Chapter 3
Monoclonal Antibodies Approved for Cancer Therapy

For many years, the mainstay of cancer therapy was the range of so-called chemotherapeutic drugs, small cytotoxic molecules demonstrating nonspecific toxicity, used together with radiation therapy in a rather crude and nondiscriminatory attempt to destroy rapidly dividing malignant cells. This approach often carried with it therapeutic as well as safety limitations since the broad strategy of killing rapidly dividing cells also adversely affected some other normal, healthy cells such as mucosal lining cells and those in the bone marrow and hair follicles. The consequences of this nonspecific therapeutic approach producing an array of toxic effects for patients were often poor tolerance of chemo- and radiation therapies, poor patient compliance, delays and interruptions to therapy, discontinuation of therapy, and ultimately poor survival outcomes. In addition, tumors can become resistant to chemotherapy and radiation treatments, and these resistances may extend to drugs not yet administered to the patient. Effective targeted therapies for cancers without concurrent toxicities have long been desired by oncologists, and from their earliest examples, mAbs specifically directed to selected antigens on many different tumors appeared to offer great promise for both clinicians and patients. Here we cover those mAbs developed as targeted antineoplastic agents that have gone on to receive regulatory approval for specific cancer indications. In keeping with the aims of this monograph, emphasis is placed on the nature of the antibodies, the strategies underlying their use, their mechanisms of action, and their safety issues.

Of the 50 monoclonal antibodies (mAbs) currently approved by the FDA and/or EMA (as at June 2016), half are indicated for the treatment of hematologic, cutaneous, or solid tumor malignancies. This is a reflection of the always-pressing need to make headway against the many different cancers that affect and kill humans of all ages; the knowledge that both innate and adaptive immunities can contribute to the recognition and elimination of malignant cells; the potential for success promised by the relatively nontoxic, specific, and targeted approach

B.A. Baldo, *Safety of Biologics Therapy*, DOI 10.1007/978-3-319-30472-4_3

offered by mAbs; and the potentially large commercial rewards that might follow development of a successful therapy. A combination of different modes of action has been demonstrated or proposed for the approved mAbs used in cancer treatments. Molecular mechanisms involved are predominately a direct cytotoxic action against cancer cells, an inhibitory effect on promitogenic signaling pathways, and immunomodulatory effects leading to the indirect destruction of cancer cells. For a small number of approved mAbs and a larger number in the development pipeline, a direct cytotoxic action is effected by a so-called antibody-drug conjugate (ADC) (Chap. 2, section "Antibody-Drug Conjugates"), whereby cell killing is imparted by an attached bioactive payload of a potentially lethal toxin, drug, cytokine, or radionuclide. Examples are ibritumomab tiuxetan (section "Ibritumomab Tiuxetan"), brentuximab vedotin (section "Brentuximab Vedotin"), and trastuzumab emtansine (section "Ado-trastuzumab Emtansine"). Examples of cell-destructive immunomodulatory effects include antibody-dependent cell cytotoxicity (ADCC) and modulation of immune checkpoints by the targeting of inhibitory pathways regulating signaling between T cells and antigen-presenting cells. Antibodies that induce ADCC and those that modulate immune checkpoints, namely, mAbs that target cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1), are discussed later in this chapter.

As well as the importance of understanding the mechanisms of action of mAbs for comprehending tumor recognition and the processes involved in cell destruction, the identification of biomarkers that predict patient's responses is important to help select the patients most likely to benefit from treatment with a particular mAb. Cancer patients are therefore often tested for specific biomarkers known to be predictive of a beneficial or poor response to a targeted agent. Examples are the selection of patients for trastuzumab therapy by testing for HER2 gene amplification and identification of patients unlikely to respond to cetuximab by detection of a mutation in codon 12 of the *KRAS* (Kirsten rat sarcoma 2 viral oncogene homolog) gene. Unfortunately, not all patients predicted to respond on the basis of a biomarker result actually do so. For example, only 25–30 % of breast cancer patients who are HER2 amplification positive respond to trastuzumab. This highlights the need for continued efforts to identify additional biomarkers that select those cancer patients most likely to benefit from therapy with a particular mAb.

Approved Monoclonal Antibodies for Cancer Therapy

Of the 50 mAbs currently approved by the FDA and/or EMA (Table 2.1), Table 3.1 lists 24 different antibodies with regulatory approval for cancer therapy indications together with their targets, warnings, precautions, risks, and safety concerns associated with their use and their recorded common and serious adverse events. Approval of atezolizumab was too recent for inclusion in the table. Extra detail on the safety of each mAb is set out in the following summaries.

Table 3.1 Adverse events associated with approved monoclonal antibodies used for cancer therapy (as at June 2016)

Monoclonal antibody [a] INN and trade names[b]	Target[c]	Warnings, precautions, risks, and safety concerns	Other adverse events,[d] serious and common
Catumaxomab[e] (Removab®)	EpCAM/CD3[f]	Monitor and evaluate for: CRS, SIRS, HAMA/HARA, GI hemorrhage, hepatic disorders, abdominal infection, ileus/intestinal perforation, decreased lymphocyte count	*Systemic*: cytopenias, hepatotoxicity, abdominal disorders, pyrexia, chills, nausea, vomiting, infections, immunogenicity, dyspnea. *Cutaneous*: rash, erythema, allergic dermatitis, hyperhidrosis, pruritus
Blinatumomab[g] (Blincyto®)	CD19/CD3[h] epsilon	*Boxed warning*: CRS, neurological toxicities. *Others*: infections, neutropenia and febrile neutropenia, TLS, elevated liver enzymes, leukoencephalopathy	*Systemic*: HLH, pyrexia, lymphopenia, leukopenia, chills, headache, CNS symptoms (disorientation, confusion, tremor, speech disorders), hypokalemia, pneumonia, sepsis, constipation, peripheral edema. *Cutaneous*: rash
Ibritumomab tiuxetan[i] (Zevalin®)	CD20[j]	*Boxed warning*: serious IR, severe cytopenias,[k] severe mucocutaneous and cutaneous reactions. *Others*: MDS and AML, extravasation, immunization	*Systemic*: infections, asthenia, musculoskeletal symptoms, GI, hemorrhage, hypersensitivity. *Cutaneous*: exfoliative dermatitis, bullous dermatitis, EM, SJS, TEN
Obinutuzumab[l] (Gazyva®, Gazyvaro®)	CD20	*Boxed warning*: hepatitis B virus reactivation, PML. *Others*: IR, TLS, neutropenia, thrombocytopenia, infections, immunization	*Systemic*: anemia, pyrexia, febrile neutropenia, neutropenia, sepsis, pneumonia, thrombocytopenia, arthralgia, respiratory and urinary infections, GI, decreased appetite, sinusitis musculoskeletal disorders, headache, cough
Ofatumumab (Arzerra®)	CD20	IR, hepatitis B virus reactivation, PML, cytopenias, intestinal obstruction, immunization	*Systemic*: infections, pneumonia, neutropenia, pyrexia, dyspnea, cough, diarrhea, URTI, nausea, fatigue, bronchitis. *Cutaneous*: rash, urticaria, hyperhidrosis
Rituximab (MabThera®, Rituxan®)	CD20	*Boxed warning*: fatal IRs, TLS, potentially fatal PML, and severe mucocutaneous reactions. *Others*: hepatitis B virus reactivation, infections, cardiac arrhythmias, bowel obstruction and perforation	*Systemic*: pulmonary events, renal toxicity, neutropenias, serum sickness, anaphylaxis, fever, lymphopenia, chills, asthenia. *Cutaneous*: paraneoplastic pemphigus, lichenoid dermatitis, vesiculobullous dermatitis, SJS, TEN

(continued)

Table 3.1 (continued)

Monoclonal antibody [a] INN and trade names[b]	Target[c]	Warnings, precautions, risks, and safety concerns	Other adverse events,[d] serious and common
Brentuximab vedotin[m] (Adcetris®)	CD30[n]	*Boxed warning*: PML. *Others*: peripheral neuropathy, IR and anaphylaxis, neutropenia, infections, fetal harm, hepatotoxicity, TLS, SJS	*Systemic*: cytopenias, immunogenicity, URTI, pyrexia, nausea, vomiting, fatigue, cough, anaphylaxis. *Cutaneous*: rash, pruritus, SJS, alopecia
Alemtuzumab[o] (Campath®, MabCampath®)	CD52[p]	*Boxed warning*: cytopenias, IR, immunosuppression/ infections[q]. *Others*: immunization	*Systemic*: pulmonary events, immunogenicity, cardiac events, diarrhea, nausea, emesis, insomnia. *Cutaneous*: rash, urticaria, erythema, pruritus
Cetuximab (Erbitux®)	EGFR	*Boxed warning*: serious IR and cardiopulmonary arrest. *Others*: pulmonary toxicity, dermatologic toxicity, hypomagnesemia	*Systemic*: electrolyte imbalance, infection, GI, anaphylaxis, headache, diarrhea. *Cutaneous*: acneiform rash, nail changes, xeroderma, paronychial inflammation, pruritus
Panitumumab[r] (Vectibix®)	EGFR	*Boxed warning*: dermatologic toxicity, IR. *Others*: increased toxicity with bevacizumab and chemotherapy, pulmonary toxicities, electrolyte depletion, ocular events	*Systemic*: pulmonary events,[s] pulmonary embolism, GI, fatigue, abdominal pain, hypomagnesemia. *Cutaneous*[t]: rash, dermatitis "acneiform," erythema, exfoliation, paronychia, skin fissures, photosensitivity, xerosis, pruritus
Necitumumab (Portrazza®)	EGFR	*Boxed warning*: cardiopulmonary arrest, hypomagnesemia. *Others*: venous and arterial thromboembolic events, infusion reactions, dermatologic toxicities, ↑ toxicity and mortality in patients with non-squamous NSCLC, embryofetal toxicity	*Systemic*: vomiting, diarrhea. *Cutaneous*: rash, dermatitis acneiform
Bevacizumab (Avastin®)	VEGF	*Boxed warning*: GI perforation, surgery/wound healing, hemorrhage. *Others*: non-GI fistula, RPLS, IR, CHF, hypertension, arterial/venous thromboembolism, eye disorders, proteinuria, neutropenia/infections, ONJ	*Systemic*: pulmonary events, epistaxis, headache, rectal hemorrhage, dry skin, necrotizing fasciitis, taste alteration, lacrimation disorder, ovarian failure *Cutaneous*: exfoliative dermatitis, alopecia

Ramucirumab (Cyramza®)	VEGFR-2	*Boxed warning*: hemorrhage, GI perforation, impaired wound healing. *Others*: arterial thromboembolic events, IR, RPLS, hypertension, deterioration in patients with cirrhosis, proteinuria including nephrotic syndrome, thyroid dysfunction, embryofetal risk	*Systemic*: hypertension, diarrhea, headache, hyponatremia, neutropenia, epistaxis, stomatitis, immunogenicity[u]
Pertuzumab (Perjeta®)	HER2	*Boxed warning*: cardiomyopathy, embryofetal toxicity. *Others*: IR, hypersensitivity/anaphylaxis	*Systemic*: neutropenias, LVD, peripheral neuropathy, fatigue, GI, asthenia. *Cutaneous*: rash, paronychia, pruritus, alopecia, PPE (in combination therapy)
Trastuzumab (Herceptin®)	HER2	*Boxed warning*: cardiomyopathy,[v] IR, pulmonary toxicity. *Others*: exacerbation of chemotherapy-induced neutropenia, embryofetal toxicity	*Systemic*: neutropenia,[w] anemia, thrombocytopenia, pulmonary events, LVD[v], GI, chills, fever, URTI, anaphylaxis/angioedema, headache, cough, stomatitis, mucosal inflammation. *Cutaneous*: rash, nail disorders, pruritus
Ado-trastuzumab emtansine[x] (Kadcyla®)	HER2	*Boxed warning*: hepatotoxicity, cardiotoxicity, embryofetal toxicity. *Others*: IR, pulmonary toxicity, extravasation, hemorrhage, thrombocytopenia, neurotoxicity	*Systemic*: pulmonary events, fetal harm, LVD, hypersensitivity/IR, nausea, fatigue, anemia, headache, musculoskeletal pain, increased transaminases, constipation. *Cutaneous*: rash, pruritus
Denosumab (Xgeva®, Prolia®)	RANKL	Hypocalcemia, osteonecrosis of the jaw, embryofetal toxicity	*Systemic*: osteomyelitis, hypophosphatemia, dyspnea, fatigue/asthenia, back pain, nausea, extremity pain. *Cutaneous*: rash, pruritus, dermatitis, eczema
Ipilimumab (Yervoy®)	CTLA-4[y]	*Boxed warning*: immune-mediated adverse reactions[z]	*Systemic*: diarrhea, fatigue, colitis. *Cutaneous*: rash, pruritus, dermatitis
Siltuximab (Sylvant®)	IL-6	Not for patients with severe infections or live vaccines, IR, cautionary use in patients with GI perforation risk	*Systemic*: hyperuricemia, URTI, increased weight. *Cutaneous*: rash, pruritus

(continued)

Table 3.1 (continued)

Monoclonal antibody [a] INN and trade names[b]	Target[c]	Warnings, precautions, risks, and safety concerns	Other adverse events,[d] serious and common
Nivolumab (Opdivo®)	PD-1	Immune-mediated adverse reactions,[aa] embryofetal toxicity	*Systemic*: increased ALT, AST, and AP; hyponatremia; hyper- and hypokalemia; hyper- and hypocalcemia; lymphopenia; fatigue; asthenia; musculoskeletal and abdominal pain; dyspnea; cough; GI. *Cutaneous*: rash, pruritus
Pembrolizumab (Keytruda®)	PD-1	Immune-mediated adverse reactions,[ab] embryofetal toxicity	*Systemic*: fatigue, peripheral edema, chills, pyrexia, renal failure, cellulitis, decreased appetite, dyspnea, arthralgia, nausea, diarrhea, cough. *Cutaneous*: rash, pruritus, vitiligo
Dinutuximab (Unituxin®)	GD2	*Boxed warning*: serious IR, neuropathy. *Others*: CLS and hypotension, infection, neurological disorders of the eye, BMS, electrolyte abnormalities, AHUS, embryofetal toxicity	*Systemic*: hypokalemia, pain, fever, hypocalcemia, hyponatremia, anemia, thrombocytopenia, lymphopenia, neutropenia, increased AST and ALT, GI. *Cutaneous*: urticaria
Daratumumab (Darzalex®)	CD38	IR, interference with serological testing,[ac] interference with determination of patient's response and disease progression[ad]	IR, infections, thrombocytopenia, pyrexia, fatigue, nausea, back pain, cough[ae]
Elotuzumab (Empliciti®)	SLAMF7[af]	IR,[ag] infections, second primary malignancies, hepatotoxicity, interference in monitoring M-protein impacting determination of complete response in patients with IgGκ myeloma protein	Fatigue, diarrhea, pyrexia, constipation, cough, peripheral neuropathy, URTI, nasopharyngitis, decreased appetite, pneumonia, headache, pain in the extremity, vomiting, lymphopenia, neutropenia, muscle spasms

Atezolizumab was approved in May 2016, too late to be included in this table. See later this chapter, "Recent Approval: Atezolizumab"
ADCC antibody-dependent cell-mediated cytotoxicity, *AHUS* atypical hemolytic uremic syndrome, *ALT* alanine transaminase, *AML* acute myelogenous leukemia, *AP* alkaline phosphatase, *AST* aspartate transaminase, *BMS* bone marrow suppression, *CHF* congestive heart failure, *CLS* capillary leak syndrome, *CNS* central nervous system, *CRS* cytokine release syndrome, *CTLA-4* cytotoxic T lymphocyte-associated antigen 4, *EGFR* epidermal growth factor receptor (HER1, ErbB1), *EM* erythema multiforme, *EpCAM* epithelial cell adhesion molecule, *GD2* disialoganglioside expressed on tumors of neuroectodermal origin, *GI* gastrointestine/gastrointestinal symptoms, e.g., nausea, diarrhea, vomiting, constipation, etc., *HAMA* human anti-mouse antibody, *HARA* human anti-rat

antibody, *HER2* human epidermal growth factor 2, also known as neu, ErbB2, CD340, or p185, *HLH* hemophagocytic lymphohistiocytosis, *IR* infusion reaction, *LVD* left ventricular dysfunction, *MDS* myelodysplastic syndrome, NSCLC non-small cell lung cancer, *ONJ* osteonecrosis of the jaw, *PD-1* programmed cell death protein 1, *PML* progressive multifocal leukoencephalopathy, *PPE* palmar-plantar erythrodysesthesia, *RANKL* receptor activator of nuclear factor kappa-B ligand (CD254), *RPLS* reversible posterior leukoencephalopathy syndrome, *SIRS* systemic inflammatory response syndrome, *SJS* Stevens-Johnson syndrome, *TEN* toxic epidermal necrolysis, *TLS* tumor lysis syndrome, *URTI* upper respiratory tract infection, *VEGF* vascular endothelial growth factor, *VEGFR-2* vascular endothelial growth factor receptor 2

[a]Nomenclature: mAbs of murine origin are given the suffix, or stem, *-omab*; chimeric antibodies in which the V region is spliced into human C region are given the *-ximab* stem; humanized antibodies with murine hypervariable region spliced into human antibody have the *-zumab* stem; and antibodies with complete human sequence are given the *-umab* stem

[b]Approved by FDA or EMA or both

[c]Specificity of antibody

[d]Adverse events in addition to those mentioned as occurring, or potentially likely to occur, and shown in column 3

[e]Registered by EMA, Health Canada, and Ministry of Health, Israel, but not FDA. Catumaxomab is a bispecific mouse—rat hybrid (given suffix *-axomab*) recognizing both EpCAM and CD3

[f]EpCAM (CD326), expressed on epithelial and epithelial-derived neoplasms; CD3—part of TCR complex on T lymphocytes

[g]A BiTE (bispecific T-cell-engaging) fusion protein

[h]CD19, a B-cell antigen; CD3, part of the T-cell receptor

[i]With yttrium-90 or indium-111. Tiuxetan is a chelator

[j]Expressed on B lymphocytes where it aids optimum B-cell response to T-independent antigens

[k]Severe neutropenia, thrombocytopenia, anemia, and lymphopenia. Incidences of thrombocytopenia grades III and IV in ibritumomab tiuxetan-treated non-Hodgkin lymphoma patients were 87 % and 13 %, respectively

[l]Glycoengineered to enrich Fc carbohydrate with non-fucosylated sugars and higher binding to FcγRIII with consequent enhanced ADCC

[m]Conjugated to cytotoxic monomethyl auristatin E (MMAE)

[n]CD30—a cell membrane protein of the tumor necrosis receptor family. Expressed on activated T and B lymphocytes

[o]Withdrawn from the USA and Europe in 2012 to be relaunched for multiple sclerosis

[p]CD52—present on the surface of mature lymphocytes and associated with some lymphomas

[q]In particular *Pneumocystis jirovecii*, CMV, EBV, and herpesvirus

[r]Not indicated for use in combination with chemotherapy due to increased toxicity

[s]Should be discontinued in patients developing interstitial lung disease, pneumonitis, and lung infiltrates

[t]Most common drug-induced reactions following this mAb are skin toxicities

[u]Neutralizing antibodies detected in 1 of 33 patients

(continued)

Table 3.1 (continued)

[v]Greatest risk (LVD) when administered with anthracyclines
[w]Highest risk with myelosuppressive therapy
[x]Called ado-trastuzumab emtansine in the USA to distinguish it from trastuzumab. Trastuzumab linked to the cytotoxin mertansine (DMI), a tubulin inhibitor. Also known as trastuzumab emtansine and T-DM1
[y]Binds CD80/CD86 on antigen-presenting cells
[z]Immune-mediated reactions due to T-cell activation and proliferation—enterocolitis, hepatitis, dermatitis, neuropathies, endocrinopathies, and other immune-mediated reactions including cutaneous and ocular manifestations
[aa]Immune-mediated pneumonitis, colitis, hepatitis, nephritis and renal dysfunction, hypothyroidism, and hyperthyroidism
[ab]Immune-mediated colitis, hepatitis, nephritis, hypothyroidism, and hyperthyroidism
[ac]Daratumumab binds to CD38 on red cells producing a positive Coombs test masking detection of minor antigens in patient's serum
[ad]Daratumumab is a human IgG kappa mAb detected by assays used to monitor endogenous M-protein. In patients with IgG kappa myeloma, this interference may influence the determination of the patient's response and the disease progression
[ae]Postmarketing usage is likely to reveal the occurrence of cytopenias
[af]Signaling lymphocytic activation molecule receptor family member 7, also known as CS1, CD2 subunit 1, and CD319
[ag]Premedicate with dexamethasone, H1 and H2 antihistamines, and acetaminophen

Catumaxomab

The dual antigen recognition specificity of this mouse-rat hybrid mAb (Removab®) (Tables 2.1 and 3.1) is effected by a mouse kappa light chain and IgG2a heavy chain and a rat lambda light chain and IgG2b heavy chain. Binding of the Fc region with Fcγ receptors provides a third functional binding site. The mouse Fab binds to EpCAM, the rat Fab binds to CD3, and the hybrid Fc fragment binds to FcγRI (CD64), FcγRIIa (CD32), and FcγRIIIa (CD16a) on macrophages, NK cells, dendritic cells, and mononuclear cells (Fig. 3.1). EpCAM (CD326), a transmembrane glycoprotein, is shielded by tight junctions in normal tissue but expressed over the whole surface of tumor cells. It promotes tumor growth and metastasis and is overexpressed on epithelial tumors of the gastrointestinal tract, esophagus, head, neck, lung, liver, kidney, ovary, pancreas, and prostate and a number of other organs and tissues. Being so widely and strongly expressed, EpCAM has become a potentially useful target for antibody therapy of various carcinomas. Overexpression of EpCAM tends to be seen in advanced cases of cancer and those with a poor outcome. In relation to its approved indication for malignant ascites, EpCAM is expressed on most epithelial cancers of this type as well as the tumor cells in malignant effusions. The trifunctional action of

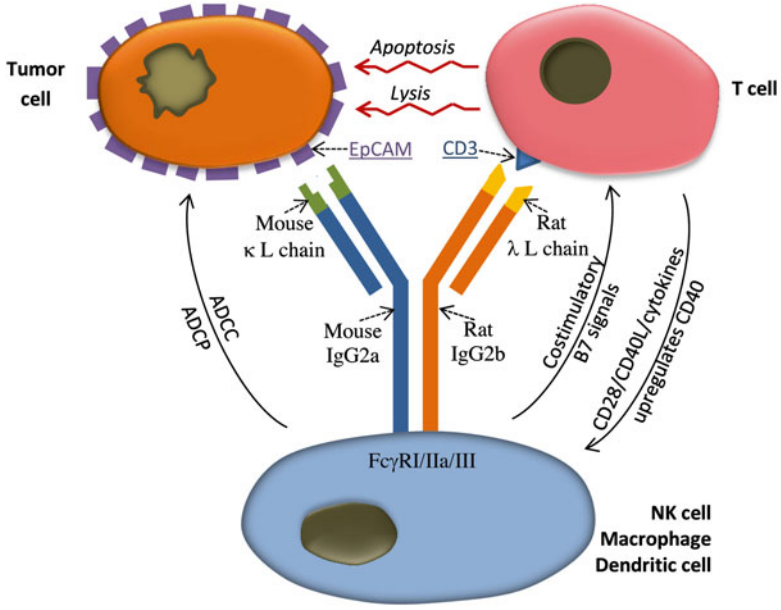


Fig. 3.1 Mechanism of action of the trifunctional hybrid monoclonal antibody catumaxomab summarizing the interactive cellular processes when the combining site of the mouse Fab binds its complementary cell surface antigen, EpCAM on a tumor cell; the rat Fab combining site binds its complementary surface antigen, CD3 antigen on a T cell; and the Fc fragment of the monoclonal antibody interacts with FcγRIIIa receptors on natural killer (NK) cells, macrophages, or dendritic cells

catumaxomab involving antibody recognition of cancer cells expressing EpCAM, CD3 on activated lymphocytes, and effector functions mediated via Fc binding results in T-cell-induced killing, ADCC, complement-dependent cytotoxicity (CDC), and antibody-dependent cellular phagocytosis (ADCP) through interaction with FcγRs on effector cells (Fig. 3.1).

When used to treat malignant ascites, the commonly seen side effects of catumaxomab of fever, nausea, vomiting, and abdominal pain are the result of cytokine release syndrome (CRS) (Chap. 1, section "Cytokine Release Syndrome"; Table 3.1). The cytokines TNF and IL-6, for example, are elevated above normal levels in 60 and 80 %, respectively, of treated patients. Cytokine release is therefore a reflection of both T-cell activation and the mode of action of catumaxomab. Pyrexia, nausea, and vomiting tend to be limited to the duration of therapy and manageable with standard symptomatic treatment. Abdominal pain is generally managed by standard pain medication, while antibody-induced grade 4 adverse events such as ileus are isolated occurrences often related to the malignancy. Lymphopenia, reported in up to 14 % of patients, is reversible, usually within a week. Cutaneous reactions of rash, erythema, pruritus, and catheter-related reactions such as erythema and infection also occur and can be serious. Up to the end of 2012, postmarketing reports from Europe listed eight cases of respiratory disorders, 11 cases of cutaneous reactions, 11 reports of infections, and eight cases of systemic inflammatory response syndrome (SIRS) (Chap. 1, section "Systemic Inflammatory Response Syndrome") with associated pyrexia, tachypnea, tachycardia, and leukocytosis.

As might be expected with rodent antibodies, both human anti-mouse and human anti-rat antibodies are induced by catumaxomab. Neutralizing antibodies, usually after the fourth infusion, have been reported, but no clear safety issues have been linked to their appearance.

Blinatumomab

There is substantial evidence that cytotoxic T lymphocytes can have a major role in controlling the growth of tumors, but therapies aimed at utilizing this observation, for example, the use of CTLA-4-blocking antibodies, have shown only limited success, presumably because of immune avoidance by the cancer cells. Although T cells can show high cytotoxic potential, they lack Fcγ receptors and therefore cannot be recruited by conventional antibodies. This fact led to an approach based on single-chain antibodies that can link T cells and tumor cells bringing them into close proximity for cell killing to occur. Blinatumomab (Blincyto®) (Tables 2.1 and 3.1), a so-called bispecific T-cell-engaging (BiTE) fusion protein composed of two antibody single-chain variable fragments each from an H and L chain, is an ~55 kDa protein derived from the linkage of the four peptide chains from four different genes. One of the two binding specificities is directed to the B-cell antigen CD19, while the other targets CD3, part of the T-cell receptor. Reaction with both antigens is exploited to link malignant B cells of patients with acute lymphoblastic leukemia

to cytotoxic T cells, activating them to destroy the tumor cells via production of perforin and granzymes that induce apoptosis. Blinatumomab induces tumor regression at very low doses, for example, partial and complete tumor regressions have been first observed at doses as low as 15 ng/sq m/day. FDA approval for blinatumomab in December 2014 was based on results of a phase II multicenter open-label study of 185 patients with Philadelphia chromosome-negative relapsed or refractory B-cell precursor acute lymphoblastic leukemia. The study revealed that 42 % of participating patients achieved complete or partial remission within two cycles of the drug, the majority improving within the first treatment cycle. Thirty-two percent of patients achieved complete remission for about 6.7 months.

Blinatumomab approval by the FDA was issued with a Risk Evaluation and Mitigation Strategy (REMS). The most common adverse events seen with blinatumomab (Table 3.1) in trials included pyrexia (62 %); neurological toxicity (50 %); headache (36 %); peripheral edema, febrile neutropenia, and nausea (each 25 %); hypokalemia (23 %); rash (21 %); and constipation (20 %). Neurological toxicities, ranging from confusion to tremors, convulsions, and speech disorders and all apparently reversible, frequently interrupted therapy. The majority of adverse events occurred within the first week of treatment and usually faded to grade 1 or less upon further treatment. CRS, sometimes life-threatening or even fatal, was reported in 11 % of patients (see boxed warning, Table 3.1). It has been suggested that CRS, in severe form at least, may be due to activation of macrophages triggered by cytokines released from T cells activated by blinatumomab. This is thought to lead to hemophagocytic lymphohistiocytosis (HLH) (Chap. 1, section "Hemophagocytic Lymphohistiocytosis"), a condition seen in a patient with CRS 36 h after infusion with blinatumomab. Symptoms of fever, circulatory collapse, and respiratory failure accompanied cytopenias, hypofibrinogenemia, and hyperferritinemia. HLH continued after withdrawal of blinatumomab but improved rapidly upon treatment with the IL-6 and inflammation blocker tocilizumab. It has subsequently been suggested that patients given blinatumomab therapy should be monitored for HLH. Unlike antibodies that block CTLA-4, no autoimmune reactions have so far been observed following blinatumomab.

Seen as an "ultra-orphan drug," the pool of potential patients for the mAb is estimated to be ~1000 acute lymphoblastic leukemia patients. This fact is being reflected in the cost of treatment, recently estimated to be approximately $178,000 for two courses of the drug.

Monoclonal Antibodies Targeting CD20: Rituximab, Ibritumomab, Ofatumumab, and Obinutuzumab

CD20 (human B lymphocyte-restricted differentiation antigen Bp35), a 33–35 kDa transmembrane glycosylated phosphoprotein that is part of the MS4A family of proteins, is expressed on the surface of B cells, except for plasmablasts, at all stages of their development until the memory B-cell stage. CD20 also occurs on

B-cell lymphomas, B-cell chronic lymphocytic leukemia, hairy cell leukemia, and melanoma cancer stem cells but is absent from mature plasma cells and other tissues. The antigen may have a functional role in one or more of B-cell growth, differentiation, activation, and proliferation via intracellular signaling or as a calcium channel in association with the B-cell receptor, but, as yet, no CD20 ligand has been identified. In addition to the effector processes initiated when bound to antibody, CD20 may be involved in transmembrane signaling controlling growth and cell death in some tumors. Due to its non-Hodgkin lymphoma (NHL) B-cell expression and the fact that it is not normally shed from cells and is internalized after binding to antibody, CD20 has been seen as an exploitable target for mAbs for the treatment of lymphomas. Four mAbs directed to CD20 are currently approved by the FDA and EMA as antitumor agents. In the order of regulatory approval, these are rituximab, ibritumomab, ofatumumab, and obinutuzumab.

Rituximab

Rituximab (MabThera®, Rituxan®) (Tables 2.1 and 3.1), a human-mouse chimeric IgG1κ antibody, was the first mAb approved by the FDA to treat relapsed or refractory NHL and in 1997 was, in fact, the first mAb approved specifically for cancer therapy. Given in combination with some selected small molecule chemotherapeutic drugs, it is now a first-line therapy for several NHLs, including follicular lymphoma and diffuse large B-cell lymphoma. In 2002, the FDA authorized the use of rituximab as a component of ibritumomab therapy. The mechanisms underlying rituximab's cytotoxicity in cancer therapy are not completely understood. Tumor cell death is attributed to ADCC, CDC, and the induction of apoptosis, but other mechanisms, such as a vaccine-like action of increasing the cytotoxic T-cell response to idiotype antigens on the malignant cells, may also be operative. Rituximab is relatively successful as a treatment for B-cell malignancies, but the mAb is not effective in all patients where variable degrees of tumor resistance occur. This resistance remains poorly understood although many suggested mechanisms have been advanced including loss of CD20 expression, blockade of ADCC, expression of proteins that inhibit CDC, and expression of anti-apoptotic proteins. Several strategies have been devised to overcome tumor cell resistance to rituximab but also to improve anti-CD20 mAb efficacy. In relation to the latter, mAb has been conjugated to a radionuclide as seen with the development of ^{90}Y-ibritumomab tiuxetan (section "Ibritumomab Tiuxetan") and ^{131}I-tositumomab.

By 2012, FDA and EMA official safety data reported for rituximab was based on a total of nearly 6000 exposed patients. A long list of side effects has been reported for the antibody including the serious events detailed in an FDA black box warning, namely, infusion reactions, progressive multifocal leukoencephalopathy (PML) (Chap. 1, section "Progressive Multifocal Leukoencephalopathy"), tumor lysis syndrome (TLS) (Chap. 1, section "Tumor Lysis Syndrome"), skin and mucocutaneous reactions (these four all potentially fatal), infections, hepatitis B reactivation, cardiac arrhythmias, and bowel problems as well as a host of often less serious systemic

and cutaneous events and toxicities (Table 3.1). Often seen as CRS, infusion reactions occur on the first infusion in up to 77 % of malignant patients, and this decreases to approximately 10 % after the second infusion. Potentially serious symptoms including hypotension and bronchospasm may complicate the reaction in about 10 % of cases. An early relationship between rituximab administration, the lymphocyte count, and CRS suggested that the appearance of the syndrome correlated with lymphocyte counts higher than 50×10^9/L. Patients with counts exceeding this figure experienced more reactions than patients with lesser numbers of tumor cells. A survey of hypersensitivity reactions to rituximab in patients at the Massachusetts General Hospital between 2006 and 2010 found immediate hypersensitivity in 79 of 901 patients (8.8 %). Approximately three-quarters of the patients showed symptoms after the initial infusion, and 46 % experienced moderate or severe reactions after subsequent infusions. An increased risk of hypersensitivity reactions occurred in patients with advanced disease, and obscurely, Waldenström's macroglobulinemia patients accounted for 10 % of the reactions despite making up only 1 % of the rituximab-treated patients. Other signs of hypersensitivity following rituximab administration are urticaria, cardiovascular and respiratory distress typical of an anaphylactoid/anaphylactic reaction, serum sickness, vasculitis, interstitial pneumonitis, some cutaneous manifestations, and acute respiratory distress syndrome (ARDS). Cough, dyspnea, and bronchospasm are fairly common but pulmonary events can be serious, for example, pulmonary fibrosis which has proved fatal. A review of 62 cases of severe respiratory reactions thought to be caused by rituximab implicated interstitial pneumonitis in three-quarters of the subjects together with other respiratory disorders, bronchiolitis obliterans, organizing pneumonia, pulmonary fibrosis, hypersensitivity pneumonitis, and ARDS. It has been suggested that rituximab should not be administered to patients with lung diseases such as pneumonia, pleural effusion, and collapsed lung.

Infections, bacterial, viral, and fungal, occurring with an incidence of up to 17 %, may be seen during monotherapy with rituximab. Infections during or after rituximab may be rare as well as opportunistic and they are always a concern since they can be serious and even fatal. Cytomegalovirus (CMV) encephalitis is mainly seen in HIV-positive individuals, but it also occurs in occasional malignant melanoma patients treated with rituximab. Another example of a rare organism in a patient receiving rituximab is infection due to *Capnocytophaga bacteraemia*, a gram-negative bacillus found in dog saliva. Fulminant myocarditis due to enterovirus is a further rare pathogenic example associated with the use of the mAb. Pneumocystis pneumonia, an opportunistic fungal infection in rituximab-treated patients caused by *Pneumocystis jirovecii*, is the subject of a number of reports. Mayo Clinic, Rochester, records for the period 1998–2011 revealed 30 patients had the infection, most developed acute hypoxemic respiratory failure, half required admission to intensive care, and 30 % of the patients died. Other reports of *P. jirovecii* pneumonia are numerous and alarming enough for the suggestion to be made that *P. jirovecii* pneumonia prophylaxis should be considered following rituximab therapy.

An incidence of 19 % has been reported for cardiovascular disorders during rituximab monotherapy, and cytopenias, usually mild, occur with only low

incidences of severe neutropenia, anemia, and thrombocytopenia. TLS may manifest as renal failure and a 2010 examination of the WHO Collaborating Centre for International Drug Monitoring Adverse Event Data Bank revealed 114 reports associated with rituximab out of 182 case reports of PML. Table 3.1 lists the serious and sometimes fatal mucocutaneous reactions reported for rituximab, most of which appear within approximately the first three months of treatment. Combining rituximab with chemotherapy has produced adverse responses in up to 86 % of malignant patients with 57 % showing severe or serious signs. Hematologic events showed the highest incidence (67 %, 48 % severe or serious), followed by dermatologic reactions (44 %, 2 %), respiratory symptoms (38 %, 4 %), gastrointestinal symptoms (37 %, 2 %), musculoskeletal effects (26 %, 3 %), and cardiovascular disorders (25 %, 3 %). Overall, the incidences of a range of adverse events were higher in patients receiving rituximab plus chemotherapy than in patients receiving either therapy alone. Postmarketing surveillance of rituximab administration has confirmed the importance of infections and respiratory and hematologic events in the safety profile of this widely and heavily used mAb. Infections, with an incidence of 6.6 %, led the number of reports of adverse events in the FDA Adverse Event Reporting System (FAERS) database containing more than 16,700 reports. Hematologic (5.7 %), respiratory (3.7 %), and gastrointestinal symptoms (2.8 %) followed in that order. Infections were again the most commonly reported event (13 %) in the European Pharmacovigilance EudraVigilance Database Management System followed by respiratory events (11 %), hematologic events (10 %), nervous system disorders (7 %), gastrointestinal disorders (6 %), and infusion reactions (4 %). Interesting and noteworthy totals for individual disorders were CRS (44 reports), TLS (145), PML (423), JC virus infections (37), leukoencephalopathy (33), and bone marrow failure (137). Recorded fatalities totaled about 6 %. Other events reported by the FDA under the heading of postmarketing experience are late onset neutropenia; hyperviscosity syndrome in Waldenström's macroglobulinemia; fatal cardiac failure; viral infections; immune/autoimmune events including uveitis, optic neuritis, vasculitis, lupus-like syndrome, serum sickness, pleuritis, and polyarticular arthritis; disease progression of Kaposi's syndrome; bowel obstruction and perforation; fatal bronchiolitis obliterans and interstitial lung disease; and the nervous system disorder, posterior reversible encephalopathy syndrome (PRES) (Chap. 1, section "Posterior Reversible Encephalopathy Syndrome").

Antichimeric antibodies were detected in 4 of 356 (1.1 %) patients with low-grade or follicular NHL receiving rituximab alone. In patients with Wegener's granulomatosis and microscopic polyangiitis treated with rituximab, 23 of 99 (23 %) tested positive for antichimeric antibodies at 18 months. The clinical relevance of the immunogenicity of antichimeric antibodies generally remains unclear although of 11 patients given rituximab for severe pemphigus, two patients who developed antibodies to the mAb experienced an increase in disease activity over an extended time. Of the nine patients who did not develop antibodies, the lesions healed in five, and partial remission was achieved in the other four patients. In

rheumatoid arthritis patients receiving rituximab, 273 of 2578 (10.6 %) developed antichimeric antibodies, but there appeared to be no association between the appearance of antibodies and infusion or other adverse reactions.

Ibritumomab Tiuxetan

Ibritumomab tiuxetan (Zevalin®) (Tables 2.1 and 3.1), a murine IgG1κ mAb covalently linked to the chelator tiuxetan by a stable thiourea bond, is radiolabeled with yttrium-90 for therapy or indium-111 for imaging. In the initial dosing schedule set out by the FDA, premedication with acetaminophen and diphenhydramine preceded infusion of rituximab on day 1 and days 7, 8, or 9. Within 4 h of completing the first rituximab infusion, ibritumomab labeled with indium-111 was given as an intravenous injection over 10 min prior to a bioscan for checking biodistribution. The requirement for the bioscan was discontinued in November 2011. Four hours after the second rituximab infusion, yttrium-labeled ibritumomab was given by intravenous injection. Rituximab infusion should be immediately ceased if serious infusion reactions occur and temporarily slowed or interrupted for less severe reactions. Patients should be monitored closely for extravasation during ibritumomab-yttrium-90 therapy, and infusion should be immediately stopped and restarted in another limb if signs of extravasation occur. The two-day half-life of ibritumomab is shorter than that of rituximab (7 days), but this is not necessarily a drawback since prolonged exposure to the beta emissions of the yttrium isotope is not desirable.

Results from clinical trials and an FDA black box warning highlight severe, and potentially fatal, infusion reactions and severe cytopenias as the most serious adverse events experienced with ibritumomab therapy (Table 3.1). Clinical trial results showed almost 60 % of patients experienced cytopenias with severe neutropenia and thrombocytopenia occurring with the highest incidences followed by anemia and hemorrhage. Other serious events are infections, mainly bacterial but some fungal and viral, and potentially fatal myeloid malignancies or dysplasias. Hypersensitivity reactions, generally manifesting as bronchospasm or angioedema, are another potentially dangerous adverse effect. Of the non-hematologic events, gastrointestinal symptoms are commonly seen as well as rare toxidermias such as bullous dermatitis, erythema multiforme, Stevens-Johnson syndrome, and toxic epidermal necrolysis. Postmarketing surveillance has revealed fatal cases of infection, infusion-related cardiac arrest, and cerebral hemorrhage; reports of the severe cutaneous and mucocutaneous reactions were already mentioned, as well as patients with exfoliative dermatitis; and 19 cases of acute myelogenous leukemia or myelodysplastic syndrome were reported in 746 NHL patients (an incidence of 2.5 %). For the term “progressive multifocal leukoencephalopathy,” a search in 2010 of the WHO Collaborating Centre for International Drug Monitoring Adverse Event Data Bank retrieved 182 reports, five of which related to ibritumomab tiuxetan. As a comparison, note that the retrieved figure for rituximab was 114.

Being a mouse mAb, it is not surprising that immunization is included in the FDA warnings and precautions for ibritumomab. In fact, human anti-mouse and human antichimeric antibodies have been observed in ~4 % of patients treated with the mAb, but antibody titers do not seem to increase with time and the antibodies generally do not mediate hypersensitivities.

Ofatumumab

Ofatumumab (Arzerra®) (Tables 2.1 and 3.1) is a fully human IgG1κ mAb originally granted orphan drug status by the EMA (2008) and FDA (2009) for the treatment of B-cell CLL. Subsequent accelerated approvals by the FDA, EMA, Australian Therapeutics Goods Administration (TGA), and Health Canada were restricted to CLL patients refractory to fludarabine and alemtuzumab. The reasoning behind this indication is based on the poor survival outcomes of patients who become refractory to fludarabine (normally the cornerstone of treatment of CLL) and alemtuzumab treatments. Although CD20 is sometimes described as an ideal target for mAbs in allowing effector recruitment for ADCC and CDC, different CD20-reactive mAbs can differ in their capacities to trigger programmed cell death. CD20 lodged in the cell membrane is thought to have two extracellular loops, a large one composed of about 44 amino acids and a smaller one which is thought to remain within the plasma membrane. Ofatumumab binds an epitope composed of both the large and small loops, which is a different region recognized by rituximab, and it is this different recognition that helps to increase ofatumumab-induced complement activation and complement-mediated lysis. Ofatumumab's more potent CDC action than that of rituximab is thought to result from the close proximity of the CD20 small loop-binding site to the cell surface which aids the deposition of complement on the cell surface. Ofatumumab induces lysis in cells with high or low CD20 expression (such as freshly isolated CLL cells) and even in complement-resistant B-cell lines and cells expressing complement inhibitory molecules. In addition to its efficient binding of C1q and activation of the complement pathway, ofatumumab induces cell death through ADCC and there is some evidence that binding of the mAb also recruits NK cells.

From clinical trials of ofatumumab, an average of nine adverse events per patient was recorded; serious events occurred in 30–54 % of patients, fatalities in 16 % of patients, and drug-related discontinuations in 14 % of participants. Most adverse events, particularly severe ones, occurred in 27 patients given the highest dose of antibody. Although there are no boxed warnings for ofatumumab, infusion reactions, the possibility of hepatitis B virus reactivation, cytopenias, intestinal obstruction, and PML comprise the warnings, precautions, and risks issued for this mAb (Table 3.1). Infusion reactions following ofatumumab may manifest as bronchospasm, dyspnea, laryngeal and pulmonary edema, flushing, hypotension, hypertension, syncope, cardiac ischemia/infarction, back and abdominal pain, angioedema, pyrexia, rash, and urticaria. Premedication with acetaminophen, an antihistamine, and a corticosteroid may be necessary, and interruption of infusion is recommended for reactions of any severity. Prolonged severe neutropenia and thrombocytopenia may occur, making necessary the regular monitoring of blood and platelet counts.

Carriers of hepatitis B should be monitored for hepatitis B virus infection during treatment with ofatumumab and for 6–12 months following the last infusion.

In an ofatumumab-as-single-agent study of 138 fludarabine-refractory CLL patients (59 fludarabine and alemtuzumab refractory and 79 fludarabine refractory with bulky lymphadenopathy), infusion reactions, nearly all grades 1 or 2, were seen in ~60 % of patients, predominantly during the first and second infusions. The most common adverse events (≥10 % of patients) during treatment were infections (67 %); cough (18 %); anemia and diarrhea (each 16 %); fatigue, fever, and neutropenia (each 15 %); dyspnea (13 %); nausea (11 %); and rash (10 %). As in other trials with ofatumumab, infections were prominent with 189 events in 92 patients; 139 (74 %) were grade 1 or 2 and 37 grade 3 or 4 with pneumonia and other respiratory tract infections the most common. Thirteen infections (sepsis 6, pneumonia 5, *Fusarium* infection 1, and PML 1) led to death.

In 2013, the FAERS database of 1056 reports listed infections (11 %) and white blood cell (10 %), respiratory (5 %), gastrointestinal (3 %), and neurological (3 %) disorders as the most common events provoked by ofatumumab. There were 157 reports of febrile neutropenia, 60 of pneumonia, 27 of sepsis, 37 of thrombocytopenia, 109 of anaphylaxis, 16 of TLS, and 12 of PML. Mucocutaneous events included 14 cases of paraneoplastic pemphigus and 19 of urticaria and 23 patients with rash.

As anticipated from a fully human antibody, ofatumumab has so far shown a relative lack of immunogenicity. In clinical trial investigations, no antibodies to the agent were found in 46 patients after the eighth infusion or in 33 patients after the 12th infusion.

Obinutuzumab

Obinutuzumab (Gazyva®, Gazyvaro®; also known as GA101 and afutuzumab) (Tables 2.1 and 3.1) was approved by the FDA in November 2013 for the treatment of CLL in combination with chlorambucil after earlier receiving Breakthrough Therapy Designation. In combination with bendamustine, approval for the mAb was extended to the treatment of follicular lymphoma in February 2016. Obinutuzumab is a humanized anti-CD20 mAb of the IgG1κ class, MW ~150 kDa, glycoengineered and prepared in Chinese hamster ovary cells. “Glycoengineered” in this case means the presence of non-fucosylated sugars that impart higher binding affinity for FcγIII receptors which, in turn, enhances ADCC and caspase-independent apoptosis (Chap. 2, sections “Glycosylation of Monoclonal Antibodies” and “Antibody-Dependent Cell-Mediated and Complement-Dependent Cytotoxicities”). Modification of elbow hinge sequences in the variable region may also contribute to the apoptotic activity of obinutuzumab. Like its fellow mAbs targeting CD20, obinutuzumab mediates B-cell lysis by engaging immune effector cells, directly activating intracellular pathways signaling cell death, activating the complement cascade, and effecting ADCC and antibody-dependent cellular phagocytosis (ADCP). Although exhibiting less CDC than rituximab, obinutuzumab has demonstrated superior efficacy. Patients with CLL and coexisting illnesses unable to tolerate combined intravenous chemotherapy are likely to be

more tolerant of obinutuzumab in its approved indication of the mAb's combination with chlorambucil.

Early observational studies in a phase I investigation of obinutuzumab induction followed by 2 years of maintenance in 22 patients with relapsed B-cell malignancies showed the main adverse events to be infusion reactions, infections, neutropenia, pyrexia, headache, and nausea. These findings were essentially supported by a comparison of obinutuzumab and rituximab, each combined with chlorambucil, in a study of 781 randomly assigned patients with previously untreated CLL. Adverse events were seen more frequently with the obinutuzumab-chlorambucil treatment. Grade 3–4 neutropenia, infusion reactions, anemia, thrombocytopenia, pyrexia, and musculoskeletal pain were the most common events recorded. In addition to a black box warning for hepatitis B virus reactivation and PML, warnings and precautions issued by the FDA cover severe and life-threatening infusion reactions, TLS, the risk of infections, and cytopenias (Table 3.1). Two-thirds of patients experienced infusion reactions by the time of the infusion of the first gram of obinutuzumab. Symptoms included hypotension, tachycardia, dyspnea, and other respiratory problems such as bronchospasm, wheezing, and throat irritation. Late reactions (up to 24 h) have occurred. Premedication with acetaminophen, an antihistamine, and a corticosteroid may be necessary. As is well known for TLS, patients with high tumor burden are at greater risk of acute renal failure, hyperkalemia, hyperuricemia, and hyperphosphatemia which can occur within 12–24 h of the first infusion. Infections, sometimes serious, may be bacterial, fungal, or viral in origin. Grade 3 or 4 cytopenias may occur in patients given obinutuzumab in combination with chlorambucil; incidences of 34 % for neutropenia and 12 % for thrombocytopenia, respectively, were found in trials. Neutropenia may be of late onset and acute thrombocytopenia, occurring within 24 h of infusion, has been seen in up to 5 % of patients. Treatment-related neutropenia also appears to be more common than with other anti-CD20 mAbs. Apart from a few other common, and less serious, adverse events (Table 3.1), more recent clinical trial experience has revealed the possibility of worsening of preexisting cardiac conditions with some fatal cardiac events occurring in patients treated with obinutuzumab. In a recently published nonrandomized, parallel-cohort, phase Ib, multicenter study of the safety and efficacy of obinutuzumab plus fludarabine and cyclophosphamide and obinutuzumab plus the nitrogen mustard bendamustine (SDX-105) used in the treatment of CLL and lymphomas, obinutuzumab in both combinations showed manageable toxicity. In the obinutuzumab-fludarabine/cyclophosphamide cohort, 6 of 21 patients (29 %) experienced serious adverse events, 86 % experienced a grade 3–4 adverse event, 43 % experienced at least one grade 3–4 hematologic event (neutropenia, febrile neutropenia, anemia, or thrombocytopenia), and 52 % had at least one infection. An infusion-related reaction occurred in 91 % of patients. In the obinutuzumab-bendamustine cohort, 9 of 20 patients (45 %) experienced serious adverse events, 85 % had a grade 3–4 event, and 60 % experienced at least one grade 3–4 hematologic event. Ninety percent of patients had an infusion-related reaction, four of which were serious and most reactions occurred with the first dose. Although cutaneous side effects of rituximab are well known and cover a wide spectrum of effects from mild sweating and pruritus to severe toxic epidermal necrolysis, paraneoplastic pemphigus, and lichenoid eruptions, reports of dermatologic

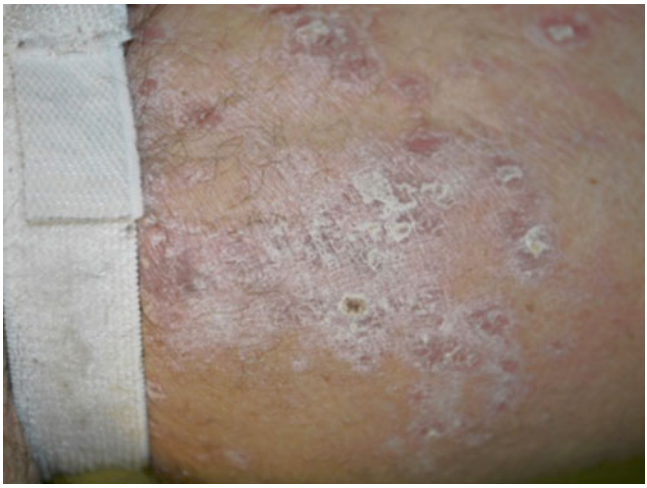


Fig. 3.2 A lichenoid eruption in a 62-year-old man treated with obinutuzumab for follicular non-Hodgkin lymphoma. The reaction, of psoriasiform appearance, was a widespread violaceous lichenoid maculopapular eruption on the trunk, back, arms, and legs. Reproduced from Bakkour W and Coulson IH. GA101 (a novel anti-CD20 monoclonal antibody)-induced lichenoid eruption. Dermatol Ther 2012;2:3. doi:10.1007/s13555-012-0003-9, an open-access article distributed under the terms of the Creative Commons Attribution License

reactions to other anti-CD20 mAbs are surprisingly rare. A fairly recent case of lichenoid eruption (Fig. 3.2) ascribed to obinutuzumab was claimed to be the first case report of a cutaneous side effect to the mAb.

For the recently FDA-approved indication of follicular lymphoma, obinutuzumab with bendamustine is followed by obinutuzumab maintenance in patients refractory to, or who have relapsed after, treatment with a rituximab-containing regimen. The most common adverse reactions seen in treated patients were neutropenia, pyrexia, anemia, thrombocytopenia, infusion reactions, respiratory and urinary infections, arthralgia, asthenia, gastrointestinal symptoms, fatigue, and sinusitis. The most common grade 3/4 adverse reactions were febrile neutropenia, neutropenia, pneumonia, infusion reactions, sepsis, and pyrexia (Table 3.1).

Up to 1 year after obinutuzumab therapy, 9 of 70 treated patients (12.9 %) tested positive for antibodies to the mAb. Neutralizing activity of the antibodies was not investigated.

Brentuximab Vedotin

Brentuximab vedotin (Adcetris®) (Tables 2.1 and 3.1) is a chimeric IgG1κ mAb conjugated to the cytotoxic agent monomethyl auristatin E (MMAE) and targeted to CD30 or TNFRSF8, a 120 kDa cell membrane glycoprotein of the tumor necrosis receptor family expressed on activated T and B lymphocytes. Recognized in 2007

as an orphan drug for Hodgkin lymphoma, later for systemic anaplastic large cell lymphoma (sALCL), and recently for mycosis fungoides, brentuximab vedotin received accelerated approval by the FDA in 2011 for Hodgkin lymphoma after failure of autologous stem cell transplant (ASCT) or after failure of at least two prior multiagent chemotherapy regimens in patients who are not ASCT candidates and for sALCL after failure of at least one prior multiagent chemotherapy regimen. In September 2015, the FDA expanded its approval of brentuximab vedotin to post-autologous hematopoietic stem cell transplantation consolidation treatment of patients with Hodgkin lymphoma who are at risk of relapse or progression.

CD30 is overexpressed in Hodgkin lymphoma, ALCL, cutaneous T-cell lymphoma, and mediastinal B-cell lymphoma. This relatively restricted expression and its lack of expression on most healthy cells make CD30 an attractive target for immunotherapy. CD30 limits the proliferative potential of autoreactive CD8 T cells protecting against autoimmunity and regulates apoptosis through nuclear factor kappa-light-chain-enhancer of activated B-cell (NF-κB) activation. TNF receptor-associated factors 2 and 5 (TRAF2 and TRAF5) interact with the CD30 receptor expressed by activated T and B cells, mediating the signaling that leads to activation of NF-κB. After binding to cells expressing CD30, the antibody-drug conjugate brentuximab linked to its toxic payload MMAE is internalized before MMAE reaches the lysosomes where it is released, disrupting microtubules and ultimately inducing apoptosis. Free MMAE is also capable of exerting a toxic effect on bystander cells. Note that the half-life of the antibody-drug conjugate is 4–6 days compared to 3–4 days for MMAE. Brentuximab vedotin does not exert its action via CDC or ADCC but it does induce phagocytosis.

The list of warnings and precautions issued by the FDA for brentuximab vedotin covers 11 concerns related to neuropathies, infusion-related reactions, hematologic toxicities, serious infections, TLS, hepatotoxicity, PML, embryofetal toxicity, and serious dermatologic reactions. In clinical trials with Hodgkin lymphoma and sALCL patients, 54 % of subjects experienced neuropathy, predominately peripheral neuropathy which is cumulative. Of these patients, only about half had complete resolution of the condition with 20 % showing no improvement. Although infusion reactions to brentuximab vedotin are mostly mild-moderate, a small number of cases of anaphylaxis have been reported. Severe neutropenia and grade 3 or 4 thrombocytopenia or anemia as well as febrile neutropenia occur. In clinical trials, myelosuppression in severe form showed an incidence of 9–21 %. Serious bacterial, fungal, and viral infections and opportunistic infections such as pneumonia and sepsis occur, and in patients with high tumor burden or a rapidly proliferating tumor, TLS may result. In clinical trials, the incidences of severe reactions and death were higher in patients with severe renal impairment; this may be due to higher exposure to MMAE compared to patients with normal renal function. It is a similar situation for patients with moderate to severe hepatic impairment, but in any case, it should be recognized that serious cases of hepatotoxicity, including some fatalities, have resulted from brentuximab vedotin therapy. Cases have occurred after the first dose or after rechallenge. A black box warning has been issued for PML after 13 post-marketing surveillance reports and three cases among 2000 brentuximab vedotin-

treated clinical trial patients. PML and the mucocutaneous toxidermias, Stevens-Johnson syndrome and toxic epidermal necrolysis, constitute three serious adverse events that, although rare, are potentially lethal, therefore requiring awareness and vigilance in assessing new onset signs and symptoms of central nervous symptom and mucocutaneous abnormalities. Common adverse reactions (incidence ≥20 % and regardless of causality) recorded in clinical trials with Hodgkin lymphoma patients given brentuximab vedotin were neutropenia, peripheral sensory neuropathy, URTI, pyrexia, anemia, thrombocytopenia, fatigue, cough, nausea, vomiting, diarrhea, abdominal pain, and rash (Table 3.1). When brentuximab vedotin was given with bleomycin, pulmonary toxicity manifesting as interstitial infiltration and/or inflammation occurred indicating that this combination of drugs is contraindicated. The most common serious events experienced by Hodgkin lymphoma patients given brentuximab vedotin include peripheral motor neuropathy (4 %), abdominal pain (3 %), and pulmonary embolism, pneumonitis, pneumothorax, pyelonephritis, and pyrexia, all with an incidence of 2 %. Experience with sALCL patients given brentuximab vedotin showed that the most commonly occurring adverse reactions were neutropenia, peripheral sensory neuropathy, pyrexia, fatigue, nausea, diarrhea, pain, and rash. The most common serious events experienced by sALCL patients were septic shock, supraventricular arrhythmia, pain in extremity, and urinary tract infection, all with an incidence of 3 %. Other serious adverse events, but with a much lower incidence, were PML, TLS, and Stevens-Johnson syndrome. The safety of brentuximab vedotin was evaluated in 25 Hodgkin lymphoma patients with relapsing disease after allogeneic stem cell transplantation. The most frequent adverse events were cough, fatigue, and pyrexia (each 52 %), nausea and peripheral sensory neuropathy (each 48 %), and dyspnea (40 %). The most common adverse events of severity ≥grade 3 were neutropenia, anemia, thrombocytopenia, and hyperglycemia in that order. CMV was detected in five patients. Overall, the safety data for brentuximab vedotin shows that neutropenia, peripheral neuropathy, the risk of infections, and the worrying occasional case of PML are perhaps the most important adverse events provoked by the antibody-drug conjugate. Reports in the FAERS database at the end of 2012 included 40 cases of peripheral neuropathy (65 neurological disorders overall), 131 cases of infections, and 13 reports of PML. Other adverse events identified during postapproval use include febrile neutropenia, hepatotoxicity, serious and opportunistic infections, hyperglycemia, pancreatitis (including fatal cases), and toxic epidermal necrolysis (including fatal outcomes).

In phase II trials on patients with Hodgkin lymphoma and sALCL, ~7 % of subjects were found to develop serum antibodies to brentuximab vedotin that persisted, whereas 30 % of patients developed antibodies of a transient nature. In all cases the antibodies were directed to the mAb and not the attached MMAE. Patients who developed persistently positive antibodies experienced a higher incidence of infusion reactions; two such patients had to discontinue treatment. Thirty-six (62 %) of 58 patients with anti-brentuximab vedotin antibodies had at least one serum sample with neutralizing antibodies. Whether or not such antibodies affect the efficacy and safety of the antibody-drug conjugate is not yet known.

Alemtuzumab

Alemtuzumab (Campath®, MabCampath®), a humanized IgG1κ mAb with complementarity-determining regions from a rat mAb, is targeted to CD52 (Campath-1 antigen) (Tables 2.1 and 3.1), an abundantly expressed 21–28 kDa 12-amino acid glycoprotein of unknown function with a single *N*-linked oligosaccharide. The early name for alemtuzumab, Campath-1, came from the original rat antibodies utilized in the Pathology Department, University of Cambridge. The name Campath-1H was adopted after the rat antibody hypervariable sequences were grafted onto a human antibody framework. CD52 is expressed on mature normal and malignant B and T lymphocytes, monocytes, macrophages, NK cells, a subpopulation of granulocytes, and dendritic cells as well as lymphoid and male sexual organs but not on erythrocytes, platelets, and hematopoietic stem cells. A figure of 5×10^5 CD52 molecules/cells has been reported for human lymphocytes. Approved by both the FDA and EMA in 2001 for the treatment of B-cell CLL resistant to alkylating agents and later as a single agent for the disease, alemtuzumab as Campath® and MabCampath® was widely used in cancer therapy until 2012 when it was withdrawn from US and European markets although a number of CLL patients could still receive it through specific access programs and some off-label usage in cancer therapy remains. Since 2012, alemtuzumab has been relaunched as Lemtrada® for multiple sclerosis (see Chap. 2, Table 2.1, and Chap. 4, section "Alemtuzumab"). After binding to CD52 on leukemic cells, alemtuzumab exerts its antitumor action primarily by ADCC with a contribution from CDC and induction of apoptosis.

The list of generally nonserious adverse effects occurring in patients given alemtuzumab, many similar to other mAbs, is summarized in Table 3.1. Serious adverse effects do, however, occur. In addition to the immunosuppression that accompanies CLL, cytopenia, resulting from alemtuzumab's mode of action in destroying white blood cells, is also the major adverse event resulting from the mAb's use in treating CLL, hence the FDA boxed warning (Table 3.1). As well as a high incidence of neutropenia (75–85 %), febrile neutropenia and thrombocytopenia (serious in 57 % of cases) may occur, the latter causing hemorrhagic problems and purpura. Infections related to alemtuzumab therapy have been reported with incidences of up to 80 % and serious events have shown incidences as high as 50 %. Bacterial and viral infections are common and are a significant cause of death; in fact, fatalities among patients on alemtuzumab therapy are mostly connected to infections. Protozoal infections are occasionally seen, and opportunistic infections, including pneumocystis pneumonia, herpesvirus and CML infections, candidiasis, JC virus activation, aspergillus, and mucormycosis, are relatively frequent with incidences up to ~40 %. *Mycobacterium tuberculosis*, another possible risk organism for opportunistic infections, has been implicated in cases of tuberculosis in alemtuzumab-treated patients receiving a renal transplant from deceased patients. A retrospective study of nonbacterial infections in 182 Asian patients treated with alemtuzumab showed that the most common infections were reactivation of CML (36.3 %), fungal infections (17 %), and varicella zoster virus (13.7 %).

The authors recommended routine prophylaxis with antiviral drugs, especially for patients receiving allogeneic stem cell transplants. The possibility of infection and a life-threatening outcome during alemtuzumab therapy was emphasized.

The destruction of T cells by alemtuzumab with resultant lymphopenia and consequent release of cytokines can lead to CRS, and potentially nephrotoxic TLS may occur as a result of the rapid and massive destruction of neoplastic cells. Infusion reactions to alemtuzumab, manifesting as pyrexia, chills, nausea, emesis, dyspnea, rash, and urticaria, predictably occur most commonly during the first week of treatment. Serious reactions that can be fatal are known. Signs and symptoms include bronchospasm, ARDS, pulmonary infiltrates, cardiac disorders, angioedema, and anaphylaxis. In neoplastic off-label applications of alemtuzumab therapy, for example, in T-cell lymphoma patients, severe pancytopenia associated with hemophagocytosis and viral reactivations has been noted. Of 4000 reports to the FAERS database during postmarketing surveillance, the largest group covered various infections with 40 % due to viruses, particularly CML, and with fungal infections also prominent. Other frequently reported events were white blood cell abnormalities and immune disorders (Goodpasture's syndrome, Graves' disease, aplastic anemia, Guillain-Barré syndrome, serum sickness, chronic inflammatory demyelinating polyradiculoneuropathy). Idiopathic thrombocytopenic purpura, TLS, and PML each made up from 0.7 to 1.1 % of the reports. Other reported adverse events were cardiomyopathy, decreased ejection fraction, and optic neuropathy.

Antihuman antibodies have been detected in 11 of 133 (8.3 %) patients previously untreated with alemtuzumab and in 4 of 211 (1.9 %) previously treated patients. Two patients in the former group proved positive for neutralizing antibodies to the mAb. The limited available data suggest that human antibodies to alemtuzumab do not adversely affect the mAb's antitumor action. In a novel approach to reduce the immunogenicity of alemtuzumab, an attempt was made to tolerize patients by pretreatment with a high dose of a noncell-binding variant of the mAb differing by a single point mutation in the H2 loop of the H chain. After two cycles of alemtuzumab treatment with and without the variant antibody, anti-alemtuzumab antibodies were found in 145 of 197 patients (74 %) who did not receive the variant protein and in 4 of 19 patients (21 %) in which tolerization was attempted. See also alemtuzumab (Lemtrada®), Chap. 4, section "Alemtuzumab."

Monoclonal Antibodies Targeting Epidermal Growth Factor Receptor: Cetuximab, Panitumumab, and Necitumumab

Tyrosine kinases are important targets for cancer therapy because of their central role in growth factor signaling leading to cell proliferation, differentiation, and survival. One of the four related growth factor receptors, epidermal growth factor receptor (EGFR, HER1, ErbB1), is a member of the ErbB family of receptors, a subfamily of closely related receptor tyrosine kinases. EGFR is a 170 kDa

transmembrane glycoprotein cell surface receptor activated by the binding of its specific ligands, epidermal growth factor (EGF), transforming growth factor alfa (TGFα), heparin-binding EGF-like growth factor (HB-EGF), amphiregulin, betacellulin, epigen, and epiregulin. After ligand binding, EGFR undergoes homodimerization although EGFR may also form a heterodimer with another member of the ErbB receptor family, for example, ErbB2 (HER2). After dimerization, the receptor-ligand complex is internalized, the autophosphorylation occurs, and the tyrosine kinase signal transduction pathways lead to regulation of gene transcription involved with cell growth and survival, motility, and proliferation. Signal transduction, resulting from natural ligand-induced activation of the EGFR, leads to activation of the wild-type KRAS protein. In tumors with *KRAS* mutations, the mutant KRAS protein is continually active and appears to be independent of regulation by EGFR. In addition to the importance of EGFR in the processes of normal cellular functions and survival, expression of EGFR may contribute to the development of cancerous cells through effects on angiogenesis, cell cycle progression, inhibition of apoptosis, and metastasis. In a number of different tumors, EGFR and its ligands are associated with growth of the cells, and elevated EGFR tyrosine kinase activity is found in many, if not most, solid tumors including breast, renal, head and neck, colon, non-small cell lung cancer, pancreatic, ovarian, prostate, glioma, and bladder carcinomas. The expression of EGFRs on malignant cells is often greater than the numbers expressed on normal cells. Table 3.2 lists the percentages of different types of tumors overexpressing the EGFR. For cancers such as head and neck, non-small cell lung cancer, and renal carcinoma, most of the tumors express the receptor. The EGFR-binding mAbs cetuximab and panitumumab bind the receptor on both normal and tumor cells, competitively inhibiting binding of the normal ligands. Ligand-induced

Table 3.2 EGFR overexpression of some of the most common human solid tumors

Tumor	Percentage of tumors overexpressing EGFR
Breast	15–37[a]
Renal carcinoma	50–90
Head and neck	80–100
Colorectal	25–100[b]
Non-small cell lung carcinoma	40–80
Pancreatic	30–50
Ovarian	35–70
Glioma	40–92[c]
Bladder	31–48
Gastric	33–81
Prostate	40–90

EGFR epidermal growth factor receptor
[a]Other percentages quoted, 14–91 %
[b]25–77 % also quoted for colon cancer
[c]Other percentages quoted, 40–63 %

autophosphorylation of the receptor and activation of receptor-associated kinases are thus prevented, resulting in inhibition of cell growth and decreases in proinflammatory cytokines and the production of vascular growth factor.

Resistance to EGFR-Targeted Monoclonal Antibodies

Anti-EGFR mAbs such as cetuximab bind to the EGFR with a 5–10 times higher affinity than the natural ligands, preventing both binding of these ligands and subsequent activation of tyrosine kinase-mediated signal transduction pathways. Monoclonal antibodies targeting the EGFR were therefore seen as a new approach for treating a range of solid tumors, one that could potentially avoid some of the limitations and safety issues associated with conventional cytotoxic chemotherapy and radiation therapy. As it turned out, toxicity profiles of the mAbs are different to the toxicities of the conventional therapies (see below), but, disappointingly, mAbs along with other targeted cancer therapies have often proved efficacious in only a minority of patients with beneficial response rates varying from 10 % to about 90 %. This situation is clearly apparent with cetuximab and panitumumab both of which bind to, and inhibit, EGFR signaling. The absence of a significant association between EGFR levels and a clinically improved response to cetuximab in colorectal cancer patients led to the findings that patients with mutations in codons 12 and 13 of the *KRAS* gene almost never benefit from cetuximab and panitumumab treatments, but 10–30 % of patients with the wild-type gene respond to the mAbs. As a result of this understanding, regulatory agencies restricted the use of cetuximab and panitumumab to colorectal cancer patients expressing the wild-type *KRAS* gene, and the American Society of Clinical Oncology issued a provisional clinical opinion stating: "patients with metastatic colorectal cancer who are candidates for anti-EGFR antibody therapy should have their tumor tested for *KRAS* mutations. If a *KRAS* mutation in codon 12 or 13 is detected, then patients with metastatic colorectal carcinoma should not receive anti-EGFR antibody therapy as part of their treatment." Note, however, the codon 12 and 13 mutations of *KRAS* account for only ~40 % of the unresponsive patients, so it must be assumed that other mutations might be conferring resistance, and this appears to be the case with mutations in the *BRAF* (B-RAF proto-oncogene serine/threonine kinase), *PI3KCA* (phosphatidylinositol-4-5-biphosphate 3-kinase, catalytic subunit alfa), *NRAS* (neuroblastoma RAS viral oncogene homolog), and *PTEN* (phosphatase and tensin homolog) genes being significantly associated with low response rates. Mutational analysis of *KRAS* and *PI3KCA* and evaluation of the PTEN protein status in patients with metastatic colorectal cancer treated with cetuximab and panitumumab showed 29 % *KRAS* and 13.6 % *PI3KCA* mutations. The latter were significantly associated with clinical resistance to the mAbs and patients had a worse clinical outcome for survival. The analysis revealed that up to 70 % of patients with metastatic colorectal cancer are unlikely to benefit from treatment with anti-EGFR antibodies.

Cetuximab

Cetuximab (Erbitux®) (Tables 2.1 and 3.1) is a recombinant chimeric human-mouse IgG1κ mAb, MW 152 kDa, that binds the extracellular domain of human EGFR. It is prepared by incorporating the Fv regions of a mouse anti-EGFR antibody with the constant regions of human IgG1 H and L immunoglobulin chains. The structural basis of cetuximab's potent inhibition of the EGFR has been identified and shown to be due to two effects: X-ray crystal structure of the Fab antigen-binding fragment of cetuximab in complex with the soluble extracellular region of EGFR (sEGFR) revealed that the mAb interacts with domain III of sEGFR, interfering with access to the ligand-binding region and sterically preventing the receptor from undergoing dimerization.

Cetuximab is approved by the FDA and EMA for the treatment of metastatic colorectal cancer and head and neck cancer. Specifically, cetuximab's approval for colorectal cancer is as a single agent for treating EGFR-expressing tumors after failure of irinotecan- and oxaliplatin-based regimens or in patients intolerant to irinotecan. Additionally, cetuximab is approved for use in combination with irinotecan for patients' refractory to irinotecan therapy. In head and neck cancer, approval is specified for the treatment of squamous cell carcinoma in combination with radiotherapy and for recurrent or metastatic squamous cell carcinoma in combination with platinum- and 5-fluorouracil-based therapy or carcinoma progressing after platinum therapy. The addition of cetuximab in these combination therapies produces an increase in antitumor effects compared to the nonantibody therapies alone. Binding of cetuximab prevents natural ligand-induced receptor autophosphorylation and the activation of receptor kinases, resulting in inhibition of cell growth, apoptosis, and decreases in matrix metalloproteinases, proinflammatory cytokines, the chemokine IL-8 (CXCL8), and vascular endothelial growth factor production. Lowering of VEGF serum levels by cetuximab indicates that antibody-induced VEGF inhibition may be associated with some antitumor activity.

Serious infusion reactions and the possibility of cardiopulmonary arrest make up an FDA boxed warning for cetuximab. The severity and risk of many of the infusion reactions are underlined by the rapid onset of symptoms of airway obstruction, hypotension, shock, cardiac arrest, myocardial infarction, and loss of consciousness, severe reactions (grades 3 and 4) that occurred in 2–5 % of 1373 patients, and the fact that 90 % of the severe cases occurred with the first infusion despite premedication. In relation to anaphylaxis to cetuximab, IgE antibodies to α-D-galactose-(1-3)-β-D-galactose were found in an alarming number of patients, especially in Southern USA, who experienced severe immediate hypersensitivity reactions after receiving the mAb. The disaccharide recognized by the IgE antibodies was shown to be present on the Fab portion of the chimeric antibody at asparagine 88 of the heavy chain, and, interestingly, most of the allergic patients already had the antibodies in their serum before receiving the mAb. The discovery of delayed-onset anaphylaxis, angioedema, and urticaria after consuming red meat and being linked to tick bites and the presence of the alfa-linked disaccharide in red meat provide a possible explanation for this intriguing phenomenon. At

least one protocol for the successful desensitization of cetuximab-induced immediate reactions has been published. Premedication was commenced with prednisolone 12 h and 1 h before and diphenhydramine 30 min before the start of the procedure. The initial infusion dose of cetuximab was 1 μg, doses were doubled every 15 min until a total of 64 mg was achieved, and then a final dose of 325 mg was administered to give a total cumulative dose of 844 mg. The appearance of cutaneous reactions was managed with diphenhydramine, dose and infusion reductions, and a 30 min pause in the ongoing procedure.

Cardiopulmonary arrest and/or sudden death, the second subject of the boxed warning, occurred in 2–3 % of patients treated with cetuximab and radiation or cetuximab and platinum therapy. Most of the fatal cases were in patients with a history of cardiac disease. Other warnings and precautions issued by regulatory agencies are for the possibility of interstitial lung disease which was recorded in 4 of 1570 patients (0.25 %); a variety of dermatologic events already known to occur, for example, "acneiform" rash (with an incidence of 76–88 %, up to 17 % serious), xerosis and fissuring, paronychial inflammation, hypertrichosis, and infectious sequelae such as cellulitis and conjunctivitis; and hypomagnesemia and electrolyte abnormalities. Hypomagnesemia occurred in 55 % of 365 clinical trial patients being given cetuximab, and the condition proved severe (grades 3 and 4) in 6–17 % of participants. Of the more commonly reported adverse events (Table 3.1), gastrointestinal symptoms, infections, electrolyte imbalance, and respiratory and cutaneous disorders rank amongst the highest incidences in both clinical trial results and postmarketing surveillance reports. As with small molecule tyrosine kinase inhibitors such as erlotinib and gefitinib, anti-EGFR antibodies like cetuximab and panitumumab frequently provoke so-called "acneiform" rashes (more correctly, papulopustular eruptions) in a large proportion of patients. These reactions are generally more severe with the mAbs than with the small molecule drugs and tend to be confined to seborrheic regions of the face, scalp, neck, shoulders, and upper trunk (Fig. 3.3a–c). EGFR is expressed by cells in these regions and it is thought that its inhibition leads to defects in the epithelial barrier, allowing the entry of bacteria and ultimately the development of the characteristic rash. Addition of EGFR inhibition to radiotherapy may lead to radiation dermatitis enhancement, producing wet or dry desquamation, necrosis, or cutaneous ulceration (Fig. 3.3d, e). Other far less frequent mucocutaneous adverse effects include pruritus, palmar-plantar rash, telangiectasia, trichomegaly, alopecia, hyperkeratosis, pyrogenic granuloma, skin hyperpigmentation, and mucositis. Severe mucositis is uncommon with EGFR inhibition therapy alone, but it is more likely to occur in combination with cytotoxic chemotherapy or radiotherapy (Fig. 3.3f, g). EGFR inhibition can also affect differentiation of keratinocytes, leading to a decrease in loricrin and the development of xerosis and skin fissures (Fig. 3.3h). Paronychia, which may become superinfected, is a risk for all patients receiving anti-EGFR mAb therapy. As well as paronychia, other nail changes such as periungual pyogenic granuloma may occur (Fig. 3.3i).

Of about 47,000 adverse events recorded in the FAERS database, pneumonia is the most frequently reported event followed by febrile neutropenia. Hypersensitivities include 66 reports of anaphylaxis, and there are 88 cases of

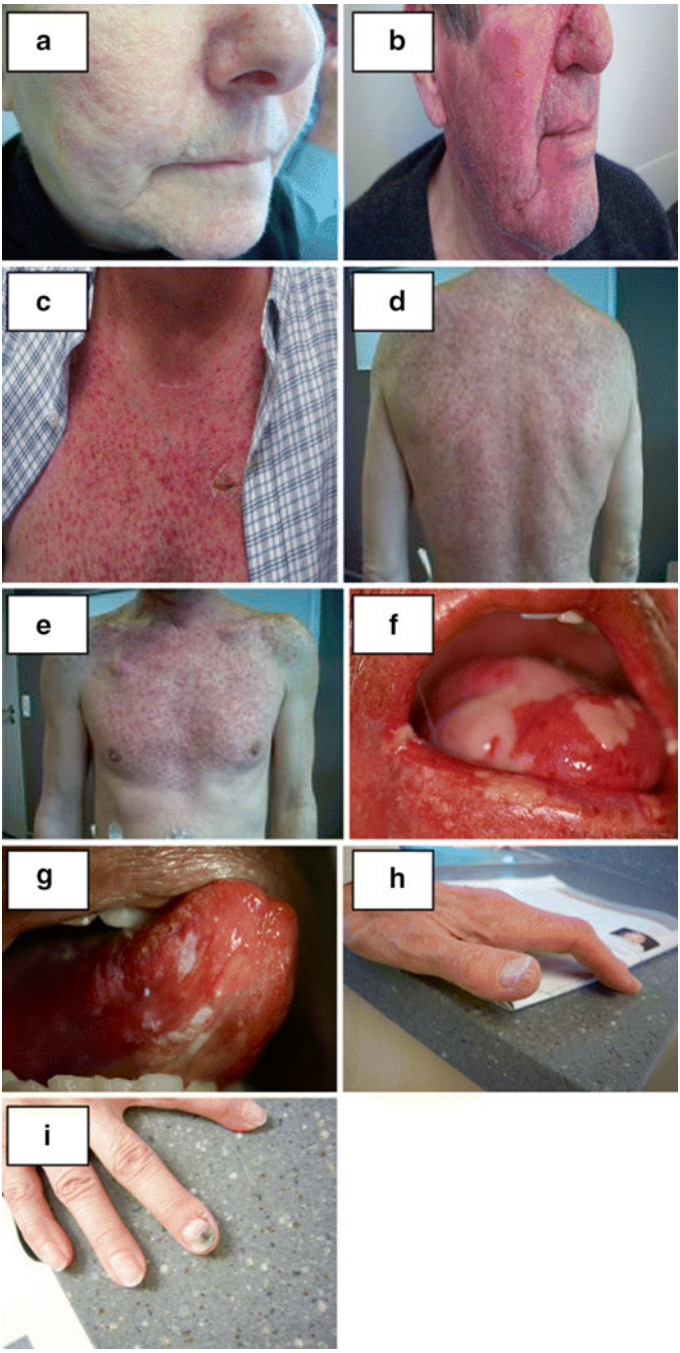


Fig. 3.3 The typical acneiform rash or papulopustular eruption caused by tyrosine kinase inhibitors that bind EGFR manifests as erythematous pruritic papules and pustules. The rash tends to be

papulopustular eruptions, three cases of aseptic meningitis, and two cases of PML. Some obvious discrepancies from these results are found in the European pharmacovigilance database covering nearly 6000 reports and 13,656 adverse events, in particular, records of 997 infusion-related reactions, 585 hypersensitivity responses, 354 anaphylactic reactions, and 38 anaphylactoid reactions. The surprisingly high number of cases of anaphylaxis calls into question the criteria employed to classify the reactions as true, type I IgE antibody-mediated hypersensitivities. Without skin test and rituximab-reactive IgE antibody data on each individual, a diagnosis of anaphylaxis could not be firmly established. Aseptic meningitis is mentioned in 19 reports and PML in two.

Although cetuximab has been reported to trigger a relatively large number of hypersensitivity responses, especially during first infusion sessions, the mAb appears to exhibit rather low immunogenicity. Non-neutralizing human antichimeric antibodies appear to have an incidence of ~4–5 % in treated patients. At least some of these antibodies may be preexisting, in particular so-called "natural" antibodies, mostly of the IgG class specific for the α-1,3-linked D-galactose disaccharide (see above) and antibodies to murine-derived *N*-glycolylneuraminic acid, a structure present in cetuximab but absent in humans.

Panitumumab

Panitumumab (Vectibix®) (Tables 2.1 and 3.1) is a recombinant, fully human IgG2κ mAb that binds the EGFR with high affinity. Like cetuximab, panitumumab is approved for the treatment of colorectal cancer but not yet for head and neck cancer, and it is currently under investigation for the treatment of malignant glioma. For colorectal cancer, both mAbs are equally effective. The antigenic structures recognized by cetuximab have been identified as a large surface conformation on domain III of the EGFR (see above, section "Cetuximab"), and although the complementary structures to the panitumumab combining sites remain poorly defined, it is already clear that both epitopes are not identical. This conclusion is supported by instances of effective treatment with panitumumab in patients with disease progression under cetuximab and the development of resistance to treatment with cetuximab in a colorectal cancer patient who acquired a point mutation in the EGFR domain (Arg

Fig. 3.3 (continued) confined to seborrheic regions of the face, scalp, neck, shoulders, and upper trunk (**a**–**c**). Reactions may occur in 50–100 % of treated patients and tend to be more severe and widespread with EGFR-targeted monoclonal antibodies than small molecule tyrosine kinase inhibitors. When EFGR inhibitors are administered with radiotherapy, a high incidence of radiation dermatitis showing erythema, desquamation, skin necrosis, or ulceration with bleeding may occur (**d**, **e**). Oral complications, most commonly mucositis presenting as stomatitis, are infrequent (**f**, **g**). Skin fissures and cracks (rhagades) may result from xerosis (**h**). Nail changes in the form of paronychia (nail fold inflammation) and periungual pyrogenic granuloma-like lesions may also be seen (**i**). Reproduced from Lacouture ME et al. Clinical practice guidelines for the prevention and treatment of EGFR inhibitor-associated dermatologic toxicities. Support Care Cancer 2011;19:1079–95. Reprinted with permission from Springer Science + Business Media

for Ser at position 468), while panitumumab binding and efficacy remained. It has been suggested that characterization of the binding sites of the two anti-EGFR mAbs could help predict the response to the mAbs in patients with mutations leading to resistance. In an effort to achieve this for panitumumab, epitope recognition was assessed by screening phage display peptide libraries. This approach identified a discontinuous epitope that overlapped with the cetuximab epitope. The overlapping epitopes were shown to consist of 17 amino acids, four of which are targeted by cetuximab and four others by panitumumab. The authors believe that these results have the potential to improve treatments by using the recognition findings to help select patients for EGFR-targeted therapies.

Granted accelerated approval by the FDA in 2006 as a single agent "for the treatment of metastatic colorectal carcinoma with disease progression on or following fluoropyrimidine, oxaliplatin, and irinotecan chemotherapy regimens," panitumumab was subsequently approved by the EMA in 2007 for treatment of wild-type KRAS metastatic colorectal cancer as monotherapy after failure of fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy regimens. In 2009 the FDA restricted the indication to patients with wild-type KRAS tumors, and in 2011 the EMA extended the indication to first-line treatment in combination with FOLFOX (folinic acid [leucovorin], fluorouracil, oxaliplatin) or FOLFIRI (folinic acid, fluorouracil, plus the topoisomerase inhibitor irinotecan) and as second-line treatment in combination with FOLFIRI for patients who have received first-line fluoropyrimidine-based chemotherapy, excluding irinotecan. In 2014, the FDA revised its indications for panitumumab to include a combination of the mAb with FOLFOX for first-line treatment.

At first approval of panitumumab in 2006, the FDA issued a boxed warning for dermatologic toxicity and infusion reactions stating that dermatologic toxicities occurred in 89 % of patients with 12 % being CTC grade 3 or higher severe reactions (Chap. 1, section "Terminology: Adverse Reactions and Adverse Events"). Severe infusion reactions were said to occur in ~1 % of patients. In the revised prescribing information issued by the FDA in August 2014, reference to infusion reactions in the boxed warning was removed and dermatologic toxicities were stated to be severe in 15 % of patients. Mucocutaneous diseases provoked by panitumumab contribute to an extensive range of clinical manifestations including erythema, rash, pruritus, skin exfoliation, acneiform dermatitis, xerosis, paronychia, and skin fissures as well as life-threatening infectious complications such as necrotizing fasciitis and abscesses. Life-threatening bullous mucocutaneous diseases with erosions, blisters, and skin sloughing following panitumumab are known, but it is not always easy to ascribe these to antibody-induced inhibition of EGFR or to immune-related drug-induced toxidermias, for example, Stevens-Johnson syndrome or toxic epidermal necrolysis. Other warnings and precautions associated with the use of panitumumab (Table 3.1) relate to the possibilities of severe infusion reactions (grade 3–4) with the reminder that fatal reactions have occurred; severe hypomagnesemia (which showed a 7 % incidence in clinical trials) as well as hypocalcemia and hypokalemia; acute renal failure resulting from severe diarrhea and dehydration when panitumumab is used in combination with chemotherapy; cases, some fatal, of interstitial lung disease and pulmonary

fibrosis; ocular toxicities such as keratitis and ulcerative keratitis; dermatologic toxicities caused by sunlight exposure as a result of panitumumab-induced photosensitivity; and the possibility of increased toxicity and mortality when panitumumab is administered in combination with bevacizumab and chemotherapy. In the EMA summary of the safety profile of panitumumab, adverse reactions occurring in ≥20 % of patients are made up of cutaneous disorders, gastrointestinal disorders (diarrhea, nausea, vomiting, constipation, abdominal pain), general disorders (fatigue, pyrexia), infections and infestations, and anorexia. Importantly, when panitumumab was used in combination with chemotherapy, the safety profile was assessed as the adverse events seen with the mAb as monotherapy plus the toxicities of the chemotherapy regimen. When this was done, no new toxicities or worsening of previously recognized toxicities beyond the expected additive effects were reported by the EMA. The most common adverse reactions (≥20 %) listed by the FDA are skin rashes of variable presentation, paronychia, fatigue, nausea, and diarrhea. Other common adverse reactions showing a ≥5 % difference compared to the reactions seen in patients given the best supportive care alone were stomatitis, mucosal inflammation, dyspnea, cough, and an extensive range of skin and subcutaneous tissue disorders.

An interesting recent case report describes the safe treatment with panitumumab of a patient in need of anti-EGFR therapy for rectal carcinoma but who was also allergically sensitized to the disaccharide α-D-galactose-(1-3)-β-D-galactose (see section "Cetuximab"). After allergological testing showed that the patient had α-D-galactose-associated red meat and gelatin allergy and was skin test negative to panitumumab, intraoperative use of gelatin-derived colloids and treatment with cetuximab, both known to carry α-D-galactose residues, were prohibited and panitumumab as anti-EGFR therapy was successfully initiated.

Postmarketing surveillance reports on panitumumab to the FAERS database at the end of 2012 totaled 3987 with 13,830 events. Dermatologic events showed the highest incidence (8.1 %), followed by infections (7.2 %), electrolyte problems (4.7 %), and gastrointestinal disorders (4.2 %). In order of the most frequent events, diarrhea headed the list with 605 reports followed by acneiform dermatitis (595), interstitial lung disease (310), febrile neutropenia (277), hypomagnesemia (276), rash (193), and sepsis (108). Six cases of anaphylaxis, 12 of maculopapular rash, and six of toxic epidermal necrolysis were recorded and angioedema appears to have been reported for the first time. Eye disorders included 25 cases of conjunctivitis and six of keratitis. Postmarketing surveillance reports to the European pharmacovigilance database showed a similar spectrum of adverse events, but incidences of cutaneous reactions (25.8 %), gastrointestinal disorders (9.8 %), and respiratory disorders (8.4 %) were higher.

Immunogenicity of panitumumab appears to be low, a conclusion in keeping with the fully human nature of the antibody. Detection of antibodies to the mAb in 1123 patients receiving panitumumab monotherapy showed an incidence of 0.4 % (five patients) when measured by an ELISA and 3.2 % (36 patients) in a Biacore® assay. Nine patients (0.8 %) had anti-panitumumab neutralizing antibodies. In combination with chemotherapy, the ELISA assay detected an incidence of anti-panitumumab antibodies of 0.9 % (12 of 1297), while the incidence found with

the Biacore® assay was 0.7 % (9 of 1296). Only 0.15 % of patients (2 of 1297) had neutralizing antibodies, and, overall, no safety concerns were found in the patients who developed antibodies. In four clinical trials assessing panitumumab plus chemotherapy, antibodies to the mAb, including neutralizing antibodies, were detected with two different immunoassays and a bioassay. Twenty patients (1.8 %) treated with panitumumab in combination with oxaliplatin or irinotecan therapy developed antibodies and two (0.2 %) developed neutralizing antibodies. Patients with tumors expressing wild-type or mutant *KRAS* and patients receiving oxaliplatin or irinotecan therapies showed similar incidences of anti-panitumumab antibodies. The presence of anti-panitumumab antibodies had no noticeable effect on safety or pharmacokinetic profiles.

Necitumumab

Necitumumab (Portrazza®) (Tables 2.1 and 3.1), approved by the FDA in November 2015, is a recombinant human IgG1κ mAb, MW ~144.8 kDa, targeted to human EGFR. Indicated for first-line treatment of patients with metastatic squamous non-small cell lung cancer in combination with gemcitabine and cisplatin, necitumumab carries FDA black box warnings for cardiopulmonary arrest and/or sudden death and for hypomagnesemia (Table 3.1). The former has been seen in ~3 % of patients treated with the mAb plus gemcitabine and cisplatin; the latter in more than 80 % of patients treated with the mAb-small molecule drug combination. Cardiopulmonary arrest or sudden death occurred in 15 of 538 patients (2.8 %) treated with necitumumab-gemcitabine-cisplatin compared to three of 541 (0.6 %) of patients given gemcitabine and cisplatin alone. Hypomagnesemia occurred in 83 % of 461 patients treated with necitumumab (20 % severe) compared to 70 % treated with the small MW drugs alone (7 % severe). Patients should be monitored for hypomagnesemia, hypocalcemia, and hypokalemia. Five other warnings/precautions issued for necitumumab are the possible occurrence of venous and arterial thromboembolic events; some fatal, infusion-related reactions; embryofetal toxicity; increased toxicity and mortality in patients with non-squamous non-small cell lung cancer treated with the mAb plus pemetrexed and cisplatin; and dermatologic toxicities. Skin toxicity, generally developing within the first 2 weeks and severe in 8 % of patients, manifests as generalized rash, dermatitis acneiform, xerosis, pruritus, maculopapular rash, and erythema in up to ~79 % of patients receiving necitumumab. Other adverse reactions to necitumumab recorded so far (Table 3.1) are essentially those seen in clinical trials; postmarketing experience will undoubtedly add to this so far small list.

Bevacizumab

Bevacizumab (Avastin®) (Tables 2.1 and 3.1) is a recombinant humanized IgG1κ mAb, MW ~149 kDa, that binds to, and inhibits, the biological action of human vascular endothelial growth factor-A (VEGF-A) (Fig. 3.4). VEGFs are a family of

secreted proteins with a highly conserved receptor-binding cystine-knot structure comprising five members. Discovered in 1983 and often described as the "founding member" of the family, VEGF-A (also called VEGF and vascular permeability factor [VPF]) is regarded as the most important regulator of the formation of blood vessels in health and disease. The five members of the VEGF family are distinguished as VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placenta growth factor (PlGF, PGF). Before the discovery of the different family members, VEGF-A was simply called VEGF. VEGF-A binds to receptors VEGFR-1 (also called Flt-1) and VEGFR-2 (KDR/Flk-1) although it is the latter receptor that seems to mediate most, if not all, of the cellular responses to VEGF (Fig. 3.4). In promoting angiogenesis, VEGF-A (and other VEGFs) acts in endothelial cells through a family of cognate receptor tyrosine kinases. As well as its function of promoting blood vessel formation in healthy subjects, VEGF-A-induced angiogenesis has a major role in the pathogenesis of a wide range of human diseases, for example, cancers, rheumatoid arthritis, and eye diseases (see also ranibizumab, Chap. 4, section "Ranibizumab," and aflibercept Chap. 6, sections "Aflibercept" and "Aflibercept"). It was this understanding of the functions of VEGF-A that led to the development of bevacizumab and its subsequent application to an impressive range of approved cancer indications and off-label treatments.

VEGFR-1 recruits hematopoietic stem cells, while VEGFR-2 regulates vascular endothelial function. During VEGFR-2 intracellular signaling and according to MJ Cross, L Claesson-Welsh, and others, binding of VEGF-A to the receptor extracellular domain induces dimerization and autophosphorylation of specific intracellular tyrosine residues. Several intracellular proteins, for example, VEGFR-associated protein (VRAP), Sck, and phospholipase C (PLC-γ), bind to specific phosphorylated tyrosine residues in the receptor via their Src homology-2 (SH2) domains leading to phosphorylation and activation of these proteins. PLC-γ activation leads to hydrolysis of membrane phosphatidylinositol 4,5-biphosphate (PIP_2), a generation of second messenger diacylglycerol (DAG) an activator of protein kinase C, and inositol 1,4,5-triphosphate (IP_3) which binds to a receptor on the endoplasmic reticulum releasing intracellular stored Ca^{2+}. Many other proteins are activated including Src, phosphoinositide 3-kinase (PI3K), and p38 mitogen-activated protein kinase (p38 MAPK), and signal transduction downstream produces several physiologic and pathologic effects including proliferation, migration, permeability, and survival. To grow and proliferate, tumors need a matching blood supply. Increased expression of VEGF has been found in many human solid tumors, presumably fueling angiogenesis and assisting tumors to grow aggressively. By binding to VEGF-A, bevacizumab prevents activation of VEGFR-2 (Fig. 3.4) and the generation of new tumor vasculature. It also appears that bevacizumab enhances cytotoxic effects of concurrent chemotherapy.

Bevacizumab, in combination with fluoropyrimidine-based chemotherapy, was approved for metastatic colorectal cancer first by the FDA in 2004 and then by the EMA the following year. In subsequent years, approvals by both agencies were extended to metastatic breast cancer (with paclitaxel or capecitabine); non-squamous, non-small cell lung cancer (with a platinum agent); metastatic renal cell carcinoma (with interferon alfa-2a); and cervical cancer (with paclitaxel and cisplatin or pacli-

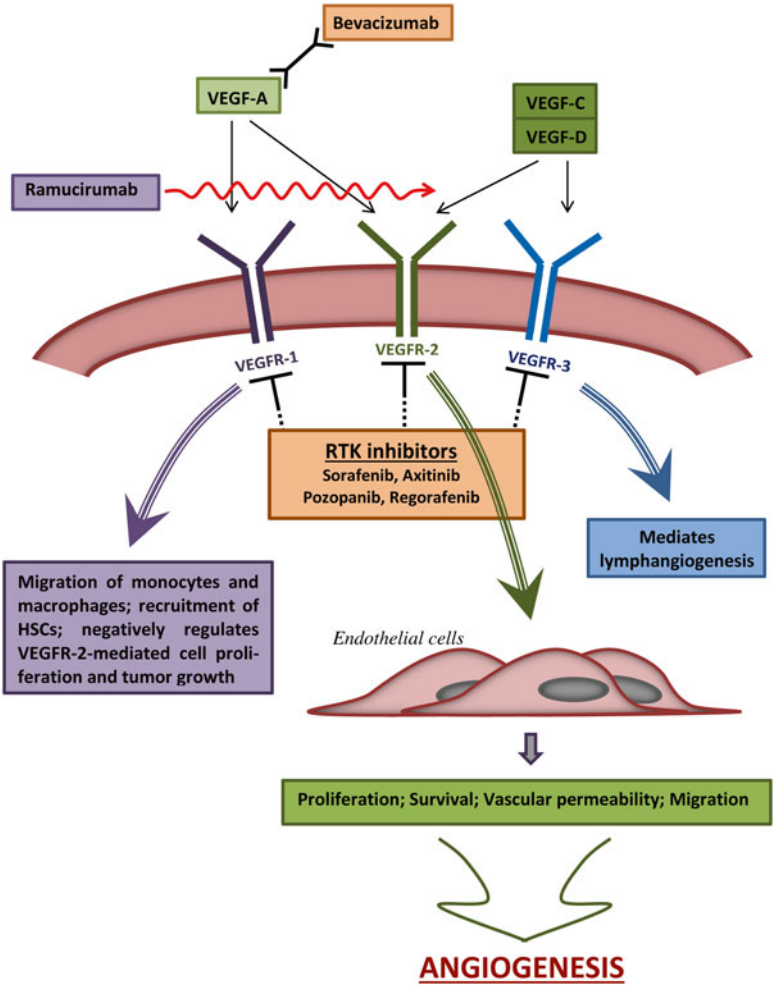


Fig. 3.4 Binding of VEGFR-2 (Flk1) to its ligand initiates receptor dimerization followed by autophosphorylation; activation of multiple downstream pathways including PLC-γ, MAPK, Akt, and Src; proliferation of endothelial cells; and ultimately, via PI3K, increased cell survival, cell migration, and vascular permeability mediated via endothelial nitric oxide synthase. VEGFR-1 (Flt1) is structurally similar to VEGFR-2 but unlike VEGFR-2 is kinase impaired. It may influence angiogenesis by acting as a decoy receptor, binding VEGF-A and preventing it from binding to VEGFR-2, or by heterodimerization with VEGFR-2. VEGFR-1 also binds exclusively VEGF-B and placental growth factor (PlGF). VEGFR-3 (Flt4) mediates lymphangiogenesis in response to VEGF-C and VEGF-D. Targets for bevacizumab, ramucirumab, and small molecule receptor tyrosine kinase inhibitors are shown. *HSCs* hematopoietic stem cells, *RTK* receptor tyrosine kinase

taxel and topotecan). The indication for metastatic breast cancer was withdrawn in 2010 by the FDA due to safety and efficacy concerns. Four adverse events, proteinuria, hypertension, left ventricular dysfunction, and hemorrhagic events, were found to show a statistically significant bevacizumab-associated risk in breast cancer therapy. Glioblastoma was added by the FDA (but not by the EMA) as an indication for bevacizumab monotherapy in 2009, and in 2012 the EMA, with certain requirements, approved bevacizumab in combination with chemotherapy for the treatment of epithelial ovarian, fallopian tube, and primary peritoneal cancers.

Although the lists of approved indications for bevacizumab vary between different regulatory agencies, the relatively wide variety of tumors (about 30, including investigative and preliminary studies) treated by this mAb and its combination with chemotherapy more or less ensures a large number and variety of consequent adverse events (Table 3.1). An extensive list of warnings and precautions is headed by an FDA black box warning for gastrointestinal perforation (which has an incidence of up to 3.2 % in treated patients), complications of surgery and wound healing, and severe or fatal hemorrhage. The incidence of bevacizumab-induced gastrointestinal perforation is estimated to be 0.7–1.2 % with a mortality rate of 11.5–37 %. Because bevacizumab has a half-life of 11–50 days, a delay of 5–8 weeks between bevacizumab treatment and surgery is recommended, and for postoperative initiation of bevacizumab therapy, an interval of 28 days after surgery with a fully healed incision is recommended. Although these delays were observed, a patient treated with bevacizumab before surgery and a second cycle of bevacizumab treatment developed a diaphragmatic rupture, a previously unreported serious adverse event. The case highlights the need for careful monitoring after postoperative administration of bevacizumab. Non-gastrointestinal fistulae, some fatal, involving vaginal, vesical, female genital tract, tracheoesophageal, biliary, renal, and bladder sites, also occur but are uncommon. Hemorrhage may be minor such as epistaxis or severe and sometimes fatal in the form of hemoptysis, gastrointestinal and vaginal bleeding, hematemesis, epistaxis, and CNS hemorrhage. Serious and fatal cases of pulmonary hemorrhage have been reported. Both arterial and venous thromboembolic events, some fatal, have been seen in patients receiving bevacizumab. Recorded arterial events include cerebral infarction, ischemic attacks, myocardial infarction, and angina. Cases of grade 3 or 4 hypertension have an incidence of 5–18 %, the incidence of proteinuria (frequency 21–63 %, serious 3 %) is increased in patients receiving the mAb, PRES has been reported with an incidence of <0.5 %, and although infusion reactions are not a major problem, some results indicate that anaphylactic and anaphylactoid reactions to bevacizumab occur more frequently in patients given the protein in combination with chemotherapy. Frequencies of severe neutropenia and serious infections are 21–26 % and 4–5 %, respectively, with pneumonia and wound and catheter infections prominent. Ovarian failure has been seen in premenopausal women receiving bevacizumab in combination with FOLFOX, emphasizing the need to inform females of reproductive age of the fertility risk prior to commencing treatment with the antibody. Table 3.1 lists some of the most common adverse events caused by bevacizumab administration at a rate of >10 % and at least twice the control rate; additions to this list include

rhinitis, dysgeusia, and back pain. Other commonly seen adverse events (any grade) associated with bevacizumab include asthenia, pain, headache, diarrhea, nausea, vomiting, constipation, and stomatitis.

Considering postmarketing surveillance, an analysis of all 351 serious cases associated with bevacizumab and recorded in the French pharmacovigilance database up to the end of 2010 revealed that reactions of the gastrointestinal tract (21.9 %), thromboembolic events (4 %), pulmonary embolism (3.2 %), hypertension (2.7 %), gastrointestinal hemorrhage (2.7 %), and cerebral hemorrhage or vascular accident (2.6 %) were the most frequently reported adverse events. Whereas adverse reactions occurred within a median duration of four cycles, nine of 18 deaths due to an adverse reaction occurred after only one cycle. Reactions causing disability were mainly neurologic in origin (frequency 40 %), especially neuropathy, paralysis, and paresis. A search of the FAERS database for the period 2004–2009 for novel adverse events to bevacizumab revealed the highest number of reports for electrolyte abnormalities followed by cardiovascular events and pneumonitis. Clinically important but unlabeled disorders included necrotizing fasciitis, vessel wall disorders, arrhythmia and conduction disorder, and autoimmune thrombocytopenia. Of 37,000 reports on bevacizumab in the FAERS database at the end of 2012, infections (6.2 %), gastrointestinal (5.5 %), hematologic (3.8 %), and respiratory (3.5 %) events showed the highest incidences. More specifically, the postmarketing period has seen reports for polyserositis, PRES, venous occlusion, gallbladder perforation, nasal septum perforation, arterial thromboembolic events, hemorrhage, and numerous eye disorders (from unapproved intravitreal use), for example, permanent vision loss, endophthalmitis, intraocular inflammation, retinal detachment, increased intraocular pressure, hemorrhage, vitreous floaters, and ocular hyperemia. In the European pharmacovigilance database at the end of 2012, gastrointestinal (incidence 20.4 %), neurologic (8 %), infectious (5 %), hematologic and malignant (4 %), cutaneous (3 %), and renal (2.5 %) disorders made up the most frequent adverse events of the 17,672 mostly serious reports. Hypertension contributed 597 cases, deep vein thrombosis 367, gastrointestinal perforation 333, proteinuria 243, sepsis 172, pneumonia 138, and acute renal failure 133, and there were 86 reported cases of nephrotic syndrome. Hypersensitivity or hypersensitivity-like responses showing the highest frequencies were anaphylaxis with 89 cases and anaphylactoid reactions with 39 cases. For mucocutaneous reactions, palmar-plantar erythrodysesthesia was the most common adverse event with 136 cases. Other less commonly seen adverse events reported during the postmarketing period include various ocular disorders, mesenteric venous occlusion, gastrointestinal ulcer, intestinal necrosis, anastomotic ulceration, gallbladder perforation, osteonecrosis of the jaw, nasal cavity lesions including septum perforation, and dysphonia.

There appears to be a dearth of data on the immunogenic activity of bevacizumab in clinical trials and over the postmarketing years. It has been stated that high titers of antibodies to the mAb were not found in 500 treated patients and the FDA has referred to the detection of anti-bevacizumab serum antibodies in 14 of 2233 (0.63 %) colon cancer patients. Three of the patients had neutralizing antibodies for the mAb, but their clinical significance was not determined.

Ramucirumab

Ramucirumab (Cyramza®) (Tables 2.1 and 3.1), a recombinant fully human IgG1κ mAb, MW ~147 kDa, prepared using a phage display library and affinity maturation selection, has binding specificity for VEGFR-2 [CD309, also known as kinase insert domain-containing receptor (KDR)], the receptor-mediating angiogenesis. VEGF-A and VEGFR-2 are often upregulated in a number of human diseases including cancers, and since uncontrolled and sustained angiogenesis is a major contributor, if not promoter, of tumor growth, targeted inhibition of the development of blood vessels is an accepted antitumor strategy. This strategy can be pursued in at least four ways: by administering small molecule, usually multitargeted, tyrosine kinase receptor inhibitors with anti-angiogenic specificity (e.g., sorafenib, sunitinib, axitinib, pazopanib, and regorafenib) (Fig. 3.4); by the selective VEGF antagonist, the pegylated oligonucleotide pegaptanib (Macugen®; indicated for wet age-related macular degeneration and the only aptamer currently approved for clinical use by the FDA), which binds to extracellular VEGF thereby inhibiting its binding to its receptor; by mAbs binding VEGF as practiced with bevacizumab binding VEGF-A (section "Bevacizumab") or the fusion protein aflibercept which targets VEGF-A, VEGF-B, and PlGF; or by targeting the VEGFR-2 receptor, blocking not only the VEGF-A but also other growth factors including VEGF-C, VEGF-D, and VEGF-E. Ramucirumab is such an antibody, targeting VEGFR-2 with high affinity and thereby inhibiting an array of biological activities including receptor activation and signaling, intracellular Ca^{2+} mobilization, and proliferation and migration of endothelial cells (Fig. 3.4). Tumor angiogenesis, a highly complex process, is the ultimate downstream result of VEGFR-2 activation and signaling. It begins with binding of VEGFR-2 to its ligand which initiates receptor dimerization and leads to intracellular autophosphorylation; activation of multiple downstream pathways including PLC-γ, MAPK, Akt, and Src; proliferation of endothelial cells; and, ultimately, via PI3K, increased cell survival, cell migration, and vascular permeability mediated via endothelial nitric oxide synthase.

Approved by the FDA in April 2014, ramucirumab has had a history of rapid additions to its original indication as a single agent for advanced gastric cancer or gastroesophageal junction adenocarcinoma after fluoropyrimidine- or platinum-containing chemotherapy. In November 2014, the FDA added approval for the option of the addition of paclitaxel to the monotherapy; in December 2014 approval was granted for combination therapy with docetaxel for treatment of metastatic non-small cell lung cancer (on or after platinum-based chemotherapy); and, most recently, in April 2015, approval was forthcoming for the use of ramucirumab with FOLFIRI in second-line treatment of colorectal cancer after prior treatment with bevacizumab, oxaliplatin, and fluoropyrimidine. Granted orphan drug status for gastric cancer by the EMA in 2012, ramucirumab in monotherapy and combination therapy with paclitaxel was, as with the FDA, approved by the agency in December 2014 for advanced gastric and gastroesophageal junction cancers. Ramucirumab is currently undergoing evaluation for hepatocellular carcinoma and breast cancer in phase III studies, so its list of approved indications and usage may be further expanded.

Safety data accumulated so far for ramucirumab is headlined by an FDA boxed warning for an increased risk of hemorrhage (including gastrointestinal hemorrhage that may be severe and sometimes fatal), gastrointestinal perforation, and impaired wound healing. Risk of the latter two adverse events is known to sometimes increase during anti-angiogenic therapy. Both the FDA and EMA have issued a number of other warnings. Serious and sometimes fatal arterial thrombotic events, including myocardial infarction, cardiac arrest, cerebrovascular accident, and cerebral ischemia, were seen in clinical trials, for example, in 1.7 % of 236 patients who were given ramucirumab monotherapy for gastric cancer. On the basis of results obtained with ramucirumab showing an increase in patients with severe hypertension after monotherapy or when combined with paclitaxel or docetaxel, it is recommended that blood pressure should be monitored at least fortnightly. Infusion-related reactions may occur, usually during or following the first or second infusion. An initial incidence of reactions of 16 % in a small number of patients prompted the institution of premedication protocols across clinical trials of the mAb. In severe cases, symptoms include bronchospasm, tachycardia, and hypotension. FDA warnings/precautions also apply for PRES (incidence so far <0.5 %), proteinuria including nephrotic syndrome (incidence 3 % in patients given ramucirumab/FOLFIRI), and thyroid dysfunction (incidence of hypothyroidism in patients given ramucirumab/FOLFIRI, 2.6 %). There have been some reports of clinical deterioration in patients with Child-Pugh scores B or C for cirrhosis following ramucirumab monotherapy, leading to the recommendation that ramucirumab should only be used in such patients if the potential benefits are judged to outweigh the risks.

With ramucirumab now approved by the FDA/EMA as monotherapy or combination therapy for a range of different cancers, safety assessments need to take the different treatment regimens into account. When given as a single agent for gastric cancer, the most commonly seen adverse reactions to ramucirumab were hypertension, diarrhea, headache, and hyponatremia in that order; the most common serious events proved to be anemia and intestinal obstruction. Reactions reported as clinically relevant were neutropenia, epistaxis, intestinal obstruction, arterial thrombotic events, and rash. Combination of the mAb with paclitaxel commonly induced fatigue, diarrhea, and neutropenia with the latter event and febrile neutropenia, constituting the most serious adverse events. Events resulting in discontinuation of therapies were neutropenia and thrombocytopenia, while sepsis and gastrointestinal perforations were assessed as clinically relevant adverse reactions. Safety assessments of the administration of ramucirumab in combination with docetaxel to non-small cell lung cancer patients revealed neutropenia, fatigue/asthenia, and stomatitis/mucosal inflammation as the most common adverse responses. Events most commonly implicated in treatment discontinuations were infusion reactions and epistaxis. The incidences of pulmonary hemorrhage in mAb plus docetaxel-treated patients compared to placebo plus docetaxel patients were not significantly different. The most common serious adverse events with the mAb-docetaxel therapy were febrile neutropenia, pneumonia, and neutropenia. The most common adverse reactions in patients with colorectal cancer given ramucirumab plus FOLFIRI and compared to

placebo plus FOLFIRI were diarrhea, neutropenia, decreased appetite, epistaxis, and stomatitis. Neutropenia and thrombocytopenia were the most common causes of treatment discontinuation in the ramucirumab-FOLFIRI group; the main adverse reactions leading to treatment discontinuation due to ramucirumab alone were proteinuria and gastrointestinal perforation. The most serious adverse events following ramucirumab plus FOLFIRI were diarrhea, intestinal obstruction, and febrile neutropenia.

The EMA and FDA refer to the immunogenicity data on ramucirumab assembled so far from clinical trials. As summarized by the EMA, in 527 ramucirumab-treated patients, 11 (2.2 %) developed anti-ramucirumab antibodies compared to 2 (0.5 %) of control patients. No neutralizing antibodies were detected and no infusion-related reactions occurred. The FDA has reported a 3 % incidence (86 of 2890 patients) of anti-ramucirumab antibodies in 23 clinical trials. Fourteen of the 86 antibody-positive patients had neutralizing antibodies.

While anti-VEGFR-2 therapy has shown some encouraging signs of efficacy, the often-seen modest survival benefits are a reminder that our understanding of angiogenic resistance mechanisms remains poor. This may be because although VEGFR-2 is undoubtedly a major, if not *the* major, signaling pathway for the biological processes and events of angiogenesis and VEGF-C and VEGF-D signaling through VEGFR-2 is blocked by ramucirumab (Fig. 3.4), the VEGFR-2 pathway is not the only one—a number of others, for example, P1GF, platelet-derived growth factor (PDGF), angiopoietin, and ephrin pathways, may contribute to the formation of tumor blood vessels. New approaches are clearly needed to overcome tumor resistance to anti-angiogenesis therapies, and given ramucirumab's long half-life (up to ~15 days), it has been suggested that combination anti-angiogenesis strategies should be sought. Investigations to identify suitable angiogenic biomarkers should also be pursued to optimize patient selection, treatment efficacy, and toxicity minimization.

Monoclonal Antibodies Targeting Human Epidermal Growth Factor 2 (HER2): Pertuzumab, Trastuzumab, and Ado-trastuzumab Emtansine

Human epidermal growth factor receptor 2 or HER2 (also known as HER2/neu, ErbB2, CD340, and p185) is a member of the erythroblastic leukemia viral oncogene (gene ErbB) family of four members, the others being HER1 (EGFR, ErbB1), HER3 (ErbB3), and HER4 (ErbB4). The ErbB proteins are each receptor tyrosine kinases related to EGFR (section "Monoclonal Antibodies Targeting Epidermal Growth Factor Receptor: Cetuximab, Panitumumab, and Necitumumab"). The structure of the HER2 receptor, a 185 kDa protein, consists of an extracellular ligand-binding domain, a transmembrane spanning section, and an intracellular protein tyrosine kinase domain with a regulatory carboxy-terminal. An intracellular

tyrosine kinase domain also exists for HER1 and HER4 but not HER3. HER2 is inactive in the monomeric state and needs to be in the dimeric or oligomeric state for activation. Although there are 11 growth factors that activate the ErbB receptors (epidermal growth factor [EGF], transforming growth factor alfa [TGFα], heparin-binding EGF-like growth factor [HB-EGF], amphiregulin, betacellulin, epigen, epiregulin, and neuregulins 1, 2, 3, and 4), there is no known natural ligand for HER2. Activation of receptor kinase function proceeds mainly via ligand-mediated hetero- or homodimerization. In addition to the ligand-dependent pathway for activation, ligand-independent receptor activation can occur with activation being triggered by overexpression of HER2 and a high concentration of cell surface receptors that results in the formation of HER2/HER2 homodimers. Overexpression of HER2 leads to constitutive activation of the growth factor signaling pathways with a consequent favorable environment for breast cancer cell growth. Heterodimers with EGFR, HER3, and HER4 also form. For receptor dimerization, HER2 is not only the preferred but also the most important partner. HER3 has a high affinity for HER2 and the HER2/HER3 heterodimer appears to be the most potent in promoting the signal transduction process and tumor promotion. Autophosphorylation of the tyrosine residues on the intracellular domain of HER2 activates the MAPK, PI3K/Akt, PLC-γ, PKC, and signal transducer and activator of transcription (STAT) pathways leading to cell survival and proliferation.

Overexpression of HER2 as a result of gene amplification is found in ~15–25 to 30 % of human breast cancers, and this overexpression tends to correlate with tumors that are more aggressive and with poorer prognosis. Three mAbs targeting HER2 are currently approved by the FDA and EMA, pertuzumab, trastuzumab, and the ADC prepared by conjugating trastuzumab to the cytotoxin, mertansine.

Pertuzumab

Pertuzumab (Perjeta®, 2C4) (Tables 2.1 and 3.1) is a recombinant humanized IgG1κ mAb, MW ~148 kDa, targeting the extracellular dimerization domain (subdomain II) of the HER2 protein thereby blocking heterodimer formation and ligand-dependent signaling via two major pathways, MAPK and PI3K. Inhibiting downstream signaling arrests cell growth and leads to apoptosis, while ADCC further contributes to the mode of action of pertuzumab. The action of pertuzumab is independent of the level of expression of HER2. Although pertuzumab and trastuzumab target the same receptor, they recognize different binding sites. Trastuzumab (section "Trastuzumab," below) binds to subdomain IV of the HER2 extracellular (ligand-binding) domain inhibiting ligand-independent signaling and complementing the mechanism of action of pertuzumab which inhibits ligand-dependent signaling, particularly between HER2 and HER3. This combination of actions potently activates cell survival and proliferation (Fig. 3.5b). The clinical efficacy of pertuzumab alone is not impressive, but together with trastuzumab, the combination produces a more complete and effective, if not synergistic, blockade of the HER2-driven signaling pathways.

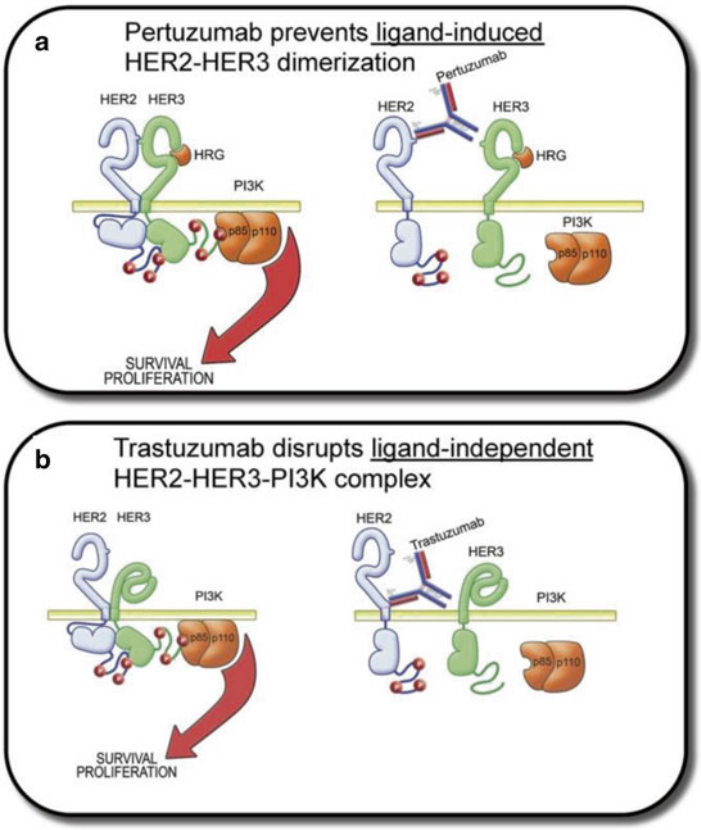


Fig. 3.5 Diagrammatic representation of the general mechanisms of action of pertuzumab and trastuzumab, respectively, in disrupting ligand-independent and ligand-dependent HER2/HER3 interactions that result in antiproliferative effects in HER2-positive tumor cells. (**a**) Pertuzumab blocks the ligand-induced HER2–HER3 dimerization by binding to HER2 extracellular subdomain II. (**b**) Ligand-independent HER2/HER3 interaction is followed by HER3 phosphorylation activating the PI3K signaling pathway leading to cell survival and proliferation. Trastuzumab disrupts the HER2–HER3 interaction by binding to extracellular subdomain IV of HER2. Reproduced from Junttila TT et al. Ligand-independent HER2/HER3/PI3K complex is disrupted by trastuzumab and is effectively inhibited by the PI3K inhibitor GDC-0941. Cancer Cell 2009;15:429–40. Modified and reprinted with permission from Elsevier Limited

Pertuzumab was granted approval by the FDA in June 2012 for use in combination with trastuzumab and docetaxel in patients with HER2-positive metastatic breast cancer who have not received prior anti- HER2 therapy or chemotherapy. The approved indications and usage also cover the same combination as neoadjuvant therapy of patients with HER2-positive, locally advanced, inflammatory, or early stage breast cancer as part of a complete treatment regimen for early breast cancer. The EMA gave final approval for pertuzumab in the same combination therapy in March 2013.

The FDA has issued a boxed warning for pertuzumab covering cardiomyopathy manifesting as congestive heart failure and decreased left ventricular ejection fraction (LVEF) and for embryofetal toxicity that may result in fetal death and birth defects. It is believed that patients who have received prior treatment with anthracyclines or prior radiotherapy to the chest may be at higher risk of decreased LVEF. The incidence of left ventricular systolic dysfunction (LVSD) was found to be higher in pertuzumab-treated patients compared to trastuzumab- and docetaxel-treated patients, and there was an increased incidence of declines in LVEF in those treated with the mAb-drug combination. Administration of pertuzumab to pregnant women can harm the fetus, and the need for this warning is backed up by the observation of oligohydramnios, delayed development of fetal kidneys, and embryofetal death in pregnant monkeys. Infusion-related reactions and hypersensitivity responses including anaphylaxis are the subjects of the remaining FDA warnings for pertuzumab. Described as "hypersensitivity, anaphylactic reaction, acute infusion reaction, or cytokine release syndrome occurring during an infusion or on the same day as the infusion," the frequency of reactions to pertuzumab alone when the mAb was given on day one without trastuzumab and docetaxel was 13 % compared to 9.8 % in the placebo group. Grade 3 and 4 reactions made up less than 1 % of the infusion reactions. Pyrexia, chills, fatigue, headache, asthenia, hypersensitivity, and vomiting were the main symptoms. In a clinical trial involving 804 patients with HER2-positive metastatic breast cancer, patients were randomized to receive pertuzumab in combination with trastuzumab and docetaxel or placebo in combination with trastuzumab and docetaxel. After a median study treatment time of 18.1 months for the pertuzumab group, the most common adverse reactions (>30 %) were diarrhea, neutropenia, alopecia, nausea, fatigue, rash, and peripheral neuropathy. The most common grade 3–4 adverse reactions (>2 %) were neutropenia, febrile neutropenia, leukopenia, diarrhea, peripheral neuropathy, anemia, asthenia, and fatigue. Reactions showing a clear higher incidence in the pertuzumab-treated group compared with the placebo-treated group were leukopenia, diarrhea, rash, pruritus, dry skin, and febrile neutropenia. There was an increased incidence of febrile neutropenia in Asian patients in both treatment arms although it was significantly higher in the pertuzumab-treated group (26 %) than the placebo-treated group (12 %). Reactions judged to be clinically relevant in the pertuzumab-treated group and reported in <10 % of patients were LVSD, pleural effusion, hypersensitivity, and paronychia. Results of trials of the pertuzumab drug combination in neoadjuvant treatment of breast cancer showed a similar spectrum of adverse reactions with upper respiratory tract infections as the main addition.

Because of the known trastuzumab-related cardiotoxicity and black box warnings of cardiomyopathy for both pertuzumab and trastuzumab, particular attention was paid to possible cardiac effects when the two mAbs were used together. In fact, cardiotoxicity to pertuzumab has been documented in several studies. In a phase I clinical study, two of 21 patients showed a reduction in LVEF and one developed congestive heart failure after two cycles of pertuzumab. Decreases in LVEF have also been observed in trials of ovarian and advanced prostate cancer patients treated

with pertuzumab. In a study to evaluate safety and efficacy of pertuzumab with trastuzumab in patients with HER2-positive metastatic breast cancer, 11 patients were given 64 cycles of the mAb combination. Echocardiograms and magnetic imaging studies showed LVSD in six patients, three with grade 1, two with grade 2, and one with grade 3 reactions. Although the observed cardiotoxicity was asymptomatic and reversible (and trastuzumab-induced cardiac dysfunction is generally reversible), it must be said that the long-term cardiac effects of pertuzumab-trastuzumab treatments are yet to be determined. More recently, a database of 598 patients treated with pertuzumab was used to examine the incidence of asymptomatic LVSD and symptomatic heart failure. Of 331 patients treated with pertuzumab alone, 23 (6.9 %) developed LVSD and one (0.3 %) displayed symptomatic heart failure. The corresponding figures for 175 patients treated with pertuzumab in combination with a non-anthracycline cytotoxic agent were six (3.4 %) and two (1.1 %) and for 93 patients treated with trastuzumab, six (6.5 %) and one (1.1 %). The results revealed that pertuzumab given as a single agent or in combination with cytotoxic agents, including trastuzumab, showed a low incidence of cardiac dysfunction similar to trastuzumab. In response to the mAb, patients experienced relatively low incidences of both LVSD and symptomatic heart failure, and there was no significant increase in cardiac side effects when pertuzumab was given in combination with other anticancer agents. Importantly, no synergistic effect between the two mAbs was apparent.

Because of the recent approval of pertuzumab by the FDA and EMA, there have so far been relatively few postmarketing reports on the mAb's safety. At mid-2013, 234 reports to the FAERS were made up of adverse gastrointestinal symptoms (9 %), infection (6 %), and respiratory/cardiorespiratory/pulmonary vascular disorders (9.5 %). Of only 90 reports to the European pharmacovigilance database, most dealt with gastrointestinal signs; constitutional, respiratory, and hematologic disorders; and infections.

In the trial with HER2-positive metastatic breast cancer patients mentioned above, patients were randomized to receive pertuzumab in combination with trastuzumab and docetaxel or placebo in combination with trastuzumab and docetaxel. Eleven of 386 (2.8 %) patients in the pertuzumab-treated group and 23 of 372 (6.2 %) patients in the placebo-treated group developed antibodies reactive with pertuzumab, suggesting that the assay might have detected antibodies that cross-react with both mAbs. An additional uncertainty is the possibility that the presence of pertuzumab in patients' sera might interfere with the detection of anti-pertuzumab antibodies.

Trastuzumab

Trastuzumab (Herceptin®) (Tables 2.1 and 3.1), the first biologic agent developed and approved for the treatment of breast cancer, is a recombinant humanized IgG1κ mAb with high-affinity binding specificity for the extracellular domain of HER2. Pertuzumab (see above, section "Pertuzumab") is known to bind to the extracellular

subdomain II of HER2, blocking ligand-induced HER2/HER3 dimerization (Fig. 3.5b). Trastuzumab binds to HER2 subdomain IV causing downregulation of PI3K/AKT signaling in tumor cells overexpressing HER2 in the absence of ligands, but it seemed accepted that this mAb did not block dimerization of HER2 with ligand-activated HER1 (EGFR) or HER3. In the early 2000s, it was shown that preventing the expression of HER3 or its interaction with HER2 produced an antiproliferative effect in HER2-positive breast cancer cells. Following up these observations, researchers at Genentech, Inc., San Francisco, used RNA silencing of HER receptor expression and a tumor-inducible HER3 knockdown model to show that EGFR has no role in HER2-positive breast cancer and, of greater significance, constitutively active HER2 is dependent on HER3 for mediating its effects. While it is clear that trastuzumab exerts its antitumor activity via a number of different mechanisms, it is also apparent that a detailed understanding of the mAb's mode of action is far from complete. A brief summary of the current understanding is as follows. Trastuzumab binds to the extracellular domain of HER2, downregulating the expression of HER2 on the cell surface and blocking cleavage of the domain preventing the formation of the constitutively active membrane-bound protein, p95-HER2. These events ultimately reduce PI3K and MAPK signaling. The cyclin-dependent kinase inhibitor p27kip1 has an important role in trastuzumab-induced cell cycle arrest and tumor inhibition by binding to, and inhibition of, cyclin E/Cdk2 complexes and inhibition of G1/S progression. It now seems that trastuzumab also selectively inhibits ligand-independent HER2–HER3 heterodimerization and disrupts the HER2-HER3-PI3K complex (Fig. 3.5) in both trastuzumab-sensitive and trastuzumab-insensitive cells. In the light of these findings, it was suggested that blocking the HER2/HER3 receptor complex with a combination treatment of trastuzumab and pertuzumab might be particularly efficacious in HER2-amplified breast cancer. Finally, in addition to the above actions, trastuzumab has anti-angiogenic effects lowering the threshold for tumor cell killing by cytotoxic drugs, and it displays significant ADCC activity by Fc interaction with FcγRIIIa receptors on immune effector cells such as NK cells and macrophages.

Despite the advance in treatment offered by trastuzumab for aggressive breast cancer, only about 30 % of patients with advanced disease benefit from monotherapy with the drug. Furthermore, a large proportion of patients (~70 %) who experience an initial beneficial response become resistant within a year. Resistance is especially marked in advanced gastric cancer. Possible mechanisms of resistance have been studied. One proposed mechanism, so-called epitope masking, involves overexpression of membrane mucins such as Muc4 which, to some extent, masks the epitope recognized by the mAb on HER2. Precipitation experiments demonstrated that HER2 and Muc4 interact, knockdown of Muc4 increases trastuzumab binding, and upregulation of Muc4 in HER2-positive breast cancer cells results in acquired resistance to trastuzumab. A second possible mechanism of trastuzumab resistance may be the failure of the antibody to suppress the progression of tumors expressing truncated HER2 isoforms. Trastuzumab blocks cleavage of the extracellular domain of HER2 and prevents the formation of constitutive p95-HER2, but oncogenic HER2 exists in multiple isoforms and in some cancers the extracellular

domain-binding epitope may be lacking. Cross-signaling is another potential mechanism of trastuzumab resistance. Several molecules appear to activate HER2 in the presence of trastuzumab including insulin-like growth factor-1 receptor (IGF-1R), hepatocyte growth factor and its receptor Met, growth differentiation factor 15 (GDF15), and members of the ErbB family. The precise mechanisms underlying such cross-signaling generally remain unclear. Activation of, or increased downstream signaling, for example, by PI3K, focal adhesion kinase (FAK), and Src, may also counter the upstream inhibition exerted by trastuzumab. Activation of PI3K may occur as a result of mutations of reduced expression of the PTEN gene, deregulated signaling from upstream, or mutations of the PI3KCA (phosphatidylinositol-4,5-biphosphate 3-kinase, catalytic subunit alfa) gene. Little or no contribution from the normally present ADCC activity of trastuzumab is yet another possible mechanism of resistance to the mAb. This may occur because of impairment or loss of Fc and Fc gamma receptors and/or disruption of antibody—target cell or antibody—NK cell interaction (Chap. 2, section "Antibody-Dependent Cell-Mediated and Complement-Dependent Cytotoxicities").

With approved indications for breast cancer, metastatic gastric, and gastroesophageal junction adenocarcinomas overexpressing HER2, trastuzumab is the subject of an FDA black box warning for cardiomyopathy, infusion reactions, and pulmonary toxicity. For patients receiving trastuzumab as a single agent or in combination therapy, there is a four- to sixfold increase in the incidence of symptomatic myocardial dysfunction. Incidences are highest when an anthracycline drug is coadministered. Trastuzumab therapy can result in cases of left ventricular cardiac dysfunction, hypertension, arrhythmias, cardiomyopathy, cardiac failure, and cardiac death. Cardiac monitoring including determination of LVEF is recommended. Reported incidences of trastuzumab-related cardiotoxicity are 2–7 % for monotherapy, 2–13 % for trastuzumab combined with paclitaxel, and up to 27 % when given in combination with anthracyclines. In an early clinical trial, 136 of 844 patients (16 %) discontinued trastuzumab due to myocardial dysfunction or a decline in LVEF. In two other trials, 2.6 and 2.9 % discontinued the mAb due to cardiotoxicity. Infusion reactions may be relatively mild but serious and fatal reactions have been reported. Serious and fatal pulmonary toxicities following trastuzumab include dyspnea, interstitial pneumonitis, pulmonary infiltrates, pleural effusions, pulmonary insufficiency and hypoxia, non-cardiogenic pulmonary edema, ARDS, and pulmonary fibrosis. Other warnings and precautions refer to exacerbation of chemotherapy-induced neutropenia and febrile neutropenia and the possibility of fetal harm with trastuzumab increasing the risk of oligohydramnios during the second and third trimesters of pregnancy. Common and serious adverse events caused by trastuzumab are listed in Table 3.1. One important adverse event in need of closer analysis became apparent in early results from trials when a surprisingly high incidence of congestive heart failure was noticed in patients treated concurrently with the anthracycline doxorubicin. Early observations showed that approximately 5 % of patients treated with trastuzumab and chemotherapy developed evidence of systolic cardiac dysfunction with an incidence 4–5 times higher than controls. In a retrospective analysis of long-term cardiac

tolerability of trastuzumab in metastatic breast cancer in 218 patients who received trastuzumab for at least 1 year, 28 % of patients experienced some type of cardiac event, 10.9 % had grade 3 cardiotoxicity, and there was one cardiac death. Forty-five percent of patients with breast cancer are older than 65 years, and bearing in mind that recent reports indicate that trastuzumab-induced cardiotoxicity is now more often seen than indicated in the early clinical trials, there is a concern that older patients are at higher risk. This concern was reinforced in 2012 by results of an Italian study of 490 patients treated with trastuzumab in the adjuvant setting; whereas the overall rate of congestive heart failure was 27 %, it was 38 % in patients over 68. In a larger study of trastuzumab-related cardiotoxicity in older patients, the incidence of congestive heart failure among those given trastuzumab was 29.4 % of 2203 patients (median age 71 years) compared with 18.9 % of those who did not receive the mAb. Patients given trastuzumab were also more likely to develop congestive heart failure, and in this group there was an increased risk of congestive heart failure in older patients, in those given trastuzumab weekly, and in patients with coronary artery disease and hypertension. Note that in the pivotal clinical trials, only 4 % of patients given trastuzumab experienced a cardiac event and this compared with 1.3 % in the control groups. The finding that the frequency of administration of trastuzumab, namely, once a week, was associated with a higher risk leaves some questions, in particular, is more frequent administration associated with more myocyte damage and is the finding unique to older patients? Of course, it must be remembered that trastuzumab cardiotoxicity usually appears to be reversible—89 % of patients showing an asymptomatic decrease in LVEF apparently recovered after stopping therapy with the mAb. As to the likely mechanism of trastuzumab-related cardiac dysfunction, it has been speculated that since HER2 may be involved in a number of different biological processes of cardiomyocytes, trastuzumab may have a direct effect on myocytes.

In relation to apparent anthracycline toxicity, some recent evidence indicates that patients treated with anthracycline-based chemotherapies exhibit poor performance of some cognitive skills compared to those who received no, or other, chemotherapies.

In the postmarketing setting for trastuzumab, the most frequently reported adverse events to the FAERS have been infections (5 %), gastrointestinal disorders (4.2 %), respiratory disorders (3.7 %), white blood cell abnormalities (3.5 %), and cutaneous reactions (2.9 %). Some attention has also been drawn to infusion reactions and oligohydramnios. Of 7116 reports to the European pharmacovigilance database, cardiac events (11 %), respiratory disorders (10 %), gastrointestinal disorders (6.8 %), cutaneous reactions (5.9 %), and infections (4.8 %) were most frequently reported. There were 95 reports of anaphylactic/anaphylactoid reactions, three cases of CRS, 39 cases of stomatitis, and 29 cases of pancreatitis. Adverse pulmonary events were made up largely of interstitial lung disease (181 cases) and lung infiltrations (49 cases). Of 673 hematologic events, 25 % were for neutropenia, 13 % for each of anemia and thrombocytopenia, and 11 % for febrile neutropenia.

Data on the immunogenicity of trastuzumab are hard to find although a number of assays for the detection of antibodies to trastuzumab in human sera are commercially available. The FDA reports the detection of human antihuman antibodies to

the mAb in one of 903 women with metastatic breast cancer. The patient exhibited no adverse effects. Anti-trastuzumab antibodies were monitored in the so-called HannaH study, an investigation of a subcutaneous formulation of trastuzumab utilizing recombinant hyaluronidase to enhance tissue permeation and facilitate absorption of the antibody. The incidence of antibodies was found to be low and no clinical relevance was observed judging by efficacy and safety assessments that remained unaffected.

Ado-trastuzumab Emtansine

Ado-trastuzumab emtansine (Kadcyla®; trastuzumab emtansine, T-DM1) (Tables 2.1 and 3.1) is a humanized IgG1κ mAb-drug conjugate made by covalently coupling the microtubule inhibitory compound mertansine (DM1, *N*2′-deacetyl-*N*2′-(3-mercapto-1-oxypropyl)maytansine), a cytotoxic, thiol-containing maytansinoid (shown in red in Fig. 3.6), to trastuzumab via a bifunctional cross-linker, SMCC (succinimidyl *trans*-4-(maleimidylmethyl)cyclohexane-1-carboxylate) (shown in blue in Fig. 3.6). Each molecule of trastuzumab may be linked to as many as eight DM1 molecules although the average number is 3.5. In an examination of the drug (toxin) distribution profile of prepared DM1-trastuzumab conjugates by mass spectrometry and peptide mapping, up to 20 chemically modified lysine residues linked via ε-amino groups were found on

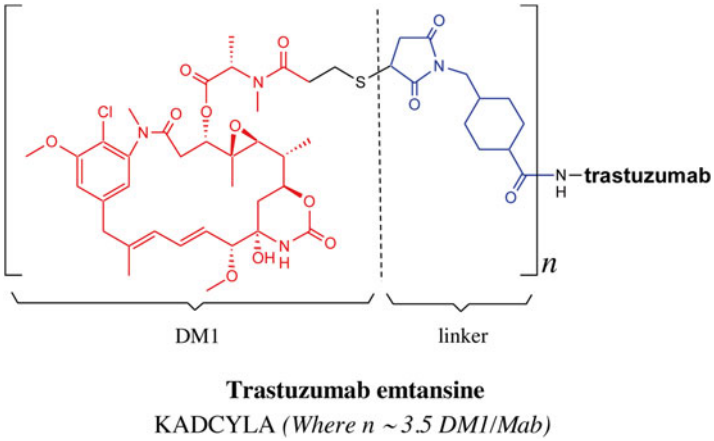


Fig. 3.6 Structure of ado-trastuzumab emtansine formed by covalently coupling trastuzumab to the cytotoxic microtubule inhibitor, mertansine, or DM1 (shown in *red*), via the bifunctional cross-linker SMCC (succinimidyl *trans*-4-(maleimidylmethyl)cyclohexane-1-carboxylate) that contains a reactive succinimide ester and a reactive maleimide that links to thioethers (shown in *blue*). The succinimide reacts with a trastuzumab lysine; the maleimide group covalently links to the free sulfhydryl group of DM1. The mertansine structure of the resultant complex together with the linkage group is designated the emtansine component. Ado-trastuzumab emtansine contains an average of 3.5 DM1 molecules for every molecule of trastuzumab

both light (~7 residues) and heavy chains (~13 residues) of the conjugated mAb. The conjugated lysines were freely accessible and none were found in the antibody complementarity-determining regions (CDRs). Random conjugation of the mAb lysine residues led to heterogeneity reflected in the number of attached toxin molecules and the different sites of their linkage. Crystal structures of the Fab and Fc fragments (Fig. 3.7) containing the modified lysine residues revealed that most of the residues are structurally and solvent accessible in the surface loops of the mAb. Only two modified lysines, Lys323 and Lys 393 in the heavy chain, are located in β-sheets (Fig. 3.7). The methodology used in this study should prove applicable to the identification of modified amino acid residues in

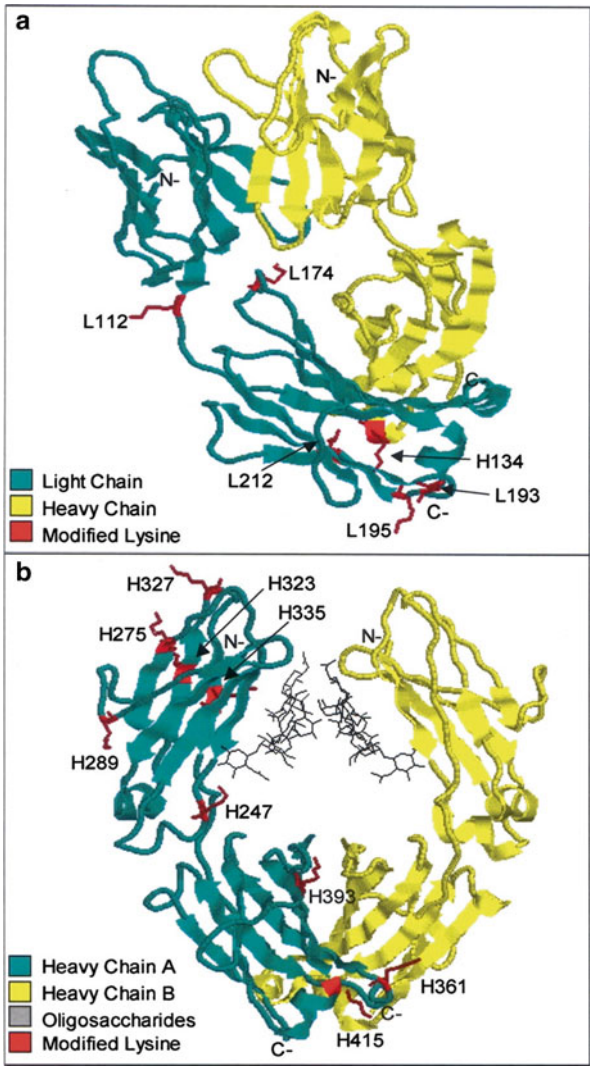


Fig. 3.7 Cartoon plots of the crystal structures of human IgG1 Fab (**a**) and Fc (**b**) fragments of the trastuzumab antibody-drug conjugate covalently linked to the cytotoxin mertansine (DM1) via lysine residues (shown in *red*). Modified lysines are shown on only one chain of the Fc fragment. N- and C-termini are labeled N and C, respectively. Images were created using Rasmol V2.6. Reproduced from Wang L et al. Structural characterization of the maytansinoid-monoclonal antibody immunoconjugate, huN901-DM1, by mass spectrometry. Protein Science 2005;14:2436–46. Reprinted with permission from John Wiley and Sons

other ADCs and different immunoconjugates. Linkage of DM1 to trastuzumab does not impair the binding of the mAb to HER2, it does not reduce its antitumor action, the linkage is nonreducible and non-cleavable, and there has been no evidence of toxic systemic exposure to free DM1 as a result of accumulation after repeated doses of the ADC. The covalent conjugate remains stable in both the circulation and tumor environment with the release of DM1 occurring only upon proteolytic degradation of the coupled mAb.

Ado-trastuzumab emtansine has a dual mechanism of action, combining trastuzumab's antitumor activity of inhibition of HER2-mediated signaling and ADCC (section "Trastuzumab") with the cytotoxic effect of DM1 on HER2-positive tumor cells. After binding of ado-trastuzumab emtansine to HER2, the HER2-T-DM1 complex enters the cell via receptor-mediated endocytosis where it is internalized by passage through endocytic vesicles to early endosomes and to mature lysosomes (Fig. 3.8). At the early endosome stage, some receptor and labeled mAb can be recycled back to the cell membrane. In the lysosome, the DM1 toxin released from T-DM1 as a result of proteolytic degradation of trastuzumab is liberated as a DM1-linker-lysine complex. DM1 inhibits the assembly of microtubules leading to cell cycle arrest and ultimately cell death.

Ado-trastuzumab emtansine is indicated as a single agent for the treatment of HER2-positive metastatic breast cancer patients who previously received trastuzumab and a taxane, separately or in combination. In its indications and usage, the FDA stipulates that patients should have received prior therapy for metastatic disease or developed disease recurrence during or within 6 months of completing adjuvant therapy.

An FDA black box warning for ado-trastuzumab emtansine mentions hepatotoxicity (including liver failure and death), cardiotoxicity (specifically reduction in LVEF), and the potential risk of fetal harm, plus a reminder that the ADC should not be substituted for, or with, trastuzumab. Warnings and precautions have also been issued for pulmonary toxicity, infusion-related and hypersensitivity reactions, thrombocytopenia, neurotoxicity, and extravasation. Signs and symptoms of dyspnea, cough, fatigue, and pulmonary infiltrates may indicate pulmonary toxicities such as interstitial lung disease, including pneumonitis, and cases of ARDS and death which were seen in clinical trials. The frequency of infusion-related reactions in clinical trials was 1.4 %; anaphylactic-like reactions may also occur. In two separate clinical trials, incidences of thrombocytopenia were as follows: any grade, 32 % and 31.2 %, and ≥grade 3, 11.7 and 14.5 %. In Asian patients the incidence of ≥grade 3 reactions in ado-trastuzumab emtansine-treated patients was 45.1 %. For peripheral neuropathy (predominately sensory), recorded incidences for any grade of reaction and ≥grade 3 reactions are ~20 % and 1.5–2 %, respectively. Reactions secondary to extravasation following infusion of the mAb may occur, usually within 24 h. No satisfactory treatment appears to be available, but reactions consisting of erythema, tenderness, skin irritation, pain, and swelling are usually mild. Commonly occurring adverse events following ado-trastuzumab emtansine are listed in Table 3.1.

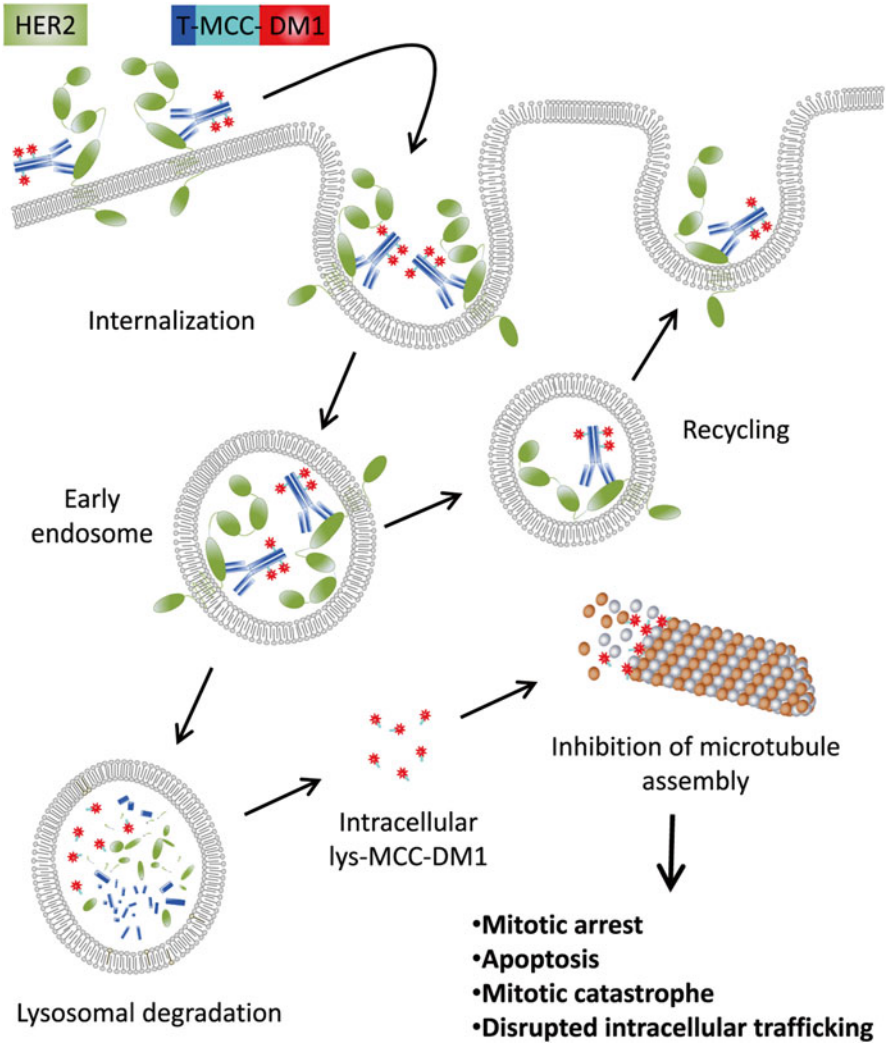


Fig. 3.8 Intracellular events involved in the antitumor action of ado-trastuzumab emtansine (T-MCC-DM1). After binding of the antibody-toxin conjugate to HER2, receptor-mediated endocytosis, some recycling of the complex back to the cell membrane, and transport via endosomes to lysosomes, the DM1-MCC linker-lysine complex released by proteolytic degradation acts to inhibit microtubule assembly. This results in cell cycle arrest, disruption of intracellular trafficking, and apoptosis. *MCC* 4-[*N*-maleimidomethyl] cyclohexane-1-carboxylate; *T* trastuzumab. Reproduced from Barok M et al. Trastuzumab emtansine: mechanisms of action and drug resistance. Breast Cancer Res 2014;16:209, an open-access article distributed under the terms of the Creative Commons Attribution License

Data on the immunogenicity of ado-trastuzumab emtansine are lacking and details of findings so far are vague. The FDA reports studies described as "tested at multiple time points for antitherapeutic antibody responses" to the ADC. Of 836 patients, 44 (5.3 %) tested positive "at one or more post-dose time points."

Denosumab

See also Chap. 4, section "Denosumab."

Denosumab (Xgeva®, Prolia®) (Tables 2.1 and 3.1), produced in transgenic mice, is a fully human IgG2κ mAb, MW ~147 kDa, with high specificity and affinity for receptor activator of nuclear factor kappa-B ligand, otherwise known as RANKL. Produced and expressed or released principally by cells of the osteoblast lineage and activated T cells, RANKL, a cytokine and member of the tumor necrosis factor (TNF) family (also called TNFSF11) (Chap. 5) responsible for bone resorption, is expressed in both soluble and membrane-bound forms. Expression is controlled by a number of cytokines and hormones, mainly IL-1, IL-6, TNF, $1,25(OH)_2$ vitamin D_3, parathyroid hormone, and parathyroid hormone-related peptide (Chap. 7, section "Parathyroid Hormone"), prolactin, prostaglandin E2, and corticosteroids. RANKL stimulates osteoclast formation, activation, adherence, survival, and ultimately resorption of the bone. Inhibition of RANKL results in an increase in bone density, volume, and strength. The actions of RANKL are effected via its cognate receptor RANK which is expressed on osteoclasts and their precursors. Binding of RANKL with its receptor occurs by interaction between extracellular domains of the ligand and extracellular cysteine-rich domains of the receptor. This activates several signaling pathways, in particular, protein kinase and NF-κB pathways, the latter upregulating c-fos in inducing gene transcription. The balance and coordination between the activities of osteoclasts and osteoblasts control bone formation, loss, and remodeling; osteoclasts first resorb the bone, osteoblasts follow, and regenerate it. A second member of the TNF family, osteoprotegerin, is also a player in the osteoclast-RANK-RANKL-induced balance of bone formation and loss. Osteoclast activity, at least in part, ultimately depends on the ratio of RANKL to osteoprotegerin since the latter binds RANKL preventing its binding and activation of RANK and leading to the inhibition of osteogenesis and resorption. Osteoprotegerin has been shown to markedly reduce the numbers of osteoclasts in bone lesions, for example, when neoplastic cells metastasize in the bone. In fact, superficially at least, osteoprotegerin and denosumab have similar mechanisms of action. Expression of osteoprotegerin is induced by the cytokines transforming growth factor-β (TGF-β) and platelet-derived growth factor (PDGF) and by estrogen, calcitonin, and calcium.

Marketed under two trade names, denosumab as Prolia® (Chap. 4, section "Denosumab") has approved cancer use indications for the treatment of men at high risk of fracture receiving androgen deprivation therapy for nonmetastatic prostate cancer and for the treatment of women at high risk of fracture receiving adjuvant

aromatase inhibitor therapy for breast cancer. As Xgeva®, denosumab is approved for the prevention of skeletal-related events in patients with bone metastases from solid tumors and the treatment of giant cell tumor of the bone.

For Xgeva®, warnings and precautions consist of hypocalcemia (fatal cases have been recorded), osteonecrosis of the jaw, and embryofetal toxicity. The nadir for the level of calcium in serum after a dose of denosumab is reached on about day 10. More frequent monitoring of serum calcium concentrations at the baseline and during the first two weeks of treatment, especially in patients with an estimated glomerular filtration rate <30 mL/min, has been recommended. Osteonecrosis of the jaw may manifest as jaw pain, osteomyelitis, osteitis, bone erosion, tooth or periodontal infection, toothache, gingival ulceration, or gingival erosion. Persistent pain or slow healing of the mouth or jaw after dental surgery may also be seen. In clinical trials, 2.2 % of patients with osseous metastasis receiving denosumab after median exposure of 13 doses developed osteonecrosis of the jaw. This figure increased to 5.4 % in patients with prostate cancer, a non-approved indication. Taking results of studies in monkeys, fetal harm after administration of denosumab to pregnant women may manifest as fetal loss, stillbirths, postnatal mortality, the absence of lymph nodes, abnormal bone growth, and decreased neonatal growth. Commonly occurring adverse events are listed in Table 3.1. Cutaneous adverse events are not uncommon and include rashes, eczema, dermatitis, exanthema, photosensitivity and possibly rare eruptions, exfoliative reactions, and bullous conditions. For Prolia®, hypersensitivity, including anaphylaxis, is listed as a warning together with hypocalcemia, osteonecrosis of the jaw, serious infections, atypical femoral fractures and suppression of bone turnover, cutaneous reactions, and severe bone, joint, and muscle pain. The most common adverse events in prostate cancer and breast cancer patients with bone loss are arthralgia, back pain, pain in extremity, and musculoskeletal pain. RANKL is expressed on T and B cells and especially CD4+ T cells, and there has been speculation that, on the one hand, denosumab may be immunosuppressive leading to increased infections and, on the other hand, that RANKL may have an immune accessory role generating costimulatory signals for dendritic cells which activate T cells after the CD40L/CD40 primary signal. As yet, there is no compelling evidence for either suggestion.

Recent serious postmarketing surveillance reports for Xgeva® cover calcium and bone disorders, gastrointestinal symptoms, and musculoskeletal and dermatologic conditions. These events, plus respiratory disorders, are also the most commonly reported reactions. Reports on Prolia® relate to musculoskeletal disorders, dermatologic conditions, infections, and gastrointestinal disorders. The most frequently mentioned infections include pneumonia, cellulitis, urinary tract infections, and sepsis.

Immunogenicity of denosumab has not proved to be a major problem. The incidence of hypersensitivity reactions appears to be similar to control groups. Human antihuman antibodies were shown to have an incidence of <1 % in 13,000 tested patients. Of 2758 patients with osseous metastases treated with denosumab (as Xgeva®) for up to 3 years, seven (0.25 %) tested positive for antibodies to the mAb. None of the antibodies detected were neutralizing for denosumab. Similar results were obtained in assessments of the immunogenicity of Prolia®.

Ipilimumab

Ipilimumab (Yervoy®, MDX-010) (Tables 2.1 and 3.1) is a recombinant fully humanized IgG1κ mAb, MW ~148 kDa, that binds with high affinity to the extracellular domain of the protein receptor human cytotoxic T lymphocyte antigen 4 or CTLA-4 (also known as CD152), a member of the immunoglobulin superfamily. CTLA-4 has a critical role as an inhibitory regulator during the early stages of T-cell expansion. For T lymphocyte activation to occur, a naïve T cell needs to receive two precise signals from an antigen-bearing, antigen-presenting cell (APC) (dendritic cell, macrophage, or B cell). The first activation signal comes via the membrane-associated major histocompatibility complex (MHC) in its interaction with the T-cell receptor (TCR), while the second activation event is the provision of costimulatory signals effected by the APC membrane protein ligand CD80 (B7-1) working in tandem with membrane ligand CD86 (B7-2). The complementary receptor for these ligands is CD28, a protein constitutively expressed on naïve T cells that enhances IL-2 production and allows the cells to undergo clonal expansion. CTLA-4 is expressed on the lymphocyte surface but only becomes functional after the start of T-cell activation when it competitively interacts with the B7 ligands (Fig. 3.9) resulting in interference with IL-2 secretion and receptor expression and downregulation of the T-cell response. When bound to the B7 complex on APCs where it binds with greater affinity than CD28, CTLA-4 has been described as an immune "off" switch playing an important role in modulating overactivity of T cells and maintaining tolerance to self-antigens. However, another consequence of suppressing the immune response can be to allow cancer cells to be recognized as self and multiply in the absence of antitumor immune challenge. Recognition of the crucial role of CTLA-4 as an inhibitory regulator of the T lymphocyte response and studies of murine models of cancer led to the strategy of blockade of the receptor by specific antibody (Fig. 3.9) and this in turn resulted in the development of the mAb ipilimumab. Blocking antibodies such as ipilimumab, an example of immune checkpoint targeting, exert an antitumor action by at least two mechanisms: blockade-induced clonal expansion of activated CD3+ and CD4+ T lymphocytes (and also probably CD8+ cells) and depletion of regulatory T cells (Tregs) CD4+ CD25+ which are induced by the tumor and act to inhibit immune recognition of tumor antigens. In addition, an increase in Th17 CD4+ T cells producing cytokines IL-17 and IL-22 was found to be associated with fewer relapses in a trial of patients treated with ipilimumab. Ipilimumab does not exhibit CDC activity, but at high concentrations it exerts weak to moderate ADCC activity on activated T cells probably via IgG Fc FcγRI (CD64) rather than FcγRIII receptors.

With an approved indication for the treatment of unresectable or metastatic melanoma (Table 2.1), a number of adverse events induced by ipilimumab are related to the mAb's mode of action of T-cell-mediated immune aggression against cancer cells. In fact, the FDA has a black box warning for severe and fatal immune-mediated adverse reactions due to T-cell activation and proliferation. Although these reactions may involve any organ system, the most common ones include enterocolitis; hepatitis; neuropathy (e.g., Guillain-Barré syndrome, myasthenia gravis); endocrinopathy

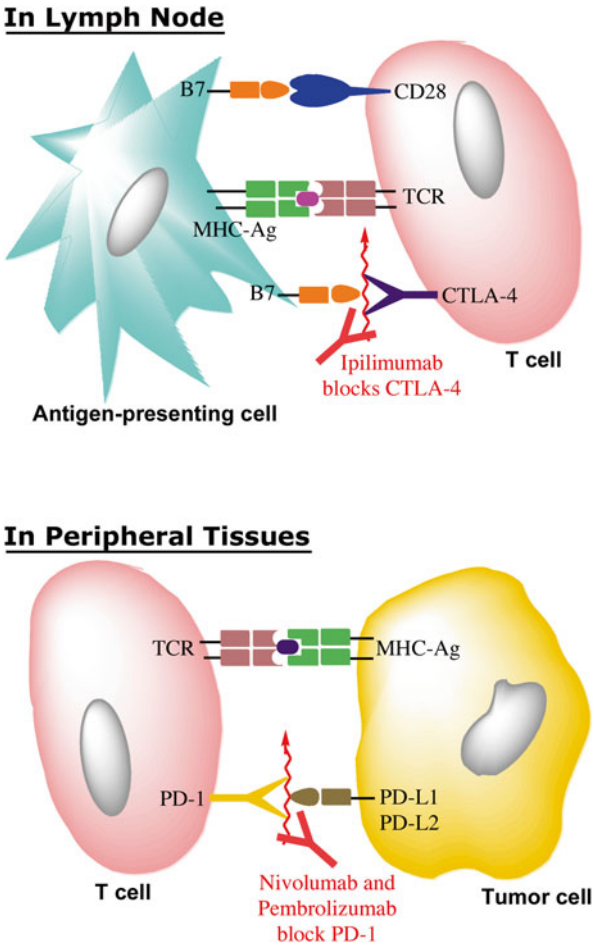


Fig. 3.9 Immune checkpoint targeting with anti-CTLA-4 and anti-PD-1 monoclonal antibodies. The cytotoxic T lymphocyte antigen 4 (CTLA-4) acts at the first checkpoint to modulate overactive T cells by competing for the B7 ligands. CTLA-4 only becomes functional after the start of T-cell activation when it competitively interacts with the B7 ligands resulting in downregulation of the T-cell response. Suppressing the immune response allowing cancer cells to be recognized as self and thus multiplying in the absence of antitumor immune challenge led to the strategy of blockade of the receptor by the anti-CTLA-4 immunostimulatory monoclonal antibody ipilimumab. The programmed cell death 1 (PD-1) receptor acts as another important immune checkpoint. Binding of PD-1 to its ligands PD-L1 and PD-L2 suppresses T-cell proliferation and activity allowing the tumor cells to avoid immune recognition and attack. Blocking at this second checkpoint by anti-PD-1 monoclonal antibodies nivolumab and pembrolizumab results in the reactivation of T cells including those with specificity for the tumor cells

(hypopituitarism, adrenal insufficiency, hypogonadism, hypothyroidism); severe cutaneous events such as Stevens-Johnson syndrome and toxic epidermal necrolysis; and immune-mediated ocular manifestations, uveitis, iritis, or episcleritis. A number of studies have led to and supported claims that ipilimumab elicits a high rate of immune-related adverse events. A recent review of records from 298 melanoma

patients treated with ipilimumab revealed that 85 % experienced an immune-related adverse event of any grade, 19 % had to discontinue therapy due to the event, and 35 % of patients examined required systemic corticosteroids for their adverse event. In 31 of the 298 patients (10.4 %), symptoms did not resolve, necessitating the administration of anti-TNF therapy. One conclusion reached by the investigators was that ipilimumab can activate immune reactions against normal tissues leading to diarrhea, hepatitis, hypophysitis, and rash.

In late 2015, the FDA extended indications and usage of ipilimumab to adjuvant treatment of patients with cutaneous melanoma with pathological involvement of regional lymph nodes of more than 1 mm who have undergone complete resection, including lymphadenectomy.

The list of less common, but often potentially serious, adverse events provoked by ipilimumab is increasing as indicated by efforts to identify the spectrum of rare, often surprising, reactions. Patient files from 19 skin cancer centers revealed drug reaction (rash) with eosinophilia and systemic symptoms, otherwise known as DRESS, granulomatous inflammation of the central nervous system, and aseptic meningitis. Other rare, often life-threatening or even fatal, adverse events were myopathy, neuropathy, leukopenia, sarcoidosis, uveitis, and Guillain-Barré syndrome. Recent reports continue to highlight apparently rare reactions to ipilimumab, for example, the first case of organizing pneumonia; Guillain-Barré syndrome or acute polyradiculoneuritis; polyradiculoneuropathy with multifocal motor blocks; facial diplegia, tetraplegia, and areflexia; immune-mediated thrombocytopenia; autoimmune lymphocytic hypophysitis with anterior panhypopituitarism; hyponatremia; and Schwartz-Bartter syndrome. Usually less serious adverse events seen with a high frequency (≥5 %) following ipilimumab are diarrhea, fatigue, colitis, rash, and pruritus (Table 3.1).

At the end of 2012, gastrointestinal disorders, principally diarrhea and colitis, were the most frequently mentioned adverse events reported to the FAERS and European pharmacovigilance databases. Infections, mainly sepsis, pneumonia, and urinary tract infections, were near the top of the FAERS list, while in Europe, tumor progression, nervous and endocrine disorders, and skin reactions were prominent. There was one case of toxic epidermal necrolysis, 20 cases of intestinal perforation, 10 of hepatotoxicity, 11 of pancreatitis, two of anaphylaxis, and one case of CRS.

Immunogenicity does not appear to be a significant problem for ipilimumab therapy regardless of dosage levels. In a study of 1024 patients, 1.1 % had anti-ipilimumab serum antibodies, none neutralizing, and reactions suggestive of hypersensitivity were not seen.

Siltuximab

Siltuximab (Sylvant®, CNTO 328) (Tables 2.1 and 3.1) is a chimeric human-mouse IgG1κ mAb targeted to interleukin-6 (IL-6), forming high-affinity complexes with the cytokine's soluble bioactive forms. IL-6, produced by many different cells including lymphocytes, monocytes, fibroblasts, and endothelial and cancer cells, is a pleiotropic cytokine with a complex action producing both proinflammatory and

anti-inflammatory effects. Promotion of inflammation by IL-6 results from its role in the activation and proliferation of T cells, stimulation of B cells, induction of acute phase proteins such as C-reactive protein (CRP) in the liver, and stimulation of hematopoietic precursor cell proliferation and differentiation. IL-6's anti-inflammatory role appears to be centered on inhibitory effects in turning off or modulating the synthesis of TNF, IL-1, and IL-10. The intricate interplay of pro- and anti-inflammatory effects of IL-6 suggests that the cytokine may be an important, if not sometimes crucial, participant in the parthenogenesis and/or response to some diseases. In fact, increased levels of IL-6 are known to be associated with a variety of diseases including rheumatoid arthritis, autoimmunities, Alzheimer's disease, neoplasia, atherosclerosis, Paget's disease, osteoporosis, and others. Cancers associated with increased production of IL-6 include renal cell carcinoma, prostate and bladder cancers, some neurologic cancers, and particularly multiple myeloma and the B-cell lymphoproliferative disorder, Castleman's disease. Castleman's disease may be localized or unicentric, or multicentric when it is characterized by generalized lymphadenopathy with systemic symptoms. Approximately half of the cases of multicentric Castleman's disease are caused by human herpesvirus-8 (HHV-8 or KSHV, Kaposi's sarcoma-associated herpesvirus), but the cause(s) of the other 50 % is unknown. Regardless, IL-6 has a central role in the pathophysiology of the multicentric form with its excess production leading to production of B lymphocytes and plasma cells, autoimmune reactions, and secretion of VEGF.

Interleukin-6 binds to a type I 80 kDa cytokine receptor (IL-6R) on target cells. The resultant complex associates with the protein gp130 (CD130), expressed on almost all cells, which dimerizes and initiates intracellular signaling. CD130 is the common signal transducer for several cytokines of the gp130 cytokine family including IL-11, leukemia inhibitory factor (LIF), and oncostatin M. Signaling proceeds through the JAK/STAT and Ras-Raf-MAPK signaling pathway after activation by phosphorylation of the phosphatase SHP-2 by JAK1. Cells that express gp130 only, do not respond to IL-6; IL-6 can only bind to cells expressing IL-6R. The receptor IL-6R, also found in soluble form (sIL-6R), binds IL-6 with an affinity comparable to the membrane-bound receptor. Importantly, cells expressing gp130, even in the absence of IL-6R, can respond to the complex of IL-6-sIL-6R. This process is called trans-signaling. Siltuximab blocks the binding of IL-6 to both the membrane-bound and soluble IL-6 receptors thereby preventing the formation of the signaling complex with gp130 on the cell surface.

Approved by both the FDA and EMA in 2014, siltuximab is indicated for the treatment of multicentric Castleman's disease in patients who are human immunodeficiency virus (HIV) negative and HHV-8 negative. Siltuximab has received orphan drug designation for multicentric Castleman's disease in both US and European Union. Warnings and precautions for the mAb issued by the FDA mention the risks inherent in administering siltuximab to patients with severe infections, including pneumonia and sepsis, and the possibility that siltuximab may mask signs of acute inflammation and suppress fever and acute phase reactants such as CRP. By extension, live vaccines should not be administered concurrently or within 4 weeks of commencing siltuximab therapy. Other warnings issued by the FDA are for infusion-related reactions and hypersensitivity and gastrointestinal perforation. An

increased risk of malignancy and the development of hyperlipidemia and hepatic impairment are additional warnings issued by the EMA.

A summary of the safety profile of siltuximab reveals that anaphylaxis is the most serious adverse event associated with its use, while infections, including those of the upper respiratory tract, pruritus, and maculopapular rash are the most commonly occurring events (Table 3.1). In clinical trials, the incidence of infusion or hypersensitivity reactions was 4.8 % with 0.8 % of patients experiencing severe reactions. An evaluation of long-term exposure to siltuximab, namely, 3.4–7.2 years (median 5.1 years), showed that the most common adverse events (>20 %) were upper respiratory tract infections (63 %), diarrhea (32 %), and pain in extremities, arthralgia, and fatigue (21 % each). There were no deaths and no cumulative toxicities identified.

Sera from 411 patients on siltuximab mono- or combination therapy tested for anti-siltuximab antibodies proved positive in only one patient (0.24 %). Antibodies were present in low titer, they were non-neutralizing, and the patient's safety profile remained unaltered.

Monoclonal Antibodies Targeting Programmed Cell Death Protein 1 (PD-1): Pembrolizumab and Nivolumab

Pembrolizumab (Keytruda®) (Tables 2.1 and 3.1), approved by the FDA for advanced melanoma in September 2014 through a Breakthrough Therapy Designation, was also recently approved by the UK's Medicines and Healthcare Products Regulatory Agency (MHRA) via the UK's Early Access to Medicines Scheme (EAMS). FDA indications and usage are for patients with unresectable or metastatic melanoma and disease progression following ipilimumab. It is stated that the mAb should be administered with a BRAF inhibitor if the patient is positive for the BRAF V600 mutation. Pembrolizumab was approved by the EMA for marketing in Europe in July 2015. Breakthrough Therapy Designation for pembrolizumab in the USA for the treatment of non-small cell lung cancer was announced in October 2014, and the mAb is said to be showing encouraging trial results in the treatment of PD-L1-positive patients with advanced triple-negative breast cancer. In early June 2015, the FDA accepted for review the supplemental Biologics License Application for pembrolizumab for the treatment of advanced non-small cell lung cancer whose disease has progressed on or after platinum and EGFR therapy. The FDA granted priority review under its accelerated approval program with a target action date of early October 2015 and approval was finally granted on October 2. Nivolumab (Opdivo®), also targeted to PD-1 and approved in Japan in July 2014, was first granted breakthrough therapy, priority review, orphan product designation, and FDA approval in December 2014 before receiving accelerated approval for metastatic melanoma by the EMA in June 2015. Nivolumab appears to demonstrate improvement over other therapies including peginterferon alfa-2b, B-Raf enzyme inhibitors, and the mAbs ipilimumab and pembrolizumab. In early March 2015, nivolumab received its second FDA approval, this time for the treatment of patients

with metastatic squamous non-small cell lung cancer with progression on or after platinum-based chemotherapy. In October 2015, approval was given for the treatment of non-squamous non-small cell lung cancer, and then in November, approval for nivolumab was extended to advanced metastatic renal cell carcinoma in patients who have received prior anti-angiogenic therapy.

Checkpoint Inhibitors and PD-1

In its vital balancing role of protecting against self-antigens while retaining an activated state to protect against foreign antigens, the immune system requires a number of checks and balances to maintain the necessary healthy equilibrium of self-tolerance, prevention of autoimmune reactions, the defense against invading organisms, and elimination of aberrant cells. The bases of these checks and balances are immune cells, particularly the T cell, together with an array of ligands and receptors that effect the many activation and inhibitory processes required for the stimulation and curtailment of immunostimuli. In relation to cancer, the terms "immune checkpoints" and "checkpoint inhibitors" refer to a number of molecules, usually proteins, on cell surfaces that exert a co-inhibitory effect on the immune response, for example, the cytotoxic T lymphocyte antigen 4 (CTLA-4) which acts at the first checkpoint (see section "Ipilimumab") to modulate overactive T cells by competing for the B7 ligands (Fig. 3.9). The programmed cell death protein 1 (PD-1, CD279) receptor which plays a critical role in cancer immunology acts as another important immune checkpoint. PD-1 is a transmembrane protein receptor of the Ig superfamily expressed on T cells during thymic development and on CD4+ and CD8+ T cells, B lymphocytes, NK cells, B cells, monocytes, and some dendritic cells during antigen signaling. PD-1 has two ligands, PD-L1 (CD274, B7-H1) and PD-L2 (CD273, B7-DC). Whereas the affinity of PD-L2 for PD-1 is three times higher than the affinity of PD-L1, it is expressed on fewer cells and cell types, for example, it is not present on resting cells but is inducibly expressed on dendritic cells, macrophages, and some B cells. PD-L1 is expressed on both hematopoietic and non-hematopoietic cells, including dendritic cells, macrophages, and B cells and cells of solid tumors. High expression of PD-L1 appears to be associated with increased aggressiveness of cancers and death. During PD-1 signaling in T cells, recruited phosphatases SHP-1 and SHP-2 cause dephosphorylation of associated signaling molecules downstream from the T-cell receptor (TCR) complex. Akt activation is inhibited via the PI3K pathway, and Akt signaling leads to suppression of T-cell proliferation, decreased protein synthesis, and survival and suppression of IL-2 production which further inhibits T-cell proliferation and survival.

Attempts to improve T cell function are proving promising in strategies aimed at complementing cancer immunotherapy. Reactivating CD8+ killer T cells , normally suppressed in many cancers, is yielding encouraging results. Enhanced proliferation of CD8+ but not CD4+ T cells was demonstrated by modulating cholesterol metabolism via inhibition of cholesterol esterification in T cells. In a mouse melanoma model, the esterification enzyme acetyl-CoA acetyltransferase 1 (ACAT1) was inhibited by the selective inhibitor avasimibe, leading to an increase in CD8+ mem-

brane cholesterol levels and decreased melanoma growth and metastasis. When used with anti-PD-1 therapy, increased efficacy in controlling tumor progression was observed.

Targeting PD-1 in Cancer Therapy

Like CTLA-4, PD-1 negatively regulates T-cell activation. While remaining free of its complementary ligands, PD-1 does not interfere with the normal immune response, but, upon binding PD-L1 and PD-L2, signaling is induced, suppressing T-cell proliferation and activity. Malignant cells, including melanoma cells, often express PD-L1 which, upon binding, induces inhibitory signaling through the receptor preventing expansion of activated T cells and allowing the tumor cells to avoid immune recognition and attack. In other words, T-cell targeting of tumor cells can be subverted by cancer cells expressing PD-1 ligands and effectively using the PD-1 inhibitory pathway to blunt the immune antitumor response. Thus, it follows that selectively blocking the pathway should reverse the checkpoint inhibition and restore the T-cell-mediated response to the tumor. This strategy has, in fact, been adopted with the development and subsequent approval of mAbs designed to bind to PD-1 and prevent interaction of the receptor with its ligands (Fig. 3.9). Recent trials have shown that two of the mAbs, pembrolizumab and nivolumab, show encouraging clinical benefits with impressive high response rates and toxicity profiles superior to ipilimumab, all leading to the impression, so far, that PD-1 may be a better target for cancer therapy.

Pembrolizumab

Pembrolizumab (MK-3475, lambrolizumab) (Tables 2.1 and 3.1) is a humanized IgG4κ mAb, MW ~149 kDa, targeted to PD-1 and indicated for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor. Since the IgG4 subtype does not activate complement (Chap. 2, section "IgG Antibody Subclasses"), pembrolizumab is not cytotoxic when it binds to T cells. In addition to its approval for advanced non-small cell lung cancer whose disease has progressed on or after platinum-containing chemotherapy, FDA-approved usage for pembrolizumab extends to patients with EGFR or ALK (anaplastic lymphoma kinase) genomic tumor aberrations whose disease has progressed on FDA-approved therapy.

Pembrolizumab is generally well tolerated, but a spectrum of adverse events may be seen, and the drug's sizeable list of immune-related adverse events can be severe. Warnings and precautions issued by the FDA are for immune-mediated pneumonitis, colitis, hepatitis, hypophysitis, hyperthyroidism, hypothyroidism, renal failure, and nephritis, all seen with an incidence in the range ~0.5–3 %. Most of the data so far on these immune-mediated conditions were derived from an uncontrolled, open-label, multiple cohort trial involving 411 patients with unresectable or metastatic melanoma receiving the mAb. Adverse reactions that led to discontinuations were pneumonitis, renal failure, and pain. Serious effects occurred in 36 % of patients

with the most frequent serious reactions being renal failure, dyspnea, pneumonia, and cellulitis. Some other immune-mediated adverse reactions, namely, uveitis, arthritis, myositis, pancreatitis, hemolytic anemia, and exfoliative dermatitis, were clinically significant but occurred in less than 1 % of patients. Based on its mechanism of action, a warning/precaution for pembrolizumab has also been issued for fetal harm. The most common adverse events reported are listed in Table 3.1.

In an early study of pembrolizumab's potential for immunogenicity, no treatment-emergent anti-pembrolizumab antibodies were detected in 97 patients examined.

Nivolumab

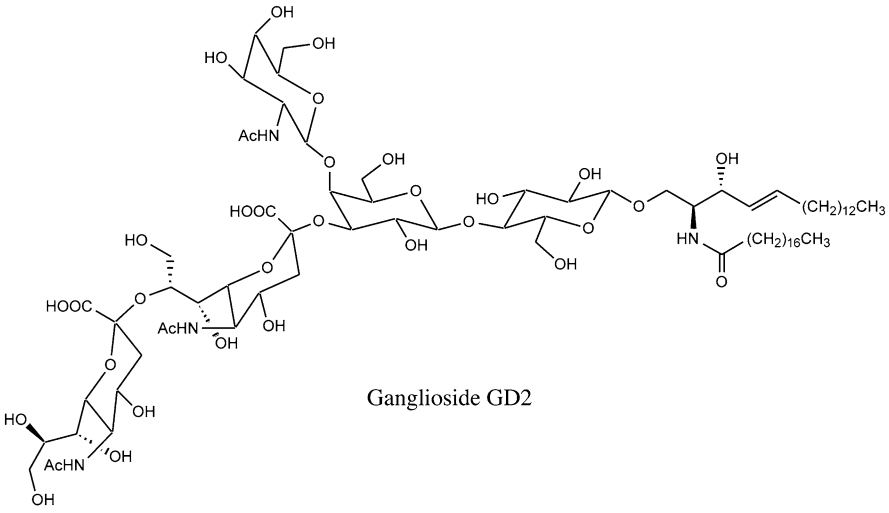
Nivolumab (BMS-936558, MDX-1106, ONO-4538) (Tables 2.1 and 3.1) is a fully human IgG4κ mAb, MW ~149 kDa, targeted to PD-1 and indicated for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab, and, if BRAF V600 mutation positive, a BRAF inhibitor should also be given. FDA approvals for the treatment of patients with metastatic squamous non-small cell lung cancer, metastatic non-squamous non-small cell lung cancer with progression on or after platinum-based chemotherapy, and advanced metastatic renal cell carcinoma have recently been announced. Like pembrolizumab, nivolumab is subject to warnings for immune-mediated adverse events of pneumonitis, colitis, hepatitis, hyperthyroidism, hypothyroidism, renal failure, and nephritis in melanoma patients. Other clinically significant, immune-mediated adverse events occurring in less than 1 % of treated melanoma patients were pancreatitis, uveitis, autoimmune neuropathy, demyelination, adrenal insufficiency, facial and abducens nerve paresis, hypophysitis, diabetic ketoacidosis, hypopituitarism, Guillain-Barré syndrome, and myasthenic syndrome. Awareness of the potential risk of embryofetal toxicity is a necessary additional precaution for pregnant women given nivolumab. Clinically significant nivolumab-induced adverse events were also assessed in 574 patients with solid tumors enrolled in clinical trials. Grade 3 and 4 adverse reactions occurred in 41 % of patients receiving nivolumab with abdominal pain, hyponatremia, and increases in aspartate transaminase and lipase reported in 2–5 % of patients. In addition to the adverse events listed here and in Table 3.1, ventricular arrhythmia, iridocyclitis, infusion-related reactions, neuropathies, and a number of different cutaneous disorders including exfoliative dermatitis, erythema multiforme, psoriasis, and vitiligo have been reported. Extensive safety data are not yet available for the use of nivolumab in patients with non-small cell lung cancer and renal cell carcinoma.

Of 281 nivolumab-treated patients, anti-nivolumab antibodies were detected in 24 patients (8.5 %) and neutralizing antibodies were found in two patients (0.7 %). There was no evidence that any of the antibodies influenced the effectiveness of the mAb or its toxicity profile.

Other mAbs directed against PD-1 are in the development pipeline. For example, in February 2016, the FDA granted Breakthrough Therapy Designation to the investigational human IgG1 mAb durvalumab for patients with inoperable/metastatic urothelial bladder cancer whose tumor has progressed on a platinum regimen.

Dinutuximab

Aberrant glycosylation is often characteristic of malignant cellular transformation. It is known that tumors with some carbohydrate antigens expressed at high levels show accelerated rates of metastasis and progression. The sialic glycosphingolipid (ganglioside), disialoganglioside (GD2), a short sialylated polysaccharide linked to ceramide through a β-glycosidic linkage (Fig. 3.10) and found highly expressed on neuroectoderm-derived cancers such as neuroblastoma, melanoma, brain tumors, osteosarcoma, and Ewing's sarcoma in children, is one such tumor-associated carbohydrate antigen. Significantly from the safety viewpoint, GD2 is not present on normal tissues and minimally expressed in the brain and peripheral nerves. The incidence of GD2-positive tumors in the USA is estimated to be in excess of 200,000 annually, and considering the high mortality rate of these cancers and the high priority given to GD2 as a potential target for cancer therapy, a number of strategies to exploit the therapeutic possibilities of this antigen have been investigated over the last three decades. One of the most promising approaches has been the development of mAbs targeted to GD2 for use in passive antibody therapy, but this strategy has faced the dual challenges of antibody affinity and tumor cell killing on the one hand and potential toxicities with pain inducement toward nerves and melanocytes on the other hand. Dinutuximab (ch14.18, Unituxin®) (Tables 2.1 and 3.1), a human-mouse chimeric IgG1κ mAb that binds GD2, received approval to treat neuroblastoma from the FDA in March 2015 and



Ganglioside GD2

Fig. 3.10 Structure of ganglioside (G2), an antigen expressed on human tumors of neuroectodermal origin. The disialylated polysaccharide structure is linked to ceramide through a β-glycosidic linkage. The structure contains one molecule each of D-glucose, *N*-acetyl-D-galactosamine, and D-galactose with two attached *N*-acetylneuraminic acid residues. Only one of the various lipoforms is shown

from the EMA in May 2015. After being shown to prevent the outgrowth of experimental melanoma and neuroblastoma tumors, this mAb was found to have an acceptable safety profile in clinical trials on patients with neuroblastoma, melanoma, and osteosarcoma. Dinutuximab had been given an orphan product designation and received priority review and a pediatric disease priority review. A combination of the antibody with the cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) (Chap. 5, section "Colony-Stimulating Factors: Filgrastim, Sargramostim, and Tbo-filgrastim") was judged to enhance efficacy. Approved indications for dinutuximab now state that it should be used in combination with GM-CSF, IL-2, and 13-cis-retinoic acid for the treatment of pediatric patients with high-risk neuroblastoma who achieve at least a partial response to prior first-line multiagent, multimodality therapy.

FDA approval for dinutuximab comes with a boxed warning for potentially life-threatening infusion reactions requiring prior hydration and premedication and a warning for neuropathy, in particular severe neuropathic pain and nerve damage requiring prior, and subsequent, intravenous opioid treatment. In one study of more than 100 patients, severe (grade 3 or 4) infusion reactions occurred in just over one-quarter of the patients; urticaria was seen in 13 % and anaphylaxis resulted in 1 % of patients. One patient died from cardiac arrest within 24 h of receiving dinutuximab. Despite pretreatment with analgesics (including morphine), 85 % of 114 patients treated with dinutuximab experienced pain which was judged severe in 51 % of patients. Neuralgia and arthralgia occur but pain is most commonly reported as generalized or in the extremities, back, abdomen, and chest. Peripheral sensory neuropathy and severe peripheral motor neuropathy each occurred in 1 % of patients. The neuropathic effects of dinutuximab are generally more severe in adults than in children. Other warnings are for capillary leak syndrome (Chap. 1, section "Capillary Leak Syndrome"), found in one study in 23 % of patients (6 % severe reactions), hypotension (16 % grades 3 or 4), infection (bacteremia 13 % grades 3 or 4, sepsis 18 %), neurological eye disorders (blurred vision, photophobia, mydriasis, ptosis), bone marrow suppression (thrombocytopenia 39 %, anemia 34 %, neutropenia 34 %, febrile neutropenia 4 %), electrolyte abnormalities (hyponatremia, hypokalemia, hypocalcemia), atypical hemolytic uremic syndrome, and embryofetal toxicity. The most commonly seen adverse reactions to dinutuximab are listed in Table 3.1; the most common serious reactions are infections, infusion reactions, hypokalemia, hypotension, pain, fever, and capillary leak syndrome.

In two clinical studies in 284 and 103 patients, 18 % and 13 % of patients, respectively, tested positive for anti-dinutuximab antibodies. Neutralizing antibodies were found in 3.6 % of the patients.

Daratumumab

Daratumumab (HuMax-CD38, Darzalex®) (Tables 2.1 and 3.1), accepted for priority review by the FDA in September 2015 and approved by the agency in November 2015 for the treatment of multiple myeloma, is the first-in-class

immunotherapy for this currently incurable disease in which almost all patients relapse or become resistant to therapy. Daratumumab, an IgG1κ human antibody, MW ~148 kDa, is the first mAb targeted to the 48 kDa glycoprotein CD38 (cyclic ADP ribose hydrolase), a surface antigen expressed by multiple myeloma cells and found on many immune cells including CD4+, CD8+, B lymphocytes, and natural killer (NK) cells. The mAb acts by inhibiting the growth of tumor cells expressing CD38, leading to apoptosis by Fc-mediated cross-linking and cell lysis induced via CDC, ADCC, and ADCP. Daratumumab is approved for the treatment of patients with multiple myeloma who have received at least three prior treatments including a proteasome inhibitor, an immunomodulatory agent, and who are double refractory to a proteasome inhibitor and an immunomodulatory agent.

Warnings and precautions for daratumumab are the occurrence of infusion reactions, interference with cross-matching and red blood cell antibody screening, and interference by the mAb when determining patient's response and disease progression in patients with IgG kappa myeloma (Table 3.1). As well as infusion reactions, adverse events recorded so far include infections, particularly pneumonia, thrombocytopenia, fatigue, and nausea. It is predicted that postmarketing usage will reveal cytopenias as one of the main adverse events (Table 3.1).

In an open-label clinical trial of patients with relapsed or refractory multiple myeloma treated with daratumumab, none of 111 patients proved positive for anti-daratumumab antibodies up to 8 weeks after completion of the treatment.

Elotuzumab

In September 2015, the humanized recombinant IgG1κ mAb elotuzumab (Empliciti®, HuLuc63) (~MW 148.1 kDa) targeting the cell surface glycoprotein receptor CS1, a member of the signaling lymphocytic activation molecule (SLAM) receptor family, was granted FDA priority review based on a phase III trial of the mAb together with lenalidomide and dexamethasone for the treatment of relapsed or refractory multiple myeloma. After its assisted pathway through orphan drug designation, breakthrough therapy, and priority review, formal approval for the mAb was granted by the FDA on November 30, 2015. Receptor CS1 (also known as CD2 subunit 1, SLAMF7, and CD319) is highly expressed on myeloma cells but not on other tissues, including hematopoietic stem cells. It has been predicted that treatment of elotuzumab with lenalidomide and dexamethasone may prove to be one of the new standards of care for patients with relapsed or refractory multiple myeloma with no additional toxic effects. In its approved indications for elotuzumab, the FDA specifies that the mAb should be given in combination with lenalidomide and dexamethasone to patients who have received one to three prior therapies. Elotuzumab targets SLAMF7 on myeloma cells and natural killer (NK) cells, facilitating the latter to kill myeloma cells through ADCC. The addition of lenalidomide to the mAb therapy results in enhanced NK cell-mediated killing.

Warnings and precautions issued by the FDA for elotuzumab are listed in Table 3.1. Infusion reactions have been reported in ~10 % of patients treated when

the mAb is given in combination with lenalidomide and dexamethasone. As a result, premedication with dexamethasone, H1 and H2 antihistamines, and acetaminophen should be administered. Infections occurred commonly in clinical trials with opportunistic infections, including herpes zoster and fungal infections, seen at a significantly higher incidence in the elotuzumab-lenalidomide arm than the lenalidomide arm of the trial. Hematologic malignancies, solid tumors, and skin cancers reported in the elotuzumab-lenalidomide and lenalidomide arms of the trial were 1.6 % and 1.6 %, 3.5 % and 2.2 %, and 4.4 % and 2.8 %, respectively. Elevation in liver enzymes (ALT, AST), total bilirubin, and alkaline phosphatase consistent with hepatotoxicity indicates the need to periodically monitor liver enzymes during elotuzumab therapy. Being a humanized IgG kappa antibody, elotuzumab may interfere with assays used to monitor endogenous M-protein and determine the complete response of patients with IgG kappa myeloma protein. Other adverse reactions to elotuzumab determined so far are listed in Table 3.1.

Of 390 patients treated with elotuzumab, 72 (18.5 %) proved positive for anti-elotuzumab antibodies. In 63 of these patients, antibodies were detected within the first 2 months of treatment, but by 2–4 months, antibodies resolved in 49 of the 63 patients. In a multiple myeloma randomized trial, 19 of 299 patients (6.4 %) were found to have neutralizing antibodies.

Recent Approval: Atezolizumab

During the production stage of this book, the FDA granted mAb approval number 50 to the humanized IgG1 kappa antibody atezolizumab (Tecentriq™) for treatment of urothelial carcinoma. Targeted to PD-L1 and blocking interaction with PD-1 and B7.1 receptors, the mAb has a long list of warnings and precautions: immune-related pneumonitis, hepatitis, colitis, pancreatitis, endocrinopathies, and myasthenic syndrome as well as ocular inflammatory toxicity, infection, infusion reactions, and embryo-fetal toxicity.

Range of Side Effects of Monoclonal Antibodies Used for Cancer Therapy

Although mAbs used for cancer immunotherapy are generally better tolerated than small molecule chemotherapeutic drugs, their range of adverse effects is still wide, varying from headaches, mild gastrointestinal symptoms, and transient rashes to severe cytopenias; anaphylaxis; autoimmunity; pulmonary, cardiac, hepatic, kidney, neurological, and embryofetal toxicities; and rare life-threatening toxidermias. Some adverse events are clearly immune-mediated, but many others do not have, or appear to not have, an immune basis. Direct cytotoxic effects account for some reactions.

Types I–IV Hypersensitivities and Cytopenias

As discussed in Chap. 2, immunogenicity is always a safety concern for mAbs, even those that are fully humanized, since the possibility of generating anti-idiotype antibodies remains. The likelihood of immune-mediated adverse events following mAb administration therefore cannot be totally eliminated, and this covers the full range of hypersensitivity responses from type I IgE antibody-mediated immediate reactions such as anaphylaxis, urticaria, and angioedema to type II drug-induced thrombocytopenia, hemolytic anemia, and agranulocytosis; type III serum sickness and drug-induced vasculitis; a range of type IV cutaneous hypersensitivities mediated by Th1, Th2, and Th17 lymphocytes; and effector mechanisms involving cytotoxic lymphocytes, macrophages, eosinophils, and a number of other cell types. In fact, "hypersensitivity" is a much misused and often poorly understood term, and there is a pressing need for the acceptance of a common definition across the medical disciplines (see Chap. 1, section "Hypersensitivities"). It is not always clear that a reaction is a true hypersensitivity response or if any immune process, direct or indirect, is involved. In the first instance, the terminology used to describe hypersensitivities needs to be standardized to enable accurate interpretations of many adverse events.

Because of their immunogenic potential, mAbs generally carry warnings of immune reactions, especially anaphylaxis, but the observed incidences of such reactions are actually quite small. IgE-mediated reactions to chimeric proteins used for cancer therapy containing mouse and/or rat sequences (catumaxomab, blinatumomab, ibritumomab tiuxetan, brentuximab vedotin, cetuximab, rituximab, siltuximab, and dinutuximab) (Table 3.1) are considered to be of greater risk, and overall, and not unexpectedly, this has proved to be true. Anaphylaxis has been reported for, at least, cetuximab, rituximab, brentuximab, bevacizumab, trastuzumab, pertuzumab, ibritumomab, and dinutuximab, but the real incidences of reactions for each of the mAbs are hard to establish for a number of reasons including common misunderstanding and misuse of the term hypersensitivity, the clinician's ability/inability to distinguish true type I IgE-mediated anaphylaxis from some severe infusion and anaphylactoid reactions, the frequent failure to test for the presence of mAb-specific IgE antibodies, and differences in the frequency and extent of the use of different mAbs. For example, a study of 901 patients showed that 79 (9%) experienced what was described as an immediate hypersensitivity reaction, while 76% developed symptoms during their initial infusion leading the authors to conclude that immediate hypersensitivity to rituximab commonly occurs during or after the first infusion. Nonetheless, no evidence for the presence or absence of specific IgE antibodies either as skin test or serum immunoassay results was presented.

Cytopenias occur in some patients treated with mAbs during anticancer immunotherapy, but the underlying mechanisms frequently remain unexplored. Type II and III hypersensitivities induced by mAbs may be underdiagnosed. The FDA has issued a boxed warning for the risk of severe cytopenias with ibritumomab tiuxetan and alemtuzumab, while general warnings and precautions are set down for obinu-

tuzumab (thrombocytopenia, neutropenia), ofatumumab (cytopenias), brentuximab vedotin (neutropenia), trastuzumab (neutropenia), and ado-trastuzumab emtansine (thrombocytopenia).

Listed among the other warnings/adverse events for the 24 anticancer mAbs are cytopenias for catumaxomab, brentuximab vedotin, and pertuzumab; lymphopenia for elotuzumab; lymphopenia and leukopenia for blinatumomab; neutropenia for rituximab; thrombocytopenia for daratumumab; thrombocytopenia and anemia for trastuzumab; and thrombocytopenia, lymphopenia, and neutropenia for dinutuximab (Table 3.1). Autoimmune forms of thrombocytopenia and hemolytic anemia are type II hypersensitivities, and reductions in the platelet, erythrocyte, and neutrophil counts, especially in the lymphoproliferative diseases, may sometimes have an immune basis. Thrombocytopenia, well known following the use of many small molecule chemotherapeutic drugs, is much more rarely seen during and after mAb treatments. Rituximab, implicated in thrombocytopenia as often as any of the approved mAbs, appears to show an incidence of ~1.7 %, and this figure is similar for mono- and combination therapies. However, an incidence as high as 10.4 % was reported in one study of 72 patients with non-Hodgkin lymphoma given a total of 317 rituximab infusions. From the study of a case of thrombocytopenia induced by rituximab and a review of the literature, it was not possible to implicate rituximab-dependent antibodies and IL-1 and IL-6 were not increased, but complement levels were elevated. This led the authors to conclude that mAb-induced transient thrombocytopenia might be mediated by complement activation and associated with CRS. Two cases of transient severe thrombocytopenia during rituximab therapy, one in a patient with mantle cell lymphoma and the other with hairy cell leukemia, reversed a few days after the antibody was withdrawn, but, again, the underlying mechanisms were not investigated. Other mAbs implicated in treatment-related thrombocytopenia include trastuzumab and alemtuzumab. When the latter was given for early multiple sclerosis, ~3 % of patients developed potentially fatal thrombocytopenia, and of 11 patients with peripheral T-cell lymphoproliferative disorders given alemtuzumab, five developed lymphopenia, neutropenia, and thrombocytopenia.

Rituximab has been implicated in both early and late neutropenia. Late onset neutropenia manifests at least 4 weeks after the cessation of therapy; it occurs with a comparatively high incidence (4–23 %) and appears to be caused by a different mechanism than the early form of the disorder. While the mechanism underlying late onset neutropenia is poorly understood, results suggest that direct cytotoxicity is unlikely and immune mechanisms, including autoantibodies, may be responsible for the rituximab-induced disease.

Incidences of 1.1 and 5.2 % for severe anemia have been reported for patients receiving rituximab monotherapy. Other serious cases involving rituximab include severe autoimmune hemolytic anemia in a patient with a lymphoproliferative disorder; a case of intravascular hemolysis, rhabdomyolysis, renal failure, and bone marrow necrosis; and multiple organ ischemia due to an anti-Pr cold agglutinin in a patient with mixed cryoglobulinemia after treatment with rituximab. At least two mAbs, rituximab and alemtuzumab, have been implicated in the induction of pure red cell aplasia and autoimmune hemolytic anemia.

Hypersensitivity vasculitis induced by drugs is a manifestation of a type III response, and a few mAbs including rituximab have been implicated in cases of cutaneous vasculitis. Serum sickness reactions to mAbs, another type III hypersensitivity, are probably underdiagnosed and reported. Chimeric antibodies in particular have the potential to induce the reactions, and, again, rituximab has been the mAb most implicated. It has been claimed that rituximab serum sickness-like reactions can occur in up to 20 % of treated patients, especially in those with autoimmune diseases (particularly autoimmune thrombocytopenia) and hypergammaglobulinemia.

Although autoimmune diseases induced by anticancer mAbs are rare, occasional cases occur with ipilimumab standing out as the most common cause. By targeting CTLA-4 and acting as an immunostimulatory agent, ipilimumab produces an antitumor response and augments T-cell activation that sometimes leads to immune-mediated colitis, hepatitis, nephritis, hypothyroidism, and hyperthyroidism. By blocking the PD-1 receptor, mAbs nivolumab and pembrolizumab may provoke a similar range of autoimmune reactions as well as autoimmune pneumonitis.

Type IV cutaneous reactions to drugs, including mAbs, generally become apparent 7–21 days after exposure, but subsequent reactions may appear only a day or two after reexposure. Specificity of the culprit antigen is established by patch and intradermal testing, usually read after a delay of at least 48 h, although great caution, or preferably avoidance, should be exercised in skin testing cases of cutaneous toxidermias such as Stevens-Johnson syndrome and toxic epidermal necrolysis. Besides these two rare and potentially life-threatening conditions, type IV cutaneous reactions include allergic contact dermatitis, psoriasis, maculopapular exanthema, fixed drug eruption, acute generalized exanthematous pustulosis, erythema multiforme, and drug reaction with eosinophilia and systemic symptoms (DRESS). Being mediated by lymphocytes, sensitivity to the provoking antigen(s) can be transferred by lymphocytes in type IV reactions. Such delayed cutaneous hypersensitivity reactions to anticancer mAbs are rare with most reported cases restricted mainly to ibritumomab tiuxetan, brentuximab vedotin, and rituximab. Lichenoid dermatitis, vesiculobullous dermatitis, and paraneoplastic pemphigus have also occurred in response to rituximab, and a case of lichenoid eruption was recently seen after obinutuzumab (section “Obinutuzumab”). A number of other mAb-induced cutaneous manifestations with features seemingly common to a type IV response may be true type IV hypersensitivities, but mechanisms remain to be established, for example, cases of dermatitis induced by catumaxomab, bevacizumab, denosumab, ipilimumab, and panitumumab.

Infusion Reactions and Cytokine Release Syndrome

The FDA has issued boxed warnings for the possibility of serious or even fatal infusion reactions to ibritumomab vedotin, rituximab, alemtuzumab, cetuximab, panitumumab, trastuzumab, and dinutuximab and a general warning for the risk of infusion reactions during or following treatment with obinutuzumab, ofatumumab, brentuximab vedotin, bevacizumab, ramucirumab, pertuzumab, ado-trastuzumab emtansine,

and siltuximab (Table 3.1). Infusion reactions provoked by mAbs usually begin within hours of the initial infusion. Reactions are typically mild to moderate manifesting as "flu"-like symptoms of fever, chills, rigor, headache, nausea, asthenia, pruritus, and rash. In a small number of patients, severe, life-threatening symptoms common to type I IgE antibody-mediated anaphylaxis, in particular, hypotension, bronchospasm, cardiac arrest, and urticaria, may occur, usually during the first or second infusion (Chap. 1, section "Hypersensitivities"). The similarity of the signs and symptoms can make it difficult to distinguish an infusion reaction from a true allergic hypersensitivity although IgE-mediated reactions generally have a faster and more severe onset, usually within minutes. Severe reactions have been reported for all, or almost all, the mAbs although some show a much higher incidence with the chimeric rituximab and humanized trastuzumab antibodies being the leading offenders. The incidence of reactions for cetuximab, another human-mouse chimera, is ~15–20 % (grade 3–4, 3 %); for trastuzumab, first infusion ~40 % (grade 3–4, <1 %); and for rituximab, first infusion ~77 % (grade 3–4, 10 %). Approximately 80 % of fatal infusion reactions to rituximab occurred after the first infusion, and 30 and 14 % of patients still reacted after the fourth and eighth infusions, respectively. Even though trastuzumab is a humanized mAb, it induces a relatively high incidence of infusion reactions, but bevacizumab, another humanized antibody, shows a reaction incidence of only <3 % (grades 3–4, 0.2 %) which is similar to the fully humanized panitumumab (3 %, grades 3–4, ~1 %). Elotuzumab, recently approved for the treatment of multiple myeloma, provokes infusion reactions in a large proportion of patients and needs to be given with premedication (Table 3.1). The mechanisms of mAb-induced infusion reactions are not yet fully understood. Cytokines, especially TNF and interleukins such as IL-6, may be involved since the symptoms they produce resemble those seen in infusion (and type I allergic) reactions. An important finding was the observation that the severity of infusion reactions is related to the number of circulating lymphocytes. For example, a severe reaction is thought to require a lymphocyte count of $>50 \times 10^9$/L. CRS may be seen after the use of mAbs directed to malignant immune cells, for example, rituximab. It is thought that the systemic inflammatory response produced together with a high fever is a consequence of antibody binding to and activating the cells. The distinguishing features in the literature between CRS and severe infusion reactions are often not clear, and in many reported cases, the two designations may be interchangeable.

Pulmonary Adverse Events

Classified under the heading drug-induced lung diseases (DILDs), these pulmonary adverse events make up a heterogeneous group of diseases, most still of unknown, or poorly understood, mechanism of action. They have been grouped into four categories: interstitial pneumonitis and fibrosis, acute respiratory distress syndrome (ARDS), bronchiolitis obliterans organizing pneumonia (BOOP), and hypersensitivity pneumonitis. There are, however, a number of other classifications in the literature. Interestingly, hypersensitivity pneumonitis to some anticancer agents is

Table 3.3 Pulmonary adverse events caused by approved monoclonal antibodies used for cancer therapy

Monoclonal antibody	Pulmonary adverse events
Rituximab (MabThera®, Rituxan®)	ARDS, BOOP,[a] bronchiolitis obliterans,[b] bronchospasm, diffuse alveolar hemorrhage, hypersensitivity pneumonitis, interstitial lung disease,[b] interstitial pneumonitis[c]
Brentuximab vedotin (Adcetris®)	With bleomycin[d]: cough dyspnea, interstitial filtrations
	In Hodgkin lymphoma: pneumonitis, pneumothorax
Alemtuzumab (Campath®, MabCampath®)	Bronchospasm,[e] diffuse alveolar hemorrhage, pulmonary infection[f]
Cetuximab (Erbitux®)	Interstitial lung disease
Panitumumab (Vectibix®)	Interstitial lung disease,[g] lung infiltrates, pneumonitis, pulmonary fibrosis
Bevacizumab (Avastin®)	Bronchospasm/anaphylaxis, pneumonitis, pulmonary hemorrhage from the site of tumor
Trastuzumab (Herceptin®)	ARDS, BOOP, dyspnea, interstitial pneumonitis, pleural effusions, pulmonary infiltrates/fibrosis/edema/insufficiency and hypoxia
Ado-trastuzumab emtansine (Kadcyla®)	Interstitial lung disease—includes pneumonitis, ARDS, pulmonary infiltrates
Nivolumab (Opdivo®)	Severe pneumonitis or interstitial lung disease including fatal cases
Pembrolizumab (Keytruda®)	Immune-mediated pneumonitis

ARDS acute respiratory distress syndrome, *BOOP* bronchiolitis obliterans organizing pneumonia
[a]BOOP is the most common clinical diagnosis followed by interstitial pneumonitis, ARDS, and hypersensitivity pneumonitis
[b]Fatal cases have occurred
[c]Also called interstitial pneumonia or Hamman-Rich syndrome
[d]Concomitant use of brentuximab vedotin with bleomycin is contraindicated, e.g., with ABVD (Adriamycin, bleomycin, vinblastine, dacarbazine) combination therapy
[e]Serious fatal infusion reactions may include bronchospasm, ARDS, pulmonary infiltrates, and anaphylaxis
[f]For example, tuberculosis and aspergillosis
[g]Discontinue panitumumab in patients developing interstitial lung disease

increasingly looking like a combined type III and type IV hypersensitivity reaction in a Th1/Th17 response. Table 3.3 lists the mAbs used in cancer therapies that have been implicated in pulmonary adverse reactions. At least ten of the currently approved mAbs for cancer therapy have some recorded pulmonary toxicity in treated cancer patients. Once again, rituximab is the main offender inducing a wide range of adverse events with BOOP most often seen followed by interstitial pneumonitis, ARDS, and hypersensitivity pneumonitis. It has been suggested that early onset organizing pneumonia is a hypersensitivity reaction to the mAb, whereas the late onset condition is either related to mAb toxicity or to immune system restoration. ARDS symptoms appearing within a few hours of infusion may be a manifestation of CRS or TLS with no relationship to hypersensitivity although ARDS has also been linked to the release of proinflammatory cytokines. The incidence of rituximab-associated interstitial lung disease has been estimated to be 0.01–0.03 %. Its pathogenesis remains largely unknown although complement and TNF may be involved. Besides rituximab,

Table 3.4 Cardiac adverse events caused by approved monoclonal antibodies used for cancer therapy

Monoclonal antibody	Cardiac adverse events
Ibritumomab tiuxetan (Zevalin®)	Cardiac arrest related to infusions
Obinutuzumab (Gazyva®, Gazyvaro®)	Worsening of preexisting cardiac conditions leading to fatal cardiac events
Rituximab (MabThera®, Rituxan®)	Cardiac arrhythmias and angina,[a] fatal cardiac failure
Brentuximab vedotin (Adcetris®)	Supraventricular arrhythmia in systemic anaplastic large cell lymphoma
Alemtuzumab (Campath®, MabCampath®)	Cardiomyopathy, decreased LVEF,[b] cardiac arrhythmias associated with infusions[c]
Cetuximab (Erbitux®)	Cardiopulmonary arrest/sudden death[d]
Bevacizumab (Avastin®)	CHF: incidence of grade 3 reaction for LVD 1 %
Ramucirumab (Cyramza®)	Serious, sometimes fatal, myocardial infarction
Pertuzumab (Perjeta®)	Cardiomyopathy manifesting as CHF and decreased LVEF[b]
Trastuzumab[e] (Herceptin®)	Cardiomyopathy manifesting as CHF and decreased LVEF[b]
Ado-trastuzumab emtansine (Kadcyla®)	Decreased LVEF[b]

CHF congestive heart failure, *LVD* left ventricular dysfunction, *LVEF* left ventricular ejection fraction

[a]Can be life threatening. Discontinue infusions. Perform cardiac monitoring after each infusion for patients with arrhythmia/angina

[b]Incidence is highest when mAb is administered with cardiotoxic agents such as anthracyclines

[c]In ~14 % of previously untreated patients. Most reaction temporarily associated with infusions

[d]In patients treated with cetuximab and radiation therapy

[e]Patients receiving trastuzumab alone or in combination therapy show a four- to sixfold increase in the incidence of myocardial dysfunction

serious and/or fatal cases have occurred following administrations of alemtuzumab, trastuzumab, bevacizumab, panitumumab, and cetuximab.

Cardiac Adverse Events

Cardiac adverse events have occurred with at least 11 of the mAbs used for cancer therapy (Table 3.4). Cardiac arrhythmias and angina are reported for rituximab; obinutuzumab may lead to a worsening of preexisting cardiac conditions; brentuximab vedotin has been linked to supraventricular arrhythmia in some lymphoma patients; ramucirumab is implicated in serious and even fatal myocardial infarction; and bevacizumab is associated with congestive heart failure with an incidence of 1 % for LVD. Cardiopulmonary arrest and/or sudden death occurred in 4 (1.9 %) of 208 patients given cetuximab and radiation therapy, and cardiac arrest after ibritumomab tiuxetan and arrhythmia after alemtuzumab were each associated with infusions. Cardiomyopathy manifesting as congestive heart failure and decreased LVEF may

occur following treatment with pertuzumab, trastuzumab, ado-trastuzumab emtansine, and alemtuzumab. Decreases in LVEF are well known for mAbs and other drugs that block HER2 activity, and this risk is increased in patients given anthracyclines or radiotherapy to the chest. In fact, patients administered trastuzumab show a four- to sixfold elevation in the incidence of myocardial infarction, and, again, this risk is highest when the mAb is given with an anthracycline. Interestingly, trastuzumab inhibits neuregulin 1, a growth factor for cardiac development and maintenance of heart structure and integrity. Necitumumab carries an FDA black box warning for cardiopulmonary arrest.

Mucocutaneous Reactions to Monoclonal Antibodies Targeted to Epidermal Growth Factor Receptor

These cutaneous reactions are not immune-mediated, that is, they are not genuine hypersensitivities. Skin reactions appear as a papulopustular eruption (sometimes less precisely called an acneiform rash), often in a large proportion of patients (50–100 %) and in a more severe form than seen with small molecule tyrosine kinase inhibitors. Eruptions tend to be confined to seborrheic regions (Fig. 3.3a–c), areas that maintain their integrity via EGFR expressed in the epidermis, sebaceous glands, and hair follicles. In the presence of inhibitors of EGFR, the epithelial barrier may be weakened allowing bacterial access and ultimately the development of the characteristic rash. Other adverse effects induced by mAbs targeted to EGFR include paronychia (Fig. 3.11), fissures, xerosis (Fig. 3.3h),

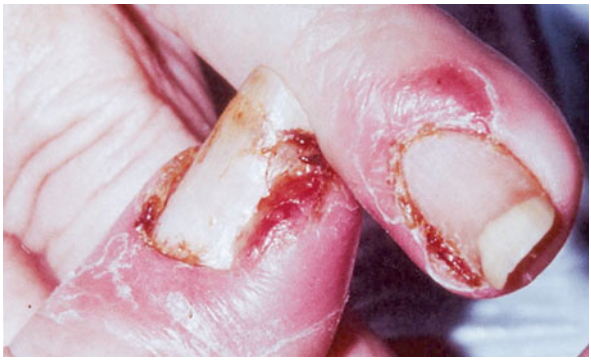


Fig. 3.11 A case of periungual granulation type of paronychia with edema and erythema caused by cetuximab. Reproduced from Boucher KW, et al. Paronychia induced by cetuximab, an antiepidermal growth factor receptor antibody. J Amer Acad Dermatol 2002;47:632–3. Modified and reprinted with permission from Elsevier Limited

palmar-plantar rash, hair changes, hyperkeratosis, mucositis (Fig. 3.3f, g), nail pyrogenic granuloma (Fig. 3.3i), and skin hyperpigmentation.

Other Rare Adverse Events Following Antitumor Monoclonal Antibody Therapy

See Chap. 1, sections "Tumor Lysis Syndrome" and "Progressive Multifocal Leukoencephalopathy," for a discussion of these two syndromes.

Tumor Lysis Syndrome

Depending on the anticancer agent used and the tumor load, within 48–72 h of starting the therapy, large numbers of malignant cells may be destroyed in a short time resulting in hyperkalemia, hypercalcemia, hyperphosphatemia, and hyperuricemia. Especially in patients with high tumor load, this can produce profound ionic imbalances in potassium, calcium phosphate, and uric acid and progress to acute renal failure, cardiac arrhythmias, seizures, and death. The condition is known as tumor lysis syndrome (TLS), and, unlike CRS, the response is easy to distinguish from type I immediate hypersensitivity reactions. TLS usually occurs in patients with leukemias and high-grade lymphomas and is rarely seen in association with solid tumors. The syndrome is well known to occur with the use of the CD20-targeted mAbs and brentuximab vedotin targeted to CD30, but the reaction elicited by rituximab appears to be somewhat atypical and remains to be further characterized. The FDA has issued a TLS boxed warning for rituximab and warning and precautions for obinutuzumab, brentuximab vedotin, and blinatumomab.

Progressive Multifocal Leukoencephalopathy

The polyomavirus JC virus which persists asymptomatically in about one-third of the population causes PML in severely immunodeficient individuals such as transplant and AIDS patients. PML is a progressive, usually fatal, disease resembling multiple sclerosis in which the myelin sheath of nerve cells is destroyed affecting nerve transmission. Although rare, the disease is occasionally seen upon the administration of some mAbs directed to B cells, in particular, rituximab, ofatumumab, obinutuzumab, and brentuximab vedotin, and there are currently FDA boxed warnings for potentially fatal PML in patients treated with these mAbs and a warning for ofatumumab (Table 3.1). In 2009, 57 cases of PML following rituximab therapy in HIV-negative patients were reported. A 2010 report of the WHO Collaborating Centre for International Drug Monitoring Adverse Event Data Bank revealed that rituximab was responsible for 114 of 182 cases of PML.

Summary

- Of the 50 monoclonal antibodies (mAbs) currently approved by the FDA and/or EMA, 24 are indicated for the treatment of hematologic, cutaneous, or solid tumor malignancies.
- **Catumaxomab** (**Removab**®), a mouse-rat hybrid mAb composed of a mouse kappa light chain and IgG2a heavy chain and a rat lambda light chain and IgG2b heavy chain, shows dual antigen recognition specificity for EpCAM and CD3, while the hybrid Fc fragment binds to FcγRI, FcγRIIa, and FcγRIIIa on macrophages and NK cells.
- Used to treat malignant ascites, catumaxomab may induce cytokine release syndrome (CRS), a reflection of T-cell activation, and the mAb's mode of action. Lymphopenia, reported in up to 14 % of patients, is reversible. Cutaneous reactions of rash, erythema, pruritus, and catheter-related reactions (erythema and infection) can be serious. Both human anti-mouse and human anti-rat antibodies are seen.
- **Blinatumomab** (**Blincyto**®), a bispecific T-cell-engaging (BiTE) fusion protein, is composed of two antibody single-chain variable fragments each from an H and L chain. One of the two binding specificities is directed to the B-cell antigen CD19, while the other targets CD3, part of the T-cell receptor. Reaction with both antigens is exploited to link malignant B cells of patients with acute lymphoblastic leukemia to cytotoxic T cells, activating them to destroy the tumor cells.
- Blinatumomab approved by the FDA is issued with a Risk Evaluation and Mitigation Strategy (REMS). Neurological toxicities range from confusion to tremors, convulsions, and speech disorders but appear to be reversible. CRS, sometimes life-threatening or even fatal, has been reported in up to 11 % of patients and may lead to hemophagocytic lymphohistiocytosis.
- CD20 is expressed on the surface of B cells, except for plasmablasts, at all stages of their development. It also occurs on B-cell lymphomas, B-cell chronic lymphocytic leukemia, hairy cell leukemia, and melanoma cancer stem cells. Due to its non-Hodgkin lymphoma (NHL) B-cell expression and the fact that it is not normally shed from cells and is internalized after binding to antibody, CD20 has been seen as an exploitable target for mAbs for the treatment of lymphomas. Four mAbs directed to CD20 are currently approved as antitumor agents—rituximab, ibritumomab, ofatumumab, and obinutuzumab.
- **Rituximab** (**MabThera**®, **Rituxan**®), a human-mouse chimeric IgG1κ antibody, was the first mAb approved to treat relapsed or refractory NHL and the first mAb approved specifically for cancer therapy. It is now a first-line therapy for several NHLs, including follicular lymphoma and diffuse large B-cell lymphoma. In 2002, the FDA authorized the use of rituximab as a component of ibritumomab therapy.
- A long list of side effects has been reported for rituximab ranging from the serious events detailed in an FDA black box warning, namely, infusion reactions, PML, TLS, and severe mucocutaneous reactions (all potentially fatal). Other adverse events are infections, hepatitis B reactivation, cardiac arrhythmias, lung

toxicities, and bowel problems plus a host of often less serious systemic and cutaneous events and toxicities.

- Infusion reactions to rituximab occur on the first infusion in up to 77 % of malignant patients and this decreases to approximately 10 % after the second infusion. Lymphocyte counts suggest that the appearance of CRS correlates with counts higher than 50×10^9/L.
- Postmarketing surveillance of rituximab administration has confirmed the importance of infections and respiratory and hematologic events in its safety profile. A 2010 report of the WHO Collaborating Centre for International Drug Monitoring Adverse Event Data Bank revealed that rituximab was responsible for 114 of 182 cases of the JC virus-induced PML.
- **Ibritumomab tiuxetan** (**Zevalin**®), a murine mAb covalently linked to the chelator tiuxetan by a stable thiourea bond, is radiolabeled with yttrium-90 for therapy or indium-111 for imaging. A boxed warning highlights severe, and potentially fatal, infusion reactions and severe cytopenias as the most serious adverse events experienced with ibritumomab therapy.
- Other serious events following ibritumomab tiuxetan are infections and potentially fatal myeloid malignancies or dysplasias. Hypersensitivity reactions, generally manifesting as bronchospasm or angioedema, are another potentially dangerous side effect. Of the non-hematologic events, gastrointestinal symptoms are commonly seen as well as rare toxidermias such as bullous dermatitis, erythema multiforme, Stevens-Johnson syndrome, and toxic epidermal necrolysis.
- The possibility of hepatitis B virus reactivation, cytopenias, intestinal obstruction, and PML comprise the warnings, precautions, and risks issued for **ofatumumab** (**Arzerra**®). Prolonged severe neutropenia and thrombocytopenia may occur, making necessary the regular monitoring of blood and platelet counts.
- Although exhibiting less CDC than rituximab, **obinutuzumab** (**Gazyva**®, **Gazyvaro**®) has demonstrated superior efficacy. Patients with CLL and coexisting illnesses unable to tolerate combined intravenous chemotherapy are likely to be more tolerant of obinutuzumab in its approved indication of the mAb's combination with chlorambucil.
- Grade 3–4 neutropenia, infusion reactions, anemia, thrombocytopenia, pyrexia, and musculoskeletal pain are the most common events induced by obinutuzumab. In addition to a boxed warning for hepatitis B virus reactivation and PML, warnings and precautions cover severe and life-threatening infusion reactions, TLS, the risk of infections, and cytopenias.
- **Brentuximab vedotin** (**Adcetris**®) is a chimeric mAb conjugated to the cytotoxic agent monomethyl auristatin E (MMAE) and targeted to CD30 which is overexpressed in Hodgkin lymphoma and some other lymphomas. After binding to cells expressing CD30, brentuximab's toxic payload MMAE is internalized. After reaching the lysosomes, MMAE is released, disrupting microtubules and ultimately inducing apoptosis.

- The list of warnings and precautions issued by the FDA for brentuximab vedotin covers 11 concerns related to neuropathies (predominately peripheral), infusion-related reactions, hematologic toxicities, serious infections, TLS, hepatotoxicity, PML, embryofetal toxicity, and serious dermatologic reactions. A small number of cases of anaphylaxis have been reported.
- Overall, the safety data for brentuximab vedotin shows that neutropenia, peripheral neuropathy, the risk of infections, and the occasional case of PML are perhaps the most important adverse events. Stevens-Johnson syndrome and toxic epidermal necrolysis constitute two other serious adverse events that, although rare, are potentially lethal.
- **Alemtuzumab** (**Campath®**, **MabCampath®**), a humanized mAb with complementarity-determining regions from a rat mAb, is targeted to CD52 which is expressed on mature normal and malignant B and T lymphocytes, monocytes, macrophages, NK cells, a subpopulation of granulocytes, and dendritic cells.
- Approved for the treatment of B-cell CLL resistant to alkylating agents, alemtuzumab was widely used in cancer therapy until 2012 when it was withdrawn from US and European markets. Patients can still receive it through specific access programs and some off-label usage in cancer therapy remains.
- Cytopenia, resulting from alemtuzumab's mode of action in destroying white blood cells, is also the major adverse event, hence the FDA boxed warning. As well as a high incidence of neutropenia (75–85 %), febrile neutropenia and thrombocytopenia (serious in 57 % of cases) may occur. Infections related to alemtuzumab therapy have been reported with incidences of up to 80 % and serious events as high as 50 %.
- The destruction of T cells by alemtuzumab can lead to CRS, and potentially nephrotoxic TLS may occur as a result of the rapid and massive destruction of neoplastic cells. Infusion reactions to alemtuzumab occur most commonly during the first week of treatment. Serious reactions can be fatal.
- In a number of different tumors, EGFR and its ligands are associated with the growth of the cells, and elevated EGFR tyrosine kinase activity is found in many, if not most, solid tumors including breast, renal, head and neck, colon, and non-small cell lung cancer. **Anti-EGFR mAbs** such as cetuximab bind to the EGFR with higher affinity than the natural ligands, preventing subsequent activation of tyrosine kinase-mediated signal transduction pathways. Monoclonal antibodies targeting the EGFR were therefore seen as a new approach for treating a range of solid tumors.
- Patients with mutations in codons 12 and 13 of the *KRAS* gene almost never benefit from cetuximab and panitumumab treatments, but 10–30 % of patients with the wild-type gene respond to the mAbs. As a result, regulatory agencies restricted the use of cetuximab and panitumumab to colorectal cancer patients expressing the wild-type *KRAS* gene.
- Serious infusion reactions and the possibility of cardiopulmonary arrest make up an FDA black box warning for **cetuximab** (**Erbitux®**). The severity and risk of many of the infusion reactions are underlined by the rapid onset of symptoms of

airway obstruction and other serious effects. Cardiopulmonary arrest and/or sudden death, the second subject of the boxed warning, occurred in 2–3 % of patients treated with cetuximab.

- Other warnings and precautions for cetuximab are for the possibility of interstitial lung disease; a variety of dermatologic events, for example, papulopustular rash (with an incidence of 76–88 %, up to 17 % serious); xerosis and fissuring; paronychial inflammation; hypertrichosis; infectious sequelae such as cellulitis and conjunctivitis; and hypomagnesemia and electrolyte abnormalities.
- **Panitumumab** (**Vectibix**®) binds the EGFR with high affinity. The antigenic structures recognized by cetuximab and panitumumab are not identical. This conclusion is supported by effective treatment with panitumumab of patients with disease progression under cetuximab and the development of resistance to cetuximab in a colorectal cancer patient who acquired a point mutation in the EGFR domain (Arg for Ser at position 468), while panitumumab binding and efficacy remained.
- At the first approval of panitumumab in 2006, the FDA issued a black box warning for dermatologic toxicity and infusion reactions. In the revised prescribing information issued in 2014, reference to infusion reactions in the boxed warning was removed, and dermatologic toxicities were stated to be severe in 15 % of patients.
- Mucocutaneous diseases provoked by panitumumab contribute to an extensive range of clinical manifestations including erythema, rash, pruritus, skin exfoliation, acneiform dermatitis, xerosis, paronychia, and skin fissures as well as life-threatening infectious complications such as necrotizing fasciitis and abscesses. Life-threatening bullous mucocutaneous diseases with erosions, blisters, and skin sloughing following panitumumab are known.
- Other warnings and precautions for panitumumab are for severe, including fatal, infusion reactions; severe hypomagnesemia, hypocalcemia, and hypokalemia; acute renal failure resulting from severe diarrhea and dehydration when panitumumab is used in combination with chemotherapy; interstitial lung disease and pulmonary fibrosis; ocular toxicities such as keratitis and ulcerative keratitis; dermatologic toxicities caused by sunlight exposure; and the possibility of increased toxicity and mortality when panitumumab is administered in combination with bevacizumab and chemotherapy.
- **Necitumumab** (**Portrazza**®) is a recombinant human mAb targeted to human EGFR. Indicated for first-line treatment of patients with metastatic squamous non-small cell lung cancer in combination with gemcitabine and cisplatin, necitumumab carries FDA black box warnings for cardiopulmonary arrest and/or sudden death and for hypomagnesemia.
- Five other warnings/precautions issued for necitumumab are the possible occurrence of venous and arterial thromboembolic events; some fatal, infusion-related reactions; embryofetal toxicity; increased toxicity and mortality in patients with non-squamous non-small cell lung cancer treated with the mAb plus pemetrexed and cisplatin; and dermatologic toxicities.
- **Bevacizumab** (**Avastin**®) binds to and inhibits the biological action of human vascular endothelial growth factor-A (VEGF-A). As well as its function of pro-

moting blood vessel formation in healthy subjects, VEGF-A-induced angiogenesis has a major role in the pathogenesis of a wide range of human diseases, for example, cancers, rheumatoid arthritis, and eye diseases.

- The relatively wide variety of tumors (about 30, including investigative and preliminary studies) treated by bevacizumab ensures a large number and variety of consequent adverse events. An extensive list of warnings and precautions is headed by a boxed warning for gastrointestinal perforation, complications of surgery and wound healing, and severe or fatal hemorrhage. Other serious adverse events seen are hypertension, arterial and venous thromboembolic events, proteinuria, PRES (<0.5 %), and anaphylaxis.
- **Ramucirumab** (**Cyramza**®) specifically binds VEGFR-2, the receptor-mediating angiogenesis, a major contributor, if not promoter, of tumor growth.
- Ramucirumab carries an FDA boxed warning for an increased risk of hemorrhage (including gastrointestinal hemorrhage that may be severe and sometimes fatal), gastrointestinal perforation, and impaired wound healing. Other warnings are for serious and sometimes fatal arterial thrombotic events including myocardial infarction, cardiac arrest, cerebrovascular accident, and cerebral ischemia; infusion-related reactions, usually during or following the first or second infusion; PRES; proteinuria including nephrotic syndrome; thyroid dysfunction; and the risk of deterioration in patients with cirrhosis.
- Overexpression of HER2 leads to constitutive activation of the growth factor signaling pathways with a consequent favorable environment for breast cancer cell growth. Overexpression, found in ~15–25 to 30 % of human breast cancers, tends to correlate with tumors that are more aggressive and show poorer prognosis. Three mAbs targeting HER2 are currently approved by the FDA and EMA, pertuzumab, trastuzumab, and the antibody-drug conjugate prepared by conjugating trastuzumab to the cytotoxin, mertansine.
- Although **pertuzumab** (**Perjeta**®) and **trastuzumab** (**Herceptin**®) target the same receptor, they recognize different binding sites. Trastuzumab inhibits ligand-independent signaling, complementing the mechanism of action of pertuzumab which inhibits ligand-dependent signaling between HER2 and HER3, a combination that potently activates cell survival and proliferation. The efficacy of pertuzumab alone is not impressive, but, together with trastuzumab, a more effective blockade of the HER2-driven signaling pathways results.
- There is an FDA boxed warning for **pertuzumab** for cardiomyopathy manifesting as congestive heart failure and decreased LVEF and for embryofetal toxicity that may result in fetal death and birth defects. Patients who have received prior treatment with anthracyclines or prior radiotherapy to the chest may be at higher risk of decreased LVEF. Infusion-related reactions and hypersensitivity responses including anaphylaxis are also the subject of a warning.
- Pertuzumab, given as a single agent or in combination with cytotoxic agents or trastuzumab, shows a low incidence of cardiac dysfunction similar to trastuzumab. Patients experience relatively low incidences of both LVSD and symptomatic heart failure. There was no significant increase in cardiac side effects

when pertuzumab was given in combination with other anticancer agents. No synergistic effect between pertuzumab and trastuzumab is apparent.

- **Trastuzumab** was the first biological agent developed and approved for the treatment of breast cancer, but only about 30 % of patients with advanced disease benefit from monotherapy with the drug. A large proportion of patients (~70 %) who experience an initial beneficial response become resistant within a year.
- Cross-signaling is a potential mechanism of trastuzumab resistance. Several molecules appear to activate HER2 in the presence of trastuzumab including insulin-like growth factor 1 receptor, hepatocyte growth factor and its receptor Met, growth differentiation factor 15, and members of the ErbB family. The precise mechanisms underlying such cross-signaling generally remain unclear.
- Trastuzumab is the subject of a boxed warning for cardiomyopathy, infusion reactions, and pulmonary toxicity. For patients receiving trastuzumab as a single agent or in combination therapy, there is a four- to sixfold increase in the incidence of symptomatic myocardial dysfunction. Incidences are highest when an anthracycline drug is coadministered. Trastuzumab therapy can result in cases of left ventricular cardiac dysfunction, hypertension, arrhythmias, cardiomyopathy, cardiac failure, and cardiac death. Trastuzumab may have a direct effect on myocytes.
- Other warnings and precautions for trastuzumab refer to exacerbation of chemotherapy-induced neutropenia and febrile neutropenia and the possibility of fetal harm with an increased risk of oligohydramnios during the second and third trimesters of pregnancy.
- **Ado-trastuzumab emtansine** (**Kadcyla**®) is an ADC made by covalently coupling the microtubule inhibitory compound mertansine (DM1), a cytotoxic, thiol-containing maytansinoid, to trastuzumab via a bifunctional cross-linker. DM1 inhibits the assembly of microtubules leading to cell cycle arrest and ultimately cancer cell death.
- An FDA black box warning for ado-trastuzumab emtansine mentions hepatotoxicity, cardiotoxicity (specifically reduction in LVEF), and the potential risk of fetal harm, plus a reminder that the ADC should not be substituted for, or by, trastuzumab. Warnings and precautions have been issued for pulmonary toxicity, infusion and hypersensitivity reactions, thrombocytopenia, neurotoxicity, and extravasation.
- **Denosumab** (**Xgeva**®, **Prolia**®) has high specificity and affinity for receptor activator of nuclear factor kappa-B ligand, otherwise known as RANKL. Responsible for bone resorption, RANKL stimulates osteoclast formation, activation, adherence, survival, and ultimately resorption of the bone. Inhibition of RANKL results in an increase in bone density, volume, and strength.
- Marketed under two trade names, denosumab as Prolia® has approved cancer indications for the treatment of men at high risk of fracture receiving androgen deprivation therapy for nonmetastatic prostate cancer and for the treatment of women at high risk of fracture receiving adjuvant aromatase inhibitor therapy for

breast cancer. As Xgeva®, denosumab is approved for the prevention of skeletal-related events in patients with bone metastases from solid tumors and the treatment of giant cell tumor of the bone.

- For Xgeva®, warnings and precautions consist of hypocalcemia, osteonecrosis of the jaw, and embryofetal toxicity. For Prolia®, hypersensitivity, including anaphylaxis, is listed as a warning together with hypocalcemia, osteonecrosis of the jaw, serious infections, femoral fractures and suppression of bone turnover, cutaneous reactions, and severe bone, joint, and muscle pain.
- Recent serious postmarketing surveillance reports for Xgeva® cover calcium and bone disorders, gastrointestinal symptoms, and musculoskeletal and dermatologic conditions; these events, plus respiratory disorders, are also the most commonly reported reactions. Reports on Prolia® relate to musculoskeletal disorders, dermatologic conditions, infections, and gastrointestinal disorders. The most frequently mentioned infections include pneumonia, cellulitis, urinary tract infections, and sepsis.
- **Ipilimumab** (**Yervoy®**) binds with high affinity to the extracellular domain of the protein receptor human cytotoxic T lymphocyte antigen 4 or CTLA-4. When bound to the B7 complex on APCs, CTLA-4 acts as an immune "off" switch modulating overactivity of T cells and allowing cancer cells to multiply in the absence of an antitumor challenge. This led to the development of ipilimumab, the first mAb example of immune checkpoint targeting.
- A number of adverse events induced by ipilimumab are related to the mAb's mode of action of T-cell-mediated immune aggression against cancer cells. These events are reflected in a boxed warning for immune-mediated reactions including enterocolitis, hepatitis, neuropathy, endocrinopathy, severe cutaneous events such as Stevens-Johnson syndrome and toxic epidermal necrolysis, and a number of immune-mediated ocular manifestations.
- **Siltuximab** (**Sylvant®**) targets interleukin-6 (IL-6), a proinflammatory and anti-inflammatory pleiotropic cytokine produced by many different cells including lymphocytes, monocytes, fibroblasts, and endothelial and cancer cells. Cancers associated with increased production of IL-6 include renal cell carcinoma, prostate and bladder cancers, some neurologic cancers, and particularly multiple myeloma and the B-cell lymphoproliferative disorder, Castleman's disease.
- Siltuximab is approved for the treatment of multicentric Castleman's disease in patients who are HIV and HHV-8 negative. Warnings and precautions mention the possibility of siltuximab masking signs of acute inflammation and suppressing fever and acute phase reactants such as CRP. By extension, live vaccines should not be administered concurrently or within four weeks of commencing siltuximab therapy. Other issued warnings are for infusion-related reactions and hypersensitivity and gastrointestinal perforation. An increased risk of malignancy and the development of hyperlipidemia and hepatic impairment are additional warnings issued by the EMA.

- The programmed cell death protein 1 (PD-1) receptor which plays a critical role in cancer immunology acts as another important immune checkpoint. PD-1 is expressed on CD4+ and CD8+ T cells, B lymphocytes, NK cells, B cells, and monocytes during antigen signaling. PD-1 has two ligands, PD-L1 and PD-L2. High expression of PD-L1 appears to be associated with increased aggressiveness of cancers and death.
- Like CTLA-4, PD-1 negatively regulates T-cell activation. PD-1 does not interfere with the normal immune response, but, upon binding PD-L1 and PD-L2, signaling is induced, suppressing T-cell proliferation and activity. Malignant cells often express PD-L1 which induces inhibitory signaling preventing expansion of activated T cells and allowing the tumor cells to avoid immune attack. Selectively blocking the pathway with anti-PD-1 antibodies reverses the checkpoint inhibition and restores the T-cell-mediated response to the tumor.
- **Pembrolizumab** (**Keytruda**®), targeted to PD-1, and indicated for metastatic melanoma and non-small cell lung cancer, can provoke a number of immune adverse reactions. Warnings have been issued for immune-mediated pneumonitis, colitis, hepatitis, hypophysitis, hyperthyroidism, hypothyroidism, renal failure, and nephritis.
- **Nivolumab** (**Opdivo**®), targeted to PD-1, is indicated for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab, and, if BRAF V600 mutation positive, a BRAF inhibitor should also be given. Other indications are non-small cell lung cancer and metastatic renal cell carcinoma.
- Nivolumab is also subject to warnings for immune-mediated adverse events of pneumonitis, colitis, hepatitis, hyperthyroidism, hypothyroidism, renal failure, and nephritis in melanoma patients.
- The sialic ganglioside disialoganglioside (GD2), a short sialylated polysaccharide linked to ceramide through a β-glycosidic linkage, is found highly expressed on neuroectoderm-derived cancers such as neuroblastoma, melanoma, brain tumors, osteosarcoma, and Ewing's sarcoma in children. **Dinutuximab** (**Unituxin**®), a human-mouse chimeric mAb that binds GD2, has been shown to prevent the outgrowth of experimental melanoma and neuroblastoma tumors.
- Approval for dinutuximab comes with a boxed warning for potentially life-threatening infusion reactions and neuropathy. Severe neuropathic pain and nerve damage requiring prior, and subsequent, intravenous opioid treatment may occur.
- Other warnings issued for dinutuximab are for capillary leak syndrome, hypotension, infection, neurological eye disorders, bone marrow suppression, electrolyte abnormalities, atypical hemolytic uremic syndrome, and embryofetal toxicity.
- **Daratumumab** (**Darzalex**®) is the first-in-class immunotherapy for multiple myeloma targeted to CD38 (cyclic ADP ribose hydrolase), a surface antigen expressed by multiple myeloma cells and found on many immune cells including CD4+, CD8+, B lymphocytes, and natural killer (NK) cells. The mAb acts by inhibiting the growth of tumor cells expressing CD38, leading to apoptosis by Fc-mediated cross-linking and cell lysis induced via CDC, ADCC, and ADCP.

- Warnings and precautions for daratumumab are the occurrence of infusion reactions, interference with cross-matching and red blood cell antibody screening, and interference by the mAb when determining patient's response and disease progression in patients with IgG kappa myeloma. As well as infusion reactions, adverse events recorded so far include infections, fatigue, and nausea. It is predicted that postmarketing usage will reveal the occurrence of cytopenias.
- The humanized mAb **elotuzumab** (**Empliciti**®), targeting the cell surface receptor CS1 (SLAMF7), a member of the signaling lymphocytic activation molecule receptor family, was recently approved for use with lenalidomide and dexamethasone for the treatment of relapsed or refractory multiple myeloma.
- Warnings and precautions associated with elotuzumab include infusion reactions (premedication is required), risk of infections, hepatotoxicity, and malignancies.
- In May 2016 FDA-approval was granted to the PD-L1-targeted mAb **atezolizumab** (Tecentriq™) for treatment of urothelial carcinoma.
- The mAbs approved for cancer therapy show the full range of hypersensitivity responses: type I IgE antibody-mediated immediate reactions such as anaphylaxis, urticaria, and angioedema; type II drug-induced thrombocytopenia, hemolytic anemia, and agranulocytosis; type III serum sickness and drug-induced vasculitis; and type IV cutaneous hypersensitivities mediated by Th1, Th2, and Th17 lymphocytes.
- Immunogenicity is always a safety concern for mAbs, even those that are fully humanized since the possibility of generating anti-idiotype antibodies remains. Warnings are often issued for anaphylaxis, but the observed incidences of such reactions are actually quite small. IgE-mediated reactions to chimeric proteins containing mouse and rat sequences are considered to be of greater risk. Anaphylaxis has been reported for, at least, cetuximab, rituximab, brentuximab, bevacizumab, trastuzumab, pertuzumab, ibritumomab, and dinutuximab.
- Cytopenias occur in some patients treated with mAbs during anticancer immunotherapy, but the underlying mechanisms frequently remain unexplored. Types II and III hypersensitivities induced by mAbs may be underdiagnosed.
- Incidences of 1.1 and 5.2 % for severe anemia have been reported for patients receiving rituximab monotherapy. At least two mAbs, rituximab and alemtuzumab, have been implicated in the induction of pure red cell aplasia and autoimmune hemolytic anemia.
- Hypersensitivity vasculitis induced by drugs is a manifestation of a type III response, and a few mAbs including rituximab have been implicated in its cause. Serum sickness reactions to mAbs are probably underdiagnosed and reported. Chimeric antibodies in particular have the potential to induce the reactions.
- Delayed, type IV cutaneous hypersensitivity reactions to anticancer mAbs are rare with most reported cases restricted mainly to ibritumomab tiuxetan, brentuximab vedotin, and rituximab. Mechanisms remain to be established for some type IV-like cutaneous reactions induced by catumaxomab, bevacizumab, denosumab, ipilimumab, and panitumumab.
- The mechanisms of mAb-induced infusion reactions are not yet fully understood. Cytokines, especially TNF and interleukins such as IL-6, may be involved

since the symptoms they produce resemble those seen in infusion and type I allergic reactions. An important finding was the observation that the severity of infusion reactions is related to the number of circulating lymphocytes.

- Cytokine release syndrome, also called cytokine storm, may be seen after the use of mAbs directed to malignant immune cells, for example, rituximab.
- At least ten of the currently approved mAbs for cancer therapy have some recorded pulmonary toxicity in treated cancer patients. Adverse pulmonary events provoked by mAbs include interstitial pneumonitis and fibrosis, ARDS, bronchiolitis obliterans organizing pneumonia (BOOP), and hypersensitivity pneumonitis. Hypersensitivity pneumonitis to some anticancer agents appears to be a combined type III and type IV hypersensitivity reaction in a Th1/Th17 response.
- Cardiac adverse events have occurred with at least 11 of the mAbs used for cancer therapy.
- Cutaneous reactions elicited by mAbs targeted to EGFR are generally not immune-mediated. Skin reactions appear as a papulopustular eruption, often in a large proportion of patients (50–100 %) and in a more severe form than seen with small molecule tyrosine kinase inhibitors. Eruptions tend to be confined to seborrheic regions such as the epidermis, sebaceous glands, and hair follicles. Other adverse effects induced by mAbs targeted to EGFR include paronychia, fissures, xerosis, palmar-plantar rash, hair changes, hyperkeratosis, mucositis, pyrogenic granuloma, and skin hyperpigmentation.
- Tumor lysis syndrome usually occurs in patients with leukemias and high-grade lymphomas. Large numbers of malignant cells may be destroyed in a short time by the mAb resulting in hyperkalemia, hypercalcemia, hyperphosphatemia, and hyperuricemia. This may produce profound ionic imbalances in potassium, calcium phosphate, and uric acid and progress to acute renal failure, cardiac arrhythmias, seizures, and death.
- Progressive multifocal leukoencephalopathy, caused by the polyomavirus JC virus in severely immunodeficient individuals, is occasionally seen upon the administration of some mAbs directed to B cells, in particular, rituximab, ofatumumab, obinutuzumab, and brentuximab vedotin.

Further Reading

Ahmed M, Hu J, Cheung N-KV. Structure based refinement of a humanized monoclonal antibody that targets tumor antigen disialoganglioside GD2. Front Immunol. 2014;5:372. doi:10.3389/fimmu.2014.00372.

Alinari L, Lapalombella R, Andritsos L, et al. Alemtuzumab (Campath-1H) in the treatment of chronic lymphocytic leukemia. Oncogene. 2007;26:3644–53.

Baldo BA. Adverse events to monoclonal antibodies used for cancer therapy. Focus on hypersensitivity responses. OncoImmunology. 2013;2(10):e26333. doi:10.4161/onci.26333

Baldo BA, Pagani M. Adverse events to nontargeted and targeted chemotherapeutic agents. Immunol Allergy Clin N Am. 2014;34:565–96.

Bargou R, Leo E, Zugmaier G, et al. Tumor regression in cancer patients by very low doses of a T cell-engaging antibody. Science. 2008;321:974–7.
Boross P, Leusen JHW. Mechanism of action of CD20 antibodies. Am J Cancer Res. 2012;2:676–90.
Bryan LJ, Gordon LI. Releasing the brake on the immune system: the PD-1 strategy for hematologic malignancies. Oncology. 2015;29:431–9.
Callahan MK, Wolchok JD. At the bedside: CTLA-4- and PD-1-blocking antibodies in cancer immunotherapy. J Leukoc Biol. 2013;94:41–53.
Castellano D, Sepulveda JM, Garcia-Escobar I, et al. The role of RANK-Ligand inhibition in cancer: the story of denosumab. Oncologist. 2011;16:136–45.
Chavez-MacGregor M, Zhang N, Buchholz TA, et al. Trastuzumab-related cardiotoxicity among older patients with breast cancer. J Clin Oncol. 2013;31:4222–8.
Cheung CH. Managing premedications and the risk for reactions to infusional monoclonal antibody therapy. Oncologist. 2008;13:725–32.
Clarke JM, Hurwitz HI. Targeted inhibition of VEGF Receptor-2: an update on ramucirumab. Expert Opin Biol Ther. 2013;13:1187–96.
De Roock W, Claes B, Bernasconi D, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol. 2010;11:753–62.
Duffy MJ, O'Donovan N, Crown J. Use of molecular markers for predicting therapy response in cancer patients. Cancer Treat Rev. 2011;37:151–9.
Ellis LM. Mechanisms of action of bevacizumab as a component of therapy for metastatic colorectal cancer. Semin Oncol. 2006;33 Suppl 10:S1–7.
Goltsov A, Deeni Y, Khalil HS, et al. Systems analysis of drug-induced receptor tyrosine kinase reprogramming following targeted mono- and combination anti-cancer therapy. Cells. 2014;3:563–91.
Holmes K, Roberts OLI, Thomas AM, et al. Vascular endothelial growth factor receptor-2: structure, function, intracellular signalling and therapeutic inhibition. Cell Signal. 2007; 19:2003–12.
Holstead Jones D, Nakashima T, Sanchez OH, et al. Regulation of cancer cell migration and bone metastasis by RANKL. Nature. 2006;440:692–6.
Jelinek T, Hajek R. Monoclonal antibodies -- A new era in the treatment of multiple myeloma. Blood Rev. 2016;30:101–10.
Junttila TT, Akita RW, Parsons K, et al. Ligand-independent HER2/HER3/PI3K complex is disrupted by trastuzumab and is effectively inhibited by the PI3K inhibitor GDC-0941. Cancer Cell. 2009;15:429–40.
Kimby E. Tolerability and safety of rituximab (MabThera®). Cancer Treat Rev. 2005; 31:456–73.
Lioté H, Lioté F, Séroussi B, et al. Rituximab-induced lung disease: a systematic literature review. Eur Resp J. 2010;35:681–7.
Liu Y-C, Szmania S, van Rhee F. Profile of elotuzumab and its potential in the treatment of multiple myeloma. Blood Lymphat Cancer. 2014;4:15–27. doi:10.2147/BLCTT.S49780.
Lokhorst HM, Plesner T, Laubach JP, et al. Targeting CD38 with daratumumab monotherapy in multiple myeloma. N Engl J Med. 2015;373:1207–19. doi:10.1056/NEJMoa1506348.
Lonberg N. Fully human antibodies from transgenic mouse and phage display platforms. Curr Opin Immunol. 2008;20:450–9.
Marmor MD, Skaria KB, Yarden Y. Signal transduction and oncogenesis by ErbB/HER receptors. Int J Radiat Oncol Biol Phys. 2004;58:903–13.
McDermott DF, Atkins MB. PD-1 as a potential target in cancer therapy. Cancer Med. 2013;2:662–73.
McKinney MS, Beavan AW. Yttrium-90 ibritumomab tiuxetan in the treatment of non-Hodgkin lymphoma. Blood Lymphat Cancer Targets Ther. 2014;4:45–59.

Merelli B, Massi D, Cattaneo L, et al. Targeting the PD-1/PD-L1 axis in melanoma: biological rationale, clinical challenges and opportunities. Crit Rev Oncol Hematol. 2014;89:140–65.
Miao H-Q, Hu K, Jiminez X, et al. Potent neutralization of VEGF biological activities with a fully human antibody Fab fragment directed against VEGF receptor 2. Biochem Biophys Res Commun. 2006;345:438–45.
Ohaegbulam KC, Assal A, Lazar-Molnar E, et al. Human cancer immunotherapy with antibodies to the PD-1 and PD-L1 pathway. Trends Mol Med. 2015;21:24–33.
Rose-John S. IL-6 trans-signaling via the soluble IL-6 receptor: importance for the pro-inflammatory activities of IL-6. Int J Biol Sci. 2012;8:1237–47.
Peggs KS, Quezada SA, Chambers CA, et al. Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of anti-CTLA-4 antibodies. J Exp Med. 2009;206:1717–25.
Portera CC, Walshe JM, Rosing DR, et al. Cardiac toxicity and efficacy of trastuzumab combined with pertuzumab in patients with trastazumab-insensitive human epidermal growth factor receptor 2-positive metastatic breast cancer. Clin Cancer Res. 2008;14:2710–6.
Sartore-Bianchi A, Martini M, Molinari F, et al. PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. Cancer Res. 2009;69:1851–7.
Senter PD, Sievers EL. The discovery and development of brentuximab vedotin for use in relapsed Hodgkin lymphoma and systemic anaplastic large cell lymphoma. Nat Biotechnol. 2012;30:631–7.
Tarhini A. Immune-mediated adverse events associated with ipilimumab CTLA-4 blockade therapy: the underlying mechanisms and clinical management. Scientifica. 2013;Article ID 857519:19 p. doi:10.1155/2013/857519
Teeling JL, Mackus WJM, Wiegman LJJM, et al. The biological activity of human CD20 monoclonal antibodies is linked to unique epitopes on CD20. J Immunol. 2006;177:362–71.
Thatcher N, Hirsch FR, Luft AV, et al. Necitumumab plus gemcitabine and cisplatin versus gemcitabine and cisplatin alone as first-line therapy in patients with stage IV squamous non-small-cell lung cancer (SQUIRE): an open-label, randomised, controlled phase 3 trial. Lancet Oncol. 2015;16:763–74.
van der Donk NW, Moreau P, Plesner T, et al. Clinical efficacy and management of monoclonal antibodies targeting CD38 and SLAMF7 in multiple myeloma. Blood. 2016;127:681–95.
Voigt M, Braig F, Göthel M, et al. Functional dissection of the epidermal growth factor receptor epitopes targeted by panitumumab and cetuximab. Neoplasia. 2012;14:1023–31.
Voskens CJ, Goldinger SM, Loquai C, et al. The price of tumor control: an analysis of rare side effects of anti-CTLA-4 therapy in metastatic melanoma from the ipilimumab network. PLoS One. 2013;8(1):e53745. doi:10.1371/journal.pone.0053745.
Zhang B. Ofatumumab. mAbs. 2009;1:326–31.