Boosting Cancer Immunotherapy with Anti-CD137 Antibody Therapy

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Abstract

In the last five years, immunomodulatory antibodies have revolutionized cancer immunotherapy. CD137, a member of the tumor necrosis factor receptor superfamily, represents a promising target for enhancing anti-tumor immune responses. CD137 helps regulate the activation of many immune cells, including CD4+ T cells, CD8+ T cells, dendritic cells and natural killer cells. Recent studies indicate that the anti-tumor efficacy of therapeutic tumor-targeting antibodies can be augmented by the addition of agonistic antibodies targeting CD137. As ligation of CD137 provides a co-stimulatory signal in multiple immune cell subsets, combination therapy of CD137 antibody with therapeutic antibodies and/or vaccination has the potential to improve cancer treatment. Recently, clinical trials of combination therapies with agonistic anti-CD137 mAbs have been launched. In this review, we discuss the recent advances and clinical promise of agonistic anti-CD137 monoclonal antibody therapy.
Introduction

Antibody-based strategies to cancer treatment have dramatically advanced in the last 20 years (1). Since rituximab was approved as the first monoclonal antibody (mAb) for the treatment of cancer in 1997 (2, 3), several mAbs have become standard of care for the treatment of both solid tumors and hematologic malignancies (Table 1). Most of the approved mAbs (e.g. rituximab, trastuzumab, cetuximab) target tumor-associated antigens on the surface of cancer cells and inhibit cell growth. Although several effective antibodies have emerged, long-term, durable responses remain elusive and resistance and relapse remain major problems (4-6). Immunomodulatory antibodies have revolutionized cancer immunotherapy and helped garner the breakthrough distinction (7-11). In 2011, the FDA approved the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)-specific mAb, ipilimumab, for the treatment of metastatic melanoma, representing a major milestone in cancer immunotherapy (12). The second FDA-approved immunomodulatory agent, pembrolizumab, is anti-programmed cell death 1 (PD-1, PDCD1 or CD279) mAb, which was approved in 2014 (13). In the same year, blinatumomab, a novel bispecific T cell engager (BiTE) antibody specific to CD19
and CD3 was approved for patients with acute lymphoblastic leukemia (14). Most cancer immunotherapy strategies stimulate the patient’s immune system to overcome immunosuppression induced by tumor cells and generate an anti-tumor immune response. The clinical data and recent FDA approvals validate mAb-mediated cancer immunotherapy as a valuable therapeutic strategy.

In addition to checkpoint blockade agents like ipilimumab and pembrolizumab, agents targeting the tumor necrosis factor (TNF) superfamily of co-stimulatory receptors have entered development (8). CD137 is one of the TNF receptor family targets that has advanced into clinical trials. CD137 regulates many immune cells, including CD4+ and CD8+ T cells, regulatory T cells (Tregs), dendritic cells (DCs) and natural killer (NK) cells (15, 16). Recent studies indicate that the addition of anti-CD137 mAbs can augment the anti-tumor efficacy of immunomodulatory antibodies.

**CD137**

CD137 (4-1BB) or TNF receptor superfamily member 9 (TNFRSF9) is a costimulatory receptor that belongs to the TNF receptor superfamily (9, 16, 17). The cDNA of CD137
was cloned in 1989 as an inducible gene from stimulated T cells (18). Follow-up studies showed that CD137 is also detectable on Tregs, DCs and NK cells. The functional role of CD137 in enhancing cytotoxic T cell responses was established in 1997 and soon anti-CD137 mAbs were being explored as cancer therapies (19). Melero et al. (20) first reported that the administration of anti-CD137 mAbs could eradicate established large tumors in mice, including the poorly immunogenic Ag104A sarcoma and the highly tumorigenic P815 mastocytoma. The immune response induced by anti-CD137 mAbs was shown to be mediated by CD8+ cells and accompanied by a marked augmentation of tumor-selective cytolytic T-cell activity. CD137 signaling also promotes some CD4+ helper T cell functions that facilitate a CD8+ CTL response. Interestingly, the efficacy of anti-CD137 mAbs was long lasting and generated memory responses as mice survived rechallenge with the same tumor. The role of CD137 in anti-tumor responses was also demonstrated in CD137−/− mice in the B16F10 melanoma model (21). The knockout mice displayed increased metastasis in the lungs and shorter survival time compared with wild type mice. Anti-CD137 mAbs elicit several immune responses on different types of immune cells. The mechanism of anti-cancer effects mediated by these cells is
described below (Fig. 1). In addition, the role of CD137 signaling has been studied in several autoimmune processes (16), including rheumatoid arthritis, experimental autoimmune encephalomyelitis, and systemic lupus erythematosus. These studies showed significant protection against the autoimmune disorders. This dual immunoregulatory activity of CD137 offers the possibility to enhance antitumor activity without autoimmune side effects associated with immunotherapy approaches.

**Co-stimulation through CD137**

**T cell**

The immune response induced by anti-CD137 mAbs is mediated by both CD8+ and CD4+ T cells and is accompanied by a significant increase in tumor-selective cytolytic T-cell activity, including increased T cell proliferation, resistance to apoptosis, and increased interferon (IFN)γ secretion (16). Several TNF receptors including CD137, OX40 (also known as TNFSF4), glucocorticoid-induced TNFR-related protein (GITR or TNFRSF18) and CD30 (TNFRSF8) function primarily as co-stimulatory molecules for T cells activation (7, 8). One of the best-characterized costimulatory activities in T cells is mediated by CD137. *In vitro* studies showed that CD137 agonistic antibody can
co-stimulate both CD4+ and CD8+ T cells, and induce IL-2 and IL-8 secretion by DCs and macrophages, leading to enhanced T cell proliferation and cytokine secretion (22). Anti-CD137 therapy was ineffective in B6 mouse embryo C3 tumors, TC-1 lung carcinoma and B16.F10 melanoma models, when CTLs were depleted (23). In melanoma tumor models, anti-CD137 antibodies not only prevented activation-induced cell death, but also augmented CD8+ T cell proliferative potential and enhanced cytolytic activity against tumor cells (24). In addition, co-stimulation through CD137 and OX40 activates Akt to promote cell cycling through regulation of cyclins and cyclin-dependent kinases (25).

**Regulatory T cell**

Regulatory T cells (forkhead box P3 (FOXP3)+ or CD4+CD25+) downregulate the functions of T cells to prevent autoimmunity. They also suppress the cytotoxic response of T cells, which leads to immune tolerance to cancer. Recently, we have demonstrated that surface expressions of both OX40 and CTLA-4 are limited to the tumor-specific Tregs subset (26). Local immunomodulation by the injection of anti-OX40 and anti-CTLA-4 mAbs into one tumor elicited potent antitumor immune response that led
to eradication of distant tumors. Thus, Tregs may control local tumor immunomodulation and also mediate systemic tumor eradication. CD137 is also expressed on Tregs (15). Curran et al. (27) and Guo et al. (28) reported that anti-CD137 mAb reduced Treg infiltration in tumors. Guo et al. asserted that anti-CD137 mAb directly reduced Tregs. Curran et al. claimed that Tregs were reduced as a percentage of the tumor T cell pool which did not necessarily involve any change to the Tregs themselves. It was also reported that only CD137 negative Tregs infiltrated tumor sites and provided protection, while the population of CD137 positive Tregs consisted primarily of activated Tregs (29). Houot et al. (30) demonstrated depletion of Tregs dramatically enhanced anti-CD137 therapy in mice. Based on these reports, we suggest that suppression or elimination of Tregs may be a valuable component of future therapeutic strategies.

**Dendritic cells**

Dendritic cells (DCs) represent unique antigen-presenting cells capable of sensitizing T cells to both new and recall antigens. DCs have been shown to play an important role in CD137-mediated anti-tumor immunity (31); their removal eliminated the efficacy of
anti-CD137 in tumor in vivo (32). Anti-CD137 mAb, when combined with vaccination with tumor cell lysate-pulsed dendritic cells (TP-DC), accelerated tumor regression, and enhanced the survival of tumor-bearing mice (33), suggesting a role for vaccinated DCs with upregulated CD137 in enhancing CTL anti-tumor activity. In the presence of human CD137L extracellular domain (exCD137L), antigen-loaded human DCs markedly increased the functions of anti-tumor CTL as measured by T lymphocyte proliferation, IL-2 and IFNγ secretion, cell viability and cytotoxicity (34). Recently, DCs were shown to be negatively regulated by immunosuppressive invariant natural killer T cells (iNKT) in 4T1 mouse mammary tumors and the selective elimination of DCs by iNKT immunosuppressive cells were shown (35). Here, priming of T cells to a tumor-specific CD8+ T cell epitope in mice treated with radiotherapy and anti-CTLA-4 or anti-CD137 mAbs was markedly enhanced in iNKT−/− compared to WT mice. These data suggest DCs play a critical role in the regulation of CD137-mediated CTL activation by enhancing co-stimulation.

NK cell
NK cells (CD3+CD56+ cells) initiate innate immune responses toward tumor and virus-infected cells (36, 37). One of the primary mechanisms of anti-tumor activity of monoclonal antibodies is antibody dependent cell-mediated cytotoxicity (ADCC) whereby NK cells bearing an Fc receptor (CD16) bind to the antibody-targeted tumor cell and mediate tumor cell lysis. CD137 was also detected on NK cells. It was reported that selective depletion of NK cells in mice by the anti-AsialoGM1 or anti-NK1.1 antibodies completely abrogated the antitumor effect of anti-CD137 mAb, implying an immunoregulatory function of CD137 on NK cells (30, 38). Expression of CD137 on NK cells increases significantly when NK cells encounter mAbs bound to tumor cells (39-41). Anti-CD137 mAbs potentiated the antitumor activity of anti-CD20 and anti-HER2 (also known as ERBB2) mAbs in the mouse models of lymphoma and breast cancer, respectively. We reasoned that the addition of an agonistic mAb against CD137 would further stimulate activated NK cells and result in enhanced ADCC towards a mAb bound to tumor cells (17). Therefore, combination therapy of anti-CD137 mAb with mAbs targeting tumor-associated antigens is appealing.

**Preclinical Studies of CD137 Antibody**
Anti-tumor efficacy was also observed in several tumor models such as MCA205 sarcoma, MC38 colon carcinoma, GL261 glioma, TC1 carcinoma, J558 myeloma, and A549 human alveolar adenocarcinoma cell lines (16). However, anti-CD137 mAb monotherapy did not eradicate some poorly immunogenic tumors, namely C3 tumor and B16/D5 melanoma (23, 42). To improve the therapeutic efficacy of anti-CD137 mAbs, several combination therapies were investigated.

**Combination with other immunomodulators**

Overcoming regulatory mechanisms of T cells can enhance anti-tumor responses. For example, PD-1, CTLA-4, T cell immunoglobulin mucin-3 (TIM-3; also known as HAVCR2) and lymphocyte-activation gene 3 (LAG-3; also known as CD223) negatively regulate T cell function, while CD137, OX40 and CD40 provide co-stimulation (7). Combination therapies can potentiate T cell–based cancer immunotherapy (Table 2). Agonistic CD137 mAbs with anti-CTLA4 or anti-CD40 mAbs increased the survival of mice injected with MC38 murine colon cancer cells (43, 44). Uno et al. (45) reported that an agonistic monoclonal antibody to death receptor 5 (DR5; also known as TNFRSF10B), the apoptosis-inducing receptor for TNF-related
apoptosis-inducing ligand, combined with agonistic monoclonal antibodies to the costimulatory molecules CD40 and CD137, rapidly stimulated tumor-specific effector CD8^+ T cells that led to eradication of pre-established tumors. This combination was named “trimAb combinations”. In addition to trimAb, the effects of anti–CTLA-4 mAb, anti-glucocorticoid-induced TNF-receptor mAb, or anti–PD-1 mAb were examined (46). Blockade of CTLA-4-mediated signals by an antagonistic mAb substantially increased the tumor rejection rate of trimAb therapy, although the immune responses of draining lymph node cells were not augmented. Anti-DR5 and anti-CD1d mAb with anti-CD137 mAb (1DMab therapy) were also reported to show enhanced effectiveness (47). Interestingly, 1DMab therapy was more effective than trimAb in tumor models regulated by CD1d-restricted type II NKT cells, but less efficacious against tumors where Tregs were critical. Simultaneous dual costimulation through CD137 and OX40 induced a massive burst of CD8 T cell effector function sufficient to therapeutically treat established tumors (48). Remarkably, combination of anti-PD-1 mAb also led to the long-term survival of mice with established TC1 lung tumors, B16.F10 murine melanoma, and CT26 cells (49-51). On the other hand, their combination significantly
increased some markers for liver toxicity and hematological parameters, compared with the corresponding anti-CD137 alone groups (50). Based on these preclinical studies, several clinical studies of anti-CD137 and anti-PD-1 mAb have been launched.

**Combination with vaccination**

Dendritic cells can be pulsed with tumor-associated antigen by a variety of methods that result in the ability of DCs to prime naive T cells, and DCs can mediate regression of established tumor when given as a vaccine in animal models (Table 2). Ito et al. (33) and Lee et al. (52) examined the role of anti-CD137 administration in modulating the immune responses induced by tumor lysate-pulsed DC (TP-DC) vaccinations. Combined therapy with TP-DC plus anti-CD137 mAb resulted in lower local recurrence rates and improved survival after surgical resection of subcutaneous tumors. Similarly, immunizations in combination with the costimulatory agonistic anti-CD137 mAb significantly enhanced the immune responses in Her-2/neu mice, resulting in complete tumor rejection (53). Using vaccines that stimulate a broad immune response in combination with costimulatory molecules could significantly improve the antitumor immune response in tolerant hosts. CpG vaccination and oncolytic viruses, as well as
adoptive transfer of tumor-specific cytotoxic T lymphocytes (CTLs), were also potentiated by agonistic anti-CD137 mAb (54-57). Thus, agonistic anti-CD137 mAb can modulate immune responses to several vaccinations and enhance anti-tumor efficacy.

Combination with mAb therapy targeting tumor antigens

One of the primary mechanisms of anti-tumor activity of monoclonal antibodies is ADCC (58). Kohrt et al. (39-41) demonstrated that an anti-CD137 agonistic mAb enhances the anti-tumor activity of therapeutic mAbs rituximab, trastuzumab and cetuximab by enhancing ADCC (Table 2). In addition, human NK cells up-regulate CD137 after encountering mAbs and tumor cells in vitro and in the patients, and subsequent stimulation of these NK cells with anti-CD137 mAb enhances mAb-dependent cytotoxicity against tumor cells (41). Therefore, sequential administration of therapeutic antibodies and CD137 mAb with a 24-hour gap would be better than concurrent administration. Stagg et al. (59) also reported interesting results showing that not only anti-CD137 mAb but also anti-PD-1 mAb enhanced the anti-tumor activity of a Her2-targeting mAb in mice. In a clinical trial, a combination
therapy of anti-PD-1 mAb with rituximab achieved a 66% objective response in patients with relapsed follicular lymphoma who were previously treated with rituximab (60). This strongly suggests that combination of immunomodulators including anti-CD137 mAbs with tumor targeting mAbs can enhance the clinical efficacy of therapeutic antibodies (61).

**Clinical Trials of CD137**

Two fully humanized mAbs of CD137, urelumab (BMS-663513) and PF-05082566 have been developed for clinical use. Urelumab is a fully human IgG4 mAb developed by Bristol-Myers Squibb, and PF-05082566 is a fully human IgG2 mAb developed by Pfizer. They are agonistic mAbs, which bind to the extracellular domain of human CD137. Clinical trials of anti-CD137 mAbs are summarized in Table 3.

**Urelumab (BMS-663513)**

The NCT00309023 study was a first-in-human open-label, ascending, multidose Phase I–II trial conducted in patients with locally advanced or metastatic solid tumors (62). In the dose-escalation phase of the study, patients were sequentially assigned to one of six dose cohorts (0.3–15 mg/kg) to receive urelumab once every 3 weeks. Eighty-three
patients (54 melanoma, 15 renal cell carcinoma, 13 ovarian and 1 prostate) have been treated. Dose limiting toxicities were reported in the 0.3 mg/kg (Grade 3 neutropenia), and 15 mg/kg (Grade 4 neutropenia) cohorts. Overall, fatigue (All: 26%, Grade 3–4: 3%), reversible Grade 3–4 transaminitis (11%) and Grade 3–4 neutropenia (5%) were the most common agent-related adverse events. Three Partial Response (PR) and 4 Stable Disease (SD) cases occurred at all three doses tested in expansion cohorts. Preliminary biomarker analysis demonstrated increased expression of IFN-inducible genes in peripheral blood, serum neopterin levels, and percentage of circulating activated CD8+ and CD4+ T cells following a single treatment. These data suggest that urelumab was tolerable across a wide dose range (0.3–15 mg/kg). Based on the Phase I study, a randomized, multi-dose, open-label, Phase II study of urelumab as a second-line monotherapy was designed in the patient with metastatic melanoma. However, the study was terminated in May 2009 due to fatal hepatotoxicity. The mechanism of anti-CD137 mAb-induced hepatotoxicity remains unclear, although the relationship between CD137 pathway and hepatotoxicity was suspected (50, 63, 64). Therefore, careful dosing of anti-CD137 mAb is needed to avoid the risk for severe
hepatotoxicity.

Following the first clinical trial, several combination therapies with chemotherapy (NCT00351325), chemoradiation (NCT00461110), ipilimumab (NCT00803374), rituximab (NCT01775631) (65), cetuximab (NCT02110082), and elotuzumab (NCT02252263) have been launched as Phase I or I/II studies. We have initiated a biomarker study (NCT01471210) using the novel technology of mass cytometry time of flight (CyTOF) (66, 67). Preliminary findings from 4 patients showed an increase in CD8+ T cells and NK cells with a decrease in CD4+ T cells and regulatory CD4+ T cells. These preliminary data are consistent with anti-CD137 agonism. Although the studies (NCT00351325, NCT00461110, NCT00803374) were terminated or withdrawn, low-dose therapies (<0.1 mg/kg) of urelumab in combination with approved mAbs are worthy of attention.

**PF-05082566**

Clinical trials of PF-05082566 are also on-going. NCT01307267 is an open-label, dose escalation study that was conducted in patients with advanced malignancies, and the preliminary data was reported (68). Cohorts of 3-6 patients were enrolled initially using
a 3+3 design (0.006–0.3 mg/kg), then a Time-To-Event continual reassessment method design for higher doses (0.6–5 mg/kg). Patients received PF-05082566 via intravenous infusion every 4 weeks (one cycle) with an 8 weeks period for assessment of dose-limiting toxicity (DLT). Twenty-seven patients have been treated with PF-05082566 up to the 0.3 mg/kg dose level, including colorectal cancer (n=11), Merkel cell carcinoma (n=6), and pancreatic adenocarcinoma (n=2). Twenty-five patients completed the DLT assessment period and 7 patients remain on therapy. All discontinuations from treatment were due to disease progression. One patient treated at 0.06 mg/kg had Grade 3 elevation in alkaline phosphatase. No additional significant elevations in liver enzymes and no DLTs have occurred to date. The best overall response of stable disease was observed in 22% (6/27) patients. These results suggested that PF-05082566 was well tolerated, with evidence of disease stabilization in multiple patients.

**Combination with anti-PD-1 mAbs**

In 2014, one of most interesting combination therapies with anti-CD137 mAbs is with nivolumab or MK-3475 (anti-PD-1 mAbs) for patients with advanced solid tumors or
advanced B cell non-Hodgkin lymphoma. The safety and tolerability of urelumab administered in combination with nivolumab is being assessed in a phase 1/2 dose escalation and cohort expansion study (NCT02253992). Nivolumab and urelumab were administered every 2 weeks up to 12 cycles and every 4 weeks up to 3 cycles, respectively. NCT02179918 is a Phase 1b study of PF-05082566 in combination with MK-3475. Both are administered every 3 weeks. A preclinical study indicated that combination of anti-CD137 and anti-PD-1 mAbs enhanced hepatic toxicity, compared with single alone (50). Although this combination has potential for good efficacy, the toxicity of this combination should be evaluated in humans.

Conclusions

More than 20 years have passed since the identification of CD137 as an immune modulator (18). One of the most promising findings is the anticancer efficacy of agonistic anti-CD137 mAb (20). The strong preclinical successes underscore the importance of CD137 in cancer therapy, especially in combination therapy. Several clinical trials of urelumab (BMS-663513) had been terminated or withdrawn, because of hepatitis. However, clinical trials of combination therapies using low-dose urelumab
with rituximab, cetuximab and anti-PD-1 mAbs have been launched. In addition, another anti-CD137 mAb PF-05082566 has been developed. We believe anti-CD137 mAbs hold great clinical promise. Its clinical potential should be tested in conjunction with other FDA-approved immunomodulators and antibody therapeutics. It is anticipated that “Combination Cancer Immunotherapy” with CD137 will make significant contributions to the field of cancer immunotherapy.

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Table 1. Therapeutic antibodies approved in the United States

<table>
<thead>
<tr>
<th>Approval</th>
<th>Antibody (Trade name; Company)</th>
<th>Type</th>
<th>Target</th>
<th>Tumor types</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td>Rituximab (Rituxan; Genentech)</td>
<td>chimeric IgG1</td>
<td>CD20</td>
<td>NHL, CLL</td>
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<tr>
<td>1998</td>
<td>Trastuzumab (Herceptin; Genentech)</td>
<td>humanized IgG1</td>
<td>HER2</td>
<td>Breast, gastric</td>
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<tr>
<td>2000</td>
<td>Gemtuzumab ozogamicin (Mylotarg; Wyeth Pharms)</td>
<td>humanized IgG4, calicheamicin</td>
<td>CD33</td>
<td>AML</td>
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<td>2001</td>
<td>Alemtuzumab (Campath; Ilex Pharmaceuticals)</td>
<td>humanized IgG1</td>
<td>CD52</td>
<td>CLL</td>
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<tr>
<td>2002</td>
<td>⁹⁰⁸Y-labeled ibritumomab tiuxetan (Zevalin; Spectrum Pharms)</td>
<td>murine IgG1, tiuxetan</td>
<td>CD20</td>
<td>NHL</td>
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<td>2003</td>
<td>¹³¹I-labeled tositumomab (Bexxar; SmithKline)</td>
<td>murine IgG2, tositumomab</td>
<td>CD20</td>
<td>NHL</td>
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<td>2004</td>
<td>Cetuximab (Erbitux; Bristol-Myers Squibb)</td>
<td>chimeric IgG1</td>
<td>EGFR</td>
<td>Colon, head, and neck</td>
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<td>2004</td>
<td>Bevacizumab (Avastin; Genentech)</td>
<td>humanized IgG1</td>
<td>VEGF</td>
<td>Colon, NSCL, glioblastoma, kidney, cervix, ovarian</td>
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<td>2006</td>
<td>Panitumumab (Vectibix; Amgen)</td>
<td>human IgG2</td>
<td>EGFR</td>
<td>Colon</td>
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<tr>
<td>2009</td>
<td>Ofatumumab (Arzerra; Genmab)</td>
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<td>CD20</td>
<td>CLL</td>
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<td>2011</td>
<td>Brentuximab vedotin (Adcetris; Seattle Genetics)</td>
<td>chimeric IgG1, MMAE</td>
<td>CD30</td>
<td>Hodgkin’s lymphoma</td>
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<td>2011</td>
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<td>2012</td>
<td>Pertuzumab (Perjeta; Genentech)</td>
<td>humanized IgG1</td>
<td>HER2</td>
<td>Breast</td>
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<td>2013</td>
<td>Ado-trastuzumab emtansine (Kadcyla, Genentech)</td>
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<td>HER2</td>
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<td>PD-1</td>
<td>Melanoma</td>
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<td>CD19, CD3</td>
<td>ALL</td>
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<td>2014</td>
<td>Nivolumab (Opdivo; Bristol-Myers Squibb)</td>
<td>human IgG4</td>
<td>PD-1</td>
<td>Melanoma, NSCL</td>
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<td>2015</td>
<td>Dinutuximab (Unituxin; United Therapeutics)</td>
<td>chimeric IgG1</td>
<td>GD2</td>
<td>Neuroblastoma</td>
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Table 2. Combination therapy with anti-CD137 mAb in mice

<table>
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<tr>
<th>Combination with mAb therapy targeting to immune cell</th>
<th>Materials</th>
<th>Tumor cell</th>
<th>Cell type</th>
<th>Ref</th>
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<tr>
<td>anti-CTLA-4 mAb</td>
<td>4F10</td>
<td>MC38 cell</td>
<td>murine colon cancer cell</td>
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<tr>
<td>anti-CD40 mAb</td>
<td>FGK-45</td>
<td>MC38 cell</td>
<td>murine colon cancer cell</td>
<td>(44)</td>
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<td>anti-DR5, CD40 mAbs</td>
<td>MD5-1, FGK45</td>
<td>4T1 cell</td>
<td>murine mammary carcinoma</td>
<td>(45)</td>
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<tr>
<td>“trimAb therapy”</td>
<td>MD5-1, FGK45, UC10-4F10</td>
<td>4T1 cell</td>
<td>murine mammary carcinoma</td>
<td>(46)</td>
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<tr>
<td>anti-DR5, CD1d mAbs</td>
<td>MD5-1, 1B1</td>
<td>4T1 cell</td>
<td>murine mammary carcinoma</td>
<td>(47)</td>
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<td>“1DMab”</td>
<td>RMT3-23</td>
<td>ID8 cell</td>
<td>murine ovarian carcinoma</td>
<td>(28)</td>
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<td>anti-OX40 mAb</td>
<td>MRC OX86</td>
<td>MethA cell</td>
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<td>anti-PD-1 mAb</td>
<td>RMP1-14</td>
<td>ID8 cell</td>
<td>murine ovarian carcinoma</td>
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<td>anti-PD-1, anti-CTLA4 mAbs</td>
<td>RMP1-14 + 9D9</td>
<td>TC1 cell</td>
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<td>anti-PD-1 mAb + platinum agent</td>
<td>RMP1-14 + cisplatin</td>
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<td>anti-PD-1 mAb</td>
<td>RMP1-14</td>
<td>B16.F10 cell</td>
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<td>anti-PD-1 mAb</td>
<td>RMP1-14</td>
<td>CT26 cell</td>
<td>murine colon adenocarcinoma cells</td>
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Table 2. (cont.) Combination therapy with anti-CD137 mAb in mice

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<thead>
<tr>
<th>Combination</th>
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<td><strong>Combination with vaccination</strong></td>
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<tr>
<td>DC vaccine</td>
<td>tumor lysate pulsed-DCs</td>
<td>MCA205 cell</td>
<td>murine fibrosarcomas</td>
<td>(33)</td>
</tr>
<tr>
<td>DC vaccine</td>
<td>tumor lysate pulsed-DCs</td>
<td>CT26 cell</td>
<td>murine metastatic colon cancer cell</td>
<td>(52)</td>
</tr>
<tr>
<td>DC vaccine</td>
<td>tumor lysate pulsed-DCs</td>
<td>N202.1A cell</td>
<td>murine mammary cell line</td>
<td>(53)</td>
</tr>
<tr>
<td>&amp; anti-OX40 mAb</td>
<td>OX86</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adoptive CTL therapy</td>
<td>tumor-specific CTL</td>
<td>B16.F10 cell</td>
<td>murine melanoma cell</td>
<td>(69)</td>
</tr>
<tr>
<td>B16-Flt3L vaccine</td>
<td>B16-Flt3-ligand</td>
<td>B16-sFlt3L cell</td>
<td>murine melanoma cells</td>
<td>(27)</td>
</tr>
<tr>
<td>&amp; anti-CTLA-4 mAb</td>
<td>9D9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CpG vaccine</td>
<td>CpG1826</td>
<td>Renca cell</td>
<td>murine renal cell carcinoma</td>
<td>(54)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MC38 cell</td>
<td>murine colon tumor cell</td>
<td></td>
</tr>
<tr>
<td>Peptide &amp; CpG vaccine</td>
<td>Trp2 peptides plus CpG</td>
<td>B16 BL6</td>
<td>murine melanoma</td>
<td>(55)</td>
</tr>
<tr>
<td>Oncolytic virus</td>
<td>Oncolytic Vvdd vaccinia virus</td>
<td>AT-3 cell</td>
<td>murine breast carcinoma</td>
<td>(56)</td>
</tr>
<tr>
<td>IL-12 gene therapy</td>
<td>adenovirus expressing IL-12</td>
<td>B16.F10 cell</td>
<td>murine melanoma cell</td>
<td>(70)</td>
</tr>
<tr>
<td>Virus vaccine</td>
<td>adenovirus with LCMV gene</td>
<td>B16.F10-GP cell</td>
<td>murine melanoma cell</td>
<td>(57)</td>
</tr>
<tr>
<td>&amp; anti-CTLA-4 mAb</td>
<td>9H10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. (cont.) Combination therapy with anti-CD137 mAb in mice

<table>
<thead>
<tr>
<th>Combination with mAb therapy targeting to tumor antigen</th>
<th>Materials</th>
<th>Tumor cell</th>
<th>Cell type</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-CD20 mAb</td>
<td>rituximab</td>
<td>Raji cell</td>
<td>human CD20⁺ B cell</td>
<td>(39)</td>
</tr>
<tr>
<td>anti-CD20 mAb</td>
<td>MB20-11</td>
<td>BL3750 cell</td>
<td>murine CD20⁺ B cell</td>
<td></td>
</tr>
<tr>
<td>anti-HER2 mAb</td>
<td>trastuzumab</td>
<td>BT474M1, MCF7</td>
<td>human breast tumor cell</td>
<td>(40)</td>
</tr>
<tr>
<td>anti-ErbB-2 mAb &amp; anti–PD-1 mAb</td>
<td>7.16.4, RMP1-14</td>
<td>HER18 cells</td>
<td>HER18 cells</td>
<td></td>
</tr>
<tr>
<td>anti-EGFR mAb</td>
<td>cetuximab</td>
<td>SCC6, T84, HCT116</td>
<td>human colon cancer cell</td>
<td>(41)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NCT number</th>
<th>Phase</th>
<th>Condition</th>
<th>Combination</th>
<th>Start year</th>
<th>Status (Feb, 2015)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT00309023</td>
<td>I/II</td>
<td>Metastatic or Locally Advanced Solid Tumors</td>
<td>-</td>
<td>2005</td>
<td>Terminated</td>
</tr>
<tr>
<td>NCT00351325</td>
<td>I</td>
<td>Advanced Solid Malignancies</td>
<td>Chemotherapy</td>
<td>2007</td>
<td>Terminated</td>
</tr>
<tr>
<td>NCT00461110</td>
<td>I</td>
<td>Non Small Cell Lung Cancer</td>
<td>Chemoradiation</td>
<td>2008</td>
<td>Terminated</td>
</tr>
<tr>
<td>NCT00612664</td>
<td>II</td>
<td>Melanoma</td>
<td>-</td>
<td>2008</td>
<td>Completed</td>
</tr>
<tr>
<td>NCT00803374</td>
<td>I</td>
<td>Advanced Malignant Melanoma</td>
<td>Ipilimumab (anti-CTLA-4 mAb)</td>
<td>2010</td>
<td>Withdrawn</td>
</tr>
<tr>
<td>NCT01471210</td>
<td>I</td>
<td>Advanced and/or Metastatic Solid Tumors</td>
<td>-</td>
<td>2012</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT01775631</td>
<td>Ib</td>
<td>Relapsed/Refractory B-cell Non-Hodgkin's Lymphoma</td>
<td>Rituximab (anti-CD20 mAb)</td>
<td>2013</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02110082</td>
<td>Ib</td>
<td>Colorectal Cancer, Head and Neck Cancer</td>
<td>Cetuximab (anti-EGFR mAb)</td>
<td>2014</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02252263</td>
<td>I</td>
<td>Multiple Myeloma</td>
<td>Elotuzumab (anti-CS1 mAb)</td>
<td>2014</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02253992</td>
<td>I/II</td>
<td>Advanced Solid Tumors, Advanced B-cell NHL</td>
<td>Nivolumab (anti-PD-1 mAb)</td>
<td>2014</td>
<td>Recruiting</td>
</tr>
<tr>
<td>PF-05082566: fully human type IgG2, Pfizer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT01307267</td>
<td>I</td>
<td>CD20 positive Non-Hodgkin's Lymphoma</td>
<td>Rituximab (anti-CD20 mAb)</td>
<td>2011</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02179918</td>
<td>Ib</td>
<td>Advanced Solid Tumors</td>
<td>MK-3475 (anti-PD-1 mAb)</td>
<td>2014</td>
<td>Recruiting</td>
</tr>
</tbody>
</table>
**Figure 1.** Immunomodulatory mechanisms of CD137. CD137 is expressed in several immune cells. Agonistic anti-CD137 mAb increases T cell proliferation, differentiation to memory cells and resistance to apoptosis in CD8+ T cells. In addition, anti-CD137 mAb can depress regulatory T cells (Tregs) function. In dendritic cells, anti-CD137 mAb with vaccination enhances tumor antigen presentation and co-stimulation to increase the functions of anti-tumor cytotoxic T lymphocytes (CTL). One of the primary mechanisms of anti-tumor activity of monoclonal antibodies is antibody dependent cell-mediated cytotoxicity (ADCC). On NK cells, stimulation of CD137 enhances ADCC. Agonistic anti-CD137 mAb stimulates CD8+ T cells, Tregs, DCs and NK cells to induce potent antitumor immune response. MHC: major histocompatibility complex, TCR: T cell receptor.
Figure 1:

Gain of effector functions: increases T-cell proliferation, differentiation to memory cells and resistance to apoptosis

Depresses regulatory T-cell function

Spontaneous cytotoxicity enhances ADCC

Enhances tumor antigen presentation and costimulation

Vaccination

ADCC

Antitumor mAb

Agonistic anti-CD137 mAb

CD8^+ T cell

Tumor

FOXP3

Treg

CD137

CD16

CD56

NK

PD-1

PD-L1

CTLA-4

TCR

MHC

CD3

CD8

CD25

CD16

CD56

CD137

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Boosting Cancer Immunotherapy with Anti-CD137 Antibody Therapy

Atsushi Yonezawa, Suparna Dutt, Cariad Chester, et al.

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