated separately, independent of previous antigen exposure or homeostatic mechanisms (15). In the context of cancer therapy, long-lasting response to tumor antigen is critical, hence the importance of developing immunotherapies that stimulate these responses via CD4⁺ TM cells.

**TH CELLS: FUNCTIONAL CLASSIFICATION**

Activated CD4⁺ T cells differentiate into several functional classes based on the cytokine milieu, antigen presentation, and expression of costimulatory molecules. Combinations of environmental stimuli and autocrine cytokine production lead to the induction of several signaling pathways to regulate the expression of lineage-specific transcription factors. CD4⁺ TH cells are polarized to one of several effector types defined by cytokine profiles and immune functions: TH1, TH2, TH17, TH9, Treg and Th0 (16–20). Here we discuss the differentiation and secretion profile of each TH cell subtypes (Figure 1), before delving deep into the molecular mechanism of signaling crosstalk between DC, CD4⁺ and CD8⁺ T cells and its therapeutic implication.

**TH1 Immune Response**

T helper type 1 (TH1) and type 2 (TH2) are the two predominant classes of CD4⁺ TH cells and were the first to be characterized by the production of interferon gamma (IFN-γ) and interleukin-4 (IL-4) cytokines, respectively (18). Specifically, generation of a TH1 effector subset is dependent on IL-12 and IFN-γ cytokines. IL-12 recruits natural killer (NK) cells to produce IFN-γ and together leads to activation of the signal transducer and activator of transcription-1 (STAT1) and STAT4 signaling pathways to induce the expression of the major transcription factor T-box expressed in T cells (T-bet), which drives TH1 differentiation by suppressing TH2/TH17 differentiation (17–20). Positive feedback regulation by IFN-γ secreted from these CD4⁺ TH1 cells support further TH1 differentiation (18, 19). The major cytokines and chemokines secreted from TH1 immune cells are the primary effector molecules downstream of immune cell signaling and will be discussed in detail later in this review.

**TH2 Immune Response**

Polarization to the TH2 effector lineage is dependent on production of IL-4, stimulating STAT6 signaling to upregulate the GATA3 transcription factor (18, 20). Similar to TH1 differentiation, a positive feedback loop is supported by autocrine IL-4 secreted from TH2 cells, while combined IL-6 production by antigen presenting cells and GATA3 expression suppresses TH1 differentiation (17–20). The balance between
IFN-γ and IL-4 feedback loops is critical to the balancing act between TH1 and TH2 CD4+ T cell immune responses.

TH2 differentiation has been shown to be dependent on IL-4 signaling via STAT6 signaling and transcriptional upregulation (21, 22). Once believed to solely derive from TH2 cells, IL-4 has since been known to be secreted by B cells, natural killer T cells (NKT), naïve CD4+ T cells and mast cells that can induce TH2 differentiation (23). Regulation of TH2 differentiation and cytokine profile has been comprehensively discussed previously (22, 24, 25). Binding of IL-4 to IL-4 receptors on immune cells leads to STAT6 phosphorylation, nuclear translocation, and expression of GATA3 transcription factor, resulting in TH2 cytokine secretion and eventual tumor growth and metastasis (26, 27). In studies ranging from lymphoma, melanoma, colorectal, breast, and lung cancer, STAT6 is overexpressed within the tumor microenvironment (TME) as an immunosuppressive signal to promote the function of M2 macrophages to assist in tumor growth and inflammation (28). To prevent dominance over each other, IL-12 expression from TH1 cells inhibits the differentiation of TH2 cells, while IL-4 inhibits TH1 differentiation (29).

Following differentiation, TH2 cells secrete IL-4, IL-5, IL-10, IL-13, and IL-17, not all of which are beneficial in cancer and have been shown to contribute to the tumor promoting role of this subtype. While IL-4, IL-5 and IL-13 have been documented to contribute to cancer growth and metastasis (21, 30, 31), a dual pro- and anti-tumorigenic role of IL-10 has been reported in recent literature, as reviewed elsewhere (32, 33). IL-10 elicits an anti-inflammatory immune response, downregulates TH1 cytokine function and MHC class II antigen presentation (29). Simultaneously, binding of IL-10 with its cognate receptor activates STAT3 signaling and transcription of anti-apoptosis and cell cycle progression genes that further strengthen the protumorigenic effect (34).

**TH9 Immune Response**

Expanding our view of CD4+ TH1 and TH2 cells, there are some less explored TH cell subsets which have unique potential in adaptive anti-tumor immunity. TH9 CD4+ T cells were once believed to be included within the TH2 subset, before being recognized as an individual population (35). Differentiation from naïve T cells to TH9 cells is facilitated by TGFβ and IL-4, mostly secreted from TH2 cells and these TH9 cells can stimulate uptake and presentation of antigens by DC for CD8+ T cell activation by secretion of IL-9 and signaling via CCL20-CCR6 axis (36, 37).

While the functional profile of TH9 cells appears like that of TH1 cells, TH9 cells were found to be less exhausted in the TME of lung carcinoma patients (38). This could offer a possible improvement to immunotherapy treatments if TH9 cell proliferation can be increased, driven by the secretion of CCL20-CCR6 and IL-3. In tumor models, activation of this CCL20-CCR6 axis by TH9 significantly drives DC generation and the proliferation of CD8+ T cells to attack cancer cells in the TME (35). IL-3 is also involved in the prevention of DC apoptosis, allowing prolonged CTL activation within draining lymph nodes (36). IL-21 secretion by TH9 has also been shown to...
increase during anti-tumor response, which stimulates IFN-γ production by CD4+ T cells and is also involved in NK cell activation (36). An adoptive cell therapy study comparing the effects of T_17, T_11, and T_9 cell transfer determined that T_9 can induce a powerful enough immune response to fully regress B16 melanoma tumors in C57BL/6 mice. T_9 cells outcompeted both T_11 and T_17 responses, both of which were able to temporarily regress the tumor, but eventually succumbed to tumor growth relapse (38).

T_9 effector differentiation results from STAT6 signaling to express the IRF-4 transcription factor through TGF-β and IL-4 cytokine production (17, 20). Significance of Notch1 developmental signaling to induce T_9 differentiation has been investigated in recent years. Notch1a activates the transcription factor SMAD3, which binds to the IL-9 cytokine promoter, and increases T_9 proliferation (35). The primary concern regarding T_9 function in the TME is the inconsistency within various tumor types. While the increase of the CCL20-CCR6 interaction is beneficial in antigen uptake by DC, the expression of CCL20 can also promote tumor cell migration as seen in a study involving lung carcinoma. IL-9 secretion can suppress immune cell response as well. However, a study found that the neutralization of secreted IL-9 limits the effect to only migration of the immune cells without affecting other immune functions (39).

As previously stated, T_11 and T_9 cells share many transcription factors involved in the differentiation of these subsets. While T_11 and T_9 both share the STAT6 signaling pathway, there are some other transcriptional differences that set them apart. For instance, PU.1, part of the EST family of transcription factors, is more highly expressed in T_9 cells compared with T_11 cells and is linked to targeted IL-9 secretion from T_9 cells, while constraining T_11 differentiation. On the same note, the IL-4R-STAT6-GATA3 axis in T_9 cells is functionally different than in T_11 cells. In T_9 cells, its role is to act on FoxP3 expression induced by TGF-β, while the same axis drives IL-4 expression from T_11 subsets (36). Therefore, despite shared transcription factors between the subsets of T_11 cells, each one has a distinct role to play in one subset that is separate from the other, leading to polarization and functional differences.

**T_17 Immune Response**

Following T_11 and T_12 effector classifications was the recognition of T_17 and regulatory T cells (T_reg), which differentiate through similar cytokine production profiles. T_17 lineage is characterized by the production of IL-17A-F, IL-21, IL-22, IL-10, IL-23, and CCL20. Polarization proceeds through three stages with TGF-β and IL-6 driving T_17 differentiation via STAT3 activation and expression of major transcription factor RORγt (18–20). Autocrine amplification by IL-21 production and secretion of IL-23 from antigen presenting cells (APC) stabilizes the T_17 lineage (19, 20). IRF-4 is also important for T_17 subtype induction, amplification and stabilization by IL-21 and subsequent IL-17 production (16). While TGF-β is important to T_17 differentiation, high concentrations of TGF-β can result in the activation of STAT5 signaling and upregulation of the FOXP3 transcription factor to drive T_reg differentiation (18, 20). T_17 immune cells display plasticity during immune response and induce immune regulatory functions (40), contributing to impaired immune functions by targeting granzyme B production, a dominant marker for cytolytic CD4+ activity (1, 41).

In the context of tumor immune response, T_17 cells can not only use these similarities to T_11 as an effector memory cell, but its stem-like properties can allow them to elicit immune response for a longer duration than T_11 cells, positing the question of further research into their future use in cellular immunotherapy (42, 43). During tumor development, T_17 promoting chemokines and cytokines are expressed within the TME, such as CCL4, CCL17, CCL22, IL-1β, IL-6, IL-23, and TGF-β (44). While T_17 exhibits anti-tumor immune responses, the increase of these promoters is driven by tumor-associated macrophages (TAM) within the tumor to assist with tumor growth. Evidence of this can be found in melanoma, breast, ovarian, hepatocellular, pancreatic, and renal cancers and can be attributed to the role of cytokine IL-17 in angiogenesis by increasing VEGF and IL-6 production and myeloid-derived suppressor cells (MDSC) production resulting in immunosuppression within the tumor (45).

**T Follicular Helper Cells**

T follicular helper cells (T_fh) are considered the fifth major lineage of CD4+ T cells and are involved in the generation of high-affinity antibody responses by supporting B cell proliferation and helping to facilitate immunoglobulin class switching (19). The production of IL-6 and IL-21 induces the expression of the Bcl-6 transcription factor through STAT3 signaling and leads to the polarization to a T_fh effector class (19, 20). In systemically untreated breast cancer patients, CD4+ T cells were found to be the principal component of the tumor infiltrating lymphocytes (TIL) and along with T_11, T_12 and T_17 subtypes, were also enriched for T_fh populations (46). Purified CD4+ T cells from a cohort of non-small-cell lung carcinoma (NSCLC) patients showed a T_fh signature associated with heightened CTL proliferation and adoptive transfer of T_fh cells in a murine tumor model augmented CTL function and inhibited tumor growth in vivo (47). Expression of ICOS and PD-1 as markers of activated T_fh cells in breast cancer has been reported while RNA analysis showed enhanced expression of IL-21, IFN-γ, and CXCL13 on sorted T_fh TIL, and only ICOS/PD-1+T_fh TIL from HER2+ and triple negative breast cancer were capable of inducing in vitro IgG secretion by B cells (46). Details of T_fh cells differentiation, signaling and functional profile has been reviewed comprehensively elsewhere (48, 49).

**Regulatory T Cells**

CD4+ regulatory T (T_reg) cells exhibit critical roles in maintaining self-tolerance and preventing various autoimmune diseases. In contrast, T_reg cells also play a detrimental role in promoting cancer progression via regulating immune surveillance and suppressing anti-tumor immune response (50). Elevated levels of T_reg cells is associated with disease progression and poor survival in patients with various types of
cancer (51, 52) as it is postulated that the reduced efficacy of various targeted therapies and immunotherapies is hindered by the activation of $T_{reg}$ cells. $T_{reg}$ cells has the ability to limit the function of antigen presenting cells by CTLA-4 dependent downregulation of CD80 and CD86 expressions, thereby evading tumor antigen presentation and tumor-specific T cell activation (53). In addition, the interlink between the expression of PD-1 on Treg cells was observed as a negative regulator, and showed preferential ability for TH2 polarization (63). The secretion in a calcineurin phosphatase-independent manner ionophore during DC maturation step antagonized IL-12 human PBMC-derived myeloid DC, presence of a calcium sensitization in T cells through an apparently calmodulin-activation in Treg cells utilizing IL-2/IL-2 receptor signaling is CD83+ and costimulatory molecule expression) and antigen presentation.

ROLE OF DC IN CD4$^+$ TH CELL DIFFERENTIATION AND FUNCTION

DC can be characterized as conventional (cDC) and plasmacytoid DC (pDC) where classical DC include all DC except pDC even though they are derived from the same origin (2, 23, 61). Our lab has previously shown calcium mobilization induces mature, activated DC phenotype acquisition (i.e. CD83+ and costimulatory molecule expression) and antigen sensitization in T cells through an apparently calmodulin-dependent mechanism in normal and transformed myeloid-derived cells (62). Our research group has also reported that in human PBMC-derived myeloid DC, presence of a calcium ionophore during DC maturation step antagonized IL-12 secretion in a calcineurin phosphatase-independent manner and showed preferential ability for $T_{h}2$ polarization (63). The inherent plasticity of DC further segregates these functional classes based on expression of surface receptors, secreted stimuli, and migratory capabilities. Plasmacytoid DC express surface markers including CD123, CD202, CD303 and CD304, which are absent from the surface of cDC, and function to monitor viral infections with capacity to secrete IFN-α and IFN-β (64, 65). Specifically, cDC are known for antigen presentation and classified as cDC1 and cDC2 based on functional activity, activation of adaptive T-cell response, and expression of MHC-I and MHC-II, respectively (2, 23, 61, 66). cDC1 express transcription factors IRF8, Btaf3, and Id2 in both human and mouse and exhibit CD141, CLEC9a, CADM1, BTLA, CD26, and XCR1 surface expression; while cDC2 polarization is driven by IRF4 and ZEB2 transcription factors and primarily express CD1c, CD11b, CD11c, CD2, FcγR1, SIRPA, and CLEC1I0A (64, 67, 68). As in humans, mice also demonstrate phenotypic distinction between cDC1 and cDC2 by CD8c+ (lymphoid) and CD103+ (migratory) expression on cDC1, and CD4+ (lymphoid) and CD11b+ (migratory) expression on cDC2 (65, 68, 69).

Role of DC in $T_{h}1$ and $T_{h}2$ Differentiation

Functionally, cDC1 are involved in antigen cross-presentation to stimulate CD8$^+$ T cell cytotoxicity, and additionally play a role in CD4$^+$ $T_{h}1$ differentiation and recruitment of NK cells through IL-12 production (23, 61). Expression of Notch ligand delta on DC upon LPS exposure has also been shown to stimulate $T_{h}1$ polarization, whereas exposure of DC to cAMP up regulators such as prostaglandin-E2 can direct CD4$^+$ T cells towards a $T_{h}2$ phenotype in an IL-4 independent manner via expression of notch ligand Jagged. Similarly, CD70 expressed on mouse DEC-205+ DC can act as a $T_{h}1$ phenotype inducer, independent of IL-12 (70). Conventional DC activating CD4$^+$ T cells is a controversial topic where recent studies have implicated cDC1 as being capable of activating CD4$^+$ T cell responses in cancer (2, 61, 64, 71, 72); however, previously it has been understood that cDC1 secrete lower levels of IL-12 in comparison to cDC2, and cDC2 are recognized as the predominant activators of CD4$^+$ T cell anti-tumor immunity (23, 64, 67, 72). This is in line with observations demonstrating that cDC2 are better equipped for CD4$^+$ T cell differentiation due to preferential expression of MHC-II (64, 72, 73). Additionally, past research has demonstrated the preferential activation of CD8$^+$ cytotoxic T cells by cDC1 through studies with Batf3-deficient mice unable to reject highly immunogenic tumor cell lines (74), while cDC2 have the capacity to stimulate CD4$^+$ T cell differentiation and polarization into $T_{h}1$, $T_{h}2$, and $T_{h}17$ effector populations via production of an array of cytokines such as IL-23, IL-1, TNF-α, IL-6, and IL-10, cytokines (64, 67).

Role of DC in $T_{h}9$ Differentiation

Dectin-1 is a β-glucan receptor present on DC, macrophages and neutrophils and dectin-1-activated DC have been shown to secrete IL-6, IL-12p40 and TNF-α, leading to $T_{h}1$ and $T_{h}17$ polarization (75). However, Zhao et al. have described dectin-1-activated DC promote a potent $T_{h}9$ polarization in vitro by overexpression of TNFSF15 and OX40L via Syk, Ral1 and NF-κB signaling pathways. While they observed a significant increase in IL-9 and $T_{h}9$-associated transcription factor IRF4, no changes were observed for other $T_{h}$ subtype-associated cytokines and transcription factors in vivo. Anti-tumor effects of Dectin-1-activated DC in melanoma and myeloma preclinical model relied heavily on this $T_{h}9$/IL-9 response while microarray analysis identified more than 40 cytokines, chemokines and costimulatory molecules such as TNF-a, TNFSF15, OX40L, TNFSF8 and low IL-12 (76, 77).
Role of DC in T\(_H\)17 Differentiation

Ability to produce cytokines IL-6, TGF-\(\beta\), IL-1b and IL-23 support a critical role of DC in polarizing T\(_H\)17 phenotype, as observed ex vivo in human DC isolated from inflammatory fluids, equivalent to monocyte-derived DC in mice. Likewise, monocyte-derived DC cultured in vitro with lymphoid tissue-resident bacteria secrete T\(_H\)17-polarizing cytokines (78). In an experimental allergic encephalitis mouse model, CD11b\(^+\) myeloid DC in the central nervous system produce IL-23, TGF-\(\beta\) and IL-6, thereby inducing T\(_H\)17 cells. Similarly, stimulation of human monocyte-derived DC with intact E. coli and ATP stimulates IL-23 secretion that further activates IL-17 producing T\(_H\)17 cells (70). Significance of DC differentiation and antigen exposure on T\(_H\)17 cell polarization has been further highlighted in a study by Khayrullina et al. as DC differentiated in presence of prostaglandin E2 promotes an IL-23 balance and inhibition of T\(_H\)1/T\(_H\)2 polarization, both in vitro and in vivo.

Role of DC in T\(_f\)h Differentiation

Co-operation between DC and B cells induces and ensures differentiation into T\(_f\)h phenotype and lymph node-resident cDC2s in van Gogh mouse model has been shown to be sufficient for such T\(_f\)h priming. The unique localization of cDC2 in the interfollicular zone at the T cell-B cell border makes them ideally positioned to be the dominant T\(_f\)h-priming DC subset in both human and mouse, which is also consistent with preferential antigen presentation on MHC-II by cDC2s and stronger antigenic stimulation favoring T\(_f\)h cell differentiation. Mice deficient in cDC2 (Cd11cCre Irf4\(^{-/-}\) and Cd47\(^{-/-}\)), but not cDC1 (Batf3\(^{-/-}\)), demonstrate impaired T\(_f\)h responses to sheep RBCs and loss of DC in the T cell-B cell border leads to loss of T\(_f\)h polarization as well. However, cDC2 is not the sole determinant of the T\(_f\)h phenotype as a specific subset of cDC2 dependent on transcription factor krueppel-like factor 4 (KLF4) and expressing transcription factor factor 3 (KLF4) and expressing CD301b can induce only T\(_H\)2 but not T\(_f\)h polarization, highlighting the diversity of T cell fate determinants, that also includes recruitment of various cytokines such as IL-6, IL-12 and IL-21 not secreted by cDC2s (61, 79).

The role of DC to selectively develop adaptive regulatory T cells has been highlighted as an inducer of peripheral tolerance. Simultaneously, negative regulation by IL-10 resulting in downregulation of MHC-II expression, IL-12 secretion and maturation of DC leads to an indirect preference for immune tolerance, and can induce regulatory DC that promote an IL-10-producing T\(_{reg}\) phenotype (80).

Studies have noted the contribution of antigenic density in determining CD4\(^+\) T cell fate where higher antigen doses are associated with T\(_H\)1 differentiation in contrast to lower antigen doses leading to T\(_H\)2 differentiation (23, 61). Overall, antigenic stimulation combined with simultaneous interactions between costimulatory molecules and cytokine stimuli produced by DC induce downstream signaling pathways that lead to T cell effector differentiation as discussed above. As tumor cells more readily express MHC-I molecules, DC play a pivotal role in the activation and priming of CD4\(^+\) T cells to initiate the CD4\(^+\) anti-tumor response.

Molecular ‘HELP’ by CD4\(^+\) T\(_H\) Cells Are Necessary for Cytotoxic Function of CD8\(^+\) T Cells

Cytotoxic and memory CD8\(^+\) T cell response as a principal component of immunity requires priming and expansion, both of which demand active help by CD4\(^+\) T cells. Even though the supporting role of CD4\(^+\) T\(_H\) cells to promote effector and memory function of CD8\(^+\) T cells have been well-established by late 1990s, recent research have generated crucial supporting evidence of the necessity of CD4\(^+\) T\(_H\) cells for anti-tumor CD8\(^+\) T cell function (81, 82). Neoantigen-specific vaccination has often showed largely CD4\(^+\) T cell response, and not CD8\(^+\) T cell response, in multiple pre-clinical models and clinical trials. In MMTV-PyMT spontaneous mammary carcinoma model, a unique T\(_H\)1 CD4\(^+\) subset was identified in non-tumor peripheral tissues that rendered protective benefit when transferred into treatment-naïve tumor hosts challenged with 4T1 tumors (83). In an aggressive B16F10 murine melanoma model, IL-21 secretion stimulated by CD4\(^+\) T\(_H\) cells drives CD8\(^+\) T cell differentiation towards CX3CR1\(^+\) cytotoxic effector phenotype and anti-tumor activity (84). T\(_H\)1 polarized CD4\(^+\) T cells offer long-term protection against tumor re-challenge and is required for response to immune checkpoint blockade therapy in a T3 murine sarcoma model (85). Based on their study with melanoma patients who showed prevalence of CD4\(^+\) neoantigen-reactive T cells, Ott et al. suggested two mechanisms underlying this unexpected dominance of CD4\(^+\) over CD8\(^+\) T cells, namely: 1) more efficient priming of CD4\(^+\) T cells compared to CD8\(^+\) T cells due to restriction of cross-presentation and 2) relatively higher promiscuity of MHC Class II epitopes owing to relaxed binding requirements, unlike MHC Class I epitopes (86).

Role of DC to Relay CD4\(^+\) ‘HELP’ to CD8\(^+\) T Cells

Priming of CD8\(^+\) T cells for effector function requires antigen cross-presentation with help from CD4\(^+\) T cells. The primary mechanism is via ‘licensing’ of DC that allows cross-presentation, essential for two-step priming of CD8\(^+\) T cells (2). To understand the spatiotemporal distribution and activation of CD4\(^+\) vs CD8\(^+\) T cells, in vivo imaging has demonstrated that after immunization, in the first priming step, CD4\(^+\) and CD8\(^+\) T cells encounter antigen in an independent and non-synchronous manner, presented by different subsets of cDC. Interaction between CD40 costimulatory protein on cDC and its cognate ligand CD40L (CD154) on CD4\(^+\) T cells is the key step in the licensing process that enhances antigen presentation on DC and allows direct interaction with CD8\(^+\) T cells.

The second step of priming these licensed type 1 cDC (cDC1) acts as a common platform where both CD4\(^+\) and CD8\(^+\) T cells
encounter the same cDC1. XC-chemokine ligand 1 produced by CD8+ T cells recruits resident XC-chemokine receptor XCR1+ cDC in a prime position for receiving cross antigen presentation and thus, molecular help from CD4+ T cells is delivered to CD8+ T cells (2). Ahrends et al. demonstrated by RNAseq in ‘helped’ vs ‘non-helped’ CD8+ T cells that there is a differential expression of a multitude of genes associated with lymphocyte activation, differentiation, cell motility, and migration. Significantly enhanced mRNA and protein expression of cytotoxic effector molecules such as TNF-α, IFN-γ, FASL and granzyme B, as well as IL-2 and its receptor CD25, are regulators of CTL survival and memory. They also reported high levels of co-inhibitory immune receptors, e.g. PD-1, lymphocyte activate gene 3 (LAG3) and B and T lymphocyte attenuator (BTLA) on ‘helpless’ CTLs, rendering them unable to kill tumor cells even though they are able to exit the lymph node and enter circulation (87). These helpless T cells subsequently undergo activation-induced cell death due to TRAIL expression upon re-stimulation (88). In therapeutic pre-clinical models, vaccination with short MHC class I binding peptides hinders CTL priming and induce tolerance, whereas combination with CD40 agonist antibody infusion or DC stimulated in vitro with antigen-specific CD4+ T cell resulted in CTL-based anti-tumor immune response (2).

Secretion of CCL3 and CCL4 from the licensed DC guide the naïve CD8+ T cells to the site of antigen specific DC-CD4+ T cell interaction, that allows rapid interaction with the antigen presenting cDC1 even with a low frequency of both immune cell subtypes (89). CD4+ T cells also stimulate clonal expansion of antigen-specific CD8+ T cells and IFN-γ secretion, whereas ‘helpless’ memory CTLs primed without help from CD4+ T cells show deficiency in secondary expansion (90, 91). CD4+ T cells are a major source of IL-2, a key molecular help that is critical for imprinting the secondary responsiveness on CD8+ T cells. IL-15 is secreted from licensed DC and is considered to be necessary for imprinting secondary responsiveness even in absence of CD4+ T cells (92).

CD4+ Help in CD8+ T Cell Differentiation and Memory Function

Recent research has highlighted that the impact of CD4+ T cell help reflects on enhanced recruitment, proliferation, and effector function of CD8+ T cells intratumorally. In a murine tumor model, IL-2 secreted from tumor-resident CD4+ T cells increased CD8+ T cell proliferation and granzyme B expression (93). Poor survival and clonal expansion of CD8+ T cells in absence of CD4+ help has been reported, and the help was necessary for survival of memory T cells during recall expansion (81). During clonal expansion and differentiation of T cells into short-lived effector or persistent memory phenotypes, CD4+ T cells help in intrinsic function of CD8+ T cells by altering gene expression profile. The transcriptomic analysis by Ahrends et al. also showed CD4+ T cell help induces transcription factors and epigenetic modulators, such as T-BET, eomesdermin homologue, and inhibitor of DNA binding 3, in a preventive model that received vaccines encoding MHC class I vs MHC class II-restricted epitope-expressing HPV E7 protein. Elevated expression of CXCR4 and CX3CR1 chemokine receptors and matrix metalloprotease proteins on ‘helped’ CTLs augment their extravasation and infiltration into the tumor (87). Another study using a similar mouse model showed CD4+ ‘help’ has been shown to impact transcriptional landscape to support formation and maintenance of CD8+ effector and central memory phenotypes, and recall response in these memory T cells were help-independent (94). Defective recall response mounted by CD8+ memory T cells from a CD4−/− mouse host indicated necessity of CD4+ help for CD8+ T cell functionality previously (91), and a recent study using an Influenza A virus infection model showed CD4+ T cell help promotes metabolic programming of CD8+ T cells to benefit recall response as well (95).

Molecular Nature of the ‘HELP’ Signal

Cytokine Signals

The key cytokine signals that deliver CD4+ T cell help to CD8+ T cells are IFN-γ and IL-12 secreted from conventional and CD40-stimulated DC, respectively. It appears contribution of these two cytokines may work in a partially redundant manner, as they both promoted survival and differentiation of effector and memory CTLs by increased expression of transcriptional regulators in a mouse model (96). CD4+ T cells are a major source of IL-2, a key molecular signal that is critical for imprinting the secondary responsiveness on CD8+ T cells. IL-2 induces NAB2 protein expression by CD8+ T cells, inhibits TRAIL expression and promotes expansion (97). Simultaneously, IL-12p70 from licensed DC also upregulates IL-2Rα/CD25 expression on CD8+ T cells and therefore, enhances their responsivity to IL-2 (98). Our group reported a novel function of IL-12 to enhance recognition of tumor by T cells along with 10- to 100-fold increases in peptide sensitivity and functional avidity (99). As reviewed by Kalia and Sarkar, IL-2 promotes differentiation into effector phenotype and contributes to the development and maintenance of short-lived effector responses by interaction with CD25 receptor (100). IL-15 is secreted from licensed DC and is considered to be necessary for imprinting secondary responsiveness even in absence of CD4+ T cells (92).

Co-Stimulatory Signals

Along with cytokines, costimulatory signaling between ligands and receptors expressed on DC, CD4+ and CD8+ T cells relay and implement CD4+ T cell help for T cell priming and effector function. Upregulated CD40L on CD4+ T cells interacts with its cognate receptor CD40 on DC and is the first step in relaying molecular help (4, 101, 102). Similarity of the cytokine profiles between CD4+ T cells and CD8+ T cells expressing CD40L has been reported and can potentially augment licensing of DC to enhance antigen cross-priming (103). CD70/CD27 costimulatory signaling has been reported to be the key mechanism to deliver CD4+ T cell help from DC to CD8+ T cells, and contribute to their clonal expansion and differentiation into effector and memory CTL in cancer and viral infections (104, 105). In a murine lung tumor model, CD27 agonism combined with anti-PD1 antibody treatment eradicated tumors and recapitulated CD4+ T cell help when vaccinated without helper epitopes (106), even though CD70/CD27 interaction alone may not stimulate sufficient CTL response and a combined, non-redundant role of CD27 and CD28 may
Contribute to the help. CD40-CD40L interaction stimulates CD80 and CD86 costimulatory molecule expression on DC and its subsequent binding with the CD28 costimulatory receptor on CD8+ T cells can deliver the CD4+ T cell help required for CTL activity, as observed in recent pre-clinical and clinical studies including anti-PD-1 and other immune checkpoint inhibitors (107, 108).

Opposing Action of Anti- and Pro-Tumorigenic CD4+ TH Cells in Cancer

Research in past decades have revealed the critical and opposing role of TH1 and TH2 cells in determining the fate of intratumoral immune response, including therapies targeting oncodrivers and neoantigens. As shown in Figure 2, the regulatory network is multi-faceted and is governed by a range of cytokines and chemokines secreted from different TH subtypes and hence, need to be coordinated in a balancing act for optimum efficiency of immunotherapy. We discuss the most well-known cytokines and chemokines secreted primarily from TH1 and TH2 cells that confer their anti- and pro-tumorigenic effects, respectively, to understand the mechanism of their opposing actions. The cytokines and chemokines secreted from the other TH subsets have been summarized in Table 1.

Anti-Tumorigenic TH Cytokines

Interferon-γ (IFN-γ)

CD4+ TH1 effector cells are characterized by the production of dominant cytokines IFN-γ, TNF-α and IL-2. IFN-γ is a pleotropic cytokine and an important player in anti-tumor immunity with the ability to directly mediate tumor rejection as well as recruit and activate both innate and adaptive immune cells in the TME (109–112). The direct tumoricidal effects of IFN-γ result in cell death signaling and inhibition of angiogenesis. Increased expression of cell cycle regulators p21 and p27 induced by IFN-γ leads to cell cycle arrest, cell dormancy, and apoptosis in tumor cells via signaling pathways that induce the expression of tumor suppressor gene IRF-1, leading to caspase activation and apoptosis (109, 112, 113). Activation of the anti-proliferative STAT1 pathway by IFN-γ can lead to sensitization of tumor cells to FAS (CD95) and TRAIL resulting in apoptosis, and hindered tumor cell growth by inhibiting angiogenesis to induce a state of cellular dormancy (17, 109, 111–113). Our lab has recently elucidated a novel mechanism of IFN-γ action via ubiquitin proteasomal degradation pathway, mediated by zinc RING finger E3 ubiquitin ligase cullin-5, to facilitate proteasomal degradation of HER2 membrane receptor and improve response in HER2+ breast cancer in vitro and in vivo (114).

In the TME, IFN-γ enhances the immunogenicity of tumor cells by upregulating MHC class I and II expression to make them more susceptible to immune recognition (109, 111, 112) and influences the stromal cells in the TME including macrophages, myeloid-derived suppressor cells (MDSC), and DC (109). IFN-γ production leads to enhanced proinflammatory functions and tumoricidal activity of type I macrophages (M1) by increasing nitric oxide production and upregulates expression of MHC and costimulatory molecules on DC. Anti-tumor immune response by IFN-γ can also be elicited
by recruitment of additional effector cells, namely NK cells and M1 macrophages to the TME, facilitating T-cell homing through CXCL9 and CXCL10 chemokines, and via enhanced CD8+ cytotoxicity in the TME (109, 111, 112).

Interleukin 2 (IL-2)
IL-2 plays a crucial role in driving T and NK cell proliferation and activation and in regulating their effector functions, such as cytolytic activity and cytokine production. IL-2 binds to IL-2 receptor (IL-2R), composed of three subunits: IL-2Rα (CD122), IL-2Rβ (CD122), and IL-2Rγ (CD132) (115, 116). The heterotrimetric complex of IL-2αβγ is essential to regulate T cell expansion, are expressed on regulatory T cells, and binds IL-2 with the highest affinity, while T cells and NK cells express only the receptor dimer IL-2αβγ (115–118). IL-2 is produced primarily by activated CD4+ T cells after antigen exposure, binds to its cognate receptors and drives differentiation to CD4+ Treg immunosuppressive population that leads to immune tolerance. Research in the last decade has identified the role of IL-2 in promoting both Th1 and Th2 differentiation, and inhibiting Th9 and Th17 development (Liao et al., 2013). IL-2 induced activation of AKT and mTORC1 signaling pathways have been shown to steer the differentiation preference towards Th1 cells and away from Th2 subtypes (119). Binding of IL-2 to these receptor complexes induces signal transduction through three proliferative pathways: JAK/STAT, PI3K/AKT, and MAPK (115, 117, 118). Additionally, the positive feedback from CD4+ Th1 produced IL-2 plays a crucial role in driving T cell effector differentiation and in the recruitment of activated cytotoxic NK and CD8+ T cells to the TME (116). While IL-2 was the first FDA approved immunotherapy for metastatic melanoma and metastatic renal cancer, the dual functionality of IL-2 has been approved immunotherapy for metastatic melanoma and hepatocellular cancer (111, 120). Similar to the double-edged sword of IFN-γ, TNF-α has shown a dual tumor suppressing and tumor promoting role dependent on concentration and localization of the soluble cytokine. As TNFR1 is ubiquitously expressed on tumors as well as healthy endothelial cells and blood vessels, chronic exposure to TNF-α can cause non-specific tissue damage and has been linked to hemorrhagic necrosis (111). Additionally, TNFR2 is expressed primarily on immune cells including Threg and MDSC (111, 120), where acute production of TNF-α is associated with Threg expansion and increased infiltration of Threg and MDSC populations in the TME, leading to tumor progression and decreased efficacy of immunotherapies (111, 121). Administration of even a low dose of TNF-α has shown increased expression of immunosuppressive molecules PDL-1 and TIM-3 on TIL and activate cell death pathways in tumor-infiltrating CD8+ CTL (121). Studies have shown that administration of TNF-α as an immunotherapy has resulted in high levels of toxicity, but localized delivery in isolated limb perfusion showed anti-tumor abilities in soft tissue sarcomas, melanoma, and hepatocellular carcinoma (111, 120).

| TABLE 1 | Role of cytokines and chemokines in Th cell differentiation. |
| --- | --- |
| **Th1** | **Th2** | **Th9** | **Th17** | **ThN** |
| **Differentiation Factors** | IL-2, IL-12, IFN-γ, IFN-α | GATA3, IL-4, IL-6 | GATA3, IL-6, PU.1, TGF-β | TGF-β, IL-6, RORγt | BO6, ICOS, IL-6, IL-21, STAT3 |
| **Secreted Cytokines** | IL-1β, IL-2, IL-12, TNF-α, IFN-γ | IL-4, IL-5, IL-10, TGF-β | IL-9, IL-3, IL-21 | IL-17A, IL-17F, IL-21, IL-22, IL-23 |
| **Chemokines and cognate receptors** | CXC5R3, CCR5, and CCR7, CXCL9, CXCL10, CXCL11, IL-4 | CXC5R3, CCR4, and CCR8, MDC, TCA3, TARG | CCL20, CCR6, CCL4, CCL17, CCL22 | IFN-γ/IFN-1 | IL-12, IFN-γ, IL-2 |
| **Classical Negative** | IL-4 | IFN-β, IFN-α, TGF-β | IL-12 | IL-2 |
| **Regulatory Cytokines** | IL-10, IL-23, IL-27, IL-31 | IL-2, IL-15, IL-21, IL-22, IL-23 | IL-10, IL-27 | IL-15, IL-21, IL-22, IL-23 |
| **Significant Downstream Signaling Pathways** | IFN-α, IFN-β, IL-12, IL-18, IL-23 | GSK3β, AKT, MAPK, JAK/STAT | GSK3β, AKT, MAPK, JAK/STAT | GSK3β, AKT, MAPK, JAK/STAT | GSK3β, AKT, MAPK, JAK/STAT |

**Anti-Tumorigenic Th Chemokines**
In addition to Th1 cytokines, the production of related chemokines has direct implications in shaping the immune landscape and TME of various cancer types. CD4+ T cell IFN-γ-inducible chemokines CXCL9, CXCL10, and CXCL11 recruit effector T cells to the TME, direct tumor infiltration, and are key players in T cell homing (122–125). CXCL9-11 bind to their cognate chemokine receptors CXCR3, which is expressed on cytotoxic CD8+ T cells, NK cells, and CD4+ Th1 cells (123, 124). Upregulation of CXCR3 on activated CD4+ T cells is associated with optimal production of IFN-γ and a Th1 effector phenotype (122). Additionally, CD40/CD40L signaling increases expression of CXCL10 and has been implicated in licensing DC and supporting the interactions of DC and naïve T cells in lymphoid organs (122, 126). CCL3 and CCL4 chemokines are released after interaction of DC with antigen specific CD4+ T cells and act as a chemoattractant for CCR5+ naïve CD8+ T cells for activation (89). Interaction of CCL19 and CCL21 with CCR7 receptor recruits Treg, CD4+ Th1, TCM, and monocyte-derived dendritic cells (mDC) to the TME. Upregulation of CXCR3 and CXCR5 chemokine receptors has been correlated with Th1 differentiation while Th2 cells express pro-apoptotic signaling via MAPK and NFκB activation (111, 113, 120).
CCR4 receptors, induced by IL-4, to bind CCL17 and CCL22 chemokines (125). Simultaneously, CXCL9 and CXCL10 have been shown to increase levels of tumor infiltrating CD8+ effector T cells and NK cells, minimize metastasis, and are correlated with improved responses to checkpoint blockade and adoptive cell transfer therapies (124, 125).

**ANTI- VS PRO-TUMOR TH IMMUNE RESPONSE IN CANCER: MOLECULAR MECHANISM OF OPPOSING ACTIONS**

TH1 and TH2 cells are often discussed in tandem in relation to cancer and tumor immune response, as TH1/TH2 balance, regulated by the factors summarized in Table 1, is paramount in tumor-specific immune response versus pro-tumor immune regulation. Typically, a shift in favor of TH1 response results in dissipated TH2 response and vice versa, resulting in either anti- or pro-tumorigenic consequences, respectively, and this shift is typically accomplished by antagonistic interaction of the cytokines produced by the TH cells themselves (127). Depending on the TME and other external signals, the initial shift to either TH1 or TH2 can then become a positive feedback loop that continues to favor the specific TH immune response (40).

TH1 and TH2 cells and their related cytokines have been studied in multiple cancer types and proved to play a pivotal role in prognosis, tumor fate, and patient disease-free survival. In one such study of patients with hepatocellular carcinoma, an increase in detectable IL-6 in whole blood after treatment with transarterial chemoembolization corresponded with a poorer prognosis and decreased overall survival rate. In the same study, a higher IFN-γ/IL-10 ratio increased overall survival rates, as did a higher IL-1/IL-10 ratio (128). In a similar study analyzing serum levels of cytokines in patients with invasive uterine cervical cancer, TH2, TH17, and Treg cells were increased in peripheral blood mononuclear cells (PBMC) with a concurrent increase in their related cytokines IL-4, IL-10, IL-17, IL-23, and TGF-β (129). In a study using The Cancer Genome Atlas (TCGA) looking at glioblastoma multiforme, a low TH1/TH2 balance correlated with better overall survival (130). These studies exemplify the importance of maintaining a TH1-high/TH2-low balance and the ability to use relative TH cell prevalence and their related cytokine levels as a predictor of patient survival. However, incidence of cancer is not necessarily indicative of a pro-tumor high ratio of TH2 over TH1, as shown in a study looking at patients with ovarian cancer before receiving treatment. When TH2 and TH1 cytokine levels in the serum and cancer tissues were compared, TH1 cytokines IL-2, TNF-α, IFN-γ, and IL-13 were significantly increased in patients compared to healthy controls. Additionally, IFN-γ/IL-4 and TNF-α/IL-4 ratios were significantly higher in cancer patients (131).

It has been previously shown that elevated TH2 cytokines (IL-4, IL-10) and decreased TH1 cytokines (IFN-γ, IL-2 and IL-12) correlate with poorer prognoses in breast cancer patients than those with elevated TH1 and suppressed TH2 cytokines. A recent study showed that alteration in TH1/TH2 cytokines can correlate with different subclasses of breast cancer as well. Shift in the TH1/TH2 balance towards a higher ratio of TH1/TH2 cytokines resulting in increased IL-4, IL-5, and IL-10 has been reported in TNBC. On the other hand, ER+ and luminal-like breast cancers were found to have lower TH2 cytokines and a general shift towards TH1 immune response. In the context of disease prognosis, such TH1/TH2 distribution is reflected in a better overall survival rate and prognosis in ER+ and luminal-like breast cancers (BC), and a worse prognosis in TNBC (132). TH1/TH2 balance in normal and cancer-associated immune response has been reviewed in general extensively elsewhere (29, 133). With respect to the cancer milieu, treatments that ensure a shift towards the anti-tumor TH1 response are essential, while maintaining a low TH2 response is critical to ensure a tumor-specific immune response is maintained.

In the context of pro-tumorigenic immune response in the TME, TH2 response has been viewed as controversial, due to their possible role in tumorigenesis, along with another CD4+ TH cell subset, TH17 cells. TH2 cells are responsible for the increase in population of tumor infiltrating M2 macrophages and eosinophils in the TME, via their expression of IL-5 and IL-13, which regulate TGF-β secretion and immunosuppressive responses (28). TH2-induced tumorigenesis is further driven by their expression of IL-7, which can act as a pro-angiogenic factor, resulting in leaky vasculature and allowing the tumor microenvironment to expand and migratory tumor cells to enter the surrounding tissue (134). A study involving luminal breast cancer found that the presence of chemokine receptor CCR5 activates TH2 differentiation, and the TH2 cells in turn, help increase MDCS production within the TME, a characteristic feature shared by TH17 cells as well but implemented via IL-17 secretion. A large enough population of MDCSmigrates to the edge of the tumor in order to prevent TIL from entering the tumor region and can severely diminish the immune response, allowing the tumor to thrive (135). A comprehensive understanding of how these TH cells induce pro-tumorigenic immune response requires further research, to identify efficient strategies to repress the immunosuppressive populations and expand therapeutic benefit of TH cell-based immunotherapy in cancer.

**REGULATION OF B CELLS BY CD4+ T CELLS: A BIDIRECTIONAL SYSTEM**

Discovery of tumor infiltrating B cells (TIB) and tertiary lymphoid structures have reinforced interest in studying the significance of TIB subtypes and success of immunotherapy in cancer. Such studies have identified a dual role of TIB subtypes in stimulating or dampening of anti-tumor immune response, orchestrated by secreted antibodies, cytokines and chemokines, B cell receptor signaling, and interaction with T cells. CD4+ T cells act in a bidirectional regulatory network with B cells to induce differentiation of B cells which in turn, stimulates CD4+
T_{H}1 and T_{H}2 differentiation, suggesting the clinical significance of these immune cells for anti-tumor response. Similar to the conventional APC, B cells express MHC class II molecules on their surface and are, hence, capable of antigen presentation to CD4+ T cells for activation, and cognate interaction between T and B cells induce differentiation of anti-tumor T_{H}2 cells (49, 136). Activated B cells secrete chemokines and costimulatory factors such as CCL2, CXCR4, CCL5, CXCL5, and CXCL10 to induce CD4+ and CD8+ T cell activation. Using B cell deficient and IFN-γ-knockout mice along with other transgenic models of immune cell function and CD4+ and CD8+ depletion studies, Park et al. showed that anti-HER2/neu antibodies are necessary and sufficient for protection from tumor challenge, a temporary necessity for CD4+ T cells for 36-48h after immunization to provide help for B cells, and no requirement for CD8+ T cells at all. While tumor growth in immunized B cell-deficient mice was comparable to controls and showed no detectable antibodies in their serum, treatment of mice with anti-HER2 serum prevented tumor growth in vivo as effectively as adenoviral vaccination, supporting the necessity and sufficiency of antibodies for anti-tumor protection (137). In a later study, Berzofsky’s group demonstrated that even in a large and well-established subcutaneous TUBO tumor model (tumor size >2cm), vaccination with a recombinant adenovirus expressing a truncated ErbB2 antigen cured primary tumors and distant lung metastases in mice by antibody-mediated blockade of HER2/neu activity, in an Fc receptor-independent mechanism. Adoptive transfer of serum from vaccinated BALB/c mice to TUBO tumor-bearing mice resulted in significantly delayed tumor growth and showed considerable presence of anti-HER2/neu antibodies which were not observed upon deletion of CD4+ T cells (138), reinforcing the therapeutic benefits of anti-oncodriver antibodies and significance of CD4+ T cells.

On the other hand, inhibition of CTL activity in tumors by B cells can be associated with a subset of B regulatory cells (B_{reg}), that contribute to immunosuppression in the TME. B_{reg} inhibit proliferation of CD4+ T_{H}1 cells by secretion of suppressive cytokines, such as IL-10 and TGF-β, and promote conversion of CD4+CD25+ T cells to CD4+CD25-FoxP3+ T_{reg} with high expression of CTLA-4 and FoxP3, and the anti-tumor effects of B cell deficiency has been shown to be mediated by enhanced T cell and NK cell infiltration, vigorous T_{H}1 and CTL activity and reduced T_{reg} proliferation (139). These studies underline the relevance of B cell mediated antibody production and CD4+ T cell activation in anti-tumor immune response to encourage further research in understanding the complete therapeutic potential of B and T cell interaction.

**Oncodriver-Specific T_{H}1 Immune Response in Breast Cancer: HER2-DC1 Vaccine**

Interaction between oncodrivers and immune response has been documented in HER2+ BC, where trastuzumab induces antibody-dependent cell-mediated cytotoxicity (ADCC) by facilitating cross-link between tumor antigen with its antigen-binding fragment and recruitment of effector cells by interaction with the Fc region (fragment crystallizable region), resulting in cytokine release and cytotoxic cell death (156). Susceptibility to ADCC correlates with infiltration of CD16 and CD56-expressing lymphocytes in the tumor, suggesting recruitment of NK cells (157). Trastuzumab has also been shown to stimulate HER2 uptake by DC for enhanced antigen presentation and activation of antigen-specific T cells (158). Higher levels of chemokines, infiltration of T cells and monocytes, and PD-1 expression has been documented on trastuzumab sensitive breast tumors, compared to non-responding tumors (159). Our lab has reported a gradual and progressive loss of HER2-specific CD4+ T_{H}1 immune response in peripheral blood in HER2+ BC patients (160). Restoration of this T_{H}1 immune response with neoadjuvant HER2 peptide-pulsed type I DC (HER2-DC1) vaccination resulted in pathologic complete response in 30% of HER2+ DCIS patients in a randomized trial (161). Co-operation
between CD4+ T \(_{\text{H}1}\) cytokines IFN-\(\gamma\)/TNF-\(\alpha\) and trastuzumab has been shown to be necessary for restoration of class I MHC molecule expression on HER2\(^{\text{high}}\) cells, critical for recognition and lysis of the cells by HER2-specific CD8+ T cells in these patients (162). In HER2+ IBC completely treated with trastuzumab and chemotherapy, anti-HER2 T \(_{\text{H}1}\) immune responsivity independently correlates with disease recurrence and is mediated by anti-HER2 CD4+ T-bet\(^+\)IFN-\(\gamma\) (T \(_{\text{H}1}\)) phenotypes but not CD4+ GATA-3\(^+\)IFN-\(\gamma\) (T \(_{\text{H}2}\)) or CD4+CD25\(^+\)FoxP3\(^+\) (T \(_{\text{reg}}\)) response (163). At the cellular level, T \(_{\text{H}1}\) cytokine treatment up-regulated apoptosis and senescence in HER2+ BC cells (164), suggesting molecular communication between immune and oncodriver signaling. In a pre-clinical model of HER2+ BC, sequential anti-PD1 antibody treatment with murine HER2-DC1 vaccination significantly improves mouse survival and supports an essential role of CD4+ T \(_{\text{H}1}\) immune response for the observed effect (165). Therapeutic success of HER2-DC1 vaccination in HER2+ BC supports the notion that targeting other oncodrivers employing the DC vaccine platform can have far-reaching beneficial effects in breast and other cancers dependent on oncodrivers, such as TNBC, which otherwise lacks effective therapeutic strategy. HER3 deserves attention in this context as our lab has reported progressive loss of HER3-specific T \(_{\text{H}1}\) immune response in TNBC patients, and patients with residual or recurrent disease showed significant suppression of immune response compared to patients without recurrence or complete response after neoadjuvant chemotherapy (166). Future research will be essential for a comprehensive understanding of the interaction between oncodrivers and immune cell signaling in tumors, for developing efficient targeted immunotherapies with improved therapeutic success.

### Neoantigens and Neoantigen-Driven Immune Response

While T cell activity towards tumor-derived neoantigen has been reported in mouse models as early as in the 1980s, they have gained renewed interest in recent years as significance of neoantigens to enhance ‘foreignness’ of tumors has been shown to be critical for success of immunotherapy, including immune checkpoint blockade therapeutics. Neoantigens are distinct from the tumor-associated antigens (TAA) which are proteins present in normal tissues and overexpressed in tumors, and therefore, peptides of TAA can be recognized by T cells following interaction with human leukocyte antigen (HLA). The most prominent TAA are HER2, MAGE, MUC1, NY-ESO-1, MART-1 and mammaglobin-A, among others. Neoantigens, on the other hand, are unique non-autologous proteins expressed in tumor, due to somatic DNA alterations such as non-synonymous point mutations, insertion/deletion, gene fusion and frameshift mutations (167). Compared to TAA, neoantigens present a more appealing target for targeted immunotherapy development due to their higher immunogenicity that is enhanced because of increasing difference between the mutated and normal peptide sequence, strong individual tumor specificity, higher affinity towards MHC, and reduced risk of autoimmunity as they are recognized as foreign antigens and not affected by central immunological tolerance (168). Targeting TAA of low abundance and weak immunogenicity versus neoantigens that are abundant and highly immunogenic may not alter the intratumoral balance of anti- and pro-tumorigenic CD4+ T \(_{\text{H}1}\) immune response, and the overall success of immunotherapy, to the same extent. As shown in Figure 3, low antigenic load presented by oncodrivers/self-antigen/TAA may require a more comprehensive shift in the balance, including both activation of anti-tumorigenic T \(_{\text{H}1}\)/T \(_{\text{H}}\) and suppression of pro-tumorigenic T \(_{\text{H}2}\)/T \(_{\text{H}}\)/T \(_{\text{reg}}\)/MDSC function, for effective immunotherapy; while the high abundance and immunogenicity of neoantigens may be enough to drive up one side of the balance, by either hyperactivating anti-tumorigenic response or severe suppression of immunoinhibitory populations in favor of anti-tumor immune response to result in superior therapeutic efficacy.

A series of studies have demonstrated correlation between tumor mutational burden and/or predicted neoantigen ‘load’ (abundance of neoantigens) and patient survival. Reports of a positive association between higher predicted neoantigen load and increased intratumoral lymphocyte infiltration (CD3+ and CD8+ T cells) and improved overall survival in colorectal, endometrial and ovarian cancer (169–171) led to studies addressing the relationship between neoantigen abundance and success of immune checkpoint blockade therapy in cancer. Indeed, in melanoma patients treated with anti-CTLA4 antibody, NSCLC patients receiving anti-PD1 antibodies and urothelial carcinoma patients receiving anti-PD-L1 therapy, the extent of DNA damage (that corresponds to tumor mutational burden and neoantigen load) correlates with therapeutic response (172–174). Even though a large number of studies have focused on teasing out the role of neoantigen-targeted CD8+ T cell activity in cancer, significant and preferential CD4+ T cell activation by neoantigens have been recognized in multiple pre-clinical and clinical studies (175). Current pre-clinical and clinical trials employ multiple platforms of neoantigen-targeted vaccines such as synthetic long peptide vaccine, DNA and RNA vaccine, and DC vaccine, along with adoptive T cell therapy. As reviewed previously, pre-clinical studies have demonstrated significant therapeutic benefit of neoantigen-targeted vaccines (167, 168).

In three murine models of melanoma (B16F10), breast (4T1) and colon (CT26) cancer, the majority of the mutated neo-epitopes were recognized by CD4+ T cells, and vaccination with such mutations elicited robust tumor rejection (176). A recent study published with 4T1 and B16F10 murine models tested therapeutic efficacy of a novel cryo-thermal therapy with respect to conventional radiofrequency ablation and showed strong neoantigen-specific CD4+ T-cell response induced by cryo-thermal therapy, resulting in anti-tumor immune response and long-lasting protection against tumor re-challenge (177). Combination of local radiotherapy with an RNA-LPX vaccine that encodes CD4+ T cell-recognized neoantigens resulted in a poly-antigenic, potent CD8+ T cell response and memory that rejected CT26 tumor re-challenge, had higher number of polyfunctional IFN-\(\gamma\)/CD4+ T \(_{\text{H}1}\) cells specific for the
immunodominant CD4 neoantigen ME1, elevated numbers of activated gp70-specific CD8+ T cells, and lower PD-1/LAG-3 expression. Follow-up immunotherapy with anti-CTLA4 antibody resulted in complete remission of gp70-negative CT26 tumors in all mice in this study (178). In an inducible lung adenocarcinoma mouse model, vaccination using the G12D KRAS mutations as neoantigens and a novel synthetic long peptide-containing cationic lipoplex-based delivery platform stimulated both CD4+ and CD8+ T cell response and suppressed tumor growth, while combination with checkpoint inhibitor furthered such suppression (179). Similarly, both CD4+ and CD8+ T cell response has been reported in recent clinical studies following neoantigen-specific vaccination, across multiple cancer types. Whole-exome sequencing demonstrated that TIL in metastatic cholangiocarcinoma contained CD4+ T H1 cells specifically responsive against a mutation in ERBB2 interacting protein (ERBB21P) and adoptive transfer of TIL containing mutation specific polyfunctional T H1 cells resulted in a decrease in target lesions with prolonged survival (180). CD4+ T cells capable of recognizing the recurrent KRASG12V and the ERBB2 internal tandem duplication oncodriver mutations were identified in PBMC samples collected from a small cohort of NSCLC patients (181). Frequent recognition of neoantigens by CD4+ TH1 cells have been reported in melanoma as well (182). In a phase I/ib study reported last year, personalized neoantigen vaccination in glioblastoma patients increased tumor infiltrating cells, accompanied by a circulating polyfunctional neoantigen-specific CD4+ and CD8+ T cell responses enriched in memory phenotype, in patients who did not received dexamethasone (183). In treatment-naïve epithelial ovarian cancer patients, whole-exome and transcriptome sequencing analysis to identify neoantigen candidates and vaccination thereafter showed spontaneous CD4+ and CD8+ T-cell responses against neoepitopes from autologous lymphocytes in 50% of the

FIGURE 3 | Therapeutic targeting of tumor associated antigens and neoantigens activate anti- vs. protumorigenic CD4+ TH cell subtypes. Therapeutic targeting of oncodrivers/TAA/self-antigens may stimulate tumor immune response differently than strategies involving neoantigens. (A) Intratumoral balance of anti- and protumorigenic CD4+ TH immune cell population maintain the equilibrium of inflammatory (IFN-γ, TNF-α, IL-2, IL-6, IL-9, IL-21) and inhibitory cytokines (IL-4, IL-5, IL-10, TGF-β) in cancer cell and determine overall immune response to therapy. (B) When tumor cells express self-antigens/TAA/oncodrivers (blue spheres), due to low abundance and weak immunogenicity of these antigens, effective immunotherapy targeting these proteins may require a more extensive shift in the balance of anti- vs pro-tumor immune effector populations, including recruitment and activation of all anti-tumorigenic T H1/9/Thpopulations (green arrow) and suppression of all pro-tumorigenic T H2/Th17/Threg/MDSC function (red arrow). Conversely, highly antigenic and abundant neoantigens (green spheres) may be sufficient to stimulate anti-tumor immune response either by (C) driving up infiltration and hyperactivation of primarily T H1, along with T H9 and Thpop immunostimulatory response (green arrow) with minimal changes in the inhibitory immune cell function (grey arrow) or by (D) drastic downregulation of immunosuppressive response by T H2/Th17/Threg/MDSC cells (red arrow) without a significant change in the immunostimulatory population of T H1/9/Thpop cells (grey arrow) (light green, T H1; red, T H2; orange, T H9; blue, Thpop; dark green, T H17; grey, Threg, purple, MDSC).
patients, along with enhanced antigen processing and presentation machinery present in those specific tumors (184).

Therefore, along with further optimization of neoantigen prediction algorithm and targeting, a comprehensive understanding of the neoantigen recognition by CD4+ T cells and how that stimulates intratumoral effector and helper function of these T cells will be of utmost importance for the development of personalized immunotherapy targeting individual tumor neoantigens and demands extensive research.

**IMMUNE CHECKPOINT MODULATORS AND T_H CELL REGULATION**

The tumor microenvironment weighs heavily on T cell differentiation. An intratumoral meshwork of regulatory immune cells and immunosuppressive cytokines/chemokines act as one of the central modulators of T cell differentiation and function. TGF-β produced by tumors can convert CD4+ T cells into Treg cells in situ (185). Recruitment of MDSC in the TME aid in this suppression of T_H immune cells, where TNF-α, IL-1, IL-6, colony stimulating factor 1 (CSF-1), IL-8, IL-10, and type I interferons can also play a role in the regulation of T_H immune response to tumor cells. VEGF, IL-10, and TGF-β have been shown to inhibit DC maturation, leading to poor antigen presentation and co-stimulation of T cells, which favors T_H2 differentiation and shifts the balance from T_H1 to T_H2 phenotype (185). Manipulation of the TME, immune checkpoint regulation and cytokine levels may ensure long term tumor free survival in patients. Immune checkpoint blockades with anti-PD-1, anti-PDL1, and anti-CTLA4 antibodies to combat the inhibitory effects of TME on the immune system have been studied and showed promising therapeutic efficacy (186). Immune checkpoints are the gatekeepers of immune response and has garnered significant attention in the field of cancer immunosurveillance and immunotherapy in recent years. These inhibitory receptors/co-stimulatory molecules target T cell receptor (TCR) signaling activation, induce T cell exhaustion and anergy, and suppress proinflammatory cytokines (e.g. IFN-γ, TNF-α) secretion, ultimately resulting in immunosuppression in the TME and has been targeted with antibody-mediated checkpoint blockade therapy in recent years, as discussed comprehensively in recent reviews (187–189). Therefore, expression of these checkpoint regulators on T_H1 as well as cytotoxic T cells, while negatively impacting their proliferation and function, can be critical in determining success of checkpoint blockade therapy in high versus low density immune checkpoint-bearing tumors. In classic Hodgkin’s Lymphoma, where MHC-I expression is lost but MHC-II expression is intact, CD4+ T cell infiltration in tumor was correlated with better prognosis in patients and showed improved efficacy of a PD-1 blocking antibody in MHC-II-expressing lymphoma. In the same study, Nagasaki et al. showed that CD4+ T cell cytotoxicity played a critical role in delivering anti-tumor effects of anti-PD1 antibody which was observed in MHC-I−MHC-II+ tumors, but not on MHC-

\[ \Gamma \text{MHC-II}^\gamma \text{ tumors}, \text{in murine models of lymphoma and solid tumors (190). Kagamu et al.} \text{investigated NSCLC patients receiving nivolumab immunotherapy and found that treatment responders had higher circulating level of effector, CD62L}^\text{low} \text{CD4+ T cells prior to PD-1 blockade that correlated with effector CD8+ T cell abundance, and these cells expressed surface markers indicative of T_H1 phenotype (191). In a study with healthy subjects and glioblastoma multiforme (GBM) patients, PD1+ CD4+ T cells were found to be unable to proliferate but secrete IFN-γ and display exhaustion markers in RNA sequencing analyses. In GBM samples, enrichment of both PD1+ CD4+ and PD1+ TIM3+ CD4+ T cells suggest combined blockade of multiple checkpoints can be a requirement to tackle aggressive cancers like GBM (192). Varying levels of PD-1 expression on follicular lymphoma cells reflect on the T_H phenotype of intratumoral CD4+PD-1^high T cells with no TIM3 expression that supports B cell growth, while CD4+PD-1^low T cells elicit an exhausted phenotype, express TIM3 with reduced cytokine secretion and cellular signaling, and significantly correlate with a reduced overall survival in follicular lymphoma patients (193). Another checkpoint modulator, CTLA-4, is constitutively expressed on CD4+CD25+ Treg cells leading to trans-endocytosis of B7 ligands and interference with the CD28 co-stimulatory signaling, and has been deemed necessary for secretion of anti-inflammatory cytokines by Treg cells (194, 195). Future research will be crucial to elaborate immune checkpoint regulation of CD4+ T_H cell differentiation and function and identify new nodes in the network for therapeutic targeting in cancer.

**T_H IMMUNE CELLS IN CANCER CELL DISSEMINATION, DORMANCY AND METASTASIS**

It is widely accepted that cancer cells disseminate from non-invasive or primary tumor sites into the circulation and reach various distant organs to form overt metastasis (196). T_H1 cytokines such as IFN-γ, TNF-α and IL-2 produced by T_H1 cells contribute to inhibit tumor growth and activation of tumorspecific immune mechanisms (155, 197). On the other hand, T_H2 cytokines IL-10, IL-4 and TGF-β from T_H2 cells can promote dissemination of cancer cells dissemination and metastasis in various cancers (198). The imbalance between the ratio of T_H1/T_H2 cells and their associated cytokines correlates with decreased progression-free survival and overall survival in patients with breast, melanoma, ovarian, esophageal and colon cancers (199). Previous studies in breast cancer patients have shown that presence of cancer cells in the systemic circulation are associated with alteration of CD4+ T_H cells (200, 201). After dissemination, cancer cells can remain dormant for a prolonged period until they emerge for metastatic colonization in secondary organs (202, 203). T_H1 cells can reduce proliferation and mediate dormancy in these disseminated cancer cells (DCC) via IFN-γ dependent STAT1 signaling pathway activation and anti-tumor immunity (113). A mouse model of melanoma showed presence
of DCC in various organs, such as the lungs, skin and reproductive tract, and regulation of their non-proliferative status by T_{H1} immune cells (204). Another study using a mouse model for sarcoma also supports a role of CD4^{+} T cells to induce dormancy in cancer cells and tumor relapse (205). These reports suggest the regulatory role of T_{H1} immune cells in controlling tumor dormancy and metastasis. Since maintaining T_{H1}/T_{H2} immune cell balance is critical in anti-tumor immunity, therapeutics that enhance T_{H1} response and prevent T_{H2} activation and associated cytokines may simultaneously help to eradicate disseminated cancer cells, preventing recurrence and metastasis.

Tumor infiltrating T_{H9} and T_{H17} cells are observed to promote epithelial to mesenchymal transition (EMT) and migration potential of lung cancer cells and metastasis outgrowth. IL-9 and IL-17 cytokines from T_{H9} and T_{H17} cells can stimulate cytokine signaling and alter various genes linked to EMT and drive metastasis (39). In addition, high accumulation of T_{H9} and T_{H17} cells in lung cancer patients with poor survival further support their multifaceted role in cancer progression and metastasis (39). Another study has demonstrated high serum level of IL-9 and IL-17 cytokines with increased frequency of T_{H9} and T_{H17} cells in hepatic carcinoma patients with malignant ascites (206). This finding suggests that T_{H9} and T_{H17} cells may play a significant role in metastatic spread through IL-9 cytokine signaling.

**CLINICAL EXPERIENCE WITH CD4^{+} T_{H} CELLS: CURRENT STATUS**

In the past decade, it has become clear that CD4^{+} T cells play a multifaceted role and are crucial for generating effective anti-tumor immunity. Therapeutic approaches designed to target CD4^{+} T cell responses can be broadly divided into passive immunotherapy (antibody-based therapies, adoptive cell therapy, and chimeric antigen receptor T cell therapy) and active immunotherapy (peptide vaccines, DC-based immunotherapies, immune checkpoint blockade). Here, we review current status of these immunotherapeutic approaches to stimulate tumor specific CD4^{+} T cell responses, focusing on peptide vaccines, adoptive T cell transfer and chimeric antigen receptor T cell therapy (Figure 4). Ongoing clinical trials utilizing these immunotherapy strategies have been summarized in Table 2.

**VACCINES**

Cancer vaccines were developed to stimulate specific anti-tumor T cell responses by (1) developing antigen-loaded DC ex vivo prior to vaccination (DC vaccines) and/or (2) directly administering immunogenic peptides (epitope-based vaccines). Antigens used in vaccination may span from use of peptide fragments or full proteins, DNA and mRNA, or even bulk cancer cell lysates to stimulate CD4^{+} TH1 responses in vivo (207, 208).

![FIGURE 4](#) | CD4^{+} T cells in cancer immunotherapy. Immunotherapeutic strategies that activate CD4^{+} T cells and their downstream effector immune cells for cancer treatment are depicted. Therapeutic vaccination includes tumor antigenic peptides, viral vector-based vaccine and DNA based vaccine that can mediate CD4^{+} T cells immune responses. DC-based vaccines can prime CD4^{+} T cells and create signals to activate cytotoxic CD8^{+} T cells differentiation and anti-tumor function. Adoptive transfer of tumor specific CD4^{+} T cells is another attractive immunotherapy approach which helps to develop specific and strong anti-tumor immune reaction. Chimeric antigen receptors can also redirect CD4^{+} T cells and provide activation signals to recognize cancer cells to eliminate them. Blockade of immune checkpoints PD1, PD-L1 and CTLA4 by antibodies can prevent tumor associated immunosuppressive environment and enhance tumor specific CD4^{+} and CD8^{+} T cells immune responses.
| ClinicalTrials.gov Identifier | Intervention | Target patients | Immune Outcome measure | Primary endpoint |
|-------------------------------|--------------|-----------------|------------------------|------------------|
| NCT03946358 Phase II          | Atezolizumab (anti PD-L1) and UCPVax (vaccine), Blood sample collection, Tumor biopsies, CT scan anti-PD-1 antibody-activated TILs | Squamous Cell Carcinoma of the Head and Neck Anal Canal Cancer | CD3+, CD8+, CD4+ or CD56+ T cells | Objective response rate at 4 months 6 months |
| NCT03904537 Phase I/II        | Avelumab (anti PD-L1), Radiation, and CTX (cyclophosphamide) | Head and Neck Cancer | | |
| NCT03734692 Phase I/II        | Cisplatin, Pembrolizumab, Rintatolimod | Ovarian Cancer Recurrent | Pre-and post-treatment CD3+, CD4 Tbet+, CD8+, NK cells and granzyme B | 13 weeks |
| NCT03698461 Phase II          | Atezolizumab, Bevacizumab, Oxaliplatin, Levoeleucovorin, 5-fluorouracil | Colorectal Neoplasm Metastasis Colonic Neoplasms Rectal Neoplasms | CD3, CD4, CD8 T cells, PD-L1, PD-1, CD45RO, FOXP3, CD68, Granzyme B | End of treatment |
| NCT03410732 Phase II          | activated DCs, radical surgery only | Gastric Cancer | CD4/CD8 T cell percentage change | Progression free survival (3 years) 1 year |
| NCT03067155 Phase II          | CMV-specific T cells, Standard anti viral therapy | Hematological Malignancies | CMV Infection | 57 days (phase I) 73 days (phase II) 29 days |
| NCT02818426 Phase I/II        | UCPVax (peptide vaccine) | Metastatic Non-small Cell Lung Cancer | | |
| NCT02957968 Phase II          | Doxorubicin, Cyclophosphamide, Paclitaxel, Carboplatin, Decitabine, Pembrolizumab | Breast Adenocarcinoma Estrogen Receptor-Negative Breast Cancer Estrogen Receptor-positive Breast Cancer HER2/Neu Negative Invasive Breast Carcinoma Progesterone Receptor Negative Progesterone Receptor Positive Tumor Stage IIA Breast Cancer Stage IIIB Breast Cancer Stage IIIA Breast Cancer Stage IIIB Breast Cancer Triple-negative Breast Carcinoma | TIL %, CD3, CD4, CD8 T cells, Treg, MDSC, B cell, PD-1, PDL-1 | |
| NCT01868490 Phase I/II        | cytokine induced killer cells | Cholangiocarcinoma | | 6 weeks |
| NCT03384914 Phase II          | WOKVAC Vaccine, DC1 Vaccine | HER-2 Positive Breast Cancer | Immunogenicity (IFN-γ ELISPOT) | Up to 7 years |
| NCT04552886 Phase I           | TH-1 Dendritic Cell Immunotherapy | Glioblastoma | | 2 years |
| NCT04157127 Phase I           | Autologous DC vaccine | Pancreatic Adenocarcinoma Pancreatic Cancer | | 6 weeks |
| NCT02846103 Phase I           | blood and tumor tissue samples (Immune monitoring) | Lung Cancer | UCP-specific Th1 response (IFN-γ ELISPOT) HER2-specific Th1 response (IFN-γ ELISPOT) | 2 years 28 weeks |
| NCT03387553 Phase I           | Neoadjuvant Chemotherapy, Curative Surgery | HER2-positive Breast Cancer | | |
| NCT03977103 Phase II          | High dose irradiation conditioning + Treg/Tcon | Acute Myeloid Leukemia Acute Lymphoid Leukemia Myeloproliferative Disorders Lymphoma Multiple Myeloma Other Hematologic Malignant Neoplasms | Myeloid, B cells, T cells (including subtypes) in CSF, blood and TME cytokines in CSF and blood | 21 days up to 15 years 3 months |
| NCT03696030 Phase I           | Chimeric Antigen Receptor T-Cell Therapy | Metastatic Malignant Neoplasm in the Leptomeninges Breast Cancer HER2-positive | | |
| NCT04433221 Phase I/II        | Multiple sarcoma-specific CAR-T cells and sarcoma vaccines | Sarcoma Osteosarcoma Ewing Sarcoma | | |
| NCT01955460 Phase I           | Aldesleukin (Recombinant Human IL-2) Cyclophosphamide Fludarabine Phosphate | Metastatic Melanoma | | Up to 5 years |

(Continued)
The first efforts to active CD4⁺ T_{H1} anti-tumor immunity were actually implemented through the generation of peptide-based vaccines, whereby immunogenic class II peptide fragments from tumor associated antigens such as MUC1 (209), NY-ESO1 (210), MAGE-A3 (211), and HER2 (212) have been injected to induce antigen-specific CD4⁺ T_{H1} response (1). Additionally, research into a universal cancer vaccine examined the efficacy of stimulating CD4⁺ T_{H1} responses through vaccination with promiscuous epitopes from surviving and telomerase proteins (213, 214). Recently, research focus has shifted to generate cancer vaccines through stimulation of CD4⁺ T_{H1} responses to mutated antigens, or neoantigens, selectively expressed by malignant tissue (168), and with the known synergistic effects of tumor specific CD4⁺ and CD8⁺ T cells, peptide epitopes capable of binding both MHC-I and MHC-II show potential to optimize vaccination efficiency (1, 207, 215). In 2017 Ott et al. demonstrated the efficacy of personalized neoantigen vaccines for the treatment of melanoma patients where specific mutations were identified in patients and synthetic long peptides were used in vaccination to stimulate both CD4⁺ and CD8⁺ responses. While CD4⁺ T_{H1} cells demonstrated the highest rates of tumor specific response and targeted 60% of the unique neoantigens used across patients, resulting in no recurrence in four out of six patients 25 months after vaccination, two recurrent disease subsequently treated with anti-PD1 therapy led to complete tumor regression and expansion of the repertoire of neoantigen-specific T cells (1, 86). Similarly, Tondini et al. developed a poly-neoantigen vaccine composed of a fusion gene incorporating three neoepitopes derived from mouse colorectal tumor in combination PD-1 IBC to target both CD4⁺ and CD8⁺ responses in murine colorectal cancer models (216).

The pivotal role of DC to generate T cell mediated tumor immunity via activation, priming, and induced rapid expansion of antigen-specific T cells implicates therapeutic potential of DC-targeted immunotherapy development (215). Loading of patient autologous DC with previously identified immunogenic epitopes from tumor antigens and reinfusion of antigen-loaded DC can lead to the induction of specific anti-tumor CD4⁺ T_{H1} responses (1). The first FDA approved DC vaccine (Sipuleucel-T) was approved in 2010 for metastatic prostate cancer (217). Following this, several trials have evaluated the therapeutic benefits of DC vaccines for the treatment of various cancer types (Table 2). Findings from multiple trials testing therapeutic efficacy of a DC vaccine primed with WT-1, a TAA overexpressed in glioblastoma, or autologous tumor lysate to treat patients with glioblastoma, has been reviewed by Eagles et al. (218). In 2016, De La Cruz et al. demonstrated the efficacy of a HER2-DC vaccine in HER2⁺ breast cancer patients, where treatment induced a significant increase in anti-HER2 CD4⁺ T_{H1} response and improved rates of pathological complete response (160, 163, 219). A clinical trial (NCT00910650) adoptively transferring MART-1 T cell receptor (TCR) transgenic lymphocytes together with MART-1 peptide-pulsed DC vaccination in HLA-A2.1 patients with metastatic melanoma showed evidence of tumor regression (220).

There are several cancer vaccines showing great promise for the treatment of different cancer types but there are still challenges in using this approach to treat advanced disease, and vaccination alone may be insufficient to control tumor progression. The efficacy of these vaccines is highly dependent on the identification of proper stimulatory antigens and functional status of the individual immune response in patients. Future research can optimize this immunotherapeutic strategy for eliciting tumor-specific CD4⁺ T cell responses by considering antigen dosing and immunogenicity, timing of the therapy, the role of adjuvants, immunosuppressive TME, and combinational strategies (221).

**ADOPTIVE CELL THERAPY**

Adoptive cell therapy (ACT) involves the generation of tumor specific T cells ex vivo that can be reinfused into patients. Clinical success of ACT depends greatly on the expansion of tumor specific T cells ex vivo, homing to the tumor site, and persistence following infusion. Although most cell therapies focus on CD8⁺ CTLs due to their tumor killing capabilities, considering the molecular ‘help’ by CD4⁺ T_{H1} cells is required for CD8⁺ cytotoxicity and recruitment, adoptive transfer of CD4⁺ T cells may play an important role in overall tumor immune response (84). Interestingly, transfer of CD8⁺ T cells alone has shown to have low tumor free survival rates whereas transfer of both CD4⁺ T_{H1} cells and CD8⁺ CTLs has shown a synergistic anti-tumor response resulting in complete regression in 80% of mice (222). Previous expansion methods focus primarily on the use of the cytokine IL-2 to expand T cells ex vivo. CD4⁺ T cells can be transformed into regulatory T cells in the presence of IL-2 and TGF-β secreted in the TME, allowing immune evasion. A study by K.L. Knutson et al. showed that IL-2 alone resulted in the loss of proliferation of antigen specific
CD4+ T cells; however, addition of IL-12 was able to overcome the loss of proliferation (223). This suggests there is a pressing need to explore other cytokines that can successfully expand tumor specific CD4+ T cells without generating regulatory T cells. Elimination of immune suppressive cells such as MDSC has been shown to greatly enhance the efficacy of ACT (224), suggesting that ACT must also overcome the immunosuppressive effects of the TME and other innate immune cells to amplify the therapeutic efficacy.

Success of ACT also relies heavily on the generation of T cells that can persist long after infusion. K.A. Read et al. showed that IL-7 and IL-15 maintain important memory phenotypes in CD4+ TIL cells which aid in long term survival (225). IL-7 is also believed to have a role in T cell trafficking to secondary lymphoid organs. Peter Cohen and his group described tumor specific CD4+ and CD8+ T cells expansion from unfractionated PBMCs using an activator of innate immunity. Addition of toll-like receptor agonists, LPS and R848 (resiquimod), followed by addition of synthetic long peptides (>20aa) derived from widely expressed oncoproteins (MUC1, HER2/neu and CMVpp65) enhanced the processing and presentation of exogenous TAA. Addition of IL-7 enhanced the antigen-driven outgrowth of CD4+ and CD8+ T cells (226). Therefore, IL-7 and IL-15 can be potential alternatives to using IL-2 in the generation of tumor specific T cells.

Adoptive cell therapy alone is not enough in providing long term effects that can prevent relapse in patients. Combination therapies using immune checkpoint blockade, migratory molecules such as CXCR2, and stimulatory cytokines such as IFN-γ and ACT have been studied widely in melanoma models with great promise (186). Toxicity, however, can be a potential hindrance to combination therapies. Thus, finding a safe adoptive cell therapy that can harness the full effects of both CD4+ and CD8+ T cells is paramount in future immunotherapies.

CHIMERIC ANTIGEN RECEPTOR T CELL THERAPY

Chimeric antigen receptor T cell (CAR-T) therapy entails genetic engineering of a patient’s own T cells to express membrane spanning fusion receptors with defined specificities for tumor associated antigens. In humans, CD4+ T cells as part of the CAR has been shown to induce target cell apoptosis in an MHC and Fas-independent manner, via cytolytic degranulation by perforin and granzyme (227, 228). However, reportedly low granzyme and perforin expression on CD4+ T cells, compared to CD8+ T cells, may contribute to their limited cytotoxicity (229). Equal tumor cell killing capacity of CD4+ and CD8+ CAR-T cells, albeit longer conjunction and delayed kinetics in CD4+ cells (230), and apoptosis and anergy in CD8+ T cells without the molecular help from CD4+ T cells in the vicinity suggest CD4+ CAR-T can potentiate the effects of the therapy in cancer (231). Between GBM-associated antigen-targeting CD4+ and CD8+ CAR-T cells, CD4+ CAR T cells showed effector persistency after tumor challenge and similarly in orthotopic GBM model, CD4+ CAR-T outcompeted CD8+ CAR-T in terms of durable anti-tumor response (232). In GBM in vitro and in vivo models, Brown et al. (233) tested anti-tumor effects of IL13Rα2-specific CAR T cells engineered from purified CD4+ or CD8+ TCM pools and showed superior tumor killing by CD4+ CAR-T cells, along with higher cytokine production and persistent effector function upon tumor challenge, when compared with CD8+ CAR-T cells. Intracranial injection of CD4+ CAR-T in an NSG model of GBM showed durable anti-tumor efficacy and prolonged survival, while mice receiving CD8+ CAR-T cells recurred following an initial response (233). In a preclinical NSG mouse model, administration of CD19-CAR-T using CD4+-targeted lentiviral vector (CD4-LV) displayed T111/T112 phenotype of the CAR-T, with a superior and faster tumor killing ability than CD8-LV CAR-T cells alone or in combination with CD4-LV. Such prolonged response by CD4+ CAR-T cells in preclinical and clinical models can be attributed to higher exhaustion in CD8+ cells (234). Overall, navigating the immune suppressive effects of the TME and reducing clinical toxicities, while maintaining a durable anti-tumor response, will be of paramount importance to successful CAR-T cell therapy for solid tumors.

FUTURE PROSPECTS OF TH CELLS IN CANCER IMMUNOTHERAPY

Recent research has underscored the significance of CD4+ TH cells as a component of anti-tumor immune response. In this review we discuss why CD4+ TH cells are considered an integral component of current immunotherapy research and how the shifting balance between TH1 and TH2 cells, along with other TH cell subtypes, modulate the intratumoral immune response. Notably, current research has pointed out how these other CD4+ TH subtypes, such as immunostimulatory TH9, TH17 while immunosuppressive TH1, TH17, THreg cells and inhibitory function of MDSC, can sway the anti- vs pro-tumorigenic balance of TH immune response and suggest the clinical relevance of targeting these CD4+ TH subtypes. Therapeutic intervention to regulate not only TH1 and TH2 functional response, but other stimulatory/suppressive immune populations may be critical for more efficacious therapy design.

Review of recent literature points out the potential advantages of CD4+ TH cell-based immunotherapy in comparison with strategies focused on CD8+ T cells. These cells are necessary and sufficient to activate CD8+ T cells for amplified anti-tumor response, along with their own contribution to cytotoxicity of tumor cells. The requirement for specific peptide recognition and HLA class matching can limit the therapeutic success of CD8+ T cells targeting neoantigens which are primarily derived by point mutations, since those mutations can significantly alter the interaction kinetics with CD8+ T cells and diminish CTL activity. The more promiscuous nature of CD4+ T cells permits interaction with a broader variety of neoantigens and mutated oncogenes. Simultaneously, a reciprocal regulation of CD4+ T cells targeting neoantigens which are primarily derived by point mutations, since those mutations can significantly alter the interaction kinetics with CD8+ T cells and diminish CTL activity. The more promiscuous nature of CD4+ T cells permits interaction with a broader variety of neoantigens and mutated oncogenes. Simultaneously, a reciprocal regulation of CD4+ T cells targeting neoantigens which are primarily derived by point mutations, since those mutations can significantly alter the interaction kinetics with CD8+ T cells and diminish CTL activity. The more promiscuous nature of CD4+ T cells permits interaction with a broader variety of neoantigens and mutated oncogenes. Simultaneously, a reciprocal regulation of CD4+ T cells targeting neoantigens which are primarily derived by point mutations, since those mutations can significantly alter the interaction kinetics with CD8+ T cells and diminish CTL activity. The more promiscuous nature of CD4+ T cells permits interaction with a broader variety of neoantigens and mutated oncogenes.
enhanced anti-tumor response. However, studies in various pre-clinical models and clinical trials that have pointed out potential obstacles such as a hostile TME, presence of inhibitory T cell populations and immune checkpoint receptors suggest that stimulating a single subpopulation of CD4\(^+\) T\(_H\) function alone may not be adequate for robust anti-tumor response. Combinations of therapies that can drive multiple subtypes of CD4 T\(_H\) may better overcome this inadequacy and improve therapeutic efficacy in cancer. Targeted inhibition of oncodrivers by blocking/neutralizing antibodies and small molecule inhibitors, cell cycle kinases CDK4/6 inhibitors and standard-of-care therapeutics in combination with immunotherapy that drive CD4\(^+\) T\(_H\) cells are currently being tested in various stages of clinical trials and warrant future research to delve into the mechanism that these therapies have on anti-vs pro-tumorigenic CD4\(^+\) T\(_H\) responses for refined synergy. Developing DC-based vaccine platforms to stimulate onco-driver-specific T\(_H\) immune response can facilitate immunotherapy and future synergistic combination for effective cancer treatment. Understanding the role of immunosuppressive T\(_H\) cells, such as T\(_{H12}\), T\(_{H17}\) and T\(_{reg}\), in the TME is equally critical to identify nodes in this regulatory network for therapeutic intervention. A successful cancer immunotherapy will require careful balancing of the CD4\(^+\) T\(_H\) compartment in order to orchestrate essential efforts that mediate tumor regression. A comprehensive overview of the CD4\(^+\) T\(_H\) cells, as discussed in this review, will help to elucidate the framework of CD4\(^+\) T\(_H\) function and highlight the clinical relevance of harnessing CD4\(^+\) T\(_H\) cells in cancer immunotherapy to encourage future translational research.

IN MEMORIAM

Dedicated to Peter Cohen, MD

Peter Cohen, MD was an early advocate for CD4\(^+\) T cell therapy for cancer, at a time when most of the field was focused almost exclusively on CD8\(^+\) CTL. Beginning as a Physician Scientist at UC San Diego, he spent many years at NCI, in both Surgery and Medicine Branches, working on advancing the concept that CD4\(^+\) T cells were critical to immunotherapy for cancer. Some of his early predictions are being borne out in the recent literature, confirming the critical importance of the CD4\(^+\) T cell populations in eliminating tumors. Dr. Cohen was also instrumental in developing technologies to facilitate the use of dendritic cells as vaccine platforms for cancer immunotherapy. Dr. Cohen went on to work with Dr. Suyu Shu at the Cleveland Clinic, and for the past 10 years has worked as a medical oncologist at the Mayo Clinic in Arizona. Those of us privileged to have Dr. Cohen as a mentor or colleague were charmed by his irresistible humor and quick wit, inspired by his intellect and drive, and humbled by his compassion and dedication to the care of his patients. It is in sincere appreciation for Dr. Cohen's enriching contributions to science, to our lives, and to our careers that we dedicate this article to his memory.

AUTHOR CONTRIBUTIONS

A Ba, BC, and KK contributed to concept, outline and writing of the review. A Ba, GR, GA, CG, ABe, CS, and KK contributed to writing. GK, MD, BC, and KK reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by Department of Defense (Award# W81XWH-16-1-0385) awarded to BC, GK and MD. This work was also supported by Pennies in action to BC and GK. MD is also supported by the Helen B. Slonaker Endowed Professor for Cancer Research.

REFERENCES

1. Tay RE, Richardson EK, Toh HC. Revisiting the Role of CD4(+) T Cells in Cancer Immunotherapy: New Insights Into Old Paradigms. Cancer Gene Ther (2020) 1–25. 10.1038/s41417-020-0183-x
2. Borst J, Ahrends T, Babala N, Melief CJM, Kastenmüller W. Cd4(+) T Cell Dedifferentiation: A Balancing Act. Cancer Res (2016) 272
3. Raskov H, Orhan A, Christensen JP, Gögenur I. Cytotoxic Cd8(+) T Cells in Cancer and Cancer Immunotherapy. Br J Cancer (2020) 124:539–67. 10.1038/s41416-020-01048-4
4. Bedoui S, Heath WR, Mueller SN. Cd4(+) T-cell Help Amplifies Innate Signals for Primary Cd8(+) T-cell Immunity. Immunol Rev (2016) 272(1):52–64. 10.1111/imr.12426
5. Byrne A, Savas P, Sant S, Li R, Virassamy B, Luen SJ, et al. Tissue-Resident Memory T Cells in Breast Cancer Control and Immunotherapy Responses. Nat Rev Clin Oncol (2020) 17(6):341–8. 10.1038/s41571-020-0333-y
6. Farber DL, Yudanin NA, Restifo NP. Human Memory T Cells: Generation, Compartmentalization and Homeostasis. Nat Rev Immunol (2014) 14(1):24–35. 10.1038/nri3567
7. Menares E, Galvez-Cancino F, Caceres-Morgado P, Ghorani E, Lopez E, Diaz X, et al. Tissue-Resident Memory Cd8(+) T Cells Amplify Anti-Tumor Immunity by Triggering Antigen Spreading Through Dendritic Cells. Nat Commun (2019) 10(1):4401. 10.1038/s41467-019-12319-x
8. Gray JI, Westerhof LM, MacLeod MKL. The Roles of Resident, Central and Effector Memory CD4 T-Cells in Protective Immunity Following Infection or Vaccination. Immmunology (2018) 154(4):574–81. 10.1111/imn.12929
9. Clarke J, Panwar B, Madrigal A, Singh D, Gjiu R, Wood O, et al. Single-Cell Transcriptomic Analysis of Tissue-Resident Memory T Cells in Human Lung Cancer. J Exp Med (2019) 216(9):2128–49. 10.1084/jem.20190249
10. Savas P, Virassamy B, Ye C, Salim A, Mintoff CP, Caramia F, et al. Single-Cell Profiling of Breast Cancer T Cells Reveals a Tissue-Resident Memory Subset Associated With Improved Prognosis. Nat Med (2018) 24(7):986–93. 10.1038/s41591-018-0078-7
11. Yau C, Wolf DM, Campbell M, Savas P, Lin S, Brown-Swigart L, et al. Abstract P3-10-06: Expression-Based Immune Signatures as Predictors of Neoadjuvant Targeted-Chemo-Therapy Response: Experience From the I-SPY 2 TRIAL of 1000 Patients Across 10 Therapies. Cancer Res (2019) 79(4 Supplement):P3-10-06. 10.1158/1538-7445.SABCS18-P3-10-06
56. Chaudhry A, Samstein RM, Treuting P, Liang Y, Pilis MC, Heinrich JM, et al. Interleukin-10 Signaling in Regulatory T Cells is Required for Suppression of Th17 Cell-Mediated Inflammation. *Immunity* (2011) 34(4):566–78. doi: 10.1016/j.immuni.2011.03.018

57. Chen ML, Pettit MJ, Gorelik L, Flavell RA, Steiner D, von Boehmer H, et al. Regulatory T Cells Suppress Tumor-Specific CD8+ T Cell Cytotoxicity Through TGFB-beta Signals In Vivo. *Proc Natl Acad Sci USA* (2005) 102(2):419–24. doi: 10.1073/pnas.0408197102

58. Pandiyan P, Zheng L, Ishihara S, Reij J, Lenardo MJ. Cd4+ Cd25+ Fopx+ Regulatory T Cells Induce Cytokine Deprivation-Mediated Apotosis of Effector Cd4+ T Cells. *Nat Immunol* (2007) 8(12):1353–62. doi: 10.1038/nl11536

59. Chikam Tannan AK, Levine AG, Fan X, Klein U, Zheng Y, et al. An Essential Role for the IL-2 Receptor in T Cell Function. *Nat Immunol* (2016) 17(11):1332–33. doi: 10.1038/nl3540

60. Ahmadzadeh M, Pasetto A, Jia L, Deniger DC, Stevanovic S, Robbins PF, et al. Identi

61. Eisenbarth SC. Dendritic Cell Subsets in T Cell Programming: Location State and the In

62. Koski GK, Schwartz GN, Weng DE, Czerniecki BJ, Carter C, Gress RE, et al. Calcium Mobilization in Human Myeloid Cells Results in Acquisition of Individual Dendritic Cell-Line Characteristics Through Discrete Signaling Pathways. *J Immunol* (1999) 163(1):82–92.

63. Faries MB, Bedrosian I, Xu S, Koski G, Oroz MA, et al. Calcium Signaling Inhibits interleukin-12 Production and Activates CD83(+)-Dendritic Cells That Induce Th2 Cell Development. *Blood* (2001) 98(8):2489–97. doi: 10.1182/blood.V98.8.2488

64. Collin M, Bigley V. Human Dendritic Cell Subsets: An Update. *Immunology* (2018) 154(1):3–20. doi: 10.1111/imn.12888

65. Amon L, Lehmann CHK, Baranska A, Schoen J, Heger L, Dudziak D, et al. Transcriptional Control of Dendritic Cell Development and Functions. *Int Rev Cell Mol Biol* (2019) 349:55–151. doi: 10.1016/bs.ircmb.2019.10.001

66. Merad M, Sathe P, Helft J, Miller J, Lenardo MJ. Dendritic Cell Subsets in T Cell Programming: Location State and the In

67. Peng Q, Qiu X, Zhang Z, Zhang Y, Liang Y, et al. Pd-L1 on

68. Hildner K, Edelson BT, Purtha WE, Diamond M, Matsushita H, Kohyama T, et al. Dendritic Cells: A Conductor of T Cell Differentiation. *Nat Immunol* (2018) 19(7):892–4.

69. Kramer J, Klimpel K, Schiessl S, Ploegk HJ, Hsu-Z, et al. An

70. Kadowaki N. Dendritic Cells: A Conductor of T Cell Differentiation. *Nat Immunol* (2016) 17(8):787–9. doi: 10.1038/nri.2016.18

71. Krzysztowiak JS, Alsen S, Yrlid U, Eisenbarth SC, Williams A. Determination of T Follicular Helper Cell Fate by Dendritic Cells. *Front Immunol* (2016) 7(2169):1829–35. doi: 10.3389/fimmu.2016.02169

72. de Jong EC, Smits HH, Kapsenberg ML. Dendritic Cell-Mediated T Cell Polarization. *Springer Semin Immunopathol* (2005) 26(3):289–307. doi: 10.1007/s00281-004-0167-1

73. Novy P, Quigley M, Huang X, Yang Y. Cd4 T Cells are Required for CD8 T Cell Survival During Both Primary and Memory Recall Responses. *J Immunol* (2007) 179(12):8243–51. doi: 10.4049/jimmunol.179.12.8243

74. Laidlaw BJ, Craft JE, Kaech SM. The Multifaceted Role of CD4(+) T Cells in CD8(+) T Cell Memory. *Nat Rev Immunol* (2016) 16(2):102–11. doi: 10.1038/nri.2015.10

75. Spitzner MH, Carmi Y, Reticker-Flynn NE, Kwek SS, Madhireddy D, Martins MM, et al. Systemic Immunity is Required for Effective Cancer Immunotherapy. *Cell* (2017) 168(3):487–502 e15. doi: 10.1016/j.cell.2016.12.022

76. Zander R, Schauder D, Xin G, Nguyen C, Wu X, Zajac A, et al. Cd4(+) T Cell Help is Required for the Formation of a Cytolytic Cd8(+) T Cell Subset That Protects Against Chronic Infection and Cancer. *Immunity* (2019) 51(6):1028–1042 e4. doi: 10.1016/j.immuni.2019.10.009

77. Alspach E, Lussier DM, Miceli AP, Kuzhiyavot I, DuPage M, Luoma AM, et al. Mhc-II Neutangents Shape Tumour Immunity and Response to Immunotherapy. *Nature* (2019) 574(7780):696–701. doi: 10.1038/s41586-019-1671-8

78. Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ, et al. An Immunogenic Personal Neotangents Vaccine for Patients With Melanoma. *Nature* (2017) 547(7662):217–21. doi: 10.1038/nature22991

79. Ahrends T, Spaanjaard A, Pulzczer B, Bahala N, Bovens A, Xiao Y, et al. Cd4(+) T Cell Help Confers a Cytotoxic T Cell Effector Program Including Cohluyibot Receptor Downregulation and Increased Tissue Invasiveness. *Immunity* (2017) 47(5):848–861 e5. doi: 10.1016/j.immuni.2017.10.009

80. Sacks JA, Bevan MJ. TRAIL Deficiency Does Not Rescue Impaired Cd8+ T Cell Memory Generated in the Absence of Cd4+ T Cell Help. *J Immunol* (2008) 180(7):4570–6. doi: 10.4049/jimmunol.180.7.4570

81. Castellino F, Huang AY, Alton-Bonnet G, Soll S, Scheincke C, Germain RN. Chemokines Enhance Immunity by Guiding Naive Cd8+ T Cells to Sites of Cd4+ T Cell-Dendritic Cell Interaction. *Nature* (2006) 440(7086):890–5. doi: 10.1038/nature04651

82. Janssen EM, Lemmens EE, Wolfe T, Christen U, von Herrath MG, Schoenberger SP. Cd4+ T Cells are Required for Secondary Expansion and Memory in Cd8+ T Lymphocytes. *Nature* (2003) 421(6925):852–6. doi: 10.1038/nature01441

83. Shedlock D, Shen H. Requirement for Cd4 T Cell Help in Generating Functional Cd8+ T Cell Memory. *Science* (2003) 300(5617):337–9. doi: 10.1126/science.1082305

84. Oh S, Perera LP, Terabe M, Ni L, Waldmann TA, Berzofsky JA. Il-15 as a Mediator of Cd4+ Help for Cd8+ T Cell Longevity and Avoidance of TRAIL-mediated Apoptosis. *Proc Natl Acad Sci USA* (2008) 105(13):5201–6. doi: 10.1073/pnas.0801030105

85. Bos R, Sherman LA. Cd4+ T-cell Help in the Tumor Miliue is Required for Recruitment and Cytotoxic Function of Cd8+ T Lymphocytes. *Cancer Res* (2010) 70(21):8368–77. doi: 10.1158/0008-5472.CAN-10-1322

86. Ahrends T, Busselaar J, Sewerson TM, Babala N, de Vries E, Bovens A, et al. Cd4(+) T Cell Help Creates Memory Cd4(+) T Cells With Innate and Help-Independent Recall Capacities. *Nat Commun* (2019) 10(1):5531. doi: 10.1038/s41467-019-1348-1

87. Cullen JG, McQuilten H, Quinn RM, Ohlshansky M, Russ BE, Morey A, et al. Cd4(+) T Cell Help Promotes Influenza Virus-Specific Cd8(+) T Cell
Memory by Limiting Metabolic Dysfunction. Proc Natl Acad Sci USA (2019) 116(10):4481–8. doi: 10.1073/pnas.1808849116
96. Agarwal P, Raghavan A, Nandiwada SL, Cartisnger JM, Bohjanen PR, Mueller DL, et al. Gene Regulation and Chromatin Remodeling by IL-12 and Type I IFN in Programming for CD8 T Cell Effector Function and Memory. J Immunol (2009) 183(3):1695–704. doi: 10.4049/jimmunol.0900592
97. Wolkers MC, Gerlach C, Arens R, Janssen EM, Fitzgerald P, Schumacher TN, et al. NAb2 Regulates Secondary CD8+ T-Cell Responses Through Control of TRAIL Expression. Blood (2012) 119(3):798–804. doi: 10.1182/blood-2011-08-373910
98. Wiesel M, Joller N, Ehert A-K, Crouse J, Spöri R, Bachmann MF, et al. Th Cells Act Via Two Synergistic Pathways To Promote Antiviral CD8+ T Cell Responses. J Immunol (2010) 185(9):5188–97. doi: 10.4049/jimmunol.1001990
99. Xu S, Koski G, Faries, Bedrossian I, Rick C, Cheever M, et al. Rapid High Efficiency Sensitization of CD8+ T Cells to Tumor Antigens by Dendritic Cells Leads to Enhanced Functional Avidity and Direct Tumor Recognition Through an IL-12-Dependent Mechanism. J Immunol (Baltimore Md. 1950) (2003) 171:2251–61. doi: 10.4049/jimmunol.171.5.2251
100. Kalia V, Sarkar S. Regulation of Effector and Memory CD8 T Cell Differentiation by IL-2-A Balancing Act. Front Immunol (2018) 9:2987. doi: 10.3389/fimmu.2018.02987
101. Ara A, Ahmed KA, Xiang J. Multiple Effects of CD40-CD40L Axis in Immunity Against Cancer. Cancer Immunother (2018) 7:55–61. doi: 10.2174/IT.5163614
102. Schoenberger SP, Toes RE, van der Voort EI, Offringa R, Meliefs CJ. T-Cell Functions. Immunotherapy (2015) 7(6):655–67. doi: 10.2217/imt.15.32
103. Frentsch M, Stark R, Matzmohr N, Meier S, Durlanik S, Schulz AR, et al. 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 Cancer Resection of Human Melanoma Infiltration. J Virol (2013) 87(12):6851–65. doi: 10.1128/JVI.03305-12
104. Ahrends T, Babala N, Xiao Y, YagitC, van Eenennaam H, Borst J, Dc27 Agonism Plus PD-1 Blockade Recapitulates CD4+ T-cell Help in Therapeutic Anticancer Vaccination. Cancer Res (2016) 76(10):2921–31. doi: 10.1118/0008-5472.CAN-15-3130
105. Hui E, Cheung J, Zhu J, Xu S, Taylor MJ, Wallweber HA, et al. T Cell Cytotoxicity Receptor CD28 Is a Primary Target for PD-1-mediated Inhibition. Science (2017) 355(6332):1428–33. doi: 10.1126/science.aaf1292
106. Kim KH, Kim HK, Kim HD, Kim CG, Lee H, Han JW, et al. J Immunol (Baltimore Md. 1950) (2015) 195(7):1540–7. doi: 10.1186/s40364-020-00228-x
107. Chen B, Wu S, Wang J, Yang Z, Yuan J, Wu J, et al. Bcl9 Promotes Cancer Progression and Immunotherapy. Oncoimmunology (2016) 5(5):e1163462. doi: 10.1080/2162402X.2016.1163462
108. Ray JP, Staron MM, Shyer J, Marshall HD, Gray SM, et al. The Interleukin-2-mTORc1 Kinase Axis Defines the Signaling, Differentiation, and Metabolism of T Helper 1 and Follicular B Helper T Cells. Immunity (2015) 43(4):690–702. doi: 10.1016/j.immuni.2015.08.017
109. Josephs SF, Ichim TE, Prince SM, Kesari S, Marincola FM, Escobed RO, et al. Unleashing Endogenous TNF-alpha as a Cancer Immunotherapeutic. J Trans Med (2016) 14(1):242. doi: 10.1182/blood-2016-01-6117-1
110. Montfort A, Colacics J, Levade T, Andreie-Ahadie N, Meyer N, Segui B. The TNP Paradox in Cancer Progression and Immunotherapy. Front Immunol (2019) 10:1818. doi: 10.3389/fimmu.2019.02515
111. Groom JR, Richmond J, Murooka TT, Sorensen EW, Sung JH, Bankert K, et al. CXCR3 Chemokine Receptor-Ligand Interactions in the Lymph Node Optimize CD4+ T Helper 1 Cell Differentiation. Immunity (2012) 37(6):1091–103. doi: 10.1016/j.immuni.2012.08.016
112. Mauldin IS, Wages NA, Stowman AM, Wang E, Smolkin ME, Olson WC, et al. Intratumoral Interferon-Gamma Increases Chemokine Production But Fails to Increase T Cell Infiltration of Human Melanoma Metastases. Cancer Immunol Immunother (2015) 64(10):1189–99. doi: 10.1007/s00262-016-1881-y
113. Nagarsrath N, Wicha MS, Zou W. Chemokines in the Cancer Microenvironment and Their Relevance in Cancer Immunotherapy. Nat Rev Immunol (2017) 17(9):559–72. doi: 10.1038/nri.2017.49
114. Vilgelm AE, Richmond A. Chemokines Modulate Immune Surveillance in Tumorgenesis, Metastasis, and Response to Immunotherapy. Front Immunol (2019) 10:333. doi: 10.3389/fimmu.2019.00333
115. Lebre MC, Burwell T, Vieira PL, Lora J, Coyle AJ, Kaspengen ML, et al. Differential Expression of Inflammatory Chemokines by Th1- and Th2-cell Populating Dendritic Cells: A Role for Different Mature Dendritic Cell Populations in Attracting Appropriate Effector Cells to Peripheral Sites of Inflammation. Immunol Cell Biol (2005) 83(5):325–35. doi: 10.1111/j.1440-1711.2005.01365.x
116. Bretscher P. On Analyzing How the Th1/Th2 Phenotype of an Immune Response Is Determined: Classical Observations Must Not Be Ignored. Front Immunol (2019) 10:1234. doi: 10.3389/fimmu.2019.01234
117. Lee HL, Tang JW, Lee SW, Yoo SH, Kwon JH, Nam SW, et al. Inflammatory Cytokines and Change of Th1/Th2 Balance as Prognostic Indicators for Hepatocellular Carcinoma in Patients Treated With Transarterial Chemoembolization. Sci Rep (2019) 9(1):3260. doi: 10.1038/s41598-019-40078-8
118. Lin W, Liu Z, Zhang H, Kong Y, Wang Z, Yang Z, et al. Imbalance of Th1/Th2 and Th17/Treg During the Development of Uterine Cervical Cancer. Int J Clin Exp Pathol (2019) 12(9):3604–12.
119. Takashima Y, Kawaguchi A, Kanayama T, Hayano A, Yamanaka R. Correlation Between Lower Balance of Th2 Helper T-cells and Expression of PD-L1/PD-1 Axis Genes Enables Prognostic Prediction in Patients With Glioblastoma. Oncotarget (2018) 9(27):19065–78. doi: 10.18632/oncotarget.24897
120. Hao CJ, Li J, Liu P, Li XL, Hu YQ, Sun JC, et al. Effects of the Balance Between Type 1 and Type 2 T Helper Cells on Ovarian Cancer. Genet Mol Res (2016) 15(2). doi: 10.4238/gmr.15027936
121. Hong CC, Yao S, McCann SE, Dolnick RY, Wallace PK, Gong Z, et al. Pretreatment Levels of Circulating Th1 and Th2 Cytokines, and Their Ratios,
are Associated With ER-negative and Triple Negative Breast Cancers. Breast Cancer Res Treat (2013) 139(2):477–88. doi: 10.1007/s10549-013-2549-3

133. Ruterbusch M, Prinzen KB, Shchata L, Pepper M. In Vivo Cd4+ T Cell Differentiation and Function: Revisiting the Th1/Th2 Paradigm. Ann Rev Immunol (2020) 38(1):705–25. doi: 10.1146/annurev-immunol-103019-085803

134. Chen K, Kolls JK. Interleukin-17A (Il17a). Gene (2017) 614:8–14. doi: 10.1016/j.gene.2017.01.016

135. Zhang Q, Qin J, Zhong L, Zhang B, Zhang Y, et al. Cd35-Mediated Th2 Immune Polarization Promotes Metastasis in Luminal Breast Cancer. Cancer Res (2015) 75(20):4312–21. doi: 10.1158/0008-5472.CAN-14-3590

136. Crotty S. T Follicular Helper Cell Differentiation, Function, and Roles in Disease. Immunity (2014) 41:529–42. doi: 10.1016/j.immuni.2014.10.004

137. Park JM, Park JH, Lee S, Kim GL, Oh JH, Lee JY, et al. Early Role of Cd4+Itsgf4+ &lt;/sup&gt; Th1 Cells and Antibodies in Her-2 Adenovirus Vaccination Protection Against Autochthonous Mammary Carcinomas. J Immunol (2005) 174(7):4228. doi: 10.4049/jimmunol.174.7.4228

138. Park JM, Terabe M, Sakai Y, Munasinghe J, Forni G, Morris JC, et al. Therapy of Her-2 Status, and Prognosis of Patients With Luminal B Breast Cancer. Cancer Res (2015) 75(4):1016–25. doi: 10.1158/0008-5472.CAN-14-0798

139. Schwartz M, Zhang Y, Rosenblatt JD. B Cell Regulation of the Anti-Tumor Response and Role in Carcinogenesis. J Immunother Cancer (2016) 4:40–6. doi: 10.1186/s40425-016-0145-x

140. Mishra R, Patel H, Alansiz S, Yuan L, Garrett JT. Her3 Signaling and Targeted Therapy in Cancer. Oncol Rev (2018) 12(1):355. doi: 10.4081/oncol.2018.355

141. Scharpenseel H, Mutha S, Fracol M, McMillan MT, Berk E, Xu S, et al. Loss of Anti-Her2 Antibody-Dependent Immunity Occurs in Breast Tumorigenesis. J Natl Cancer Inst (2009) 101(10):736–50. doi: 10.1093/jnci/djp082

142. Tovey SM, Brown S, Doughty JC, Mallon EA, Cooke TG, Edwards J. Poor Correlation of Her2 Expression With Clinicopathological Characteristics and is Negatively Associated With Outcomes. J Immunother Cancer (2016) 4(1):e012301. doi: 10.1186/s40428-015-022301

143. Lowenfeld L, Mick R, Datta J, Xu S, Fitzpatrick E, Fisher CS, et al. Dendritic Cell Vaccination Enhances Immune Responses and Induces Regression of Her2(pos) Dcis Independent of Route: Results of Randomized Selection Design Trial. Clin Cancer Res (2017) 23(12):2961–71. doi: 10.1158/1078-0432.CCR-16-1924

144. Rosenthal C, Datta J, Lowenfeld L, Xu S, Fitzpatrick E, Fisher CS, et al. Dendritic Cell Vaccination Enhances Immune Responses and Induces Regression of Her2(pos) Dcis Independent of Route: Results of Randomized Selection Design Trial. Clin Cancer Res (2017) 23(12):2961–71. doi: 10.1158/1078-0432.CCR-16-1924

145. Gravalos C, Jimeno A. Her2 in Gastric Cancer: A New Prognostic Factor and is Negatively Associated With Outcomes. Ann Oncol (2017) 28(1):xii11. doi: 10.1093/annonc/mdx081

146. Peng M, Mo Y, Wang Y, Wu P, Zhang Y, Xiong F, et al. Neoantigen Vaccine: An Emerging Tumor Immunotheraphy. Mol Cancer (2019) 18(1):128. doi: 10.1186/s12934-019-1553-6

147. Giltman JM, Moeder CB, Campbell RL. Combined HER2-EGFR Score in Triple-Negative Breast Cancer Provides Prognostic and Predictive Significance Superior to Individual Biomarkers. Sci Rep (2020) 10(1):3009. doi: 10.1186/s13246-020-06954-1

148. Basu A, Ramamootthi G, Jia Y, Faughin J, Wiener D, Awshah S, et al. Immunotherapy in Breast Cancer: Current Status and Future Directions. Adv Cancer Res (2019) 143:295–349. doi: 10.1016/bs.acr.2019.03.006

149. Ogden A, Bhattachar S, Sahoo B, Mongan NP, Alsaleem M, Green AR, et al. Combined Her2-Efgr Score in Triple-Negative Breast Cancer Provides Prognostic and Predictive Significance Superior to Individual Biomarkers. Sci Rep (2020) 10(1):3009. doi: 10.1186/s13246-020-06954-1

150. Zhang Q, Qin J, Zhong L, Zhang B, Zhang Y, et al. Cd35-Mediated Th2 Immune Polarization Promotes Metastasis in Luminal Breast Cancer. Cancer Res (2015) 75(20):4312–21. doi: 10.1158/0008-5472.CAN-14-3590

151. Bae SY, La Choi Y, Kim S, Kim M, Kim J, Jung SP, et al. Her2 Status by Immunohistochemistry is Correlated With Poor Prognosis in Hormone Receptor-Negative Breast Cancer Patients. Breast Cancer Res Treat (2013) 139(3):741–50. doi: 10.1007/s10549-013-2570-6
