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# Current modalities in cancer immunotherapy: immunomodulatory antibodies, CARs and vaccines

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## Abstract

Successes of immune checkpoint inhibitors (ICIs) and chimeric antigen receptor (CAR) T cell therapy in curing patients with otherwise lethal cancers have validated immunotherapy as a treatment for cancer and have inspired excitement for its broader potential. Most promising is the ability of each approach to eliminate bulky and advanced-stage cancers and to achieve durable cures. Despite this success, to date only a subset of cancer patients and a limited number of cancer types respond to these therapies. A major goal now is to expand the types of cancer and number of patients who can be successfully treated. To this end a multitude of immunotherapies are being tested clinically in new combinations, and many new immunomodulatory antibodies and CARs are in development. A third major immunotherapeutic approach with renewed interest is cancer vaccines. While over 20 years of therapeutic cancer vaccine trials have met with limited success, these studies have laid the groundwork for the use of therapeutic vaccines in combination with other immunotherapies or alone as prophylactic cancer vaccines. Prophylactic vaccines are now poised to revolutionize cancer prevention as they have done for the prevention of infectious diseases. In this review we examine three major cancer immunotherapy modalities: immunomodulatory antibodies, CAR T cell therapy and vaccines. For each we describe the current state of the art and outline major challenges and research directions forward.

### Keywords

cancer immunotherapy; cancer vaccine; immune checkpoint inhibitor; immunomodulator; chimeric antigen receptor (CAR); adoptive cell therapy

## 1. Introduction

Within the last two decades cancer immunotherapy, the therapeutic modulation or targeting of the immune response against cancer, has surged to the forefront of cancer research and treatment (Couzin-Frankel, 2013). Renewed interest in the field has been inspired by the

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dramatic success of two major cancer immunotherapies that achieve durable responses in patients with terminal stages of disease: immune checkpoint inhibitor (ICI) antibodies targeting the PD-1/CTLA-4 axes in advanced melanoma (now also in lung and renal cell carcinomas) and adoptive cell therapy (ACT) with chimeric antigen receptor (CAR) T cells targeting CD19 on B-cell leukemias and lymphomas (J. R. Brahmer et al., 2012; Brentjens et al., 2013; Brentjens et al., 2011; Hodi et al., 2010; Porter, Levine, Kalos, Bagg, & June, 2011; Topalian et al., 2012). Clinical responses to these therapies have highlighted the general power and promise of stimulating an immune response to treat cancer. Immune-based therapies can eliminate large tumor masses in advanced-stage cancer patients and elicit immunological memory that can lead to prolonged protection from cancer relapse (Chapman, D'Angelo, & Wolchok, 2015; Pedicord, Montalvo, Leiner, & Allison, 2011). These results stand in contrast to other targeted therapies that typically only extend lives by weeks and more rarely months (Maemondo et al., 2010; Maude et al., 2014; Schadendorf et al., 2015; Shaw et al., 2013). The results with CARs and ICIs validate different but complementary general therapeutic mechanisms. ICIs lead to the activation and expansion of existing tumor-specific immune cells that are otherwise suppressed in the tumor microenvironment (TME) while for CAR therapy, patients' immune cells are genetically engineered to acquire new tumor-targeting specificity and potency.

Despite the justified excitement about unprecedented clinical results, current immunotherapies are still only able to achieve durable responses in subsets of cancer patients. In the case of ICIs, only 15-25% of patients with selected tumor types (NSCLC, RCC and Merkel cell carcinoma) experience complete responses (J. Brahmer et al., 2015; Motzer et al., 2015; Nghiem et al., 2016; Postow et al., 2015). One of the biggest questions in cancer immunotherapy now is whether new ICIs or combinations of existing ICIs with other treatments can vastly improve the number of treatable patients and cancer types. To this end there has been an influx of clinical trials in which existing ICI antibodies are being combined with various standard of care therapies such as chemotherapy and radiotherapy or new immunomodulators and other immunotherapies such as vaccines (Khalil, Smith, Brentjens, & Wolchok, 2016). Currently there are >350 ongoing or planned clinical trials registered on *clinicaltrials.gov* in which an ICI is combined with one or more different treatments and this number is steadily growing. In the case of CAR therapy, the major success to date has been in hematologic malignancies targeting a single antigen, CD19. The predominant goal for the field now is to expand CAR therapy to additional patients and cancers by targeting other antigens including those on solid tumors (Morello, Sadelain, & Adusumilli, 2016). Successful treatment of solid tumors with CARs presents an additional challenge of creating cells that can function in the immune suppressive TME. Many CARs targeting antigens other than CD19 are already in preclinical or clinical development, and there are several therapeutic design strategies being tested to overcome TME immune suppression.

The successes of ICI and CAR therapies have led to mainstream realization that cancer is an immunological disease (Hanahan & Weinberg, 2011). It is now established that even the activity of chemotherapy and radiation depends on the patient's immune system and the ability of these standard therapies to induce immunogenic cell death (Galluzzi, Buque, Kepp, Zitvogel, & Kroemer, 2015). The idea that there is an immunological component to

cancer has been posited for over 100 years beginning with the development of Coley's toxin and the work of Paul Ehrlich (Coley, 1910; Kaufmann, 2008). Observation of the abscopal effect in 1953, in which local radiation treatment of a tumor led to elimination of metastases outside the treatment area, is now appreciated to be through activation of anti-tumor immunity (Mole, 1953; Postow et al., 2012). Around this time Burnet and Thomas put forward the cancer immunosurveillance hypothesis which posits that our immune system protects us from cancer as well as from pathogens (Burnet, 1957). This hypothesis was validated with pre-clinical experiments in the 1990s, and it was more conclusively shown in the 2000s with the elucidation of the process of tumor immunoediting defined by three outcomes: tumor elimination, equilibrium and escape (Dunn, Old, & Schreiber, 2004a, 2004b). The wide acceptance of this immune function supports the development of prophylactic cancer vaccines that would strengthen or boost anti-tumor immune memory that could be reactivated in the presence of early premalignant or malignant cells leading to their elimination and cancer prevention. After over 20 years of cancer vaccines being applied in advanced-stage cancer patients, only now are the first prophylactic cancer vaccines being tested in at-risk patients. Many existing vaccines that failed in clinical trials with late-stage cancer patients are appropriate candidates for testing in the prophylactic setting (Finn & Beatty, 2016).

## 2. Immunomodulatory antibodies

A major shift in antibody-based immunotherapy of cancer has been in targeting immune cells in the TME instead of cancer cells. Monoclonal antibodies (mAbs) for cancer therapy have been in development since the 1970s and early efforts were focused solely on targeting tumor-associated antigens (TAAs) and directly killing tumor cells (Kohler & Milstein, 1975). Antibodies can kill target cells through a variety of mechanisms such as antibody dependent cell cytotoxicity (ADCC), complement dependent cytotoxicity, interfering with cell signaling pathways, or facilitating tumor cell phagocytosis by macrophages (Scott, Wolchok, & Old, 2012). They can also be chemically conjugated to cytotoxic drugs or radioisotopes to deliver toxic payloads, or even fused to immunomodulatory antibodies to create bi-specific molecules that target immune cells to tumor antigens. Although the development of antibodies that target TAAs is still a very promising area of investigation it is noteworthy that of the ~20 total FDA-approved mAbs and conjugates for cancer therapies, the 5 antibodies targeting non-tumor cells in the TME were approved within the last five years (Redman, Hill, AlDeghaither, & Weiner, 2015). The first clinical application of an antibody targeting non-tumor cells in the TME targeted vascular endothelial growth factor (VEGF) affecting tumor vasculature with the goal of blocking tumor blood supply and angiogenesis, which is important for delivery of nutrients for tumor growth and metastasis (Bennouna et al., 2013). More recently immunomodulatory antibodies targeting the PD-1/PD-L1 and CTLA-4 inhibitory receptors on patients' T cells have moved to the forefront of antibody therapy showing objective responses with survival benefit in a large percentage of treated patients and complete durable responses of over 10 years for small subsets of patients (Ascierto & Marincola, 2015).

ICI antibodies function by reactivating patient's anti-tumor T cells to kill tumor cells. T cells are the major immune effector cells that mediate anti-tumor immunity, and understanding T

cell activation signaling is key to understanding the activity of immunomodulatory antibodies. T cell activation is regarded as a two-signal process requiring recognition of a specific MHC-presented antigenic peptide by the T cell receptor (TCR) and a second accessory co-stimulatory signal from a co-receptor on the target cell (L. Chen & Flies, 2013). If T cells receive only the TCR signal they enter into an anergic state, become suppressive or apoptose. T cells are also susceptible to suppressive signals such as those mediated through inhibitory receptors CTLA-4 and PD-1 that engage their ligands B71/2 and PD-L1, respectively, on antigen presenting cells (APCs). Activation of these checkpoint inhibition pathways plays an important role in dampening a potentially overactive immune response when fighting pathogens in order to prevent excessive immune pathology. These same inhibitory pathways can also be detrimental to antitumor T cell responses. The ligands for CTLA-4 and PD-1 (B71/2 and PD-L1, respectively) can be co-opted by tumor cells to inhibit tumor antigen-specific T cells. Antibodies that block inhibitory receptors or their ligands on T cells, APCs, or tumor cells enhance the effectiveness of tumor-specific T cells and lead to tumor rejection (Pardoll, 2012).

With the dramatic effectiveness of this approach, a major research effort was stimulated to expand the use of these antibodies to different cancers in combinations with other immunotherapies and to develop additional immunomodulatory antibodies targeting other known T cell suppression and co-stimulation pathways. In part, this effort involves identification of patients likely to respond and identifying predictive biomarkers of response (Topalian, Taube, Anders, & Pardoll, 2016). Additionally, efforts are underway to define molecular events and therapies that would turn initial non-responders into potential responders (Gajewski, 2015).

### 2.1 Modulating the tumor microenvironment with immune checkpoint inhibitors: anti-CTLA-4 and anti-PD1/PD-L1 antibodies

The first T cell inhibitory receptor to be discovered was the cytotoxic T-lymphocyte associated protein 4 (CTLA-4) (Krummel & Allison, 1995; Walunas et al., 1994). As part of the process of T cell activation, CTLA-4 translocates to the cell surface and acts to limit TCR signaling by activating inhibitory signaling and by blocking the CD28 co-stimulatory receptor from binding to its ligands B7-1/2. The importance of normal immune regulation by CTLA-4 can be seen in *Ctla-4* knockout mice, which develop fatal autoimmune disease with uncontrolled expansion of T cells (Waterhouse et al., 1995). CTLA-4 is also one of the key molecules used by regulatory T cells ($T_{regs}$) to suppress activation of effector T cells. In the TME it is expressed on tumor cells and on $T_{regs}$, both of which can suppress T cell lysis of tumors (Wing et al., 2008).

Allison et al. were the first to discover that antibodies blocking CTLA-4 binding to B71/2 could expand and activate anti-tumor T cells to inhibit growth of certain mouse tumors (Leach, Krummel, & Allison, 1996). This approach was tested in several Phase I and II trials leading to a Phase III validation trial in melanoma with two treatment arms – anti-CTLA-4 alone or in combination with the gp100 peptide plus adjuvant vaccine (Hodi et al., 2010). The results showed that the anti-CTLA-4 antibody improved survival even without the addition of the vaccine indicating that the antibody was relieving the suppression of

preexisting tumor-specific T cells. This trial was a major success as it was the first Phase III study to show durable improvement for patients with advanced stage melanoma, increasing median survival time by 4 months. Another striking feature of the response was that patients who survived beyond 2 years continued to respond for the length of the trial suggesting a potential cure. CTLA-4 blockade has since been shown to increase the breadth of the T cell repertoire targeting the tumor instead of simply expanding a few clones (L. Robert et al., 2014). The anti-CTLA4 antibody ipilimumab received FDA approval for treatment of metastatic melanoma in 2011. Currently anti-CTLA-4 antibodies are being tested in hundreds of registered clinical trials for several cancers including bladder, prostate and lung cancer and in combinations with other therapies such as PD-1 blockade, and other immunomodulatory antibodies and small molecules as well as radiation, chemotherapy, viral therapy, vaccines, and cryoablation (Postow et al., 2012; Waitz, Fasso, & Allison, 2012; Zamarin et al., 2014).

Programmed death 1 (PD-1) was the second major inhibitory receptor discovered on T cells for which an antagonistic antibody was developed. PD-1, expressed on activated and exhausted T cells, inhibits T cell function after binding to its ligands PD-L1 and PD-L2 expressed on cancer cells and APCs. This binding acts to down-regulate T cell signaling and inhibit IL-2 production and T cell expansion. PD-L1 can also suppress T cell function by interfering with CD28-mediated co-stimulation by binding to its ligand CD80 (J. J. Park et al., 2010). Antibodies blocking either PD-1 on T cells or PD-L1 on tumor cells were therapeutic in pre-clinical mouse tumor models (Ohigashi et al., 2005; Okudaira et al., 2009). Humanized antibodies targeting PD-1 were generated (nivolumab and pembrolizumab) and showed very successful clinical results in advanced stage melanoma where 40% of patients had objective responses compared to 12% for ipilimumab (C. Robert et al., 2015). This trial led to the approval in 2014 of anti-PD-1 antibody therapy for advanced stage melanoma. Anti-PD-1 therapy has since been approved for several other cancers including NSCLC refractory to platinum therapy with a 15% overall response rate (ORR) and RCC (21.5% ORR) (J. Brahmer et al., 2015; Motzer et al., 2015). An antibody targeting PD-L1, atezolizumab, was also approved in 2016 for the treatment of bladder cancer after a Phase III trial showed a 22% ORR and is now being evaluated in trials for several other cancers (Powles et al., 2014). Importantly the combination of CTLA-4 and PD-1 ICI antibodies is also FDA-approved given an unprecedented 58% ORR in a Phase III melanoma trial including a 11.5% complete response rate (Larkin et al., 2015). The combination of these agents is considered by many to be the primary treatment modality moving forward.

While effective for many patients, it is important to note that ICI therapy can also cause serious immune-related adverse events (irAE) (Michot et al., 2016). Serious irAEs include grade III to grade IV autoimmune toxicities that are most commonly dermatologic, gastrointestinal, hepatic, or endocrine related. In a study comparing ICI treatments targeting PD-1, CTLA-4, and the combination of PD-1 and CTLA-4 for metastatic melanoma, it was found that 16.3%, 27.3%, and 55.0% of patients receiving these therapies experienced serious grade III/IV irAEs, respectively. This result indicated that the toxicities as well as the efficacies from the combined therapies appear to be additive. Higher toxicities are consistently observed for CTLA-4-targeting ICIs compared to those targeting PD-1 (T. W.

Chen, Razak, Bedard, Siu, & Hansen, 2015). While most of the irAE toxicities can be managed or reversed using corticosteroids without negatively impacting tumor response, they are sometimes serious enough to warrant discontinuation of therapy (Larkin et al., 2015).

There is an ongoing effort to determine why the large majority of patients treated with anti-CTLA-4 and anti-PD-1/PD-L1 therapies do not respond. For both therapies some key biomarkers of response have emerged (Topalian et al., 2016). Expression of CTLA-4 and PD-L1 or PD-L2 pathway proteins on the tumor and/or tumor infiltrating T cells (TIL) for each respective ICI has been shown to correlate with response to therapy with the best responders being the ones with the highest levels of these molecules. The presence of a T cell infiltrate in tumors has also been shown to be important. Together these biomarkers fit with the mechanism of action of the ICIs as there must be an existing immune response that the antibodies can reactivate by blocking the immune checkpoints. Another interesting correlation with ICI response appears to be the extent of DNA mutations in the tumor (Rizvi et al., 2015; Snyder et al., 2014; Van Allen et al., 2015). This correlation suggests that the more highly mutated cancers are more likely to be under immune surveillance. However, there is no obvious threshold number of mutations and there are significant exceptions where patients with only a few mutations respond and some with highly mutated tumors do not. Looking at gene expression profiles of responders versus non-responders, type I interferon gene signature correlated with a better response (Diamond et al., 2011; Fuertes et al., 2011). It has also been demonstrated in pre-clinical studies that the gut microbiome plays a role in identifying patients who will respond to both PD-1 and CTLA-4 therapies (Sivan et al., 2015; Vetizou et al., 2015).

For patients who do not respond to PD-1 and CTLA-4 ICI antibody therapy, one major research focus is to induce conditions in the tumor that are associated with a favorable response prior to checkpoint inhibitor treatment, such as the presence of high numbers of T cells, primarily $CD8^+$ T cells, where previously there were very few (Gajewski, 2015). Strategies include radiation, activation of innate immune signaling pathways such as stimulator of interferon genes (STING), local delivery of type I interferons, inhibition of immunosuppressive oncogene pathways such as STAT-3 and beta-catenin, and induction of tertiary lymphoid structures in the tumor (Burdelya et al., 2005; Deng et al., 2014; Oh et al., 2012; Spranger, Bao, & Gajewski, 2015). Another strategy being investigated is the identification of probiotics that will enhance therapy and induce more favorable responses as suggested by pre-clinical studies in mice that showed the importance of the gut microbiome (Iida et al., 2013). In total there are over 240 registered clinical trials investigating the effects of ICIs targeting PD-1 or PD-L1 in combination with other therapeutic modalities.

### 2.2 Next-generation immune checkpoint inhibitors

Immunomodulatory antibodies acting on pathways other than CTLA-4 and PD-1 are also under development. With the combination of CTLA-4 and PD-1 blockade leading to additive responses in a subset of patients, it is tempting to consider that the non-responders could be successfully treated with antibodies that suppress other inhibitory checkpoints or agonize stimulatory immune response pathways (Larkin et al., 2015). It is too early to tell whether

these new therapies will lead to an incremental change in outcomes or if there will be breakthrough drugs or combinations that will significantly expand the number of treatable patients and tumor types. As the number of these agents and their potential targets grow, identification of biomarkers that can predict effectiveness of therapy will be paramount for creating rational treatment combinations. Table 1. lists and categorizes clinical trials with some of the most promising new immunomodulators that are reviewed below.

One T cell inhibitory receptor being targeted in the clinic is T-cell membrane protein 3 (TIM-3). This receptor is up-regulated on exhausted T cells and has been shown to suppress T cell functions and induce apoptosis (Y. H. Huang et al., 2015; Sabatos et al., 2003; Zhu et al., 2005). In preclinical studies in mice with colon cancer, melanoma, and sarcoma, TIM-3 blockade in combination with PD-L1 blockade has shown a synergistic improvement in tumor elimination and control (Kim et al., 2016; Sakuishi et al., 2010). Because TIM-3 is known to interact with several ligands, such as galectin-9, HMGB1, phosphatidyl serine and CEACAM-1, the precise mechanism of TIM-3 blockade that is leading to favorable outcomes is still unclear. TIM-3 blocking antibodies are being tested in clinical trials in combination with anti-PD-1 therapy.

Another therapeutic target with an antagonistic antibody already in clinical trials is the lymphocyte-activation gene-3 (LAG-3) molecule. LAG-3 is expressed on activated effector T cells, $T_{regs}$, B cells and plasmacytoid DC (pDCs) (Baixeras et al., 1992). Mechanistically, LAG-3 acts as a direct competitor for MHC-II binding to CD4, which leads to suppression of T cell activation and increase of the suppressive abilities of $T_{regs}$ (C. T. Huang et al., 2004). Anti-LAG-3 antibodies work to block this interaction. LAG-3 is co-expressed with PD-1 on exhausted T cells and the two act synergistically in tumor models (Blackburn et al., 2009; Woo et al., 2012). Thus, efforts are focused on simultaneously blocking the effect of both molecules. Several trials are underway testing LAG-3 and PD-1 dual blockade in a variety of solid tumors as well as one trial in combination with an anti-4-1BB agonist antibody.

KIR2DL1/2L3 is an inhibitory member of the Killer-cell immunoglobulin-like receptor (KIR) family of receptors found on NK cells and some T cells. Binding to MHC Class I leads to inhibition of NK-mediated cell killing, and antibodies targeting this molecule have been shown in pre-clinical work to reactivate this NK function. Multiple trials are underway testing the anti-KIR antibody lirilumab in combination with other ICIs as well as TAA-targeting antibodies believed to act via NK-mediated ADCC (Kohrt et al., 2014; Romagne et al., 2009).

Yet another ICI antibody in clinical trials targets V-domain Ig suppressor of T cell activation (VISTA). VISTA is found on hematopoeitic cells including T cells and DCs as well as on tumor cells. VISTA was shown to inhibit T cell proliferation and cytokine production *in vitro* as well as anti-tumor immunity in pre-clinical mouse experiments. Pre-clinical experiments show that treatment with a VISTA-specific antagonist antibody relieves this inhibition and controls tumor growth in several mouse tumor models. The anti-VISTA ICI has also been shown to synergize with PD-1 blockade (Le Mercier et al., 2014; Lines et al., 2014; L. Wang et al., 2011).

A complementary strategy to inhibiting immune checkpoint pathways is to instead target co-stimulatory receptors with agonistic antibodies. Several of these antibodies are currently in clinical trials, and antibodies targeting molecules such as CD40, OX40 and 4-1BB have been in trials since the mid-late 2000s (Table 1.). The main advance in the new trials has been targeting these molecules in combination with other therapies based on extensive pre-clinical work showing synergy with other therapeutic modalities.

One co-stimulatory target protein is the 4-1BB receptor that is expressed on T cells, NK cells and monocytes (Shuford et al., 1997). Binding of 4-1BB on T cells to its ligand 4-1BBL on DCs leads to enhanced proliferation and up-regulation of anti-apoptotic proteins, ultimately protecting T cells from activation induced cell death (AICD) (H. W. Lee et al., 2002; Starck, Scholz, Dorken, & Daniel, 2005). Pre-clinical studies have been carried out investigating the effects of anti-4-1BB agonist therapy alone and in combination with several other modalities and found it to improve tumor regression (May, Chen, Zheng, & Liu, 2002; Melero et al., 1997). While initial clinical testing of anti-4-1BB was halted due to liver toxicity, there are new ongoing trials testing it in combination with various drugs (Bartkowiak et al., 2015; Kocak et al., 2006; A. Yonezawa, Dutt, Chester, Kim, & Kohrt, 2015).

OX40 is a co-stimulatory receptor expressed on T cells, NK cells and neutrophils. It interacts with OX40L found on APCs and various other immune cells (Linch, McNamara, & Redmond, 2015). Activation of OX40 leads to T cell proliferation, survival and cytokine secretion and was also found to induce AICD in $T_{regs}$ (Hirschhorn-Cymerman et al., 2009; Rogers, Song, Gramaglia, Killeen, & Croft, 2001). Anti-OX40 antibodies have been shown to mediate T cell-dependent anti-tumor activity and establishment of tumor-specific memory in pre-clinical experiments in mice (Pan, Zang, Weber, Meseck, & Chen, 2002). Anti-tumor activity was recently observed in a Phase I clinical trial for melanoma and RCC, and other trials are ongoing (Curti et al., 2013).

Glucocorticoid-induced TNFR-related protein (GITR) is another co-stimulatory receptor up-regulated on activated T cells and constitutively expressed on $T_{regs}$ (Nocentini & Riccardi, 2005). Unlike the similarly regulated immune checkpoint proteins, GITR acts to enhance function of effector T cells and confers resistance to the inhibitory effects of $T_{regs}$ (Kanamaru et al., 2004; Ronchetti et al., 2007). In pre-clinical studies, agonistic GITR antibodies mediated tumor rejection in several mouse cancer models (Cohen et al., 2010; Ko et al., 2005; Schaer et al., 2013). Anti-GITR antibodies are currently in early phase clinical trials for several different cancers.

CD40 is normally expressed on macrophages, monocytes and B cells, as well as on various cancers including melanoma, lymphoma, leukemia and carcinomas. The ligand for CD40, CD40L, is expressed on $CD4^+$ T cells and plays a role in the activation of T cells by APCs (Eliopoulos & Young, 2004). There is promising pre-clinical data for the effects of anti-CD40 agonistic antibodies that are being tested in several clinical trials (Hamzah et al., 2008; Horton et al., 2010; Luheshi et al., 2016; White et al., 2011). Currently the most promising results have been with anti-CD40 antibodies combined with chemotherapy to treat pancreatic cancer (G. L. Beatty et al., 2011). Unexpectedly, in mouse models the

predominant anti-tumor response appears to depend on anti-tumor effects of activated macrophages.

Another new target being explored is the CD73 ecto-5′-nucleotidase that leads to the degradation of AMP into adenosine, a small molecule metabolite that promotes immunosuppression and angiogenesis in the TME (Antonioli, Yegutkin, Pacher, Blandizzi, & Hasko, 2016). It is found primarily on $T_{regs}$ and is a major contributor to their immunosuppressive activity. It is also found on tumor cells and other stromal cells in the TME. Pre-clinical models with antibodies targeting CD73 alone or in combination with other modalities such as anti-CTLA-4 and anti-PD-1, have shown significant anti-tumor activity (Allard, Pommey, Smyth, & Stagg, 2013; Loi et al., 2014). Finally, a trial looking at anti-CD73 monotherapy or combined therapy with PD-L1 ICI therapy is underway.

CD27 is a co-stimulatory molecule in the TNF-super family related to CD40, 4-1BB and OX40 that is constitutively expressed on T cells and some B and NK cells. Its only known ligand is CD70. Activation of CD27 leads to activation of the NF-kB pathway and cell survival, activation and proliferation. CD27 activation has also been shown to promote broadening of the T cell repertoire. Anti-CD27 agonist antibodies have shown activity in pre-clinical models enhancing $CD8^+$ T cell responses and having a synergistic effect with anti-PD-1 therapy. Clinically the agonist antibody varlilumab has been shown to be well-tolerated and to have several positive effects in subsets of patients including decreasing the number of $T_{regs}$ and mediating $CD8^+$ T cell responses. Interestingly, varlilumab can also act to directly kill tumor cells that are over-expressing CD27 by mediating ADCC (He et al., 2013; Thomas, He, Marsh, & Keler, 2014).

An additional co-stimulatory molecule targeted clinically by an immunomodulatory antibody is inducible T-cell co-stimulator (ICOS) (Hutloff et al., 1999). ICOS is expressed on activated T cells and is especially important for Th2 T cell responses. Pre-clinical experiments showed that ICOS knockout dramatically decreases the efficacy of anti-CTLA-4 treatment in controlling and eliminating tumors. Agonistic anti-ICOS antibody can synergize with CTLA-4 blockade in the anti-tumor effect (Fan, Quezada, Sepulveda, Sharma, & Allison, 2014).

## 3. CAR T cell therapy

CARs are engineered antigen receptor proteins consisting of an antigen binding region and TCR signaling domains. When expressed on a patient's T cells, CARs act to re-direct T cells' effector functions, target cell lysis and cytokine production upon binding to antigens on tumor cells (Sadelain, Brentjens, & Riviere, 2013). T cells are genetically modified, most commonly via a retrovirus or a lentivirus that encodes the CAR, expanded and then adoptively transferred back into the patient (Barrett, Singh, Porter, Grupp, & June, 2014).

The major clinical success of CAR T cell therapy so far has been with CARs targeting CD19 in the treatment of refractory pediatric and adult B-ALL. Across multiple trials conducted by various groups, CD19-CAR therapy has resulted in astounding ~70-95% complete response rates compared to an expected 7% 5-year survival rate for standard therapy for refractory

adult ALL (Davila et al., 2014; Fielding et al., 2007; Kebriaei et al., 2016; D. W. Lee et al., 2014; D. W. Lee et al., 2015; Maude et al., 2014; J. H. Park et al., 2014). CD19-targeting CARs have also shown modest success in treating adult chronic lymphoblastic leukemia (CLL) (28% complete response rate) and are showing early promise in clinical trials for other hematological malignancies including B-NHL and MM (Garfall et al., 2015; Kochenderfer et al., 2015; J. H. Park et al., 2014; Savoldo et al., 2011; Schuster et al., 2014). The first CARs were made as early as 1989, however it took 22 years to attain this first clinical success with CAR therapy (Gross, Waks, & Eshhar, 1989). This success was the culmination of several technological advancements including those that facilitate gene delivery, enhance T cell growth and expansion, as well as CAR design considerations including the choice of CD19 antigen and most importantly the addition of co-stimulatory signaling domains such as CD28 and 4-1BB that led to enhanced CAR T cell persistence, proliferation and potency (Maher, Brentjens, Gunset, Riviere, & Sadelain, 2002; Zhong, Matsushita, Plotkin, Riviere, & Sadelain, 2010).

In addition to further optimizing CD19-CAR therapy to treat other $CD19^+$ malignancies, a major goal in the CAR therapy field is to select new tumor antigens for a broader range of cancers, including additional hematological malignancies and solid tumors (Morello et al., 2016). This research is still focused on the overarching issues of antigen choice, CAR T cell persistence *in vivo*, proliferation and potency, although the TME of solid cancers poses additional engineering challenges. In response, researchers are using new design strategies that are being tested both pre-clinically and/or in clinical trials. Table 2. contains a list of registered clinical trials using CAR T cells organized by the different antigens that they target.

### 3.1 CAR-targeted antigens: beyond CD19

The focus on CD19 was paramount to the CAR therapy success, as CD19 has many ideal characteristics of a prototype antigen. It is present on nearly all cancer cells within a patient, thus targeting it is likely to eliminate the cancer; it plays an active role in tumorigenesis so cancer cells are less likely to escape; and it is specific to cancer cells and non-essential tissues. While the downside of the incomplete tumor specificity is that tumor rejection is accompanied with deletion of normal cells in the B cell lineage, this toxicity is manageable clinically and has been well tolerated by patients when treated with regular intravenous immunoglobulin (Davila et al., 2014; Maude et al., 2014).

The tumor-specificity of a CAR is especially important to consider. Clinical trials with adoptively transferred T cells expressing a MAGE-A3 targeted affinity-enhanced TCR and CAR cells targeting ErbB2 resulted in lethal on-target/off-tumor toxicities (Cameron et al., 2013; Morgan et al., 2010). There are currently over 30 tumor antigens being targeted by CARs in registered clinical trials and even more are in pre-clinical development. There are very few antigens that are either entirely tumor-specific or specific to tumors and non-essential tissues, like CD19. For hematological cancers these antigens include CD22 and CD20 (Haso et al., 2013; Till et al., 2012). For solid tumors they include abnormally glycosylated MUC1 and CSPG4 proteins and the abnormal splice variant of EGFR, EGFRvIII (Beard et al., 2014; Johnson et al., 2015; Lohmueller et al., 2016; Wilkie et al.,

2008). Search for other abnormally glycosylated or abnormally spliced targets could yield exciting new antigen targets for CAR therapy. The great majority of tumor targets being tested show over-expression on tumors, and lower levels of expression on normal tissues (hTERT, CEA, PSMA, GD2, MUC16, HER2/ERBB2, MSLN, IL-13R, alphaFR, EpCAM, RORgamma, CD4, CD33, FR, kappa-light chain and VEGFR-2). For each of these antigens, extensive safety testing will be required and potentially a transient CAR T treatment as in the case of MSLN targeted CAR (G. L. Beatty et al., 2014). Most CAR clinical trials are set up as dose-escalation studies testing a wide range of T cell numbers. It has been observed that lower numbers of potentially harmful CAR T cells show less toxicity, indicating that perhaps off-target toxicity can be managed by transferring fewer cells. However, none of these trials so far has shown a durable anti-tumor response. Alternative methods being explored to lower toxicity are limiting the CAR T cell activity to the tumor by either intratumoral injection and/or local activation or by affinity-tuning the antigen binding domain of the CAR (Adusumilli et al., 2014; Liu et al., 2015; Roybal et al., 2016).

In addition to tumor specificity there are other major considerations when choosing CAR targets. Many of these characteristics were emphasized in the report from the 2009 NCI workshop in which 75 tumor antigens were systematically evaluated and prioritized as candidates for cancer vaccines (Cheever et al., 2009). This prioritization was based on several criteria including proven therapeutic function, immunogenicity, role in oncogenicity, specificity, expression level, expression on cancer stem cells, the number of patients with tumors expressing the antigen, the number of epitopes and cellular localization. Similar criteria apply to CAR antigens and many of these antigens could be prioritized for targeting by CARs.

Another exciting approach that effectively increases the tumor-specificity of CAR therapy is based on antigen combinations using logic gate CARs. The first of these gates demonstrated was a logical “AND gate” for which only cells expressing antigens A AND B are lysed by CAR cells (Kloss, Condomines, Cartellieri, Bachmann, & Sadelain, 2013). This behavior was accomplished by creating a $1^{st}$ generation CD3-zeta CAR with a weakened antigen binding domain for one target antigen, co-expressed with a co-stimulatory CD28 CAR recognizing the second antigen. Another logical circuit designed was an A AND NOT B circuit in which cells with antigen A but not antigen B were killed, using an inhibitory CAR to inhibit CAR signaling in response to antigen B (Fedorov, Themeli, & Sadelain, 2013). A more recent approach uses a receptor to sense the first signal and then turn on the production of a CAR to sense the second signal (Roybal et al., 2016). Finally, while most CARs target tumor-expressed antigens, there are also emerging examples of CARs targeting molecules specific to immunosuppressive cells in the TME. In 2014 Wang et al. created a CAR to target fibroblast activation protein (FAP) expressed on cancer-associated fibroblasts (CAF), leading to better tumor control without major toxicities (L. C. Wang et al., 2014). The recent case report of CD19 CAR T cells leading to a complete remission of a $CD19^-$ cancer suggests that the CD19 CAR could be functioning not only by destroying $CD19^+$ tumor cells but possibly also by eliminating $CD19^+$ immunosuppressive regulatory B cells (Garfall et al., 2015).

### 3.2 Optimizing CAR design and therapy

Further work is being applied to optimize the CAR protein design as well as other parts of CAR therapy including immunodepletion pre-conditioning therapy, using different defined T cell subtypes as therapeutic cells and finally the addition of accessory proteins to augment the CAR T cells' activity. All parts of the CAR, the antigen binding region, extracellular spacer, transmembrane domain, co-signaling domain and activation domain, have undergone some level of design optimization (Sadelain et al., 2013). From both pre-clinical and clinical studies principles of CAR design have emerged, although aspects of each are still active areas of investigation. Ideally one could imagine the creation of a universal “best CAR” architecture that could be used interchangeably with any new antigen binding region. However, it is unclear whether such an architecture is possible. For one, it has been shown that the immune synapse, the space between the T cell and target cell, is important for CAR efficiency, and the extracellular spacer length for each new antigen targeted might need to be tailored to that antigen (Haso et al., 2013; Hudecek et al., 2015). It is also possible that certain co-signaling domain configurations will be better suited to different TMEs requiring personalized CAR architectures for individual patients, similar to ICI antibody combinations (Condomines et al., 2015). Co-signaling domains to date include CD28, 4-1BB, ICOS, DAP10 and OX40 (Altvater, Pscherer, Juergens, & Rossig, 2005; Guedan et al., 2014; Maher et al., 2002; Zhong et al., 2010). Each co-stimulatory domain drives distinct biomolecular pathways leading to unique effects on CAR persistence, survival, metabolism and T cell fate (Kawalekar et al., 2016; Long et al., 2015). When over-stimulated these co-receptors can also have negative effects such as causing AICD and T cell exhaustion (Hombach & Abken, 2011). Interestingly, in the case of CD19 CAR trials, several different architectures including different extracellular spacer domains and co-stimulatory domains have had very similar clinical outcomes. These results suggest that for treating some cancers the specific CAR architecture does not have a major impact on outcome. So far the CARs in clinical testing have been developed through rational empirical testing of between 2-10 different designs at one time (Alonso-Camino et al., 2013; Duong et al., 2013). Future implementation of these high-throughput CAR generation methods will also require new high-throughput assays to screen larger numbers of CARs in ways that will be relevant to *in vivo* CAR activity. Such methods have the capability of further clarifying a set of optimal CAR design rules and lead to more rapid CAR development, especially for new antigen targets.

Most clinical trials and pre-clinical experiments to date have been performed using bulk and undefined T cell populations expressing the CAR which have been variable among patients (Brentjens et al., 2011). There is some focus now on better defining the optimal T cell subsets for use in therapy (Riddell et al., 2014). Pre-clinical studies have shown that there are subsets of T cells that lead to longer term CAR T cell persistence and the ability to control tumor growth. Combinations of $CD4^+$ and $CD8^+$ T cells together have been shown to be better than $CD8^+$ T cells alone, and $CD4^+$ and $CD8^+$ naïve and central memory T cells ($T_{cm}$) appear to be more effective than effector memory T cells ($T_{em}$). Another general approach to augment CAR T cell function is to provide an accessory protein along with the CAR, either constitutively or inducibly-expressed upon T cell activation, creating so-called “armored CARs” (Morello et al., 2016; Pegram, Park, & Brentjens, 2014). Some of these

accessory proteins include cytokines such as IL-12 or switch receptors that can bind to immunosuppressive proteins on tumor cells and lead to CAR T-cell co-stimulation (Liu et al., 2016). It is possible that certain accessory proteins will be optimal for patients with certain TMEs requiring a personalized CAR approach. Another common set of accessory genes is CAR suicide switches which allow for drug-mediated depletion of CAR T cells. These switches will be especially useful for Phase I trials testing the safety of new CARs with unknown toxicities and in the cases where the CARs target antigens found on normal cell populations that can be re-populated only after the CAR T cells are eliminated from the patient (Budde et al., 2013; Narayanan et al., 2011; X. Wang et al., 2011).

Finally, while CAR therapy is not yet FDA-approved, such approval is expected for CD19 CARs. There are currently over 50 registered CD19 CAR clinical trials across various institutions accounting for a treatment population of over 3300 patients. As CAR therapy is very complex involving genetic modification, culturing and expansion of a patient's cells, it will require major efforts for process standardization in specialized centers before bringing it to widespread use (Kaiser et al., 2015; X. Wang & Riviere, 2016).

## 4. Therapeutic cancer vaccines

The general purpose of a cancer vaccine is to elicit, expand or boost patients' tumor antigen-specific T cells and antibodies. In the therapeutic setting, vaccines are intended to activate the patient's immune cells that have already effectively lost the battle with cancer. Therapeutic cancer vaccines have been tested for many different cancers, in a variety of antigen and adjuvant combinations and delivery methods (Banday, Jeelani, & Hruby, 2015; Melero et al., 2014). They have targeted a plethora of different antigens including non-mutated shared tumor antigens as well as patient-specific mutated antigens. Many of these vaccines have elicited antigen-specific immune responses as defined by the production of antigen-specific T cells and antibodies in both pre-clinical and clinical settings. However, clinical outcomes have been largely disappointing. The major reason for this is now known to be the immune suppression established during the many years of tumor growth, which affects the ability of the vaccine to induce strong immune responses systemically and the ability of the induced immune response to function at the tumor site (Finn, 2003).

The first vaccine formulations were created from irradiated whole tumor cells, often also transfected to express immunostimulatory cytokines, that were expected to present shared antigens as well as patient-specific (unique) tumor antigens (Dranoff et al., 1993; Hanna & Peters, 1978). This approach had the advantage of not requiring knowledge of specific antigens expressed on the tumor and also of potentially eliciting a response to many different tumor antigens. Unfortunately these vaccines were largely unsuccessful as tumor cells likely had been previously immune-edited and/or may have even co-expressed immune suppressive molecules rendering them unlikely to elicit robust immune responses (Eager & Nemunaitis, 2005). There was some early clinical success using a related anti-idiotype vaccine approach for B-cell lymphomas in which antibodies generated from a patient's lymphoma cells were used as the vaccine antigen in combination with an adjuvant. Several patients were able to mount an immune response to their lymphomas and some even had complete disease

regressions, however the vaccine ultimately did not meet clinical endpoints in a Phase III trial (Kwak et al., 1992; Levy et al., 2014).

As more and more tumor antigens were being identified, the field shifted to vaccines based on defined tumor antigens and one of a variety of immunostimulatory adjuvants, such as those that stimulate toll-like receptors (TLRs) or other pattern recognition receptors (PRRs) (Goutagny, Estornes, Hasan, Lebecque, & Caux, 2012; Parmiani et al., 2014; Steinhagen, Kinjo, Bode, & Klinman, 2011). The antigens were expected to be taken up by a patient's APCs, processed and presented to T cells. The soluble antigen was expected to stimulate antibody production by B cells. It became obvious that the patients' APC function was also compromised in the TME. This realization has led to new approaches, facilitated by the discovery of conditions under which human dendritic cells (DCs) could be generated and matured *in vitro* (Steinman & Banchereau, 2007). Patients' DCs were grown *in vitro* from peripheral blood monocytes, loaded with antigen, fully matured and re-infused into patients to stimulate anti-tumor immunity, primarily T cells (Nair, Archer, & Tedder, 2012; Tedder & Jansen, 2001). Other vaccine approaches have included DNA encoding the antigen to be taken up by DC in vivo, or loaded on DCs in vitro and presented to T cells (Rice, Ottensmeier, & Stevenson, 2008). Viruses, bacteria and yeast, engineered to express tumor antigens have also been used. Many of these approaches were capable of stimulating antigen-specific T cell and antibody responses and some even showed a marginal therapeutic benefit (Melero et al., 2014).

The first therapeutic cancer vaccine to be approved by the FDA, Sipleucel-T (trade-name Provenge) for prostate cancer combines *in vitro* generation and antigen (prostatic acid phosphatase, PAP) loading of patients' DCs with *in vitro* stimulation of patients' T cells. The whole mixture, antigen-loaded DC and *in vitro* stimulated T cells is then reinfused into the patient. FDA-approval was based on the overall survival benefits in 3 Phase II trials and a median 4.1 month survival increase in a Phase III clinical trial for patients with metastatic, asymptomatic hormone-refractory prostate cancer. There are ongoing clinical trials trying this vaccine in combination with various ICIs (Wei, Fong, & Small, 2015). While the positive clinical results with Provenge are notable, the company developing it, Dendreon, filed for bankruptcy soon after this FDA approval, highlighting the difficulty in bringing an expensive and patient-personalized therapy into widespread use.

For the future of therapeutic cancer vaccines active areas of investigation include the generation of new adjuvants, enhanced antigens and personalized peptide neoantigens (Banday et al., 2015; Gubin, Artyomov, Mardis, & Schreiber, 2015). Other promising approaches include the optimization of vaccine antigens to remove potential immune inhibitory epitopes and the use of multiple antigens to increase the breadth of the vaccine-elicited immune response (Cecil et al., 2014; Disis et al., 2013). Finally, the path forward for therapeutic cancer vaccines will depend on a more thorough understanding of the inhibitory signals in the TME and their application in combination with other immunomodulatory agents (van der Burg, Arens, Ossendorp, van Hall, & Melief, 2016).

## 5. Prophylactic cancer vaccines

The frontier in the field of cancer vaccines is the development of vaccines for the prevention of cancer (Finn & Beatty, 2016). The first FDA-approved prophylactic cancer vaccines prevent cancer indirectly by preventing infection with viruses known to cause cancer. Two of these vaccines, Gardasil and Cervarix, target and prevent human papilloma virus (HPV) infection, responsible for over 70% of cervical cancers, and the third, Recombivax HB, is for the prevention of hepatitis B infection, a major cause of liver cancer. The vaccines block infection largely through the induction of neutralizing antibodies. The results of several large clinical trials demonstrate their success at preventing cancer and the safety of this approach - 10 years for HPV and 25 years for hepatitis B (Schiller, Castellsague, & Garland, 2012; Trepo, 2014). As most cancers are not known to be caused by viruses there is great need to create vaccines to prevent non-viral cancers.

Currently, it is thought that ideal antigens for preventative cancer vaccines are those found on pre-malignant lesions and/or cancer stem cells (Kensler et al., 2016). Vaccinating individuals diagnosed with premalignant lesions who therefore are at an increased risk for cancer would strengthen immunosurveillance and lead to elimination of nascent tumors and their initiating cells at an earlier stage of tumor progression before a suppressive TME is likely to form. In addition to this requirement, it is also favorable to target antigens that are able to drive tumorigenesis, as cells that lose these driver genes would be less likely to survive.

In Table 3. we present a list of the subset of the top 75 tumor antigens from the NCI 2009 Workshop found to be expressed on premalignant lesions and/or stem cells, or are oncogenes and list the premalignant lesions that can be targeted. Many of these antigens have already been targeted by therapeutic vaccines. These antigens include the major families of the cancer/testis (CT) antigens including MAGE-A1, -A3 and –A4, NYESO-1 and GAGE which are found on early-stage invasive ductal carcinomas and ductal carcinoma *in situ*, squamous dysplasia leading to head and neck cancer, as well as esophageal squamous cell carcinoma *in situ* (Y. T. Chen, Panarelli, Piotti, & Yantiss, 2014; Piotti, Scognamiglio, Chiu, & Chen, 2013). Three other extensively studied tumor antigens found on various adenocarcinomas as well as on premalignant lesions are mesothelin, abnormally glycosylated Mucin 1 (MUC1) and hTERT. These antigens are expressed on pancreatic mucinous cysts and MUC1 and hTERT are also found in pancreatic intraepithelial neoplasms, precursors to pancreatic cancer (Adsay et al., 2002; Luttges, Feyerabend, Buchelt, Pacena, & Kloppel, 2002; Paini et al., 2014; S. Yonezawa, Higashi, Yamada, & Goto, 2008). Abnormally glycosylated MUC1 has also been found on breast ductal carcinomas *in situ*, as well as adenoma of the colon, a precursor to colon cancer (Ajioka, Watanabe, & Jass, 1997; Mommers et al., 1999). Recent studies to find new antigens expressed on cancer stem cells and premalignant lesions looking at colon adenoma and colorectal cancer microarrays identified 160 up-regulated genes compared to normal colon (Broussard et al., 2013). In another study researchers found that a multi-partite vaccine targeting three antigens on pre-invasive breast disease (Neu, IGFBP2, IGF-IR) could prevent breast cancer in a spontaneous mouse tumor model in which mice were treated after developing premalignant lesions (Disis et al., 2013). Other pre-clinical studies in mice with

premalignant lesions have shown that common oncogene mutations that appear early and drive cancer formation could perhaps also be targeted by vaccines in animals including H-ras in carcinogen-induced tumors and EGFR in a lung cancer model (Ebben, Lubet, Gad, Disis, & You, 2016; Nasti et al., 2015). These common oncogenes in addition to others such as K-ras and p53 have been tested in therapeutic vaccines with limited efficacy but warrant further investigation as prophylactic vaccines (Carbone et al., 2005). Finally, another promising source of vaccine antigens are those targeted by spontaneous anti-tumor immune responses. Two such antigens, Cyclin B1 and SOX2, are found to be common targets of spontaneous immune responses for lung and prostate premalignant lesions and MGUS and MM, respectively (Dai et al., 2014; Kao et al., 2001; Spisek et al., 2007).

Patients at an increased risk for cancer stand to benefit most from prophylactic cancer vaccines. Statistics are available for many cancers indicating risk and timeframe for progression of premalignant lesions to cancer. Pre-clinical studies in mice engineered to develop spontaneous tumors show that such lesions either do not develop in vaccinated mice or do not progress to cancer (P. L. Beatty, Narayanan, Gariepy, Ranganathan, & Finn, 2010; Ebben et al., 2016). The increasing focus on early detection of cancer, including pre-malignant lesions, as well as identifying genetic and behavioral risk factors for cancer, can define candidate patient populations for prophylactic vaccination. A vaccine is a compelling alternative to current preventative measures, many of which rely on surgical and other invasive approaches (e.g. prophylactic mastectomies for patients with the BRCA1/2 mutation to prevent breast cancer and removal of CINs to prevent cervical or vulvar cancer) (S. Adams et al., 2011; Husemann et al., 2008; Singh et al., 2013). Indeed, when women diagnosed with premalignant vulvar neoplasias were given an HPV vaccine comprised of long peptides encoded by oncogenes E6 and E7 mixed with incomplete Freund's adjuvant, over 75% had significant responses with 47% clearing the lesions and maintaining a complete response for at least 24 months (de Vos van Steenwijk et al., 2012; de Vos van Steenwijk et al., 2014; Kenter et al., 2009). These responses correlated with the appearance of vaccine-induced HPV-specific $CD4^+$ and $CD8^+$ T cells. In 2013 our group undertook the first prophylactic cancer vaccine clinical trial targeting a non-viral antigen, MUC1, in patients with $MUC1^+$ advanced adenomas of the colon (Kimura et al., 2013). The vaccine was well-tolerated with no adverse events occurring, and 47% of the patients produced high titers of anti-MUC1 antibodies and durable immune memory, an important requirement for a prophylactic vaccine. Of note the patients who did not respond already had high levels of circulating immunosuppressive myeloid derived suppressor cells (MDSCs), highlighting the great potential benefit to administering the vaccines even earlier. We are currently following up this trial with a larger scale, randomized Phase II trial for vaccine efficacy in preventing polyp recurrence (NCT02134925), and based on the documented safety we are seeking to carry out future trials even earlier in the pre-malignant process.

It would also be possible, albeit expensive, for patients with risk for cancer to receive a personalized prophylactic vaccine based on mutations discovered by sequencing of biopsies of pre-malignant lesions (Gubin et al., 2015). However, treating a patient prophylactically with a mutated antigen vaccine would require knowledge of the expression of antigen and its processing by APCs and presentation to T cells. Given the large number of shared tumor antigens for which this information is already known, it is much more practical for now to

test the immunogenicity and safety of those tumor antigens as vaccines in the prophylactic setting (Finn, 2014).

## 6. Conclusions

In recent years, immunotherapy has been validated as an approach to treat cancer with many FDA-approved reagents becoming standard of care and current standard of care treatments being relegated to auxiliary roles. These successes have stimulated development of a multitude of new immunomodulatory drugs, drug combinations and advances in adoptive T cell therapy that promise to change the dire prognosis for the broad group of late-stage cancer patients. It is worth noting that several other promising immunotherapy approaches not covered in this review are being developed and are reviewed elsewhere including small molecule drugs capable of immune modulation and oncolytic viruses (J. L. Adams, Smothers, Srinivasan, & Hoos, 2015; Kaufman, Kohlhapp, & Zloza, 2015). The correct implementation of these therapies and determination of optimal therapeutic combinations will continue to be strengthened by basic research on the TME and the changing state of the immune system during tumor progression. Finally, a focused re-purposing of already developed therapeutic cancer vaccines to the prophylactic setting to treat patients at risk for cancer has the potential for the greatest overall impact by eliminating the cancer epidemic through immune prevention.

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## Abbreviations

**AAH** atypical adenomatous hyperplasia

| | |
|---|---|
| **AK** | actinic keratosis |
| **AML** | acute myelogenous leukemia |
| **AMM** | asymptomatic multiple myeloma |
| **APC** | antigen presenting cell |
| **B-ALL** | B cell acute lymphocytic leukemia |
| **B-CLL** | B cell chronic lymphocytic leukemia |
| **BD** | Bowen's disease |
| **BE** | Barrett's esophagus |
| **B-NHL** | B cell non-Hodgkin's lymphoma |
| **BPH** | benign prostatic hyperplasia |
| **B-SLL** | B cell small lymphocytic lymphoma |
| **CAR** | chimeric antigen receptor |
| **CIN** | cervical intraepithelial neoplasia |
| **CIS** | carcinoma in situ |
| **CLL** | chronic lymphocytic leukemia |
| **CML** | chronic myelogenous leukemia |
| **DCIS** | ductal carcinoma in situ |
| **DH** | ductal hyperplasia |
| **EIN** | endometrial intraepithelial neoplasia |
| **HGPIN** | high-grade prostatic intraepithelial neoplasia |
| **HL** | Hodgkin's lymphoma |
| **HNSCC** | head and neck squamous cell carcinoma |
| **ICI** | immune checkpoint inhibitor |
| **IM** | intestinal metaplasia |
| **IN** | intraepithelial neoplasia |
| **IPMN** | intraductal papillary mucinous neoplasm |
| **mAb** | monoclonal antibody |
| **MGUS** | monoclonal gammopathy of undetermined significance |
| **MIS** | melanoma in situ |

| | |
|---|---|
| **MM** | multiple myeloma |
| **NA** | not available |
| **ND** | not determined |
| **NOS** | not otherwise specified |
| **NSCLC** | non-small cell lung carcinoma |
| **OL** | oral leukoplakia |
| **ORR** | overall response rate |
| **OSMF** | oral submucous fibrosis |
| **PanIN** | pancreatic intraepithelial neoplasia |
| **RCC** | renal cell carcinoma |
| **RIN** | renal intratubular neoplasia |
| **SD** | squamous dysplasia |
| **SSM** | superficial spreading melanoma |
| **TCR** | T cell receptor |
| **TME** | tumor microenvironment |
| **VIN** | vulvar intraepithelial neoplasia |
| **z** | CD3-zeta fragment |

**Table 1**

**Next-generation immunomodulatory antibodies in clinical trials**

| Antibody Target | Combination | Conditions | Phase(s) | Enrollment | Start Date | NCT #[a] |
|---|---|---|---|---|---|---|
| ***4-1BB*** | - | *Melanoma* | *Phase 2* | *158* | *Mar-8* | *NCT00612664* |
| **4-1BB** | rituximab | B-NHL,NSCLC,RCC,HNSCC | Phase 1 | 220 | Jun-11 | NCT01307267 |
| ***4-1BB*** | *anti-PD1* | *Advanced Solid Tumors* | *Phase 1* | *45* | *Aug-14* | *NCT02179918* |
| **4-1BB** | anti-OX40 | Neoplasms | Phase 1 | 190 | Apr-15 | NCT02315066 |
| **4-1BB** | anti-PD-L1 | Advanced Cancer | Phase 1 | 317 | Nov-15 | NCT02554812 |
| **4-1BB** | anti-PD1, TIL therapy, cytokine | Melanoma | | 12 | Mar-16 | NCT02652455 |
| **4-1BB** | anti-PD1 | Urothelial Carcinoma,Bladder Cancer | Phase 2 | 44 | Sep-16 | NCT02845323 |
| ***CD27*** | - | *CD27 Expressing B-cell Malignancies, T-cell Malignancies, Solid Tumors* | *Phase 1* | *90* | *Oct-11* | *NCT01460134* |
| **CD27** | anti-CTLA-4, CDX-1401 | Unresectable Stage III or Stage IV Melanoma | Phase 1,2 | 100 | Apr-15 | NCT02413827 |
| **CD27** | anti-PD-1 | NSCLC, SCCHN, Ovarian Carcinoma, Colorectal Cancer, RCC, GBM, Melanoma | Phase 1,2 | 190 | Jan-15 | NCT02335918 |
| **CD27** | anti-PD-L1 | Kidney Neoplasms | Phase 1,2 | 55 | Oct-15 | NCT02543645 |
| **CD27** | glembatumumab vedotin | Melanoma | Phase 2 | 90 | Nov-14 | NCT02302339 |
| ***CD27*** | *ONT-10 vaccine* | *Advanced Breast Carcinoma, Advanced Ovarian Carcinoma* | *Phase 1* | *22* | *Nov-14* | *NCT02270372* |
| **CD27** | radiotherapy | Prostate Cancer | Phase 1 | 21 | Nov-14 | NCT02284971 |
| **CD27** | sunitinib | Kidney Neoplasms | Phase 1,2 | 58 | May-15 | NCT02386111 |
| ***CD40*** | - | *Advanced Solid Tumors* | *Phase 1* | *null* | *Jan-4* | *NCT02225002* |
| ***CD40*** | - | *Multiple Myeloma* | *Phase 1* | *44* | *Mar-4* | *NCT00079716* |
| ***CD40*** | - | *B-NHL* | *Phase 1* | *50* | *Dec-4* | *NCT00103779* |
| ***CD40*** | - | *CLL* | *Phase 1* | *26* | *Apr-5* | *NCT00108108* |
| ***CD40*** | - | *CLL* | *Phase 1,2* | *12* | *Jul-5* | *NCT00283101* |
| ***CD40*** | - | *MM* | *Phase 2* | *33* | *Sep-5* | *NCT00231166* |
| ***CD40*** | - | *B-NHL* | *Phase 2* | *46* | *Dec-6* | *NCT00435916* |
| ***CD40*** | - | *B-NHL* | *Phase 1* | *29* | *Jul-7* | *NCT01561911* |
| ***CD40*** | *chemotherapy, dexamethasone* | *MM* | *Phase 1* | *36* | *Aug-7* | *NCT00525447* |
| ***CD40*** | *rituximab, chemotherapy* | *B-NHL* | *Phase 2* | *151* | *Sep-7* | *NCT00529503* |

| Antibody Target | Combination | Conditions | Phase(s) | Enrollment | Start Date | NCT #[a] |
|---|---|---|---|---|---|---|
| ***CD40*** | *chemotherapy, dexamethasone* | *Neoplasms* | *Phase 1* | *34* | *Nov-7* | *NCT00607048* |
| ***CD40*** | *rituximab* | *B-NHL* | *Phase 1* | *22* | *Dec-7* | *NCT00556699* |
| ***CD40*** | - | *B-NHL, HL* | *Phase 1,2* | *111* | *Mar-8* | *NCT00670592* |
| ***CD40*** | *rituximab, chemotherapy* | *B-NHL* | *Phase 1* | *33* | *Apr-8* | *NCT00655837* |
| ***CD40*** | *bortezomib* | *MM* | *Phase 1* | *18* | *May-8* | *NCT00664898* |
| ***CD40*** | *chemotherapy* | *Pancreatic Neoplasm* | *Phase 1* | *22* | *Jun-8* | *NCT00711191* |
| ***CD40*** | *anti-CTLA-4* | *Recurrent Melanoma, Stage IV Melanoma* | *Phase 1* | *32* | *Feb-10* | *NCT01103635* |
| ***CD40*** | *chemotherapy* | *Follicular Lymphoma* | *Phase 1* | *1* | *Feb-11* | *NCT01275209* |
| **CD40** | anti-PD-L1 | Solid Cancers | Phase 1 | 160 | Dec-14 | NCT02304393 |
| **CD40** | - | Neoplasms | Phase 1 | 32 | May-15 | NCT02482168 |
| **CD40** | vanucizumab | Advanced/Metastatic Solid Tumors | Phase 1 | 170 | Jan-16 | NCT02665416 |
| **CD40** | anti-PD-1 | Melanoma | Phase 1,2 | 41 | Oct-16 | NCT02706353 |
| **CD73** | PD-L1 | Solid Tumours | Phase 1 | 188 | Jul-15 | NCT02503774 |
| **GITR** | - | Unresectable Stage III or Stage IV Malignant Melanoma or Other Solid Tumor Malignancies | Phase 1 | 40 | Oct-10 | NCT01239134 |
| **GITR** | anti-PD-1 | Advanced Solid Tumor | Phase 1 | 96 | Nov-15 | NCT02553499 |
| **GITR** | - | Advanced Solid Tumors | Phase 1 | 45 | Nov-15 | NCT02583165 |
| **GITR** | - | Solid Tumors | Phase 1 | 44 | Dec-15 | NCT02628574 |
| **GITR** | - | Advanced Cancer, Metastatic Cancer | Phase 1,2 | 146 | Apr-16 | NCT02697591 |
| **GITR** | anti-PD-1 | Solid Tumors, Lymphomas | Phase 1 | 264 | Jun-16 | NCT02740270 |
| **ICOS** | - | Lymphomas | Phase 1 | 46 | 16-Apr | NCT02520791 |
| **ICOS** | anti-PD-1 | Cancer | Phase 1 | 304 | Jun-16 | NCT02723955 |
| **KIR** | anti-CTLA-4, anti-PD-1 | B-NHL, HL, MM | Phase 1 | 375 | Jun-12 | NCT01592370 |
| **KIR** | anti-PD-1 | CANCER,NOS | Phase 1 | 162 | Oct-12 | NCT01714739 |
| ***KIR*** | *anti-CTLA-4* | *CANCER, NOS* | *Phase 1* | *22* | *Dec-12* | *NCT01750580* |
| **KIR** | anti-PD-1 | Solid Tumors | Phase 1,2 | 260 | Oct-15 | NCT02598960 |
| **LAG-3** | anti-PD-1 | Neoplasms by Site | Phase 1 | 360 | Oct-13 | NCT01968109 |
| **LAG-3** | anti-PD-1 | Hematologic Neoplasms | Phase 1,2 | 132 | Feb-14 | NCT02061761 |
| **LAG-3** | anti-PD-1 | Advanced Solid Tumors | Phase 1,2 | 416 | Jun-15 | NCT02460224 |

*Pharmacol Ther*. Author manuscript; available in PMC 2018 October 01.

| Antibody Target | Combination | Conditions | Phase(s) | Enrollment | Start Date | NCT #[a] |
|---|---|---|---|---|---|---|
| **LAG-3** | anti-4-1BB, anti-PD-1 | Glioblastoma,Gliosarcoma, Recurrent Brain Neoplasm | Phase 1 | 68 | Mar-16 | NCT02658981 |
| **LAG-3** | anti-PD-1 | Advanced Cancer | Phase 1 | 70 | May-16 | NCT02720068 |
| ***OX40*** | *vaccine* | *Advanced Cancer* | *Phase 1* | *30* | *Nov-3* | *NCT01644968* |
| ***OX40*** | *chemotherapy, radiotherapy* | *Prostate Cancer* | *Phase 1,2* | *10* | *Oct-10* | *NCT01303705* |
| **OX40** | radiotherapy | Metastatic Breast Cancer,Lung Metastases,Liver Metastases | Phase 1,2 | 40 | Feb-13 | NCT01862900 |
| ***OX40*** | *rituximab, anti-CTLA-4, anti-PD-L1* | *Advanced Solid Tumors,Aggressive B-cell Lymphomas* | *Phase 1,2* | *58* | *Aug-14* | *NCT02205333* |
| ***OX40*** | *anti-PD-L1* | *Recurrent or Metastatic Solid Tumors* | *Phase 1* | *39* | *Sep-14* | *NCT02221960* |
| **OX40** | - | Head and Neck Cancer | Phase 1 | 55 | Oct-14 | NCT02274155 |
| **OX40** | - | Advanced Solid Tumors | Phase 1 | 196 | Mar-15 | NCT02318394 |
| **OX40** | anti-PD-L1, bevacizumab | Solid Tumor | Phase 1 | 762 | Apr-15 | NCT02410512 |
| **OX40** | - | Colorectal Neoplasms | Phase 1 | 44 | Sep-15 | NCT02559024 |
| **OX40** | anti-CTLA-4, anti-PD-L1 | Select Advanced Solid Tumors | Phase 1 | 364 | Mar-16 | NCT02705482 |
| **TIM-3** | anti-PD-1 | Advanced Malignancies | Phase 1,2 | 250 | Nov-15 | NCT02608268 |
| **TIM-3** | anti-PD-1 | Advanced or Metastatic Solid Tumors | Phase 1 | 402 | Jul-16 | NCT02817633 |
| **VISTA** | - | Advanced Cancer | Phase 1 | 150 | Jan-16 | NCT02671955 |

[a]Trials with recruitment listed as “Completed,” “Active but not recruiting,” “Terminated” or “Suspended” on clinicaltrails.gov are *italicized*. Trials were obtained by bulk data download from clinicaltrials.gov on August 1st 2016.

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**Table 2**

**CAR clinical trials by target antigen**

| Antigen | Signaling and accessory | Cell type[a] | Delivery method[b] | Disease(s) | Primary institution(s) | Phase(s) | Enrollment | NCT #[c] |
|---|---|---|---|---|---|---|---|---|
| BCMA | 41BBz | T cells | NA | MM | UPenn | Phase 0 | 30 | NCT02546167 |
| BCMA | 41BBz | T cells | Lenti | MM | bluebird bio | Phase 1 | 50 | NCT02658929 |
| BCMA | 41BBz | T cells | Lenti | MM | bluebird bio | | 50 | NCT02786511 |
| CD7 | CD28-41BBz | NK-92 | NA | AML | PersonGen | Phase1,2 | 10 | NCT02742727 |
| *CD19* | *41BBz* | *T cells* | *Retro* | *B-ALL, B-CLL, B-NHL* | *UPenn* | | *110* | *NCT01029366* |
| *CD19* | *41BBz* | *T cells (allo)* | *Lenti* | *B-ALL* | *UPenn* | *Phase 1* | *10* | *NCT01551043* |
| *CD19* | *41BBz* | *T cells* | *Lenti* | *B-ALL, B-CLL, B-NHL* | *UPenn* | *Phase 1* | *20* | *NCT01626495* |
| CD19 | 41BBz | *T cells* | Lenti | B-CLL, B-SLL | UPenn | Phase 2 | 65 | NCT01747486 |
| CD19 | 41BBz | T cells 1:1 $CD4^+$ and $CD8^+$ | Lenti | B-ALL | Seattle Children's | Phase1,2 | 80 | NCT02028455 |
| CD19 | 41BBz | T cells | Lenti | B-NHL | UPenn | Phase 2 | 51 | NCT02030834 |
| CD19 | 41BBz | T cells | Lenti | B-ALL | UPenn | Phase 2 | 24 | NCT02030847 |
| *CD19* | *41BBz* | *T cells* | *RNA (electro)* | *HL* | *UPenn* | *Phase 0* | *16* | *NCT02277522* |
| CD19 | 41BBz | T cells | Lenti | B-NHL | Novartis | Phase 2 | 100 | NCT02445248 |
| CD19 | 41BBz | T cells | RNA (electro) | HL | UPenn\|CHOP | Phase 0 | 10 | NCT02624258 |
| CD19 | 41BBz | T cells | Lenti | B-CLL, B-SLL | UPenn | | 15 | NCT02640209 |
| CD19 | 41BBz | T cells | Lenti | B-NHL | UPenn | Phase1,2 | 12 | NCT02650999 |
| CD19 | 41BBz | T cells | Lenti | B-CLL | Shanghai GeneChem | Phase1,2 | 30 | NCT02672501 |
| CD19 | 41BBz | T cells | Lenti | MM | UPenn | Phase 2 | 25 | NCT02794246 |
| CD19 | 41BBz and zeta-only | T cells | Retro | B-ALL, B-CLL, B-NHL | Chinese PLAGH | | 12 | NCT01864889 |
| CD19 | 41BBz and zeta- only | T cells | Retro | B-NHL | Chinese PLAGH | Phase1,2 | 2 | NCT02081937 |
| CD19 | 41BBz,CD20/34t | T cells (allo, gen mod) | Lenti | B-ALL, B-CLL, B-NHL | Servier | | 200 | NCT02735083 |
| CD19 | 41BBz,CD20/34t | T cells (allo, gen mod) | Lenti | B-ALL, B-CLL | Servier | Phase 1 | 12 | NCT02746952 |
| CD19 | 41BBz,EGFRt | T cells | Lenti | B-ALL, B-CLL, B-NHL | FHCRC\|NCI | Phase1,2 | 145 | NCT01865617 |
| CD19 | 41BBz,EGFRt | T cells | Lenti | B-NHL | Juno | Phase 1 | 70 | NCT02631044 |
| CD19 | 41BBz,EGFRt | T cells | Lenti | B-NHL | FHCRC\|NCI\|Juno MedImmune AstraZeneca | Phase 1 | 42 | NCT02706405 |
| CD19 | CD27z,iCasp9 | T cells | Lenti | B-NHL | PKU\|UF | Phase1,2 | 20 | NCT02247609 |

| Antigen | Signaling and accessory | Cell type[a] | Delivery method[b] | Disease(s) | Primary institution(s) | Phase(s) | Enrollment | NCT #[c] |
|---|---|---|---|---|---|---|---|---|
| CD19 | CD28-41BBz | T cells | Retro | B-CLL, B-NHL | Uppsala Univ. | Phase1,2 | 15 | NCT02132624 |
| *CD19* | *CD28-41BBz,EGFRt* | *CMV or EBV $T_{cm}$ cells (allo)* | *NA* | *B-ALL, B-CLL, B-NHL* | *FHCRC\|\|NCI* | *Phase1,2* | *1* | *NCT01475058* |
| *CD19* | *CD28z* | *T cells* | *Retro* | *B-NHL* | *NCI\|NIHCC* | *Phase 1* | *43* | *NCT00924326* |
| CD19 | CD28z | T cells | NA | B-ALL | MSKCC | Phase 1 | 60 | NCT01044069 |
| *CD19* | *CD28z* | *T cells (allo)* | *Virus* | *B-ALL, B-CLL, B cell lymphomas* | *NCI\|NIHCC* | *Phase 1* | *42* | *NCT01087294* |
| *CD19* | *CD28z* | *T Cells* | *Transpos (electro)* | *B-NHL* | *M.D. Anderson Intrexon/Ziopharm* | *Phase 1* | *60* | *NCT00968760* |
| CD19 | CD28z | EBV-CTLs (allo) | NA | B-ALL, B-NHL | MSKCC | Phase 1 | 12 | NCT01430390 |
| *CD19* | *CD28z* | *T Cells (allo)* | *Transposon (electro)* | *B-ALL, B-CLL, B-NHL* | *M.D. Anderson Intrexon/Ziopharm* | *Phase 1* | *140* | *NCT01497184* |
| *CD19* | *CD28z* | *T cells* | *Retro* | *B-ALL, B-NHL* | *NCI\|NIHCC* | *Phase 1* | *52* | *NCT01593696* |
| CD19 | CD28z | T cells | NA | B-ALL, B-CLL, B-NHL | M.D. Anderson ZiopharmIIntrexon | Phase 1 | 30 | NCT02529813 |
| *CD19* | *CD28z* | *T cells* | *NA* | *B-NHL* | *MSKCC* | *Phase 1* | *17* | *NCT01840566* |
| CD19 | CD28z | T cells | NA | B-ALL | MSKCC\|Dana-Farber | Phase 1 | 24 | NCT01860937 |
| CD19 | CD28z | T cells (allo) | Retro | B-ALL, B-CLL, B-NHL | Baylor | Phase 1 | 40 | NCT02050347 |
| CD19 | CD28z | T cells | Retro | B-NHL | Jichi Med. Univ.\|Takara | Phase1,2 | 18 | NCT02134262 |
| CD19 | CD28z | T cells | Retro | B-NHL | Kite Pharma | Phase1,2 | 124 | NCT02348216 |
| CD19 | CD28z | T cells | Retro | B-ALL | Juno | Phase 2 | 90 | NCT02535364 |
| CD19 | CD28z | T cells | Retro | B-NHL | Kite Pharma | Phase 2 | 70 | NCT02601313 |
| CD19 | CD28z | T cells | Retro | B-ALL | Kite Pharma | Phase1,2 | 75 | NCT02614066 |
| CD19 | CD28z | T cells | Retro | B-ALL | Kite Pharma | Phase1,2 | 75 | NCT02625480 |
| CD19 | CD28z | T cells | Retro | B-NHL | Xuzhou Med. College | Phase1,2 | 20 | NCT02652910 |
| CD19 | CD28z | T cells | Retro | B-ALL | Juno | | 500 | NCT02813252 |
| CD19 | CD28z | T cells | Retro | B-ALL, B-NHL | Sheba Med. Center | Phase1,2 | 40 | NCT02772198 |
| CD19 | CD28z and 41BBz | T cells | Lenti | B-ALL | AMMS\|PKU | Phase 1 | 5 | NCT02186860 |
| CD19 | CD28z and 41BBz | T cells | NA | B-ALL, B-CLL, B-NHL | Xuzhou Med. College | Phase1,2 | 20 | NCT02685670 |
| CD19 | CD28z and CD28-41BBz | T cells | Retro | B-ALL, B-CLL, B-NHL | Baylor | Phase 1 | 14 | NCT01853631 |
| *CD19* | *CD28z and zeta-only* | *$T_{cm}$-enriched T cells* | *Lenti* | *B-NHL* | *City of Hope\|NCI* | *Phase1,2* | *57* | *NCT01318317* |
| *CD19* | *CD28z,EGFRt* | *T cells* | *Lenti* | *B-ALL* | *Seattle Children's* | *Phase 1* | *18* | *NCT01683279* |
| CD19 | CD28z,EGFRt | T cells $T_{cm}$ or $T_n$,$T_{nem}$ | Lenti | B-ALL | City of Hope\|NCI | Phase 1 | 48 | NCT02146924 |

| Antigen | Signaling and accessory | Cell type[a] | Delivery method[b] | Disease(s) | Primary institution(s) | Phase(s) | Enrollment | NCT #[c] |
|---|---|---|---|---|---|---|---|---|
| CD19 | CD28z,undefined safety switch | T cells | Retro or Lenti | B-ALL, B-CLL, B-NHL | Shenzhen Second People's Hospital | Phase 1 | 36 | NCT02456350 |
| CD19 | NA | T cells | NA | B-ALL, B-CLL, B-NHL | Southwest Hospital, China | Phase1,2 | 45 | NCT02349698 |
| CD19 | NA | T cells | NA | B-ALL, B-CLL, B-NHL | ICT | | 30 | NCT02813837 |
| CD19 | NA | T cells | Lenti | B-ALL, B-CLL, B-NHL | Tongji Univ. Med. | Phase1,2 | 40 | NCT02537977 |
| CD19 | NA | T cells | NA | B-CLL | SMMU | Phase1,2 | 20 | NCT02644655 |
| CD19 | NA | T cells (allo) | NA | B-ALL | AMMS\|Chinese PLAGH | Phase 1 | 10 | NCT02799550 |
| CD19 | NA | T cells | NA | B-NHL | Sinobioway Cell Therapy | Phase1,2 | 24 | NCT02728882 |
| CD19 | NA | T cells | NA | B-ALL | Sinobioway Cell Therapy | Phase1,2 | 24 | NCT02735291 |
| CD19 | NA | T cells | NA | B-NHL | Univ. College, London | Phase 1 | 12 | NCT02431988 |
| CD19 | NA | T cells | NA | B-ALL, B-CLL, B-NHL | Beijing Doing Biomed. | Phase 1 | 100 | NCT02546739 |
| CD19 | NA | Yδ T-cells (allo) | NA | B-ALL, B-CLL, B-NHL | Beijing Doing Biomed. | Phase 1 | 48 | NCT02656147 |
| CD19 | NA | T cells | Retro | B-ALL, B-CLL, B-NHL | NCI\|NIHCC | Phase 1 | 64 | NCT02659943 |
| CD19 | NA | T cells | NA | B-ALL | Guangdong General Hospital\|CAS | Phase 1 | 30 | NCT02822326 |
| CD19 | NA | T cells | NA | B-ALL, B-NHL | Univ. College, London | Phase 1 | 18 | NCT02443831 |
| CD19 | NA | T cells | NA | B-CLL | iCarTAB Xuzhou Med. | Phase1,2 | 50 | NCT02782351 |
| CD19 | NA | T cells | NA | B-NHL | PKU\|Marino | Phase 1 | 20 | NCT02842138 |
| CD19 | NA | T cells | NA | B-ALL, B-CLL, B-NHL | PersonGen | Phase1,2 | 10 | NCT02851589 |
| CD19 | NA | T cells | NA | B-ALL, B-CLL, B-NHL | PersonGen | Phase1,2 | 10 | NCT02819583 |
| *CD19* | *zeta-only* | *T cells* | *Retro* | *B-NHL* | *Christie NHS Foundation* | *Phase 1* | *24* | *NCT01493453* |
| CD19, CD20 | NA | T cells | NA | B-NHL | Southwest Hospital, China | Phase1,2 | 40 | NCT02737085 |
| *CD19, Mesothelin* | *41BBz* | *T cells* | *Lenti* | *Pancreatic Cancer* | *UPenn\|UCSF* | *Phase 1* | *12* | *NCT02465983* |
| CD20 | NA | T cells | Virus | B-ALL, B-CLL, B-NHL | Southwest Hospital, China | Phase1,2 | 45 | NCT02710149 |
| CD22 | 41BBz | T cells | Lenti | B-ALL, B-NHL | NCI\|NIHCC | Phase 1 | 57 | NCT02315612 |
| *CD22* | *41BBz* | *T cells* | *Lenti* | *B-ALL* | *UPenn* | | *15* | *NCT02588456* |
| CD22 | 41BBz | T cells | Lenti | B-ALL | UPenn\|CHOP | Phase 1 | 15 | NCT02650414 |
| CD22 | 41BBz | T cells | Retro | B-NHL | Xuzhou Med. | Phase 1 | 20 | NCT02721407 |
| CD22 | NA | T cells | Virus | B-ALL, B-CLL, B-NHL | iCarTAB\|Xuzhou Med. | Phase1,2 | 10 | NCT02794961 |
| CD30 | CD27z,iCasp9 | T cells | Lenti | HL, B-NHL | PKU\|UF | Phase1,2 | 20 | NCT02274584 |
| *CD30* | *CD28z* | *T cells* | *Virus* | *HL, B-NHL* | *UNC Lineberger\|Baylor* | *Phase 1* | *18* | *NCT01316146* |

| Antigen | Signaling and accessory | Cell type[a] | Delivery method[b] | Disease(s) | Primary institution(s) | Phase(s) | Enrollment | NCT #[c] |
|---|---|---|---|---|---|---|---|---|
| CD30 | CD28z | T cells | Virus | HL, B-NHL | UNC Lineberger | Phase 1 | 18 | NCT02663297 |
| CD30 | CD28z | T cells | Virus | HL, B-NHL | UNC Lineberger | Phase1,2 | 31 | NCT02690545 |
| CD30 | NA | T cells | NA | HL, B-NHL | Chinese PLAGH | Phase1,2 | 30 | NCT02259556 |
| CD33 | 41BBz and zeta-only | T cells | Retro | AML | Chinese | Phase1,2 | 10 | NCT01864902 |
| CD33 | 41BBz and zeta-only | T cells | Retro | AML | AMMS\|Chinese PLAGH | Phase 1 | 12 | NCT02799680 |
| CD70 | NA | T cells | Retro | CD70$^+$ cancers | NCI\|NIHCC | Phase1,2 | 113 | NCT02830724 |
| *CD123* | *41BBz* | *T cells* | *RNA (electro)* | *AML* | *UPenn* | *Phase 0* | *7* | *NCT02623582* |
| CD123 | CD28z,EGFRt | T cells | Lenti | AML | City of Hope NCI | Phase 1 | 30 | NCT02159495 |
| CD133 | 41BBz and zeta-only | T cells | Retro | AML, B-ALL, various solid tumors | Chinese PLAGH | Phase 1 | 20 | NCT02541370 |
| CD138 | 41BBz and zeta-only | T cells | Retro | MM | Chinese PLAGH | Phase1,2 | 10 | NCT01886976 |
| CD171 | CD28-41BBz,EGFRt and 41BBz,EGFRt | T cells 1:1 CD4$^+$ and CD8$^+$ | Lenti | Neuroblastoma | Seattle Children's | Phase 1 | 80 | NCT02311621 |
| *CEA* | *CD28z* | *T cells* | *NA* | *Various solid tumors* | *Roger Williams* | *Phase 2* | *48* | *NCT01723306* |
| CEA | NA | T cells | NA | Various solid tumors | Southwest Hospital, China | Phase 1 | 75 | NCT02349724 |
| *CEA* | *zeta-only* | *T cells* | *Retro* | *Various solid tumors* | *Cancer Research UK* | *Phase 1* | *14* | *NCT01212887* |
| EGFR | 41BBz and zeta-only | T cells | Lenti | EGFR$^+$ Solid Tumors | Chinese PLAGH | Phase1,2 | 60 | NCT01869166 |
| EGFRvIII | 41BBz | T cells | Lenti | Glioma | UPenn\|UCSF | | 12 | NCT02209376 |
| EGFRvIII | 41BBz | T cells | Lenti | Glioblastoma | UPenn | | 8 | NCT02666248 |
| *EGFRvIII* | *CD28-41BBz* | *T cells* | *Retro* | *Glioma* | *NCI\|NIHCC* | *Phase1,2* | *18* | *NCT01454596* |
| EGFRvIII | NA | T cells | Lenti | Glioma | RenJi Hospital | Phase 1 | 10 | NCT02331693 |
| EGFRvIII | NA | T cells | Retro | Glioblastoma | Duke Univ. | Phase 1 | 48 | NCT02664363 |
| EGFRvIII | NA,EGFRt | T cells | Lenti | Glioblastoma | Beijing Sanbo Marino | Phase 1 | 20 | NCT02844062 |
| EPCAM | NA | T cells | NA | Stomach Neoplasms | Sinobioway | Phase1,2 | 19 | NCT02725125 |
| EPCAM | NA | T cells | NA | Liver Neoplasms | Sinobioway | Phase1,2 | 25 | NCT02729493 |
| EPHA2 | NA | T cells | NA | Glioma | Fuda, Guangzhou | Phase1,2 | 60 | NCT02575261 |
| ErbB dimers | CD28z,4aβ | T cells | Retro | Head and Neck Cancer | King's College | Phase 1 | 30 | NCT01818323 |
| FAP | CD28z | CD8+ T cells | Retro | Mesothelioma | Univ. of Zurich | Phase 1 | 6 | NCT01722149 |
| GD2 | CD28-41BB-CD27z,iCasp9 | T cells | Lenti | Neuroblastoma | Zhujiang Hospital | Phase 2 | 30 | NCT02765243 |
| *GD2* | *CD28-* | *T cells* | *Retro* | *Neuroblastoma* | *Baylor/NCI* | *Phase 1* | *11* | *NCT01822652* |

| Antigen | Signaling and accessory | Cell type[a] | Delivery method[b] | Disease(s) | Primary institution(s) | Phase(s) | Enrollment | NCT #[c] |
|---|---|---|---|---|---|---|---|---|
| GD2 | CD28-OX40z,iCasp9 | NK T cells | Retro | Sarcoma, Osteosarcoma, Neuroblastoma, Melanoma | NCI\|NIHCC | Phase 1 | 72 | NCT02107963 |
| GD2 | CD28-OX40z,iCasp9 | VZV T cells | Retro | Sarcomas | Baylor\|NCI | Phase 1 | 26 | NCT01953900 |
| GD2 | CD28-OX40z,iCasp9 | NK T cells | Retro | Neuroblastoma | Baylor | Phase 1 | 18 | NCT02439788 |
| GD2 | CD28z | T cells | Virus | Neuroblastoma | Cancer Research UK | Phase 1 | 27 | NCT02761915 |
| *GD2* | *NA* | *triVirus-CTLs (allo)* | *Retro* | *Neuroblastoma* | *Children's Mercy, KC* | *Phase 1* | *5* | *NCT01460901* |
| GPC3 | 41BBz | T cells | Virus | Hepatocellular Carcinoma | Shanghai GeneChem | Phase1,2 | 30 | NCT02715362 |
| GPC3 | CD28-41BBz | T cells | Virus | Hepatocellular Carcinoma | RenJi Hospital | Phase 1 | 20 | NCT02395250 |
| GPC3 | NA | T cells | NA | Hepatocellular Carcinoma | Fuda, Guangzhou | Phase1,2 | 60 | NCT02723942 |
| HER2 | 41BBz and zeta-only | T cells | Virus | HER-2$^+$ Solid Tumors | Chinese PLAGH | Phase1,2 | 10 | NCT01935843 |
| HER2 | CD28z | T cells | Virus | Sarcoma | Baylor | Phase 1 | 36 | NCT00902044 |
| *HER2* | *CD28z* | *CMV T cells* | *Virus* | *Glioblastoma* | *Baylor* | *Phase 1* | *16* | *NCT01109095* |
| HER2 | CD28z | T cells | Retro | Breast Cancer | Fuda, Guangzhou | Phase1,2 | 60 | NCT02547961 |
| HER2 | CD28z | T cells | Virus | Glioblastoma | Baylor | Phase 1 | 14 | NCT02442297 |
| *HER2* | *CD28z,TGFβDN* | *EBV T cells* | *Retro* | *HER2$^+$ Malignancies* | *Baylor* | *Phase 1* | *19* | *NCT00889954* |
| HER2 | NA | T cells | NA | Various solid tumors | Southwest Hospital, China | Phase1,2 | 60 | NCT02713984 |
| IL-1-RAP | NA | NA | NA | CML | Univ. Hospital of Besancon | | 40 | NCT02842320 |
| IL-13R | 41BBz,CD19t | T cells $T_{cm}$-enriched | Lenti | Glioma | City of Hope\|NCI | Phase 1 | 75 | NCT02208362 |
| Kappa | CD28z | T cells | Retro | B-CLL, B-NHL | Baylor | Phase 1 | 54 | NCT00881920 |
| *LeY* | *CD28z* | *T cells* | *Retro* | *MM, AML, MDS* | *Peter MacCallum* | *Phase 1* | *6* | *NCT01716364* |
| *Mesothelin* | *41BBz* | *T cells* | *RNA (electro)* | *Mesothelioma* | *Upenn* | *Phase 1* | *18* | *NCT01355965* |
| *Mesothelin* | *41BBz* | *T cells* | *Lenti* | *Various solid tumors* | *UPenn* | *Phase 1* | *21* | *NCT02159716* |
| Mesothelin | 41BBz | T cells | Lenti | Various solid tumors | UPenn | | 50 | NCT02388828 |
| Mesothelin | 41BBz | T cells | Retro | Various Mesothelin$^+$ Tumors | Chinese PLAGH | Phase 1 | 20 | NCT02580747 |
| Mesothelin | 41BBz | T cells | Virus | Pancreatic Cancer | Shanghai GeneChem | Phase 1 | 30 | NCT02706782 |
| *Mesothelin* | *CD28-41BBz* | *T cells* | *Retro* | *Various solid tumors* | *NCI\|NIHCC* | *Phase1,2* | *15* | *NCT01583686* |
| Mesothelin | CD28z-iCasp9 | T cells | Virus | Mesothelioma, Lung and Breast Cancers | MSKCC | Phase 1 | 24 | NCT02414269 |
| Mesothelin | NA | T cells | Virus | Breast Cancer | MSKCC | Phase 1 | 24 | NCT02792114 |

| Antigen | Signaling and accessory | Cell type[a] | Delivery method[b] | Disease(s) | Primary institution(s) | Phase(s) | Enrollment | NCT #[c] |
|---|---|---|---|---|---|---|---|---|
| MUC1 | CD28-41BBz | T cells | Virus | Various solid tumors | PersonGen | Phase1,2 | 20 | NCT02587689 |
| MUC1 | CD28-41BBz | T cells | Virus | Glioma, colorectal, gastric | PersonGen | Phase1,2 | 20 | NCT02617134 |
| MUC1 | NA | pNK cells | NA | Various solid tumors | PersonGen | Phase1,2 | 10 | NCT02839954 |
| MUC16 | CD28zfIL-12 | T cells | NA | Ovarian Cancer | MSKCC | Phase 1 | 30 | NCT02498912 |
| NKG2D | NA | T cells | Virus | AML, Myelodysplastic Syndrome, MM | Celdara Med. | Phase 1 | 21 | NCT02203825 |
| PSCA | zeta-only, iCD40/MyD88 | T cells | Retro | Pancreatic Cancer | Bellicum | Phase 1 | 30 | NCT02744287 |
| *PSMA* | *CD28z* | *T cells* | *Retro* | *Prostate Cancer* | *Roger Williams* | *Phase 1* | *18* | *NCT00664196* |
| PSMA | CD28z | T cells | Virus | Prostate Cancer | MSKCC | Phase 1 | 18 | NCT01140373 |
| ROR1 | 41BBz | T cells | NA | B-ALL, B-CLL, B-NHL, Various solid tumors | FHCRC\|NCI | Phase 1 | 60 | NCT02706392 |

[a] Unless specified cells are autologous patient-derived cells.

[b] "Virus" indicates that the trial description mentions introducing the CAR by viral transduction but does not specify the kind of virus used.

[c] Trials with recruitment listed as "Completed," "Active but not recruiting," "Terminated" or "Suspended" on clinicaltrails.gov are *italicized*. Trials were obtained by bulk data download from clinicaltrials.gov on August 1st 2016.

**Table 3**

Therapeutic cancer vaccine antigens from the NCI 2009 workshop as candidates for prophylactic vaccines: expression in precancer.

| NCI Rank[a] | Antigen | Oncogenic[a] | Cancer stem cell expression[a] | Premalignant lesion(s)[b,c] | Cancer type[c] |
|---|---|---|---|---|---|
| **1** | WT1 | yes | yes | nephrogenic rests | Kidney |
| **2** | MUC1 | yes | yes | PanIN, IPMN; BE; adenoma;bronchial preneoplasia; MGUS; AMM | pancreatic, esophageal,colorectal, lung, multiple myeloma |
| **3** | LMP2 | - | yes | *ND* | - |
| **4** | HPV E6 E7 | - | some | CIN, VIN | cervical |
| **5** | EGFRvIII | - | yes | *ND* | - |
| **6** | HER-2/neu | yes | - | DCIS; adenoma | breast, colorectal |
| **8** | MAGE A3 | - | yes | DCIS; SD; esophageal SCCIS | breast, head and neck, esophageal |
| **9** | p53 non- mutant | yes | yes | OL, OSMF; CIN; hyperplasia and dysplasia; schistosomal cystitis; SDIS; DH; dysplasia; DCIS | oral, cervical, head and neck, bladder, lung, pancreatic, breast |
| **10** | NY-ESO-1 | - | yes | DCIS; SD; SCIS | breast, head and neck, esophageal |
| **11** | PSMA | - | - | HGPIN | prostate |
| **13** | CEA | - | - | dysplasia, CIS; adenoma; IM | cervical, colorectal, gastric |
| **14** | MelanA/MART1 | - | - | BD, AK | melanoma |
| **15** | Ras-mutant | yes | yes | adenoma; EIN; BE; DH and dysplasia | colorectal, endometrial, esophageal, pancreatic |
| **17** | p53 mutant | yes | some | OL, OSMF; CIN; hyperplasia and dysplasia; schistosomal cystitis; SDIS; DH, dysplasia; DCIS; BE; AK, BD | oral, cervical, head and neck, bladder, lung, pancreatic, breast, esophageal, melanoma |
| **19** | Bcr-abl | yes | yes | *ND* | - |
| **20** | Tyrosinase | - | - | MIS, SSM | melanoma |
| **21** | Survivin | yes | - | OL; RIN; IPMT; BD | oral, renal, pancreatic, melanoma |
| **23** | hTERT | yes | yes | PanIN, IPMN; BD, AK | pancreatic, melanoma |
| **24** | Sarcoma translocation breakpoints | yes | yes | *ND* | - |
| **25** | EphA2 | yes | - | AAH, noninvasive bronchioloalveolar component of adenocarcinoma | lung |
| **27** | ML-IAP | yes | - | *ND* | - |
| **28** | AFP | - | yes | *ND* | - |
| **29** | EpCAM | - | yes | IM; IN | gastric and esophageal |

| NCI Rank[a] | Antigen | Oncogenic[a] | Cancer stem cell expression[a] | Premalignant lesion(s)[b,c] | Cancer type[c] |
|---|---|---|---|---|---|
| **30** | ERG (TMPRSS2 ETS fusion gene) | yes | - | HGPIN | prostate |
| **32** | PAX3 | yes | - | neurofibroma | nerve sheath, melanoma |
| **33** | ALK | yes | yes | *ND* | - |
| **34** | Androgen receptor | yes | - | OL, OSMF; BPH, PIN | oral, prostate |
| **35** | Cyclin B1 | yes | - | bronchial preneoplasia; SD; DCIS | lung, head and neck, breast |
| **36** | Polysialic Acid | - | - | CIN; OL, OSMF | cervical, oral |
| **37** | MYCN | yes | - | *ND* | - |
| **38** | RhoC | yes | - | *ND* | - |
| **39** | TRP-2 | - | yes | *ND* | - |
| **42** | Mesothelin | - | - | PanIN, IPMN | pancreatic |
| **43** | PSCA | - | - | HGPIN | prostate |
| **44** | MAGE A1 | - | yes | DCIS; SD; SCCIS | breast, head and neck, esophageal |
| **45** | sLe(a) | - | - | adenoma; CIN | colorectal, cervical |
| **46** | CYP1B1 | - | - | PIN | prostate |
| **47** | PLAC1 | yes | - | DCIS | breast |
| **49** | BORIS | yes | - | *ND* | - |
| **50** | Tn | - | - | AK, BD; Paget's disease | melanoma, breast |
| **52** | ETV6-AML | yes | - | *ND* | - |
| **55** | SART3 | - | - | CIS | gastric |
| **56** | STn | - | - | adenoma, Crohn's colitis; IM | colorectal, gastric |
| **57** | Carbonic anhydrase IX | - | - | columnar cell metaplasia; BE, dysplasia | colorectal, esophageal |
| **58** | PAX5 | yes | - | *ND* | - |
| **59** | OY-TES1 | yes | - | *ND* | - |
| **66** | B7H3 | - | - | PIN | prostate |

[a] Rankings from 2009 NCI Workshop reported in Cheever et al. 2009.

[b] Premalignant lesions with validated expression on human tissue samples.

[c] Listed cancer types correspond in order to listed premalignant lesions. Premalignant lesions of the same cancer tissue type are separated by commas while lesions of different tissue types are separated by semi-colons.