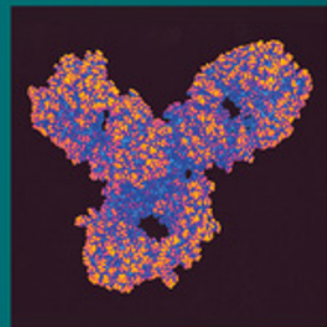
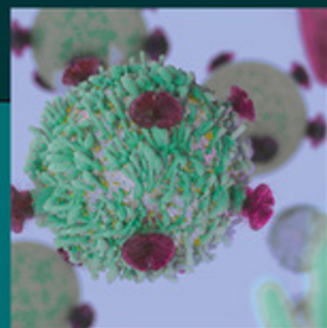
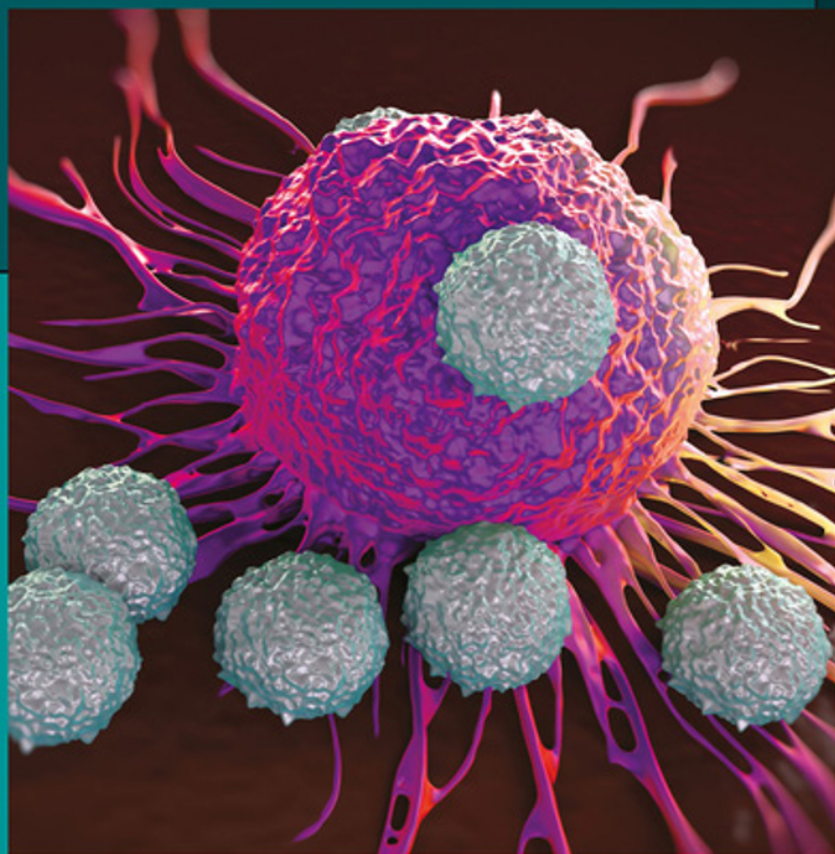


# Immunotherapy in Translational Cancer Research

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**Translational  
Oncology**

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Immunotherapy  
in Translational  
Cancer Research



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# Contents

- Editors, vi
- Contributors, vii
- Introduction, ix
- 1 Translation in Immunology: The Role of Translational Biomarkers to Guide Clinical Use of Immunotherapy for Cancer, 1  
*Saranya Chumsri and Keith L. Knutson*
  - 2 Monoclonal Antibody Therapy, 12  
*Elizabeth A. Mittendorf and Sabitha Prabhakaran*
  - 3 Somatic Mutations and Immunotherapy, 24  
*Eric Tran*
  - 4 Checkpoint Blockade, 37  
*Tracy A. Proverbs-Singh, Jedd Wolchok, and Alexandra Snyder*
  - 5 Myeloid Leukemia Vaccines, 54  
*Paul M. Armistead and Jonathan S. Serody*
  - 6 Cancer Vaccines for Solid Tumors, 72  
*Timothy J. Vreeland, Garth S. Herbert, and George E. Peoples*
  - 7 Immunotherapy with Non-Genetically Modified T Cells, 91  
*Cassian Yee*
  - 8 Immunotherapy with Genetically Modified T Cells, 101  
*Liora Schultz and Crystal Mackall*
  - 9 Immunomodulation with Adjuvants and Cytokines, 115  
*Dae won Kim and Adi Diab*
  - 10 Immune Cell Migration in Cancer and Immunotherapy, 132  
*Timothy Murray and Daniel E. Speiser*
  - 11 Repurposing of Drugs for Immunotherapy, 143  
*Jyothishmathi Swaminathan and Vidya Gopalakrishnan*
  - 12 Immunomodulation with Radiation, 161  
*Jonathan E. Schoenhals, Taylor R. Cushman, Alexandra P. Cadena, Sandra Demaria, and James W. Welsh*
  - 13 Good Manufacturing Practices Facilities for Cellular Therapy, 177  
*Andrew D. Fesnak and Bruce L. Levine*
  - 14 T Cell Receptor Mimic Antibodies, 186  
*Gheath Alatrash and Jeffrey J. Mollidrem*
  - 15 Stem Cell Transplantation for Treatment of Malignancy, 201  
*Lohith S. Bachegowda and Richard E. Champlin*
  - 16 Natural Killer Cell-Based Immunotherapy, 215  
*Jennifer A. Foltz, Jeffrey S. Miller, and Dean A. Lee*
  - 17 Immunologic Monitoring in Immunology-Oncology, 228  
*William R. Gwin and Mary L. Disis*
  - 18 Cell-Based Therapies for Canine Cancer, 252  
*Nicola J. Mason and M. Kazim Panjwani*
  - 19 Interaction between Targeted Therapy and Immunotherapy, 268  
*Peter A. Prieto, Miles C. Andrews, Alexandria P. Cogdill, and Jennifer A. Wargo*
- Index, 286



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# Introduction

We live and work in the age of immunotherapy. The modality is now firmly affixed to the triad of chemotherapy, radiation therapy, and surgery. This book captures the translation of immunology into therapies. The migration of bench research to bedside experimentation has been largely driven by academia and amplified by industry; however, in the current age, there is equipoise between the not-for-profit and for-profit enterprises regarding advancements in the human applications of immunotherapies.

The breadth of treatments reflects the complexity of the immune system itself. The coordinated response of the multiple components of an endogenous immune response has generated a portfolio of immunotherapy options that are reflected in the names of the chapters. Not all chapters, though, are created equally. Some immunotherapies are just beginning their human experimentation and some are seasoned and perhaps even seen as out of vogue. Nevertheless, as a whole, these components of immune-based therapies provide patients with therapeutic optimism and some with therapeutic impact.

This book is a sum of its chapters and thus individual immunotherapies. What is not yet evident is how combinations of immune-based therapies can be harnessed. This is undoubtedly

needed in order to secure long-term and complete treatment for the majority of malignancies, especially arising as solid tumors. The coordinated response of the endogenous immune system will be mirrored by the corradicated application of immunotherapies. However, that will be the topic of a future book. What is present and is remarkable, is that monotherapies based on harnessing the immune response have resulted in Lazarus-type moments, are used to prevent cancer, and have provided responses in tumors that were previously considered untreatable.

Immunotherapy as presently wielded is a relatively blunt tool. Yet the immune system is built on precision. Academics and industry investigators are only beginning to understand how to sharpen the therapeutic edge of an applied immune response. The proving ground is the human experience as preclinical models by and large do not yield sufficient information regarding efficacy and toxicity. Thus, immunotherapy practitioners and patients alike are risk takers. Together, they will advance the clinical application of the immune system so that its complexity can be harnessed as an instrument to treat cancer on an individualized basis.

This is a good time to be studying immunotherapy, and we hope this book rewards your interest.



# Translation in Immunology: The Role of Translational Biomarkers to Guide Clinical Use of Immunotherapy for Cancer

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## Introduction

For over a century, the role of the immune system in controlling and eradicating tumors has been a subject of intense debate. Since the 1800s, it has been recognized that the immune system also plays an important pathologic protumor role in tumor initiation and progression. Virchow commented on the interaction between inflammation, leukocytes, and cancer in his article from 1863 [1]. More than a hundred years later, we are still extricating the complexities of the interaction between cancers and the host immune system. More recently, Schreiber, Old, and Smyth described the process in which cancer and the immune system interact with each other, termed “cancer immunoediting” [2]. Cancer immunoediting describes a contiguous process that the immune system influences and shapes developing tumors. This process can result in successful rejection of the tumor or generate a tumor through immunologic evasion, the latter of which we now know can occur by multiple mechanisms and more often than not through any one of a number of immune suppressive pathways [3].

Despite the long-standing interest in host antitumor immunity, it was only recently that immunotherapy emerged as one of the effective treatment options for cancer. In the past decade, several new immunotherapies, such as immune checkpoint blockade agents, tumor antigen-targeted monoclonal antibodies, and a cell-based dendritic vaccine, were approved by the U.S. Food and Drug Administration (FDA) for the treatment of multiple cancer types. In particular, the immune checkpoint blockade agents, which are treatments that target cytotoxic T-lymphocyte associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), and programmed cell death ligand 1 (PDL-1), have gained impetus as potent anti-cancer therapies and have shown promising results across several tumor types, leading to a widespread revolution in cancer treatments and a massive shift in laboratory investigations. Since this form of therapy targets the host’s regulatory components of the immune system rather than specific oncogenic mutations or tumor cells themselves, immune checkpoint blockade has been shown to be effective across multiple cancer types. Furthermore, given that the immune system has the capacity for

long-term memory, patients who respond to this form of immunotherapy frequently have durable responses, which can protect against disease progression for months and years [4–6].

While the early results of immune checkpoint blockade have been quite promising, only about a third of patients benefit from single agent therapy, accounting for both partial and complete responses, defined by the FDA as the objective response rate (ORR). Not all tumor types are equally responsive to immune checkpoint blockade, for reasons that as of yet remain unclear. Emerging studies suggest that combination treatments adding additional immunotherapies or other modalities to immune checkpoint blockade results in ORRs that appear to be higher in many cases. However, in most cases the superiority of combination therapy over monotherapy is still not well proven. Chen and Mellman *et al.* introduced the concept of the cancer-immunity cycle, which describes the interactions and processes of how the immune system recognizes and eradicates cancer cells [7]. To ensure effective antitumor activities, a series of stepwise events, including release of cancer cell antigens, antigen presentation, priming and activation, trafficking of T cells to tumors, infiltration of T cells into tumors, recognition of cancer cells by T cells, and killing of cancer cells, must be initiated and properly expanded. This cancer-immunity cycle hypothesis provides potential opportunities to intervene, and provides rationale for combination therapy consisting of multiple immunotherapies to improve clinical responses [8]. Additionally, several other combination approaches, including with chemotherapy, anti-angiogenic therapy, and hormonal therapy, are being considered [5, 9, 10]. In this chapter, potential and established biomarkers that can be used as prognostic indicators or as identifiers of patients who will benefit more from these immune checkpoint blockade agents are reviewed. Thus, the impressive therapeutic activity of immune checkpoint blockade, seen in recent years, has solidified the science of translational biomarkers, which enable more rapid, sensible deployment of novel clinical approaches for the select groups of patients who are most likely to benefit.

## Biomarkers for anti-CTLA-4

Cytotoxic T-lymphocyte associated protein 4 (CTLA-4) is an immune checkpoint that down-regulates immune responses. CTLA-4 functions predominantly early in the cancer-immunity cycle during T cell activation and enhances the immunosuppressive activity of regulatory T cells ( $T_{reg}$  cells) [11, 12]. In contrast to PD-1 or PDL-1, which is typically thought to modulate antigen-experienced effector cells in inflammatory environments, CTLA-4 engages in the priming phase and regulates the amplitude of early activation of naïve and memory T cells [13]. Ipilimumab was the first immune checkpoint blockade agent approved by FDA, is a humanized monoclonal antibody against CTLA-4, and is indicated for advanced melanoma. However, the response rate for single-agent ipilimumab is merely 10%, and ipilimumab has several concerning mechanistic-based toxicities [14]. Common serious toxicities associated with ipilimumab are dermatitis, enterocolitis, endocrinopathies, liver abnormalities, and uveitis [15]. Therefore, it is critical to identify biomarkers that can be used to select patients who are more likely to benefit from this toxic therapy.

Several serum biomarkers, such as lactate dehydrogenase (LDH), C-reactive protein (CRP), vascular endothelial growth factor (VEGF), and soluble CD25 (sCD25), have been shown to be associated with ipilimumab treatment in patients with advanced melanoma [16–19]. Higher baseline levels of LDH and VEGF were associated with reduced ipilimumab treatment response in patients with metastatic melanoma. However, subsequent reductions in LDH, CRP, and  $T_{regs}$  as well as an increase in absolute lymphocyte count after ipilimumab treatment were significantly associated with improved overall survival (OS) and disease control rate. sCD25 acts as a decoy receptor for IL-2. While recombinant IL-2 improves efficacy of ipilimumab, sCD25 inhibits the anticancer effects of ipilimumab, and the high level of baseline sCD25 appears to confer resistance to ipilimumab [16]. However, most of these studies were small retrospective database reviews, and at this time, no confirmatory clinical trials have been done to support the routine use of these biomarkers for the selection of patients who should receive ipilimumab.

Given that ipilimumab exerts its antitumor activity through activation and increasing proliferation of T cells, serial measurements of absolute lymphocyte counts (ALC) in the blood after treatment have also been investigated as a pharmacodynamic biomarker of ipilimumab [20, 21]. After ipilimumab therapy, an ALC  $\geq 1000/\mu\text{L}$  at week seven or an increase in ALC from baseline at week twelve was significantly associated with improved OS [18, 22, 23]. Besides a simple absolute count of lymphocytes, which can be heterogeneous, CD4<sup>+</sup>ICOS<sup>+</sup> T cells, an activated T cell subset, have been used to track immune response after ipilimumab therapy as a pharmacodynamics marker. Four independent studies demonstrated that patients who had a sustained increase in CD4<sup>+</sup>ICOS<sup>+</sup> T cells over twelve weeks after ipilimumab therapy had significant improvement in OS [24–28]. This consistent finding is intriguing because ICOS (inducible T cell costimulatory) costimulation is associated with Th2 immune responses, suggesting the possibility that antibodies are involved in the clinical activity of CTLA-4 blockade [29].

Since T cells recognize processed peptides presented by host major histocompatibility complex molecules, mutations in cancers can produce unique peptides that can be recognized by T cells, termed mutated neoantigens [30]. The antigenicity of these neoantigens may affect the function of the protein, and a passenger mutation with no functional role may still generate sufficient immune responses, although the potential for immune escape based on antigen loss is still possible. However, a greater mutational load in the tumors can potentially produce more neoantigens, which will result in a larger repertoire of existing tumor-specific T cells, and less chances of antigen-loss variant escape. Given the fact that immune checkpoint blockade agents exert their activity by unleashing these preexisting tumor-specific T cells, it was initially hypothesized that tumors with higher mutational loads would respond better to this form of therapy [30]. This hypothesis was substantiated based on the early results of studies with ipilimumab, which has activity in the cancer with the highest mutational load, melanoma. In two melanoma studies of ipilimumab, patients who responded to ipilimumab had a statistically significant higher median mutation load in their

tumors compared to patients who did not respond. However, there appeared to be no distinct cutoff that can be used to identify patients who would not benefit from ipilimumab therapy [31, 32]. The inability to establish a cutoff may reflect important variations such as HLA allelic variation and immunogenicity of the putative neoantigens, both of which may limit the utility of the mutational load as a response indicator [33].

Despite years of trials and retrospective studies, to date no companion diagnostic test has been approved by the FDA to identify patients who are more likely to benefit from ipilimumab. Thus, additional translational studies of patients undergoing therapy should be designed and implemented to aid in identifying the patients most likely to respond.

### Biomarkers for anti-PD-1/PDL-1 therapies

Programmed cell death protein 1 or PD-1 (also known as PDCD1) and its ligand PD-1 ligand 1 or PDL-1 (also known as B7-H1) are key immune checkpoints that down-regulate antitumor effects of T cells in the tumor microenvironment [34, 35]. PDL-1 engages PD-1 and inhibits proliferation and cytokine production of T cells [36]. Several preclinical studies demonstrated that inhibition of the PD-1/PDL-1 interaction enhances T cell responses and augments their antitumor activities [34, 37, 38]. The potential translational biomarkers for anti-PD-1/PDL-1 can be categorized into either immune-related or genomic-related biomarkers [39].

### Immune-related biomarkers

PD-1 and PDL-1 immune checkpoint blockade agents are thought to exert their activity mainly by enhancing the antitumor activities of preformed host immune responses [40]. Thus, the amount of preexisting immune infiltrate in the tumor at baseline prior to anti-PD-1/PDL-1 treatment was one of the first translational biomarker candidates to be explored. In melanoma, higher numbers of preexisting CD8<sup>+</sup> T cells, particularly at the invasive tumor margin, have been shown to associate with tumor regression in patients treated with anti-PD-1 therapy (pembrolizumab) [40]. Comparing between responders and nonresponders, responding

patients had significantly higher numbers of CD8<sup>+</sup>, PD-1<sup>+</sup>, and PDL-1<sup>+</sup> cells at the invasive tumor margin and a more clonal T cell antigen receptor repertoire. Furthermore, patients who responded to therapy had significant increases in CD8<sup>+</sup> T cells both inside the tumors and at the invasive margins. Similar findings, in which an increase in CD8<sup>+</sup> T cell infiltration after anti-PD-1 therapy correlates with tumor regression, were also observed in another study with pembrolizumab in melanoma and nivolumab in solid tumors in a phase I study [41, 42].

Another immune-related biomarker that has received a great deal of attention is tumor cell-associated PDL-1 expression. PDL-1 is widely expressed in the tumor microenvironment not only on the tumor cells but also in subsets of immune cells, particularly macrophages, dendritic cells, and activated T, B, and NK cells as well as other nonmalignant cells, including endothelial cells as part of a physiological process to down-regulate host immune responses in inflammatory microenvironment [43–45]. The distribution of PDL-1 expression differs among tumor types. In certain type of cancers, PDL-1 is expressed on both tumor cells and immune infiltrating cells. These types of cancers include squamous cell carcinoma of the head and neck (SCCHN), melanoma, breast cancer, and renal cell carcinoma [46–50]. However, in other forms of cancers such as colorectal (CRC) and gastric cancer, PDL-1 is expressed almost exclusively on the immune-infiltrating cells but rarely on the tumor cells [51, 52].

In the initial phase I trial of nivolumab, an anti-PD-1 antibody, in 39 patients with advanced solid malignancies, 9 biopsied samples were available for PDL-1 assessment by immunohistochemistry. Among these 9 patients, 3 out of 4 patients with membranous expression of PDL-1 responded to nivolumab. Objective responses were not observed in the other 5 patients without PDL-1 expression [42]. Similar findings were subsequently observed in a larger trial of nivolumab, which demonstrated no objective response in patients with PDL-1-negative tumors. In contrast, patients with PDL-1 expression of  $\geq 5\%$  of tumor cells were twice as likely to respond compared to the overall study population [39, 53]. While PDL-1 expression can be used to identify patients who are more likely to

respond to anti-PD-1 therapy, subsequent studies have shown that objective responses could still be observed in some patients with PDL-1-negative tumors [54]. In an analysis of multiple anti-PD-1 trials, the average ORR of anti-PD-1 therapy was approximately 29% across 15 trials in various solid malignancies. Among patients with PDL-1-positive tumors, the ORR was 48% compared to 15% in PDL-1-negative tumors [55]. These findings exemplified that PDL-1 negativity cannot be used to exclude patients from anti-PD-1 therapies but rather to enrich patients who are more likely to benefit from this therapy.

Of note, while PD-1 is the actual target of anti-PD-1 therapy, expression of PD-1 does not appear to provide any additional predictive value [50]. Tumeh *et al.* demonstrated that more complex parameters, such as close proximity of PD-1<sup>+</sup> cells to PDL-1<sup>+</sup> cells, proliferation of CD8<sup>+</sup> T cells measured by Ki67 and CD8 costaining, and markers of IFN $\gamma$  signaling, provided superior predictive value compared to a single marker [40].

There are several technical difficulties and limitations of using PDL-1 expression as a biomarker for anti-PD-1/PDL-1 therapies. First, the expression of PDL-1 is variable in multiple tumor biopsies collected over time and/or from different anatomical sites in each individual patient [39]. This variable expression represents a potential pitfall of developing PDL-1 IHC as an absolute biomarker based on a single biopsied tumor specimen. Moreover, the tumors used to evaluate PDL-1 expression were collected after varied duration of treatment among multiple clinical trials. Some of the trials used tumors collected right before the initiation of therapy, and some trials used the tumors from the initial diagnosis. The tumors that were collected after the initial diagnosis, which could have been months or years before the initiation of therapy, may not have reflected the PDL-1 status at the time of therapy. Furthermore, the expression of PDL-1 is not uniform within the tumors. Focal expression of PDL-1 could be missed in small core needle biopsy specimens, resulting in false negative results [56].

### Genomic-related biomarkers

To date, no specific oncogenic mutations have been shown to associate with outcome in patients treated with anti-PD-1/PDL-1 therapy as an independent

variable. However, several aberrant oncogenic drivers and signaling pathways have been shown to associate with PDL-1 expression. PTEN mutations resulting in constitutive activation of the PI3K-AKT pathway have been shown to associate with higher PDL-1 expression in glioma cells [57]. Similar findings were observed with constitutive ALK signaling activation, which was found to associate with increased PDL-1 expression via activation of STAT3 in certain lymphomas and lung cancers [58]. Additionally, in a subset of lung adenocarcinomas, KRAS mutations were associated with increased PDL-1 expression and denser inflammation compared to wild-type tumors [59]. Nevertheless, there appeared to be no significant difference in PDL-1 expression in non-small-cell lung cancer (NSCLC) tumors with mutant EGFR and those with wild-type EGFR [60]. Furthermore, in melanoma, a previous study also demonstrated no significant difference in PDL-1 expression between BRAF-V600E mutated vs. wild-type tumors [61]. Consistent with this finding, the response to anti-PD-1 therapy appeared to be similar in patients with BRAF-V600E mutation and BRAF wild-type tumors [6, 62].

Given that genes encoding for both PDL-1 and another PD-1 ligand, PDL-2, are located on the 9p24.1 locus, translocations or amplifications of 9p24.1 locus also have been shown to increase PDL-1 and PDL-2 expression on the surface of tumors. Amplification of 9p24.1 has been observed in several tumor types, including Hodgkin lymphomas [63, 64], mantle cell lymphomas [65], gastric cancers [66], and breast cancer [67]. Up to 97% of classical Hodgkin's lymphomas have alterations of the PDL-1 and PDL-2 loci: either polysomy, copy number gain, or amplification resulting in PDL-1 overexpression. Furthermore, consistent with the known capability of virus-caused up-regulation of the PD-1/PDL-1 pathway, Epstein-Barr virus infection, which is common in Hodgkin's lymphoma, also contributes to overexpression of PDL-1. As a result of these two mechanisms, a large proportion of classical Hodgkin's lymphoma have increased PDL-1 expression [68]. Corresponding to these findings, the initial phase I study of nivolumab in 23 patients with relapsed or refractory Hodgkin's lymphoma, with the majority progressing after autologous

stem-cell transplantation and brentuximab vedotin, showed a remarkable ORR of 87%, including 17% with a complete response, 70% partial response, and 13% with stable disease [63, 64]. Similar findings were observed in a subsequent multi-center, single arm phase II trial of nivolumab in 80 patients with classical Hodgkin's lymphoma after failure of both autologous stem-cell transplantation and brentuximab vedotin. However, the expression of PDL-1 on Reed-Sternberg cells was not required and patients were enrolled regardless of their PDL-1 expression status. ORR was observed in 66.3% of patients, with 9% complete response, 58% partial response, and 23% stable disease [69]. Based on these promising results, the FDA granted the accelerated approval of nivolumab for the treatment of patients with Hodgkin's disease in this setting.

Similar to that reported with ipilimumab, mutational burden is another key factor that has been found to be associated with clinical response to anti-PD-1/PDL-1 therapies. Early studies of anti-PD-1 indicated that these agents appear to have activity across all cancers with the highest median mutation loads, namely, melanoma, NSCLC, SCCHN, bladder cancer, and gastric cancer. The ORR for anti-PD-1 in these cancer types was more than 15% across the board [53, 70, 71]. In contrast, the ORR is relatively low in cancers with low mutational loads, such as prostate and pancreatic cancers. In a small study of patients with lung cancer receiving pembrolizumab, higher mutational burden was associated with improved response to this agent [72]. Nevertheless, and much like that observed for ipilimumab, there is no clear cutoff for the number of actual mutations that can be used for the purpose of patient selection. Currently, beyond the number of mutations, there are several computational algorithms that can be used to predict the numbers of potential neoantigens. However, to date, these algorithms are still highly imperfect and at present not suitable for use for routine clinical management.

Another specific genetic subset that has been shown to associate with higher mutation burden and better response to anti-PD-1/PDL-1 is tumors with DNA mismatch repair (MMR) defects. Genes in the MMR complex are often found to be mutated, deleted, or epigenetically silenced in several

cancers, including CRC, gastric, endometrial, ampullary, duodenal, and prostate cancers. MMR-deficient genotypes account for approximately 4% of all solid tumors and can be identified by detecting microsatellite instability (MSI) or by immunohistochemical staining of MMR proteins [39]. These tumors with MMR defect have a 10- to 100-fold increase in mutational burden compared to MMR-proficient tumors. Furthermore, colon cancers with MSI exhibit several other features that predict sensitivity to anti-PD-1/PDL-1 therapy. These features include high CD8<sup>+</sup> T cell infiltration, CD4<sup>+</sup> T cells with the Th1 phenotype, high levels of PD-1, PDL-1, CTLA-4, lymphocyte activation gene (LAG3), and IFN $\gamma$ -inducible immune inhibitory metabolic enzyme (IDO1) [51, 73]. Despite a generally low rate of response in CRC patients, there was a patient with CRC who had a durable complete response in the initial phase I trial of nivolumab [42]. Subsequent analysis of this patient's tumor demonstrated an MSI<sup>hi</sup> phenotype [74]. This finding was confirmed in a larger phase II trial of pembrolizumab in patients with tumors harboring MMR defects. In this particular trial, patients with MMR-deficient and -proficient CRC were enrolled. The ORR was 40% in MMR-deficient CRC compared to 0% in MMR-proficient CRC. Similar high response rates were also observed in another cohort of patients with MMR-deficient non-CRC with an ORR of 71% [60].

Besides somatic mutations, integration of oncogenic viruses in cancer genomes represents another form of genetic alterations that can produce neoantigens. There are several human cancers that are driven by viruses, namely, Epstein-Barr virus, human papillomavirus, Merkel cell polyomavirus (MCPyV), human T-lymphotropic virus 1 (HTLV-1), Kaposi sarcoma-associated herpes virus (KSHV), hepatitis B, and hepatitis C viruses. Early studies demonstrated that these viral-associated cancers might have high response rates to anti-PD-1/PDL-1 therapies. Approximately 80% of Merkel cell carcinomas are associated with MCPyV infection, and patients with Merkel cell carcinoma often produce MCPyV T-antigen-specific T cells and antibodies [75, 76]. A high ORR of 56% was observed in a phase II trial of pembrolizumab in this group of patients, which might be indicative of activation of latent MCPyV-specific immune

effectors [77]. Similar findings were also observed in hepatocellular carcinoma, in which the ORR was 36% among hepatitis C infected patients compared to 15% in noninfected patients [78].

### Approved anti-PD-1/PDL-1 blockade agents and biomarkers in clinical use

Since 2014, several agents targeting this particular pathway have been approved or are under consideration by the FDA. Presently, three PD-1-PDL-1 targeting agents have been approved by the FDA, namely, pembrolizumab, nivolumab, and atezolizumab. Multiple other agents targeting this particular pathway are currently under clinical development. The agents targeting PD-1 currently in clinical development include pidilizumab, AMP-224, and AMP-514, as well as agents targeting its ligand, PDL-1, including BMS-936559, durvalumab, and avelumab [79].

Pembrolizumab, a humanized monoclonal IgG4 antibody against PD-1, was the first PD-1/PDL-1 targeting agent approved by the FDA. It was approved in September 2014. Pembrolizumab is currently indicated for the treatment of unresectable or metastatic melanoma patients, whose tumors express PDL-1, either as an initial treatment or subsequent treatment after progressing on ipilimumab and/or a BRAF inhibitor, the first or later line treatment of patients with metastatic NSCLC, and the treatment of patients with recurrent or metastatic SCCHN after progressing on platinum-containing chemotherapy [80–83].

Similar to pembrolizumab, nivolumab is a humanized monoclonal IgG4 antibody against PD-1. Currently, nivolumab is indicated as a single agent for the first-line treatment of patients with BRAFV600 wild-type unresectable or metastatic melanoma, metastatic NSCLC progressing after platinum-based chemotherapy, advanced renal cell carcinoma with prior antiangiogenic therapy [84], relapsed Hodgkin lymphoma after autologous hematopoietic stem cell transplantation and posttransplantation brentuximab vedotin, and recurrent or metastatic SCCHN progressing after platinum-based therapy [64, 85–89]. In addition, nivolumab is also indicated in combination with ipilimumab in unresectable or metastatic melanoma patients with BRAF wild-type [90, 91].

**Table 1.1** Summary of approved biomarkers for anti-PD-1/PDL-1 blockade agents in clinical use.

Assay	Agent	Disease Setting	Cutoff	Reference
<b>PDL-1 IHC 22C3 PharmDx assay</b>	PDL-1 IHC 22C3 PharmDx assay	1st-line NSCLC without EGFR or ALK mutation	TPS $\geq$ 50%	82
		For patient selection $\geq$ 2nd-line NSCLC	TPS $\geq$ 1%	60
<b>PDL1 IHC 28-8 PharmDx assay</b>	Nivolumab	Nonsquamous NSCLC For prognostic purpose	TPS $\geq$ 1%	89

Note: TPS = tumor proportion score.

In contrast to pembrolizumab and nivolumab, atezolizumab is a humanized monoclonal IgG1 antibody against PDL-1. Atezolizumab is indicated for the treatment of patients with locally advanced or metastatic urothelial carcinoma progressing after platinum-based chemotherapy [92] and patients with metastatic NSCLC progressing after platinum-based chemotherapy [93].

At present, two established biomarkers are currently in routine clinical use. They are the PDL-1 IHC 22C3 pharmDx assay for pembrolizumab in NSCLC and the PDL-1 IHC 28-8 pharmDx assay for nivolumab in nonsquamous NSCLC and melanoma. Upon the approval of pembrolizumab in NSCLC, the FDA also simultaneously approved the companion diagnostic test, PDL-1 IHC 22C3 pharmDx assay, to guide patient selection. PDL-1 IHC 22C3 pharmDx is a qualitative immunohistochemical assay using mouse monoclonal anti-PDL-1 clone 22C3 in formalin-fixed, paraffin-embedded samples. Tumor proportion score (TPS) is used to determine the expression level of PDL-1. PDL-1 is considered positive if TPS  $\geq$  1% and high PDL-1 expression is defined as TPS  $\geq$  50%. Currently, pembrolizumab has two indications in metastatic NSCLC, including the first-line therapy for NSCLC patients whose tumors have high PDL-1 expression (TPS  $\geq$  50%) and no EGFR or ALK genomic aberrations [82]. This approval was based on a large phase II trial of pembrolizumab in patients with squamous and nonsquamous NSCLC, which demonstrated significantly higher ORR, improved PFS, and OS in patients with tumors expressed PDL-1  $\geq$  50% [60]. The second indication includes the second or later line of therapy in NSCLC patients progressing on platinum-based chemotherapy. In this

indication, the cutoff for TPS is lower than the first indication at  $\geq$  1% rather than  $\geq$  50%. This lower cutoff may be due to enhanced sensitivity to immune checkpoint blockade agents among patients with platinum resistance. In contrast, PDL-1 IHC 28-8 pharmDx for nivolumab in NSCLC and melanoma was approved as a complementary companion diagnostic test rather than a required test for patient selection. In two phase III trials of nivolumab, NSCLC patients whose tumors expressed PDL-1  $\geq$  1% using PDL-1 IHC 28-8 pharmDx assay had improved OS, but only in the nonsquamous NSCLC group [88, 89]. These assays in current clinical use are summarized in Table 1.1.

## Conclusion

Immunotherapy, particularly with immune checkpoint blockade, represents a revolutionary paradigm shift in cancer treatment. By enhancing endogenous host immune responses, rather than specifically targeting particular aberrant signaling pathways intrinsic to the tumor cell, this form of treatment has proven to be effective across multiple tumor types. Nonetheless, the response to immunotherapy is not universal and specific translational biomarkers are needed to identify patients who are more likely to benefit from this therapy. To date, there are only two PDL-1 immunohistochemistry assays that are approved by the FDA and are currently in clinical use. However, as our understanding of the interplay between immune system and tumor microenvironment grows, novel mechanistic-based biomarkers and combination therapy will emerge to improve patient selection for this form of therapy.

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# Monoclonal Antibody Therapy

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## Introduction

Monoclonal antibody (mAb)-based therapy is one of the most important and successful therapeutic strategies used for treating patients with solid tumors and hematologic malignancies. The origins of this treatment modality began with the observation of antigen expression by tumor cells made using serological techniques in the 1960s [1]. These antigens were thought to be “targets” that could be addressed therapeutically. The development of hybridoma technology in 1975, whereby mice were immunized against a specific epitope on an antigen, led to the first generation of murine antibodies targeting surface antigens [2]. Subsequently, approaches were developed to humanize antibodies, which allowed for the creation of mAbs not recognized as foreign by the human immune system [3, 4]. This represented a critical step in advancing mAbs in the clinic. In the following decades, serologic, genomic, proteomic, and bioinformatics techniques were used to identify numerous cell surface antigens that are mutated, overexpressed, or selectively expressed in tumor tissues versus normal tissues. Concomitantly, additional technologies to generate human antibodies, including use of transgenic mice, phage display

techniques, and innovative antibody engineering approaches, as well as the development of large-scale production techniques, allowed for the transition of mAb therapy from the laboratory to widespread clinical use [5–8].

This chapter summarizes the mechanisms of action of mAbs, characteristics of ideal tumor antigens to serve as antibody targets, clinical development of mAb therapy, and mechanisms of resistance. We focus on mAbs targeting antigens involved in cancer cell proliferation and survival. Monoclonal antibodies that have been developed to either activate or antagonize immunologic pathways are discussed in a subsequent chapter of this book. Similarly, mAbs that have been used in the construction of chimeric antigen receptor T cells are the focus of another chapter. Finally, mAbs that target peptide-major histocompatibility complexes, referred to as TCR mimics, have also been developed and are the subject of another chapter.

## Mechanisms of action

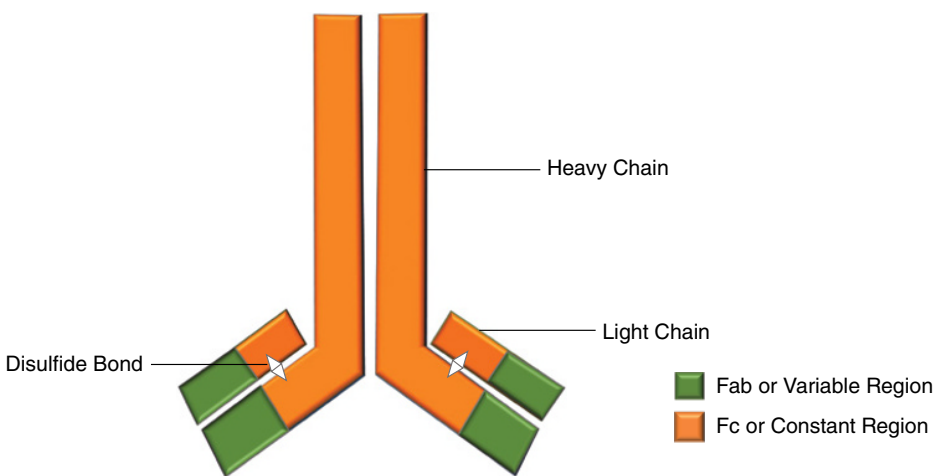
Antibodies, which are secreted by B cells, are key components of the adaptive immune system capable of recognizing antigens with high specificity. They

share similar basic structural units that include two large heavy chains and two small light chains. These structural units are organized into two distinct regions, the Fab variable region, which recognizes and binds specific antigens, and the Fc region, which is capable of interacting with specific Fc receptors (Figure 2.1).

The mechanisms by which antibodies can kill tumor cells can be categorized broadly as direct tumor cell killing and immune-mediated tumor cell killing. Direct tumor cell killing can be accomplished by an agonistic mAb binding to a cell surface receptor and inducing apoptosis. In addition, antagonistic mAbs can bind to a cell surface receptor blocking dimerization, kinase activation, and downstream signaling. This in turn leads to inhibition of cell growth and induction of apoptosis. Inhibition of cell signaling is one mechanism by which the mAbs cetuximab and trastuzumab work [9, 10]. Cetuximab is a mAb that targets the extracellular domain of the epidermal growth factor receptor (EGFR). It is approved by the U.S. Food and Drug Administration (FDA) for the treatment of KRAS wild-type, EGFR-expressing metastatic colorectal cancer and recurrent or metastatic head and neck cancer. The approval in metastatic colorectal cancer was based on tumor samples from patients enrolled in the CRYSTAL trial and two supporting studies analyzed retrospectively [11–13]. In patients with KRAS wild-type

tumors, the addition of cetuximab to chemotherapy or best supportive care resulted in improvements in overall survival, progression-free survival, and overall response rate. There was no benefit in patients with KRAS mutant tumors. The initial approval in head and neck cancer was for use in combination with radiation in patients with locally or regionally advanced squamous cell carcinoma or as a single agent for patients with recurrent or metastatic disease, for whom prior platinum-based therapy had failed. This approval was based on a significant improvement in overall survival and duration of locoregional disease control when cetuximab was added versus radiation therapy alone [14]. It was subsequently approved for use in combination with platinum-based therapy plus 5-fluorouracil for first-line treatment of patients with recurrent locoregional and/or metastatic disease. This approval was based on the results of a multicenter study that enrolled patients with metastatic or locally recurrent head and neck cancer not suitable for potentially curative treatment with surgery or radiation [15]. The addition of cetuximab to platinum-based therapy plus 5-fluorouracil resulted in significantly improved progression-free and overall survival [16].

Trastuzumab is a mAb that targets the extracellular portion of HER2. It received initial FDA approval in 1998 for the treatment of metastatic breast cancer that overexpresses the HER2 protein.



**Figure 2.1** General antibody structure. The basic structural units of an antibody are organized into two distinct regions, which include two large heavy chains and two small light

chains linked by disulfide bonds. The Fab variable region (green) recognizes and binds specific antigens and the Fc region (orange) interacts with specific Fc receptors.

Approval was based on data from a phase III trial that showed that the addition of trastuzumab to chemotherapy in heavily pretreated patients improved response rates and extended time to disease progression [17]. Trastuzumab is now routinely used in the treatment of HER2-positive breast cancer in the metastatic, adjuvant, and neo-adjuvant settings.

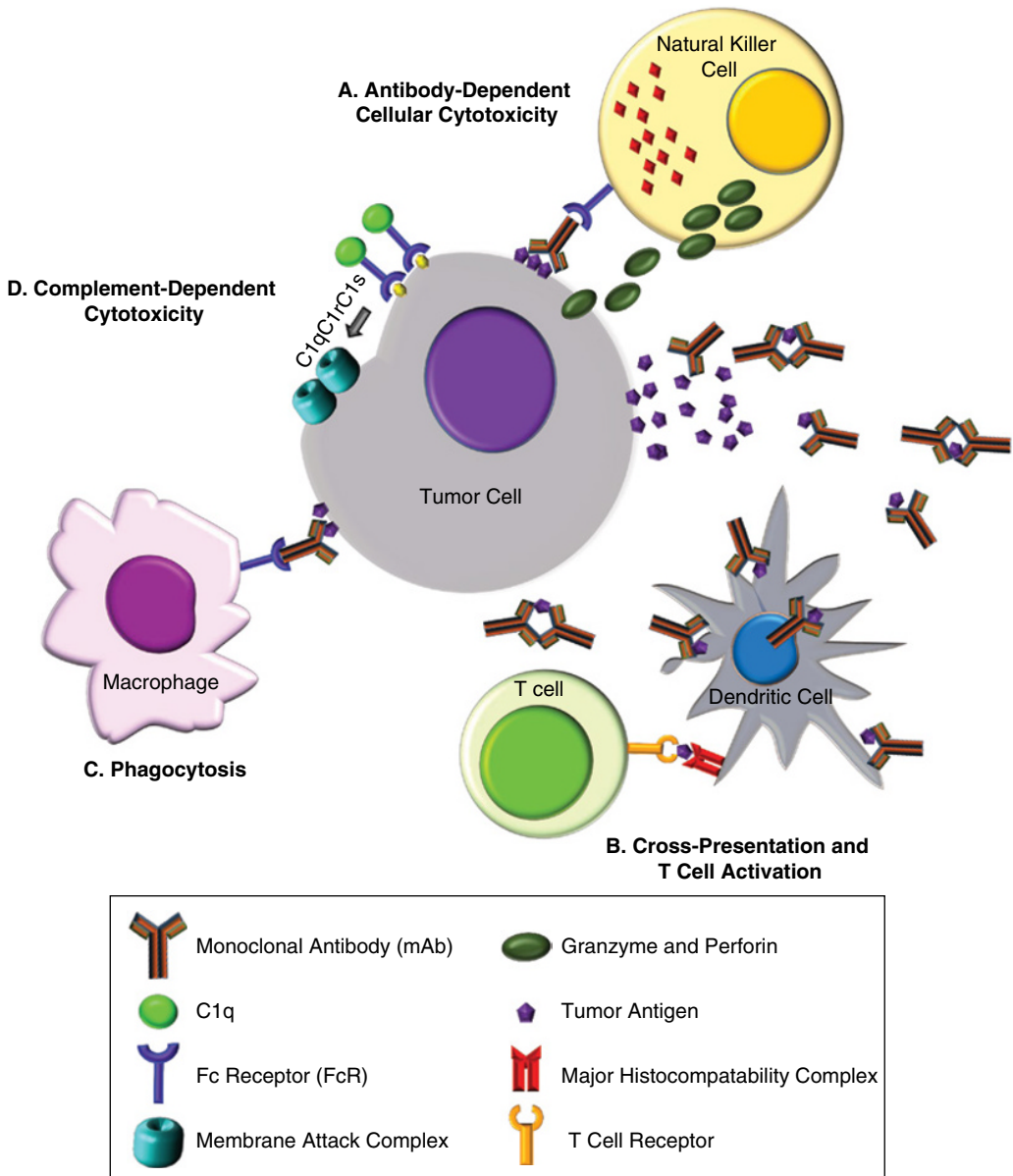
In addition to these direct tumor cell killing mechanisms of action of “naked” mAbs, antibodies can be conjugated to cytotoxic drugs and used to deliver these drugs directly to the tumor site. The first antibody drug conjugate (ADC) to receive FDA approval was gemtuzumab ozogamicin, an ADC that links the cytotoxic agent calicheamicin to an anti-CD33 antibody. It was approved for use in 2000 for patients at least 60 years of age with CD33-positive acute myeloid leukemia. This was based on a single-arm phase II trial showing an overall response rate of 26% in these patients [18]. This approval was conditioned on completion of a subsequent phase III clinical trial; unfortunately, that confirmatory study was negative, leading to withdrawal of the approval in 2010 [19]. More recently, the results of additional randomized studies adding gemtuzumab ozogamicin to various induction regimens have suggested improved overall survival in specific patients with acute myeloid leukemia with intermediate cytogenetic characteristics, leading to renewed interest in this agent [20, 21, 22].

The second ADC to receive regulatory approval was brentuximab vedotin, which combines an anti-CD30 antibody conjugated to monomethyl auristatin E, a microtubule disrupting agent. Brentuximab vedotin is approved for use in treating patients with relapsed or refractory Hodgkin's lymphoma based on a single-arm, multicenter study that enrolled patients who relapsed after autologous stem cell transplant [23]. In this study, brentuximab vedotin showed a 75% objective response rate with a median duration of response of 20.5 months in patients with relapsed or refractory Hodgkin's lymphoma [23]. A subsequent randomized phase III trial (AETHERA) showed that consolidation with brentuximab vedotin after autologous stem cell transplant improved progression-free survival in Hodgkin's lymphoma patients with high-risk factors for relapse or disease progression after transplantation [24]. The approval for

systemic anaplastic large-cell lymphoma was based on a single-arm, phase II, multicenter study that demonstrated an 86% objective response rate in patients with CD30-positive systemic anaplastic large cell lymphoma who had previously received multiagent chemotherapy [25].

One early ADC used routinely in the clinic is trastuzumab emtansine (T-DM1), which includes the mAb trastuzumab linked to emtansine (DM1), a highly potent microtubule polymerase inhibitor. T-DM1 was approved for use in HER2-positive breast cancer based on the results of the EMILIA clinical trial that enrolled women with advanced HER2-positive breast cancer who were resistant to trastuzumab alone [22]. T-DM1 improved median overall survival compared to the combination of the HER2 tyrosine kinase inhibitor lapatinib and capecitabine. Many additional ADCs are currently being evaluated in clinical trials.

Other mechanisms by which antibodies can kill tumor cells are categorized immune-mediated tumor cell killing. Much of immune-mediated tumor cell killing is due to immune cell engagement with the Fc portion of the mAb (Figure 2.2) [26]. Specifically, Fc receptors (FcR) on natural killer (NK) cells binding to the Fc portion of a mAb engaged with a surface receptor can lead to antibody-dependent cellular cytotoxicity (ADCC). ADCC involves the FcR on an NK cell (FcRIII; CD16) recognizing cell-bound antibodies and cross-linking the antibodies, which leads to the release of granzyme and perforin into the synapse, promoting apoptosis [27, 28]. Although FcR genotypes are not completely predictive of response to therapy, there is evidence that FcR polymorphisms enhance response rates for rituximab in follicular lymphoma, cetuximab in colorectal cancer, and trastuzumab in breast cancer [29–31]. ADCC can also be mediated by macrophages. In addition, macrophage binding to antibodies coating the cell surface can promote phagocytosis. Furthermore, tumor antigen-targeted mAbs can trigger an antigen-specific T cell response via a process known as cross-presentation. Briefly, the mAb-coated tumor antigens released by dying cells are taken up by dendritic cells, which process and present the antigen to T cells [32, 33]. These activated T cells are then able to recognize the antigen expressed by the tumor cells complexed with an MHC molecule. Efforts



**Figure 2.2** Immune-mediated mechanisms of action for monoclonal antibodies. **A.** Antibody-dependent cellular cytotoxicity. Fc gamma receptors on the surface of immune effector cells such as natural killer cells bind to the Fc region of antibodies interacting with tumor cell surface antigens, leading to the release of granzyme and perforin that mediate tumor cell killing. **B.** Cross-presentation and T cell activation. Tumor antigens released by dying

tumor cells and coated with mAbs are taken up by dendritic cells, processed, and presented to T cells. **C.** Phagocytosis. Macrophage binding to mAb via its FcR mediates phagocytosis. **D.** Complement-dependent cytotoxicity. C1q complement proteins bind to antibodies interacting with tumor cell surface antigens, triggering the complement cascade. Membrane attack complexes form on the surface of the cell, leading to cell death.

have been made to modify the Fc region of mAbs to increase their ability to interact with the immune system. Approaches used include changing the amino acid sequence or altering the

glycosylation pattern in a manner that enhances interaction with the FcR on effector cells [34, 35]. Obinutuzumab is an example of a glycomodified mAb that was shown to be safe and effective,

leading to FDA approval for the treatment of chronic lymphocytic leukemia [36, 37].

Finally, mAbs can also induce complement-dependent cytotoxicity (CDC) [38, 39]. The exact role of CDC in the clinical response to mAb therapy is unknown, as the effects of CDC are very rapid, whereas the response to mAb-based therapy occurs over weeks. It has been suggested that CDC may contribute most to the efficacy of mAbs in hematologic malignancies, where target cells are exposed to complement proteins in the circulation [40]. Consistent with this, it is generally accepted that CDC is limited as a mechanism of action for mAb treatment of solid tumors [41]. It has also been suggested that CDC plays a role in some of the adverse effects observed with mAb therapy [42].

There is evidence of interactions between various mechanisms of action of a single mAb. These interactions can be synergistic or antagonistic and can impact the antitumor effects of the mAb. For example, the effects of complement fixation are complex [43]. The anti-CD20 mAb rituximab can promote rapid target cell killing via CDC. However, complement fixation can also block the interaction between the mAb and the FcR on NK cells, thereby decreasing ADCC [44]. The mAb, trastuzumab, also has multiple described mechanisms of action, including inhibition of cell signaling by preventing dimerization and promoting receptor internalization, which inhibits kinase activation [10]. ADCC is another described mechanism of action [32]. ADCC requires that the mAb complexed with the target antigen remain on the cell surface for recognition by the FcR of NK cells. Therefore, the effect of trastuzumab promoting receptor internalization could decrease the extent of ADCC.

## Tumor antigens

The efficacy and safety of therapeutic mAbs depends on the target antigen. An ideal target antigen is abundant and has consistent expression by malignant cells [45]. In addition, there should be limited tendency for antigen-negative tumor variants to emerge [41]. If the desired mechanism of action is ADCC or CDC, then it is preferable that the mAb-antigen complex not be internalized rapidly to allow maximal exposure of the Fc region of the mAb to immune effector cells or complement

proteins [45]. In contrast, for mAbs for which cell surface receptor internalization is a primary mechanism of action or for conjugated mAbs that are designed to deliver a payload into the cancer cell, then rapid, efficient internalization is preferable [45]. Another consideration, specifically when considering mAbs for the treatment of solid tumors, is whether the antigen is secreted. Secreted antigens bind the mAb in the circulation, thereby limiting the availability of mAb for tumor binding.

Several different categories of tumor antigens that can be recognized by therapeutic mAbs exist (Table 2.1). Antigens involved in growth and differentiation signaling typically are growth factors or growth factor receptors, including EGFR, HER2, ERBB3, MET, insulin-like growth factor 1 receptor (IGF1R), ephrin receptor A3 (EPHA3), tumor necrosis factor (TNF)-related apoptosis-inducing ligand receptor 1 (TRAILR1), TRAILR2, and receptor activator of nuclear factor- $\kappa$ B ligand (RANKL). Antigens involved in angiogenesis include growth factors and proteins that support the formation of microvasculature, including vascular endothelial growth factor (VEGF), VEGF receptor (VEGFR), integrin  $\alpha$ 5 $\beta$ 1, and  $\alpha$ V $\beta$ 3. Stromal and extracellular matrix antigens are critical in that they provide structural support for the tumor. Stromal and extracellular matrix antigens that can be targeted include fibroblast activation protein and tenascin. Glycoproteins can be found on the surface of solid tumor cells as well as malignant hematologic cells. Examples of glycoproteins expressed by solid tumors include epithelial cell adhesion molecule (EPCAM), carcinoembryonic antigen (CEA), mucins, prostate-specific membrane antigen (PSMA), and folate-binding protein (FBP). Hematopoietic differentiation antigens are typically associated with cluster of differentiation (CD) groupings and include CD20, CD30, CD33, and CD52.

## Clinical development of monoclonal antibodies

The initial step in developing mAbs for clinical use involves *in vitro* and *in vivo* preclinical studies [8, 45, 46]. First, the physical and chemical properties of the antibody must be characterized. In addition, detailed analyses must be performed to determine

**Table 2.1** Categories of tumor antigens recognized by therapeutic mAbs.

Type of Antigen	Target	Tumors Expressing Antigens	Therapeutic Monoclonal Antibody	Type	U.S. FDA-Approved*
<b>Carbohydrate</b>	Lewis-Y	Breast, colon, lung, and prostate	Hu3S193 and IgN311	Naked antibody	No
	<b>Epidermal growth factor receptor</b>	EGFR	Colorectal carcinoma	Panitumumab	Naked antibody
<b>Glycolipid</b>	GD2	Neuroblastoma	Dinutuximab	Naked antibody	Yes
	GD2	Neuroblastoma and osteosarcoma	Hu3F8 – Y – mAbs[[check]]	Naked antibody	Yes
<b>Glycoproteins expressed by solid tumors</b>	EPCAM	Breast, colon, and lung	Adecatumumab	Naked antibody	No
	CA IX	Renal cell carcinoma	Girentuximab	Naked antibody	Yes
	gpA33	Colorectal carcinoma	huA33	Naked antibody	No
	PSMA	Prostate carcinoma	J591	Naked antibody	No
	CEA	Breast, colon, and lung	Labetuzumab	Naked antibody	No
	FBP	Ovarian	MOv18 and MORAb-003	Naked antibody	No
	<b>Growth and differentiation signaling</b>	HER2	Breast, colon, lung, ovarian, and prostate	Ado-trastuzumab emtansine	Naked antibody
VEGF		Lung, colon, and kidney	Bevacizumab	Naked antibody	Yes
EGFR		Glioma, lung, breast, colon, and head and neck tumors	Cetuximab	Naked antibody	Yes
IGF1R		Breast, colorectal, and pancreatic	Dalotuzumab	Naked antibody	No
CD38		Myeloma	Daratumumab	Naked antibody	Yes
RANKL		Breast and bone	Denosumab	Naked antibody	Yes
MET		Non-small cell lung cancer, acute myeloid leukemia, and head and neck cancer	Ficlatuzumab	Naked antibody	No
EPHA3		Acute myeloid leukemia, myelodysplastic syndromes, and myelofibrosis	Ifabotuzumab	Naked antibody	No
TRAILR1		Cervical	Mapatumumab	Naked antibody	No
EGFR		Lung	Necitumumab	Naked antibody	Yes
EGFR		Pancreatic cancer	Nimotuzumab	Naked antibody	Yes
PDGFR $\alpha$		Sarcoma	Olaratumab	Naked antibody	Yes
HER2		Breast, colon, long, ovarian, and prostate	Pertuzumab	Naked antibody	Yes
ERBB3		Breast, non-small cell lung cancer, and ovarian	Seribantumab	Naked antibody	Yes
ERBB2		Breast, colon, lung, ovarian, and prostate	Trastuzumab	Naked antibody	Yes

(Continued)

Table 2.1 (Continued)

Type of Antigen	Target	Tumors Expressing Antigens	Therapeutic Monoclonal Antibody	Type	U.S. FDA-Approved*
<b>Hematopoietic differentiation antigen</b>	CD52	Leukemia	Alemtuzumab	Naked antibody	Yes
	CD19	Leukemia	Blinatumomab	Bispecific antibody	Yes
	CD3				
	CD30	Hodgkin's lymphoma	Brentuximab vedotin	Conjugated antibody	Yes
	CD33	Leukemia	Gemtuzumab ozogamicin	Conjugated antibody	Yes
	CD20	Lymphoma	<sup>90</sup> Y-labeled ibritumomab tiuxetan	Conjugated antibody	Yes
	CD20	Leukemia	Obinutuzumab	Naked antibody	Yes
	CD20	Leukemia	Ofatumumab	Naked antibody	Yes
	VEGFR-2	Gastric and adenocarcinoma	Ramucirumab	Naked antibody	Yes
	CD20	Non-Hodgkin's lymphoma	Rituximab	Naked antibody	Yes
	CD20	Lymphoma	<sup>125</sup> I-labeled tositumomab	Conjugated antibody	Yes
<b>Immunostimulatory</b>	SLAMF7 receptor	Myeloma	Elotuzumab	Naked antibody	Yes

\*As of August 2, 2017.

Note: Monoclonal antibodies targeting immune-checkpoint inhibitors are discussed in Chapter 4. Checkpoint blockades are therefore not included in this table.