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Mechanisms of Action of Therapeutic Antibodies for Cancer

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Abstract

The therapeutic utility of antibodies and their derivatives is achieved by various means. The FDA has approved several targeted antibodies that disrupt signaling of various growth factor receptors for the treatment of a number of cancers. Rituximab, and other anti-CD20 monoclonal antibodies are active in B cell malignancies. As more experience has been gained with anti-CD20 monoclonal antibodies, the multifactorial nature of their anti-tumor mechanisms has emerged. Other targeted antibodies function to dampen inhibitory checkpoints. These checkpoint inhibitors have recently achieved dramatic results in several cancers, including melanoma. These and related antibodies continue to be investigated in the clinical and pre-clinical settings. Novel antibody structures that target two or more antigens have also made their way into clinical use. Tumor targeted antibodies can also be conjugated to chemo- or radiotherapeutic agents, or catalytic toxins, as a means to deliver toxic payloads to cancer cells. Here we provide a review of these mechanisms and a discussion of their relevance to current and future clinical applications.

1. Introduction

Antibodies have proven to be powerful additions to the therapeutic armamentarium for a wide range of human diseases, including many types of cancer. The class of antibody most frequently used clinically is IgG. IgG is further divided into subclasses, each with unique and sometimes overlapping properties, including the ability to not only target and interfere with cell signaling but also induce CDC, ADCC, and ADPh[1-3]. While antibodies are commonly thought of in terms of their antigen specificities, native IgG is a bifunctional

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Conflicts of Interest

Louis M. Weiner, MD Merrimack Pharmaceuticals (Scientific Advisor, Stock Options) Celldex Pharmaceuticals (Scientific Advisor, Stock Options) TDT Therapeutics (Stock) Jounce Therapeutics (Scientific Advisor, Stock Options) Abbvie (Scientific and Clinical Advisor) Immunome (Scientific Advisor) Sandoz Pharmaceuticals (Consultant) CytoMx (Consultant)

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protein. It is becoming increasingly evident that the anti-tumor effects of Ab are driven both by their antigen-binding regions and by the properties of their Fc domains.

Several FDA approved antibodies target the receptors of the epidermal growth factor family and are mainstays of some breast and colon cancer treatment algorithms. By directly binding to these membrane bound receptors, these Abs inhibit their activity, resulting in dampened function of the downstream signaling cascades that promote cell cycle and function. However, in addition to signaling blockade, some members of this family of antibodies can also mediate ADCC of tumor cells[4, 5]. Other antibodies such as rituximab, targeting CD20 expressed on B cells/B cell malignancies, are also capable of inducing a signaling mediated death. However a growing body of work has demonstrated that both the variable and constant regions mediate the effects of rituximab by inducing CDC, ADPh and ADCC[6-12]. This information has led to development of novel anti-CD20 Abs selected for their superiority in inducing CDC and ADCC based on their physical properties that may alter binding with Fc receptors on immune effector cells.

Immune checkpoint inhibiting antibodies have produced some of the most striking results within recent years. By essentially taking the brakes off of T cells, treatment with these antibodies is creating durable responses in patients with advanced melanoma[13-16] and other diseases, including renal cell carcinoma, non-small cell lung cancer and Hodgkin's Disease. Agonist antibodies to immune activating molecules are also under investigation.

Antibody structural derivatives also contribute to the growing clinical immunotherapy arsenal. The first clinically approved bispecific T cell engager is able to redirect the killer T cells of cancer patients directly to tumor cells via two engineered antigen binding sites. Various other platforms are in development and clinical trials for multiple malignancies. Anti-tumor agents can also be ferried by antibodies to tumor cells and exert their effects with decreased collateral damage to healthy tissue.

We will discuss the many antibodies relevant to cancer therapy with the aim of highlighting their basic mechanisms of action. Bearing in mind that several antibodies have multiple mechanisms of action, we have grouped antibodies into sections, based upon predominant mechanism or structure. This approach provides a glance at the rapidly evolving clinical landscape.

We searched for relevant articles on PubMed. In order to guide selection of PubMed searches regarding agents under development by private companies terms, Google searches were also employed. References to clinical trials are verified as cited or by searching clnicaltrials.gov.

2. Antibody Structure

Antibodies, or immunoglobulins (Igs) exist in five distinct forms: IgA, IgD, IgE, IgG and IgM. Each of these has unique properties and functions determined by the constant region of the Ig. IgG is the class of Ig most often used in cancer therapy[17]. IgG consist of two identical antigen binding fragments (Fab) and one Fc region. While the Fab regions bind the target of the antibody (Ab), the Fc region binds to multiple molecules. These include

components of the complement cascade, neonatal Fc receptors and Fcγ receptors present on neutrophils, monocytes, eosinophils, NK cells, and DCs[2, 18]. Classes of antibodies are further divided into subtypes. Different subtypes of Ig vary in their ability to mediate antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). For example, human IgG2 can recruit myeloid cells for ADCC but does not activate complement[1]. Human IgG4 does not activate ADCC or CDC, while human IgG1 can activate complement and recruit immune effector cells for ADCC[2, 3]. Further, posttranslational modification of the Fc region can also influence the function of antibodies[19]. Therapeutic antibodies are also distinguished by the method in which they were produced. In addition to producing purely mouse monoclonal antibodies, a wide array of contemporary antibody engineering techniques have led to the ability to generate Abs that are human and mouse chimeras, humanized or completely human. Chimeric Abs consist of murine derived variable regions and human constant regions. Humanized Abs are completely human, except for the complementarity determining regions[20].

3. Signaling Disruption

Targeted antibodies affect tumor cells via multiple mechanisms. Tumor signaling can be perturbed when targeted Abs disrupt growth signaling pathways by manipulating the activation state of membrane bound receptors or neutralizing cytokines that are critical to cellular growth and proliferation. Several targets have been extensively validated in the clinic, some of which are highlighted below. Of note, interactions between the Fc portion of these antibodies and FcR on immune effector cells play an additional role in anti-tumor activity, i.e. induction of antibody-dependent cell-mediated cytotoxicity (ADCC). See the Fc mediated section for more information regarding ADCC.

3.1 Epidermal growth factor receptor

The Epidermal growth factor receptor (EGFR) is overexpressed in many different malignancies, including those originating in the colon, head and neck, ovary, lung and brain. EGFR is a transmembrane glycoprotein, containing an extracellular ligand-binding domain and a cytoplasmic domain containing a tyrosine kinase[21]. When a ligand binds to the EGFR, receptor dimerization occurs, leading to activation of the tyrosine kinase domain and subsequent promotion of cell proliferation, migration and invasion via activation of the MAPK and AKT pathways[22]. In tumor cells that overexpress EGFR, some have rearrangements of the EGFR gene that lead to the expression of mutant, constitutively activated receptors [23]. The most common EGFR mutation in the receptor's extracellular domain is EGFRvIII, found in glioblastoma, head and neck cancers and non-small cell lung carcinoma. This mutated receptor has constitutive tyrosine kinase activity and has important prooncogenic effects including proliferation and chemotherapeutic resistance[24-26]. Cetuximab is the most thoroughly studied anti-EGFR agent. Cetuximab is a chimeric EGFR-specific IgG1 monoclonal antibody. It induces cell cycle arrest and apoptosis in tumor cells by blocking ligand binding and receptor dimerization [17]. Combining cetuximab with the widely used FOLFIRI chemotherapy regimen can be effective in patients with metastatic colon cancer characterized by the expression of a wild-type KRAS allele[27]. Colon cancer commonly carries an activating mutation in exon 2 of the KRAS gene encoding a

GTPase[28]. This GTPase functions downstream of EGFR signaling. Accordingly, adding cetuximab to standard therapy does not benefit patients with cancers with activating mutations of KRAS[29]. Panitumumab, another EGFR targeted monoclonal antibody, is a humanized IgG2 isotype antibody that is specific for EGFR. Panitumumab is also ineffective when used to treat patients with cancers that possess activating KRAS mutations[30]. However some reports show that KRAS mutational status is not a predictor of clinical response to cetuximab in patients with non-small cell lung cancer[31]. Other anti-EGFR antibodies include necitumumab and nimotuzumab. These are competitive inhibitors of EGFR's ligand. Necitumumab combined with pemetrexed and cisplatin recently failed to show a benefit in overall survival of patients with NSCLC compared to pemetrexed and cisplatin alone[32]. Nimotuzumab is approved for the treatment of various epithelial malignancies in Europe. For example, it is approved for pancreatic cancer treatment in Germany. It has been shown to provide benefit in head and neck cancer[33], and is used in India. Other clinical trial data suggest a benefit in some pediatric patients with diffuse pontine gliomas[34].

3.2 Human epidermal growth factor receptor 2

Human epidermal growth factor receptor 2 (HER2) is a tyrosine kinase receptor that can be overexpressed in some gastrointestinal, lung, prostate and ovarian adenocarcinomas and is gene amplified in approximately 30% of breast cancers[35, 36]. It is different from EGFR in that it has no known ligand and constitutively adopts an open configuration priming it for heterodimerization and increased mitogenic signaling[36]. Overexpression of HER2 leads to increased signal transduction and activation of the MAPK and P13K/AKT pathways[37]. Antibodies targeting this receptor achieve their signaling perturbation effects by inhibiting receptor homo- and hetero- dimerization and internalization, rather than by blocking ligand binding[36]. Trastuzumab, a humanized IgG1 antibody, was the first FDA approved anti-HER2 antibody. In addition to inhibition of receptor dimerization, trastuzumab induces endocytic destruction of the receptor, and inhibition of HER2 shedding[3]. Trastuzumab can also induce ADCC via Fc region binding to FcγRIIIA on NK cells[4].

Pertuzumab is a newer recombinant humanized monoclonal antibody targeting HER2. Trastuzumab binds HER2 at the juxtamembrane domain IV whereas pertuzumab binds HER2 at the extracellular dimerization subdomain II, which is critical for heterodimerization[38]. Preclinical data suggests that pertuzumab can induce cell death in the absence of HER2 overexpression, by inhibiting receptor heterodimerization[39]. Pertuzumab is also distinct from trastuzumab in that pertuzumab blocks HER2 heterodimerization with the other HER-family receptors that include EGFR, HER3, and HER4[40]. Like trastuzumab, pertuzumab is an IgG1 Ab and can induce ADCC[5]. The CLEOPATRA trial demonstrated synergy of pertuzumab plus trastuzumab leading to the FDA approval in 2012 of this combination when added to docetaxel for HER2-positive metastatic breast carcinoma in patients who had not received prior HER2-directed therapy or chemotherapy for metastatic disease[41]. The combination of pertuzumab with trastuzumab and docetaxel is approved as first line therapy for HER2 positive metastatic breast cancer[42] and for neoadjuvant treatment of HER2 positive breast cancer. Pertuzumab holds promise for the treatment of other solid tumors as well[43].

3.3 Insulin-like growth factor receptor

Signaling through the insulin-like growth factor receptor is reported to play an important role in transformation and cell growth and is over expressed in a wide range of cancers[44]. The insulin-like growth factor system is formed by three ligands, three receptors and at least six high affinity binding proteins that work cooperatively to regulate cellular metabolism, proliferation, differentiation and apoptosis in most cells[45]. The three ligands are insulin, IGF-1 and IGF-2. Correlating with each ligand are three receptors, insulin receptor (IR), IGF-I receptor (IGF-IR) and IGF-2R [46].

Several tissues including liver, bone, muscle and brain produce IGF-1. IGF-IR activation is important in tumorigenesis because of its ability to promote proliferation, protect from apoptosis and potentiate cell migration via activation of the PI3K/AKT/mTOR pathway[46, 47]. For example, IGF-IR activation is seen in hepatocellular carcinoma (HCC) cells. Increased levels of IGF-IR protein and/or excess of IGF ligands have been identified in HCC[48]. MEDI-573, a humanized antibody that neutralizes the IGF-1R ligands IGF-1 and IGF-2 has shown anti-tumor efficacy in pre-clinical models[49, 50]. In a phase I trial MEDI-573 was well tolerated but failed to achieve partial or complete responses in HCC patients[51]. A study in Japanese patients with advanced solid tumors produced similar results[52]. EM164 is a humanized anti-IGF-1R antibody that inhibits signaling through IGF-1R. It has been shown to delay growth of human pancreatic cancer and neuroblastoma cells[44, 53]. Ganitumab, an Ab targeted to IGF-1R, was well tolerated in a phase III trial but failed to improve OS in combination with gemcitabine compared to gemcitabine alone in patient s with metastatic pancreatic cancer[54]. Dalotuzumab is a humanized IGF-1R monoclonal antibody that selectively binds to IGF-IR and blocks receptor autophosphorylation and cell proliferation in vitro. It showed anti-tumor efficacy against breast and lung tumor xenografts in preclinical studies[55]. However, a phase II study showed that the addition of dalotuzumab to the EGFR-targeted tyrosine kinase inhibitor erlotinib did not improve efficacy or outcome in patients with refractory advanced NSCLC[56]. This failure is surprising in light of evidence the IGF-1R inhibition abrogated erlotinib resistance mechanisms in NSCLC cell lines[57].

3.4 Bispecific antibodies for signal disruption

Such failures to validate pre-clinical activity in clinical trials suggests that a complex interplay exists amongst the growth factor receptors and the cascades that they induce, favoring the emergence of survival mechanisms that emerge when therapies target individual receptors. Preclinical work suggests that antibodies bispecific for IGF-1R and HER3 hold promise for overcoming tumor resistance that is based on redundant signaling pathways that allow for tumor cell survival in such settings. Fitzgerald et al showed that HER3 activation can counteract the anti-proliferative effects of IGF-1R antagonism, offering a possible explanation for lack of clinical success of anti-IGF-1R agents such as MEDI-573 and dalotuzumab. To address this, they tested MM-141, an antibody bispecific for IGF-1R and HER3. Treatment with MM-141 reduced levels of HER3 and IGF-1R expressed by pancreatic cells in vitro. Furthermore, they observed that docetaxel, a mainstay in treatment of many solid tumors, caused an increase in IGF-1R and HER3 expression in DU145

prostate cancer xenografts[58]. MM-141 treatment reduced docetaxel-induced upregulation of IGF-1R and HER3, implying that this therapy may avert docetaxel resistance.

Everolimus is an mTOR inhibitor used in refractory cases of RCC. Inhibiting mTOR can also induce expression of HER3 and IGF1R [59]. The Fitzgerald et al data suggest that use of MM-141 can overcome this mTOR inhibitor induced resistance by simultaneously blocking IGF-1R and HER3. MM-141 is currently recruiting patients with advanced solid tumors for a phase I clinical trial.

Many breast cancer patients develop resistance to trastuzumab therapy. Heterodimerization amongst HER2, HER3 and IGF-1R may play a role in this resistance[60-62]. MM-111 is an Ab bispecific for HER2/HER3 that interferes with this interaction. It is attached to a modified human albumin to improve serum half-life. Clinical trials studying MM-111 activity in breast and gastroesophageal cancers are underway. To our knowledge, no peer-reviewed results have yet been published.

Chen et al developed an antibody bispecific for HER2 and IGF-1R[63]. They demonstrated that this construct inhibits tumor growth in mouse models. Interestingly, the bispecific antibody was more effective than the combination of trastuzumab and an antibody specific for IGF-1R alone. The authors speculated that this is due to steric effects resulting from bispecific binding as compared to monospecific antibodies used in concert. Furthermore, the authors suggest that ADCC might play a role in this anti-tumor mechanism, as the components of this construct have human IgG1 Fc regions, which can bind mouse FcγRIII[64].

Some tumor cell lines that are resistant to small molecule inhibitors of IGF-1R overexpress EGFR[65]. This suggests that EGFR overexpression is a survival mechanism in tumors challenged with IGF-1R inhibition. XGFR is a bispecific antibody targeted to these two receptors. It has shown promise for tumor inhibition via both signaling disruption and ADCC in a preclinical murine tumor model.

In a murine lung cancer model, tumors that developed resistance to anti-EGFR Abs (cetuximab) possessed up-regulated HER3 [66]. MEHD7945A is a dual activity Ab that binds both EGFR and HER3. It is under investigation in clinical trials studying its activity in combination with various agents in treating several solid tumors including colorectal and head/neck cancer.

3.5 c-Met

Acquired resistance to EGFR tyrosine kinase inhibitors is a common occurrence and may result due to parallel activation of downstream signaling pathways including MET[67]. For example, 5% to 22% of NSCLC patients with secondary resistance to EGFR tyrosine kinase inhibitors were found to have evidence of amplification of the MET oncogene[68, 69].

MET is a heterodimeric transmembrane receptor tyrosine kinase composed of an extracellular alpha-chain and a membrane-spanning beta-chain linked via disulfide bonds [70]. MET is also known as hepatocyte growth factor receptor (HGFR). It's ligand is hepatocyte growth factor (HGF)[71]. Binding of HGF to MET triggers receptor dimerization

and transphosphorylation leading to conformational changes in MET that activate the tyrosine kinase domain to stimulate morphogenic, proliferative, and anti-apoptotic activities[70]. Amplification of MET and HGF and/or overexpression of MET and HGF occur in multiple tumor types correlating with with poor prognosis in non small cell lung cancer and other solid tumors[72, 73]. Laboratory data suggest MET plays a role in cell motility and morphogenesis, and metastatic lesions typically exhibit higher expression levels of MET than primary tumors[71]. Onartuzumab is a monoclonal antibody developed for dual MET-EGFR inhibition as second-line therapy for NSCLC. It was well tolerated in a phase I trial[74]. A randomized phase II trial suggested that the combination of onartuzumab plus erlotinib may have more benefit than erlotinib alone in patients with MET-over expressing tumors[75, 76]. Unfortunately, a phase III trial of the drug combination in advanced NSCLC was stopped in 2014 because there was no apparent benefit to patients receiving the drug combination compared to erlotinib alone (NCT01456325).

Recent preclinical work has produced antibodies that target four regions of MET[77]. The authors demonstrated that these antibodies inhibit the HGF-dependent MET activity in a cooperative manner and hinder tumor growth in murine models. As more is learned about the complex molecular machinery of MET, this information can be used to develop novel therapeutic antibodies.

3.6 VEGF

Vascular endothelial growth factor (VEGF) and its isoforms are pro-angiogenic factors that bind and activate three different tyrosine kinase receptors VEGFR1, VEGFR2, and VEGFR3[78]. VEGFR-2, which is expressed mainly on the surface of vascular endothelial cells, has been the heavily targeted for oncologic monoclonal antibody development. VEGFR-2 and its receptor are highly expressed in many tumor types, including cancers of the gastrointestinal tract[79]. VEGF-A binding to VEGFR-2 leads to autophosphorylation of tyrosine residues at the carboxy-terminal of the receptor, initiating cell signaling and angiogensis. Blocking angiogenesis deprives tumor cells of a blood supply. Both the ligand (VEGF) and its receptor (VEGFR2) have been targets of monoclonal antibody development. Bevacizumab was the first monoclonal antibody that specifically binds to VEGF-A, sequesters it and prevents it from binding to its receptor[80]. It is routinely used in the treatment algorithms of several solid tumors, including colon, NSCLC, ovarian, cervical, glioblastoma, and metastatic renal cell carcinoma. Ramucirumab is an IgG1 monoclonal antibody that selectively binds to and inhibits VEGFR2 and blocks the VEGFR2 related signaling and activating pathways[81]. Ramucirumab is approved for use in advanced cases of gastric and gastroesophageal adenocarcinomas that have been refractory to first line treatments. Additionally, a novel adnectin (see engineered antibodies section) is currently being investigated for activity in inhibiting the VEGF network.

3.7 Apo2L/TRAIL

Tumor necrosis factor–related apoptosis inducing ligand (Apo2L/TRAIL) is a member of the tumor necrosis factor superfamily. It is expressed on immune cells, including NK cells, can be shed from the cell surface[82, 83]. It binds to many receptors, including death receptor-4 (DR4) and death receptor-5 (DR5). This interaction leads to formation of the

death induced signaling complex and ultimately an apoptotic cascade[83]. Several monoclonal agonist antibodies have been investigated as cancer therapies. Most are directed to DR5; these include Lexatumumab (Human Genome Sciences/GSK), Conatumumab (Amgen), Dorzitumab (Genentech/Roche), LBY135 (Novartis), and Tigatuzumab (Dalichi Sankyo). Mapatumumab DR4 (Human Genome Sciences/GSK) is specific to DR4. A tetrameric nanobody to DR5 was also tested but development did not proceed past phase I trials. As reviewed by Holland, the preclinical activity of apo2L/TRAIL receptor agonists was validated, yet phase II trials in hematologic and solid malignancies failed to demonstrate efficacy (alone or in combination with chemotherapy) that warranted conducting phase III trials.[83] In treatment of metastatic or unresectable pancreatic cancer, Tigatuzumab demonstrated similar PFS rates in combination with gemcitabine compared to other studies using gemcitabine alone[84]. However, this was a single arm study and any added benefit from tigatuzumab cannot be truly assessed. In a trial treating metastatic NSCLC patients, tigatuzumab plus carboplatin plus paclitaxel did not add any benefit compared to carboplatin plus paclitaxel alone[85]. These disappointing results may be explained by evidence that suggests Apo2L/TRAIL signaling can weakly activate the MAP kinase pathway and NFκB[86, 87]. Some data have raised the possibility that apoptosis resistant tumor cells may be able to take advantage of this less dominant apo2L/TRAIL signaling pathway[88, 89]. Clearly many mechanisms are at play when apo2L/TRAIL is targeted, and further investigation is warranted.

3.8 RANKL

Denosumab is a targeted human IgG2 monoclonal Ab used as treatment in unresectable giant cell tumor of the bone (GCTB). Denosumab targets receptor activator of nuclear factor-κB ligand (RANKL). Inhibition of RANKL decreases bone remodeling by blocking the signaling cascade induced by binding of receptor activator of nuclear factor-κB (RANK) [90]. GCTB cells are osteoclast-like, and express RANKL[91, 92]. Clinical studies demonstrated that denosumab treatment was effective in reducing tumor burden in GCTB patients[93-95]. Furthermore, some preclinical studies have indicated that RANKL inhibition can dampen metastasis of non-boney primary tumors[96]. Additionally, RANKL inhibition and subsequent decreased bone remodeling can prevent the skeletal adverse effects related to cancer and/or cancer treatments[97, 98]. This antibody is used to treat osteoporosis, as it decreases bone reabsorption in post-menopausal women[99]. Denosumab has FDA approval in several settings: treatment of non-resectable GCTB or as a way to decrease skeletal related adverse events in patients at risk for fractures due to boney metastatic disease or hormone modulating therapy in breast and prostate cancer.

4. Fc Domain-mediated activation

The Fc domain's therapeutic significance lies in its ability to induce effector functions, induce complement activation and to manipulate the serum half-lives of antibodies. First, we will briefly review the multiple mechanisms by which targeted monoclonal antibodies can initiate immunologic tumor destruction.

4.1 Antibody-dependent cell-mediated cytotoxicity and antibody-dependent phagocytosis

Antibody-dependent cell-mediated cytoxicity (ADCC) occurs when antibodies are bound to a target cell e.g. a tumor cell and an immune effector cell[100]. While the antibody's variable regions bind an antigen on a target cell, the antibody's Fc region can bind to Fcγ receptors (FcγR) expressed on leukocytes. The result of this interaction depends both on the IgG subclass and the type of FcγR. For the purposes of this review, discussion will be limited to the mechanisms as they occur in humans. The two subclasses of IgG that function in ADCC are IgG1 and IgG3. They bind to FcγRIIIA on NK cells. This causes recruitment of adapter proteins and activation of the NK cell, followed by a cascade that leads to destruction of the cell via release of lytic factors[101, 102]. This can only occur when target cells are opsonized by IgG. Free circulating antibodies cannot activate ADCC. FcγRI is expressed on macrophages, neutrophils, and eosinophils. Antibody-dependent cellular phagocytosis (ADPh) can occur when FcγRI binds IgG1 or IgG3 that is bound to its cognate antigen on a cancer cell. Studying phagocytosis in vitro is technically challenging, however there is some evidence that it plays a significant role in destruction of antibody coated tumor cells via FcγRI and other FcγRs expressed on macrophages[103, 104]. Interferon gamma induces FcγRI upregulation on polymorphonuclear cells[105]. As others have suggested[106], interferon gamma release by NK cells may activate other immune effector cells, which would favor ADPh.

4.2 Complement-dependent cytotoxicity

Alternatively, tumor destruction can be mediated by complement activation. IgM and IgG activate the classical complement pathway. Although IgM can activate the classical pathway, the antibodies discussed in this review are IgG and discussion will be limited to that class. The alternative and lectin mediated complement pathways are relevant to non-mammalian pathogens, and will not be discussed here. As reviewed, [100] IgG1 and IgG3 are the most efficient IgG subclasses at activating complement. The C1q subunit of complement factor 1 (C1) initiates the cascade by binding the Fc regions of IgG. C1q must be bound to multiple IgG in order to initiate the cascade. When two or more IgG are bound to the cell, the C1r subunit activates the C1s which enzymatically cleaves circulating complement factor 4 (C4) to C4a and C4b. C4a does not further participate directly in the cascade, but is released and has chemotactic activity. C4b binds to the cell surface or the antigen-antibody complex. The complex then cleaves circulating complement factor 2, with C2b being released and C2a binding to the complex to form the C3 convertase. The C3 convertase converts circulating C3 to C3a and C3b. Like C4b, C3b binds to the cell surface or the antigen-antibody complex. Once bound, it incorporates circulating factor B. This complex can then amplify the cascade by incorporating thousands of C3b molecules. The downstream result is the formation of the membrane attack complex (MAC), a pore that leads to cell lysis. For further details, complement pathways have been extensively reviewed elsewhere[107, 108].

4.3 Anti-CD20 monoclonal antibodies

Rituximab was the first widely used therapeutic monoclonal antibody to be approved by the FDA, and is perhaps the most studied. It targets CD20, which is expressed on normal and

malignant B cells, but not mature plasma cells. It was first approved in the 1997 for low grade B cell lymphoma, and subsequently was shown to improve cure rates when added to the standard CHOP chemotherapy regimen for patients with diffuse large B cell lymphoma[109]. While there were many hypotheses about the mechanisms of action of anti-CD20 antibodies at the time of rituximab's approval, better understanding of these mechanisms has emerged over the past 20 years.

Firstly, there is evidence anti-CD20 Abs exert their effects in an Fc independent fashion by virtue of binding CD20. One report described patients injected with a murine anti-CD20 IgG2a as pretreatment before injecting what was planned to be the therapeutic antibody i.e. the same Ab labeled with a radioactive isotope. Patients experienced regression with the unlabeled antibody alone[6]. Murine IgG2a does not strongly interact with human FcγR, thus implying another mechanism of this regression. A non-immune effector cell-mediated mechanism is also supported by several studies[7-9] in which CD20+ tumor cell lines were killed in vitro, a setting in which these cells are absent. Anti-CD20 antibodies altered so that they cannot mediate CDC or ADCC are still able to disrupt vital cellular proliferative and survival activity[110]. Furthermore, other work indicates that rituximab induces sensitization to cytotoxic drugs such as paclitaxel, gemcitabine, and vinorelbine[111].

With respect to CDC, preclinical studies have shown that the anti-tumor activity of rituximab is dependent on C1q[10] and that depletion of complement reduces effectiveness of rituximab in a xenograft model of human B cell lymphoma[112]. Furthermore, C1qa subunit genetic polymorphisms correlate with the clinical response in patients with follicular lymphoma receiving Rituximab[113]. In vitro studies performed by Cragg and Glennie have enabled categorization of CD20-targeted Ab as type I or type II[112, 114, 115]. Type I anti-CD20 Abs e.g. rituximab, are potent activators of CDC. Type II anti-CD20 Abs are discussed below. When type I Abs are bound to CD20, the Ab-Ag complex is shuttled to lipid rafts, favoring complement activation[116]. Ofatumumab is a type I anti-CD20 Ab approved by the FDA in 2009 for treatment of CLL. It binds to a different epitope than rituximab. Binding kinetics are superior, resulting in a lower off-rate when is Ab bound to CD20[115]. Accordingly, in vitro studies suggest that ofatumumab activates complement more efficiently than rituximab[117]. An ongoing single arm phase II trial has produced complete or partial responses in several patients receiving CHOP therapy plus ofatumumab for the treatment of Richter's transformation, a transformation of CLL to diffuse large B cell lymphoma[118].

Type II anti-CD20 Abs are able to trigger non-apoptotic cell death via an actin dependent lysosomal pathway[119]. Obinutuzumab, discussed below, is one example. Whereas type I Abs are able to bind two separate CD20 molecules, the three dimensional structure of type II Abs causes them to bind only two regions on the same CD20 tetramer, preventing the IgG aggregation needed for classical complement initiation[120, 121].

With respect to ADCC, earlier clinical studies have demonstrated clear evidence of a significant role for NK cell mediated ADCC in the mechanism of rituximab therapy. Patients with FcγRIIIA polymorphisms that conferred a higher binding affinity for the Fc region of IgG1 had improved responses to rituximab monotherapy[11]. Work in murine

models also supports this interpretation, since anti-tumor activity via tumor targeted Abs requires the presence of functioning FcγR [122]. Furthermore, mice deficient in the inhibitory FcγR, FcγRIIb, showed increased tumor inhibition when treated with anti-tumor Abs.[122]. However, later studies in CLL and follicular lymphoma showed no correlation between FcγRIIIA polymorphisms and response to rituximab[123, 124] thus suggesting that affinity is not always the predominant driving mechanism for NK cell-mediated ADCC. For example, ex vivo studies have shown that rituximab induces ADPh of CLL cells by macrophages[12]. Furthermore, other in vitro studies support a role for immune effector cells bearing various other FcγRs inducing Fc-mediated ADCC as well[125-127]. Unfortunately, establishing the relevance of in vitro ADCC studies to clinical outcomes is challenging. As previously reviewed[106], available assays are technically challenging and can require experimental conditions that are not physiologically relevant. However, the prevailing evidence supports the interpretation that NK cell-mediated ADCC plays a role in the effects of anti-CD20 Abs. Altering the composition of the Fc domain changes its binding affinity for Fc receptors, and has implications for therapeutic applications[128-130]. Obinutuzumab (formerly GA-101) is a glycoengineered anti-CD20 Ab. Its Fc region has been engineered to contain less fucose[131]. This confers increased affinity for the FcγRIIIA expressed on NK cells, which should hypothetically increase activity. The FDA approved obinutuzumab for the treatment of CLL, and its activity in various B cell malignancies is under clinical investigation. Of much relevance to its design aimed at increasing NK cell-mediated ADCC in patients, a trial comparing it to rituximab in indolent lymphoma has been completed (NCT00576758). Results are not yet published.

In the setting of cell death, the induction of ADCC and CDC by anti-CD20 Abs indirectly contributes to tumor destruction by cross priming antigen-presenting cells (APC). In this well described process, APCs phagocytize and present processed peptides to CD8+ and CD4+ T cells via MHC-I and MHC-II, respectively. This can induce tumor-specific CD8+ cytotoxic T lymphocytes (CTL) and CD4+ Helper T cells capable of priming B cells to produce tumor-specific Ab[132-134]. Correspondingly, data show that the greatest effect of rituximab is seen months after treatment[135, 136]. This suggests that the generation of CTL by rituximab, and potentially other CD20 targeted Abs is important.

ADCC, CDC, ADPh, cross priming to generate CTL, and lysosomal mediated non-apoptotic death are each components of anti-CD20 anti-tumor activity. However, these roles are altered by different characteristics of the Abs themselves and the physiologic environment in ways that remain to be fully elucidated. Nonetheless, these agents possess remarkable clinical utility.

Rituximab and other anti-CD20 antibodies represent important clinical triumphs. However it is important to understand why anti-CD20 therapies fail, and investigate methods to avoid these pitfalls. For example, nearly 30% of B-cell NHL will not respond to, or relapse after treatment with rituximab[109, 137]. Levy and colleagues showed that variation in levels of complement defense molecules had no correlation with rituximab activity[138]. Other data illustrate trogocytosis as a mechanism of tumor cell resistance. Through this process, target cells are able to evade opsonization when immune effector cells phagocytize Ab/Ag complexes that have translocated to lipid rafts [139-141]. Tumor cells may also evade

opsonization by internalization of the Ag/Ab/FcγRIIB complex[142]. Further, it has been shown that malignant B cells express a variable level of FcγRIIB, and that increased expression correlates with rituximab internalization. These observations make FcγRIIB a potential target for future therapeutics[109].

4.4 Other targets

Other monoclonal Abs targeted to malignant cells can induce tumor cell death by ADCC and CDC, or work in synergy with CD20 Abs. For example, daratumumab targets CD38, highly expressed on hematologic malignancies, and has been shown to augment both ADCC and CDC in multiple myeloma[136]. Elotuzumab targets CS1, which is overexpressed on multiple myeloma cells. It received breakthrough status from the FDA in 2014. Recent published data demonstrated that it induces NK-cell mediated ADCC which is enhanced via tumor necrosis factor α and interleukin-2 signaling pathways when combined with lenalidomide in vitro[143]. Trials testing Elotuzumab plus lenalidomide plus prednisone in multiple myeloma are underway. A planned trial will recruit subjects to test Elotuzumab plus urelumab (anti-4-1BB agonist Ab) plus lirilumab (anti-KIR Ab). One study showed that anti-KIR Abs can increase rituximab-mediated cytolysis[144] by NK cells in mouse models. Agonism of CD137 (4-1BB) augments NK cell lysis of rituximab-coated cells[145]. In combination with rituximab Levy and colleagues showed that CD137 agonist antibodies have activity in combination with rituximab in murine and human xenograft preclinical studies.[145]. Other preclinical studies suggest activity in combination with chemotherapy and radiotherapy, while one clinical trial CD137 agonist urelumab was hampered by liver toxicity.[146].

Alemtuzumab is a humanized monoclonal antibody targeted to CD52. By binding CD52, it induces ADCC of CLL cells[147]. It was withdrawn from the market in 2012, and rebranded to treat multiple sclerosis. However it is still available to patients with refractory CLL who have failed therapy with alkylating agents and second line therapy with fludarabine. Dinutuximab is a monoclonal Ab targeted to the GD2 glycoprotein expressed on neuroblastoma cells[148]. It can induce ADCC of target cells[149, 150] and was approved by the FDA in March 2015 for pediatric high-risk neuroblastoma.

5. Checkpoint blockade

In recent years, therapeutic antibodies targeted to various immune checkpoint molecules have progressed from preclinical studies to clinical deployment with impressive results. CTLA-4 and members of the PD-1/PD-L1 axis have proven to be important clinical targets for monoclonal antibodies. The relevant mechanisms have been reviewed previously in great detail [17, 151, 152] and will be briefly summarized here, followed by a review of the antibodies' performances in clinical trials.

5.1 CTLA-4

CTLA-4 is a receptor that is solely expressed on T cells. When the T cell receptor (TCR) of a T cell is bound to an MHC on an APC, co-stimulatory molecules expressed on each cell engage one another (**Figure 1**). Some of these interactions are stimulatory to the T cell,

while others are inhibitory. CTLA-4's ligands, CD80 and CD86, are expressed on APCs. CD28 is expressed on T cells, and also binds CD80 and CD86. Those interactions have an activating effect on the T cell. However, the binding affinity of CTLA-4 for CD80 and CD86 is higher than that of CD28[153] allowing for the activating effects of CD28 to be offset as CTLA-4 outcompetes CD28.

The intracellular signaling following CTLA-4 binding to it's ligand is not completely understood, however evidence suggests that activation of the protein phosphatases SHP2 and others play an important role in the cascade leading to disruption of activating signals produced by TCR and CD28 crosslinking[154]. CTLA-4 also inhibits T cell activation by trans-endocytosis, the internalization and degradation of CD80 and CD86[155]. The administration of anti-CTLA-4 Ab dampens inhibitory currents within the T cell. In regards to the mechanism of anti-tumor activity, it is thought that this influence is most important in splaying the balance between Helper T cells and Regulatory T cells (Tregs). Blocking CTLA-4 on helper T cells enhances the generation of immune effector cells[151, 156, 157]. Interestingly, activation of CTLA-4 on Tregs augments their immunosuppressive abilities by an unknown mechanism[157]. Conversely, blockade of CTLA-4 on T regs will inhibit immunosuppressive function.

5.2 Anti-CTLA-4 in Clinical Trials

The current clinical progress of checkpoint blockade antibodies has been extensively summarized in recent reviews[16, 158]. In a phase II trial, ipilumimab-treated melanoma patients had a mean 2-year survival of 18% compared to 5% in control group patients[13]. In treatment naïve patients with metastatic melanoma, addition of ipilumimab to dacarbazine improved overall survival compared to dacarbazine plus control[159]. When pooling data from several trials, 20% of patients who received ipilumimab had survival of 3 years[14-16]. These results represent an immunologic response driven by memory mechanisms. The average time for patients to clinically meet the criteria for a response to treatment was 30 months[15]. This observation would be consistent with the gradual expansion of an arsenal of tumor specific effector T cells in the setting of a dampened immunosuppressive environment. Ipilumimab has also demonstrated activity in NSCLC and prostate cancer[16]. Ongoing clinical studies are investigating its activity in other malignancies. In order to guide the selection of patients for checkpoint blockade therapies, identification of biomarkers correlated to a favorable response would be useful. Some evidence suggests that ICOS expression[160] and peripheral blood absolute lymphocyte count [161] correlate with response to ipilumimab.

5.3 PD-1/PD-L1 axis

PD-1 provides a potent inhibitory signal to T cells. Extensive preclinical work has demonstrated the blockade of PD-1 enhances immune function. In contrast to CTLA-4, PD-1 is not only expressed on T cells, but also B cells ad NK cells[162, 163]. PD-1 expression is induced on T cells when they become activated[164]. Its ligands are PD-L1 (B7-H1) and PD-L2 (B7-DC). They are members of the B7 family of costimulatory molecules. Further, when bound to PD-L1 or PD-L2, PD-1 causes phosphatases involved in effector T cell activation to become inhibited[165]. PD-L1 is expressed by many

tumors[166] and also can be expressed by APCs[167]. When PD-L1 expressed on tumor cells or dendritic cells binds to PD-1 on CD4+ helper T cells or CD8+ T cells, it can inhibit T cell function or cause anergy[168]. A portion of tumor-infiltrating lymphocytes (TIL) are regulatory T cells (Tregs) [151, 168] which contribute to immune evasion by tumor. For example, in vitro studies performed on samples taken from lymph nodes in patients with metastatic cervical cancer had increased frequency of Tregs and high levels of dendritic cells expressing PDL1[169]. As with CTLA-4, when PD-1 on Tregs is stimulated, Treg suppressive capabilities are enhanced[170]. Furthermore, PD-L1 expression is an adaptive mechanism utilized by tumor cells to evade immune attack. In the setting of inflammation, effector cells produce mediators such as interferon-gamma (IFN-γ). IFN- γ induces expression of PD-L1 on tumor cells[151]. These PD-L1 expressing tumor cells can then exert inhibitory effects on effector T cells and feed the suppressive activities of Tregs. The interactions within the PD-1/PD-L1 axis are still not completely understood. For example, PD-L1 can bind CD80[171], and behave as a receptor rather than a ligand. The significance of this biology has not yet been elucidated. Overall, it is clear that PD-1 blockade is effective by preventing inhibition of effector T cells[16].

5.4 Anti-PD-1 in Clinical Trials

Abs targeting PD-1 include nivolumab, pembrolizumab, pidilizumab. Those targeting PD-L1 are MPDL3280A (Genentech), MEDI4736 (MedImmune/Astra Zeneca), BMS-936559 (Bristol-Meyers Squibb) and MSB0010718C (EMD Serono). In phase 1 studies, pembrolizumab and nivolumumab both had durable responses in patients with melanoma, RCC, NSCLC and other cancers[172, 173]. Another anti-PD-1 Ab, pidilizumab has shown activity in hematologic malignancies alone or in combination with rituximab[174-176]. One trial comparing nivolumumab to dacrarbazine in advanced melanoma patients was stopped since patients receiving nivolumumab had dramatically increased overall survival[16]. Nivolumab has activity in treatment naïve melanoma patients as well as those who had previously received treatment. A recent Phase 3 trial showed that Nivolumab treatment increased OS and PFS in previously untreated BRAF mutation negative melanoma patients[177]. Interestingly, a trial in patients who had failed ipilumimab therapy showed that patients who then received nivolumumab had a higher overall response rate (32% vs. 11%) compared to patients received dacarbazine or carboplatin/paclitaxel[178]. Pembrolizumab also produced responses in patient who had failed ipilumumab therapy[179]. The distinct mechanisms of immune regulation by PD-1 and CTLA-4 are highlighted by these reports. The tumors of patients refractory to CTLA-4 blockade, but repsonsive to PD-1 blockade may be driven by PD-L1 expression at the tumor site. In such a case, peripheral activation of effector T cells by anti-CTLA-4 Abs would not be effective if inhbiton of T cells by PD-L1 was occurring at the tumor site.

A combinatorial effect of CTLA-4 blockade and PD-1 blockade also makes sense, and is supported by clincical data. Ipilumimab plus nivolumab achieved striking results in a phase I clinical trial treating melanoma patients. As reported by Wolchok et al, the combination yielded a greater response rate than previously shown in patients treated with either drug alone. All responses were characterized by a reduction of 80% or more in tumor burden

[180]. Phase II/III trials combining CTLA-4 and PD-1 antagonist Abs are planned or underway.

5.5 Anti-PD-L1 in Clinical Trials

Inhibition of PD-L1 in cancer patients is yet another exciting approach with distinct immulogic mechanisms yet to be completely charted. For example, B7 and PD-L1 have been shown to interact, exerting inhibitory effects on T cells[181]. A phase1 trial of an anti-PD-L1 Ab demonstrated anti-tumor activity in patients with solid tumors[182]. Other anti-PD-L1 Abs have shown activity in GI, bladder, head and neck cancers[16, 183-186]. As might be expected, PD-L1 expression by tumor cells can correlate with response to PD-1 Ab therapy. For example, NSCLC patients whose tumors where PD-L1+ had a 67% ORR, compared to zero of six patients with PD-L1 negative tumor in a trial of nivolumab as monotherapy[158, 187]. However, PD-L1 expression is not required. This was evidenced in a trial where patients with PD-L1 negative RCC had clinical responses[188]. It is also unknown what role PD-L2 (another ligand of PD-1) plays. Blockade of PD-L1 will not negate the effects of PDL2 binding to PD-1.

It is important to note the unique set of immune related adverse events associated with checkpoint inhibitors. These are most commonly GI, hepatic, endocrine or dermatologic and are described in greater detail elsewhere[16].

5.6 Other Immune Antagonist Antibodies

The inhibition of other immune checkpoint molecules is under clinical investigation. Trials testing anti-Lymphocyte-activation gene 3 (LAG-3) in hematologic malignancies and in combination with anti-PD-1 Ab for treatment of solid tumors are underway. Preclinical work has shown that anti-killer-cell immunoglobulin-like receptors (KIR) Abs can activate NK cells as monotherapy [144] as well as work in concert with daratumumab (an anti-CD38 antibody that induces ADCC), against multiple myeloma in vitro[189]. Ongoing clinical studies are testing anti-KIR Ab in combination with anti-PD-l Abs in patients with solid tumors.

5.7 Immune Agonist Antibodies

Other approaches involving T cell activation for cancer treatment utilize agonist Abs to molecules members of the TNFR family that activate T cells; these include CD40, GITR, OX40, and 4-1BB (**Figure 1**). The expression of these molecules varies by cell and timing, in relation to activating/inhibitory signals. Our understanding of the exact organization of their roles in activating immune responses is incomplete. The current body of work and gaps in our knowledge were recently reviewed[190]. Here we will touch on some of these molecules' roles and their clinical applications thus far.

CD40 is expressed on various immune cells including B cells, and most pertinently, dendritic cells. It is also expressed some malignancies, making our understanding of the role of its activation in cancer treatment a challenge. When binding to its ligand, CD40L on T cells, the interaction is important for up regulation of co-stimulatory molecules and ultimately T cell help[191, 192]. Agonism of CD40 on DCs increases antigen

presentation[193]. However agonism on CD40 expressed on malignant cells can be directly cytotoxic to them[194]. In a clinical study by Vonderheide and colleagues, some patients with pancreatic ductal adenocarcinoma achieved tumor regression with anti-CD40 agonist Ab therapy plus gemcitabine compared to gemcitabine alone. Interestingly, pathological analysis of these tumor sites was characterized by macrophage infiltration. Furthermore, in a murine model of pancreatic ductal adenocarcinoma the same treatment produced similar findings in some mice. However the frequency of this response was preserved after mice were depleted CD4+ and CD8+ T lymphocytes[195]. This work implied that the anti-tumor mechanism of CD40 agonism to be macrophage-dependent and T cell independent. Activating CD40 appears to have more than one distinct mechanism of anti-tumor activity and has recently been reviewed in more extensive detail elsewhere[146, 196].

GITR is expressed CD4+ and CD8+ T cells, and increases when they are activated. This molecule is constitutively expressed on Tregs, and was also on DC, monocytes, and NK cells[197, 198]. It functions to induce T cell proliferation and development of effectors cells[199]. GITR has been extensively studied and reviewed by Wolchok and colleagues[199]. Clinical testing of anti-GITR agonist antibodies is in progress.

Stimulation of OX40 and 4-1BB activates T cells, while dampening the activity of Tregs[200]. In a phase I trial, an OX40 agonist increased immune activity in patient tumor samples but failed to achieve any partial responses in the advanced cancer patients participating in the study[201]. Since preclinical observations suggest that synergy exists between the checkpoint inhibitors and some of these immune activators, OX40 and 4-1BB agonists may prove more useful in combination approaches. PD-1 and OX40 in combination protects against tumor growth in a mouse model of ovarian cancer[202]. PD-1 and 4-1BB together can increase effector/memory CD8+ T cells in a mouse model[203]. As seen on clinical trial.gov, studies are at various stages and include antibodies targeted to 4-1BB, OX40. A Study Of 4-1BB Agonist PF-05082566 in combination with a PD-1 inhibitor is currently recruiting patients.

6. Novel Antibody Structures

6.1 Multifunctional Antibodies

The Fab (antigen binding fragment) is the antigen-binding domain of the antibody. It exists in native form as $F(ab')_2$., and can be enzymatically cleaved into Fab(154). The three bispecific formats that will be discussed here utilize the scFv (single chain variable fragment), an artificially produced entity similar in function to the Fab[204]. These are the BiTE (Bispecific T cell Engager), the DART (Dual Affinity ReTargeting) and the TandAb (tetravalent tandem antibodies) structural formats.

BiTEs are recombinant single polypeptide chains consisting of two scFv joined together by a flexible linker[205]. As the name suggests, a BiTE specifically binds two targets: T cells and a target tumor cell. One target antigen is the TCR's (T cell receptor) co-receptor, CD3. The second is a selected tumor target. The engagement and activation of T-cells is accomplished independent of TCR binding to MHC[206]. BiTEs induce T-cell killing when crosslinked to target cells by triggering T-cells to release perforin and granzyme B[207] (**Figure 2**). The

most clinically advanced BiTE is blinatumomab. It was recently approved by the FDA for the treatment of relapsed or refractory B-cell precursor ALL. It binds CD19, expressed on normal and malignant B cells. Ongoing trials are investigating its use in other B cell malignancies [208]. One drawback is the short half-life, necessitating prolonged periods of continuous infusion[209]. Other BiTEs in the clinic include MT110, targeting CD3 and EpCAM for the treatment of solid tumors of epithelial origin including breast, colon, pancreatic and ovarian. MT110 is currently in a Phase I clinical trial against solid tumors to assess its safety and tolerability. MT111 is the third BiTE to enter clinical trials. It targets CEA, an immunoglobulin superfamily glycoprotein that is expressed on a variety of solid tumors and on the gastrointestinal track[210]. MT111 has been preclinically assessed in models of colon cancer[211, 212] and is currently in a phase I clinical trial against gastrointestinal adenocarcinoma. BAY2010112/AMG112 is specific for PSMA, overexpressed on prostate cancer cells. A corresponding BiTE is in phase I trials for prostate cancer [208]. An exciting BiTE that may be moving into the clinical pipeline is bispecific for CD3 and EGFRvIII. EGFRvIII is tumor specific for glioblastoma, and preclinical data suggest that it will be active beyond the blood brain barrier [213]. Bispecific antibodies targeting GD2 in neuroblastoma are also under preclinical investigation[214].

The DART and the TandAb molecules are similar to BiTEs in that they target two antigens simultaneously, but possess different structural properties [215] [216]. Whereas a BiTE is composed of a single polypeptide, DARTs consist of two polypeptides joined by a disulfide bridge[215]. TandAbs consist of one polypeptide chain but are dimerized, endowing them with four binding sites (two per target)[217]. Laboratory data suggest that in the CD19xCD3 format, BiTEs are superior to TandAbs in ability to lyse B cells in vitro[216]. However other platforms may hold promise. AFM13 (Affimed) is a TandAb bispecific for CD30 and CD16A (FcγRIIIa) that can redirect NK cells to CD30+ malignant cells[216, 218]. As recently presented at a national meeting[219], it recently completed a phase I clinical trial for the treatment of Hodgkin Lymphoma. A phase II trial is in development. DARTs have not been investigated clinically. However, clinical applications may be forthcoming as preclinical studies indicated that a CD19xCD3 DART is superior to a CD19xCD3 BiTE in its abilities to activate T cells and lyse B cells at lower concentrations[220]. This DART's mechanism of action is the same as that of a CD19×CD3 BiTE. However is unclear what structural property of the DART platform is responsible for its superior ability to redirect T cell lysis of CD19 positive cells. Catumaxomab is a triomAb consisting of two Fab domains, each with a different binding specificity. One binds EpCAM and the other binds CD3. An intact Fc portion allows binding to activating Fc receptors found on effector T cells (CD3), NK cells (FcγRIIIA), and macrophages (FcγRIIA), leading to cell-mediated cytotoxicity, ADCC and ADPh, respectively[221, 222]. Catumaxomab was approved in Europe for malignant ascites treatment in 2009. It continues to be evaluated in the US, not only intraperitoneally for malignant ascites, but also for intravenous dosing to treat NSCLC[223, 224]. Two similar (possessing intact Fc) triomAbs are in development. Ertumaxomab is directed against HER and CD3. It is currently recruiting subjects with Her2/neu positive solid tumors for a phase I trial. FBTA05 is specific for CD20 and CD3. A phase 1-2 trial in 2013 demonstrated activity of FBTA05 plus donor lymphocyte infusions in patients with relapsed or refractory B-cell lymphoma after allogeneic stem cell transplantation[225].

6.2 Other structures

A combody is a novel structure consisting of cartilage oligomeric protein 38 and a scFv in pentameric form specific for DR5, a target within the Apo2L/TRAIL pathway. This approach may address challenges in developing Apo2L/TRAIL inhibitors[83, 226]. Another antibody permutation is represented by peptibodies. These consist of an Fc fused to a peptide with binding affinity to a target[227] [228, 229]. Trebananib (AMG386) is a peptibody with the Fc domain of human IgG fused to peptides that interfere with the binding of Ang1 and Ang2 to the Tie2 receptor. As an alternative pro-angiogenesis pathway to the VEGF axis, this pathway can become enhanced in tumors resistant to Bevacizumab[230, 231]. A phase III trial of Trebananib showed activity in women with recurrent ovarian cancer[232]. Another phase III trial incorporating this peptibody into first line therapy for ovarian cancer is underway. Trials of trebananib in treating other solid tumors and hematologic malignancies are in various stages.

Anticalins are derived from naturally occurring proteins that are engineered to have a binding affinity for particular targets[233, 234]. These proteins are readily altered chemically, making them attractive candidates as antibody mimics.[235, 236]. Anticalins specific for c-MET, CTLA-4 and VEGF have been engineered[237, 238]. While development has not yet advanced to the clinic, anticalins represent another potential tool for targeted cancer therapy.

Adnectins represent another protein species with functional abilities akin to antibodies. Derived from human fibronectin[239], they possess qualities that make them desirable candidates for clinical use. Adnectins can be engineered into multimers, making multifunctional adnectins a feasible goal. Additionally, since they are derived from naturally occurring human proteins, adnectins would have less immunogenicity and favorable properties for serum stability and access to small biological spaces [240]. CT322 is an adnectin that binds to and inhibits VEGFR2 [241]. A recent phase II clinical trial failed to demonstrate efficacy in glioblastoma patients and was terminated early[242]. A phase II study of CT322 for NSCLC failed to show that this adnectin improved outcomes in comparison to paclitaxel/carboplatin plus Bevacizumab[243].

We have reviewed the clinical potential of other antibody-like molecules, and the reader is referred to that review for more details[244, 245].

7. Antibodies as Targeting Vehicles

Many agents i.e. drugs, radioactive materials and toxins are effective in killing tumor cells, however they are also toxic to healthy tissue thus limiting their use as therapy. Conjugating anti-tumor agents to antibodies can limit collateral damage. The ideal antibody for such a job would target an antigen that is exclusively expressed on tumor cells (tumor specific). Unfortunately most antigens that are highly expressed on tumor cells are also highly expressed on normal cells[246]. Alternatively some antigens are overexpressed on tumor cells when compared to normal tissue (tumor selective). As recently reviewed[244], many factors go into antibody selection and design when developing an immunoconjugate. Conjugates may be joined by a noncleavable linker, leading to endocytosis and complete

digestion of the conjugate[244]. Others are cleaved intracellularly by various mechanisms[247-250], or extracellularly[244].

7.1 Radioimmunoconjugates

Ibritumomab tiuxetan is an antibody specific for CD20 that delivers yttrium-90 or indium-111 to its target. This mouse IgG1 Ab is joined via tiuxetan to the radioactive isotope and is absorbed entirely by the targeted cell[244]. This immunoconjugate has been approved by the FDA since 2002 and has clinical activity against refractory follicular lymphoma[251]. The practicalities of preparing and delivering this therapy are complex, contributing to its lack of widespread use. However, many clinical trials are in various stages investigating its use in various B cell malignancies e.g. as first line therapy in follicular lymphoma. I-131-Labetuzumab is a monoclonal antibody targeted to CEA. It is used to treat colon cancer that has metastasized to the liver.

Phase III trials are currently investigating the use of clivatuzumab, an antibody specific for PAM4, conjugated to yttrium. Earlier trials had shown it to be active when combined with gemcitabine in the treatment of pancreatic adenocarcinoma[252-254]. However, development of radioimmunoconjugates for treatment of solid tumors has proven to be more challenging than in hematologic malignances. Higher doses of radiation are required to have effects against solid tumors and it is difficult to safely deliver such doses to tumor without substantial host toxicities[255]. There are several proposed strategies to address these challenges. For example, preclinical data suggest that combining radioimmunoconjugates with chemotherapy may be helpful[256]. Another approach would be to pretarget tumor with antibody, and then administer radioisotope-conjugated hapten aimed to bind Ab bound to tumor cells. This approach has yet to prove successful in clinical trials[257].

7.2 Drug Immunoconjugates

Brentuximab vedotin is an anti-CD30 Ab conjugated to a tubulin binder, monomethyl auristatin (MMAE). Its linker is cleaved within the lysosome, at which point MMAE disrupts microtubule function and leads to apoptosis[258]. Brentuximab vedotin has marked activity in some B cell malignancies and is approved by the FDA[259-261]. Its efficacy in other malignancies is yet to be validated, however preclinical studies suggest that it may have activity against mesothelioma[262].

Another FDA approved conjugate is trastuzumab emtansine (T-DM1) Trastuzumab targets HER2 expressed on breast cancer and is joined via a non-cleavable linker to emtansine (maytansinoid DM1), another tubulin binder than leads to an apoptosis-inducing cascade with the cell[263]. T-DM1 is active in treating advanced HER2 positive breast cancer[264, 265].

Glembatumumab vedotin is another immunoconjugate that delivers MMAE intracellularly; the Ab targets glycoprotein non-metastatic-b (GPNMB). GPNMB is overexpressed on triple negative breast cancer, among other solid tumors[266]. A phase I/II clinical trial demonstrated that it is active in advanced melanoma[267]. Clinical data suggest that it is particularly active in triple negative breast cancer patients with high levels GPNMB expression[266]. Ongoing phase II studies will provide more information about its activity.

Inotuzumab ozogamicin is an immunoconjugate that targets CD22. The antibody is a humanized IgG4[268]. The calicheamicin derivative is an antibiotic that binds to the minor groove of DNA causing disruption that leads to cell cycle arrest[269]. It has shown promising results in Phase 1 clinical trials. However, a Phase III trial in combination with rituximab in treating some adult non-Hodgkin lymphoma was recently discontinued as observations indicated that the primary objective of improving overall survival would be not met[270]. Its clinical use in ALL continues to be evaluated. Gemtuzumab ozogamicin is another antibody-drug conjugate that targets CD33. It was used inn the US from 2000-2010, but withdrawn from the market due to toxicity. It is still studied elsewhere. For example, a French study showed benefit in pediatric patients with AML[271]

SAR3419 is an immunoconjugate comprised of a humanized IgG1 targeted CD19 that delivers a tubulin inhibitor intracellularly[272, 273]. Phase II trials are assessing its use in patients with acute lymphocytic leukemia. MM-302 is an anti-HER2 Ab conjugated to doxorubicin containing liposomal particles. This agent is being studied in patients with HER2 positive breast cancer.

Lorvotuzumab mertansine is a humanized IgG1 targeting CD56 conjugated to DM1 that received FDA orphan status approval for treatment of Merkel-cell carcinoma in light of early evidence of its activity in that rare malignancy[274]. However, its potential as treatment for a broad range of malignancies is also promising. For example, a phase I trial in combination with lenalidomide and dexamethasone showed a response rate of 59% in patients with multiple myeloma[275]. Phase II studies in multiple myeloma and solid tumors are anticipated[276].

Other antibody drug conjugates are at various stages in clinical trials. These include conjugates that target EGFR, EGFRvIII, CD79b, and NaPi2b [276-278]. Other potential conjugates that may soon enter the clinical pipeline are drug carrying nanoparticles conjugated to antibodies. An advantage of this approach is the ability to deliver greater quantities of a drug, stored in the nanoparticle. With direct conjugation of the antibody to the drug, it may not be possible to deliver a large enough dose to trigger cell death[279]. Tamoxifen loaded PLGA nanoparticles conjugated to trastuzumab induce apoptosis in HER2 positive cell lines[280]. The features of nanoparticles have been recently been reviewed in detail elsewhere[281].

It is also important to note evidence that internalization of antibody drug conjugates may not be mandatory for anti-tumor effects. Spliced isoforms of fibronectin are expressed in the tumor vasculature[282], but not normal human tissue aside from the female reproductive tract[283]. In recent work, an antibody called F8, specific for one of thee fibronectin isoforms, was conjugated via a disulfide linker to DM1. This antibody localized to tumor tissue and produced cures in one mouse tumor model[284], suggesting that with an easily cleavable linker, cytotoxins are able to make their way into tumor cells and induce apoptosis. Tenascin-c is also commonly expressed in the tumor microenvironment. F16IL-2 is an scFv specific for tenascin-c conjugated to recombinant IL-2, designed to activate immune effector cells, e.g., NK cells, and T cells at the tumor site. It has been shown to inhibit AML progression alone and in combination with cytarabine in preclinical mouse

studies[285, 286]. A clinical trial in Merckel cell cancer patients is currently recruiting. This drug has generated responses, including a CR in post- allogeneic hematopoietic stem cell transplant AML patients[287]. Its efficacy in combination with doxorubicin is also being investigated in solid tumors[288].

7.3 Toxin Immunoconjugates

Biologic toxins represent a third category of agents that can be delivered to tumor cells via Ab. No such therapeutic modality is yet approved by the FDA. This is not surprising, as unintended targeting of these exceedingly potent toxins to normal organs can cause unacceptable toxicity. However, utilization of engineered Abs has created new possibilities and several agents are in the clinical development pipeline. A vehicle for delivery of Pseudomonas exotoxin A is SS1P, targeting mesothelin. This immunoconjugate is undergoing active clinical studies in mesothelioma and multiple solid malignancies. It can be administered with B and T cell depleting therapy in order to abate generation of anti-toxin Abs[289]. Combotox is a mixture of two antibodies (anti-CD19 and anti-CD22), each conjugated to ricin A toxin. This is currently under clinical investigation in treatment of B cell malignancies[276]. Moxetumomab pasudotox shows significant promise for the therapy of hairy-cell leukemia. It consists of an Fv antibody targeted to CD22, linked to Pseudomonas exotoxin A. The toxin is cleaved intracellularly[290, 291]. A trial in relapsed and/or refractory hairy-cell leukemia demonstrated high overall response and complete response rates, as well as a low side effect profile[291]. Other clinical trials are at investigating moxetumomab pasudotox activity in various B cell malignancies. The above list of agents is not exhaustive, and the topic recently been reviewed in more detail[244, 276].

With respect to future developments in the clinical use of immunotoxins, it is now recognized that, despite the extraordinary potency of the toxin moieties, such therapy can be impeded by cellular resistance mechanisms. Various methods can be employed to combat this and are under investigation[292-294]. Clearly, using antibodies as vehicles to target potent therapies to cancer is a promising area that will undoubtedly mature in the future.

7.4 Immunoimaging

In addition to therapeutics, antibodies conjugated to radioisotopes can serve as tools for malignancy monitoring. A diabody specific for HER2 has been studied in the preclinical setting as a means to develop immunoPET[295]. Clinical studies have shown this to be feasible[296]. In this developing field, innovations include addition of amino acid chains (PASylation) to Abs in order to impair renal filtration. This improves serum half-life, therefore optimizing tumor uptake to the result of enhanced tumor imaging[297].

8. Conclusion

Therapeutic antibodies and their many structural variants have evolved into important components of the cancer therapy arsenal. These contemporary approaches are based upon an accumulating body of knowledge that permits the translation of complex concepts to clinical implementation. Interestingly, most of the clinically tested approaches discussed

here utilize only one of these concepts. As we look toward the future, how can we combine these concepts to achieve even better efficacy in cancer patients?

As evidenced in clinical trials targeting IGF-1R, DR5, and clinical experience with trastuzumab, pertuzumab and cetuximab, tumors possess many molecular avenues for escape from suppression or activation of any particular target. By developing agents that simultaneously block signaling of redundant survival pathways, improved clinical activity may be achieved. Preclinical data studying bispecific Ab to combinations HER2/HER3, EGFR/IGF-1R and IGF-1R/HER3 appear promising. Additionally, combination of receptor inhibition with other modalities discussed here would be interesting. Combination of any of these agents with checkpoint blockade immunotherapies is one approach that is undergoing intensive evaluation. As cells tumor cells die from receptor inhibition, tumor material will be presented by APC to T cells leading to vaccine-like anti-tumor responses.

Understanding the utility of manipulating the structure of anti-CD20 Abs should provide important insights into the impact of specific anti-tumor mechanisms of action. The newest generation of these antibodies is positioned to test preclinical study-driven hypotheses in the clinical arena. Ofatumumab has the property of a of low off rate in its binding to CD20. Theoretically, the superiority in ability to induce CDC compared to that of rituximab should translate into superior clinical activity. However, in a recent trial of ofatumumab in mantle cell lymphoma[298], ofatumumab as a single agent achieved an overall response rate lower than rituximab in a similar earlier trial[299]. If this lower off rate does increase CDC, one would have expected superior clinical activity to that of rituximab. This unexpected result speaks to the incomplete understanding of these agents' mechanisms of action. Along the same lines, Obinutuzumab was also selected for properties predicted to yield superior clinical anti-tumor capabilities. Obinutuzumab's Fc region modifications increase affinity for FcγRIIIA on NK cells. Ongoing clinical trials of these agents have the potential to teach us more about the role that each mechanism plays, and potentially validate such hypotheses.

Antibodies targeted to CTLA-4, PD-1 and PD-L1 have demonstrated important clinical value. As discussed, the blockade of each molecule has effects within different immune compartments. As more is learned about PD-L1 expression by tumors and PD-1 expression by TILs, clinical strategies can be more precisely designed to test hypotheses regarding mechanisms of action. Combination with immune activating monoclonal antibodies such OX40 agonists also holds promise.

Combining the success of antibodies with chemotherapy, targeted therapies and other emerging treatment modalities such as adoptive cellular therapy, vaccines, and enzyme inhibitors clearly holds promise for the future. However, much work remains to be done to determine which types of treatment can be productively combined, and how this should be approached with respect the selections of doses and treatment schedules. Despite these complexities it should be emphasized that we now are dealing with the consequences of success, pondering transitions from identifying efficacious agents to the development of highly effective and even curative treatment regimens. We have come a long way, and the future is bright for the field of antibody-targeted therapeutics.

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Glossary

Ab	antibody
Ag	antigen
ADCC	antibody-dependent cell mediated cytotoxicity
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
AKT	Protein kinase B
APC	antigen-presenting cell
Apo2L/TRAIL	tumor necrosis factor-related apoptosis inducing ligand
BiTE	bispecific T cell engager
BTK	Bruton's tyrosine kinase
CDC	complement-dependent cytotoxicity
CEA	carcinoembryonic antigen
CHOP	cyclophosphamide, Daunorubicin, Vincristine, Prednisone
CR	complete response
CS1	signaling lymphocytic activation molecule-F7
CTL	cytotoxic lymphocyte
CTLA-4	cytotoxic T-lymphocyte-associated protein 4
DART	Dual Affinity ReTargeting
DC	dendritic cell
DR4	death receptor-4
DR5	death receptor-5
EGFR	epidermal growth factor
Fab	immunoglobulin antigen binding fragment
Fc	fragment crystallizable
FcγR	Fcγ Receptor
FOLFIRI	folinic acid, fluorouracil and irinotecan
GITR	glucocorticoid-induced tumor necrosis factor receptor
GPNMB	glycoprotein non-metastatic-b
HCC	Hepatocellular carcinoma

HER2	human epidermal growth factor
HGFR	hepatocyte growth factor receptor
HGF	hepatocyte growth factor
IGF	insulin like growth factor
IGFR	insulin-like growth factor receptor
IGF-1R	insulin like growth factor 1 receptor
IGF-2R	Insulin like growth factor 2 receptor
Ig	immunoglobulin
IR	insulin receptor
KIR	killer-cell immunoglobulin-like receptors
KRAS	V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
LAG-3	anti-lymphocyte-activation gene 3
MAC	membrane attack complex
MAPK	Mitogen-activated protein kinase
MET	oncogene
MHC	major histocompatibility complex
MM	Multiple Myeloma
MMAE	monomethyl auristatin
NFκB	nuclear factor kappa-light-chain-enhancer of activated B cells
NK cells	natural killer cells
NSCLC	non small cell lung cancer
OS	overall survival
PD-L1	programmed cell death ligand
PET	positron emission tomography
PFS	Progression free survival
PI3-K	Phosphoinositide 3-kinase
PLGA	Poly(D, L-lactic-co-glycolic acid)
RANK	receptor activator of nuclear factor-κB
scFv	short chain variable fragment
T-DM1	trastuzumab emtansine
TandAb	tetravalent tandem antibody
TCR	T cell receptor

TIL	tumor infiltrating lymphocyte
Treg	regulatory T cell
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor

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Highlights

- Therapeutic antibodies have emerged as important components of effective cancer therapy regimens.
- Unconjugated antibodies can exert their effects by manipulating malignancy-associated signaling, activating complement or inducing immune effector mechanisms.
- Immunoconjugates exploit the abilities of antibodies to selectively target tumors where they can deliver toxic payloads such as toxins, radioactive particles or drugs. Antibody-drug conjugates are demonstrating important anti-tumor activity in breast cancer and other common malignancies.
- Manipulation of immune checkpoints such as CTLA4 and PDL-1 demonstrate exciting clinical activity in numerous malignancies and provide a platform for future successful cancer therapies.

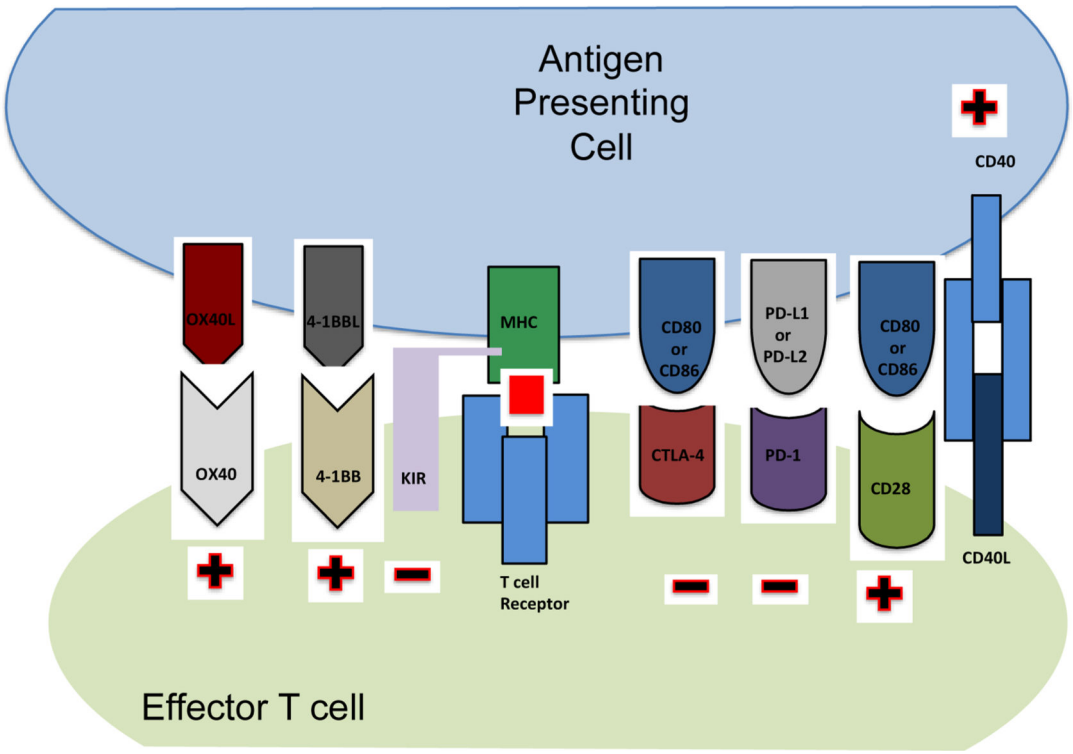

Figure 1.

Immune Checkpoint Molecular Targets. Synapse between an antigen presenting cell and effector CD4+ or CD8+ T cells involves the interaction of many receptors and ligands. When peptide antigen is presented on class I or II Major histocompatibility complex (MHC) to the T cell receptor (TCR), binding of CD80 or CD86 to CTLA-4 can lead to inhibition or anergy of the T cell. Binding of PD-1 by its ligand also inhibits the T cell. The interaction of KIR with MHC also has an inhibitory effect. Binding of CD28, OX40 and 4-1BB by their ligands has an activating effect of the T cell. The antigen presenting cell can also be influenced by these interactions. Binding of CD40 by its cognate ligand has an activating effect on the antigen presenting cell. Activating effect indicated by (+), inhibiting effect indicated by (−).

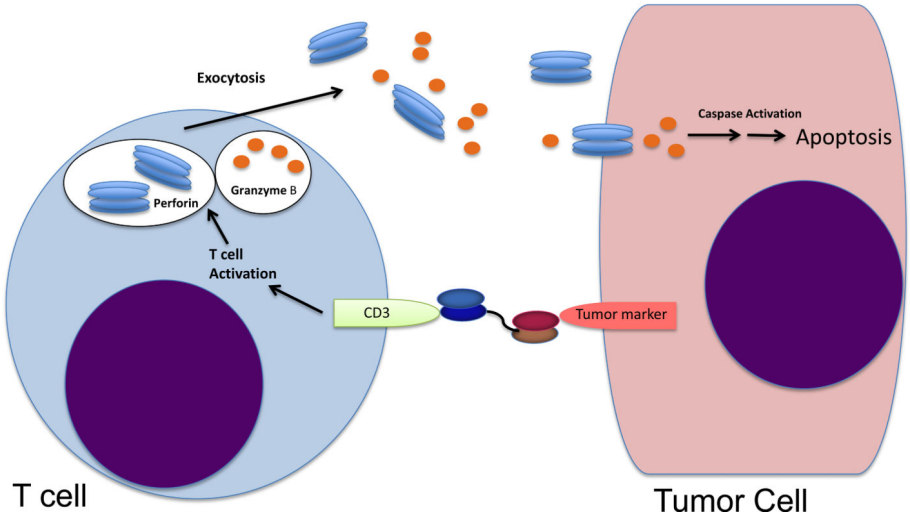

Figure 2.

Bispecific T cell Engager (BiTE). Two short chain variable fragments (scFv) joined by a flexible linker are all parts of a single polypeptide. When the CD3 targeted scFv has bound CD3 and the other scFv has bound its target, the T cell becomes activated. Cell-mediated cytotoxicity directed at the tumor cell follows.

Table 1

FDA Approved Antibodies for the Treatment of Cancer

Generic name (trade name)	Origin	Isotype (conjugate)	Target	Approved Uses/Trials	Initial Approval
Unconjugated MAbs					
Rituximab (Rituxan)	Chimeric	IgG1	CD20	Various Leukemias & Lymphomas	1997
Ofatumumab (Arzerra)	Human (XenoMouse)	IgG1	CD20	CLL/NHL	2009
Obinutuzumab (Gazyva)	Humanized	IgG1	CD20	CLL/NHL	2013
Alemtuzumab (Campath-1H)	Humanized	IgG1	CD52	CLL (no longer in clinical use)	2001
Trastuzumab (Herceptin)	Humanized	IgG1	HER2	Breast & Gastric ccancer/Esophageal	1998
Pertuzumab (Perjeta)	Humanized	IgG1	HER2	Breast/Esophageal, Neuroendocrine, Gastric	2012
Cetuximab (Erbitux)	Chimeric	IgG1	EGFR	Colorectal, HNSCC, Lung	2004
Panitumumab (Vectibix)	Humanized	IgG2	EGFR	Colorectal/Pancreatic	2006
Bevacizumab (Avastin)	Humanized	IgG1	VEGF-A	Colorectal, NSCLC, Glioblastoma, RCC, Cervical, Ovarian	2004
Denosumab (Xgeva)	Human	IgG2	RANKL	GCTB	2010
Ramucirumab (Cyramza)	Humanized	IgG1	VEGFR2	Gastric Cancer, NSCLC/HCC, RCC, Breast	2014
Ipilumimab (Yervoy)	Human	IgG1	CTLA-4	Melanoma	2011
nivolumab (Opdivo)	Human	IgG4	PD-1	Melanoma	2014
pembrolizumab (Keytruda)	Humanized	IgG4	PD-1	Melanoma/NSCLC, Glioblastoma, Ovarian, Colon, RCC	2014
Dinutuximab (UNIT UXIN)	Humanized	IgG1	GD2	High Risk Neuroblastoma	2015
Immunoconjugates					
Ibritumomab Tiuxetan (Zevalin)	Murine	IgG1 (^{90}Y)	CD20	Non-Hodgkin's Lymphoma	2002
Brentuximab vedotin (Adcetris)	Chimeric	IgG1 (MMAE)	CD30	Hodgkin's Lymphoma, SALCL	2011
Ado-trastuzumab emtansine (Kadcyla)	Humanized	IgG1 (DM1)	HER2	Breast Cancer	2013
Gemtuzumab ozogamicin (Mylotarg)	Humanized	IgG1(calicheamicin)	CD33	AML	2000*

MAb, monoclonal antibody; IgG, immunoglobulin-G; CLL, Chronic Lymphocytic Leukemia; NSCLC, Non-Small Cell Lung Cancer; 90Y, yttrium-90 ; 131I-Iodine-131; MMAE, monomethyl auristatin E; DM1, Mertansine; HNSCC, Head and neck squamous cell carcinoma; SRE, skeletal-related event; GTCB, giant cell tumor of bone; SALCL, Systemic Anaplastic Large Cell Lymphoma;

*Withdrawn in 2010, currently reentered clinical trials for AML in France

Table 2

Some Antibodies under investigation for the treatment of cancer

Name	Origin/isotype	Target	Diseases Treated	Clinical Trial phase
Onartuzumab	Humanized IgG (Fab fused to Fc)	Met/EGFR	NSCLC	III
Necitumumab	Human IgG1	EGFR	NSCLC	III
Zalutumumab	Human IgG1	EGFR	SCCHN	III
Nimotuzumab	Humanized IgG1	EGFR	Head and neck	III
MEDI-573	Human IgG1	IGF-1R and IGF-2R	HCC, Solid Tumors, Metastatic breast cancer	I I I
Dalotuzumab	Humanized IgG1	IGF-1 R	Breast Cancer, NSCLC	I-II
Ganitumab	Human IgG1	IGF-1 R	Solid tumors	II/III
Lexatumumab	Human IgG1	TRAIL-R2/Death receptor-5 (DR5)	Multiple Sarcomas	I
Conatumumab	Human IgG1	TRAIL-R2/Death receptor-5 (DR5)	Solid Tumors	I/II
Dorzitumab	Human IgG1	TRAIL-R2/Death receptor-5 (DR5)	Metastatic Colorectal Cancer	I
Tigatuzumab	Humanized IgG1	TRAIL-R2/Death receptor-5 (DR5)	Breast Cancer	II
Mapatumumab	Human IgG1	DR4	MM, Solid tumors	I/II
Clivatuzumab	Humanized IgG1	MUC1/PAM4	Pancreatic cancer	III
Tremelimumab	Human IgG2	CTLA4	Solid tumors	II
BMS-936558	Human IgG4	PD1–B7H1, PD1–B7DC	Solid tumors	III
Pidilizumab	Humanized IgG1	PD1–B7H1, PD1–B7DC	Solid tumors Multiple Myeloma	II II
BMS-936559	Human IgG4	PD1–B7H1	Solid tumors	I
MPDL3280A	Engineered human IgG1	PD1–B7H1	Solid tumors	I
MEDI4736	Engineered human IgG1	PD1–B7H1	Solid tumors	I
IMP321	Fusion protein (LAG3+ IgG1)	LAG3–MHCII	Multiple cancers	I/II
BMS-663513	Human IgG4	4-1BB	Solid tumors	I/II
PF-05082566	Human IgG2	4-1BB	NHL, Solid tumors	I
Anti-OX40	OX40-specific mouse IgG	OX40 agonist	Solid tumors	II
TRX518	GITR-specific humanized IgG1	GITR–GITRL	Melanoma	I
CP-870,893	Human IgG1	CD40	Solid tumors	I
Lucatumumab	Human IgG1	CD40	Lymphoma and leukemia	I/II
Dacetuzumab	Humanized IgG1	CD40	MM, Lymphoma	II
Labetuzumab I131-labetuzumab	Humanized IgG1	CEA	Colorectal, pancreatic scancr	I/II
Elotuzumab	Humanized IgG1	CS1	MM	III
MM-302	Human IgG1 conjugate to doxorubicin	HER2	Breast cancer	II
MM-111	Two fused human scFv linked to midified human albumin	HER2/HER3	Breast cancer	II I
MM-141	Human IgG fused to two scFv	IGF-1R/HER3	Solid tumors	I

NSCLC, Non-Small Cell Lung Cancer; SCCHN, squamous cell carcinoma of the head and neck; HCC, Hepatocellular carcinoma; B7H1, B7 homolog 1(programmed death-1 ligand-1), B7-DC, (B7 dendritic cell-programmed death-1 ligand-2); CTLA4, cytotoxic T lymphocyte antigen 4; GITR, glucocorticoid-induced TNFR-related protein; GITRL, GITR ligand; IgG1, immunoglobulin G1; LAG3, lymphocyte activation gene 3; MHCII, major histocompatibility complex class II; OX40L, OX40 ligand; PD1, programmed cell death protein 1; CEA, carcinoembryonic antigen ; CS1, CD2 subunit 1; MUC1, pancreatic cancer antigen 1.