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Commentary

Monoclonal antibodies in cancer therapy

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Introduction

Monoclonal antibody-based treatment of cancer has been established as one of the most successful therapeutic strategies for both hematologic malignancies and solid tumors in the last 20 years. The initial combining of serological techniques for cancer cell surface antigen discovery with hybridoma technology led to a series of landmark clinical trials that paved the way for new generation antibodies and subsequent clinical success. Optimization of anti-tumor immune responses through Fc modifications has also made a major contribution to clinical efficacy. The modulation of immune system interplay with tumor cells through targeting of T cell receptors has emerged as a powerful new therapeutic strategy for tumor therapy and to enhance cancer vaccine efficacy. This commentary will provide an overview of the history of antibody identification of tumor surface antigens, antigenic targets suitable for antibody-based therapy, antibody mechanisms of action, and recent successes of antibodies in the clinic.

Cancer serology - the prelude to antibody therapeutics

The concept that antibodies could serve as 'magic bullets' in the diagnosis and therapy of cancer dates back to their discovery in the late 19th century. A considerable effort over the ensuing decades involved immunization of a variety of animal species with human cancer in the hope of generating antisera with some degree of cancer specificity (1). Unfortunately, this approach had limited early success, with the notable exception of the discovery of carcinoembryonic antigen (CEA), a marker for colon and other cancers, and α-fetoprotein, a marker for hepatocellular cancer (1, 2).

The development of inbred mice initiated a new era of serological investigation of cancer with the emergence of the cytotoxic test as a powerful tool to analyze the cell surface reactivity of alloantibodies. This subsequently led to the recognition that the cell surface is a highly differentiated structure. During the 1960s and 1970s, Lloyd Old made a series of discoveries that revolutionized our understanding of the immune system. In collaboration with Ted Boyse, he introduced the concept of cell surface differentiation antigens that could distinguish lineage and functional subsets of leukocytes in mice (3). This led to major contributions at the time which include the discovery of the thymus-leukemia (TL) antigen, the linking of the major histocompatibility complex (MHC) and leukemia, and subsequently the Ly series of antigens (4). These discoveries led to the precise and systematic identification of cell surface antigens that distinguished normal cells from malignant cells, and directly to the cluster of differentiation (CD) classification.

Following the development of hybridoma technology by Köhler and Milstein (5), combined with serological techniques and analytical tools such as fluorescence-activated cell sorting (FACS), monoclonal antibodies (mAbs) were used to dissect the surface structure of human cancer cells, thus paving the way for the identification of cancer cell surface antigens suitable for targeting by antibodies. The characterization of the cancer cell "surfaceome" has been enhanced in recent times with proteomic, genomic, and bioinformatic approaches to identifying antigen targets on cancer cells, as well as in cancer stroma and vasculature.

Tumor antigens as targets for antibody therapy

The selection of tumor antigens suitable for antibody targeting and therapy requires a comprehensive analysis of tumor expression (including homogeneity of expression) and normal tissue expression, as well as an understanding of the biologic role of the antigen in tumor growth. If the desired mechanism of action is engagement with cell surface receptors (to either activate or inhibit signaling), or to activate antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC), then it is desirable that the antigen-mAb complex should not be rapidly internalized. This allows the maximization of the availability of the Fab region to appropriately engage with surface receptors, and of the Fc region to immune effector cells and complement proteins. In contrast, internalization is desirable for antibodies or proteins delivering toxins into the cancer cell and for antibodies whose action is primarily based on downregulation of cell surface receptors (2).

Tumor-associated antigens recognized by therapeutic mAbs are outlined in Table 1. Hematopoietic differentiation antigens are glycoproteins usually associated with CD groupings and include CD20, CD30, CD33, and CD52 (2, 6-8). Cell surface differentiation antigens represent a diverse group of glycoproteins and carbohydrates that are found on the surface of

both normal and tumor cells. Growth factors that are targets for antibodies in oncology patients include CEA (2), epidermal growth factor receptor (EGFR; also known as ErbB1) (9), ErbB2 (also known as HER2) (10), ErbB3 (11), MET (12), insulin-like growth factor 1 receptor (IGF1R) (13), ephrin receptor A3 (EphA3) (14), TNF receptor apoptosis-inducing ligand receptor 1 (TRAIL-R1), TRAIL-R2, and receptor activator of nuclear factor κβ ligand (RANKL) (15). Antigens involved in angiogenesis are usually proteins or growth factors that support the formation of new microvasculature, including vascular endothelial growth factor (VEGF), VEGF receptor (VEGFR), and integrins αVβ3 and α5β1 (16). Stromal and extracellular matrix antigens that are therapeutic targets include fibroblast activation protein (FAP) and tenascin (17-19).

Antibody engineering and mechanisms of action

The development of hybridoma technology led to the first generation of murine antibodies against tumor cell surface antigens. Following initial clinical trials in the 1980s with murine antibodies against CEA and CD3, a range of antibodies against solid tumor and hematologic malignancies were developed and entered clinical trials (20). The development of immune responses against these murine antibodies (human anti-mouse antibodies, HAMA) significantly limited their clinical utility, and as a consequence, apart from the FDA-approved ^{131}I-anti-CD20 antibody tositumomab, and ^{90}Y-anti-CD20 antibody ibritumomab tiuxetan, murine antibodies were not further pursued (20).

The development of humanization approaches by Winter and colleagues (21), whereby murine Fc and Fv framework regions of murine antibodies were replaced by human germline amino acids, revolutionized the field of antibody therapeutics. Through this technology, minimal immune responses to antibodies were observed, allowing the multiple infusions of engineered antibodies, leading to the successful entry of multiple antibodies into the clinic (6, 22). Additional strategies to generate fully human antibodies through phage display techniques, as well as the use of transgenic mice that produce fully human antibodies, have also been successfully implemented (17). More recently, innovative antibody engineering approaches to produce smaller antibody variants, fusion proteins, and bispecific antibodies have been utilized (6, 7, 22). Combined with improved cell line generation and larger scale production techniques, the transition from laboratory scale preclinical testing to large clinical trial batch production has been considerably enhanced.

Table 1
Tumor-associated antigens targeted by monoclonal antibodies

Antigen category	Examples of antigens	Tumor types expressing antigen
Cluster of differentiation (CD) antigens	CD20	non-Hodgkin lymphoma
	CD30	Hodgkin lymphoma
	CD33	Acute myelogenous leukemia
	CD52	Chronic lymphocytic leukemia
Glycoproteins	EpCAM	Epithelial tumors (breast, colon, lung)
	CEA	Epithelial tumors (breast, colon, lung)
	gpA33	Colorectal carcinoma
	Mucins	Epithelial tumors (breast, colon, lung, ovarian)
	TAG-72	Epithelial tumors (breast, colon, lung)
	Carbonic anhydrase IX	Renal cell carcinoma
	PSMA	Prostate carcinoma
	Folate binding protein	Ovarian tumors
Glycolipids	Gangliosides (e.g., GD2, GD3, GM2)	Neuroectodermal tumors, some epithelial tumors
Carbohydrates	Lewis-Y[2]	Epithelial tumors (breast, colon, lung, prostate)
Vascular targets	VEGF	Tumor vasculature
	VEGFR	Epithelium-derived solid tumors
	αVβ3	Tumor vasculature
	α5β1	Tumor vasculature
Growth factors	ErbB1/EGFR	Glioma, lung, breast, colon, head and neck tumors
	ErbB2/HER2	Breast, colon, lung, ovarian, prostate tumors
	ErbB3	Breast, colon, lung, ovarian, prostate tumors
	c-MET	Epithelial tumors (breast, ovary, lung)
	IGF1R	Lung, breast, head and neck, prostate, thyroid, glioma
	EphA3	Lung, kidney, colon, melanoma, glioma, hematological malignancies
	TRAIL-R1, TRAIL-R2	Solid tumors (colon, lung, pancreas) and hematological malignancies
	RANKL	Prostate cancer and bone metastases
Stromal and extracellular matrix antigens	FAP	Epithelial tumors (colon, breast, lung, head and neck, pancreas)
	Tenascin	Glioma, epithelial tumors (breast, prostate)

Table 2
Mechanisms of tumor cell killing by antibodies

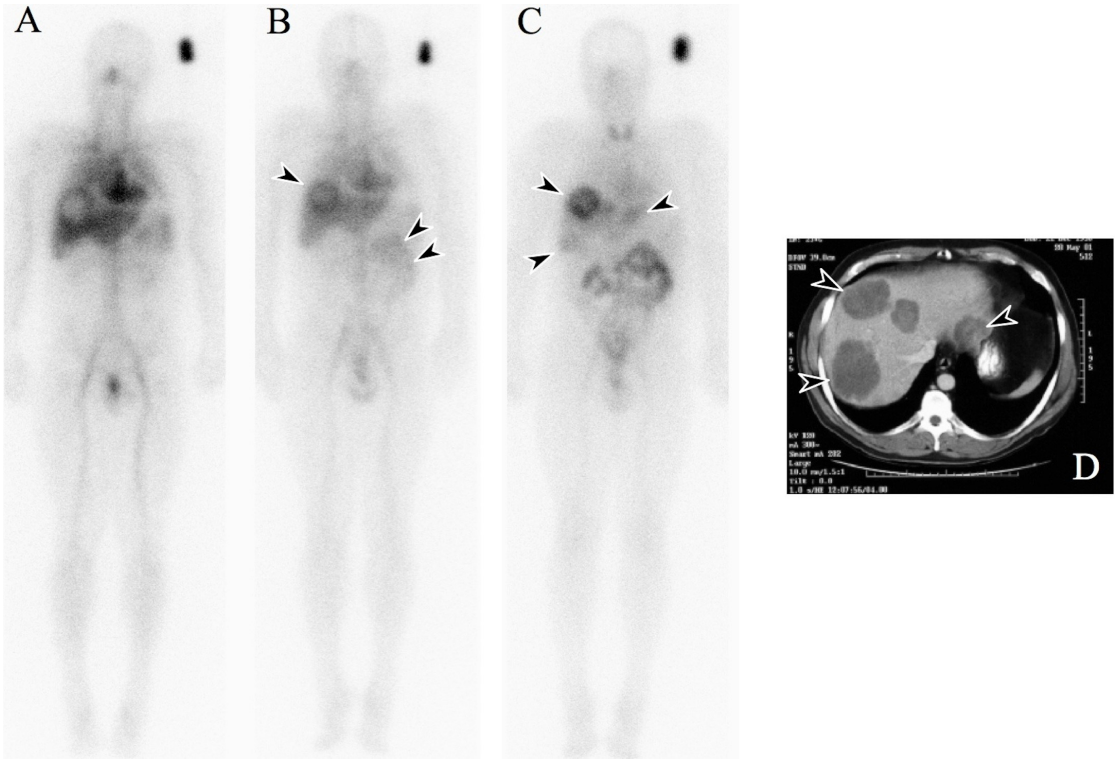
Direct tumor cell killing
• cell surface receptor agonist activity (leading to apoptosis) • cell surface receptor antagonist activity (inhibit signaling, reduce proliferation, induce apoptosis) • cell surface enzyme neutralization (leading to signaling abrogation) • conjugated antibody, delivery of payload (drug, toxin, radio-isotope, leading to cell death)
Immune-mediated tumor cell killing
• induction of phagocytosis • complement activation • antibody-dependent cell-mediated cytotoxicity (ADCC) • target gene-modified T cells • activate T cells (through inhibition of T cell inhibitory receptors, such as CTLA-4, or antibody-mediated cross presentation of antigen to dendritic cells)
Vascular and stromal ablation
• vessel receptor antagonism or ligand trap • stromal cell inhibition • conjugated antibody, delivery of payload

The mechanisms of tumor cell killing by antibodies are outlined in Table 2. These can be due to direct cell killing, such as through receptor blockade or agonist activity, induction of apoptosis, or delivery of a drug, radiation, or cytotoxic agent; immune-mediated cell killing mechanisms; regulation of T cell function; and specific effects on tumor vasculature and stroma. The Fc function of antibodies is particularly important in mediating tumor cell killing through CDC, and immune cell activation (e.g., NK cells) and tumor cell killing through ADCC. The abrogation of tumor cell signaling (e.g., cetuximab and trastuzumab) (9, 10), effector function primarily through ADCC (e.g., rituximab) (23), and immune modulation of T cell function (e.g., ipilimumab) (24) are the approaches that have been most successful and have led to approval of antibodies using these effector mechanisms.

"*In vivo veritas*" - translation of antibodies into the clinic

While the identification of novel target antigens expressed in tumors and the generation of antibodies against these antigens with optimal functional characteristics is the important initial step in developing a potential therapeutic antibody, there are many issues to address before embarking on clinical trials in cancer patients. These include the physical and chemical properties of the antibody, a detailed specificity analysis of antigen expression using panels of normal and malignant tissues, and immune effector function and signaling pathway effects of antibodies. In addition, antibody humanization and *in vivo* therapeutic activity of the antibody, either alone or conjugated with radioactive isotopes or other toxic agents, are essential in the preclinical evaluation of antibodies (6, 8, 17, 25-29).

Figure 1



Biodistribution of ^{131}I-huA33 in a patient with metastatic colorectal carcinoma. Anterior whole body gamma camera images are shown following infusion of ^{131}I-huA33 at (A) day 0, (B) day 1, and (C) day 5. A standard for quantitation of ^{131}I-huA33 uptake is present, adjacent to the left shoulder. Initial (day 0) images (A) show blood pool appearance only, with large metastatic lesions in the liver demonstrating an initial hypovascular appearance. (B) Excellent targeting of the metastatic lesions in the liver by ^{131}I-huA33 is clearly seen (arrow) as early as day 1, and increasing rapidly with time to day 5. Some central necrosis in the larger tumors is also evident (arrow), also seen on CT scan (D). Gradual bowel uptake (double arrow) of ^{131}I-huA33 is also seen, which gradually decreases with time. No other normal tissue uptake of ^{131}I-huA33 is evident.

One of the most essential steps in the clinical evaluation of a potential therapeutic antibody is *in vivo* specificity—determining the biodistribution of antibody (often radiolabeled) in patients to assess the ratio of antibody uptake in the tumor in relation to normal tissues (18, 25, 29) (Figure 1). This information is essential for the design for clinical trials, as knowledge about the targeting of normal tissues is critical for predicting toxicity and determining optimal antibody dose and schedule (8, 29). Under the leadership of Lloyd Old, at the Ludwig Institute for Cancer Research (LICR), we developed a model of a phase I antibody clinical trial that incorporates biodistribution, pharmacokinetics, and pharmacodynamics analyses with toxicity assessment (25). This trial design has been successfully applied to first-in-human clinical trials of more than 15 antibodies in cancer patients (18, 19, 25, 29-34). This approach can identify subtle changes in antibody physicochemical properties (28) that affect biodistribution, which can significantly impact efficacy. In addition, normal tissue and tumor distribution can be quantitated, thus allowing the relationship of the loading dose to tumor concentration to be accurately assessed, rather than relying on plasma concentration and clearance rates to establish an optimal dose. Examples of where this approach was successfully used include the early biodistribution studies of mouse anti-colon cancer antibody A33 (33), the anti-CD33 antibody M195 (30), anti-CAIX antibody G250 (34), anti-FAP antibody F19 (19), anti-GD3 antibody KM871 (31), and anti-Le^y antibody hu3S193 (32). This approach has also been applied to recent studies of trastuzumab (which targets ErbB2) biodistribution and *in vivo* assessment of ErbB2 expression by tumors (35). In non-Hodgkin lymphomas, assessment of the biodistribution of a radioconjugate in both the tumor and through whole body dosimetry was essential in initial trials exploring patient suitability for treatment and treatment dose for the United States Food and Drug Administration (FDA)-approved anti-CD20 radioimmunoconjugates tositumumab and ibritumomab tiuxetan (8, 28, 36).

The use of patient biopsies can also be utilized to assess the *in vivo* effect of antibody abrogation of signaling pathways (36). The evaluation of pharmacodynamics in early-phase clinical trials can also involve biological effector function of antibodies, such as ADCC (through optimized FcγR binding) and cytotoxicity (26). The assessment of antibodies as delivery vehicles for toxic agents can also be assessed using this clinical trial design approach (8, 26-29).

Table 3
Monoclonal antibodies currently FDA-approved in oncology

Antibody	Target	FDA-Approved indication	Mechanism of action
Trastuzumab (Herceptin®) humanized IgG1	HER2 (ErbB2)	HER2-positive breast cancer, as single agent or in combination with chemotherapy for (i) adjuvant or (ii) palliative treatment; HER2-positive gastric or gastroesophageal junction carcinoma, as first-line treatment in combination with cisplatin and capecitabine/5-FU	Inhibition of HER2 signaling; ADCC
Bevacizumab (Avastin®) humanized IgG1	VEGF	For the palliative treatment of colorectal cancer, non-squamous non-small cell lung cancer, glioblastoma, or renal cell carcinoma	Inhibition of VEGF signaling
Cetuximab (Erbitux®)* chimeric human/murine IgG1	EGFR (ErbB1)	In combination with radiation therapy for the initial treatment of locally or regionally advanced squamous cell cancer of the head and neck (SCCHN); As a single agent for SCCHN patients with whom prior platinum-based therapy has failed; Palliative treatment of pre-treated metastatic EGFR-positive colorectal cancer	Inhibition of EGFR signaling; ADCC
Panitumumab (Vectibix®)* human IgG2	EGFR (ErbB1)	As a single agent for the treatment of pre-treated EGFR-expressing, metastatic colorectal carcinoma	Inhibition of EGFR signaling
Ipilimumab (Yervoy®) IgG1	CTLA-4	For the treatment of unresectable or metastatic melanoma	Inhibition of CTLA-4 signaling
Rituximab (Rituxan® and Mabthera®) chimeric human/murine IgG1	CD20	For the treatment of CD20-positive B cell non-Hodgkin lymphoma (NHL) and chronic lymphocytic leukemia (CLL), and for maintenance therapy for untreated follicular CD20-positive NHL	ADCC; direct induction of apoptosis; CDC
Alemtuzumab (Campath®) humanized IgG1	CD52	As a single agent for the treatment of B cell CLL	Direct induction of apoptosis; CDC
Ofatumumab (Arzerra®) human IgG1	CD20	Treatment of patients with CLL refractory to fludarabine and alemtuzumab	ADCC; CDC
Gemtuzumab ozogamicin (Mylotarg®) humanized IgG4	CD33	For the treatment of patients with CD33-positive acute myeloid leukemia in first relapse who are 60 years of age or older and who are not considered candidates for other cytotoxic chemotherapy (withdrawn from use in June 2010)	Delivery of toxic payload, calicheamicin toxin
Brentuximab vedotin (Adcetris®) chimeric IgG1	CD30	For the treatment of relapsed or refractory Hodgkin lymphoma and systemic anaplastic lymphoma	Delivery of toxic payload, auristatin toxin
^{90}Y-Ibritumomab Tiuxetan (Zevalin®) murine IgG1	CD20	Treatment of relapsed or refractory, low-grade, or follicular B cell NHL; Previously untreated follicular NHL in patients who achieve a partial or complete response to first-line chemotherapy	Delivery of the radio-isotope yttrium-90
^{131}I-Tositumomab (Bexxar®) murine IgG2	CD20	Treatment of patients with CD20 antigen-expressing relapsed or refractory low-grade, follicular, or transformed NHL	Delivery of the radio-isotope iodine-131; ADCC; direct induction of apoptosis

*Not recommended in colorectal cancer patients whose tumors express mutated KRas

Success of antibodies in the clinic

There have been twelve antibodies that have received approval from the FDA for the treatment of a variety of solid tumors and hematological malignancies (Table 3). In addition, there are a large number of additional therapeutic antibodies that are currently being tested in early- and late-stage clinical trials. The use of therapeutic antibodies in patients with solid tumors has been most successful with classes of antibodies targeting the ErbB family (which includes EGFR) and VEGF (9, 16, 20, 37-39). Recent evidence shows that patients with colorectal cancer bearing wild-type KRas tumors who were treated with anti-EGFR antibodies have improved responses (9, 40), disease

control (40), and survival (41, 42). These findings have resulted in the FDA-approved use of these agents restricted to patients with colorectal cancer in which KRas is not mutated. The use of trastuzumab has also been restricted to patients with high levels of ErbB2 expression, as studies have shown that this is the group that derives maximum benefit from trastuzumab treatment (6, 10). As a result of the clinical success of these antibodies and preclinical data demonstrating the improved tumor response (and reversal of resistance to single agent) of combined signaling blockade with antibodies to different receptors, or to different epitopes on the same receptor (e.g., trastuzumab and pertuzumab), numerous clinical trials of antibodies as combination therapies are currently under way (20).

A number of antibodies have also been approved for the treatment of hematological malignancies, both as unconjugated antibodies and for delivery of isotopes and drugs or toxins to cancer cells (Table 3). Antibody-drug or -toxin conjugates have been shown to have high potency in hematological malignancies, and there have been two approved by the FDA: gemtuzumab ozogamicin in elderly patients with CD33-positive AML (although this drug was voluntarily withdrawn in June 2010 following a post-marketing phase III trial), and more recently brentuximab vedotin in patients with CD30-positive Hodgkin lymphoma (27, 43). A similar approach in patients with advanced ErbB2-positive breast cancer with the antibody-drug conjugate trastuzumab-emtansine (T-DM1) (44) is currently being explored in phase III trials.

There are other antibodies approved for cancer indications outside the U.S. Catumaxomab, a mouse bispecific antibody against CD3 and EpCAM, is approved in the European Union for use in patients with malignant ascites generated by an EpCAM-positive tumor (45). Nimotuzumab, a humanized IgG antibody against EGFR, is approved for use in some countries in Asia, South America, and Africa for the treatment of head and neck cancer, glioma, and nasopharyngeal cancer (46). Vivatuxin (^{131}I-chTNT), a radiolabeled IgG1κ chimeric monoclonal antibody against intracellular DNA-associated antigens, has also been approved by the Chinese drug regulator for the treatment of malignant lung cancer (47).

Immune regulation by antibodies

In addition to targeting antigens involved in cancer cell physiology, antibodies can also function to modulate immunological pathways that are critical to immune surveillance. Antigen-specific immune responses result from a complex dynamic interplay between antigen presenting cells, T lymphocytes, and target cells. Immunologic signal 1, the recognition of specific antigenic peptides bound to MHC by the T cell receptor (TCR) is insufficient for T cell activation. Signal 2, ligation of CD28 by a member of the B7 family of costimulatory molecules (CD80, CD86), initiates T cell activation via signaling pathways resulting in autocrine IL-2 production. Just after T cell activation, CTLA-4, a molecule normally found in intracellular stores, translocates to the immunologic synapse, where it serves to inhibit the activated T cell by binding with high avidity to the same B7 molecules and stopping activation signals mediated by CD28. The role for blockade of CTLA-4 with an antibody as a means to potentiating T cell activation and initiating responses to targets on tumor cells was proposed in 1996 (48) and provided the scientific foundation for the development of two fully human monoclonal antibodies blocking CTLA-4 (ipilimumab and tremelimumab). Ipilimumab was approved by the U.S. FDA, European Medicines Agency (EMA), and a number of other national regulatory agencies for treatment of patients with metastatic melanoma after a pivotal phase III trial demonstrated significant improvement in overall survival resulting from its use, making it the first treatment to be shown to enhance survival and the first newly approved medicine in 13 years for melanoma (24). CTLA-4 blockade does present new paradigms in terms of treatment-related toxicity. The immune-related adverse events are inflammatory and largely confined to the skin and gastrointestinal tract but can more rarely affect the liver and endocrine glands. With prompt diagnosis, these events are generally manageable with immunosuppressive medications such as corticosteroids, which fortunately do not seem to interfere with the anti-tumor effect (24).

The therapeutic success of ipilimumab has led to enthusiasm for the development of other immune modulating antibodies. The next most advanced products target PD-1, a marker of activated or exhausted T cells that can trigger apoptosis when bound by its ligand, PD-L1 (B7-H1) (49). Interestingly, this ligand is found not only on antigen presenting cells, but also on many tumor cells. PD-1 blockade has been shown in early clinical trials to result in durable responses in patients with melanoma, renal cell carcinoma, non-small cell lung cancer, and colorectal cancer (49). Several antibodies that target the PD-1 axis are in development. Agonistic antibodies are also being explored, including two fully human antibodies to CD137 (4-1BB), an activator of T cells, from Pfizer and Bristol-Myers Squibb (BMS). The BMS antibody has been in phase I trials, demonstrating anti-tumor efficacy across a wide dose range. Trials were temporarily suspended due to severe hepatic toxicity at high doses but are now opening again using low doses. This highlights an important aspect of antibody drug development as higher doses of a blocking antibody may yield better therapeutic results while low doses of agonistic antibodies may allow for a better risk-benefit profile. Other pathways of interest for agonistic antibodies include CD40, where favorable preclinical and clinical results have been noted particularly in pancreatic cancer (50), and the glucocorticoid-induced TNF receptor (GITR).

Antibody therapeutics might also have a role in generation of *de novo* immune responses to the antigen targeted by the antibody through promoting antigen presentation to Fc receptor-bearing cells (51-53). *De novo* induction of secondary immune responses may therefore allow for the effects of antigen-specific antibodies to persist after the dosing is completed.

Conclusion

The use of monoclonal antibodies for the therapy of cancer is one of the major contributions of tumor immunology to cancer patients. This success is built on decades of scientific research aimed at serological characterization of cancer cells, techniques for generating optimized antibodies to tumor targets, detailed investigation of signaling pathways relevant to cancer cells, and an understanding of the complex interplay between cancer cells and the immune system (20, 54). The clinical development of antibodies is inextricably linked to disciplined and detailed exploration of the properties of antibodies *in vivo* and assessment of functional effects on cancer cells. One of our major challenges is now to fully exploit antibody therapies in cancer patients by combining the two major immune-based treatment approaches—antibodies and vaccines. Trials combining ipilimumab with vaccines have shown mixed results thus far (24, 55). The Cancer Vaccine Collaborative, a joint academic clinical trials infrastructure established by LICR and

the Cancer Research Institute (CRI), is about to embark on a series of trials exploring NY-ESO-1 vaccines along with ipilimumab to further investigate this important area. In this way, the full promise of tumor immunology in controlling and treating cancer will hopefully be realized.

Abbreviations

ADCC, antibody-dependent cell-mediated cytotoxicity; MHC, major histocompatibility complex

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