“Biologics,” sometimes also referred to as “biotherapeutics” or “biopharmaceuticals,” here refers to therapies that are prepared from materials made or expressed in living organisms. They may simply be isolated proteins such as enzymes, hormones, and blood products or, as is increasingly the case, preparations produced by recombinant DNA technology. As well as proteins, biologics may be composed of nucleic acids, sugars, lipids, or combinations of these; they may be of human, animal, or microbial origin; and, in the vast majority of cases, they are created by biological processes, or biotechnology, rather than chemical synthesis. Besides recombinant therapeutic proteins such as some antibodies, cytokines, and receptors, included in a list of biologics are gene therapies, somatic cells, adult and embryonic stem cells, vaccines, tissues, and blood components and products.

Biologics by virtue of recombinant methodologies, precise targeting, exquisite specificity, and high binding affinity are continuing to provide new, or improve on, existing treatments for some diseases where, for many years, major advances in efficacy have not been forthcoming. For example, biologic therapies have sometimes provided new treatments previously seen as a long, difficult, uncertain, and costly process because of the difficulties involved in finding and developing small molecule agonists and antagonists to interact with a particular metabolite or with receptors, some newly suspected or identified. Examples are seen in the regulatory approvals and clinical efficacies of research-based biopharmaceuticals such as the anticancer checkpoint inhibitor monoclonal antibodies (mAbs) pembrolizumab; the fusion protein etanercept and anti-TNF mAbs to treat rheumatoid arthritis; cytokines interferon-beta 1a and beta 1b for relapsing multiple sclerosis; a range of different insulins for better glycemic control; truncated recombinant factor VIII preparations for hemophilia A; and the enzyme recombinant urate oxidase rasburicase for the management of tumor lysis syndrome (Chap. 14, Sect. 14.2.2.7). Further examples of the efficacy, efficiency, and speed of the modern successful application of recombinant technologies to treat diseases are seen in the assisted development and marketing of biologic therapies brought to market under Orphan Drug Designation programs. In the United States, the FDA Office of Orphan Products Development provides incentives for the development of products (drugs, biologics, devices, medicinal foods) for the diagnosis and/or treatment of rare conditions, that is, diseases or disorders, that affect fewer than 200,000 people in the United States or where developers/manufacturers are not expected to cover the total costs of developing and marketing the agents. Application of the program to an increasing number of rare diseases has led to an expanding list of successful biologic therapeutics including approved cytokines, enzymes, and other targeted agents in highly purified, well-characterized recombinant form.
There are many, and often marked, differences in characteristics and properties between biologic therapeutic agents and small molecule drugs. The foremost advantage of recombinant protein therapeutics technology over small MW drug development is the capacity to rapidly develop and treat diseases formerly regarded as beyond immediate or even longer-term reach of conventional pharmacologic therapies. To this can be added specificity of action and potent therapeutic efficiency, often more predictable actions, fewer side effects including the expected lower immunogenicity due to their human origin, faster regulatory approval time, and their uniqueness allowing better patent protection for developers and marketers. However, and not surprisingly, as shown below and in later chapters, clinical experience may sometimes reveal significant departures from the expected outcomes. Table 13.1 summarizes a comparison of the properties of biologic and conventional small molecule drugs.

In this chapter we cover the basic structural features, nomenclature, and disease applications of a range of regulatory-approved biologics including some important cytokines, fusion proteins, hormones, enzymes, and coagulation proteins and introduce mAbs, the most successful and widely applied form of biologic therapy. Because of the high incidence of the many different cancers, the relative numbers of affected patients, and high potential financial returns, a large proportion, 36 (41%), of the 88 currently approved (at April 2020) mAbs are anticancer agents, and this figure far outnumbers the mAbs indicated for each of the dif-

### Table 13.1 Comparison of properties of biologic products and small molecule drugs

| Biologics | Small molecule drugs |
|-----------|----------------------|
| Generally high molecular weight | Generally low molecular weight |
| Often heterogeneous mixtures; may include variants | Homogeneous |
| Structure may not be well-defined or known | Well-defined structure |
| Complex physicochemical characteristics | Physicochemically far less complex |
| May be synthesized (e.g., short peptides) but usually made with the aid of or from live cells and organisms | Usually organic molecules prepared by chemical synthesis |
| Usually many critical process steps in preparation and manufacture | Fewer critical steps involved |
| Usually not easily characterized | Not difficult to characterize and therefore usually well characterized |
| Often not stable; usually heat sensitive | Usually relatively stable |
| Controlled storage conditions often required to overcome instability | Often stable for storage at room temperature for long periods |
| Usually administered parenterally | Often administered orally |
| May have long half-life (days–weeks) allowing daily to monthly dosing | Relatively short half-life with dosing required every few hours |
| High selectivity and specificity for target | High potential for off-target effects |
| Catabolized to amino acids, sugars, lipids, etc. Limits toxicity | Metabolism by liver enzymes such as cytochrome P450 may lead to toxicity |
| Do not readily penetrate cells and cross the blood-brain barrier | Smaller size often allows cell entry and passage across blood-brain barrier (especially if lipid soluble) |
| Often immunogenic | Without linkage to a carrier, usually not immunogenic |
| High cost of development | Cost high but often less than a biologic |
| Cost of treatments for patients and health budgets often very high | Treatment cost usually lower, often considerably so |

Reproduced with permission from Baldo 2016 BA. Safety of biologics therapy. Monoclonal antibodies, cytokines, fusion proteins, hormones, enzymes, coagulation proteins, vaccines, botulinum toxins. Cham Switzerland: Springer Nature; 2016

*Biologics as defined in this chapter
*Generally ≥2–5 kDa but may be significantly greater
*Generally <0.5 kDa
ferent non-cancer disease categories. Accordingly, mAbs used to treat the many different cancers are considered separately in the following chapter, and the remaining antibodies covering a wide and diverse range of different diseases and conditions are considered here.

13.1 Monoclonal Antibodies

Over the last 20 years, specially designed and produced mAbs have become one of the most important and successful therapies for patients with hematologic malignancies and solid tumors. Targeted mAbs, some developed as orphan drugs, are also finding increasing application for the treatment of a wide range of other diseases, including chronic asthma, migraine, psoriasis, systemic lupus erythematosus, and macular degeneration; the prevention of the rejection of transplanted organs and graft-versus-host reactions; inhibition of platelet aggregation in some cardiovascular diseases; treatment of autoimmune diseases such as Crohn’s disease and rheumatoid arthritis; anti-infection treatments for anthrax, *Clostridium difficile*, respiratory syncytial virus, and HIV-1; paroxysmal nocturnal hemoglobinuria and cryopyrin-associated periodic syndrome; and hypercholesterolemia.

13.1.1 Evolution of Therapeutic Monoclonal Antibodies: From Mouse to Man

In 1985, muromonab-CD3 (Orthoclone OKT3), the murine IgG2a mAb directed against the CD3 (T3) receptor on the surface of human T lymphocytes, became the first therapeutic mAb to receive regulatory (FDA) approval. Approved indications were for its use as an immunosuppressant to combat steroid-resistant acute allograft rejection in patients with renal, hepatic, and cardiac transplants and for acute graft-versus-host disease. Given its murine protein composition, it soon became apparent that patient immune responses to mouse protein and the poor pharmacokinetics of the mAbs were a problem for repeated and long-term therapy. Adverse events associated with the mouse antibody were numerous and included cytokine release syndrome (Chap. 14, Sect. 14.2.2.6) and the induction of anaphylactoid/anaphylactic reactions. Approvals for two other murine mAbs, ibritumomab tiuxetan and tositumomab-131I, both indicated for non-Hodgkin lymphoma and a mouse/rat hybrid hybridoma, catumaxomab, for malignant ascites followed (Table 13.2), but it was soon clear that the mAbs for human therapy needed to be less foreign and more human in protein composition. An ongoing iterative process therefore began, first with the production of chimeric antibodies in which the variable (antigen-binding) regions of mouse antibodies were incorporated into the constant regions of human immunoglobulins (Fig. 13.1). Antibodies in this category, abciximab, basiliximab, brentuximab vedotin, cetuximab, rituximab, dinutuximab, infliximab, and siltuximab (Table 13.2), with a two-thirds reduction in murine proteins failed to eliminate adverse reactions, especially occasional potentially serious hypersensitivities. Production of so-called humanized antibodies with only ~5–10% murine proteins, achieved by substituting murine hypervariable or complementarity-determining regions (CDRs) in place of human sequences while retaining the remainder of the antibody as human (Fig. 13.1), resulted in mAbs with fewer adverse effects, but some undesirable, less immediately obvious effects soon became apparent. Two deficiencies in the path toward fully human proteins without accompanying unwanted reactions remained. Firstly, small amino acid changes, even to only a single residue, especially in the variable region, could cause slight alterations in the CDRs, perturbing antibody conformational integrity and, in turn, binding affinity and avidity. Secondly, pharmacokinetic properties and efficacy were found to be negatively influenced by defects in the posttranslational glycosylation of the mAb. Such defects manifested as poor solubility and clearance rates, lack of specificity and potency, and, importantly, excessive immunogenicity. The achievement of fully human mAbs became possible with the development of phage display and transgenic mouse technologies. Now
| INN and trade name | Type of mAb | Cell line | Targeta | Mechanism of action | Approved indications |
|--------------------|-------------|-----------|---------|---------------------|---------------------|
| **Human-mouse chimeric (-ximab)** | | | | | |
| Abciximab (ReoPro®) | Chimeric IgG1κ Fab | Sp2/0 | Glycoprotein IIb/IIIa | A receptor antagonist. Binds gp IIb/IIIa inhibiting platelet aggregation by preventing binding of fibrinogen and von Willebrand factor | Adjunct therapy for prevention of cardiac ischemic complications |
| Basiliximab (Simulect®) | Chimeric IgG1κ | Sp2/0 | α-Chain IL-2 receptor (CD25) | Competitively inhibits IL-2-mediated activation of T cells involved in allograft rejection | Prevent organ transplant rejection |
| Infliximab (Remicade®) | Chimeric IgG1κ | Sp2/0 | TNF | Inhibits binding of TNF to its receptor neutralizing its many proinflammatory actions | Crohn’s disease; ulcerative colitis; rheumatoid arthritis; ankylosing spondylitis; psoriatic arthritis; plaque psoriasis |
| Obiltoxaximab (Anthim®) | Chimeric IgG1κ rh-DNA murine cell line | Bacillus anthracis PA | Binds free PA (Kₐ 0.33 nM) inhibiting its binding to its receptors and preventing cell entry of anthrax, edema, and lethal toxins | Inhalational anthrax Bacillus anthracis PA |
| **Humanized (-zumab)** | | | | | |
| Alemtuzumab (Lemtrada®) | Humanized IgG1κ CHO | CD52 | Binds CD52 on leukemic cells and induces antibody-dependent cell-mediated lysis | Lemtrada®: Multiple sclerosis |
| Benralizumab (Fasenra®) | Humanized IgG1κ (afucosylated) CHO | IL-5Rα | Binds α-subunit of IL-5Rα on basophils and eosinophils ultimately facilitating apoptosis of these cells by ADCC | Asthma |
| Brolucizumab (Beovu®) | Humanized single chain (scFv) fragment E. coli | VEGF-A | Binds major isoforms VEGF-A 110, 121, and 165; prevents interaction with VEGFR-1 and VEGFR-2, suppressing cell proliferation, neovascularization and vascular permeability | Neovascular (wet) age-related macular degeneration |
| Caplacizumab-yhdpb (Cablivi®) | Humanized bivalent single domain nanobody E. coli | von Willebrand factor (vWF) | Targets A1-domain of vWF, inhibiting its interaction with platelets and reducing platelet adhesion and consumption | Acquired thrombotic thrombocytopenic purpura |
| Certolizumab pegolc (Cimzia®) | Humanized IgG1κ Fab, pegylated E. coli | TNF | Binds TNF neutralizing its proinflammatory actions. No Fc therefore no CDC or ADCC | Crohn’s disease; rheumatoid arthritis |
| INN and trade name                  | Type of mAb | Cell line | Target | Mechanism of action                                      | Approved indications                                                                 |
|------------------------------------|-------------|-----------|--------|-----------------------------------------------------------|---------------------------------------------------------------------------------------|
| Crizanlizumab-tmca (Adakveo®)      | Humanized IgG2κ | CHO       | P-selectin | Binds P-selectin on endothelium and platelets blocking interactions with its ligands and between endothelial cells, platelets, red cells, and leukocytes | Sickle cell disease                                                                   |
| Daclizumab (Zinbryta®)             | Humanized IgG2κ | NSO       | α-Chain IL-2 receptor (CD25) | Binds α-subunit of IL-2α-modulating IL-2 mediated-activation of lymphocytes         | Multiple sclerosis                                                                     |
| Eculizumab (Soliris®)              | Humanized IgG2/4κ | NSO       | Complement C5 | Inhibits cleavage of C5 and complement-mediated intravascular hemolysis and thrombotic microangiopathy | Paroxysmal nocturnal hemoglobinuria; atypical hemolytic uremic syndrome                  |
| Emicizumab-kxwh (Hemlibra®)        | Humanized IgG4 bispecific | CHO | Factors IXa and X | Bridges activated factors IX and X restoring function of missing activated factor VIII | Hemophilia A                                                                             |
| Fremanezumab-vfrm (Ajovy®)         | Humanized IgG4κ | CHO       | CGRP   | Binds CGRP blocking its binding to its receptor          | Migraine                                                                               |
| Galcanezumab-gnlm (Emgality®)      | Humanized IgG4κ | CHO       | CGRP   | Binds CGRP blocking its binding to its receptor          | Migraine                                                                               |
| Ibalizumab-uiyk (Trogarzo®)        | Humanized IgG4 | NSO       | CD4     | Binds domain 2 of CD4+ cells blocking entry of HIV-1 and preventing viral transmission | HIV-1 infection                                                                         |
| Idarucizumab (Praxbind®)           | Humanized IgG1 antibody fragment Fab | CHO | Dabigatran | Binds to dabigatran and its acylglucuronide metabolites neutralizing the anticoagulant effect | Reversal of anticoagulant effects of dabigatran; life-threatening or uncontrolled bleeding |
| Mepolizumab (Nucala®)              | Humanized IgG1κ | CHO       | IL-5    | Binds IL-5 and blocks receptor, reducing the production and survival of eosinophils and reducing inflammation in asthma | Asthma; eosinophilic granulomatosis with polyangiitis                                    |
| Natalizumab (Tysabri®)             | Humanized IgG4κ | NSO       | α4 integrin | Binds α4 subunit of α4β1 and α4β7 on leukocytes inhibiting adhesion to counter receptors and prevents leukocyte migration to inflamed tissue | Multiple sclerosis; Crohn’s disease                                                   |
| Ocrelizumab (Ocrevus®)             | Humanized IgG1 | CHO       | CD20    | Binds CD20 on pre- and mature B lymphocytes resulting in ADCC and CDC | Multiple sclerosis                                                                      |

(continued)
| INN and trade name | Type of mAb | Cell line | Target\(^a\) | Mechanism of action | Approved indications |
|-------------------|-------------|-----------|-------------|---------------------|---------------------|
| Omalizumab (Xolair\(^b\)) | Humanized IgG1\(\kappa\) | CHO | IgE | Inhibits binding of IgE to Fc\(\gamma\)RI on mast cells and basophils limiting mediator release and cell-bound IgE | Persistent asthma; chronic idiopathic urticaria |
| Ixekizumab (Taltz\(^b\)) | Humanized IgG4 | CHO | IL-17A | Inhibits interaction of IL-17A with its receptor inhibiting release of other inflammatory cytokines and chemokines | Plaque psoriasis; psoriatic arthritis |
| Palivizumab (Synagis\(^b\)) | Humanized IgG1\(\kappa\) | NSO | RSVF | Reduces pulmonary RSV replication and inhibits virus entering cell | Prevention of lower respiratory tract disease RSV in children |
| Ranibizumab (Lucentis\(^b\)) | Humanized IgG1\(\kappa\) Fab | E.coli | VEGF-A | Binds VEGF-A preventing its interaction with receptors and reducing angiogenesis and vascular leakage | Neovascular (wet) age-related macular degeneration; macular edema following retinal vein occlusion; diabetic macular edema |
| Ravulizumab-cwvz (Ultomiris\(^b\)) | Humanized IgG2/4\(\kappa\) | CHO | Complement C5 | Binds complement C5 inhibiting its cleavage to C5a and C5b, prevents generation of C5b9, and inhibits C-mediated intravascular coagulation | Paroxysmal nocturnal hemoglobinuria |
| Reslizumab (Cinqair\(^b\)) | Humanized IgG4\(\kappa\) | NSO | IL-5 | Binds IL-5 and blocks receptor, reducing the production and survival of eosinophils and reducing inflammation in asthma | Asthma |
| Risankizumab-rzza (Skyrizi\(^b\)) | Humanized IgG1 | CHO | IL-23 p19 | Selectively binds p19 subunit of IL-23 inhibiting its interaction with the IL-23 receptor\(^4\) | Plaque psoriasis |
| Romosozumab-aqqg (Evenity\(^b\)) | Humanized IgG2 | CHO | Sclerostin\(^1\) | Inhibits sclerostin resulting in stimulation of osteoblasts and increases in trabecular and cortical bone mass | Osteoporosis\(^a\) |
| Tildrakizumab-asmn (Ilumetri\(^b\); Ilumya\(^b\)) | Humanized IgG1\(\kappa\) | CHO | IL-23 p19 | Selectively binds p19 subunit of IL-23 inhibiting its interaction with the IL-23 receptor\(^4\) | Plaque psoriasis |
| Tocilizumab (Actemra\(^b\); RoActemra\(^b\)) | Humanized IgG1\(\kappa\) | CHO | IL-6R | Binds IL-6 receptors inhibiting signaling and proinflammatory effects of IL-6 in joints affected by inflammation | Rheumatoid arthritis; polyarticular juvenile idiopathic arthritis; systemic juvenile idiopathic arthritis |
| INN and trade name | Type of mAb | Cell line | Target$^a$ | Mechanism of action | Approved indications |
|-------------------|-------------|-----------|------------|---------------------|---------------------|
| **Vedolizumab** (Entyvio®) | Humanized IgG1κ | CHO | α4β7 integrin | Blocks interaction of α4β7 integrin with addressinn, inhibiting T cell migration into inflamed GI tissue | Adult ulcerative colitis; adult Crohn’s disease |
| **Fully human (−umab)** | | | | | |
| **Adalimumab** (Humira®) | Human IgG1κ | CHO | TNF | Binds to TNF preventing receptor activation and articular inflammation$^a$ characteristic of these diseases | Rheumatoid arthritis; psoriatic arthritis; ankylosing spondylitis; plaque psoriasis; Crohn’s disease |
| **Belimumab** (Benlysta®) | Human IgG1λ | NSO | BlyS | Blocks binding of soluble BlyS$^p$ to receptors on B cells inhibiting B cell survival | Systemic lupus erythematosus |
| **Bezlotoxumab** (Zinplava®) | Human IgG1 | CHO | *Clostridium difficile* toxin B | Binds to and neutralizes *Clostridium difficile* toxin B infection | Recurrence of *Clostridium difficile* toxin B infection |
| **Brodalumab** (Siliq®; Kyntheum®; Lumicef®) | Human IgG2κ | CHO | IL-17RA | Binds IL-17A inhibiting IL-17-induced release of proinflammatory cytokines and chemokines | Plaque psoriasis |
| **Burosumab-twza** (Crysvita®) | Human IgG1κ | CHO | FGF23 | Binds to and inhibits FGF23 restoring renal PO$_4$ reabsorption and increasing serum vitamin D | X-linked hypophosphatemia |
| **Canakinumab** (Ilaris®) | Human IgG1κ | Sp2/0 | IL-1β | Mutations in NLRP-3 gene lead to excess IL-1 β and inflammation. Antibody binds to IL-1 β neutralizing its action | Cryopyrin-associated periodic syndromes (CAPS) including familial cold autoinflammatory and Muckle-Wells syndromes; SJIA with body weight ≥7.5 kg; NOMID/CINCA; FCAS/FCU; gouty arthritis |
| **Denosumab** (Prolia®) | Human IgG2κ | CHO | RANKL | Binds RANKL preventing receptor activation, osteoclast formation, bone resorption, osteolysis, and tumor growth | Bone loss. For osteoporosis and to increase bone mass in menopausal women at high risk of fracture |
| **Dupilumab** (Dupixent®) | Human IgG4 | CHO | IL-4Rα-subunit | Binds IL-4Rα-subunit inhibiting IL-4 and IL-13 signaling and release of proinflammatory cytokines, chemokines, and IgE | Atopic dermatitis |
| **Emapalumab-lzsg** (Gamifant®) | Human IgG1 | CHO | IFNγ | Binds IFNγ which is hypersecreted and has a role in HLH | HLH |

(continued)
| INN and trade name | Type of mAb | Cell line | Targeta | Mechanism of action | Approved indications |
|--------------------|--------------|-----------|---------|---------------------|---------------------|
| Erenumab-aooe (Aimovig®) | Human IgG2 | CGRP receptor | Binds the CGRP receptor antagonizing receptor function | Migraine |
| Golimumab (Simponi®) | Human IgG1κ | Sp2/0 | TNF | Binds to TNF preventing receptor activation and articular inflammation characteristic of these diseases | Rheumatoid arthritis; psoriatic arthritis (both in combination with methotrexate); ankylosing spondylitis |
| Guselkumab (Tremfya®) | Human IgG1λ | CHO | IL-23 | Binds p19 subunit of IL-23 inhibiting its interaction with its receptor and release of proinflammatory cytokines and chemokines | Plaque psoriasis |
| Lanadelumab-flyo (Takhzyro®) | Human IgG1κ | CHO | Plasma kallikrein | Binds plasma kallikrein inhibiting its proteolytic activity, preventing formation of kallikrein and increased vascular permeability | HAE prevention |
| Raxibacumab (ABthrax®) | Human IgG1λ | NSO | *Bacillus anthracis* PA | Binds free PA inhibiting its binding to its receptors and preventing cell entry of anthrax edema and lethal toxins | Inhalational anthrax to *Bacillus anthracis* and prophylaxis in absence of alternative therapies |
| Sarilumab (Kevzara®) | Human IgG1 | CHO | IL-6R | Antibody binding to IL-6R inhibits IL-6-mediated signaling. IL-6 produced in synovial and endothelial cells in joints contributes to inflammation in RA | RA |
| Secukinumab (Cosentyx®) | Human IgG1κ | CHO | IL-17A | Inhibits interaction of IL-17A with its receptor inhibiting release of other inflammatory cytokines and chemokines | Moderate to severe plaque psoriasis |
| Teprotumumab-trbw (Tepezza®) | Human IgG1 | CHO | IGF-1R | Blocks activation and signaling of IGF-1R | Thyroid eye disease |
| Ustekinumab (Stelara®) | Human IgG1κ | Sp2/0 | IL-12, IL-23 | Binds to p40 protein subunit of IL-12 and IL-23 disrupting their signaling and immune and inflammatory responses | Plaque psoriasis |
| Alirocumab (Praluent®) | Human IgG1κ | CHO | PCSK9 | Inhibits binding PCSK9 to LDLR, LDLR expression↑, LDL clearance ↓, and LDL-C↓ in circulation | Heterozygous FH; atherosclerotic CV disease requiring additional ↓ of LDL-C |
ADCC antibody-dependent cell-mediated cytotoxicity; BAFF: B lymphocyte stimulator, also known as B cell activating factor; BAFF: C5 complement component 5; CDC complement-dependent cytotoxicity; CGRP calcitonin gene-related peptide; CHO Chinese hamster ovary cells; CGRP calcitonin gene-related peptide; CINCA chronic infantile neurological, cutaneous, articular syndrome; CV cardiovascular; EMA European Medicines Agency; FCAS familial cold autoinflammatory syndrome; FCU familial cold urticaria; FDA US Food and Drug Administration; FH familial hypercholesterolemia; FGF23 fibroblast growth factor 23; GI gastrointestinal; HAE hereditary angioedema; HIV human immunodeficiency virus; HLH primary hemophagocytic lymphohistiocytosis; IGF-1R insulin-like growth factor-1 receptor; IPP International Nonproprietary Name; LDL low-density lipoprotein; LDL-C LDL-cholesterol; LDLR LDL receptor; NLRP-3 gene cryopyrin or nucleotide-binding domain, leucine rich family, pyrin domain containing 3 gene; NOMID neonatal-onset multisystem inflammatory disease; NSCLC non-small cell lung cancer; NSO non-Ig-secreting, non-L chain-synthesizing, 8-azaguanine-resistant and HAT-sensitive mouse myeloma cell line; PA protective antigen of B. anthracis toxin; PCSK9 proprotein convertase subtilisin/kexin type 9; RANKL receptor activator of nuclear factor kappa-B ligand (CD254) a member of the TNF cytokine family; RSV human respiratory syncytial virus (F viral protein coat antigen); SJIA active systemic juvenile idiopathic arthritis; Sp2/0 BALB/c mouse spleen cells fused with P3 myeloma. Cells do not secrete Ig, are resistant to 8-azaguanine and HAT-sensitive; TNF tumor necrosis factor; VEGF vascular endothelial growth factor (a subfamily of growth factors; includes VEGF-A); VEGFR2 vascular endothelial growth factor receptor 2, also known as KDR (kinase insert domain-containing receptor), FLK1 (fetal liver kinase 1), or CD309

Table 13.2 (continued)

| INN and trade name | Type of mAb | Cell line | Targeta | Mechanism of action | Approved indications |
|-------------------|-------------|-----------|---------|---------------------|---------------------|
| Evolocumab (Repatha®) | Human IgG2λ | CHO | PCSK9 | Inhibits binding PCSK9 to LDLR, LDLR expression↑, LDL clearance ↓, and LDL-C↓ in circulation | Primary hyperlipidemia and mixed dyslipidemia; homozygous FH to reduce LDL-C and other lipids |

Note: Eptinezumab-jjmr (Vyepti™), a humanized IgG1 mAb (κ L chains) targeted to the calcitonin gene-related peptide (CGRP) was recently approved by the FDA as an injection for intravenous use for the preventative treatment of migraine (see also Table 13.9).

(Continued)
immunogenicity to at least some degree. In addition, antibodies to some drugs, including mAbs, are sometimes found in normal sera and sera of pretreated patients. These antibodies include rheumatoid factors and anti-glycan, anti-hinge, and anti-allotype antibodies, and although they do not usually interfere with treatments, they may occasionally have clinical consequences.

13.1.2 Monoclonal Antibody Nomenclature

With the exception of the first mAb, muromonab, the original nomenclature used for mAbs, was adopted by both US Adopted Names (USAN) and WHO’s International Nonproprietary Names (INN) for pharmaceuticals. All mAb names ended with the stem -mab and had a preceding substem (sometimes called a prestem) to distinguish the animal origin of the mAb. A list of these substems is shown in Table 13.3. In addition, a second substem, preceding the designation for the mAb origin, was usually included in the name as a one-, two-, or three-letter identifier to indicate the mAb target or disease state, for example, “-tu-” for a mAb directed against a tumor(s) or “-ci-” for mAbs with a cardiovascular/circulatory action. More mAbs indicated for tumors were given regulatory approval than mAbs for any other disease or general disorder, and this initially led to recommendations for further substem divisions to identify individual tumors such as “-co-” for colon, “-me-” for melanoma, “-pr-” for prostate, and so on. This practice was not always followed and is reflected in the current list of mAbs approved for non-cancer indications (Table 13.2). Lastly, each mAb had a unique prefix, a few letters not necessarily with any special meaning but selected to identify the mAb as an individual product (Table 13.3).

This INN system of nomenclature, used since the early 1990s for more than 500 mAbs, has recently been revised following increasing concerns related, firstly, to the need to identify increasing numbers of mAbs with names that are new, distinct, more easily pronounceable, and not too long and, secondly, to concerns about marketing arising from claims that some substems might be incorrectly claimed to indicate an advantageous immunogenicity profile. As a consequence, an INN Expert Group decided to revise the nomenclature scheme that assigns INNs for mAbs, and in May 2017 the WHO recommended the discontinuation of the spe-
cies substem B (also called the species or source infix), except for the pre-substem -vet- for veterinary use. Tables 13.3 and 13.4 and Fig. 13.2 summarize the old and new nomenclature schemes side by side. It was concluded that this will lead to greater diversity in names for mAbs.

To avoid confusion between the previous and new INN nomenclature schemes, some target substems will be changed, for example, -tu- for tumor will be replaced by -ta- (Table 13.5). Note that the target class substem category is said to be currently under revision.

### Table 13.3 New nomenclature for monoclonal antibodies

| INN substem (prestem) | Target |
|-----------------------|--------|
| -o                | Mouse  |
| -a                | Rat    |
| -axo               | Mouse-chimera |
| -e                | Hamster |
| -I                | Primate |
| -xi               | Chimeric-humanized |
| -zu               | Humanized |
| -u                | Fully humanized/human |

*From Baldo BA. Safety of biologics therapy. Monoclonal antibodies, cytokines, fusion proteins, hormones, enzymes, coagulation proteins, vaccines, botulinum toxins. Cham, Switzerland: Springer Nature; 2016. Reproduced with permission from Springer Nature*

*All monoclonal antibodies have the stem (suffix) -mab and a unique prefix with no special meaning used to identify the individual product*

*To identify animal species*

*To identify target or disease. Placed before substem*

*Further substem subdivision for tumors: co/col colon; go/got testis; go/gov ovary; ma/mar mammary; me/mel melanoma; pr/pro prostate; tu/tum miscellaneous tumor examples illustrating the application of these nomenclature rules:*

*Palivizumab, Pali* unique prefix identifier; -vi- targeted to a virus (respiratory syncytial virus); -zu- INN substem for humanized; -mab stem for all monoclonal antibodies

*Canakinumab, Cama* unique prefix identifier; -ki- targeted to interleukin IL-1β; -u- INN substem for fully human monoclonal antibody; -mab stem for all monoclonal antibodies

### Table 13.4 Previous INN monoclonal antibody nomenclature scheme

| Substem: target class | Substem: species |
|-----------------------|------------------|
| -b(a)                 | Bacterial        |
| -am(i)                | Serum amyloid protein (SAP)/amyloidosis (pre-substem) |
| -c(i)                 | Cardiovascular   |
| -f(a)                 | Fungal           |
| -gr(o)                | Skeletal muscle mass-related growth factors and receptors (pre-substem) |
| -ki(k)                | Interleukin      |
| -li(i)                | Immunomodulating |
| -n(e)                 | Neural           |
| -so(s)                | Bone             |
| -tox(toxa)            | Toxin            |
| -tu(t)                | Tumor            |
| -vi(v)                | Viral            |

*13.1.3 Immunoglobulin G Subclasses*

Of the five immunoglobulin classes, IgG, made up of four subclasses IgG1, IgG2, IgG3, and IgG4, is generally favored for the preparation of therapeutic mAbs. Each subclass has similar intrachain H and L domain disulfides; the interchain disulfide bridges linking the H and L chains for IgG2, IgG3, and IgG4 are the same but different to the linkage in IgG1. IgG2 and IgG3 have 4 and 11 disulfides, respectively, linking their H chains, whereas 2 disulfides link the IgG1 and IgG4 H chains. For mAbs used therapeutically, kappa L chains are overwhelmingly represented over lambda L chains. Selection of the IgG antibody subclass is important in obtaining the desired effector functions of a mAb. For example, half-lives of the different subclasses differ, ranging from ~7 days for IgG3 to ~21 days for the other three isotypes, and binding affinities for FcγRIIIa, the receptor involved in antibody-dependent cell-mediated cytotoxicity (ADCC) and important for cell killing, are greater for IgG1 and IgG3 (Table 13.6). IgG2 and IgG4 are therefore the choices when cell death is to be avoided. The IgG1 Fc fragment initiates effector functions by binding to FcγRs, C1q, and the FcRn
IgG3 also binds complement component C1q mediating complement-dependent cytotoxicity (CDC), but despite this, and its high affinity for the FcγRIIIa receptor, its relatively short half-life and susceptibility to proteolysis due to its extended hinge region and its allotypic polymorphism ensures that it is not used therapeutically, or used only rarely, for mAb production. IgG4 has little or no affinity for C1q, so it can be employed when this effector function is not wanted, for example, with natalizumab, when unwanted exchange of Fabs between endogenous IgG4 molecules and IgG4 antibody given as therapy may occur. Also, with, for example, dulaglutide, the GLP-1 analog receptor agonist is fused to the IgG4 Fc fragment to avoid antibody-ADCC and CDC. Like IgG4, IgG2 designates bacterial, and so on. The suffix -mab, representing the common stem for antibody therapeutics, remains the same for both schemes. (Reproduced from Parren PWHI, Carter PJ, Plückthun A. Changes to International Nonproprietary Names for antibody therapeutics 2017 and beyond: of mice and men. Mabs. 2017:9:898–906, an Open Access article distributed under the terms of the Creative Commons Attribution License)

**Fig. 13.2** Comparison of the naming schemes for antibody INNs (International Nonproprietary Names) before 2017 and the new system. Prior to 2017, the target infix was divided into substem A, indicating -t(u)- for tumor, -c(i)- for cardiovascular, etc., and substem B, indicating mAb development from chimeric (-xi-) to humanized (-zu-) and to human (-u-). In the new scheme, the infix has been simplified to avoid confusion with earlier schemes, for example, -ta- now designates tumor antigen, -ba- designates bacterial, and so on. The suffix -mab, representing the common stem for antibody therapeutics, remains the same for both schemes. (Reproduced from Parren PWHI, Carter PJ, Plückthun A. Changes to International Nonproprietary Names for antibody therapeutics 2017 and beyond: of mice and men. Mabs. 2017:9:898–906, an Open Access article distributed under the terms of the Creative Commons Attribution License)

**Table 13.5** New INN monoclonal antibody nomenclature scheme

| Target class | Substem A | Substem B |
|--------------|-----------|-----------|
| -ba-         | Serum amyloid protein (SAP)/amyloidosis (pre-substem) |
| -ami-        | Bacterial |
| -ci-         | Cardiovascular |
| -fung-       | Fungal |
| -gros-       | Skeletal muscle mass-related growth factors and receptors (pre-substem) |
| -ki-         | Interleukin |
| -li-         | Immunomodulating |
| -ne-         | Neural |
| -os-         | Bone |
| -toxa-       | Toxin |
| -ta-         | Tumor |
| -vet-        | Veterinary use (prestem) |
| -vi-         | Viral |
may be chosen when soluble antigens are targeted and neutralization without, or with reduced, effector functions is desired.

### 13.1.4 Glycosylation of Monoclonal Antibodies

In addition to its specificity and avidity for the target, posttranslational modifications that help determine antibody stability, immunogenic potential, and effector functions contribute to the efficacy of a mAb. Posttranslational glycosylation has a critical role in antibody effector functions. This can be seen in the human-type glycoform pattern produced by Chinese hamster ovary (CHO), mouse monoclonal NSO, or Sp2/0 mammalian cells. Human IgG molecules produced in these systems have a single N-linked biantennary glycan at the conserved glycosylation site, asparagine 297 (Asn297), in each of the two CH2 domains and produce the desired human glycoforms for therapeutic mAbs, mainly the heptasaccharide core plus L-fucose (G0F), and this structure plus β-D-galactose linked (1→4) to N-acetyl-D-glucosamine on each branch (G1F) (Fig. 13.3).

Fc glycosylation is composed of a biantennary heptasaccharide core formed from two β-D-N-acetyl-D-glucosamine branches linked (1→2) to D-mannose residues which are, in turn, linked α(1→6)- and α(1→3) to a branch-point D-mannose, itself linked to diacetylchitobiose attached to Asn297. Additional sugars, namely, L-fucose, D-galactose, N-acetyl-D-glucosamine, and terminally linked α(2→6) N-acetylneuraminic acid, may be attached to the core. Further modifications, including addition of D-galactose linked to the bisecting N-acetyl-D-glucosamine and different glycosylation at the two CH2 Asn297 sites, contribute to the microheterogeneity of Fc glycan structures (Fig. 13.3). Effector functions are mediated via Fc receptors FcγRI, FcγRII, and FcγRIII, the neonatal Fc receptor (FcRn), and the

| Table 13.6 | Properties of IgG subclasses relevant to their use as therapeutic monoclonal antibodies |
|---|---|---|---|---|
| IgG subclass | Percentage in serum | Half-life (days) | Binding affinity for FcγRIIIa | Complement activation |
| IgG1 | 66 | ~21 | +++ | ++ |
| IgG2 | 23 | ~21 | +/- | + |
| IgG3 | 7 | ~7 | +++ | +++ |
| IgG4 | 4 | ~21 | + to – | 

Reproduced from Baldo BA. Safety of biologics therapy. Monoclonal antibodies, cytokines, fusion proteins, hormones, enzymes, coagulation proteins, vaccines, botulinum toxins. Cham, Switzerland: Springer Nature; 2016 with permission from Springer Nature

+++ high affinity; ++ intermediate affinity; + low affinity; – no affinity

Meanwhile, the α-FcRn receptor (CD223) is present on adult natural killer (NK) cells, macrophages, and neutrophils. Receptor involved in antibody-dependent cell-mediated cytotoxicity (ADCC). For ADCC: IgG1 ≥ IgG3 > IgG4 >> IgG2

*Mediated by binding of complement component C1q to Fc to produce complement-dependent cytotoxicity (CDC). For CDC: IgG3 > IgG1 > IgG2

\[ \text{G2} \rightarrow \text{G1} \rightarrow \text{G0} \]

\[ \alpha\text{-NeuAc } (2\rightarrow6) \beta\text{-D-Gal } (1\rightarrow4) \beta\text{-D-GlcNAc } (1\rightarrow2) \alpha\text{-D-Man } (1\rightarrow6) \]

\[ \alpha\text{-L-Fuc } (1\rightarrow6) \beta\text{-D-Gal } (1\rightarrow4) \beta\text{-D-GlcNAc } (1\rightarrow2) \alpha\text{-D-Man } (1\rightarrow3) \]

\[ \alpha\text{-NeuAc } (2\rightarrow6) \beta\text{-D-Gal } (1\rightarrow4) \beta\text{-D-GlcNAc } (1\rightarrow2) \alpha\text{-D-Man } (1\rightarrow3) \]

\[ \alpha\text{-L-Fuc } (1\rightarrow6) \beta\text{-D-Gal } (1\rightarrow4) \beta\text{-D-GlcNAc } (1\rightarrow2) \alpha\text{-D-Man } (1\rightarrow3) \]

Fig. 13.3 Structure of the biantennary heptasaccharide core (in black) attached at asparagine 297 of the IgG Fc. This is termed the G0 glycoform. Additional sugars (in red), namely, L-fucose, D-galactose, N-acetylneuraminic acid, and N-acetyl-D-glucosamine, may be attached to the core structure producing the glycoforms G1 (G0 plus D-galactose on each branch) and G2 (G1 plus N-acetylneuraminic acid on each branch). Glycoforms G0F, G1F, and G2F are corresponding structures with the addition of L-fucose. (Reproduced with permission from Baldo BA. Safety of biologics therapy. Monoclonal antibodies, cytokines, fusion proteins, hormones, enzymes, coagulation proteins, vaccines, botulinum toxins. Cham, Switzerland: Springer Nature; 2016)
C1q component of complement. Glycosylation of the IgG Fc piece is necessary for the biological activities mediated by the Fcγ receptors and C1q but not the neonatal receptor. Glycoform profiles of mAbs compared to polyclonal IgG may negatively impact the Fc piece and therefore some antibody effector functions. Some evidence for this is an increase in ADCC when a bisecting N-acetyl-D-glucosamine was added to the mAb oligosaccharide of rituximab and a 40–50-fold increase in ADCC by a non-fucosylated glycoform of trastuzumab (Herceptin®).

13.1.5 Antibody-Drug Conjugates

Still an emerging technology, the so-called “magic bullet with payload” approach to specifically target and selectively kill diseased or aberrant cells without unwanted off-target effects, has been a goal since the earliest development of therapeutic antibody technology. This is especially so for the treatment of cancers (see Chap. 14), but the strategy is not necessarily restricted to tumor cells and might be potentially employed in other diseases where elimination, restriction, inhibition, or change of certain cell populations is desired. Such targeted antibody therapeutic agents have been termed antibody-drug conjugates (ADCs) where the “drug,” or payload, can be a small molecule drug aimed at a specific diseased cell type, a toxin as in inotuzumab ozogamicin, an anticancer mAb targeted to CD22 and attached to a calicheamicin cytotoxic agent, or a radioactive isotope as in ibritumomab, used for radioimmunotherapy. In the latter case, the mAb is conjugated to the chelator tiuxetan which complexes a radioactive isotope (yttrium-90 or indium-111) that kills target cells and some nearby CD20-bearing cells by radioactive beta emissions. The general structure of an ADC and the mechanism of action is illustrated in Fig. 13.4. Although the concept, strategy, and basic principles of ADCs appear simple, in practice there are many challenges in developing effective and safe therapeutic products. With four factors to consider, namely, the target, the mAb, the linking structure, and the drug/toxin payload, the manufacturing processes are difficult, complex, and specialized. Early problems encoun-

Fig. 13.4  (a) General structure of an antibody-drug complex (ADC) showing an antibody molecule with a cytotoxic payload attached via a cleavable (sometimes non-cleavable) covalent linker. (b) General mechanism of action of an ADC which is internalized in the cell after binding to its cell surface target antigen receptor. The resultant ADC-antigen complex undergoes endocytosis and lysosomal processing whereby the cytotoxic payload is released. Cell death follows binding of the payload to its target organelle. (Reproduced from Tsuchikama K, An Z. Antibody-drug conjugates: recent advances in conjugation and linker chemistries. Protein Cell. 2018;9:33–46, an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/))
tered included linkers that were too stable or not stable enough to avoid systemic toxicity, insufficient internalization and potency of the conjugate, and changes to the physicochemical and/or therapeutic properties of the final antibody-drug/toxin conjugate.

For further examples of ADCs employed against various types of cancers, see Chap. 14, Sect. 14.2.

### 13.1.6 Breakdown of Antibody Type, Target, and Indications

In categorizing the 52 approved mAbs for non-cancer therapy (as of April 2020), attention is drawn to alemtuzumab and denosumab, each of which are formulated and marketed as two separate products (Campath® and Lemtrada® for alemtuzumab, Prolia® and Xgeva® for denosumab) (Table 13.2; see also Chap. 14, Table 14.4). These are considered here as two different mAbs, not four, and mAb biosimilars are not viewed as separate from the originally approved mAb. The 52 mAbs are listed in Table 13.2 along with the antibody type, cell cultures used for production, antibody targets, mechanisms of action, and indications. Twenty-eight (54%) humanized (-zumab) and 20 (38.5%) fully human (-umab) mAbs comprise the largest groups of the 52 currently approved mAbs. The remaining four approved products (8%) are human-mouse chimeras (-ximab). Note that there are four Fab fragments, the chimeric abciximab, and the humanized idarucizumab, ranibizumab, and certolizumab pegol. Caplacizumab-yhdp is a humanized bivalent single domain nanobody and brolucizumab is a single chain scFv fragment. All 52 of the antibodies belong to the IgG isotype with the overwhelming number being subclass IgG1 with kappa light chains. Only three (belimumab, raxibacumab, evolocumab) have lambda light chains. Thirty-three of the mAbs are subclass IgG1, 7 are subclass IgG2, and 8 are IgG4, while ravulizumab-cwvz and eculizumab are IgG2/4κ hybrids, the latter containing regions from human IgG2 and IgG4 sequences and murine complementarity-determining regions grafted onto the human variable regions (Table 13.2).

A collective of 37 different targets are recognized by the 52 mAbs used for non-cancer therapies with some targets complementary to more than one mAb. The proinflammatory cytokine tumor necrosis factor (TNF) is the target of four mAbs, adalimumab, certolizumab pegol, infliximab, and golimumab, while ten other targets are each recognized by at least two mAbs (Tables 13.2 and 13.7). A classification of the currently approved mAbs used for non-cancer indications reveals that 24 antibodies are used to treat inflammatory and/or immune disorders, while 28 antibodies are indicated for a diverse range of diseases/applications (Tables 13.2 and 13.8). Some diseases/applications are the treatment target for more than one mAb; for example, ten different mAbs are indicated for psoriasis/psoriatic arthritis, and five different mAbs for each of Crohn’s disease, rheumatoid arthritis, and infectious agents (Table 13.2). Recently approved mAbs with new indications are targeted to treat hemophilia A, migraine, HIV-1 and *Clostridium difficile* infections, primary hemophagocytic lymphohistiocytosis, thyroid eye disease, and X-linked hypophosphatemia. Note that in practice, mAb off-label therapies sometimes cover a wider and more diverse range of disorders, and this is reflected in any examination of adverse events provoked by these agents.

### Table 13.7 Non-cancer targets with more than one complementary monoclonal antibody

| Target | Monoclonal antibodies |
|--------|-----------------------|
| TNF    | Adalimumab; certolizumab pegol; infliximab; golimumab |
| IL-2R α-chain | Basiliximab; daclizumab |
| Complement C5 | Eculizumab; ravulizumab |
| α4 integrin | Natalizumab; vedolizumab |
| *Bacillus anthracis* PA | Obiltoxaximab; raxibacumab |
| IL-17A | Ixekizumab; secukinumab |
| IL-6R | Tocilizumab; sarilumab |
| IL-23 p19 | Tildrakizumab-asnn; risankizumab-rraa |
| PCSK9 | Alirocumab; evolocumab |
| CGRP | Eptinezumab-jjmr; fremanezumab-vfrm; galcanezumab-gnlm; |
| IL-5 | Mepolizumab; reslizumab |

*CGRP* calcitonin gene-related peptide; *PCSK9* proprotein convertase subtilisin/kexin type 9; *TNF* tumor necrosis factor.
As with many biologics given by infusion, injection site reactions are commonly seen with mAbs. Federal Adverse Event Reporting System (FAERS) reports of injection site reactions over the 2-year period following FDA approval of five subcutaneously administered approved biologics for the treatment of plaque psoriasis, namely, the mAbs, adalimumab, ixekizumab, secukinumab, and ustekinumab, and the fusion protein etanercept, were analyzed for the most common adverse effects seen during induced injection site reactions. Injection site reaction-related preferred terms used in the FAERS database are those listed in the Medical Dictionary for Regulatory Activities (MedDRA; https://www.meddra.org/). The preferred terms for individual adverse effects monitored in the analysis were erythema, pain, pruritus, rash, reactions, urticaria, swelling, induration, bruising, hemorrhage, hematoma, irritation, and extravasation. The numbers of injection site reaction-related reports in FAERS for the 5 agents were 15,637 for adalimumab, 1771 for ixekizumab, 654 for secukinumab, 141 for etanercept, and 8 for ustekinumab. The adverse effects most often recorded were pain (23.3%), irritation (14.1%), and erythema (9.6%) for adalimumab; pain (19.8%) and reaction (13.6%) for ixekizumab; bruising (25.7%), pain (25.4%), and hemorrhage (12.5%) for secukinumab; reaction (24.1%), pain (16.3%), and erythema (11.4%) for etanercept; erythema (19.9%), and pain (75%), induration (12.5%), and swelling (12.5%) for ustekinumab (Fig. 13.5).

Immune responses to mAbs, including those that are fully human, are well-known to occur. Binding of patient antibodies to administered therapeutic mAbs can lead to loss of efficacy, hypersensitivity reactions, and poor clinical outcomes by depleting levels of free mAb and formation of immune complexes causing enhanced clearance of the treatment antibody. For example, patient antibodies occur against all four mAb TNF blockers, adalimumab, infliximab, certolizumab pegol, and golimumab, although neutralizing antibody effects on efficacy can be more apparent with the first two of these antibodies. Of the 52 mAbs considered here, ~75% are associated with “anaphylaxis” and/or “hypersensitivity” by the FDA in one or more of a boxed warning, warnings and precautions, or adverse reactions. Of course, being protein in composition, the possibility always remains that any of the 52 mAbs may provoke an immediate allergic reaction in rare patients. Type I and III hypersensitivity reactions are the true hypersensitivity responses (i.e., immune-based responses), most often observed following the administration of many mAbs. Type IV cell-mediated, delayed hypersensitivities are less common but well-known, and given

### Table 13.8 Therapeutic applications of approved non-cancer monoclonal antibodies at March 2020

| Inflammation and immune disorders | Other disorders |
|-----------------------------------|-----------------|
| Adalimumab                        | Abciximab       |
| Alemtuzumab                       | Alirucumab      |
| Belimumab                         | Basiliximab     |
| Brodalumab                        | Benralizumab    |
| Canakinumab                       | Bezlotoxumab    |
| Certolizumab                      | Brolocizumab    |
| Daclizumab                        | Burosimum-twza  |
| Denosumab(Prolica®)               | Caplacizumab-yhdp |
| Dupilumab                         | Crizanlizumab-tnca |
| Emapalumab-lzsg                   | Eculizumab      |
| Golimumab                         | Emicizumab-kxwh |
| Guselkumab                        | Eptinezumab-jjmr|
| Infliximab                        | Erenumab-aooe   |
| Lanadelumab                       | Evolocumab      |
| Natalizumab                       | Fremanezumab-vfrm |
| Ocrelizumab                       | Galcanezumab-gml |
| Risankizumab-rlza                 | Ibalizumab-uylk |
| Romosozumab-aqgg                  | Idarucizumab    |
| Sarilumab                         | Ixekizumab      |
| Secukinumab                       | Mepolizumab     |
| Tildrakizumab-asmn                | Obinutuzimab    |
| Tocilizumab                       | Omalizumab      |
| Ustekinumab                       | Palivizumab     |
| Vedolizumab                       | Ranibizumab     |
| Ranibizumab-cwvz                  | Ravulizumab     |
| Raxibacumab                       | Reslizumab      |
| Reslizumab                        | Teprotumumab-trbw |

**13.1.7 Hypersensitivities, Warnings, Precautions, and Adverse Events**

As with many biologics given by infusion, injection site reactions are commonly seen with mAbs. Federal Adverse Event Reporting System (FAERS) reports of injection site reactions over the 2-year period following FDA approval of five subcutaneously administered approved biologics for the treatment of plaque psoriasis, namely, the mAbs, adalimumab, ixekizumab, secukinumab, and ustekinumab, and the fusion protein etanercept, were analyzed for the most common adverse effects seen during induced injection site reactions. Injection site reaction-related preferred terms used in the FAERS database are those listed in the Medical Dictionary for Regulatory Activities (MedDRA; https://www.meddra.org/). The preferred terms for individual adverse effects monitored in the analysis were erythema, pain, pruritus, rash, reactions, urticaria, swelling, induration, bruising, hemorrhage, hematoma, irritation, and extravasation. The numbers of injection site reaction-related reports in FAERS for the 5 agents were 15,637 for adalimumab, 1771 for ixekizumab, 654 for secukinumab, 141 for etanercept, and 8 for ustekinumab. The adverse effects most often recorded were pain (23.3%), irritation (14.1%), and erythema (9.6%) for adalimumab; pain (19.8%) and reaction (13.6%) for ixekizumab; bruising (25.7%), pain (25.4%), and hemorrhage (12.5%) for secukinumab; reaction (24.1%), pain (16.3%), and erythema (11.4%) for etanercept; erythema (19.9%), and pain (75%), induration (12.5%), and swelling (12.5%) for ustekinumab (Fig. 13.5).

Immune responses to mAbs, including those that are fully human, are well-known to occur. Binding of patient antibodies to administered therapeutic mAbs can lead to loss of efficacy, hypersensitivity reactions, and poor clinical outcomes by depleting levels of free mAb and formation of immune complexes causing enhanced clearance of the treatment antibody. For example, patient antibodies occur against all four mAb TNF blockers, adalimumab, infliximab, certolizumab pegol, and golimumab, although neutralizing antibody effects on efficacy can be more apparent with the first two of these antibodies. Of the 52 mAbs considered here, ~75% are associated with “anaphylaxis” and/or “hypersensitivity” by the FDA in one or more of a boxed warning, warnings and precautions, or adverse reactions. Of course, being protein in composition, the possibility always remains that any of the 52 mAbs may provoke an immediate allergic reaction in rare patients. Type I and III hypersensitivity reactions are the true hypersensitivity responses (i.e., immune-based responses), most often observed following the administration of many mAbs. Type IV cell-mediated, delayed hypersensitivities are less common but well-known, and given
the number of observed cytopenias with poorly defined mechanisms induced by mAbs, apparently rare type II hypersensitivity may occur more often than currently identified. Hypersensitivity responses may manifest as infusion reactions and anaphylactic reactions. *Type I or IgE antibody-mediated reactions* cross-linking Fce receptors on mast cells and basophils have been documented for at least ten of the approved mAbs discussed here (Table 13.9 and Box 13.1). What has been described as atypical anaphylaxis after secondary exposure to a mAb is thought to result from immune complexes cross-linking Fcγ receptors on neutrophils leading to cross-linking and release of platelet-activating factor. While immediate reactions such as anaphylaxis and urticaria with IgE antibody involvement are usually readily identifiable as type I allergic responses, infusion reactions with signs and symptoms of one or more of cytokine release syndrome, an anaphylactic/anaphylactoid reaction, and direct toxicity may be less easy to define with confidence and precision. Infusion of many biologics, particularly mAbs, provokes a characteristic infusion syndrome, usually

### Fig. 13.5

Proportions of injection site reaction-related adverse effects in the FAERS database for five biologic agents indicated for plaque psoriasis, adalimumab, etanercept, ixekizumab, secukinumab, and ustekinumab. Reports covered the first 2 years after FDA approval. Note that the proportions for individual adverse effects induced by ustekinumab are obtained from only eight spontaneous reports. (From Grace E, Goldblum O, Renda L, et al. *Injection site reactions in the Federal Adverse Event Reporting System (FAERS) post-marketing database vary among biologics approved to treat moderate-to-severe psoriasis.* *Dermatol Ther (Heidelb).* 2020;10:99–106, [https://doi.org/10.1007/s13555-019-00341-2](https://doi.org/10.1007/s13555-019-00341-2), an article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License [http://creativecommons.org/licenses/by-nc/4.0/](http://creativecommons.org/licenses/by-nc/4.0/))
within one or a few hours during/after the first administration. Most reactions are mild to moderate with symptoms often described as “flu”-like with fever, chills, rigors, headache, nausea, asthenia, rash, and pruritus, but a small number of patients, mostly at the first or second infusion, show potentially fatal symptoms resembling an IgE antibody-mediated reaction with hypotension, cardiac arrest, bronchospasm, and urticaria. Alemtuzumab, approved and marketed as Lemtrada® for the treatment of adult patients with relapsing remitting multiple sclerosis, is a good example of a mAb that provokes such an infusion syndrome. The mAb carries an FDA black box warning for serious and life-threatening infusion reactions with the direction that it must be administered in a setting with appropriate equipment and personnel to manage reactions. Premedication with corticosteroids for the first 3 days of infusion together with antihistamines and/or antipyretics is recommended, but reactions may occur despite pretreatment. The human-mouse chimeric mAbs cetuximab and rituximab and trastuzumab, a humanized mAb, induce the highest incidence of infusion reactions, and, overall, reaction incidences and severity to humanized and fully human mAbs are significantly lower. Regression of symptoms seen in some cancer patients given chemotherapy coincides with decreases in serum cytokine concentrations leading to the suggestion that TNF and IL-6 may be responsible for symptoms similar to those seen in type I allergic responses. Severity of infusion reactions may also be related to the number of circulating lymphocytes with severe reactions seen when counts exceed 50 x 10⁹/L. Besides alemtuzumab, other mAbs with non-cancer indications that provoke sometimes serious infusion reactions include the human-mouse chimera infliximab, humanized ocrelizumab, and human mAbs, belimumab, emapalumab-lzsg, and raxibacumab. Urticaria is fairly often seen, occurring after, among other mAbs, certolizumab pegol, alemtuzumab, natalizumab, obinotoximab, raxibacumab, and secukinumab. The hypersensitivity response, eczema, has been reported following treatment with denosumab (Prolia®).

Box 13.1 Hypersensitivity Reactions to Monoclonal Antibodies Used for Non-cancer Therapy

- **Type I hypersensitivity**: FDA warnings, precautions, and adverse events data cover ~75% of 52 mAbs. Anaphylaxis/serious hypersensitivity reactions reported for adalimumab certolizumab pegol, infliximab, belimumab, omalizumab, palivizumab, obinotoximab, ustekinumab, reslizumab, and tocilizumab. Urticaria is often seen.
- **Serious infusion reactions** with signs and symptoms resembling, and sometimes confused with anaphylaxis, occur with some mAbs, for example, alemtuzumab. Cytokine release appears to be involved.
- **Precise mechanisms for type II hypersensitivity** (immune-mediated) neutropenia, thrombocytopenia, and anemia have generally not been determined but may occur with, for example, Lemtrada® for multiple sclerosis and abciximab.
- **Type III hypersensitivities**, serum sickness-like reactions, cutaneous vasculitis, and pneumonitis (may be both type II and III hypersensitivities) occur with, for example, infliximab, adalimumab, and alirocumab.
- **Type IV hypersensitivities**, including serious Stevens-Johnson syndrome, have been reported following adalimumab and infliximab. Other delayed cutaneous reactions seen include dermatitis, erythema multiforme, and skin exfoliation. Apart from the anti-TNF mAbs, skin manifestations for most other mAbs are rare and include mainly rash and pruritus.

Cytopenias have been observed with the use of at least 25% of the approved non-cancer mAbs (Table 13.9). Some reactions, e.g., in Lemtrada®-treated patients, have an autoimmune basis for the observed neutropenia, thrombocytopenia,
### Table 13.9  Adverse events associated with approved\(^{a}\) monoclonal antibodies used for non-cancer therapies as at April 2020

| Monoclonal antibody\(^{b}\) INN and trade names | Target\(^{b}\) | Warnings, precautions, risks, and safety concerns | Other adverse events\(^{c}\), serious and common |
|-----------------------------------------------|----------------|-------------------------------------------------|-----------------------------------------------|
| **Adalimumab (Humira\(^{b}\))**              | TNF            | Boxed warning: Serious infections\(^{d,e}\), malignancy<br>**Other:** Anaphylaxis, serious allergic reactions, hepatitis B reactivation, demyelinating disease, cytopenias\(^{f}\), heart failure, lupus-like syndrome | Systemic: Infections\(^{g}\), isr, ILD, sarcoidosis, liver failure<br>Cutaneous: SJS, EM, psoriasis, cutaneous vasculitis, alopecia |
| **Certolizumab pegol (Cimzia\(^{b}\))**       | TNF            | Boxed warning: Serious infections\(^{d,e}\), lymphoma, and other malignancies<br>**Other:** Heart failure, serious allergic reactions, hepatitis B reactivation, demyelinating disease, cytopenias, lupus-like syndrome | Systemic: URTI, cardiac disorders\(^{h}\), eye disorders\(^{i}\), isr, hepatitis and ↑ liver enzymes, nephrotic syndrome, renal failure, thrombophlebitis, vasculitis<br>Cutaneous: Dermatitis, erythema nodosum, urticaria |
| **Infliximab (Remicade\(^{b}\))**             | TNF            | Boxed warning: Serious infections\(^{d,e}\), malignancy<br>**Other:** Hepatitis B reactivation, hepatotoxicity, cytopenias, demyelinating disease, lupus-like syndrome | Systemic: Infections\(^{d,i}\), pancytopenia, anemia, cellulitis, serum sickness, thrombophlebitis, intestinal obstruction, ILD, anaphylaxis, IRs<br>Cutaneous: Cutaneous vasculitis, SJS, EM, psoriasis |
| **Golimumab (Simponi\(^{b}\))**               | TNF            | Boxed warning: Serious infections\(^{d,e}\), lymphoma, and other malignancies<br>**Other:** Invasive fungal infections, heart failure, hepatitis B reactivation, demyelinating disease, hypersensitivity | Systemic: URTI, viral infections, bronchitis, ↑ liver enzymes, sarcoidosis, ILD, paresthesia<br>Cutaneous: Skin exfoliation, rash |
| **Abciximab (ReoPro\(^{b}\))**                | Glycoprotein IIb/IIIa | ↑ Risk of bleeding, thrombocytopenia\(^{k}\) | Systemic: Bleeding\(^{j}\), intracranial hemorrhage or stroke, GI, CV\(^{m}\); anemia; NS\(^{n}\), respiratory\(^{o}\), urinary disorders\(^{p}\)<br>Cutaneous: Pruritus, generalized exanthema\(^{a}\) |
| **Alemtuzumab (Lemtrada\(^{b}\))**            | CD52           | Boxed warning: Autoimmunity, IRs, malignancies\(^{s}\)<br>**Other:** Other immune cytopenias, glomerular nephropathies, thyroid disorders, delay therapy in cases of infections, pneumonitis | Systemic: Headache, pyrexia, nausea, UTI, herpes virus infection, extremity and back pain, dizziness, flushing, cough, chills, vomiting, dyspnea<br>Cutaneous: Rash, urticarial, pruritus, dermatitis |
| **Basiliximab (Simulect\(^{b}\))**            | IL-2 receptor α-chain (CD25) | Boxed warning: General risk of immunosuppressive therapy<br>**Other:** Immunogenicity, hypersensitivity | Systemic: GI, viral infection, peripheral edema, UTI, URTI, dyspnea, wound complications, hypertension, anemia, hypo- and hyperkalemia and hyperuricemia, headache, tremor<br>Cutaneous: Rash, pruritus, hypertrichosis |
| **Daclizumab (Zinbryta\(^{b}\))**             | IL-2 receptor α-chain (CD25) | Boxed warning: Hepatic injury including autoimmune hepatitis and other immune-mediated disorders<br>**Other:** Hypersensitivity, infections, depression and suicide | Systemic: Nasopharyngitis, URTI, oropharyngeal pain, bronchitis, eczema, depression, influenza<br>Cutaneous: Dermatitis, rash |

(continued)
| Monoclonal antibody | Target | Warnings, precautions, risks, and safety concerns | Other adverse events, serious and common |
|--------------------|--------|------------------------------------------------|----------------------------------------|
| **Belimumab** *(Benlysta®)* | BLyS | Mortality, serious infection, malignancy, hypersensitivity including anaphylaxis, IR, depression, immunization | **Systemic**: Nausea, diarrhea, pyrexia, pain in extremity, bronchitis, depression, migraine |
| **Canakinumab** *(Ilaris®)* | IL-1β | Increased risk of serious infections, immunization, MAS, hypersensitivity, immunosuppression | **Systemic**: CAPS – Nasopharyngitis, diarrhea, influenza, headache, nausea, dizziness/vertigo, SJIA – Nasopharyngitis, URTI; abdominal pain, isr |
| **Eculizumab** *(Soliris®)* | Complement C5 | *Boxed warning*: Serious meningococcal infections | **Systemic**: PNH – Headache, nasophagitis, back pain, nausea. AHUS – Hypertension, URTI, GI, abdominal pain, anemia, cough, pyrexia, peripheral edema |
| **Ravulizumab-cwvz** *(Ultomiris®)* | Complement C5 | *Boxed warning*: Serious meningococcal infections | **Systemic**: URTI, headache, diarrhea, nausea |
| **Denosumab** *(Prolia®)* | RANKL | Hypersensitivity, hypocalcemia, serious infections, osteonecrosis of jaw, atypical femoral fractures; severe bone, joint, muscle pain, suppression of bone turnover, dermatologic reactions | **Systemic**: Post-menopausal osteoporosis – Back extremity, musculoskeletal pain, hypercholesterolemia, cystitis. Male osteoporosis – Back pain, arthralgia, nasopharyngitis |
| **Natalizumab** *(Tysabri®)* | α4 integrin (binds to α4β1 and α4β7 integrins) | *Boxed warning*: PML | **Systemic**: MS – Headache, fatigue, arthralgia, urinary tract infection, URTI, gastroenteritis; vaginitis; diarrhea. CD – Headache, URTI, nausea |
| **Vedolizumab** *(Entyvio®)* | α4β7 integrin | Hypersensitivity/infusion reactions, infections, PML, liver injury | **Systemic**: Headache, arthralgia, nausea, pyrexia, URTI, cough, bronchitis, influenza, back pain, pain in extremities, nasopharyngitis |
| **Omalizumab** *(Xolair®)* | IgE | Anaphylaxis, malignancy, acute asthma, ↓ CSs gradually, eosinophilia, SS-like reaction, parasitic infection | **Systemic**: Allergic asthma – Arthralgia, pain, dizziness, fracture, earache. CIU – Nausea, pharyngitis, URTI, sinusitis, arthralgia, headache, cough, virus infections |
| **Palivizumab** *(Synagis®)* | RSVF | Anaphylaxis, delay administration during moderate-severe infections, give with caution in cases of thrombocytopenia or coagulation disorders | **Systemic**: isr, pyrexia, apnea, cough, dizziness thrombocytopenia |
| **Obiltoxaximab** *(Anthim®)* | *Bacillus anthracis PA* | *Boxed warning*: Hypersensitivity and anaphylaxis | **Systemic**: URTI; headache; pruritus; IR pain, swelling, bruise |

**Table 13.9** (continued)
### Table 13.9 (continued)

| Monoclonal antibody and trade names | Target | Warnings, precautions, risks, and safety concerns | Other adverse events<sup>c</sup>, serious and common |
|------------------------------------|--------|-------------------------------------------------|---------------------------------------------------|
| **Raxibacumab (Abthrax<sup>a</sup>)** | Bacillus anthracis PA | IR | **Systemic:** Pain in extremity, somnolence, headache, URTI, nausea, cough, arthralgias  
**Cutaneous:** Rash, pruritus, urticaria |
| **Ranibizumab (Lucentis<sup>a</sup>)** | VEGF-A | Endophthalmitis and retinal detachment, increase in intraocular pressure and risk of arterial thromboembolic events after intravitreal injection | **Systemic:** Conjunctival hemorrhage, eye pain, vitreous floaters, cataracts |
| **Brolucizumab-dbll (Beovu<sup>a</sup>)** | VEGF-A | Endophthalmitis and retinal detachment, risk of arterial thromboembolic events, increase in intraocular pressure | **Systemic:** Conjunctival hemorrhage, eye pain, vitreous floaters, cataracts, blurred vision |
| **Ixekizumab (Taltz<sup>a</sup>)** | IL-17A | Infections, TB – evaluate prior, hypersensitivity, inflammatory bowel disease | **Systemic:** URTI, isr, nausea, tinea infections |
| **Secukinumab (Cosentyx<sup>a</sup>)** | IL-17A | Infections, tuberculosis activation, exacerbation of Crohn’s disease, hypersensitivity, avoid live vaccines | **Systemic:** Nasopharyngitis, diarrhea, URTI, rhinitis  
**Cutaneous:** Urticaria |
| **Brodalumab (Siliq<sup>a</sup>; Kyntheum<sup>a</sup>; Lumicef<sup>a</sup>)** | IL-17RA | Boxed warning: Suicidal ideation and behavior  
**Other:** TB, infections, Crohn’s disease, avoid live vaccines | **Systemic:** Arthralgia, headache, fatigue, diarrhea, oropharyngeal pain, nausea, myalgia, isr, influenza, neutropenia, and tinea infections |
| **Tocilizumab (Actemra<sup>a</sup>; RoActemra<sup>a</sup>)** | IL-6R | Boxed warning: Serious infections  
**Other:** GI perforation, avoid live vaccines, hypersensitivity, laboratory monitoring<sup>ad</sup> | **Systemic:** Nasopharyngitis, nausea, ↑ liver enzymes, infusion reactions, hypertension, thrombocytopenia, neutropenia, headache  
**Cutaneous:** Dermatologic reactions |
| **Sarilumab (Kevzara<sup>a</sup>)** | IL-6R | Boxed warning: Risk of serious infection  
**Other:** GI perforation, avoid live vaccines, hypersensitivity, neutropenia, thrombocytopenia | **Systemic:** ↑ALT, isr, URTI, urinary tract infections |
| **Ustekinumab (Stelara<sup>a</sup>)** | IL-12  
IL-23 | Infections, tuberculosis, RPLS, malignancies, anaphylaxis, avoid live vaccines | **Systemic:** Nasopharyngitis, headache, dental infections, URTI, isr, arthralgia, GI  
**Cutaneous:** Pruritus |
| **Guselkumab (Tremfya<sup>a</sup>)** | IL-23 | Infections, prior evaluation for TB | **Systemic:** URTI, isr, arthralgia, headache, diarrhea, tinea, gastroenteritis, herpes simplex infections |
| **Tildrakizumab-asmn (Ilumetri<sup>a</sup>; Ilumya<sup>a</sup>)** | IL-23 p19 | Infections, prior evaluation for TB, hypersensitivity | **Systemic:** URTI, isr, diarrhea |
| **Risankizumab-rzaa (Skyrizi<sup>a</sup>)** | IL-23 p19 | Infections, prior evaluation for TB, hypersensitivity | **Systemic:** URTI, isr, diarrhea |
| **Ibalizumab-uiyk (Trogarzo<sup>a</sup>)** | CD4 | IRIS | **Systemic:** Diarrhea, nausea, dizziness  
**Cutaneous:** Rash |

<sup>a</sup> INN and trade names.  
<sup>c</sup> Other adverse events, serious and common.  
<sup>ad</sup> Laboratory monitoring: Complete blood count, serum electrolytes, liver function tests, renal function tests, urinalysis, fasting glucose, fasting lipids, routine chest radiograph, screening for tuberculosis.  
<sup>IR</sup> Intraocular pressure.  
<sup>IRIS</sup> Intraocular inflammation syndrome.
| Monoclonal antibody* INN and trade names | Target | Warnings, precautions, risks, and safety concerns | Other adverse events*, serious and common |
|----------------------------------------|--------|--------------------------------------------------|------------------------------------------|
| **Alirocumab** (Praluent®)            | PCSK9  | Allergic reactions (pruritus, urticaria, rash) including some serious (including hypersensitivity vasculitis) | **Systemic:** Nasopharyngitis, isr, influenza, UTI, diarrhea, myalgia, bronchitis, muscle spasms, sinusitis, cough, contusion musculoskeletal pain, liver enzyme abnormalitiesad |
| **Evolocumab** (Repatha®)             | PCSK9  | Patients with renal and hepatic impairments have not yet been adequately studied. Cover of prefilled syringe and pen contain latex which may cause allergic reactionsad | **Systemic:** Nasopharyngitis, isr, influenza, URTI, back pain, allergic reactions (rash, hives), nausea, arthralgia, hypertensionad |
| **Erenumab-aooe** (Aimoig®)           | CGRP receptor | | **Systemic:** Constipation, isr |
| **Fremanezumab-vfrm** (Ajovy®)        | CGRP   | Hypersensitivity | **Systemic:** isr |
| **Galcanezumab-gnln** (Emgality®)     | CGRP   | Hypersensitivity | **Systemic:** isr |
| **Caplacizumab-yhdp** (Cablivi®)      | von Willebrand factor | Bleeding | **Systemic:** Epistaxis, gingival bleeding, headache, isr |
| **Enicizumab-kxwh** (Hemlibra®)      | Factors IXa and X | Boxed warning: Thrombotic microangiopathy and thromboembolism Other: mAb interference with coagulation tests | **Systemic:** Arthralgia, headache, isr |
| **Romosozumab-aqqg** (Evenity®)       | Sclerostin | Boxed warning: Potential risk of myocardial infarction, stroke, and cardiovascular death Other: Cardiac events, hypersensitivity, hypocalcemia, atypical femoral fracture | **Systemic:** Arthralgia, headache |
| **Burosumab-twza** (Crysvita®)        | FGF23  | Hypersensitivity, isr, hyperphosphataemia and risk of nephrocalcinosis | **Systemic:** Headache, isr, vomiting, pyrexia, pain in extremity; decreased vitamin D |
| **Emapalumab-lzsg** (Gamifant®)       | IFNγ   | Infections, IR, avoid live vaccines | **Systemic:** Infections, pyrexia, hypertension, IR |
| **Lanadelumab-flyo** (Takhzyro®)      | Plasma kallikrein | Hypersensitivity | **Systemic:** URTI, isr, headache, diarrhea, dizziness, myalgia Cutaneous: Rash |
| **Bezlotoxumab** (Zinplava®)          | *Clostridium difficile* toxin B | Heart failure | **Systemic:** Nausea, pyrexia, headache |
| **Ocrelizumab** (Ocrevus®)            | CD20   | Infections, IR, increased risk of malignancy | **Systemic:** Respiratory tract infections IR Cutaneous: Skin infections |
| **Dupilumab** (Dupixent®)             | IL-4Rα | Hypersensitivity, conjunctivitis and keratitis, eosinophilic conditions, helminth infections – treat prior, decrease steroids gradually | **Systemic:** Conjunctivitis, blepharitis, eye pruritus, herpes infections, keratitis, dry eye, oropharyngeal pain, isr, eosinophilia |
### Table 13.9 (continued)

| Monoclonal antibody* INN and trade names | Target* | Warnings, precautions, risks, and safety concerns | Other adverse events†, serious and common |
|------------------------------------------|---------|--------------------------------------------------|------------------------------------------|
| Mepolizumab (Nucala®)                    | IL-5    | Hypersensitivity, helminth infections – treat prior, herpes zoster infections – consider prior vaccination, decrease steroids gradually, not to be used for bronchospasm or status asthmaticus | Systemic: Headache, isr, back pain, fatigue |
| Reslizumab (Cinqair®)                    | IL-5    | *Boxed warning: Anaphylaxis.* *Other:* Helminth infections – treat prior, decrease steroids gradually, malignancy | Systemic: Oropharyngeal pain |
| Benralizumab (Fasenra®)                  | IL-5Rα  | Hypersensitivity; helminth infections – treat prior, decrease steroids gradually | Systemic: Headache; pharyngitis |
| Idarucizumab (Praxbind®)                 | Dabigatran | Thromboembolic risk, hypersensitivity, risk of adverse reaction in patients with hereditary fructose intolerance* at *, reappearance of bleeding* d | Systemic: Headache, hypokalemia, delirium, pneumonia, constipation, pyrexia |
| Crizanlizumab-tmca (Adakveo®)            | P-selectin | Infusion reactions | Systemic: Nausea, arthralgia, back pain, pyrexia |
| Teprotumunab-trbw (Tepezza®)             | IGF-1R  | Infusion reactions, exacerbation of pre-existing inflammatory bowel disease, hyperglycemia | Systemic: Muscle spasm, nausea, alopecia, diarrhea, fatigue. Hyperglycemia, hearing impairment, dry skin, dysgeusia, and headache |

Adapted from Baldo BA. *Safety of biologics therapy. Monoclonal antibodies, cytokines, fusion proteins, hormones, enzymes, coagulation proteins, vaccines, botulinum toxins.* Cham, Switzerland: Springer Nature; 2016. Adapted and reproduced with permission from Springer Nature

*Note: For the recently approved eptinezumab-jjmr (Vyepti™) (Table 13.2), the FDA has issued warnings and precautions for hypersensitivity reactions, including angioedema, urticaria, flushing, and rash.

AHUS atypical hemolytic uremic syndrome; BLyS B lymphocyte stimulator, also known as B cell activating factor (BAFF); C5 complement component 5; CAPS cryopyrin-associated periodic syndrome; CD Crohn’s disease; CIIU chronic idiopathic urticaria; CSx corticosteroids; CV cardiovascular; EM erythema multiforme; GI gastrointestinal; HSTC hematopoietic stem cell transplantation; IGF-1R insulin-like growth factor receptor-1; ILD interstitial lung disease; IR infusion reaction; IRIS immune reconstitution inflammatory syndrome; isr injection site reaction; MAS macrophage activation syndrome; MS multiple sclerosis; NS nervous system; PA protective antigen of *B. anthracis* toxin; PCSK9 proprotein convertase subtilisin/kexin type 9; PML progressive multifocal leukoencephalopathy; PNH paroxysmal nocturnal hemoglobinuria; RANKL receptor activator of nuclear factor kappa-B ligand (CD254); REMS Risk Evaluation and Mitigation Strategy; RSVF human respiratory syncytial virus (F protein coat antigen); SJIA active systemic juvenile idiopathic arthritis; SJS Stevens-Johnson syndrome; URTI upper respiratory tract infection; UTI urinary tract infection; VEGF-A vascular endothelial growth factor A

*Approved by FDA or EMA or both
*Specificity of antibody
*Adverse events in addition to those mentioned as occurring, or potentially likely to occur, and shown in column 3
*Including tuberculosis, tuberculosis reactivation, bacterial sepsis, and invasive fungal infections
*Not to be given to patients with active infections and opportunistic pathogens, e.g., histoplasmosis. Concurrent administration with anakinra or abatacept was associated with a greater proportion of serious infections
*Also rare reports of pancytopenia including aplastic anemia
*Upper respiratory tract and sinusitis
*Optic neuritis, retinal hemorrhage, uveitis
*Not to be given with live vaccines and therapeutic infectious agents
hemolytic anemia, and pancytopenia suggesting type II hypersensitivities (Box 13.1) and/or joint type II and III hypersensitivities.

The formation of large antibody-mAb immune complexes in the circulation may give rise to type III hypersensitivity reactions (Box 13.1) when complexes are not cleared and deposited in tissues such as the kidney, synovial membranes, and the choroid plexus. Deposits form in post-capillary venules, and tissue damage results from complement activation and Fc-mediated inflammatory processes with resultant organ dysfunction and cell death. Type III serum sickness-like hypersensitivities (Chap. 2, Sect. 2.4.2 and Chap. 3, Sect. 3.12.1) have occurred with omalizumab and infliximab; vasculitis has also been reported with the latter. Cutaneous vasculitis (Chap. 2, Sect. 2.4.1 and Chap. 3, Sect. 3.12.2) has also occurred with adalimumab, certolizumab pegol, and alirocumab therapies. High titers of antibodies to adalimumab have been associated with venous and thromboembolisms and also with a higher risk of developing thromboembolism. Pneumonitis (Chap. 2, Sect. 2.4.3, and Chap. 3, Sects. 3.7.1 and 3.12.3), sometimes in hypersensitivity form (probably a combination of types II and III), is also known. Again, infliximab provides a good example (Table 13.9 and Box 13.1).

Serious skin reactions in the form of Stevens-Johnson syndrome (SJS), a type IV hypersensitivity, has occurred with adalimumab and infliximab. Usually self-limiting but occasionally life-threatening, erythema multiforme has also been associated with the same two mAbs. Dermatitis and erythema nodosum are recorded for certolizumab pegol, and skin exfoliation has occurred with the fourth anti-TNF mAb, golimumab. Two other mAbs, denosumab (Prolia®) and omalizumab, have been associated with the induction of dermatitis. Overall though, apart from occasional rashes and pruritus, delayed hypersensitivity reactions are not often seen with other mAbs with non-cancer indications (Table 13.9 and Box 13.1).

### 13.2 Cytokines Used Therapeutically

Cytokines, currently more than 130 in number, are relatively small signaling proteins, usually glycosylated, of MW <30 kDa. They are pro-
duced by a variety of different cells, particularly those of the immune system, the epithelia, endothelia, and stroma. Two key features are their role as important modulators of the immune and inflammatory responses, stimulating or suppressing cellular activities in infection, innate and adaptive immunity, autoimmunity, inflammation, and malignancy, and their pleiotropism with overlapping activities, functional redundancies, and side effects. Secretion of cytokines is induced by different stimuli associated with infection, inflammation, or tumorigenesis, producing a diverse range of biological responses including proliferation, differentiation, activation, inflammation, chemotaxis, and cell death. The nature of the stimulus determines whether an immune response is humoral- or cell-mediated, cytotoxic, immunosuppressive, or allergic.

Classified here into 9 main families, most of the 21 approved cytokine preparations (Table 13.10) (17 different cytokines; 4 pegylated), all in recombinant human (rh) form, are grouped in the hematopoietic growth factor, interferon (IFN), platelet-derived growth factor (PDGF), and transforming growth factor β (TGFβ) families. In the hematopoietin family, approved cytokines are aldesleukin (rhIL-2), oprelvekin (rhIL-11), filgrastim and tbo-filgrastim (rh-granulocyte-colony-stimulating factor; rhG-CSF), sargramostim (rh granulocyte-macrophage colony-stimulating factor; rhGM-CSF), metreleptin (rh-leptin), anestim (rh-SCF), and the rh-erythropoietins, epoetin and darbepoetin alfa. Anakinra, a recombinant receptor antagonist for IL-1, is in the IL-1 family; recombinant interferons alfa-1, alfa-2, beta-1, and gamma-1 make up the interferon family. Interferons are broad-spectrum antiviral cytokines with individual, and some overlapping, activities. Seven interferons, divided into classes I, II and III, occur in humans. Of most interest for therapy are the type I interferons alfa and beta and the type II interferon, gamma interferon. Three approved interferon alfa preparations have been included in the CDER Biologic Products List. These are peginterferon alfa-2a which, together with ribavirin (Copegus®; Fig. 13.6), are indicated for the treatment of chronic hepatitis C in adults; interferon alfa-2b, administered extensively for hepatitis B and C as well as several malignancies; and peginterferon alfa-2b, given for hepatitis C and as adjuvant treatment for melanoma, often after premedication (Table 13.10). Side effects of interferons are many and varied. Interferon alfas occasionally provoke an extensive range of adverse reactions including cardiovascular, respiratory, endocrine, hematologic, metabolic, urinary tract, skin, and nervous and sensory system adverse events as well as neuropsychiatric disorders. Depression, occurring mostly in the first 3 months of treatment, cognitive dysfunction, and mania are well-known and have been intensively studied. Fatal interstitial lung disease, secondary to interferon alfa-ribavirin therapy for hepatitis C infection, reported for both interferon alfa-2a and 2b, has an incidence of 0.03–0.3% (~1.1% Japanese patients). Fatal interstitial pulmonary disease can also occur with pegylated interferon alfa-2b. Autoantibodies

13.2.1 Adverse Effects of Individual Approved Cytokines

Interferons are broad-spectrum antiviral cytokines with individual, and some overlapping, activities. Seven interferons, divided into classes I, II and III, occur in humans. Of most interest for therapy are the type I interferons alfa and beta and the type II interferon, gamma interferon. Three approved interferon alfa preparations have been included in the CDER Biologic Products List. These are peginterferon alfa-2a which, together with ribavirin (Copegus®; Fig. 13.6), are indicated for the treatment of chronic hepatitis C in adults; interferon alfa-2b, administered extensively for hepatitis B and C as well as several malignancies; and peginterferon alfa-2b, given for hepatitis C and as adjuvant treatment for melanoma, often after premedication (Table 13.10). Side effects of interferons are many and varied. Interferon alfas occasionally provoke an extensive range of adverse reactions including cardiovascular, respiratory, endocrine, hematologic, metabolic, urinary tract, skin, and nervous and sensory system adverse events as well as neuropsychiatric disorders. Depression, occurring mostly in the first 3 months of treatment, cognitive dysfunction, and mania are well-known and have been intensively studied. Fatal interstitial lung disease, secondary to interferon alfa-ribavirin therapy for hepatitis C infection, reported for both interferon alfa-2a and 2b, has an incidence of 0.03–0.3% (~1.1% Japanese patients). Fatal interstitial pulmonary disease can also occur with pegylated interferon alfa-2b. Autoantibodies
Table 13.10  Cytokines approved for human therapy*: properties, approved indications*, mechanisms, and side effects

| Generic and trade names | Properties | Approved indications* | Warnings and side effects, serious and common |
|-------------------------|------------|-----------------------|-----------------------------------------------|
| Peginterferon alfa-2a (Pegasys®) | Covalent complex of recombinant interferon alfa-2a 127 amino acids MW ~20 kDa with PEG linked by an amide bond to lysine | Chronic hepatitis C, chronic hepatitis B (HBeAg+ or − patients) | Boxed warnings: Neuropsychiatric, autoimmune, ischemic, and infectious disorders and ribavirin-associated effects Other effects: Fatigue/asthenia, pyrexia, headache, myalgia, cytopenias, autoimmunity, infection, colitis, pulmonary, CV, and cutaneous disorders |
| Interferon alfa-2b (Intron A®) | Recombinant protein MW ~19 kDa 165 amino acids with Arg 23; similar to leukocyte IFN | Chronic hepatitis B and C, MM, HCL, A-RKS, FL, condylomata acuminata | Boxed warnings: Neuropsychiatric, autoimmune, ischemic, and infectious disorders Other effects: Flu-like symptoms of fever, fatigue, chills, headache, myalgia, neutropenia, less common/PM period |
| Peginterferon alfa-2b (Pegintron®) | Recombinant protein linked to PEG | Chronic hepatitis C with or without ribavirin | Boxed warnings: Neuropsychiatric, autoimmune, ischemic, and infectious disorders and ribavirin-associated effects Other effects: Fatigue/asthenia, fever, nausea, rigor, myalgia, less common/PM period Boxed warnings: Depression and other neuropsychiatric disorders Other effects: As above plus ↑ALT and AST, less common/PM period |
| (Sylatron®) | Recombinant protein linked to PEG | Adjuvant treatment of melanoma | |
| Interferon beta-1a (Avonex®; Rebif®) | Recombinant 166 amino acid glycoprotein MW 22.5 kDa; amino acid sequence identical to natural protein | Relapsing forms of multiple sclerosis | Warnings and precautions: Depression, ↓ blood count, hepatic injury, anaphylaxis, AI disorders, seizures, monitor patients with CHF Other effects: ISR, flu-like symptoms – chills, fever, myalgia; asthenia, depression, immunogenicity, anaphylaxis, pruritus, rash |
| Peginterferon beta-1a (Plegridy®) | Recombinant 165 amino acid protein MW 18.5 kDa, gene contains ser for cys at position 17 | Relapsing forms of multiple sclerosis | Flu-like symptoms, lymphopenia, leukopenia and neutropenia, ISR, myalgia, depression, hypertonia, abdominal pain, asthenia, rash, ↑ liver enzymes, immunogenicity, anaphylaxis |
| Generic and trade names       | Properties                                                                 | Approved indications                                                                 | Warnings and side effects, serious and common                                      |
|-------------------------------|-----------------------------------------------------------------------------|---------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|
| Interferon gamma -1b          | Recombinant 140 amino acid polypeptide; non-covalent dimer of 2 identical 16.465 kDa monomers of 6 α-helices | Chronic granulomatous disease, malignant osteopetrosis                                | Most common: Flu-like symptoms – fever, headache, chills, fatigue; ISR, rash diarrhea Other effects: Neutropenia, thrombocytopenia, hepatotoxicity, CV, pulmonary, CNS and GI events, and pulmonary toxicity |
| Filgrastim (Neupogen®; Nivestim®; Zarzio®) | Recombinant hu-G-CSF; 175 amino acid MW 18.8 kDa non-glycosylated protein; differs from natural by an N-terminal methionine | Cancer patients receiving: chemotherapy for AML, myelosuppression or BMT; patients with chronic neutropenia or undergoing PBPCCT | Warnings: Splenic rupture, sickle cell crisis Other effects: Nausea/vomiting, fever, bone pain, hypersensitivity, ARDS, ISR, alveolar hemorrhage, immunogenicity, osteoporosis, rash, cutaneous vasculitis, Sweet’s syndrome |
| Pegfilgrastim (Neulasta®)      | Recombinant biosimilar nonglycosylated G-CSF expressed in E. coli. Formulated for short action | Severe neutropenia in patients with non-myeloid malignancies receiving myelosuppressive anticancer drugs | Warnings: Splenic rupture, ARDS, allergic reactions, sickle cell crisis Other effects: Bone pain, nausea/vomiting, fever, diarrhea, immunogenicity, cutaneous vasculitis, Sweet’s syndrome |
| Sargramostim (Leukine®)        | Recombinant hu-GM-CSF, three molecule species MWs 19.5, 16.8, 15.5 kDa 127 amino acids; leu23 differs from natural factor | Patients receiving: chemotherapy for AML, BMT; or undergoing PBPCCT; myeloid recovery in NHL, ALL, BMT | Warnings and precautions: Fluid retention, respiratory, CV, renal, and hepatic symptoms Other effects: Fever, headache, nausea/vomiting, myalgia, malaise, anorexia, bone pain, diarrhea, alopecia, stomatitis, rash, Sweet’s syndrome |
| Oprelvekin (Neumega®)          | Recombinant IL-11, nonglycosylated 177 amino acids MW 19 kDa; lacks N-terminal proline of 178 amino acid natural IL-11 | Prevention of thrombocytopenia and reduction of need for platelet transfusion after myelosuppressive chemotherapy | Boxed warning: Allergic reactions including anaphylaxis Warnings: Fluid retention, dilutional anemia, CV events, papilledema, stroke Other effects: Nausea, vomiting, asthenia, abdominal and bone pain, myalgia, anorexia, chills, alopecia |
| Becaplermin (Regranex®)        | Recombinant PDGF MW ~ 25 kDa; homodimer of two identical peptide chains of 109 amino acids -S-S- joined at cys43 and 52 | Treatment of diabetic neuropathic ulcers that extend into subcutaneous tissue          | Boxed warning: Increased rate of mortality secondary to malignancy Other effects: Erythematous skin rash, burning at application site, infection, URTI, skin ulceration, cellulitis, osteomyelitis, skin hypertrophy, bullous eruption |

(continued)
| Generic and trade names | Properties | Approved indications | Warnings and side effects, serious and common |
|-------------------------|------------|---------------------|---------------------------------------------|
| Palifermin (Kepivance®) | Truncated recombinant human KGF 140 amino acids, nonglycosylated, MW 16.3 kDa | Severe oral mucositis in patients with hematologic malignancies | Warning: Potential for stimulation of tumor growth. Other effects: Fever, dysesthesia, tongue discoloration/thickening, arthralgias, ↑ serum amylase, edema, rash, erythema, hand-foot syndrome, pruritus |
| Aldesleukin (Proleukin®) | Recombinant analog of human IL-2 MW 15.3 kDa; unlike IL-2, not glycosylated, ser for cys at position 125 and no N-terminal ala | Metastatic renal cell carcinoma, metastatic melanoma | Boxed warning: Restrict to patients with normal cardiac and pulmonary functions, administer in hospital with ICU facility and specialists, CLS, impaired neutrophil function, withhold in cases of lethargy and somnolence. Other effects: Chills, diarrhea, hypotension, oliguria, thrombocytopenia, erythema, rash |
| Anakinra (Kineret®) | Recombinant receptor antagonist for IL-1 (IL-1RA), 153 amino acids MW 17.3 kDa; has met added to amino terminal | Rheumatoid arthritis, cryopyrin-associated periodic syndrome | ISR, worsening rheumatoid arthritis, upper respiratory and other infections, headache, nausea, diarrhea; flu-like symptoms; arthralgia, abdominal pain, hypersensitivity (including anaphylaxis, angioedema), sinusitis |
| Epoetin alfa (Epogen®; Procrit®; Eprex®; Erypo®) | Recombinant human erythropoietin; glycoprotein, 165 amino acids (identical to natural product) MW 30.4 kDa | Treatment of anemia due to chronic kidney disease, zidovudine in HIV patients, effects of chemotherapy, reduction of allogeneic red blood cells in surgery | Boxed warning: ESAs increase the risk of death, myocardial infarction, stroke, venous thromboembolism, thrombosis of vascular access, and tumor progression or recurrence. Other effects: Pyrexia, arthralgias, nausea, hypersensitivity, headache, cough, ISR, hypertension, rash, pruritus, stomatitis, myalgia, pure red cell aplasia |
| Darbepoetin alfa (Aranesp®) | Recombinant human erythropoietin, 165 amino acids, MW ~37 kDa; two amino acids substituted to enhance glycosylation | Treatment of anemia due to chronic kidney disease, effects of concomitant myelosuppressive chemotherapy | Boxed warning: As for epoetin alfa. Other effects: Hypertension, dyspnea, peripheral edema, cough, abdominal pain, pure red cell aplasia, thrombovascular events, seizures, hypersensitivity (including anaphylaxis, angioedema, bronchospasm), rash/erythema |
and development or exacerbation of autoimmune diseases are known to occur in response to interferon alfa therapy including hypothyroidism, immune-mediated hemolysis, systemic lupus erythematosus, Raynaud’s disease, mixed connective tissue disease, and Hashimoto’s thyrotoxicosis. Other adverse events with a possible immune basis or component are interferon alfa-induced thyroid dysfunction (incidence of 5–14%) which likely has an autoimmune mechanism, neutropenia, autoimmune thrombocytopenia, pernicious anemia, bone marrow hypoplasia, and pure red cell aplasia. The list of cutaneous reactions to interferon alfa is extensive and includes injection site reactions (ery-
tHEMA, necrosis, and vasculitis), psoriasis and exacerbation of pre-existing psoriasis, pruritus, xerosis, urticaria, hyperpigmentation, alopecia, lichen planus, pityriasis rosea, sarcoid nodules, eosinophilic fasciitis, livedo reticularis, fixed drug eruption, and vitiligo (Fig. 13.7).

A flu-like illness is the most commonly occurring adverse event following administration of interferons beta-1a and beta-1b (Table 13.10), and injection site reactions are common especially with the beta-1b preparation. Neutralizing antibodies to subcutaneously administered interferon beta are found in about a quarter of treated patients. The consensus is that they neutralize or reduce the cytokine’s activity, and this has the potential to significantly reduce the effectiveness of the therapy, leading to the suggestion that the immunogenic potential of interferon beta should be considered as well as its safety. Other immunologic effects observed are cases of a lupus-like syndrome to both beta interferons and cutaneous lymphocytic vasculitis to subcutaneous interferon beta-1b. Interferon beta may induce thyroid disorders including Hashimoto’s encephalopathy. Reported skin reactions include urticaria to interferon beta-1a and an acneiform eruption to interferon beta-1b. In August 2014 the FDA granted approval for Plegridy®, a pegylated preparation of interferon beta-1a produced as a glycosylated protein in Chinese hamster ovary cells and covalently attached via the N-terminal residue to a linear 20 kDa methoxypolyethylene glycol molecule. The most common adverse reactions to Plegridy® are like those of the non-pegylated form of the cytokine with injection site reactions, an influenza-like illness, asthenia, arthralgia, and pruritus seen most commonly. Issued warnings and precautions for the preparation are also similar to those for the non-pegylated form (Table 13.10).

Interferon gamma shows a different biological activity spectrum, in particular in its action of differentiating normal and B lymphocytes, as an immunomodulator of macrophage activity, and its roles in dealing with intracellular pathogens and tumor control. Like other interferons, it induces flu-like symptoms and granulocytopenia and a suspicion of acute respiratory insufficiency in patients with idiopathic pulmonary fibrosis. Cardiovascular and renal toxicities to interferon gamma have been recorded. There appears to be only a few reports of cutaneous reactions to interferon gamma although increased levels of interferon gamma mRNA were detected in the skin of patients with vitiligo (Fig. 13.7) and inhibitors of the cytokine have proved to be beneficial treatments in some cases. In an investigation of 50 patients with vitiligo, an expansion of CD8+ cytotoxic T lymphocytes expressing interferon gamma was detected leading the authors to conclude that the CD8+ cells may have a pivotal role in the induction and maintenance of the condition (Chap. 3, Sect. 3.8.12).

Colony-stimulating factors (CSFs), filgrastim, sargramostim, and tbo-filgrastim, described by Metcalf as “the master regulators of granulocyte and macrophage populations,” are glycoprotein cytokines with multiple actions on hematopoietic cells used to treat chemotherapy-induced neutropenia, mobilize stem cells for transplantation, and enhance the immune response to cancer. Despite dissimilarities in amino acid sequences, human granulocyte-colony-stimulating factor (G-CSF) and human granulocyte-macrophage colony-stimulating factor (GM-CSF) show three-dimensional structural similarities to each other and a number of other signaling proteins. Approved members of the CSF family are filgrastim and pegfilgrastim,
both G-CSFs; sargramostim, a GM-CSF; and tbo-filgrastim, a short-acting biosimilar G-CSF (Table 13.10). G-CSF has been associated with adult respiratory distress syndrome; other pulmonary toxicities, particularly pulmonary edema which has proved fatal; interstitial pneumonitis; and capillary leak syndrome, and the factor may be a risk for the progression of myelodysplastic syndrome, but this has not been unequivocally established. Cardiovascular complications have been associated with GM-CSF and speculation that it might contribute to the development of acute coronary syndrome. Two other potentially life-threatening responses to CSFs, both the subject of FDA warnings, are anaphylactic/anaphylactoid reactions and severe adverse events such as acute chest syndrome, vaso-occlusive episodes, multi-organ failure, and death seen in patients with sickle cell disease. There is a long list of adverse skin reactions provoked by CSFs, including psoriasis flare, pyogenic granulomas, pruritic erythematous maculopapular eruptions, palmoplantar pustulosis, erythema multiforme, neutrophilic dermatoses, and Sweet’s syndrome (Fig. 13.8). Filgrastim and sargramostim are the most frequently implicated drugs in Sweet’s syndrome.

Recombinant human IL-11, or *oprelvekin* (Table 13.10), is a member of a family of human growth factors which includes human growth hormone and G-CSF. It is used to prevent chemotherapy-induced thrombocytopenia and reduce the need for platelet transfusions in patients with nonmyeloid malignancies. Oprelvekin carries an FDA black box warning for allergic reactions including anaphylaxis and warnings and precautions for dilutional edema, cardiovascular events, papilledema, and fluid retention resulting in peripheral edema, dyspnea, pulmonary edema, capillary leak syndrome, and sometimes death. Anaphylactic reactions to the drug have occurred after the first dose or subsequent doses.

**Becaplermin** is a recombinant human platelet-derived growth factor (PDGF) (Table 13.10). Naturally occurring PDGF has A and B chains in homodimeric or heterodimeric form, but becaplermin is a homodimer made up of two disulfide-bonded B chains and hence written as rhPDGF-BB. It promotes the growth of granulation tissue and wound healing via interaction with receptors on fibroblasts and has therefore found use in gel form as a topical application for patients with lower extremity diabetic neuropathic ulcers. Because growth factors cause cell proliferation, the possibility of increased cancer rates is considered. In 2008 the FDA issued a boxed warning for Regranex® Gel stating that “malignancies distant from the site of application have occurred in becaplermin users.... and an increased rate of death from systemic malignancies was seen in patients who have received 3 or more tubes” and, in addition, “becaplermin should be used with caution in patients with known malignancy” and only used “when the benefits can be expected to outweigh the risks”. In 2010, the EMA’s Committee for Medicinal Products for Human Use recommended that becaplermin should not be used in patients with a pre-existing cancer and then July 2012 the European Commission withdrew the marketing authorization for Regranex®.

**Palifermin** (Table 13.10) is a recombinant human keratinocyte growth factor genetically modified to increase stability by shortening the natural protein which belongs to the fibroblast growth factor family. Palifermin stimulates dif-

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**Fig. 13.8** Cutaneous eruption mimicking Sweet’s syndrome or acute febrile neutrophilic dermatosis following the administration of filgrastim (recombinant granulocyte-colony-stimulating factor [G-CSF]). (Reproduced from Aubin F, Carbonnel, Wendling D. The complexity of adverse side-effects to biologic agents. *J Crohn’s Colitis*. 2013;7:257–62, an open access article distributed under a Creative Commons License; reproduced with permission from Elsevier)
differentiation, proliferation, and migration of epithelial cells in numerous tissues including skin, hair follicles, tongue, stomach, intestine, lung, liver, kidney, lens of the eye, and many other tissues and organs. These properties make it a useful agent in oncological supportive care, aiding the management of mucositis in cancer patients by protecting the mucosal epithelium and aiding its regeneration after chemotherapy- and radiation-induced injury. Adverse events following palifermin include rash, pruritus, paresthesia, and edema and, interestingly, palmoplantar erythrodysesthesia (acral erythema; hand-foot syndrome) and a papulopustular (acne-like) eruption on the head and trunk. Hyperpigmented papillomatous plaques in the axillae and inguinal areas and a case of lichenoid papules have also been described. Being a growth factor, palifermin carries a warning of potential stimulation of tumor growth.

**Interleukin-2 (IL-2)** has a wide range of immune effects regulating T cells and immune activation and homeostasis. The recombinant form, aldesleukin (Table 13.10), differs from the natural cytokine by absence of glycosylation, a serine substitution for cysteine at amino acid position 125, and no N-terminal alanine. The main toxicities of aldesleukin are cardiovascular adverse events; capillary leak syndrome; pulmonary effects (usually related to the former); hematologic adverse effects, particularly anemia, leukopenia, thrombocytopenia, and eosinophilia; renal and gastrointestinal toxicities; and thyroid dysfunctions. Cutaneous reactions are numerous, ranging from mild erythema, pruritus, injection site reactions, and vitiligo to urticaria, angioedema, reactivation of eczema, exacerbation of psoriasis, vasculitis, fixed drug eruptions, exfoliative dermatitis, and severe manifestations like pemphigus, IgA bullous dermatosis, and toxic epidermal necrolysis (TEN). An acute blistered scalded skin-like reaction after IL-2 therapy in an immunocompromised patient has been reported. Erythema during IL-2 immunotherapy is common, and urticaria at the end of IL-2 therapy has also been reported. Cutaneous side effects in metastatic melanoma patients treated with IL-2 were observed in 56 of 78 treatment cycles (72%) of 25 patients. Fifty-three of the eruptions were mild with a burning pruriginous erythema and 3 were severe with urticaria, necrotic lesions, and blisters. There appear to be no reports of anaphylaxis following IL-2 immunotherapy.

**Interleukin-1 (IL-1)** is a cytokine produced in response to inflammatory stimuli in a number of immunological reactions. Its receptor (IL-1R), which occurs in membrane or soluble form, exists as two types, type I, responsible for the expression of the inflammatory effects of IL-1, and type II which may compete for IL-1 and act as a suppressor of the cytokine. Anakinra (Table 13.10) is a recombinant specific receptor antagonist (IL-1RA) for IL-1 differing from the natural receptor by the addition of a single methionine at the amino terminal end. The recombinant receptor antagonist competes with IL-1, blocking its access to its complementary receptor, thus making it a useful agent in the treatment of some inflammatory conditions such as rheumatoid arthritis where it acts as a biological response modifier rather than a disease-modifying anti-rheumatic drug. Injection site reactions are the most frequent adverse event, but overall anakinra is safe and well tolerated for up to 3 years of continuous use. Cutaneous reactions are usually at the injection site and occur in up to 73% of patients but cause cessation of treatment in less than 5% of affected individuals. Skin biopsy specimens from rheumatoid arthritis patients treated with anakinra, and with well-defined erythema and edema at the injection sites, showed marked dermal edema, an increased number of mast cells, and a lichenoid infiltrate of mainly lymphocytes together with eosinophils and CD68 macrophages. In some cases, cutaneous reactions are associated with systemic involvement. The observed skin reactions were said to resemble reactions seen in patients receiving chemotherapy and colony-stimulating factors. A cutaneous reaction in one patient was shown to be mediated by specific IgE antibodies.

A case of apparent immediate hypersensitivity was reported in a 25-year-old woman with familial Mediterranean fever who developed urticaria and angioedema on the face and diffuse erythema over the entire body after
the 12th subcutaneous daily dose of anakinra (100 mg/day). The reactions responded well to antihistamines and intradermal tests with the drug proved positive, suggesting a type I hypersensitivity response. A desensitization protocol was employed after a premedication dose of 10 mg of cetirizine given 1 h before the first subcutaneous injection of anakinra. Six doses, starting at 1.5 mg, were administered at 1 h intervals. Subsequent doses were 3, 5.5, 12.5, 25, and 52.5 mg, totaling a cumulative dose of 100 mg of anakinra. After desensitization, the intradermal test was negative, and the patient continued on daily anakinra without problems. A similar cutaneous reaction involving itching, erythema on the face and abdomen, shortness of breath, and abdominal pain was seen 3 h after administration of anakinra. The patient proved skin prick test-positive to the recombinant cytokine, again indicating a type I allergic reaction. Successful desensitization to anakinra has also been reported in a 34-year-old man who developed a delayed local injection site reaction to the protein. Systemic reactions to anakinra are rare, but an anaphylactic reaction occurred in a patient with rheumatoid arthritis, and severe systemic symptoms including urticaria, angioedema, and pruritic tongue were reported in a 7-year-old girl with juvenile idiopathic arthritis.

Human erythropoietin (or erythropoetin) (EPO), also called hematopoi etin (or hemopoi etin) (Table 13.10), is a glycoprotein hormone of 165 amino acids MW 34 kDa that controls erythropoiesis (red blood cell production). Recombinant human EPO, introduced in 1986, is available as epoetin alfa. Collectively known as erythropoietin-stimulating agents ESAs, there are also beta, delta, and omega forms, each differing from the endogenous hormone, and from each other, by the individual sugar and sialic acid residues present. Epoetin beta (Mircera®) was approved in pegylated form (methoxy polyethylene glycol-epoetin beta) by the FDA in 2007. Epoetins are administered for renal and non-renal anemias. In 2001 the FDA and European Medicines Agency approved a new epoetin, darbepoetin alfa, for treatment of anemia due to renal failure and in patients undergoing immuno therapy. Darbepoetin alfa is a recombinant epoetin molecule containing two extra N-linked oligosaccharide chains introduced to give greater stability and thus allow less frequent administrations. In recent years, there appears to have been an increase in the number of patients developing neutralizing anti-EPO antibodies during therapy, and there are now well in excess of 250 known cases of pure red cell aplasia. Serious allergic reactions including angioedema, bronchospasm, tachycardia, pruritus, rash, and urticaria and severe cutaneous reactions including erythema multiforme, SJS, and TEN have occurred with ESAs. Injection site reactions are also well-known. Cutaneous reactions at the sites of former subcutaneous injections of epoetins following the intravenous injection of different epoetins proved to be the signs of allergic skin and systemic reactions in a patient with pure red cell aplasia and anti-EPO antibodies. After switching the patient from epoetin alfa to, first, epoetin beta, and then darbepoetin alfa, a systemic anaphylactic/anaphylactoid response occurred, and anti-EPO antibodies cross-reactive with epoetin beta and darbepoetin alfa were detected in the patient’s serum. This case illustrates that continuation of epoetin therapy in patients with anti-EPO antibodies may carry the risk of a serious systemic (anaphylactic or anaphylactoid) reaction and that skin reactions at the injection site may be the first sign of sensitization which precedes the development of anemia. In three other rare cases, anaphylaxis, serum IgE antibodies, and a generalized eczematous reaction to recombinant EPO were reported in the presence of negative skin tests to the glycoprotein, and acute exanthematous pustulosis was diagnosed after epoetin alfa was replaced by darbepoetin alfa.

EPO receptors have been demonstrated in tumor tissue, and the cytokine may assist with tumor angiogenesis, suggesting the possibility of EPO initiating tumor growth or aiding tumor progression. The FDA has issued a boxed warning for both epoetin alfa and darbepoetin alfa related to a possible increased risk of death, cardiac and thromboembolic events, and tumor progression or recurrence.
Leptin, a 167 amino acid protein of MW 16 kDa, helps to control energy homeostasis and body weight by adjusting hunger and energy expenditure to regulate fat stores. It also regulates some neuroendocrine functions and other physiological processes, many yet to be defined. **Metreleptin** (Table 13.10) is a recombinant analog of leptin used as replacement therapy to treat leptin deficiency in patients with congenital or acquired generalized lipodystrophy. Adverse events specifically due to metreleptin occur with an incidence of ≥5% and include injection site reactions 66.7%, nausea 25.9%, nasopharyngitis 7.4%, headache 7.4%, hypersensitivity 7.4%, and vomiting 7.4%. Injection site reactions often include inflammation, erythema, and ecchymoses. More serious reported adverse events to metreleptin include the worsening of renal disease, the production of antimetreleptin antibodies, and development of T cell lymphomas.

**Ancestim** (*Stemgen®*) (Table 13.10) is a recombinant human stem cell factor (SCF) used with filgrastim to produce increases in circulating peripheral blood progenitor cells (PBPCs) and achieve a reduction in the number of aphereses required to reach the PBPC number target. This has resulted in the Australian Therapeutic Goods Administration (TGA) approving the combination to increase the number of PBPCs for transplant patients at risk of poor PBPC mobilization. The TGA has issued a number of important warnings and precautions for ancestim including the following: the cytokine should not be used alone; it should only be administered to patients who are at risk of inadequate PBPC mobilization; care should also be exercised in administering the combination of ancestim and filgrastim in patients given chemo-/radiotherapy; and administration should be avoided 24 h before and after the cytotoxic therapy.

Because SCF increases mast cell proliferation and promotes the release of histamine and tryptase, allergic-like symptoms sometimes occur in treated patients. Ancestim should therefore only be administered in a setting with the appropriate staff, facilities, and medications to respond to a possible life-threatening anaphylactic/anaphylactoid reaction. In addition, patients should be premedicated with H1 and H2 antihistamines and a bronchodilator. Patients with a history of anaphylaxis, asthma, recurrent urticaria and/or angioedema, and mast cell diseases such as systemic mastocytosis, urticaria pigmentosa, or diffuse cutaneous mastocytosis may be at particular risk. Systemic allergic reactions, generally moderate to severe but not life-threatening, occur more often at higher doses; at <30 μg/kg/day of ancestim, 5% of patients had such a reaction, while 27% of patients given 30–100 μg/kg/day had reactions. Approximately 10% of patients produce serum antibodies to the cytokine, but no adverse clinical consequences, including a reduced therapeutic effect or serum sickness, have been recorded. Respiratory problems, affecting 25% of recipients of combination therapy and 14% of those on ancestim alone, include cough, pharyngitis, and dyspnea.

Injection site reactions, including erythema at a previous injection site, occurring within 1–24 h, are the most often observed cutaneous adverse events with up to 84% of patients given ancestim showing mild to moderate reactions. Other reported reactions are erythema (incidence 59%), pruritus (25%), urticaria (16%), and occasional cases of hyperpigmentation and rash at the injection site.

Bearing in mind that ancestim is usually administered with filgrastim, there is usually an element of doubt as to which of the two cytokines, or both, are responsible for observed effects.

### 13.3 Fusion Proteins

Chimeric fusion proteins used for human therapy are made up of an effector domain coupled to a “carrier,” usually protein or peptide, that also contributes to the functional properties of the resultant fusion protein (Fig. 13.9). Effector peptides employed so far are ligand-binding portions of receptors of a few cytokines and growth factors, extracellular domains of some lymphocyte antigens, coagulation factors, a glucagon-like peptide, and a toxin for cell killing. Fusion partners employed have mainly been the crystallizable Fc region of the human IgG antibody or human serum albumin.
13.3.1 Adverse Effects of Individual Approved Fusion Proteins

Fusion proteins examined here are included in Table 13.11 where summaries of their properties, approved indications, and adverse effects are presented.

The TNF-targeted fusion protein etanercept (Enbrel®), a recombinant, engineered, fully human dimeric Fc-fusion protein linked to the ligand-binding portion of the human TNF receptor (TNFR) (Table 13.11), was the first chimeric fusion protein to gain regulatory approval when, in 1998, it was approved by the FDA for the treatment of rheumatoid and other forms of arthritis. Etanercept has an extensive adverse events profile, recognized by the British Society for Rheumatology Standards, Guidelines and Audit Working Group (SGAWG), who refer to six different use exclusion criteria: (1) women who are pregnant or breastfeeding, (2) the presence of active infection, (3) septic arthritis of a joint in the last year, (4) sepsis of a prosthetic joint, (5) grade 3 or 4 congestive cardiac failure, and (6) a clear history of demyelinating disease. An FDA boxed warning covers serious infections which have been recorded for a wide range of organisms including bacteria (tuberculosis, streptococcus, listeria, actinobacillus), viruses (varicella), fungi (aspergillus), protozoa (toxoplasma), and cestodes (echinococcus). Other well-known side effects are neurologic events associated with demyelinating disorders, hematological events with reports/warnings for cytopenias and pancytopenia, and respiratory events, and there is the question of the possible tumorigenicity of etanercept, the subject of a boxed warning. Of most interest here are immunological and cutaneous events induced by etanercept. Although injection site reactions are the most common adverse event of etanercept therapy, the mechanism(s) of the reaction(s) has not been well studied, and some early injection site adverse responses may be true immediate, type I hypersensitivities. There appears to be at least a half dozen possible/probable reports of immediate hypersensitivity/
| Generic and trade names | Properties | Approved indications | Side effects, serious and common |
|-------------------------|------------|---------------------|----------------------------------|
| Etanercept (Enbrel<sup>®</sup>) | A dimeric fusion protein, MW 150 kDa, of the extracellular ligand-binding portion of 75 kDa human TNFR with Fc portion of human IgG1 | Rheumatoid arthritis, polyarticular juvenile idiopathic arthritis, psoriatic arthritis, plaque psoriasis, ankylosing spondylitis | Boxed warnings: Serious infections, malignancies. *Other effects*: Fever, injection site reactions, cutaneous vasculitis, hypersensitivity (including anaphylaxis, angioedema, urticaria), pruritus, denyselinating disease, cytopenia, lupus syndrome |
| Belatacept (Nulojix<sup>®</sup>) | Differs from abatacept by 2 amino acid substitutions in the ligand-binding region of CTLA-4: alanine 29 to tyrosine and leucine 104 to glutamic acid | Prophylaxis of organ rejection in adult patients receiving a kidney transplant | Boxed warnings: ↑ risk of PTLD, ↑ susceptibility to infections and malignancies. *Other effects*: Anemia, diarrhea, peripheral edema, hypertension, urinary tract infections, cough, hypo- and hyperkalemia, graft dysfunction |
| Abatacept (Orencia<sup>®</sup>) | Homodimeric fusion protein of Fc fragment of human IgG1 and extracellular domain of CTLA-4. Fc mutated to lose its ADCC and CDC actions | Adult rheumatoid arthritis, juvenile idiopathic arthritis | Infections, malignancies, immunogenicity, hypersensitivity, reactions in patients with COPD, injection site reactions, upper respiratory tract infection, headache, nausea |
| Rilonacept (Arcalyst<sup>®</sup>) | A dimeric fusion protein of the extracellular ligand-binding domains of IL-1RI and IL-1RACP linked in line to human IgG1 Fc | Cryopyrin-associated periodic syndromes (CAPS) | Injection site reactions, upper respiratory tract infections, immunogenicity |
| Aflibercept (Zaltrap<sup>®</sup>; Eylea<sup>®</sup>) | Fusion protein of Fc portion of IgG1 and ligand-binding domain 2 of VEGFR1 and domain 3 of VEGFR2 | Zaltrap: Metastatic colorectal cancer in combination with FOLFIRI Eylea: Wet macular degeneration | Zaltrap Boxed warnings: Hemorrhage, compromised wound healing, GI perforation. *Other effects*: Cytopenias, proteinuria, hypertension, ↑ serum creatinine, acral erythema, stomatitis. Eylea: Eye pain, cataract, conjunctival hemorrhage, vitreous detachment, ↑ intraocular pressure |
| Romiplostim (Nplate<sup>®</sup>) | Dimeric fusion peptibody MW ~60 kDa; four copies of thrombopoietin mimetic peptide fused to C-terminus of aglycosylated human IgG1 Fc | Thrombocytopenia in patients with chronic ITP | Arthralgia, dizziness, insomnia, abdominal and shoulder pain, myalgia, pain in extremity; dyspepsia, paresthesia, headache |
Table 13.11 (continued)

| Generic and trade names | Properties | Approved indications | Side effects, serious and common |
|-------------------------|------------|---------------------|----------------------------------|
| Alefacept (Amevive®)c   | A dimeric fusion protein MW 91.4 kDa; consists of the first extracellular domain of LFA-3 fused to human IgG1 Fc | Moderate-severe chronic plaque psoriasis in candidates for systemic therapy or phototherapy | Warnings and precautions: Lymphopenia, malignancies, infections, hypersensitivity, hepatic injury, immunosuppression Other effects: Headache, chills, pharyngitis, URTI, dizziness, cough, nausea, infections, pruritus, inject site reactions |
| Factor VIII Fc fusion protein (Eloctate®; Elocta®) | Large Fc fusion monomer, 1890 amino acids MW ~330 kDa made up of BDD-rFVIII linked to IgG1Fc. Produced in HEK cells | Control and prevention of bleeding episodes in adults and children with hemophilia, for routine prophylaxis and surgical prophylaxis (perioperative management) to prevent or reduce the frequency of bleeding episodes | Warnings and precautions: Hypersensitivity reactions, neutralizing antibodies, monitor factor VIII activity and inhibitors' Other effects: Arthralgia, malaise, rash |
| Factor IX Fc fusion protein (Alprolix®) | Recombinant factor IX expressed in HEK cells covalently linked to Fc domain of human IgG1; fusion protein has 867 amino acids, MW ~98 kDa | Indicated in adults and children with hemophilia B for control and prevention of bleeding; perioperative management, routine prophylaxis to prevent or reduce frequency of bleeding episodes | Warnings and precautions: Hypersensitivity including anaphylaxis, development of neutralizing antibodies, thromboembolic complications Other effects: Headache, oral paresthesia |
| Tagraxofusp-erzs (Elzonris®) | Cytotoxic fusion protein of recombinant IL-3 and truncated diphtheria toxin (DT). MW ~57.7 kDa produced in E.coli cells | Cytotoxic treatment of blastic plasmacytoid dendritic cell neoplasm | Boxed warning: Capillary leak syndrome Other warnings and precautions: Hypersensitivity, hepatotoxicity |

From Baldo BA. Chimeric fusion proteins used for therapy: Indications, mechanisms, and safety. Drug Saf. 2015;38:455–79. Adapted and reproduced with permission from Springer Nature

APCs antigen presenting cells; BDDrFVIII B-domain deleted recombinant factor VIII; COPD chronic obstructive pulmonary disease; CTLA-4 cytotoxic T lymphocyte-associated antigen 4; FOLFIRI folinic acid (leucovorin), 5-fluorouracil, irinotecan; GI gastrointestinal; GLP-1 glucagon-like protein-1; HEK human embryonic kidney; IL-1RAcP interleukin-1 receptor accessory protein; IL-1RI interleukin-1 receptor type I; ITP immune thrombocytopenia; LFA-3 lymphocyte function-associated antigen 3; PTLD post-transplant lymphoproliferative disorders; TNF tumor necrosis factor; TNFR tumor necrosis factor receptor; VEGFR1 and VEGFR2 vascular endothelial growth factor receptors 1 and 2; URTI upper respiratory tract infection

aApproved by FDA or EMA or both
bZiv-aflibercept in the United States

In FDA Center for Drug Evaluation and Research’s (CDER) Discontinued Therapeutic Biologic Products list. Discontinued date, September 28, 2012

anaphylaxis to etanercept including two cases of anaphylaxis in children with juvenile idiopathic arthritis and two episodes of angioedema. In two other reported cases, one patient experienced urticaria and swelling of the tongue and periorbital regions within hours of administration of etanercept; the second patient, who had Still’s disease, experienced facial swelling, periorbital edema, diffuse pruritic rash, and difficulty swallowing. Cases of etanercept-induced cytopenias and vasculitis may be type II and type III hypersensitivities, respectively, and some pulmonary events caused by etanercept may ultimately be shown to be type III or combined type III/type IV reactions. Significant numbers of vasculitic adverse events after etanercept, particularly hypersensitivity vasculitis and necrotizing vasculitis, have been recorded by the FDA Adverse Events
Reporting System. Etanercept may provoke autoimmune reactions such as hyperthyroidism and the development of anti-synthetase syndrome, Crohn’s disease, and Henoch-Schönlein purpura. In one case of the latter, increased concentrations of IgA rheumatoid factor resulted in IgA immune complexes; in another case, Henoch-Schönlein purpura occurred with acute renal failure.

Several different cutaneous adverse events may be seen after etanercept administration. In addition to injection site reactions, the most common skin manifestations seen with etanercept are erythema, edema, pain, and pruritus, which may be seen in ~20–50% of patients. Reactions occur within the first two months of treatment and may reoccur 1–2 days after the final injection. Etanercept has been associated with a wide variety of adverse dermatological events, most mild but some serious, and a few life-threatening. Included are onset and exacerbations of psoriasis and atopic dermatitis, lichenoid reaction, erythema multiforme, angiokeratomata, pemphigus vulgaris, palmoplantar pustulosis, and acute generalized exanthematous pustulosis.

For a summary of desensitization procedures used for hypersensitivity reactions to etanercept, see Sect. 13.6.1. **Belatacept** (*Nulojix®*) (Table 13.11), a fusion protein of the modified extracellular domain of CTLA-4 (Table 13.11), binds CD80 and CD86 on antigen-presenting cells, thus blocking CD28-mediated co-stimulation of lymphocytes. Belatacept has an FDA black box warning stating that “only physicians experienced in immunosuppressive therapy and management of kidney transplant patients should prescribe Nulojix” and therapy carries increased risks of post-transplant lymphoproliferative disorder and susceptibility to infection. Acute infusion reactions, defined as a reaction within the first hour of an infusion, occurred with an incidence of 3% in one study. Epstein-Barr virus (EBV or human herpesvirus 4 [HHV-4]) seronegativity is a risk for developing post-transplant lymphoproliferative disorder (PTLD) from belatacept therapy for kidney transplantation. EBV-seronegative transplant recipients developed PTLD with an incidence of 7.3% compared to 0.6% for EBV-seropositive patients. There appears to be no reports of anaphylaxis to belatacept and skin reactions are rare.

**Abatacept** (*Orencia®*) (Table 13.11), like belatacept, also contains the extracellular domain of CTLA-4. Again, as with belatacept, acute infusion reactions occur; however, they are mostly mild to moderate. The overall frequencies of adverse events, serious adverse events, and malignancies appear similar in treated and control patients, and abatacept is associated with low immunogenicity with no associated safety or efficacy issues. Incidence rates defined as events/100 patient-years were, for serious events (8.76), infections (44.8), serious infections (1.72), malignancies (1.19), and autoimmune events (1.31). Cutaneous reactions to abatacept are rare with only a few reports of cases of induced paradoxical psoriasiform eruptions, 13 cases of psoriasis (0.67% of 1945 patients) and a case of erythema elevatum diutinum reported in a juvenile idiopathic arthritis patient.

**Rilonacept** (*Arcalyst®*) (Table 13.11), also known as IL-1 trap (target-related affinity profiling), is a dimeric Fc-fusion protein in which the IL-1R accessory protein (IL-1RAcP) ligand-binding region is fused via its C-terminus to the N-terminus of the interleukin receptor IL-1RI extracellular domain and these linked peptides are then fused via IL-1RI to the N-terminus of each of the Fc chains of human IgG1. Rilonacept thereby captures IL-1β, preventing activation of IL-1 receptors and thus reducing the inflammation and other effects due to overproduction of the interleukin. Rilonacept was originally granted orphan drug status and approved for the treatment of cryopyrin-associated periodic syndromes (CAPS). The most common adverse events to the protein are injection site reactions and upper respiratory tract infections. Warnings and precautions for rilonacept issued by the FDA state that IL-1 blockade may interfere with the immune response to infections, and hence live vaccines should not be given concurrently with the drug.

**Afibercept** (*Zaltrap®; Eylea®*) (Table 13.11), or VEGF trap, is a fusion of domain 2 from vascu-
lar endothelial growth factor receptor-1 (VEGFR-1) with domain 3 of VEGFR-2, attached to the hinge region of the Fc domain of human IgG1. Aflibercept, as ziv-aflibercept or Zaltrap®, is used for the treatment of oxaliplatin-resistant metastatic colorectal cancer and, as Eylea®, an ophthalmic intravitreal injection for the treatment of neovascular (wet) age-related macular degeneration and for macular edema. Warnings, precautions, and known side effects of ziv-aflibercept are extensive and include potentially fatal hemorrhage, gastrointestinal perforation, compromised wound healing, fistula formation, hypertension, arterial thrombotic events, proteinuria, neutropenia and its associated complications, diarrhea and dehydration, and posterior reversible encephalopathy syndrome.

Romiplostim (Nplate®) (Table 13.11) is a peptibody formed by the fusion of four identical copies of a thrombopoietin mimetic peptide to the C termini of aglycosylated human IgG1 Fc chains. Each H chain of the Fc-protein is attached to a molecule of the thrombopoietin mimetic peptide linked to another molecule of the peptide by an octaglycine bridge. Romiplostim is indicated for the treatment of thrombocytopenia in patients with chronic immune thrombocytopenia (ITP). The fusion protein is subject to regulatory agency warnings for thrombotic/thromboembolic complications, bone marrow reticulin formation, bone marrow fibrosis, and risk of progression of myelodysplastic syndromes to acute myelogenous leukemia. Reported adverse events are otherwise mild to moderate. A maculopapular rash may be the only report of an adverse cutaneous reaction.

Tagraxofusp-erzs (Elzonris®) (Table 13.11) is a CD123-directed cytotoxic fusion protein of recombinant IL-3 and truncated diphtheria toxin (DT) that acts by inhibiting protein synthesis and causing cell death in CD123-expressing cells. Approved by the FDA in 2018, it is indicated for the treatment of blastic plasmacytoid dendritic cell neoplasm. Capillary leak syndrome is the subject of a boxed warning for the cytotoxin, and there are other warnings and precautions for hypersensitivity and hepatotoxicity.

The production of alefacept (Amevive®) (Table 13.11) has been discontinued, but, nevertheless, along with another discontinued fusion protein, denileukin diftitox (see below), it provides extra interesting insights into the possible variety, nature, and actions of these new targeted therapies. Alefacept, which can be viewed as an anti-CD2, selectively targets effector memory T cells for both CD4+ and CD8+, but not naïve T cells and central memory T cells, in psoriasis vulgaris. Lymphopenia with reductions in CD4+ and CD8+ lymphocytes, malignancies, serious infections, and hypersensitivity reactions are each the subject of warnings and precautions by the FDA. These adverse effects are listed as the most serious adverse reaction of alefacept. The most common side effects of the drug include injection site reactions which are mild to moderate. There appears to be no correlation between decreased CD4+ T cell counts and the incidence of infections. Data from clinical trials revealed 4 of 1869 patients (0.2%) experienced angioedema, while urticaria occurred in 6 patients (0.3%).

The other now discontinued genetically engineered recombinant fusion protein denileukin diftitox (Ontak®), introduced in 1992 as DAB389IL2, was the first fusion toxin to be approved. The fusion construct is made up of the full-length IL-2 molecule and the catalytic domain of diphtheria toxin. Diphtheria toxin is a single polypeptide chain of 535 amino acids. For use as a targeted toxin, it has been modified by deleting the 147 amino acid residue receptor- (cell-)binding domain to produce a protein of 388 amino acids commonly referred to as DT388 or DAB389. This remaining protein consists of the adenosine diphosphate (ADP)-ribosyltransferase and membrane-translocating domains of native diphtheria toxin. Replacing the receptor-binding domain of the native toxin by the sequences encoding the IL-2 gene produced the recombinant fusion toxin then designated as DAB389-IL-2. Bound to the IL-2 receptor, the fusion toxin undergoes endocytosis and is proteolytically cleaved liberating the modified toxin and causing ADP-ribosyltransferase-mediated inhibition of protein synthesis. Denileukin diftitox was
approved by the FDA in 1999 for the treatment of cutaneous T cell lymphoma.

In the pivotal phase III trial of the fusion protein for the treatment of cutaneous T cell lymphoma, adverse effects were seen, and occurred in most patients, during the first treatment course. In the first 24 h, acute hypersensitivity-like reactions involving dyspnea, hypotension, chest tightness, and back/chest pain occurred, and approximately one third of patients experienced cutaneous infusion-related events including flushing and pruritus. Administration of antihistamines and corticosteroids were used to alleviate or prevent acute symptoms. Subsequently, the FDA issued a black box warning for the possibility of serious and even fatal infusion reactions and for vascular leak syndrome, usually seen in the first 14 days of treatment, and reported by 25% of patients. Loss of visual acuity, with or without retinal pigment mottling, was a third possible adverse event listed in the FDA black box warning. Rashes not related to infusions manifested in 25% of patients. Overall, 35% had cutaneous reactions classified as maculopapular, petechial, vesicular-bullous, urticarial, and/or eczematous. Cutaneous reactions classified as delayed hypersensitivities and including a case of exfoliative dermatitis were reported in 3 of 35 patients with psoriasis. There was at least one fatal report of TEN. Assessment of neutralizing antibodies showed that inhibited functional activity increased from 45% at baseline to 97% after three courses although the authors in the pivotal phase III trial concluded that development of antibodies to the fusion toxin did not appear to impair the response to treatment, and no clinical correlation was observed between levels of antibodies to IL-2 and any adverse event.

Two other interesting Fc fusion proteins developed as therapies for indications quite different to those discussed above are the coagulation factor preparation marketed as Eloctate®, Elocta®, and Alprolix® (Table 13.11). Eloctate® and Elocta® are trade names for a factor VIII Fc fusion protein prepared by linking B-domain deleted recombinant factor VIII (BDDrFVIII) toIgG1 Fc. Used for the control and prevention of bleeding in hemophilia A patients, the fusion protein is covered by warnings for the possibility of hypersensitivity reactions and the formation of neutralizing antibodies. Alprolix® or factor IX Fc fusion protein is a conjugate of coagulation factor IX and the Fc domain of human IgG1. Indicated for the control and prevention of bleeding in hemophilia B, relevant warnings and precautions are similar to the factor VIII preparation, namely, hypersensitivity reactions and the formation of neutralizing antibodies, but with a further warning drawing attention to a known association of factor IX with the development of thromboembolic complications.

Another novel Fc fusion protein, dulaglutide (Trulicity®), a fusion dimer of a glucagon-like protein-1 (GLP-1) analog linked to IgG4 Fc, was approved by the FDA in 2014 for the improvement of glycemic control in adults with type 2 diabetes. Safety issues included warnings and precautions for the risk of thyroid C cell tumors, pancreatitis, hypersensitivity, and hypoglycemia. Albiglutide (Tanzeum®; Eperzan®) and factor IX fusion protein rIX-FP (Idelvion®) are two currently approved albumin fusion constructs. Albiglutide, prepared in Saccharomyces cerevisiae, is a recombinant fusion protein used for glycemic control of type 2 diabetes. It is made up of two copies of a modified 30 amino acid GLP-1 sequence fused in tandem to the N-terminus of human albumin. The FDA black box warning of the risk of thyroid C-cell tumors issued for other GLP-1 receptor agonists also applies to albiglutide as do the warnings and precautions for pancreatitis, hypoglycemia, renal impairment, and hypersensitivity. Factor IX albumin fusion protein is generated by genetic fusion of recombinant human albumin to recombinant factor IX, producing a single recombinant protein designated rIX-FP. Warnings and precautions applying to the fusion protein relate to the possibility of hypersensitivities including anaphylaxis, the development of neutralizing antibodies, thromboembolism, and nephrotic syndrome following induction of immune tolerance to factor IX in hemophilia B patients with factor IX inhibitors and a history of allergic reactions to factor IX.
13.4 Enzymes Approved for Therapy

Research and drug developments fostered under orphan drug product development programs have greatly assisted the introduction of efficient and safe enzyme-based therapies for a range of disorders. The introduction and regulatory approval of more than two dozen different purified enzymes, most of them recombinant preparations, has enabled, often for the first time, effective enzyme therapies. These therapies include enzyme replacement therapies (ERT) for some lysosomal storage disorders including Gaucher disease, Fabry disease, and Pompe disease, lysosomal acid lipase deficiency, and mucopolysaccharidoses I, II, IVA, VI, and VII. Approved enzymes are also now used as therapies for myocardial infarction (alteplase, reteplase, tenecteplase, and streptokinase); hypophosphatasia (asfotase alfa); cystic fibrosis (dornase alfa); chronic gout (pegloticase); tumor lysis syndrome (rasburicase); leukemia (L-asparaginase); some collagen-based disorders such as Dupuytren’s contracture (collagenase); severe combined immunodeficiency disease (pegademase bovine); detoxification of methotrexate (glucarpidase); vitreomacular adhesion (ocriplasmin); and a spreading agent, in particular for drugs (hyaluronidase). Enzyme-induced adverse events with an immune basis, that is true hypersensitivities, are discussed.

13.4.1 Hypersensitivities to Enzymes Used as Therapy for Lysosomal Storage Diseases

In the main, enzymes used therapeutically are produced naturally in humans, so adverse reactions to them might be expected to be rather rare. Reactions, when they do occur after therapy, often tend to be mild, but being protein in nature, there is always the potential for immediate allergic reactions, including anaphylaxis. Two additional factors should be kept in mind. Firstly, many of the enzymes now used are recombinant products that, compared to the original molecule, have been altered, often only slightly, but sometimes enough to cause some rare recognition and small functional changes. Secondly, an increasing number of enzymes have been developed and administered under orphan drug programs, so at the time of their approval for use, they may have been administered to relatively small numbers of patients. This means that collected available safety data may constitute a much smaller body of results than is usually the case for newly approved medical agents.

Under an initiative of the FDA Office of Orphan Products Development, several recombinant enzymes for rare lysosomal storage disease enzyme replacement therapies have been developed, approved, and brought to market. These enzymes, their indications, and adverse effects are summarized below.

Agalsidase Beta (Fabrazyme®) for Fabry Disease Agalsidase beta is a recombinant α-D-galactosidase A. Infusion reactions, the most common adverse event seen during administration of agalsidase, occur in ~10–14% of patients. Reactions, usually mild with no sign of respiratory symptoms, urticaria, or changes in vital signs, occur with a higher frequency (~18%) in children. Symptoms tend to be easily controllable with antihistamines and corticosteroids.

Alglucosidase Alfa for Pompe Disease Although two registered commercial preparations of alglucosidase alfa, Lumizyme® and Myozyme®, are both a recombinant human glycogen-specific acid alfa-glucosidase, they are recognized by the FDA as being biologically different preparations due to a different manufacturing process. Lumizyme® is approved for late-onset, that is, non-infantile, Pompe disease without evidence of cardiac hypertrophy in patients more than 8 years old; Myozyme® is approved for infantile-onset Pompe disease. In a randomized study of alglucosidase alfa for late-onset Pompe disease in 60 patients, 3 cases of anaphylaxis occurred, 2 with respiratory and cutaneous reactions, and the third with severe tongue swelling. Myozyme®, given to 18 patients with infantile-onset Pompe disease, caused mild to moderate infusion reactions in 11 patients within 2 h of the infusion.
Imiglucerase (Cerezyme®), Taliglucerase Alfa (Elelyso®), and Velaglucerase Alfa (VPRIV®) to Treat Gaucher Disease

All three enzymes are a recombinant human β-glucocerebrosidase. Immediate type hypersensitivity reactions are the most prominent adverse events seen with these three enzymes. A post-marketing safety surveillance of more than 4500 patients treated with imiglucerase found that the most common and consistently reported adverse events could be classified into three categories: general disorders and administration site reactions (i.e., pyrexia, chills, chest discomfort); skin and subcutaneous tissue disorders (pruritus, rash, urticaria); and respiratory, thoracic, and mediastinal disorders (dyspnea, cough, throat irritation). Adverse events related to imiglucerase were most frequently associated with infusion of the enzyme. Taliglucerase alfa may have the poorest safety profile of the three recombinant enzymes used to treat Gaucher disease. The enzyme is subject to an FDA warning for anaphylaxis, allergy, and infusion reactions, events observed in a phase III trial and a study of eight patients with Gaucher disease. A fixed drug reaction was also seen. Like imiglucerase and taliglucerase alfa, velaglucerase alfa is generally well tolerated with drug-induced adverse events usually mild to moderate although there are reports of severe prolonged activated partial thromboplastin time and allergic dermatitis occurring with the enzyme. One patient of 71 treated with velaglucerase alfa who had previously had an allergic reaction to taliglucerase alfa experienced an allergic reaction during the first infusion of velaglucerase alfa, and a second switch-over patient had a fixed drug reaction at the first infusion.

Idursulfase (Elaprase®) for MPS II

An early clinical trial of idursulfase (recombinant human iduronate-2-sulfatase) showed that the most common adverse events were infusion-based reactions, the incidence of which reached a maximum between weeks 4 and 12 and declined thereafter. Other hypersensitivity-like responses were pruritus, pruritic rash, swelling at the infusion site, and urticaria. In a Japanese idursulfase MPS II ERT study, five of ten patients experienced drug-related infusion reactions occurring within 24 h of the infusion. Patients who developed urticaria and erythema showed the highest incidences. Two patients experienced serious reactions, one involving flushing, diffuse urticaria, and numbness of the tongue after commencement of the fifth infusion. A number of other studies reports mention infusion-related reactions in patients with MPS II after receiving idursulfase. Most reactions were mild to moderate and could be managed by slowing the rate of infusion or by giving antihistamines or antipyretics. In a recent Korean study, anti-idursulfase IgE antibodies were detected in 34 patients taking the drug for MPS II. IgE antibodies were detected in three patients (8.8%) by skin prick testing, an enzyme-linked immunosorbent assay, and Western blotting. All three patients with anaphylaxis proved to be skin test-positive. Given the extent of the evidence of infusion and immediate type I hypersensitivity reactions to idursulfase, it is not surprising that the enzyme has received a black box warning for the risk of anaphylaxis after/during infusion of the drug.

Laronidase (Aldurazyme®) for Mucopolysaccharidosis (MPS) I

Laronidase (a recombinant variant of human α-L-iduronidase), in the treatment of MPS I, is generally well tolerated with few treatment-related events and few, if any, serious adverse reactions. In common with other enzymes used in ERT, the FDA has issued a boxed warning for the potential of anaphylaxis to occur with laronidase. A long-term trial showed laronidase infusions provoked mostly mild and easily managed reactions in 53% of 40 patients. One patient experienced an anaphylactic reaction, but, in the main, reactions diminished markedly after 6 months.

Elosulfase Alfa (Vimizim®) for MPS IVA

Clinical trials results revealed that approximately 19% of 235 patients infused with eolsulfase alfa (recombinant N-acetylgalactosamine-6-sulfatase) experienced a hypersensitivity reaction. Eight percent of the reactions were classified as anaphylactic, a finding reflected in an FDA box warning. Data collected in a phase III-controlled study of 176 MPS IVA patients aged
≥5 years showed that 22.4% of patients experienced extended disruptions or discontinuation of infusions as a result of an adverse reaction to elosulfase alfa.

**Galsulfase (Naglazyme®) for MPSVI** Galsulfase is a recombinant N-acetylgalactosamine-4-sulfatase. Results from clinical trials and follow-up studies revealed an incidence of infusion reactions of 20–75%. A total of only 1.8% of patients experienced reactions and serious drug-related adverse events were uncommon. An assessment of the efficacy and safety of galsulfase for MPS VI in 34 children less than 5 years of age showed no serious infusion-related reactions although 8 children (24%) experienced some treatment-related adverse events. Infusions were continued in each case with the aid of slower infusion rates and antipyretics if needed.

**Vestronidase Alfa-vjbk (Mepsevii™) for MPS VII (Sly syndrome)** Vestronidase alfa-vjbk is a recombinant human lysosomal beta glucuronidase. The enzyme is the subject of an FDA boxed warning for anaphylaxis with a statement that anaphylaxis has occurred with the first dose. Premedication with a non-sedating antihistamine with or without an antipyretic is recommended 30–60 min before the start of infusion. The infusion should be administered over approximately 4 h with 2.5% of the total volume infused in the first hour. Thereafter, the remainder can be infused over 3 h as tolerated. Mepsevii™ was approved by the FDA in 2017. In addition to anaphylaxis, adverse reactions reported so far include infusion site extravasation and swelling, peripheral swelling, pruritus, and rash.

**Sebelipase Alfa (Kanuma®) for Lysosomal Acid Lipase Deficiency** The enzyme is a recombinant human lysosomal acid lipase produced in chicken egg white which accounts for the warning of the risk of possible anaphylaxis to eggs and egg products. Apart from allergic sensitivity to egg, attention is drawn to hypersensitivity reactions, including anaphylaxis. In clinical trials, ~20% of 106 infused patients experienced symptoms of hypersensitivity. In ~3% of patients the reaction was judged to be anaphylactic. Anaphylaxis has been reported as early as the sixth infusion and even after a year of treatment.

13.4.2 Hypersensitivities Associated with Other Approved Enzymes Used for Therapy

As discussed above, being proteins, an immune response in some individuals receiving enzyme therapy would be expected, with some enzymes provoking a higher incidence of allergic reactions, in particular immediate type I hypersensitivity, than others. Perusal of Table 13.12 shows this to be true. Of the 14 different enzyme preparations summarized, 11 include one or more of “allergy,” “allergic reactions,” “hypersensitivity,” and “anaphylaxis” in the list of warnings and precautions and adverse effects. Pegloticase and rasburicase each carry a black box warning for anaphylaxis. Only ocriplasmin appears to be free of evidence for, or suspicion of, allergic adverse effects. Urticaria has been observed with dornase alfa and although type I hypersensitivity, anaphylaxis, or allergic reactions do not appear to be associated with pegademase bovine, urticaria and injection site reactions occur, and possible type II hypersensitivities in the form of hemolytic anemia, autoimmune hemolytic anemia, and perhaps thrombocytopenia are known with this enzyme (Table 13.12).

The tissue plasminogen activators alteplase, reteplase, and tenecteplase (Table 13.12) show similar side effects extending to the possibility of allergic reactions including anaphylaxis; the incidence of such reactions to tenecteplase is <1%. Allergic reactions are well-known adverse events resulting from alteplase therapy. For example, at least 41 cases of orolingual angioedema after alteplase administration have been recorded. The condition, although rare, is characterized by swelling of the upper lip and tongue. It develops within minutes of injection causing airway obstruction and breathing problems, reaction characteristics that show a clear resemblance to a type I immediate hypersensitivity anaphylactic-like response mediated by IgE antibodies.
| Generic and trade names | Enzyme specificity and properties | Approved indications | Adverse effects, serious and common |
|------------------------|----------------------------------|----------------------|-----------------------------------|
| **Alteplase** *(Activase®, Actilyse®, Cathflo® Activase®)* | Recombinant tissue plasminogen activator (tPA), a serine protease; single glycosylated polypeptide chain 527 amino acids MW ~59 kDa | Myocardial infarction with ST elevation, acute ischemic stroke, pulmonary embolism | Bleeding (especially GI), sepsis, venous thrombosis, allergy including anaphylaxis |
| **Asfotase alfa** *(Strensiq®)* | Recombinant non-specific alkaline phosphatase; two identical chains of 726 amino acids MW ~161 kDa linked by two disulfide bonds | Treatment of perinatal/infantile- and juvenile-onset hypophosphatasia | **Warnings**: Hypersensitivity reactions, lipodystrophy, ectopic calcifications. Also: ISR, ectopic calcifications (eye and kidney), hypersensitivity |
| **L-Asparaginase** *(Elspar®, Erwinase®)* Pegaspargase *(Onaspar®)* | From *E. coli* (Elspar®) or *Erwinia chrysanthemi* (Erwinaze®). L-asparagine specific; four subunits MW 35kDa *E. coli* enzyme linked to monomethyl polyethylene glycol (PEG) | Acute lymphocytic leukemia. Erwinaze® administered to patients hypersensitive to *E. coli* L-asparaginase | Allergic reactions including anaphylaxis, thrombotic events, pancreatitis, glucose intolerance, hepatotoxicity, PRES, coagulopathy |
| **Collagenase** *(Xiaflex®, Xiapex®, Santyl®)* | Proteases that hydrolyze collagen; two enzymes, collagenases AUX-I (MW 114 kDa) and AUX-II (MW 113 kDa), each ~1000 amino acids | Dupuytren’s contracture with palpable cord (Xiaflex® and Santyl® ointment for wound cleaning and healing) | Peripheral edema, allergic reactions, ISR, contusion, pain in treated tissue, tendon rupture, ligament damage; pruritus |
| **Dornase alfa** *(Pulmozyme®)* | Recombinant human deoxyribonuclease I (rhDNase I); 260 amino acids, identical to natural enzyme | Cystic fibrosis | Voice alteration; sore throat; rash; pharyngitis, laryngitis; dyspnea; dyspepsia; chest pain; urticaria |
| **Glucarpidase** *(Voraxaze®)* | Recombinant pseudomonas sp. carboxypeptidase G2 produced in *E. coli* | Treatment of toxic concentrations of methotrexate due to impaired renal clearance | Allergic reactions including anaphylaxis; flushing; paresthesia; headache; nausea; vomiting; hypotension |
| **Hyaluronidase** *(Hylenex® recombinant; Amphadase®, Hydase™; Vitrase®)* | Recombinant human hyaluronidase (rhuPH20); glycoprotein 447 amino acids, MW ~61 kDa produced in CHO cells; bovine and ovine ‘purified preparations | Dispersion and absorption of injected drugs, sc fluid administration for hydration, sc urography for resorption of radiopaque agents | **Warnings and precautions**: Spread of localized infection, not to be applied to cornea, inactivated iv. Also: edema in association with hypodermoclysis, ISR, allergic reactions |
| **Ocriplasmin** *(Jetrea®)* | A recombinant truncated human plasmin, two polypeptide chains of 19 and 230 residues linked by two disulfide bonds, MW 27.24 kDa | Treatment of symptomatic vitreomacular adhesion | Vitreous floaters, macular hole, eye pain, conjunctival hemorrhage, photopsia, vision blurring and impairment |
| **Pegademase bovine** *(Adagen®)* | Extensively pegylated bovine Adenosine deaminase (ADA) [(monomethoxypolyethyleneglycol succinimidyl) 11–17-adenosine deaminase] | Enzyme replacement for ADA deficiency in patients with SCID | Hemolytic anemia, autoimmune hemolytic anemia, trombocytopenia, ISR, urticaria |
Hypersensitivity reactions to \( L\)-asparaginase (Table 13.12), occasionally fatal, are well-known especially in children where allergic sensitivity can sometimes be avoided by switching from the \( E.\ coli \) to the \( Dickeya \) (\( Erwinia \)) enzyme. The FDA has recorded that among 62 patients with relapsed acute lymphoblastic leukemia and prior hypersensitivity reactions to asparaginase, 35 patients (56%) had a history of clinical allergic reactions to \( E.\ coli \) \( L\)-asparaginase, and 27 patients (44%) had a history of clinical allergic reactions to both \( E.\ coli \) and \( Erwinia \) \( L\)-asparaginase. Twenty (32%) of these 62 patients experienced clinical allergic reactions to Oncaspar®.

Injection site reactions and allergic reactions including urticaria, angioedema, and rarely anaphylaxis are among the main adverse events listed for hyaluronidase (Table 13.12). Non-purified hyaluronidase is much more likely to elicit immediate IgE-mediated allergic reactions, especially after repeat administrations. Some animal-derived preparations of the enzyme, in

![Table 13.12 (continued)](image_url)
particular preparations of bovine and ovine origin, were reported to provoke allergic responses in up to 10% of patients. Hypersensitivity reactions, many in patients who had received animal-derived hyaluronidase before, have occurred after retrobulbar or peribulbar blocks. Symptoms include periorbital edema with erythema, itch, pain, and angioedema.

Two enzymes indicated for management of plasma uric acid levels, pegloticase, given for gout, and rasburicase, administered during anticancer therapy, are both subject to an FDA black box warning for anaphylaxis (Table 13.12). There is also a warning for infusion reactions with pegloticase. In an assessment of the safety of rasburicase and a comparison with allopurinol in adults at risk of tumor lysis syndrome, adverse events, mainly allergic in nature, were more common in the rasburicase groups. Most reactions were grade 1 or 2; no anaphylaxis or grade 4 hypersensitivity was reported.

Type I IgE antibody-mediated allergic hypersensitivities after streptokinase (Table 13.12) administration occur often enough to lead to a consensus that streptokinase should be used only once in a patient and that the patient should be provided with some sort of record in case of a future exposure to the enzyme. It is thought that the bacterial origin of the enzyme contributes to its apparent potent antigenicity/allergenicity producing immediate reactions ranging from rash and itching to full-blown angioedema and anaphylaxis. Infusion reactions with hypotension may occur if streptokinase is given too quickly. Streptokinase is a good example of a biologic drug that can provoke almost the full spectrum of hypersensitivity type reactions. The type III hypersensitivity responses, serum sickness, and leukocytoclastic vasculitis may occur with serum sickness leading to acute renal failure after both prolonged and short infusions of the enzyme. Acute tubular necrosis with structurally normal glomeruli and mesangial deposits of IgA, IgM, and complement C3 were seen in one patient. Vasculitis resembling Henoch-Schönlein purpura associated with streptokinase therapy has been described, and acute renal failure with pronounced granular staining of glomeruli and prominent deposits of IgA developed in one patient 10 days after only a single dose of streptokinase. An unusual case of crescentic glomerulonephritis, judged to be a hypersensitivity reaction and apparently associated with a streptococcal infection and streptokinase therapy, has been reported. The reaction developed 2 weeks after a β-hemolytic streptococcal throat infection and 33 days after the administration of streptokinase. The authors speculated that the prior exposure to the streptococcus-derived enzyme had sensitized the patient for the subsequent hypersensitive response. A possible type II or mixed type II and type III hypersensitivity in the form of thrombocytopenia together with acute renal failure after streptokinase administration was found to be improved by corticosteroid therapy.

13.5 Hypersensitivities to Hormones

Like human enzymes, administered human protein hormones may provoke rare hypersensitivities in the occasional patient, but of the many peptide and glycoprotein hormones used therapeutically, allergic reactions to insulin receive by far the greatest interest because of the medical importance of the hormone and the large numbers of treated patients and therefore potential reactors.

13.5.1 Insulins

Because of insulin’s essential role in the regulation of the metabolism of carbohydrates and fats, the consequences of its insufficient supply from the pancreas in some individuals, and the resultant need for ongoing supplementation, large numbers of patients depend on receiving regular injected doses of the hormone. Inevitably, rare individuals will be intolerant to the administered protein with some responses manifesting as hypersensitivity reactions. Adverse reactions to insulin resembling hypersensitivity responses were not uncommon in the past when the administered preparations were from porcine and bovine
A range of symptoms were seen, from mild cutaneous reactions such as erythema, itching, swelling at the injection site, and pruritus of soles and palms to generalized flushing, urticaria, and angioedema. Severe systemic cases with dyspnea, hypotension, and anaphylaxis, including rare deaths, also occurred. The introduction of recombinant human insulin decreased the incidence of such reactions, but allergic reactions are still occasionally seen, and insulin allergy is now reported to be less than 1% of diabetic patients treated with insulin. Most reactions usually occur after a sensitization period, but some manifest on first exposure. Early findings of IgE antibodies in one patient to porcine, bovine, and even recombinant human insulin without associated clinical relevance were puzzling, but high levels of anti-human insulin IgE reactive with all three insulins were found in a patient with gestational diabetes who showed similar reactions to porcine and bovine insulins. Further investigations demonstrated positive skin tests and cross-reactive IgE antibodies to human, porcine, and bovine insulins in a number of other patients, supporting the conclusion of insulin allergy and the presence of similar or identical allergenic determinants on all three insulin preparations.

In addition to immediate, type I allergic reactions, biphasic reactions and type III and IV hypersensitivities to insulin may occur. The appearance of an indurated lesion lasting up to 24 h, 4–6 h after the initial wheal and flare response, histopathological features typical of a late-phase reaction, and demonstration that the reaction is transferable by serum are all features of a true late-phase insulin reaction. Recorded type III hypersensitivities to bovine/porcine insulin, usually developing 4–6 h after injection, peaking at 12 h, and transferable by Prausnitz-Küstner (P-K) testing, showed histological features of a true Arthus-type reaction. Leukocytoclastic vasculitis, seen following injection of both semisynthetic and recombinant human insulins, is another example of a type III hypersensitivity response to insulin. Reactions have occurred to both semisynthetic and recombinant human insulins. In one case, symptoms of intense itching and redness but without a wheal and flare immediately after subcutaneous injection were independent of the injection site. Skin biopsies from 5-hour- and 5-day-old lesions showed perivascular and interstitial infiltration with neutrophilic and eosinophilic granulocytes, granulocytic infiltration, fibrin deposition, and erythrocytes in the vascular walls, indicating leukocytoclastic vasculitis. Multiple cases of allergy to the long-acting recombinant insulin analog, insulin detemir, have been documented including type I and immune complex (type III) hypersensitivities. The latter reactions appeared 4–6 h after insulin administration. Type IV delayed hypersensitivity to insulin detemir manifesting as subcutaneous, inflammatory, non-pruritic nodules appearing 2–12 h after injection and lasting 2–3 days have also been described. In the same study, a patient with type I hypersensitivity to the recombinant insulin also reacted to 11 other insulin preparations; the patients with type IV allergy reacted only to insulin detemir. There are a number of documented cases of patients reacting to more than one insulin preparation including, collectively, regular insulin, insulin NPH (contains protamine), and the recombinant human preparations insulin detemir, insulin glargine, insulin lispro, and insulin aspart. Severe injection site reactions, occurring up to 8 h after injection of insulin detemir, have also been observed.

Insulin-specific IgE and IgG antibodies without any apparent clinical relevance were found in up to 28% of type 1 diabetic patients treated exclusively with recombinant human insulin, an indication of sensitization only. Insulin skin test kits are provided by Novo Nordisk, Bagsvaerd, Denmark, and Sanofi-Aventis Pharma Deutschland, Bad Soden am Taunus, Germany, and specific antibodies against human insulin can be detected with the CentAK® anti-IA2 radiiligand assay. In a case where skin testing proved decisive in reaching a conclusive diagnosis, intradermal testing was undertaken with different human insulin preparations and protamine solution. The patient developed large (>15 cm diameter), persisting (days) pruritic plaques at the injection sites after receiving different insulin preparations. Intradermal tests showed positive reactions to different standard recombinant insulin.
insulin and insulin isophane preparations and to protamine solution (Fig. 13.10 a, b), and although IgG antibodies to insulin were detected, IgE antibody tests were negative for both insulin and protamine. Skin biopsy revealed an Arthus-type type III reaction, and the patient was successfully desensitized by subcutaneously administering insulin in a rush protocol (see Sect. 13.5.1.1).

13.5.1.1 Desensitization to Insulin

Although desensitization in cases of suspected hypersensitivity is usually only successful in IgE-mediated type I reactions, in the above case, successful rush desensitization to insulin was achieved after a series of subcutaneous injections of increasing doses of the hormone. On day 1, injections of 0.004, 0.01, 0.02, 0.04, 0.1, 0.2, 0.5, and 1 IU of human insulin were given at 30 min intervals. A dose of 180 mg of the anti-histamine fexofenadine was given twice daily. On day 2, doses of 1, 2, 3, and 5 IU were given every 30 min, and on day 3, two doses of Lantus® 6 IU were administered. All desensitizing doses were tolerated, and the patient subsequently tolerated normal insulin therapy. Fexofenadine was reduced to 180 mg daily and withdrawn 6 months after desensitization.

There are a number of reports of effective and well-tolerated subcutaneous injection or infusion desensitization treatments. Using a subcutaneous desensitization protocol, early attention should be directed to the starting dose which is generally in the region of $10^6$–$10^3$ IU but may be more dilute depending on the severity of the initial

![Fig. 13.10](image-url)
reaction. Table 13.13 sets out a simple 7-step procedure of Bavbek. In another procedure involving allergy to the human insulin, insulin lispro, the following schedule was adhered to: 0.7 IU/h for 2–8 h; 0.3 IU/h for 8–13 h; 0.6 IU/h for 13–18 h; 0.8 IU/h for 18–21 h; and 0.6 IU/h for 21–22 h, plus an additional bolus of 6 IU before breakfast, 5 IU before lunch, and 6 IU before dinner. Although the patient remained clinically asymptomatic, the skin prick test to insulin remained positive 3 months later. In a case of a patient with severe insulin-induced allergic symptoms, ascending single doses of insulin starting with a dose of 0.00001 units followed by progressive tenfold increases up to 1 unit and then 2, 4, 8, 12, 16, and 20 units were administered. If local reactions occurred, the causative dose was repeated until tolerated; for systemic reactions, the dose was halved. Blood sugar was monitored throughout, and the procedures were carried out in an in-patient setting with emergency measures at hand if needed. In a diabetic patient who reacted to human isophane insulin with persisting 8–12 cm flares at the injection sites together with generalized pruritic erythema, skin testing demonstrated positive reactions to regular, NPH, and lispro insulins but negative responses to insulin aspart and glargine. Rapid appearance of erythema, wheal, and pruritus at the injection site after the administration of insulin aspart together with dextrochlorpheniramine did not indicate likely success, but the administration of 1 unit of glargine 4 days later produced only a mild, non-pruritic reaction which decreased despite gradual increases in the daily dose. Reintroduction of insulin aspart proved possible on day 5, and the patient was able to continue to receive the therapy without concurrent administration of an antihistamine.

An intravenous desensitization protocol was used successfully when continuous subcutaneous injections of insulin and oral anti-allergic agents did not prevent life-threatening symptoms. Following the absence of a reaction to an initial injection of 0.05 units of regular insulin, the dose of insulin was gradually increased until the required dose was reached using a central venous catheter, a subcutaneously embedded reservoir, and a portable infusion pump. Allergic symptoms disappeared as soon as intravenous injections began, and, within a year, levels of anti-insulin IgE and IgG returned to normal without upsetting glucose control. When reversion to subcutaneous insulin injections resulted in exacerbation of allergic symptoms, continuous intravenous injections of insulin were resumed supplemented with intravenous injections before meals. Antibody levels again declined reaching normal values 10 months after the start of intravenous treatment.

### 13.5.1.2 “Allergic” Recognition of Different Insulins

In attempting to understand why many different insulins, and in particular the human preparations, are subject to immune allergic recognition by some patients, two preliminary points need to be considered. In the first place, at least some of the reports of “allergy” to different insulins may not, in fact, have been true immune-based reactions. Secondly, the number and nature of different excipients present in the different insulin preparations, and the possibility of their involvement in the observed adverse reactions, need to be taken into account. Due to

| Steps | Dose (IU) |
|-------|-----------|
| 1     | 0.001a    |
| 2b    | 0.01      |
| 3b    | 0.1       |
| 4b    | 1         |
| 5     | 2         |
| 6     | 4         |
| 7     | 10        |

From Babvek S, Lee MJ. Subcutaneous injectable drugs hypersensitivity and desensitization. Insulin and monoclonal antibodies. *Immunol Allergy Clin N Am.* 2017;37:761–71. Reproduced with permission from Elsevier

Starting at $10^{-6}$ or $10^{-3}$ IU

aStarting dose may be more dilute depending on the severity of initial reaction. Increase tenfold every 15–30 min until reaching 1 IU. After 1 IU is reached, increase about twofold (or double) every 15–30 min until reaching the goal dose

bMonitor glucose level at each step or at least 30–60 min intervals
the heterogeneity of the cases in terms of the different clinical practices, insulin preparations and patients involved, the absence of verified and standardized investigative procedures, and the variable amount of information supplied for individual cases, the possibility of the uncertainty of allergy is clear to see. Commonly included excipients in insulin preparations include meta-
cresol and phenol as preservatives, protamine as a retardant, zinc as a stabilizer, acid-base buffers, and isotonic agents including glycerol. Except for protamine (see Sect. 13.5.1 and Fig. 13.10b), each of these excipients seems unlikely to act as a sensitizing, allergenic, inflammatory, or irritating agent, but metacresol as a common component in different insulin preparations has, in fact, been identified as the culprit in causing painful, localized erythema and urticaria leading to eventual skin breakdown in a diabetic patient (Figs. 13.11a, b). However, assuming this is a likely rare case, the chemical compositions and structures of the different insulins seem the most likely source of the allergic reactions seen with the recombinant human insulins. In the structure of long-acting insulin detemir, threonine has been deleted at position 30, the C-terminal of the B chain, and myristic acid has been linked to lysine at B29. Initially bound to serum albumin by the fatty acid, the recombinant insulin molecule slowly dissociates from the complex. In the other long-acting preparation insulin glargine, asparagine has been replaced by glycine at the C-terminal position 21 on the A chain and two arginines added at the C-terminus of the B chain (Fig. 13.12). In the rapid-acting recombinants insulin aspart and insulin lispro, aspartic acid is substituted for proline at B28 in insulin aspart and proline is replaced by lysine at B28 and lysine replaced by proline at B29 in insulin lis-
pro. Despite the careful selections of these substitutions, it is highly likely that the amino acid replacements, and perhaps even myristic acid in insulin detemir, cause some small perturbations of the molecules giving rise to small localized shape distortions recognized immunologically as an antigenic/allergenic dissimilarity by the occasional rare patient. It has been suggested by some that rapid-acting insulins may be less antigenic/
including insulin. Note also that there are reports of patients allergic to one rapid-acting insulin but not another, again suggesting that individual immune recognition differences are the basis of the observed varied reactions.

13.5.2 Glucagon

Glucagon is commonly added to barium sulfate suspensions to reduce intestinal mobility in double-contrast radiologic procedures, but occasional cases of hypersensitivity to the drug, including anaphylaxis, have been reported. In a typical case, a palpable purpuric rash appeared on the legs and erythematous papules and plaques formed on the arms and trunk of a patient given a barium enema together with intravenous glucagon. Skin biopsy revealed perivascular infiltrates of lymphocytes and eosinophils consistent with a drug eruption. In a case of anaphylaxis following a barium enema with glucagon, early itching and a tingling rapidly progressed to vomiting, the patient becoming diaphoretic, and cardiopulmonary arrest and death soon followed. Hypersensitivity reactions to glucagon cover a wide spectrum of symptoms including skin rashes, urticaria, periorbital edema, erythema multiforme, breathing difficulties, and anaphylaxis. Of 11 hypersensitivity reports to a manufacturer of glucagon, 4 involved respiratory distress and/or hypotension following glucagon administration, 5 had previous exposure, and 2 had experienced a previous reaction to the hormone. Seven of the patients had received glucagon for treatment of hypoglycemia, and four experienced the reaction during a radiologic procedure.

13.6 Desensitization to Biologics-Induced Hypersensitivities

Apart from insulin, more information is becoming available on appropriate diagnostic and desensitization procedures to manage hypersensitivities to a steadily increasing number of mAbs, fusion proteins, cytokines, enzymes, coagulation proteins, vaccines, botulinum toxins. (Reproduced with permission from Baldo BA. Safety of biologics therapy. Monoclonal antibodies, cytokines, fusion proteins, hormones, enzymes, coagulation proteins, vaccines, botulinum toxins. Cham, Switzerland: Springer Nature; 2016)
protocols offers a good basis for future desensitization therapies for newly introduced agents.

Among the expanding list of biologic agents already employed in desensitization protocols, some representative examples are presented here. TNF is one of the most important mediators of inflammation, and some approved agents targeting this cytokine are widely used, well tolerated, and generally safe, but they may induce a variety of adverse reactions, local and systemic. Two such agents used to treat chronic inflammatory diseases are the subcutaneous TNF inhibitors etanercept and adalimumab, both known to provoke local infusion and injection reactions, usually termed injection site reactions which are characterized by erythema, itching, and swelling. Desensitization protocols for the treatment of injection site reactions and systemic reactions have not yet been standardized, but standardized protocols for subcutaneous administration in patients sensitized to etanercept and adalimumab have been generated by Bavbek’s group in Ankara.

13.6.1 Desensitization for the Fusion Protein Etanercept

The TNF-binding fusion protein etanercept (Sect. 13.3.1) causes injection site reactions in about one third of patients as well as hypersensitivities types I and IV, probably some type III and/or combined type III and IV reactions, and possibly some type II cytopenias. Etanercept can provoke hypersensitivity responses such as pruritus, urticaria, angioedema and anaphylaxis, and an array of delayed reactions including thromboembolic events, a serum sickness-like reaction, maculopapular rashes, vasculitis, erythema multiforme, SJS, and TEN. IgE antibody-mediated reactions are known, and desensitizations of the drug’s hypersensitivity-inducing effects have been achieved. Skin testing can be carried out with commercially available etanercept; concentrations used are 50 mg/ml for prick tests and for intradermal testing, 0.025, 0.05, and 0.5 mg/ml. A subcutaneous desensitization protocol for etanercept is shown in Table 13.14.

| Time (min) | Dose (mg) | Dilution (concentration) | Volume administered (ml) |
|-----------|----------|--------------------------|--------------------------|
| Days 1 and 3 | | | |
| 0 | 0.5 | 1:100, (0.5 mg/ml) | 1.0 |
| 30 | 1.0 | 1:10, (5 mg/ml) | 0.2 |
| 60 | 2.0 | 1:10, (5 mg/ml) | 0.4 |
| 90 | 4.0 | 1:10, (5 mg/ml) | 0.8 |
| 120 | 8.0 | 1:1, (50 mg/ml) | 0.16 |
| 150 | 9.0 | 1:1, (50 mg/ml) | 0.18 |
| Total dose | 24.5 | | |

Day 8

| Time (min) | Dose (mg) | Dilution (concentration) | Volume administered (ml) |
|-----------|----------|--------------------------|--------------------------|
| 0 | 0.5 | 1:100, (0.5 mg/ml) | 1.0 |
| 30 | 1.0 | 1:10, (5 mg/ml) | 0.2 |
| 60 | 2.0 | 1:10, (5 mg/ml) | 0.4 |
| 90 | 4.0 | 1:10, (5 mg/ml) | 0.8 |
| 120 | 8.0 | 1:1, (50 mg/ml) | 0.16 |
| 150 | 16.0 | 1:1, (50 mg/ml) | 0.32 |
| 180 | 18.5 | 1:1, (50 mg/ml) | 0.37 |
| Total dose | 50.0 | | |

Adapted from Bavbek S, Lee MJ. Subcutaneous injectable drugs hypersensitivity and desensitization. Insulin and monoclonal antibodies. *Immunol Allergy Clin N Am*. 2017;37:761–71. Reproduced with permission from Elsevier

13.6.2 Desensitization to Hypersensitivities to Monoclonal Antibodies

Adalimumab is a fully human IgG1 mAb targeted to TNF and indicated for the prevention of articular inflammation characteristic of several diseases including rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, plaque psoriasis, and Crohn’s disease (Tables 13.2 and 13.9). As with etanercept, a successful subcutaneous desensitization protocol for adalimumab (Table 13.15) enabled patients to be maintained on weekly adalimumab for 3 months with pre-
medication and then weekly adalimumab injections without further symptoms.

In addition to adalimumab, desensitizations have been successfully performed for hypersensitivities induced by a growing list of mAbs including cetuximab, rituximab, trastuzumab, alemtuzumab, infliximab, bevacizumab, brenntuximab, tocilizumab, and ofatumumab. Skin testing with mAbs is useful in helping in the assessment of patients and decision-making before proceeding with desensitization. Skin prick and intradermal test concentrations for some monoclonal antibodies are shown in Table 13.16. Premedication with antihistamines, corticosteroids, and leukotriene inhibitors is often undertaken to lessen the chance of reactions during the desensitization procedure. If a reaction occurs during the procedure, the infusion must be stopped. Reactions to biologic agents are not uncommon and occur in about one third of procedures. Most reactions are mild and occur on the skin, and 70% of reactions are seen during the final dosing step. A standard desensitization protocol for mAbs as used by the Castells group involves 12 steps, administering doubling doses every 15 min. The starting dose is usually a 1–1000 to a 1–10,000 dilution of the final dose. The patient is given the highest dose in the final step. Note that acquired tolerance needs to be re-achieved by repeating the procedure before every future infusion. Table 13.17 summarizes the details and steps in an intravenous protocol for a patient desensitized to ofatumumab.

### Table 13.15 Subcutaneous desensitization protocol with adalimumab (for 50 mg/ml, weekly injection)

| Time (min) | Dilution concentration (50 mg/ml) | Volume (ml) | Dose (mg) | Cumulative dose (mg) |
|------------|-----------------------------------|-------------|-----------|----------------------|
| 0          | 1:100 (0.5 mg/ml)                 | 1.00        | 0.50      | 0.50                 |
| 30         | 1:10 (5 mg/ml)                    | 0.15        | 0.75      | 1.25                 |
| 60         | 1:10 (5 mg/ml)                    | 0.25        | 1.25      | 2.50                 |
| 90         | 1:1 (5 mg/ml)                     | 0.50        | 2.50      | 5.00                 |
| 120        | 1:1 (50 mg/ml)                    | 0.10        | 5.00      | 10.00                |
| 150        | 1:1 (50 mg/ml)                    | 0.20        | 10.00     | 20.00                |
| 180        | 1:1 (50 mg/ml)                    | 0.40        | 20.00     | 40.00                |

From Bavbek S, Lee MJ. Subcutaneous injectable drugs hypersensitivity and desensitization. Insulin and monoclonal antibodies. *Immunol Allergy Clin N Am*. 2017;37:761–71. Reproduced with permission from Elsevier

### Table 13.16 Proposed skin testing concentrations for some monoclonal antibodies

| Monoclonal antibody | Prick testing (ml/ml) | Intradermal testing (mg/ml) |
|---------------------|-----------------------|-----------------------------|
| Rituximab           | 10                    | 0.1, 1, 10                  |
| Infliximab          | 10                    | 0.1, 1, 10                  |
| Tocilizumab         | 20                    | 0.2, 2, 20                  |
| Cetuximab           | 20                    | 0.2, 2, 20                  |
| Trastuzumab         | 21                    | 0.21, 2.1, 21               |
| Bevacizumab         | 25                    | 0.25, 2.5, 25               |
| Adalimumab          | 40                    | 0.4                         |
| Omalizumab          | 125                   | 0.00125                     |
| Pertuzumab          | 1.6                   | 0.16                        |
| Brentuximab vedotin | 0.0018                | 0.0018, 0.018, 0.18         |

Adapted from Santos RB, Galvão VR. Monoclonal antibodies hypersensitivity. Prevalence and management. *Immunol Allergy Clin N Am*. 2017;37:695–711. Reproduced with permission from Elsevier

### 13.7 Biosimilars

A biosimilar may be defined as a follow-on biologic that meets extremely high standards for comparability or similarity to the originator biologic drug that is approved for use in the same indication. They are not the same as generics. Approval for marketing is given only if any differences or variabilities do not affect efficacy and safety. The biosimilar and its reference product (an approved existing biological medicine) should have an identical amino acid sequence, but different isoforms may result due to glycosylation posttranslational modifications. In addition to mAbs, some other biologics, including cytokines, fusion proteins, hormones, and enzymes, have been developed and approved as biosimilars or are currently in the development stage. In 2006, the EMA approved the recombinant somatropin preparation, Omnitrope®, making it the first
approved biosimilar. In March 2015, the FDA approved its first biosimilar, Zarxio® (filgrastim, reference Neupogen®), approved in Europe in 2009 as Zarzio®. By 2016, 45 biosimilars had been approved including those for infliximab, trastuzumab (Herceptin®), adalimumab (Humira®), rituximab (Rituxan®), and the fusion protein etanercept (Enbrel®). At December 2019, 26 biosimilars had been approved in the United States including Avsola® (infliximab-axxq), the fourth biosimilar to Remicade® (infliximab). Other than mAbs, biologics approved as biosimilars in the United States and/or European Union include filgrastim, somatropin, erythropoietins, follitropin, and insulin glargine.

### 13.8 Summary

- “Biologics” here refers to therapies that are prepared from materials made or expressed in living organisms. They may simply be isolated proteins such as enzymes, hormones and blood products, or, as is increasingly the case, preparations produced by recombinant DNA technology.
- Advantages of recombinant protein therapeutics technology over small MW drug development are rapid development to treat diseases formerly regarded as beyond immediate or even longer-term reach of conventional therapies, specificity of action and potent therapeutic efficiency, often more predictable actions, lower immunogenicity due to their human origin, faster regulatory approval time, and their uniqueness allowing better patent protection.
- Monoclonal antibodies for non-cancer use are now available to treat a wide range of diseases, including asthma, migraine, psoriasis, systemic lupus erythematosus, macular degeneration, infections of anthrax, Clostridium difficile, respiratory syncytial virus, and HIV-1 and treatment of autoimmune

| Table 13.17 Intravenous desensitization to ofatumumab in a patient who presented a grade 2 reaction to the drug (throat tightness, cough, and angioedema) |
|---|---|---|---|---|---|
| Bag | Volume per bag (ml) | Concentration (mg/ml) | Total dose per bag (mg) | Amount of bag infused (ml) |
| 1 | 250 | 0.04 | 10 | 9.38 |
| 2 | 250 | 0.4 | 100 | 18.75 |
| 3 | 500 | 1.98425 | 992.125 | 500 |

Adapted from Galvão VR, Castells MC. Hypersensitivity to biological agents – Updated diagnosis, management, and treatment. *J Allergy Clin Immunol Pract*. 2015;3:175–85. Reproduced with permission from Elsevier

Bag 3/step 12: if the patient tolerates the final step at 80 ml/h for 15 min, the rate may be increased in 15 min intervals to 120 ml/h, to 160 ml/h, to 200 ml/h, and then to 240 ml/h until infusion is completed

Total infusion time: 8:78 h
Standard volume per bag: 250 ml
Final rate of infusion: 80 ml/h
Number of bags: 3; time per step, 15 min; total number of steps, 12; total dose, 1000 mg
diseases such as Crohn’s disease and rheumatoid arthritis.

- Selection of the IgG antibody subclass is important in obtaining the desired effector functions. Binding affinities for FcγRIIIa, the receptor involved in antibody-dependent cell-mediated cytotoxicity (ADCC) and important for cell killing, are greater for IgG1. IgG2 and IgG4 are the choices when cell death is to be avoided.

- Twenty-eight (54%) humanized (-zumab) and 20 (38.5%) fully human (-umab) mAbs comprise the largest groups of the 52% currently approved mAbs for non-cancer therapy. A collective of 37 different targets are recognized by the 52 mAbs used for non-cancer therapies with some targets complementary to more than one mAb.

- Almost equal numbers are used to treat inflammatory and/or immune disorders on the one hand and a diverse range of diseases/applications on the other. Some diseases/applications are the treatment target for more than one mAb; for example, ten different mAbs are indicated for psoriasis/psoriatic arthritis and five different mAbs for each of Crohn’s disease, rheumatoid arthritis, and infectious agents.

- Immune responses to mAbs, including those that are fully human, are well-known to occur. Hypersensitivity responses may manifest as infusion reactions and anaphylactic reactions. Type I or IgE antibody-mediated reactions have been documented for at least ten of the approved mAbs.

- Cytopenias have been observed with the use of at least 25% of the approved non-cancer mAbs. Some reactions have an autoimmune basis for the observed neutropenia, thrombocytopenia, hemolytic anemia, and pancytopenia suggesting type II hypersensitivities and/or joint type II and III hypersensitivities.

- Type III serum sickness-like hypersensitivities have occurred with omalizumab and infliximab; vasculitis and pneumonitis have also been reported with the latter. Cutaneous vasculitis may occur with adalimumab, certolizumab pegol, and alirocumab therapies. High titers of antibodies to adalimumab have been associated with venous and thromboembolisms and a higher risk of developing thromboembolism.

- Serious type IV cutaneous hypersensitivity reactions in the form of SJS and erythema multiforme have occurred with adalimumab and infliximab.

- Cytokines, currently more than 130 in number, are relatively small signaling proteins, usually glycosylated, of MW <30 kDa. There are currently 21 approved cytokine preparations all in recombinant human (rh) form.

- Side effects, some serious, occur despite cytokines being endogenous proteins. This is reflected in the relatively small number of cytokines currently approved by regulatory agencies and by the fact that 15 of the FDA-approved preparations carry warnings, with 10 being black box warnings.

- There are three approved interferon alfa preparations: peginterferon alfa-2a, interferon alfa-2b, and peginterferon alfa-2b. Side effects of interferons alfa are many and varied. Adverse reactions include fatal interstitial lung disease, autoantibodies and development or exacerbation of autoimmune diseases, thyroid dysfunction, neutropenia, thrombocytopenia, pernicious anemia, bone marrow hypoplasia, pure red cell aplasia, and depression.

- Cutaneous reactions to interferon alfa are extensive and include injection site reactions (erythema, necrosis, and vasculitis), psoriasis, pruritus, xerosis, urticaria, hyperpigmentation, alopecia, lichen planus, pityriasis rosea, sarcoid nodules, eosinophilic fasciitis, livedo reticularis, fixed drug eruption, and vitiligo.

- A flu-like illness and injection site reactions are the most commonly occurring adverse events following administration of interferons beta-1a and beta-1b. Other adverse effects are a lupus-like syndrome, cutaneous lymphocytic vasculitis, thyroid disorders including Hashimoto’s encephalopathy, urticaria, and an acneiform eruption.

- Interferon gamma induces flu-like symptoms, granulocytopenia, and a suspicion of acute respiratory insufficiency in patients with
idiopathic pulmonary fibrosis and some cardiovascular and renal toxicities.

- Granulocyte-colony-stimulating factor (G-CSF) has been associated with adult respiratory distress syndrome; other pulmonary toxicities, particularly pulmonary edema; interstitial pneumonitis, and capillary leak syndrome. Cardiovascular complications have been associated with GM-CSF (granulocyte-macrophage colony-stimulating factor).

- Anaphylactic/anaphylactoid reactions to CSFs are the subject of FDA warnings. Skin reactions to CSFs include psoriasis flare, pyogenic granulomas, pruritic erythematous maculopapular eruptions, palmoplantar pustulosis, erythema multiforme, neutrophilic dermatoses, and Sweet’s syndrome.

- Recombinant human IL-11, or oprelvekin, a growth factor used to prevent chemotherapy-induced thrombocytopenia, carries an FDA black box warning for allergic reactions including anaphylaxis.

- Becaplermin, a recombinant human platelet-derived growth factor (rhPDGF-BB), promotes the growth of granulation tissue and wound healing via interaction with receptors on fibroblasts. Because growth factors cause cell proliferation, the possibility of increased cancer rates is considered, and in 2008 the FDA issued a boxed warning for malignancies for Regranex® Gel.

- Palifermin, a recombinant human keratinocyte growth factor, stimulates differentiation, proliferation, and migration of epithelial cells in numerous tissues including the skin, hair follicles, intestine, etc., making it useful in oncological supportive care, aiding the management of mucositis in cancer patients. Adverse events following palifermin include rash, pruritus, paresthesia, and edema and, interestingly, palmoplantar erythrodysesthesia (acral erythema; hand-foot syndrome) and a papulopustular (acne-like) eruption on the head and trunk.

- Aldesleukin is the recombinant form of interleukin-2 (IL-2). Toxicities include capillary leak syndrome, pulmonary effects, and hematologic adverse effects, particularly anemia, leukopenia, thrombocytopenia, and eosinophilia. Cutaneous reactions are numerous, ranging from mild erythema, pruritus, injection site reactions, and vitiligo to urticaria, angioedema, reactivation of eczema, exacerbation of psoriasis, vasculitis, fixed drug eruptions, exfoliative dermatitis, and severe manifestations like pemphigus, IgA bullous dermatosis, and toxic epidermal necrolysis (TEN).

- Anakinra, a recombinant specific receptor antagonist (IL-1RA) for interleukin-1 (IL-1), is a useful agent in the treatment of some inflammatory conditions such as rheumatoid arthritis. Injection site reactions are the most frequent adverse event, but overall anakinra is safe and well tolerated for up to 3 years of continuous use.

- Erythropoietin (or erythropoetin) is available as recombinants, epoetin alfa, beta, delta, and omega and darbepoetin alfa (all collectively known as erythropoietin stimulating agents). Some patients develop neutralizing anti-EPO antibodies during therapy, and there are now well in excess of 250 known cases of pure red cell aplasia. Serious allergic reactions including anaphylaxis, angioedema, bronchospasm, tachycardia, pruritus, rash, and urticaria and severe cutaneous reactions including erythema multiforme, SJS, and TEN have occurred with erythropoietin stimulating agents. Injection site reactions are also well-known.

- Metreleptin is a recombinant analog of leptin used as replacement therapy to treat leptin deficiency in patients with congenital or acquired generalized lipodystrophy. Adverse events specifically due to metreleptin include injection site reactions, nausea, nasopharyngitis, and hypersensitivity.

- Ancestim is a recombinant human stem cell factor used with filgrastim to produce increases in circulating peripheral blood progenitor cells. Ancestim increases mast cell proliferation and promotes the release of histamine and tryptase sometimes producing allergic-like symptoms. It should therefore only be administered in a setting with the appropriate staff, facilities, and medications to respond to a possible life-threatening anaphylactic/anaphylactoid reaction. Patients
should be premedicated with H₁ and H₂ antihistamines and a bronchodilator.

- Injection site reactions, including erythema at a previous injection site, occurring within 1–24 h, are the most commonly observed cutaneous adverse events with up to 84% of patients given ancestim showing mild to moderate reactions. Other reported reactions are erythema, pruritus, urticaria, hyperpigmentation, and rash.
- Etanercept is a recombinant fully human dimeric Fc-fusion protein linked to the ligand-binding portion of the human TNF receptor (TNFR). Injection site reactions are the most common adverse event seen with etanercept, anaphylaxis cases are known, and the protein carries an FDA boxed warning for serious infections. Etanercept-induced cytopenias and vasculitis may be type II and type III hypersensitivities, respectively, and some pulmonary events caused by etanercept may be type III or combined type III/type IV reactions. Significant numbers of vasculitic adverse events after etanercept, particularly hypersensitivity vasculitis and necrotizing vasculitis, have been recorded, and autoimmune reactions such as hyperthyroidism, anti-synthetase syndrome, Crohn’s disease, and Henoch-Schönlein purpura have occurred.
- Belatacept, a fusion protein of the modified extracellular domain of CTLA-4, binds CD80 and CD86 on antigen-presenting cells, thus blocking CD28-mediated co-stimulation of lymphocytes. Acute infusion reactions to the drug occur with an incidence of ~3%. In belatacept therapy for kidney transplantation, EBV-seronegative transplant recipients developed post-transplant lymphoproliferative disorder (PTLD) with an incidence of 7.3% compared to 0.6% for EBV-seropositive patients. Like belatacept, abatacept contains the extracellular domain of CTLA-4. As with belatacept, acute infusion reactions occur; however, they are mostly mild to moderate. The overall frequencies of adverse events, serious adverse events, and malignancies appear similar. Cutaneous reactions are rare with only a few reports of induced paradoxical psoriasiform eruptions and psoriasis.
- Rilonacept, also known as IL-1 trap, captures IL-1β preventing activation of IL-1 receptors, thus reducing inflammation and other effects due to overproduction of the interleukin. The most common adverse events to the protein are injection site reactions and upper respiratory tract infections.
- Aflibercept (ziv-aflibercept or Zaltrap®), or VEGF trap, is a fusion of domain 2 from vascular endothelial growth factor receptor-1 (VEGFR-1) with domain 3 of VEGFR-2, attached to the hinge region of the Fc domain of human IgG1. Warnings, precautions, and known side effects of ziv-aflibercept are extensive and include potentially fatal hemorrhage, gastrointestinal perforation, and compromised wound healing.
- Romiplostim is a peptibody formed by the fusion of four identical copies of a thrombopoietin mimetic peptide to the C-termini of aglycosylated human IgG1 Fc chains. Romiplostim is indicated for the treatment of thrombocytopenia in patients with chronic immune thrombocytopenia. Romiplostim is subject to some regulatory agency warnings including for thrombotic/thromboembolic complications.
- Tagraxofusp-erzs is a CD123-directed cyto-toxic fusion protein of recombinant IL-3 and truncated diphtheria toxin (DT) indicated for the treatment of blastic plasmacytoid dendritic cell neoplasm. Capillary leak syndrome is the subject of a boxed warning for the cytotoxin, and there are other warnings and precautions for hypersensitivity and hepatotoxicity.
- Alefacept, an anti-CD2, selectively targets effector memory T cells for both CD4+ and CD8+, but not naïve T cells and central memory T cells, in psoriasis vulgaris. Lymphopenia with reductions in CD4+ and CD8+ lymphocytes, malignancies, serious infections, and hypersensitivity reactions were the subject of warnings and precautions by the FDA. The fusion protein denileukin diftitox was the first fusion toxin to be approved. The fusion construct consisted of the full-length IL-2 molecule and the catalytic domain of diphtheria toxin. FDA black box warnings for the drug covered serious and even fatal infusion
reactions, vascular leak syndrome, and loss of visual acuity. Both alefacept and denileukin diftitox were subsequently discontinued.

- Fc fusion proteins developed as therapies for hemophilia are the coagulation factor VIII protein prepared by linking B-domain deleted recombinant factor VIII (BDDrFVIII) to IgG1 Fc and the factor IX conjugate of coagulation factor IX and the Fc domain of human IgG1. Safety issues include hypersensitivity reactions and the formation of neutralizing antibodies.
- Two currently approved albumin fusion constructs are albigitide, a recombinant fusion protein used for glycemic control of type 2 diabetes, made up of two copies of a modified 30 amino acid GLP-1 sequence fused in tandem to the N-terminus of human albumin, and factor IX albumin fusion protein, generated by genetic fusion of recombinant human albumin to recombinant factor IX, producing a single recombinant protein designated rIX-FP. Warnings and precautions for the former relate to pancreatitis, hypoglycemia, renal impairment, and hypersensitivity, and, for the latter, hypersensitivities including anaphylaxis, neutralizing antibodies, thromboembolism, and nephrotic syndrome.
- Enzyme replacement therapies for some lysosomal storage disorders developed under orphan drug product development programs include recombinant enzymes to treat Gaucher disease, Fabry disease, and Pompe disease, lysosomal acid lipase deficiency, and mucopolysaccharidoses I, II, IVA, VI and VII.
- Agalsidase beta for Fabry disease is a recombinant α-D-galactosidase A. Infusion reactions, the most common adverse event seen during therapy, occur in ~10–14% of patients.
- Two commercial preparations of alglucosidase alfa, Lumizyme® and Myozyme®, are approved by the FDA for Pompe disease. Mild to moderate infusion reactions and a few cases of anaphylaxis to the enzyme have occurred.
- Imiglucerase, taliglucerase alfa, and velaglucerase alfa to treat Gaucher disease are three recombinant human β-glucocerebrosidase enzymes. Immediate type hypersensitivity reactions are the most prominent adverse event seen with these three enzymes. Other adverse reactions include administration site reactions, skin and subcutaneous tissue disorders, and respiratory, thoracic and mediastinal disorders.
- Laronidase for mucopolysaccharidosis (MPS) I, a recombinant variant of human α-L-iduronidase, is generally well tolerated with few treatment-related events and few, if any, serious adverse reactions. In common with other enzymes used in ERT, the FDA has issued a boxed warning for the potential of anaphylaxis.
- Used for MPS II, the most common adverse events for idursulfase, a recombinant human iduronate-2-sulfatase, are infusion-based reactions, pruritus, pruritic rash, swelling at the infusion site, and urticaria. Anti-idursulfase IgE antibodies have been detected in a few anaphylactic patients receiving the enzyme.
- Eloksulfase alfa (recombinant N-acetylgalactosamine-6-sulfatase) for MPS IVA induces a relatively high incidence of anaphylaxis which is reflected in an FDA box warning.
- Galsulfase for MPS VI, a recombinant N-acetylgalactosamine-4-sulfatase, shows a high incidence of infusion reactions (20–75%).
- Vestronidase alfa-vjbk for MPS VII is a recombinant human lysosomal beta glucuronidase. The enzyme is the subject of an FDA boxed warning for anaphylaxis with a statement that anaphylaxis has occurred with the first dose. Premedication with a non-sedating antihistamine with or without an antipyretic is recommended.
- Sebelipase alfa for lysosomal acid lipase deficiency is a recombinant human lysosomal acid lipase produced in chicken egg white which accounts for the warning of the risk of possible anaphylaxis to egg proteins. Apart from allergic sensitivity to egg, ~3% of patients may experience anaphylaxis.
- The tissue plasminogen activators alteplase, reteplase, and tenecteplase show similar side effects including the possibility of anaphylaxis.
Hypersensitivity reactions to L-asparaginase, occasionally fatal, are well-known especially in children where allergic sensitivity can sometimes be avoided by switching from the *E. coli* to the *Dickeya (Erwinia)* enzyme.

Injection site reactions and allergic reactions including urticaria, angioedema, and rarely anaphylaxis are among the main adverse events listed for hyaluronidase. Non-purified hyaluronidase and some animal-derived preparations (bovine and ovine origin) are more likely to elicit immediate IgE-mediated allergic reactions, especially after repeat administrations.

Two enzymes indicated for management of plasma uric acid levels, pegloticase, given for gout, and rasburicase, administered during anticancer therapy, are both subject to an FDA black box warning for anaphylaxis. Pegloticase also carries a warning for infusion reactions.

Type I IgE antibody-mediated allergic hypersensitivities after streptokinase administration have led to a consensus that streptokinase should be used only once and the patient should be provided with some sort of record in case of a future exposure to the enzyme. The type III hypersensitivity responses, serum sickness and leukocytoclastic vasculitis, may occur with serum sickness leading to acute renal failure after both prolonged and short infusions of the enzyme. A possible type II or mixed type II and type III hypersensitivity in the form of thrombocytopenia together with acute renal failure has been described after streptokinase.

In addition to immediate, type I allergic reactions, biphasic reactions and type III and IV hypersensitivities to insulin may occur. Type III hypersensitivities to bovine/porcine insulin usually develop 4–6 h after injection and peak at 12 h. Reactions such as leukocytoclastic vasculitis have occurred to both semisynthetic and recombinant human insulins. The chemical compositions and structures of the different insulins seem the most likely source of the allergic reactions seen with the recombinant human insulins.

There are effective and well-tolerated subcutaneous injection or infusion desensitization treatments for insulin hypersensitivity.

Hypersensitivity reactions to glucagon cover a wide spectrum of symptoms including skin rashes, urticaria, periorbital edema, erythema multiforme, breathing difficulties, and anaphylaxis.

Apart from insulin, desensitization procedures to manage hypersensitivities to a steadily increasing number of mAbs, fusion proteins, cytokines, enzymes, and various other approved biologics are now being used.

A biosimilar may be defined as a follow-on biologic that meets extremely high standards for comparability or similarity to the originator biologic drug that is approved for use in the same indication. In addition to mAbs, cytokines, fusion proteins, hormones, and enzymes have been developed and approved as biosimilars or are currently in the development stage.

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