Leading Edge
Minireview
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Antibody-Based Immunotherapy of Cancer

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By targeting surface antigens expressed on tumor cells, monoclonal antibodies have demonstrated efficacy as cancer therapeutics. Recent successful antibody-based strategies have focused on enhancing antitumor immune responses by targeting immune cells, irrespective of tumor antigens. We discuss these innovative strategies and propose how they will impact the future of antibody-based cancer therapy.

Introduction

Specific recognition and elimination of pathological organisms or malignant cells by antibodies were proposed over a century ago by Paul Ehrlich, who is credited for conceptualizing the "magic bullet" theory of targeted therapy. Over the past 30 years, antibody cancer therapeutics have been developed and used clinically in an effort to realize the potential of targeted therapy. The diversity of these targeted approaches reflects the versatility of antibodies as platforms for therapeutic development (Weiner et al., 2010).

Antibodies may target tumor cells by engaging surface antigens differentially expressed in cancers. For example, rituximab targets CD20 in non-Hodgkin B cell lymphoma, trastuzumab targets HER2 in breast cancer, and cetuximab targets EGFR in colorectal cancer (Table S1 available online). The antibodies can invoke tumor cell death by blocking ligand-receptor growth and survival pathways. In addition, innate immune effector mechanisms that engage the Fc portion of antibodies (Figure S1) via Fc receptors (FcR) are emerging as equally important (Jiang et al., 2011). The mechanisms include antibody-dependent cellular cytotoxicity (ADCC) and complement-mediated cytotoxicity (CMC); antibody-dependent cellular phagocytosis (ADCP) is likely relevant as well (Figure 1).

Although unconjugated antibodies have had efficacy, molecular genetics and chemical modifications to monoclonal antibodies (mAbs) have advanced their clinical utility. For example, modification of immune effector engagement has improved pharmacokinetic profiles, and conjugating cytotoxic agents to mAbs has enhanced targeted therapeutic delivery to tumors. The increasing facility of antibody modifications has made it possible to construct diverse and efficacious mAb-based therapeutics (Figure S1).

Structural engineering and alternative targets have also expanded the ability of mAbs to stimulate adaptive immune effectors, such as T cells, that can induce antitumor activity. Antibodies directly targeting receptors involved in checkpoint regulation of immune cells have exhibited preclinical and clinical successes. Ongoing studies also suggest that antibodies can indirectly elicit adaptive immunity through antibody-dependent engagement of immune effector mechanisms (Figure 1). Overall, the diverse effects of antibodies and their putative mechanisms of action suggest several exciting directions for developing therapeutic strategies. Some that have achieved recent success are discussed below.

Manipulating Antibody Structure

The natural properties of antibodies that enable specific antigen engagement can be leveraged and improved upon by engineering approaches that increase antitumor activity. One example is the creation of bispecific antibodies (bsAbs) with dual affinities for a tumor antigen and either another tumor antigen or a target in the tumor microenvironment. As the Fc domain of mAbs does not directly activate T cells, the activating receptor for T cells, CD3, is a common target of bsAbs. Catumaxomab is a bsAb that binds the tumor antigen EpCAM, CD3, and innate effector cells through an intact Fc portion (Ruf and Lindhofer, 2001). This bsAb, termed a TriomAb, effectively kills tumor cells in vitro and in vivo and induces protective immunity, most likely through the induction of memory T cells. Catumaxomab's success in a phase II/III clinical trial led to its approval by the European Commission in 2009 for the treatment of malignant ascites. This success spurred the development of other TriomAbs targeted against the tumor antigens HER2/neu (ertumaxomab), CD20 (Bi20/FBTA05; NCT01138579 [see Web Resources section below for information on full urls]), GD2, and GD3 (Ektomun).

A promising approach to directly stimulate T cell immunity with mAbs is the development of bispecific T cell engager (BiTE) molecules that target CD3 and either CD19, EpCAM, or EGFR. Low doses of BiTEs induce antitumor activity, and BiTEs have the added potential to overcome mutations in signaling pathways that classically lead to resistance. In BiTEs, the variable domains of a CD3-targeted antibody and a tumor antigen-targeted antibody are genetically linked, rendering it possible to activate a T cell when it physically engages a tumor cell (Lutterbuese et al., 2010). Lysis of bound tumor cells and the accumulation of cytotoxic T cells in the tumor microenvironment ensues, leading to tumor regression at in vivo doses three orders of magnitude less than those of the parent antibody (Lutterbuese et al., 2010). The newly characterized BiTEs directed against EGFR utilize the parental antibodies cetuximab and panitumumab, with potent antitumor abilities against KRAS- and BRAF-mutated cells that demonstrate resistance to conventional EGFR antibodies (Lutterbuese et al., 2010). The CD19-CD3 BiTE demonstrates significant clinical promise in patients with advanced non-Hodgkin lymphoma (NHL) and is currently

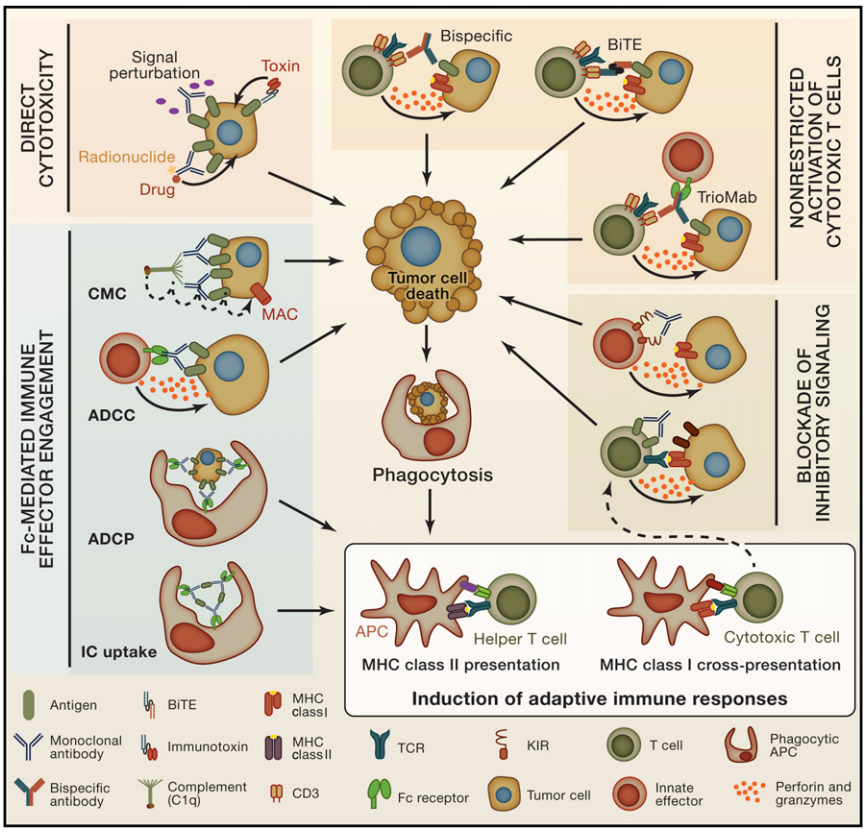

Figure 1. Mechanisms of Action of Antibody Immunotherapy in Cancer

Mechanisms of anticancer antibody therapies are diverse and represent the versatility of antibody-based approaches. Here, four different strategies are depicted. Upper left: direct cytotoxicity, in which mAbs can induce direct cytotoxicity in tumor cells by perturbing oncogenic signaling pathways or in which immunoconjugates can carry cytotoxic agents to targeted cells. Lower left: FcR-mediated immune effector engagement, in which the Fc portion of mAbs can engage immune effector functions, including soluble CMC (through the membrane attack complex MAC) as well as NK cells, macrophages, and dendritic cells, through FcRs, allowing for ADCC, ADCP, and IC uptake. Upper right: Nonrestricted activation of cytotoxic T cells, in which tumor-infiltrating CTLs can be activated against tumor cells—independent of T cell receptor (TCR) specificity—by engaging coreceptors on the T cells and tumor antigens. Lower right: blockade of inhibitory signaling, in which cytotoxic lymphocytes, including NK cells and CTLs, express inhibitory receptors for various ligands that may be expressed by tumor cells. Antagonistic antibodies that target these inhibitory receptors can block ligand-receptor interactions so that targeted cytotoxicity can ensue. These four strategies enhance tumor cell death, which can promote phagocytosis of tumor cell antigens, and induction of adaptive immune responses (bottom right) in two ways: MHC class I cross-presentation and priming of cytotoxic T cells and MHC class II presentation and priming of helper T cells. These adaptive immune responses can lead to enhanced—and possibly persistent—antitumor immunity.

being tested in six phase I/II clinical trials. The EpCAM-CD3 BiTE is in a phase I clinical trial.

An alternative method of creating bsAbs relies on the systematic analysis of binding affinities toward a second antigen after random mutation of the light-chain complementarity-determining regions (CDRs) of a parent antibody. Using this technique, bsAbs with two identical Fab regions, targeting VEGFA and HER2 or HER3 and EGFR, have been developed (Schaefer et al., 2011). MEHD7945A, an IgG1 antibody that binds to HER3 and EGFR with high affinity, exhibited equal or better antitumor efficacy than either parent antibody in 12 xenograft models (Schaefer et al., 2011). Although this method has theoretical utility for the development of bsAbs against any combination of two or more antigens, its potential for systematic applicability remains to be fully demonstrated.

The CovX-Body method is another recent technique for the rapid creation of bispecific antibodies (Doppalapudi et al., 2010). By fusing two peptide pharmacophores together and linking this complex to a universal scaffold antibody, a bispecific antibody with known Fc functions can be created. This structure, classified as a bispecific CovX-Body, is reproducible and specific and has the potential for widespread adoption. CVX-241, the first CovX-Body to enter clinical trials (NCT00911898), targets the angiogenesis ligands VEGFA and Ang2. Preclinically, CVX-241 exhibited moderate antitumor effects but, when combined with the chemotherapy agent irinotecan, significantly inhibited tumor growth (Doppalapudi et al., 2010). Another bsAb, MM-111, represents an alternative approach to bispecific engineering, based on linking the variable regions from two different antibodies. MM-111 targets HER2 and HER3 and is in phase I clinical trials. As the repertoire of cancer targets increases, dual targeting techniques may enhance clinical efficacy compared to traditional single-antigen targeting approaches.

Stimulating Persistent Immunity

Generation of a persistent antitumor immune response is a prevailing goal of cancer immunotherapy. Antibody therapy can indirectly stimulate persistent responses against tumor-associated antigens through induction of adaptive immunity (Figure 1). Hence, therapeutic antibodies can act to promote vaccine-like antitumor effects. Tumor cell death can modulate antigen uptake, maturation, and presentation in antigen-presenting cells (APCs), which are critical for initiating adaptive immunity (Sauter et al., 2000). Beyond inducing tumor cell death by blocking survival pathways, therapeutic antibodies can also coat tumor cells and mark them for recognition by immune cells. APCs, such as dendritic cells (DCs) or macrophages, can phagocytose antibody-coated tumor cells. Antibodies bound to soluble antigens in immune complexes (ICs) can also induce uptake by APCs. Through these various mechanisms of tumor antigen uptake, tumor contents—not only the antibody-targeted antigen—can be processed and presented by major histocompatibility complexes (MHCs) to activate different adaptive immune responses. Antigens presented via MHC class II can prime helper ($CD4^+$) T cell responses important for endogenous antibody (humoral) immunity. In addition, cross-presentation of

antigen and MHC class I-restricted priming of cytotoxic ($CD8^+$) T cell, or cytotoxic T lymphocyte (CTL), responses can occur (Dhodapkar et al., 2002). The induction of these T cell responses can enable immunological memory to the presented antigens, which is critical for long-term immunity.

The capacity of mAbs to induce tumor-directed CTL responses is intriguing. Intratumoral CTL composition and distribution have been associated with clinical outcomes (Galon et al., 2006), suggesting the relevance of T cells in antitumor immunity. Moreover, CTLs can target intracellular antigens that are thought to be inaccessible to antibody therapies. Therefore, antibody-initiated cross-presentation of tumor antigens can be exploited to induce adaptive immunity that may extend beyond the targeted antigen. This strategy has been described as the "vaccinal effect" in rituximab therapy of lymphoma (Hilchey et al., 2009) and has been shown to be relevant in antibody therapy of solid tumors. The antibody-dependent promotion of adaptive immunity remains an active and very promising area of research, as the induction of adaptive immunity can be accompanied by efforts to expand, shape, and prolong the host immune response. Because tumors may establish local and systemic immunosuppressive environments, concomitant efforts to neutralize immunosuppressive mechanisms may also amplify the vaccinal effect of mAbs.

Modulating the Amplitude of Immune Responses

Following activation, T cells upregulate the expression of inhibitory receptors, which protects against deleterious autoimmunity. This host-protective mechanism permits tumors to evade persistent immune control due to localized immune tolerance. This control is further manipulated by tumors through downregulation of surface immunogens or through the activation of diverse immune-suppressive mechanisms. This interplay between the immune system and tumor cells, termed immunoediting, allows tumors to escape immune elimination even when tumor-specific immunity is present (Schreiber et al., 2011).

Ipilimumab, an IgG1 mAb, antagonizes the inhibitory receptor CTLA-4, which is expressed on activated T cells. Treatment with this antibody in combination with dacarbazine correlates with a marked increase in overall survival and progression-free survival of previously untreated melanoma patients compared to treatment with dacarbazine alone (Robert et al., 2011). It is clear from the success of this landmark phase III trial that harnessing the activity of T cells will have a therapeutic antitumor benefit, even in the absence of a tumor antigen-targeted strategy. This clinical efficacy highlights the inherent ability of the immune system to recognize and eliminate abnormal self without the aid of tumor-specific therapies.

The PD-1 axis represents another promising immune checkpoint pathway to manipulate. PD-1 is expressed on activated T and B cells and provides a potent inhibitory signal when bound by ligand. Antibodies inhibiting the PD-1 checkpoint may reactivate T cells by blocking APC inhibition of T cells or by stopping tumor cells, which often overexpress PD-1 ligands, from inactivating T cells in the tumor microenvironment. In a phase I trial for refractory solid tumors, Brahmer et al. evaluated an IgG4 mAb, MDX-1106, that recognizes the extracellular domain of PD-1 with high affinity. The antibody was well tolerated in 38 of the 39 patients treated, resulting in three objective responses (two complete responses) and prompting its further evaluation in a phase II trial for clear-cell renal cell carcinoma (NCT01354431) (Brahmer et al., 2010).

Other approaches to enhancing T cell-specific immunity against tumor cells aim to activate stimulatory receptors, including 4-1BB, OX40, CD27, CD40, and DR3. Preclinical models have shown that the stimulation of 4-1BB, OX40, and CD27 leads to proliferation and cytokine production in T cells (Croft, 2009). Interestingly, activation of 4-1BB and OX40 can also inhibit the differentiation and proliferation of regulatory T cells, which contribute to tumor-derived immunosuppression (Croft, 2009). Therefore, agonistic antibodies to these TNF receptors potentially have dual and synergistic immune-promoting roles. Currently, two 4-1BB agonist antibodies are in phase I and II clinical trials for NHL and melanoma, respectively (NCT01307267, NCT00612664), and one agonistic OX40 antibody is being studied in a phase I/II and phase II trial for prostate cancer and melanoma (NCT01303705, NCT01416844).

Although the manipulation of T cells is currently a primary focus, other approaches may leverage the innate immune system. By blocking the function of inhibitory killer cell immunoglobulin-like receptors (KIRs), natural killer (NK) cells could elicit a tumor-specific cytotoxic response without harming normal self cells, as evidenced by the anti-KIR (KIR2DL1/L2/L3 and KIR2DS1/S2) antagonist antibody IPH2101, which is currently in numerous early phase clinical trials (NCT00999830). As a clinical validation of the immunoediting hypothesis, the manipulation of the immune system to elicit an antitumor response has the potential to serve as an efficacious treatment modality across all cancers.

Immunoconjugates: Targeting Cytotoxic Agents

Early efforts to enhance the antitumor effects of mAbs focused on boosting their direct cytotoxic effects on targeted cells. Conjugation of radionuclides (radioimmunotherapies, or RITs), drugs (antibody-drug conjugates, or ADCs), toxins (immunotoxins), and enzymes (antibody-directed enzyme prodrug therapy, or ADEPT) yielded a multitude of antibodies—or antibody-like molecules—with varying clinical efficacy. Three conjugated antibodies have translated into FDA-approved therapies, all for hematological malignancies. Two are RIT agents targeting CD20 and are indicated for treatment of relapsed and/or rituximab-refractory follicular or low-grade lymphomas: ^{90}Y-ibritumomab tiuxetan and ^{131}I-tositumomab. At least a dozen other RIT agents are in active development, including ten that target solid tumors (Steiner and Neri, 2011). The third approved immunoconjugate, brentuximab vedotin, is an ADC targeting CD30 and carrying the antimitotic drug monomethyl auristatin E. Brentuximab vedotin was recently approved for treatment of anaplastic large cell lymphoma (NCT00866047) and Hodgkin lymphoma (NCT00848926).

The limited translational success of immunoconjugates reflects the challenges of developing a highly cytotoxic agent with acceptable pharmacokinetics and toxicity. RITs can cause systemic toxicity, and many tumors, particularly solid tumors, are inaccessible or insensitive to deliverable doses of radiation. Although a premise of antibody immunotherapy is low toxicity imparted by specificity, "tumor-specific" antigens are often more selective than specific. Additionally, generation of neutralizing antibodies to recombinant immunoconjugates, such as

immunotoxins, may limit their clinical utility, similar to the limitations associated with murine antibodies (Kreitman et al., 2009).

These challenges have not thwarted attempts to develop immunoconjugates. Another ADC, trastuzumab-MCC-DM1 (trastuzumab-DM1 or T-DM1), has shown promise in patients with HER2-positive metastatic breast cancer. A phase III trial comparing T-DM1 against capecitabine, a prodrug of 5-fluorouracil, plus lapatinib, a tyrosine kinase inhibitor, is underway (NCT00829166). The most clinically advanced immunotoxin, BL22, contains an anti-CD22 Fv bound to a modified *Pseudomonas* exotoxin. BL22 has shown significant promise in phase II trials for the treatment of hairy cell leukemia (Kreitman et al., 2009). Beyond direct tumor cell cytotoxicity, it is possible that cytotoxic immunoconjugates could induce antigen-targeted adaptive immune responses, though this requires additional study.

A Longer-Term View

Activating FcRs involved in immune effector activities are important for antitumor effects (Clynes et al., 2000). However, two recent, independent reports have demonstrated that engagement of either activating and inhibitory FcRs (Wilson et al., 2011) or inhibitory FcR alone (FcgammaRIIB) (Li and Ravetch, 2011) can drive antitumor immune effects of agonistic antibodies targeting death receptor superfamily members. The compelling observation that inhibitory FcRs alone drive productive immune responses is unexpected. Thus, efforts to modify mAb structure to balance engagement of FcRs will remain a critical component of antibody development (Jiang et al., 2011). These findings highlight how antibody therapy can elucidate novel mechanisms of immune effector activity that may differ from fundamental understanding in immunology.

An even more basic tenet of antibody therapy is the concept of targetable antigens. The dogma has been that only soluble extracellular or cell-surface antigens are accessible targets for antibodies. Targeting intracellular antigens—particularly oncogenic or mutated cytosolic proteins specific to tumor cells—has been left to other membrane-permeable treatment modalities. However, cellular immunotherapy targeting endogenous intracellular antigens that are processed and presented at the tumor cell surface, as with the prostate cancer therapy Sipuleucel-T, has already demonstrated its worth.

Remarkably, recent findings suggest that we may have underestimated the capacity for antibodies to target intracellular antigens. Guo and colleagues have demonstrated that mAbs can effectively target intracellular antigens and inhibit tumor growth in mouse models (Guo et al., 2011). The capacity for antibodies to be internalized by tumor cells, thereby allowing for access to intracellular antigens, may explain this provocative observation. Targeting intracellular antigens would profoundly broaden antibody immunotherapy to include tumor-specific mutated intracellular proteins and other intracellular mediators of cell survival and proliferation. As this is a nascent area of research, it is only speculative that intracellular antigen targeting would provide sufficient antitumor effect to translate into clinical efficacy. In concert with targeted strategies that enhance antitumor immunity, even in the face of immune evasion, tolerance, and suppression, it is possible to envision a future where combinatorial antibody approaches transition into cancer immunotherapeutic strategies.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one figure and one table and can be found with this article online at doi:10.1016/j.cell.2012.02.034.

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WEB RESOURCES

Information about the referenced clinical trials can be found at the following url: http://clinicaltrials.gov/ct2/show/xxx, where xxx is the specific number/letter code referenced in the text.

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