

Chapter 5

Cytokines

Introduction

General Characteristics

Cytokines, currently known to be more than 130 in number, are relatively small signaling proteins of MW < 30 kDa, usually glycosylated, and produced by a variety of different cells including those of the immune system, epithelia, endothelia, and stroma. Cytokines are key modulators of the immune and inflammatory responses functioning in an autocrine, paracrine, or endocrine manner stimulating or suppressing cellular activities in infection, innate and adaptive immunity, autoimmunity, inflammation, and malignancy. Key to an understanding of these regulatory proteins is the recognition of their pleiotropism and sometimes overlapping activities, functional redundancies, and side effects. Their secretion may be induced by an array of different stimuli associated with infection, inflammation, or tumorigenesis, first releasing waves of (for example) proinflammatory molecules followed by anti-inflammatory cytokines to restore homeostasis. Cytokines therefore induce a diverse range of biological responses including proliferation, differentiation, activation, inflammation, chemotaxis, and cell death and the nature of an immune or inflammatory stimulus determines whether an immune response is humoral- or cell-mediated, cytotoxic, immunosuppressive or allergic.

Classification of Cytokines

The many attempts to classify cytokines over the last three decades and the complexities in devising classifications based on structural and/or functional parameters are not hard to understand given the sheer number of imprecisely defined “factors” identified in the early years and the difficulties and work involved in trying to accumulate details on functions and diseases. In their discussion of the evolution of cytokine biology and

nomenclature, Steinke and Borish draw attention to three phases of development in the identification and classification of cytokines. The identification of cytokines by their biologic activities (e.g., T-cell growth) occurred in the first, or factor, stage. The production of recombinant cytokines and demonstration of their pleiotropism and redundancy led to much of our current understanding and this can be called the recombinant-cloning or second phase. Currently, we are experiencing the third, or genomic phase, where cytokines are being identified on the basis of homology with known, characterized cytokines. In the more recent progressive assemblages published by Tato and Cua detailing each cytokine's receptor(s), source, targets, major function, and disease association, the first 16 interleukins were grouped in order of their discovery. Many of these interleukins form homodimeric structures and have the γ c and/or β c chains in their receptors. More recently discovered interleukins have proven more difficult to classify in relation to their function in health and disease due to the complexity of their heterodimeric ligands and receptors. For example, a homotrimeric motif for ligands and receptors and bidirectional signaling were found to be important features of the TNF family. Many original names are still in use and many of the originally described "factors" share receptors with other interleukins.

Here, we focus on the 23 FDA-approved cytokine products from the CDER-approved Biologic Products list. The cytokine classification presented is based on the Kyoto Encyclopedia of Genes and Genomes with input from Vacchelli et al. (<http://dx.doi.org/10.4161/onci.20459>). Nine main families are recognized (Table 5.1) with most of the cytokines of interest classified in the hematopoietic growth factor, interferon (IFN), platelet-derived growth factor (PDGF), and transforming growth factor β (TGF β) families. In the hematopoietin family, approved cytokines manufactured by recombinant DNA technology are aldesleukin (rh-interleukin-2 [IL-2]), oprelvekin (rhIL-11), filgrastim and tbo-filgrastim (rh-granulocyte colony-stimulating factor [G-CSF]), sargramostim (rh-granulocyte macrophage [GM]-CSF), metreleptin (rh-leptin) and rh-erythropoietins, epoetin, and darbepoetin alfa. Anakinra, a recombinant receptor antagonist for IL-1, is a representative of the IL-1 cytokine family; recombinant interferons alfa-1, alfa-2, beta-1, and gamma-1 make up the interferon family; palifermin (rh-keratinocyte growth factor [KGF]) and becaplermin (rhPDGF-BB) are in the PDGF family; and rh-bone morphogenetic protein [BMP]-2 and rhBMP-7 represent the TGF β family. Chemokines, placed here in group 9 (Table 5.1), behave as regulatory molecules for leukocytes and lymphoid tissue and have an important role in infectious, inflammatory, allergic and autoimmune responses as well as angiogenesis, hematopoiesis, and tumor growth. No members of the chemokine family are yet approved for therapy.

Adverse Effects of Individual Approved Recombinant Cytokine Analogs

A number of the characteristics and properties of cytokines provide an insight into the possibility of adverse effects when these "natural" agents are used therapeutically. These include, in particular, their pleiotropic nature; relatively short

Table 5.1 Family classification of cytokines^a relevant to this review

Family	Members
Hematopoietin ^b	IL-2; IL-6 ^c ; IL-11 ^{c,d} ; IL-12 ^e ; G-CSF ^c ; GM-CSF; leptin ^{c,f} ; EPO ^g ; TPO ^g ; SCF
IL-1	IL-1 α ; IL-1 β ; IL-18 ^h
IL-10 ⁱ	IL-10 ⁱ
IL-17 ^k	IL-17; IL-17B; IL-17C; IL-17D; IL-17E; IL-17F
Interferon ^l	IFN α -1; IFN α -2; IFN β -1; IFN γ -1
PDGF ^m	EGF; KGF; M-CSF; PDGFA-D; PGF; VEGFA-D
TGF β	BMP-2 ⁿ ; BMP-7 ^o ; TGF β 1 ^p ; TGF β 2 ^p ; TGF β 3 ^p
TNF	TNF; TNFSF4 ^q ; TNFSF5 ^r ; TNFSF6 ^s ; TNFSF10 ^t ; TNFSF11 ^u ; TNFSF12 ^v
Chemokines	CC subfamily; CXC subfamily; C subfamily; CX3C subfamily ^w

BMP bone morphogenetic protein, *EGF* epidermal growth factor, *EPO* erythropoietin, *G-CSF* granulocyte colony-stimulating factor, *GM-CSF* granulocyte macrophage colony-stimulating factor, *IFN* interferon, *IL* interleukin, *KGF* keratino-cyte growth factor, *M-CSF* macrophage colony-stimulating factor, *PDGF* platelet-derived growth factor, *PGF* placenta growth factor, *SCF* stem cell factor, *TGF β* transforming growth factor β , *TNF* tumor necrosis factor, *TNFSF* tumor necrosis factor ligand superfamily member, *TPO* thrombopoietin, *VEGF* vascular endothelial growth factor From Baldo BA. Side effects of cytokines approved for therapy. *Drug Saf* 2014;37:921–43. Adapted and reproduced with permission from Springer Science + Business Media

^aBased on the Kyoto Encyclopedia of Genes and Genomes, www.genome.jp/kegg/ and Vacchelli et al. *Onc Immunology* 2012;1:493–506. <http://dx.doi.org/10.4161/onci.20459>

^bClass I cytokines

^cMember of IL-6 receptor subfamily that also includes IL-11, G-CSF, and leptin. IL-6 involved in cytokine storm reactions

^dAlso called AGIF, adipogenesis inhibitory factor. Promotes platelet recovery after chemotherapy-induced thrombocytopenia

^ePromotes Th1 responses and stimulates production of IFN γ and TNF from T and NK cells

^fHomologous in structure to a cytokine. Included here according to Vacchelli et al. (see above text) but often described as a hormone. Produced primarily in adipose tissue; regulates fat storage

^gMember of single chain subfamily

^hProinflammatory but suppresses metastasis surveillance by NK cells

ⁱClass II cytokines. Interferons sometimes classified in this family. Family also includes IL-19, -20, -22, -24, and -26

^jAnti-inflammatory and immunosuppressive

^kProinflammatory cytokines; stimulate release of other cytokines, for example, IL-1 β , IL-6, GM-CSF, TGF β , and TNF

^lClass II cytokines. Comprise three types: type I (IFN α , IFN β , IFN ω 1, IFN κ 1, and FN τ 1), type II (IFN γ), type III (IL-28A, -28B, and -29)

^mPDGFs, PGF, and VEGFs belong to subclass I of cysteine-knot growth factors. M-CSF is included in the 4-helix bundle growth factors

ⁿBMP2 subfamily

^oBMP5 subfamily

^pMember of TGF β subfamily

^qAlso called OX40L and CD252, the ligand for CD134. Expressed on the surface of activated B, T, dendritic and endothelial cells

^rAlso called CD40L and CD154. Costimulatory molecule with T-cell receptor in activation of antigen presenting cells

^sAlso called FASL or Fas ligand. Binding with its receptor induces apoptosis

(continued)

Table 5.1 (continued)

[†]Also called CD253 or TRAIL, TNF-related apoptosis-inducing ligand

[‡]Also called RANKL, receptor activator of nuclear factor kappa- β ligand

[§]Also called TWEAK, TNF-related weak inducer of apoptosis

[¶]Small peptides divided into four subfamilies on the basis of a cysteine motif

half-lives; the presence of other cytokines; their capacity to release other cytokines producing a cytokine “cocktail”; and the existence of multiple receptors on different cells that bind the same cytokine with different affinities. Overall, and as one might expect with biological systems involving genetically diverse patients; the diverse range of biological activities of cytokines; their action in causing the release of additional cytokines; the knock-on pharmacological effects of these secondarily released agents; and different disease statuses of patients, side effects of cytokines are not unusual, are to be expected, and patient-to-patient spectra of these effects will be variable.

For the common side effects of cytokines used as therapeutic agents, as well as for the less common but important hematologic, psychiatric, endocrine, neurologic, pulmonary, and dermatologic adverse effects, space constraints and the many hundreds of relevant studies do not always allow individual consideration of the many pertinent report. Instead, general summaries and one or more selected examples or studies that are particularly germane are provided.

Individual Approved Cytokines

The main physicochemical features, FDA-approved indications, modes of action and side effects, as well as warnings, are summarized for the cytokine preparations approved by the FDA CDER (Table 5.2). They will now be considered individually.

Interferon Alfa

Interferons are a class of broad-spectrum antiviral cytokines, seven of which occur not only in humans and which have overlapping, but also in some individual, activities. They can be divided into three classes, designated types I, II, and III. Of most interest for therapy are interferons alfa, beta, and gamma. The former two, classified as type I interferons, bind to the interferon alfa receptor (IFNAR) consisting of IFNAR1 and IFNAR2 chains; interferon gamma, a type II interferon, binds the interferon gamma receptor (IFNGR) consisting of IFNGR1 and IFNGR2.

It is said that virtually all patients treated with interferon alfa experience some adverse effect(s) at some time during therapy. In fact, the literature on side effects to interferons is voluminous and probably greater than all the other approved, non-mAb biologics literatures put together. Three interferon alfa preparations are

Table 5.2 Cytokines approved for human therapy^{a,b}: properties, approved indications^a, mechanisms, and side effects

Generic and trade names	Properties	Approved indications ^a	Mechanism(s) of action relevant to indications	Warnings and side effects, serious and common
Peginterferon alfa-2a ^b (Pegasis [®])	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 ^{c,d} ; chronic hepatitis B ^c (HBeAg ⁺ ± patients)	Not fully known. † IFN α binds to its receptor, activating JAK1 and Tyk2 which phosphorylate receptors which bind STAT1 and STAT2. These combine with IRF-9 ^f leading to expression of multiple interferon stimulated genes. Type I IFNs have antiviral and proliferative effects and modulate immune responses but their relative potencies differ. IFN α binds IFN receptors less stably than IFN β	<i>Boxed warnings:</i> Neuropsychiatric, autoimmune, ischemic, and infectious disorders ^b 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		<i>Boxed warnings:</i> Neuropsychiatric, autoimmune, ischemic, and infectious disorders ^b Other effects: Flu-like symptoms of fever, fatigue, chills, headache, and myalgia; neutropenia; less common/PM period ^d
Peginterferon alfa-2b (Pegintron [®])	Recombinant protein linked to PEG	Chronic hepatitis C with or without ribavirin		<i>Boxed warnings:</i> Neuropsychiatric, autoimmune, ischemic, and infectious disorders ^b and ribavirin-associated effects; Other effects: fatigue/asthenia; fever; nausea; rigor; myalgia; and less common/PM period ^k
(Sylatron [®])	Recombinant protein linked to PEG	Adjuvant treatment of melanoma		<i>Boxed warnings:</i> Depression and other neuropsychiatric disorders. Other effects: as above plus \uparrow ALT and AST and less common/PM period ^l

(continued)

Table 5.2 (continued)

Generic and trade names	Properties	Approved indications ^a	Mechanism(s) of action relevant to indications	Warnings and side effects, serious and common
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	Not fully understood. IFNβ binds to receptor leading to complex events including ↑ expression of anti-inflammatory agents and ↓ proinflammatory cytokines; gene products and markers include 2', 5' oligoadenylate synthetase, neopterin; CD56 killer cells increase	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; and pruritus; rash
Peginterferon beta-1a (Plegridy [®])				
Interferon beta-1b (Betaseron [®] ; Extavia [®])	Recombinant 165 amino acid protein MW 18.5 kDa; gene contains ser for cys at position 17	Relapsing forms of multiple sclerosis	5 oligoadenylate synthetase, neopterin; CD56 killer cells increase	Flu-like symptoms; lymphopenia, leukopenia and neutropenia; ISR; myalgia; depression; hypertension; abdominal pain; asthenia; rash; ↑ liver enzymes; immunogenicity; and anaphylaxis
Interferon gamma-1b (Actimmune [®])	Recombinant 140 amino acid polypeptide; noncovalent dimer of two identical 16,465 kDa monomers of 6 α-helices	Chronic granulomatous disease; malignant osteopetrosis	Interacts with heterodimeric receptor IFNγR1 and IFNγR2 activating JAK-STAT pathways and altering transcription of up to 30 genes	Most common: flu-like symptoms — fever; headache, chills, fatigue; ISR; rash; and 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	Cancer patients receiving: chemotherapy for AML, myelosuppression or BMT; patients with chronic neutropenia or undergoing PBPCCT	Acts via G-CSF receptors on progenitor cells of neutrophil-granulocyte lineage. Enhances phagocytosis, chemotaxis, cytotoxicity of mature neutrophils. ⁿ Signals via JAK/STAT, Ras/MAPK, PI3K/PKB	Warnings: Splenic rupture and sickle cell crisis. Other effects: nausea/vomiting; fever; bone pain; hypersensitivity; ARDS; ISR; alveolar hemorrhage; immunogenicity; osteoporosis; rash; cutaneous vasculitis; and Sweet's syndrome
Pegfilgrastim ^m (Neulasta [®])	nonglycosylated protein; differs from natural by an N-terminal methionine			
Tbo-filgrastim (Granix [®] ; Tevagrastim [®])	Recombinant biosimilar nonglycosylated G-CSF expressed in <i>E. coli</i> . Formulated for short action	Severe neutropenia in patients with nonmyeloid malignancies receiving myelosuppressive anticancer drugs	As for filgrastim	Warnings: Splenic rupture; ARDS; allergic reactions; and sickle cell crisis. Other effects: bone pain; nausea/vomiting; fever; diarrhea; immunogenicity; cutaneous vasculitis; and Sweet's syndrome

Generic and trade names	Properties	Approved indications ^a	Mechanism(s) of action relevant to indications	Warnings and side effects, serious and common
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, and BMT	Induces progenitor cells to proliferate → neutrophils, monocytes, and macrophages and enhances neutrophil function via specific receptors and signaling through JAK2, STAT, MAPK, and PI3K pathways and transcriptional changes	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; and 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	Stimulates megakaryocytopoiesis and thrombopoiesis → ↑platelet production. Binds to IL-11R α ; gp130 activates JAK which phosphorylates tyr on gp130	<i>Boxed warning:</i> Allergic reactions including anaphylaxis. Warnings: fluid retention [†] ; dilutional anemia; CV events [‡] ; papilledema; and stroke. Other effects: nausea; vomiting; asthenia; abdominal and bone pain; myalgia; anorexia; chills; and alopecia
Becaplermin (Regranex [®]) ^y	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 ¹	Binds to and activates PDGF receptors by dimerization and autophosphorylation binding SH ₂ sites and activating signal pathways	<i>Boxed warning:</i> Increased rate of mortality secondary to malignancy. ^u Other effects: erythematous skin rash; burning at application site; infection; URTI; skin ulceration; cellulitis; osteomyelitis; skin hypertrophy; and bullous eruption
Palifermin (Kepivance [®])	Truncated recombinant human KGF: 140 amino acids, nonglycosylated, MW 16.3 kDa	Severe oral mucositis in patients with hematologic malignancies	Binds to fibroblast growth factor receptor activating Ras-MAPK signaling and transcriptional activation of cell growth and survival	<i>Warning:</i> Potential for stimulation of tumor growth. Other effects: fever; dysesthesia; tongue discoloration/thickening; arthralgias; ↑serum amylase; edema; rash; erythema; hand-foot syndrome; and pruritus

(continued)

Table 5.2 (continued)

Generic and trade names	Properties	Approved indications ^a	Mechanism(s) of action relevant to indications	Warnings and side effects, serious and common
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	Binds to IL-2 receptor → heterodimerization of IL-2Rβ and -2Rγ → activation JAK3; phosphorylation of tyr on IL-2Rβ → activated receptor, signaling molecules and T cell stimulation	<i>Boxed warning:</i> Restrict to patients with normal cardiac and pulmonary functions; administer in hospital with ICU facility and specialists; CLS ^w ; impaired neutrophil function ^m ; and withhold in cases of lethargy and somnolence. Other effects: chills; diarrhea; hypotension; oliguria; thrombocytopenia; erythema; and 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	Binds to IL-1RI receptor blocking activity of IL-1α and β and acting as a biological response modifier, for example, for cartilage degradation and bone resorption	ISR; worsening rheumatoid arthritis; upper respiratory and other infections; headache; nausea; diarrhea; flu-like symptoms; arthralgia; abdominal pain; hypersensitivity (including anaphylaxis and angioedema); and 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	Binds receptors on erythroid progenitor cells triggering conformational change, activation of JAK2 by transphosphorylation, Src signaling, STAT regulation of genes for cell division and differentiation	<i>Boxed warning:</i> 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; hypotension; rash; pruritus; stomatitis; myalgia; and pure red cell aplasia ^y
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	As for epoetin alfa	<i>Boxed warning:</i> As for epoetin alfa. Other effects: hypertension; dyspnea; peripheral edema; cough; abdominal pain; pure red cell aplasia; thrombovascular events; seizures; hypersensitivity (including anaphylaxis, angioedema, and bronchospasm); and rash/erythema

Generic and trade names	Properties	Approved indications ^a	Mechanism(s) of action relevant to indications	Warnings and side effects, serious and common
Bone morphogenetic protein 2 (InFUSE [®]) Bone Graft/LT-Cage [®] z	Recombinant human BMP-2 (rhBMP-2 and dibotermim alfa); a disulfide-linked homodimer; glycosylated subunits 114 and 131 amino acids	Spinal fusion procedures in patients with degenerative disc disease	BMP binds to ser/thr kinase types I and II receptors forming activated complexes. SMAD proteins, part of type I receptors, relay BMP signal to target genes in the nucleus. This in turn induces transcription of osteogenic genes leading to cell proliferation and differentiation	Erythema; swelling over implant site; immunogenicity; ectopic/heterotopic ossification; myositis ossificans; wound-related complications; osteolysis; infections; radiculitis; compression of airways after spine fusion; urogenital events; retrograde ejaculation; and allergy
Bone morphogenetic protein 7 (OP-1 Putty [®] , OP-1 Implant [®] , Opgenra [®] ab, Osigraft [®] ab)	Recombinant human BMP-7 (rhBMP-7; OP-1; and eptotermim alfa). 30 k Da homodimeric glycoprotein produced by CHO cells; two 139 amino acid peptides correspond to 293–431 of full length BMP-7 ^{ac}	Opgenra: posterolateral lumbar spinal fusion with spondylolisthesis and failed autograft Osigraft: tibial nonunions of at least 9 months		
Metreleptin ^{ad} (Myalept [®])	Recombinant analog of leptin, 147 amino acids, nonglycosylated, MW 16.14 kDa; 1 more met than leptin at NH ₂ terminal; 1 -S-S- at cys97-cys147	Complications of leptin deficiency in patients with congenital and acquired generalized lipodystrophy	Binds and alters conformation of homodimer receptor ^{ac} activating JAK2 which phosphorylates other tyr residues within receptor JAK2 complex to mediate downstream signaling	<i>Boxed warning:</i> Antimetreleptin antibodies with neutralizing activity worsening metabolic control and/or infection; T-cell lymphoma. <i>Warnings:</i> hypoglycemia with concomitant insulin/insulin secretagogues; autoimmunity; hypersensitivity; and benzyl alcohol toxicity. Other effect: immunogenicity

(continued)

Table 5.2 (continued)

Generic and trade names	Properties	Approved indications ^a	Mechanism(s) of action relevant to indications	Warnings and side effects, serious and common
Ancestim ^{®f} (Stemgen [®])	Recombinant human soluble SCF ^{™g} with N-terminal met, 166 amino acids, 18.5 kDa monomer ^{ah}	Use with filgrastim for PBPC transplantation to increase number of collected PBPCs	Growth factor stimulating hemopoietic progenitor cells, mast cells and melanocytes. SCF binds to c-Kit receptor ^{ai} activating signaling pathways PI3K, ras/ERK, Src kinase, and JAK/STAT	Warnings: Need for premedication; history of allergy/asthma; caution with chemotherapy/radiotherapy; and growth factor potential. Other effects: ISR; tachycardia; respiratory symptoms; paresthesia; headache; dizziness; allergic reactions; nausea; rash; and distant skin reactions

AI autoimmune, *ALL* acute lymphoblastic leukemia, *ALT* alanine aminotransferase, *AML* acute myeloid leukemia, *ARDS* acute respiratory distress syndrome, *A-RKS* AIDS-related Kaposi's sarcoma, *AST* aspartate aminotransferase, *BMP* bone morphogenetic protein, *BMT* bone marrow transplantation, *CHF* congestive heart failure, *CHO* Chinese hamster ovary, *CLS* capillary leak syndrome, *CNS* central nervous system, *CV* cardiovascular, *EM* erythema multiforme, *EMA* European Medicines Agency, *ESA* erythropoiesis-stimulating agent, *FDA* US Food and Drug Administration, *FL* follicular lymphoma, *GI* gastrointestinal, *HCL* hairy cell leukemia, *hu-G-CSF* human granulocyte colony-stimulating factor, *hu-GM-CSF* human granulocyte macrophage colony-stimulating factor, *IFN* interferon, *ISR* injection site reactions, *JAK* Janus-activated kinase, *KGF* keratinocyte growth factor, *MAPK* mitogen-activated protein kinase, *MM* malignant melanoma, *NHL* non-Hodgkin lymphoma, *PI3* phosphoinositide 3-kinase, *PBPC* peripheral blood progenitor cell, *PBPCCT* peripheral blood progenitor cell collection therapy, *PDGF* platelet-derived growth factor, *PEG* bis-monomethoxy polyethylene glycol, *PI3K* phosphatidylinositol 3-kinase, *PKB* protein kinase B, *PM* postmarketing, *SJS* Stevens–Johnson syndrome, *SLE* systemic lupus erythematosus, *STAT1*, *STAT2* signal transducer and activator of transcription proteins 1 and 2, *SCF* stem cell factor, *TEN* toxic epidermal necrolysis, *TyK2* tyrosine kinase 2

^aCytokines (and date) approved by:

FDA—Peginterferon alfa-2a (2002); Interferon alfa-2b (1986); Peginterferon alfa-2b, Pegintron[®] (2001), Sylatron[®] (2011); Interferon beta-1a, Avonex[®] (1996), Rebif[®] (2002); Interferon beta-1b, Betaseron[®] (1993), Extavia[®] (2009); Interferon gamma-1b (1999); Filgrastim, Neupogen[®] (1991), Nivestim[®] (2010); Pegfilgrastim (2002); Tbo-filgrastim (2012); Sargramostim (1991); Oprelvekin (1997); Becaplermin (2004); Aldesleukin (1992); Anakinra (2001); Epoetin alfa (1989); Darbepoetin alfa (2001); Bone morphogenetic protein 2 (2002, 2004, and 2007 for different indications); Bone morphogenetic protein 7 (2001); Metreleptin (2014) **EMA**—Peginterferon alfa-2a (2002); Interferon alfa-2b (2000); Peginterferon alfa-2b, Pegintron[®] (2001); Interferon beta-1a, Avonex[®] (1997), Rebif[®] (1998); Interferon beta-1b, Betaferon[®] (1995), Extavia[®] (2008); Filgrastim, Neupogen[®] (1991), Nivestim[®] (2010); Pegfilgrastim (2002); Tbo-filgrastim (2008); Becaplermin (1999); Palifermin (2005); Aldesleukin (2006); Anakinra (2002); Epoetin alfa (2007); Darbepoetin alfa (2001); Bone morphogenetic protein 2 (2002); Bone morphogenetic protein 7 (2001). Metreleptin designated an orphan drug in 2012

From Baldo B.A. Side effects of cytokines approved for therapy. Drug Saf 2014;37:921–43. Adapted and reproduced with permission from Springer Science + Business Media

^aApproved by FDA CDER or EMA or both *

^bPeginterferon alfa-2a and ribavirin (Copegus[®]) are indicated for the treatment of adults not previously treated with interferon alfa and with chronic hepatitis C and liver disease. This drug combination is the only FDA-approved regimen for the treatment of chronic hepatitis C infected with both hepatitis C virus and HIV

^cIn adults with compensated liver disease

^dCombination therapy with ribavirin recommended

^eHBeAg, hepatitis B “e” antigen circulating in blood when the virus is replicating

^fAll type I interferons have antiviral, antiproliferative, and immunomodulatory activities

^gIFN-regulatory factor 9

^hFatal or life threatening

ⁱReactions less commonly seen and/or seen during PM period include nephrotic syndrome; renal insufficiency and failure; pancreatitis, SJS, TEN, injection site necrosis, myositis, immune-mediated disorders including thrombocytopenia

^jRibavirin may cause birth defects; avoid pregnancy. It is a potential carcinogen

^kLess commonly seen and during PM period: thrombocytopenia; cardiac disorders; renal insufficiency and failure; hearing and eye disorders; infections; immune disorders including anaphylaxis, angioedema, urticaria, SJS, TEN, SLE, and EM; nervous system disorders such as peripheral neuropathy and seizures

^lLess commonly seen and during PM period: CV; endocrinopathies; hepatic failure; retinopathy; ear, eye, pulmonary, and immune (thrombocytopenic purpura, SLE, EM, SJS, and TEN) disorders; pancreatitis; colitis; and psoriasis

^mIndicated to decrease the incidence of infections in patients with nonmyeloid malignancies receiving myelosuppressive anticancer drugs and associated with febrile neutropenia. Extends half-life to 42 h from ~3.5 h for filgrastim

ⁿMechanisms still poorly understood

^oOther actions: regulation of intestinal epithelium growth; inhibition of adipogenesis and proinflammatory cytokines; and induction of acute phase protein synthesis (e.g., fibrinogen)

^pPulmonary and peripheral edema, dyspnea, and CLS

^qArrhythmias, pulmonary edema

^rUsed topically as a gel

^sPDGF also known as PDGF-BB

^tPromotes chemotactic recruitment and proliferation of cells for wound healing and formation of granulation tissue

^uGel should only be used when benefits are expected to outweigh the risks and used with caution in cancer patients with known malignancy

^vrhKGF differs from endogenous protein by truncation of the *N*-terminal amino acid to increase stability

^wCLS results in hypotension, reduced organ perfusion and possibly death and may be associated with cardiac arrhythmias, angina, myocardial infarction, respiratory insufficiency, edema, etc.

(continued)

Table 5.2 (continued)

^xReduced chemotaxis therefore treat pre-existing infection prior to aldesleukin therapy; patients with indwelling central lines particularly at risk

^ySevere anemia with erythrocyte count 1 and 0.5 % mature erythroblasts in bone marrow

^zInFUSE® Bone Graft consists of rhBMP-2 absorbed to a collagen sponge. The LT-Cage® titanium alloy device is a small, hollow, perforated machined cylinder with one end closed and the other open for addition of the InFUSE® Bone Graft component

^{aa}Acquired by Olympos Biotech from Stryker Corp

^{ab}Equivalent to OP-1 Putty and Implant preparations, respectively

^{ac}Several different recombinant mature forms starting at positions 293, 300, 315, and 316 have been identified

^{ad}Leptin, often called a hormone, shows some structural homology to cytokines

^{ae}Leptin receptors are members of the IL-6 class I cytokine receptor family

^{af}Approved in Australia, New Zealand, and Canada

^{ag}Also known as Kit-ligand or steel factor

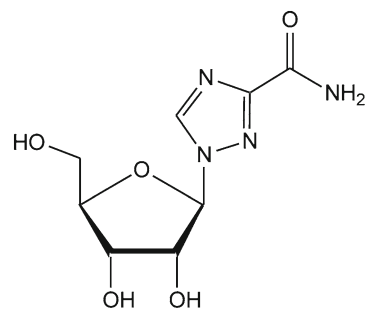
^{ah}Produced in *E. coli*. Amino acid sequence is identical to human sequence except for N-terminal methionine. Normally exists as noncovalently linked dimer

^{ai}CD117, a receptor tyrosine kinase expressed on hematopoietic and germ cells, mast cells, and melanocytes

in the CDER Biologic Products List. Peginterferon alfa-2a together with the guanosine analog and nucleoside inhibitor, ribavirin (1- β -D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide Copegus[®]; Fig. 5.1) are indicated for the treatment of chronic hepatitis C in adults who have compensated liver disease and were not previously treated with interferon alfa. This drug combination is also the approved treatment of patients infected with hepatitis C and HIV and peginterferon alfa-2a alone is approved for the treatment of patients with chronic hepatitis B who have compensated liver disease, viral replication, and liver inflammation. Interferon alfa-2b is administered extensively for hepatitis B and C as well as several malignancies (Table 5.2). It upregulates the expression of MHC I proteins enhancing activation of CD8+ T cells and cytotoxic lymphocyte-mediated killing as well as inducing synthesis of several other antiviral agents including protein kinase R. Peginterferon alfa-2a and peginterferon alfa-2b are covalent conjugates of the recombinant interferon with a single branched bis-monomethoxy polyethylene glycol (PEG) chain, MW 40 kDa. Pegylation, which is FDA approved, nontoxic and contributes to water solubility, helps to protect the protein from immune recognition, that is, it reduces the immunogenicity and antigenicity and increases the molecule's size thus extending protein half-life and circulatory time and reducing renal clearance. For interferon alfa-2a, adverse events in patients treated with the pegylated form and ribavirin occur with a similar, or significantly less, frequency than those treated with standard interferon/ribavirin. For interferon alfa-2b, a number of adverse events occur more frequently with pegylated interferon/ribavirin. Premedication with acetaminophen is often recommended prior to the first dose of peginterferon alfa-2b; thereafter, premedication is undertaken as needed. In some reports on side effects, especially in the earlier literature, interferon alfa is often not distinguished as alfa-2a or alfa-2b although this can be important as demonstrated by some of the different effects induced by alfa-2a and alfa-2b interferons mentioned below.

Interferon alfa-induced neuropsychiatric disorders, particularly depression, cognitive dysfunction, and mania are well known and have been intensively studied. Other symptoms include altered sleep pattern, anorexia, and fatigue. Of the patients who develop severe depressive symptoms, most occur within the first 3 months of

Fig. 5.1 Structure of ribavirin (1- β -D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide; Copegus[®]), the guanosine analog and nucleoside inhibitor which, in combination with interferon alfa, is the mainstay of treatment of hepatitis C infection



Ribavirin

treatment and the incidence of depressive disorders has been estimated to be 23–41 %. Symptoms may be prolonged for 6 months or more after the cessation of therapy. There is some evidence that the serotonergic system is involved in the pathophysiologic mechanism although the central opioid, dopamine, and glutamate neurotransmitter systems may also be involved. A positive correlation between depression scores and serum concentrations of soluble ICAM (intracellular adhesion molecule)-1 in patients who received interferon alfa led to the suggestion that the cytokine may induce the adhesion molecule which then increases the permeability of the blood–brain-barrier, allowing the interferon to more easily enter the brain. A number of susceptibility factors have been suggested including a history of depression; high dose of interferon; long treatment duration; female sex; and possession of the apolipoprotein Eε4 allele, said to be associated with some neuropsychiatric disorders.

The appearance of autoantibodies and development or exacerbation of autoimmune diseases are known to occur in response to interferon alfa therapy. In one study, seven cases of autoimmune disease, including one of hypothyroidism, two each of immune-mediated hemolysis and systemic lupus erythematosus, one of Raynaud's disease, and one case of mixed connective tissue disease were identified in 76 patients after a median of 19 months of treatment. Reports of autoimmune reactions to interferon alfa or its combination with ribavirin are not rare and include cases of Hashimoto's thyroid-toxicosis followed by type 1 diabetes, autoimmune thyroiditis, and development and exacerbation of a lupus-like syndrome. See also "Endocrine effects" below.

In addition to their neuropsychiatric and immune effects, interferon alfas occasionally provoke an extensive range of adverse reactions including cardiovascular, respiratory, endocrine, hematologic, metabolic, urinary tract, and skin adverse events as well as adverse effects on the nervous and sensory systems. Cardiovascular complications such as pericarditis and cardiomyopathy with left ventricular dilatation in patients with malignancies improved after withdrawal of the interferon and thereafter treatment with lower doses proved possible. Pegylated interferon alfa-2b has been associated with acute myocardial infarction, pericarditis, pericardial effusion with tamponade, and sick sinus syndrome producing arrhythmias. An orthotopic heart transplant patient died after allograft failure with death attributed to interferon toxicity. Interstitial lung disease, reported for both interferon alfa-2a and 2b, is seen more frequently with the former agent and with high doses of the latter. Potentially fatal interstitial pneumonitis, secondary to interferon alfa-ribavirin therapy for hepatitis C infection, is said to have an incidence of 0.03–0.3 % although an incidence of ~1.1 % was found in 558 Japanese patients. Fatal interstitial pulmonary disease can occur with pegylated interferon alfa-2b as shown by a patient with interstitial pneumonitis who also developing adult respiratory distress syndrome. Cases of bronchiolitis obliterans organizing pneumonia (BOOP), some fatal, are also known.

Interferon alfa may have adverse effects on the nervous system in the form of seizures in patients with no history of epilepsy, involuntary facial movements and weakness, features resembling multiple sclerosis, restless legs syndrome, 17 reports of sensorimotor polyneuropathy, and Bell's palsy. Adverse effects on

sensory systems, mainly not only the eyes but also the ears, occur particularly to interferon alfa-2b. Ocular complications include occlusive vasculitis, central retinal artery occlusion, and anterior ischemic optic retinopathy. Twenty seven of 42 patients taking interferon alfa-2b/ribavirin developed a retinopathy: cotton wool spots occurred in 27 patients, retinal hemorrhage in six, subconjunctival hemorrhage in two, and optic nerve edema in one patient. Other ocular complications described in patients treated with interferon alfa-2b include permanent loss of sight due to combined retinal artery and central retinal vein obstruction, development of an epiretinal membrane, and the T-cell-mediated autoimmune syndrome, Vogt-Koyanagi-Harada disease.

Endocrine effects of interferon alfa are probably best illustrated by thyroid dysfunction which is not yet fully understood but may have an autoimmune mechanism. Thyroid dysfunction occurs with an incidence of 5–14% in patients treated for chronic hepatitis C. Hypothyroidism occurs more often than hyperthyroidism and resolution occurs in about 60% of cases. Interferon alfa-2b can cause both conditions. Although an autoimmune reaction is the most likely mechanism, some patients develop hypothyroidism without autoimmunity. A direct inhibitory effect of thyrocytes has been suggested as the possible mechanism.

Neutropenia induced by interferon alfa is fairly commonly seen while other reported hematologic side effects include acute and autoimmune thrombocytopenia, pernicious anemia, bone marrow hypoplasia which may be immune mediated, and pure red cell aplasia.

A number of acute renal complications in response to interferon alfa have been well documented and include renal thrombotic microangiopathy, acute nephrotic syndrome, hemolytic-uremic syndrome, renal insufficiency due to interstitial nephritis, tubular necrosis, and IgA nephropathy.

The list of cutaneous reactions to interferon alfa is extensive and includes injection site reactions (erythema, necrosis, and vasculitis), pruritus, xerosis, urticaria, hyperpigmentation, psoriasis, alopecia, lichen planus, pityriasis rosea, sarcoid nodules, eosinophilic fasciitis, livedo reticularis, vitiligo, and fixed drug eruption. Interferon alfa is well known for exacerbating pre-existing psoriasis but cases of new onset, and extensive, psoriasis have been reported for both interferon alfa-2a (Fig. 5.2) and interferon alfa-2b. In one example of extensive psoriasis induced by interferon alfa-2b, an adult patient being treated for chronic hepatitis C developed a mild form of psoriasis during the third month of therapy. The condition became worse by the fifth month at which time the patient was hepatitis C virus RNA-negative. Therapy was completed at 6 months and one month later the patient's psoriasis receded spontaneously and completely with no recurrence after 4 years.

The apparent association of vitiligo with interferon therapy for hepatitis C is interesting. Vitiligo is an idiopathic acquired skin disease characterized by loss of skin pigment due to destruction, probably by apoptosis and not necrosis, of melanocytes (Fig. 5.3). Vitiligo often manifests during the first 6 months of interferon therapy, but there is conflicting evidence on the question of a relationship between presence of the virus and the skin response. Although the exact pathogenesis of vitiligo remains unclear, an autoimmune process has been implicated, perhaps with



Fig. 5.2 Extensive psoriasis in a patient with no previous history of psoriasis, treated for chronic hepatitis C with interferon alfa-2a and ribavirin. Patient presented with clearly defined erythematous plaques with scales on the chest and abdomen (a), the back (b), scalp (d), and extremities (e). The finger nails (c) showed signs of pitting and onycholysis. Reproduced from Kim G-W, Jwa S-W, Song M, et al. *Ann Dermatol.* 2013;25:479–82. doi:[10.5021/ad.2013.25.4.479](https://doi.org/10.5021/ad.2013.25.4.479), an open-access article distributed under the terms of the Creative Commons Attribution License)

Fig. 5.3 An example of vitiligo in an adult male showing loss of skin pigment of the hand due to destruction of melanocytes. The exact pathogenesis of vitiligo remains unclear (Photograph kindly provided by Dr. R. Spiewak)



the involvement of cytokines such as interferons, IL-2 (section “Aldesleukin”), soluble IL-2 receptor (sIL-2R), IL-10, IL-13, and IL-17A. Recent research on the pathophysiology of the disorder indicates an involvement of cytotoxic T lymphocytes expressing interferon gamma that ultimately leads to melanocyte apoptosis (section “Interferon Gamma”).

Interferon Beta

The transcriptional response to interferons beta-1a and beta-1b appear to be indistinguishable, but the biological and clinical responses may vary with the dosage schedules. A flu-like illness is the most commonly occurring adverse event following administration of the interferon beta proteins (Table 5.2) and injection site reactions are common. A comparison of interferon beta-1a, 30 µg, given intramuscularly (im) once per week with interferon beta-1b, 44 µg, subcutaneously (sc) every other day, showed that injection site reactions and antibodies were significantly more frequent in patients given the beta-1b preparation but after 2 years, clinical outcomes to this agent were superior. The questions of the production of neutralizing antibodies to interferon beta and whether they reduce the therapeutic effectiveness in treated patients, especially in the treatment of multiple sclerosis, are important ones. Such antibodies are found in about a quarter of patients treated with sc administered interferon beta-1b and the consensus is that they neutralize or reduce the cytokine’s activity. Some believe that this has the potential to significantly reduce the effectiveness of the therapy and it has been suggested that the immunogenic potential of interferon beta should therefore 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 sc interferon beta-1b.

Unlike interferon alfa, results from studies do not support an association of interferon beta with depression, but the FDA mention depression, suicide, and psychotic disorders in their warnings and precautions for the cytokine. Interferon beta can induce thyroid disorders notably hyperthyroidism and a severe case of hypothyroidism to interferon beta-1a resembling Hashimoto’s encephalopathy has been described. Skin reactions reported 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 then covalently attached via the *N*-terminal residue to a linear 20 kDa methoxypolyethylene glycol molecule, giving the complex a total molecular mass of approximately 44 kDa. The amino acid sequence of the recombinant cytokine is identical to its human interferon beta counterpart. The apparent molecular mass of Plegridy[®] in solution is more than 300 kDa, that is, more than 13-fold increase compared to interferon beta-1a. This ensures a significantly reduced patient clearance of the pegylated preparation. In placebo-controlled clinical studies, the most common adverse reactions to Plegridy[®] were similar to

the nonpegylated 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 the nonpegylated form (Table 5.2). Whereas less than 1 % of patients given Plegridy[®] every 14 days for 1 year developed neutralizing antibodies, 7 % of treated patients developed antibodies to PEG.

Interferon Gamma

Interferon gamma, structurally distinct from other interferons, is produced predominately by NK (TCR not expressed) and NKT cells and by CD4 and CD8 cytotoxic T lymphocytes in antigen-specific immunity. The cytokine shows a different biological activity spectrum, in particular in its action of differentiating normal and B lymphocytes, and as an immunomodulator of macrophage activity. It also has an important role in dealing with intracellular pathogens, including viruses, and tumor control.

Early phase I studies of the biological activity of, and tolerance to, recombinant interferon gamma showed the common appearance of flu-like symptoms and granulocytopenia. In another early study, a 30 % fall in peripheral blood lymphocytes was seen after 10 days of interferon gamma therapy. The occurrence of fatal acute respiratory failure in four patients treated with interferon gamma-1b for advanced idiopathic pulmonary fibrosis prompted further investigation in the form of a double blind study of the effect of the cytokine in 330 patients with that condition. No significant differences were found in lung function, gas exchange, or quality of life, but the patients experienced more frequent upper respiratory infections and pneumonia. However, acute respiratory insufficiency has been reported in a single patient with idiopathic pulmonary fibrosis 4 months after receiving interferon gamma. Cardiovascular toxicity to interferon gamma, particularly at higher doses, and including hypotension, arrhythmias, coronary vasospasm, and ventricular tachycardia and renal toxicity, namely acute renal failure, nephrotic syndrome, and tubular necrosis, have been recorded.

There appears to be few reports of cutaneous reactions to interferon gamma, but severe erythroderma occurred in 5 of 10 bone marrow transplant patients given the drug. Recent studies have found increased levels of interferon gamma mRNA in skin of patients with vitiligo (Fig. 5.3) and inhibitors of the cytokine have proved to be beneficial treatments in some cases. In an investigation of 50 patients with vitiligo, the frequency of interferon gamma-producing cells in skin and peripheral blood was determined. Significant expansions of CD8+ cytotoxic T lymphocytes expressing interferon gamma were detected and, when examined in vitro, the cytokine directly induced melanocyte apoptosis leading the authors to conclude that the CD8+ cells have a pivotal role in the induction and maintenance of the skin disease.

Colony-Stimulating Factors: Filgrastim, Sargramostim, and Tbo-Filgrastim

CSFs, produced by most tissues and cell types, are glycoprotein cytokines with multiple actions on hematopoietic cells. Described by Metcalf as “the master regulators of granulocyte and macrophage populations,” the CSFs are 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 (Figs. 5.4 and 5.5) to each other and a number of other signaling proteins, for example, human growth hormone (Chap. 7, section “Human Growth Hormone”), interferon beta (section “Interferon Beta”), IL-2 (section “Aldesleukin”), and IL-4. This conservation of tertiary structure suggests similar binding of the different ligands to their respective receptors. Currently, approved members of the CSF family are filgrastim and pegfilgrastim, both G-CSFs, sargramostim, a GM-CSF, and tbo-filgrastim, a short acting biosimilar (Chap. 13) G-CSF (Table 5.2). The latter is used for severe neutropenia in patients with lung cancer receiving platinum drug chemotherapy. GM-CSF, used as an immunostimulant following bone marrow transplantation and chemotherapy, is also viewed as a potential immunoadjuvant for anticancer vaccines. The *E. coli*-derived GM-CSF molgramostim, seen as a potential immunostimulant, showed a higher incidence of adverse effects than sargramostim and was not granted FDA approval.

As well as the most common, and usually mild and transient reactions of headache, bone pain, myalgia, fever, flushing, and rash for filgrastim and sargramostim, other more severe, but rare, respiratory, cardiovascular, hematologic, and cutaneous

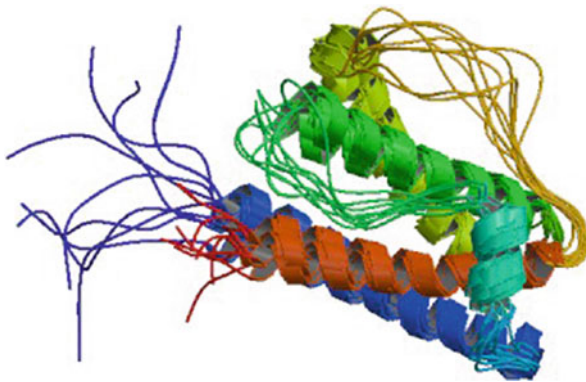
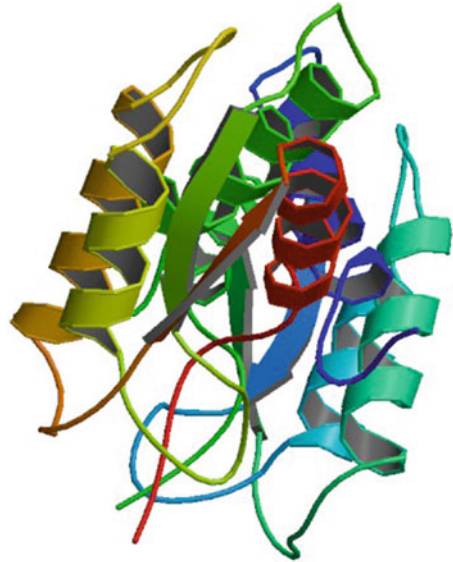


Fig. 5.4 Structure of recombinant human G-CSF (rhu-G-CSF) determined by NMR spectroscopy. Structure is predominately helical with 106 of the 175 amino acids forming a left-handed four- α -helix bundle. Helices are composed of helix A, residues 11–41 and helix B, residues 71–95, aligned parallel to each other (*up, up*), and helix C, residues 102–125 and helix D, residues 145–170, which are antiparallel (*down, down*). The structure is from Protein Data Bank RCSB PDB file 1GNC (Zink T, Ross A, Luers K, et al. *Biochemistry*. 1994;33:8453–63)

Fig. 5.5 Three-dimensional crystal structure of recombinant GM-CSF (rhu-GM-CSF) determined at 2.8 Å. Like G-CSF, the main structural feature is a four- α -helix bundle (helices A, B, C, and D) which makes up nearly half the structure. Helices are in a left-handed antiparallel arrangement with two overhand connections containing a two-stranded antiparallel beta sheet. The structure is from Protein Data Bank RCSB PDB file 1CSG (Walter MR, Cook WJ, Ealick SE, et al. *J Mol Biol.* 1992;224:1075–85)



reactions occur. Adult respiratory distress syndrome (ARDS) following G-CSF is more likely when a rapid rise in the white cells occurs in patients taking pulmonary toxic drugs, when there is concomitant infection, and in patients with HLA-B51 or HLA-B52 antigens. Other occasional respiratory side effects are pulmonary toxicities, particularly pulmonary edema which has proved fatal, and interstitial pneumonitis. There has been speculation that GM-CSF might contribute to the development of acute coronary syndrome. In fact, cardiovascular complications have been observed. These include fluid retention, pulmonary edema and weight gain, aortitis to molgramostim, and capillary leak syndrome (Chap. 1, section “Capillary Leak Syndrome”) following G-CSF which can be severe and even fatal. Recorded hematologic side effects to CSFs consist mainly of a number of cases of thrombocytopenia, some with an immune mechanism, splenomegaly, and splenic rupture (note FDA issued warning, Table 5.2). There is a belief that G-CSF may be a risk for the progression of myelodysplastic syndrome (MDS), but this has not been unequivocally established. MDS has been reported after G-CSF treatment and the incidence of MDS or acute myeloid leukemia (AML) was found to be 11 % in patients treated with G-CSF, but only 5.8 % in patients receiving immunosuppression alone. In another more recent study, patients who received G-CSF showed a 2.5-fold increased risk. Interpretation of results relevant to the alleged risk of G-CSF is not straight forward however. Findings that there is no significant relationship between G-CSF therapy and MDS/AML onset are at odds with the belief that the risk of leukemia in severe congenital neutropenia patients increases with the G-CSF therapy. Two other potentially life-threatening responses to CSFs, both the subject of warnings, are anaphylactic/anaphylactoid reactions and severe adverse events such as acute chest syndrome, vaso-occlusive episodes, multiorgan failure, and death seen in patients with sickle cell disease.



Fig. 5.6 Cutaneous eruption mimicking Sweet's syndrome or acute febrile neutrophilic dermatosis following the administration of filgrastim (recombinant granulocyte colony-stimulating factor [G-CSF]). Sweet's syndrome is characterized by an elevated neutrophil count and the presence of neutrophils in the upper dermis. Reproduced from Aubin F et al. The complexity of adverse side-effects to biological agents. *J Crohn's Colitis*. 2013;7:257–62. Distributed under a Creative Commons License; reproduced with permission from Elsevier Limited (CCC License number 3662450405678)

There is a long list of adverse skin reactions provoked by CSFs. The most commonly occurring cutaneous reaction is Sweet's syndrome seen after therapy with filgrastim (Fig. 5.6) and sargramostim. In fact, these two colony-stimulating factors are the most frequently implicated drugs in Sweet's syndrome. Other adverse cutaneous events to CSFs include psoriasis flare, pyogenic granulomas, pruritic erythematous maculopapular eruptions, palmoplantar pustulosis, erythema multiforme, and neutrophilic dermatoses.

Oprelvekin

Recombinant human IL-11, or oprelvekin (Table 5.2), is used to prevent chemotherapy-induced thrombocytopenia and reduce the need for platelet transfusions in patients with nonmyeloid malignancies. The most commonly occurring adverse events seen in placebo-controlled studies were edema, dyspnea, tachycardia, palpitations, atrial fibrillation/flutter, pleural effusions, conjunctival injection, and oral moniliasis. Fluid retention and an increase in plasma volume underlie many of the adverse events, for example, edema, dyspnea, pleural effusions, arrhythmia, dilutional anemia, and renal failure and indicate that oprelvekin should be used with caution in patients with congestive heart failure. No evidence of cumulative toxicity

or bone marrow exhaustion has been observed after sequential cycles of the cytokine and no proliferative effect on tumors has been noted. Two other clinically important adverse reactions reported are papilledema and periosteal bone formation. An incidence of 3–4% was found for antioprelvekin antibodies in treated patients.

Becaplermin

Becaplermin is a recombinant human platelet-derived growth factor (PDGF), a homodimer made up of two disulfide-bonded B chains and hence written as rhPDGF-BB. Naturally occurring PDGF has A and B chains in homodimeric or heterodimeric form. The PDGF-A chain binds to the α receptor, whereas the PDGF-B chain binds to both the α and β . rhPDGF-BB promotes the growth of granulation tissue and wound healing via interaction with receptors on fibroblasts (α and β) and endothelial cells (β receptors). Becaplermin has therefore found use in gel form as a topical application for patients with lower extremity diabetic neuropathic ulcers, lesions that are notoriously difficult to heal and a major cause of morbidity (Fig. 5.7).

Growth factors cause cell proliferation so the possibility of increased cancer rates is considered for drugs with a cell growth-promoting property. In a retrospective study by the FDA of a medical claims database, cancer rates and deaths were compared for 1622 becaplermin users and 2809 matched nonusers. The incidence rate ratios of becaplermin to matched controls for all cancers and for mortality from all cancers were 1.2 and 1.8, respectively, and the incidence rates for mortality among patients who received three or more tubes of becaplermin and controls were 3.9 and 0.9 per 1000 patient-years, respectively. The rate ratio for cancer mortality in the patient group receiving 3 or more tubes was 5.2 (95% CI 1.6–17.60). Following an

Fig. 5.7 Diabetic pressure-induced foot ulcer. Reproduced from Shapiro J, Koshimune D, Moellmer R. Diabetic foot ulcers—treatment and prevention. In: Masuo K, editor. Type 2 diabetes. InTech; 2013. Ch 12. <http://www.intechopen.com/books/type-2-diabetes/diabetic-foot-ulcers-treatment-and-prevention> Accessed October 13, 2015. An open-access article distributed under the terms of the Creative Commons Attribution License



earlier safety study in 2001, where more cancers were found in the becaplermin group than a nonuser group, the FDA in 2008 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.” As a consequence, it was stated that “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 but, at the same time, admitted that there was no evidence either way to establish, or rule out, a link between therapeutic use of the cytokine and cancer. In July 2012, the European Commission withdrew the marketing authorization for Regranex®.

In studies on the safety of becaplermin gel in the treatment of neuropathic diabetic foot ulcers, clinical findings showed little difference between the drug and placebo in relation to cardiovascular, respiratory, musculoskeletal, and nervous system disorders. No neutralizing antibodies were detected. Rash was seen in 2% of patients treated with becaplermin and 1% receiving placebo. Apart from the possibility of becaplermin-induced cancers and the drug’s known side effects listed in Table 5.2, there is a dearth of subsequent studies on the safety of becaplermin, including case reports. This is probably because clinical experience with the agent did not live up to the initial high expectations, the concerns related to cancer, and the high cost of the agent.

rhPDGF-BB, together with beta-tricalcium phosphate, is a component of Augment® Bone Graft, a combination device/drug product developed for bone repair and regeneration. Intended for the treatment of foot and ankle fusions, a major claimed advantage of the product is the elimination both of the need to harvest autologous bone and the associated risks of ongoing pain and infection. Augment® Bone Graft is indicated for use as an alternative to autograft in hindfoot and ankle fusion procedures that require supplemental graft material, for example, in tibiocalcaneal, talonavicular, and calcaneocuboid fusions. In October 2014, the FDA approved the Premarket Approval Application for Augment® Bone Graft subject to a preapproval facilities inspection. Already approved in Canada, the rationale for the product’s action is the inclusion of rhPDGF-BB for the promotion of growth and proliferation of osteoblasts and beta-tricalcium phosphate, a resorbable synthetic bone matrix, as the framework for new bone growth. Each component is packaged separately and mixed immediately before use. Already issued warnings and precautions for the product include its as yet unknown effect on fetal development; whether or not it is excreted in milk; its safety at sites other than the ankle and foot; the need for its use on well-vascularized bone; unknown safety of repeat applications; and the product’s safety in patients less than 18 years old. In the primary clinical study, Augment® Bone Graft was compared to autologous bone graft as the “gold standard” in a representative foot and ankle fusions clinical model. Safety studies revealed 973 treatment-emergent adverse events with no significant differences between the two treatment groups. Overall, the Augment group showed fewer serious adverse events and fewer complications associated with surgery and

infections. In a prospective, open-label, multicenter trial undertaken in Canada and designed to evaluate Augment® Bone Graft, 60 patients requiring hindfoot, mid-foot, or ankle fusions, were followed for 36 weeks. A total of 22 adverse events, none serious, was recorded, most arising from the surgery; 15 were general and administration site disorders (swelling, feeling hot, tenderness, and impaired healing), 5 were due to injury and procedural complications, and 2 were musculoskeletal and connective tissue disorders (muscle spasms, and pain).

Palifermin

Palifermin, a recombinant human keratinocyte growth factor produced by mesenchymal cells and fibroblasts, stimulates differentiation, proliferation, and migration of epithelial cells via interaction with its complementary receptors on epithelial cells widely distributed in numerous tissues including skin, hair follicles, tongue, stomach, intestine, lung, liver, kidney, lens of the eye, and many other tissues and organs. The recombinant molecule is a nonglycosylated, 16.3 kDa, 140 amino acid protein belonging to the fibroblast growth factor family that has been genetically modified to increase stability by shortening the natural protein at the *N*-terminal end. Palifermin is an important 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.

Reported adverse events following palifermin administration in a phase III double-blind, placebo-controlled trial were rash, pruritus, erythema, paresthesia, edema, taste alteration, rhinitis, arthralgia, thickening of the tongue, and numbness. The keratinocyte growth stimulation properties of palifermin may underlie a number of cutaneous reactions seen following its administration. Cases of palmoplantar erythrodysesthesia (acral erythema and hand-foot syndrome), 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 been described. The latter reaction consisted of a cutaneous eruption of planar papules resembling lichen planus, together with erythema, mainly in an intertriginous distribution, and confluent white plaques on the oral mucosa. Being a growth factor, palifermin carries a warning of potential stimulation of tumor growth (Table 5.2).

Aldesleukin

Interleukin-2 (IL-2) is one of the best studied cytokines after its discovery as an activator of T lymphocytes nearly 40 years ago. Because it possesses a wide range of immune effects regulating T cells and immune activation and homeostasis, IL-2 was one of the first cytokines characterized at the molecular level. The recombinant form, called aldesleukin, differs from the natural cytokine by absence of glycan

residues and at position 125 and the end terminal amino acid (Table 5.2). X-ray and NMR studies have shown that the IL-2 fold is similar to that seen in the myelopoietic stimulatory factors G-CSF (Fig. 5.4) and GM-CSF (Fig. 5.5), but it has three minor structural differences to the other four-helix bundles (Fig. 5.8).

Aldesleukin has been applied clinically in a number of ways, particularly for melanoma and renal cell carcinoma, and from its earliest applications showed a wide range of the sort of side effects often seen with cytokines including fever, chills, myalgia, nausea, vomiting, diarrhea, hypotension, oliguria, and edema (Table 5.2) plus a number of more severe cardiovascular, hematologic, endocrine, kidney, central nervous system, infectious, and cutaneous toxicities.

Cardiovascular adverse events are the main dose-limiting toxicities of aldesleukin with recorded cases of hypotension, tachycardia, peripheral edema, pleural effusions, myocarditis, myocardial infarction, heart block, arrhythmias, cardiac eosinophilic infiltration, and coronary ischemic changes. An important occasional and serious adverse event of IL-2 therapy is capillary (sometimes called vascular) leak syndrome (Chap. 1, section “Capillary Leak Syndrome”) which causes hypovolemia and fluid accumulation in the extravascular spaces and may lead to oliguria, ischemia, and confusion. Aldesleukin therapy can induce increased vascular permeability, interstitial edema, and ultimately organ failure seen as an increase in body weight, fluid retention, peripheral edema, ascites, pleural and pericardial effusions, and ultimately pulmonary and cardiovascular failure. Pulmonary side

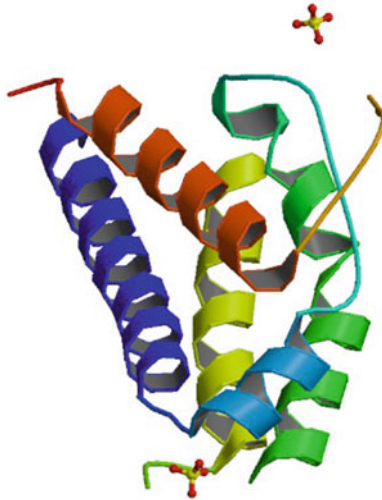


Fig. 5.8 Secondary structure of IL-2 consists of four alpha helices A, B, C, and D, with an up–up and down–down arrangement, a structure similar to G-CSF (see Fig. 5.4) and GM-CSF (Fig. 5.5). IL-2, however, differs in that it has an irregular one turn helix in the AB loop, a distortion in the B helix, and a two-stranded antiparallel beta structure formed from parts of the AB and CD loops. The structure is from Protein Data Bank RCSB PDB file 1M47 (Arkin MA, Randal M, DeLano WL, et al. Proc Natl Acad Sci USA. 2003;100:1603–8)

effects to aldesleukin are usually related to capillary leak syndrome and are more likely, and more severe (e.g., as pulmonary edema and respiratory distress), in patients with existing cardiac problems. Hematologic adverse effects, particularly anemia, leukopenia, and thrombocytopenia occur but are rarely severe or dose limiting. Thrombocytopenia is a common toxicity of high dose IL-2 therapy but rapidly reverses upon cessation of treatment. Eosinophilia may occur in the later stages of therapy accompanied by rash and pruritus. Figure 5.9 shows the hematologic changes, often rapid and dramatic, occurring in response to aldesleukin challenges over a 6 day period. Within hours of the first dose, platelets and lymphocytes fall rapidly to low levels and eosinophil numbers rise slowly, whereas neutrophils and hemoglobin are maintained at stable levels. At the cessation of treatment, lymphocytes and platelets quickly return to normal and, in fact, may exceed their baseline levels by 2- to 5-fold. Eosinophils persist and may do so for many weeks. Endocrine effects usually manifest as hypothyroidism which may affect up to one-third of patients, or as the far less common hyperthyroidism. Renal toxicity, especially oliguria, and gastrointestinal toxicities are also seen, the latter being particularly common in the form of nausea, vomiting, diarrhea, anorexia, gastritis, and mucositis. Gastrointestinal perforation has also been reported. IL-2-induced infectious toxicities may occur at venous catheter sites and in the urinary tract. Such infections, usually due to *Staphylococcus* species, are thought to arise from the known affect of IL-2 on neutrophil chemotaxis. Neurological effects, especially to high doses during IL-2 therapy, include anxiety, depression, altered sleep patterns, somnolence, emotional fragility, vivid dreams, and confusion. The list of aldesleukin-induced cutaneous reactions is extensive, ranging from mild erythema, pruritus, injection site reactions, and vitiligo, to urticaria, angioedema, reactivation

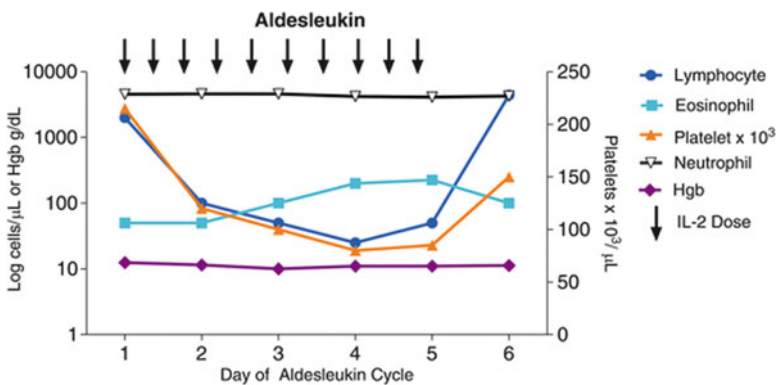


Fig. 5.9 Plot of hematologic responses to a series of IL-2 injections over a 5 day period. Platelet and lymphocyte numbers fell rapidly, the eosinophil count showed a steady but undramatic increase and the neutrophil count remained constant. After the completion of treatment, platelets and lymphocytes recovered quickly. The elevated eosinophil count may persist for weeks. Hemoglobin levels remained constant throughout (Reproduced from Dutcher JP, Schwartzentruber DJ, Kaufman HL, et al. *J Immunother Cancer* 2014;2:26. doi:10.1186/s40425-014-0026-0, an open-access article distributed under the terms of the Creative Commons Attribution License)



Fig. 5.10 A severe cutaneous reaction of erythema with blisters and bullae resembling acute scalding induced by IL-2 in an immunocompromised adult patient (Reproduced from Zhang J, Meng L-H, Zhang L-T, et al. *J Clin Exp Dermatol Res.* 2014;5:1. doi:[10.4172/2155-9554.1000204](https://doi.org/10.4172/2155-9554.1000204), an open-access article distributed under the terms of the Creative Commons Attribution License)

of eczema, exacerbation of psoriasis, generalized erythema followed by desquamation, vasculitis, and severe manifestations like pemphigus, IgA bullous dermatosis, and toxic epidermal necrolysis. An acute blistered scalded skin-like reaction after IL-2 therapy in an immunocompromised patient (Fig. 5.10) suggests that the cytokine should be given with great caution, and on an individualized basis, to immunocompromised patients. A curious case involved the implication of high-dose IL-2 therapy in the occurrence of multifocal fixed drug eruptions after the administration of other drugs, namely, ondansetron, granisetron, acetaminophen (paracetamol), and indomethacin.

Anakinra

Interleukin-1 (IL-1) is a cytokine produced in response to inflammatory stimuli in a number of immunological reactions including rheumatoid arthritis. The receptor for IL-1 (IL-1R), in membrane or soluble form, is widely expressed on tissues and organs and exists as two types, type I which is 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 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 (Table 5.2). Anakinra therefore acts as a biological response modifier in the treatment of diseases like rheumatoid arthritis and the spectrum of autoinflammatory syndromes collectively known as cryopyrin-associated periodic syndrome (CAPS) (Chap. 4, sections “CAPS

Diseases and Approved Indications for Canakinumab” and “Canakinumab: Warnings, Precautions, and Adverse Events”). CAPS encompasses Muckle–Wells syndrome, neonatal-onset multisystem inflammatory disease (NOMID), and familial cold-induced urticaria, now known as familial cold autoinflammatory syndrome (FCAS). All symptoms of Schnitzler syndrome, a rare, underdiagnosed systemic disease with many features in common with the above autoinflammatory syndromes including nonitching urticarial-like lesions, fever, and bone/joint pain, can be relieved within hours of the first injection of anakinra. Symptoms recur if treatment is stopped.

The side effects profile of anakinra is not large with two adverse events, injection site reactions (122 events per 100 patient years) and infection episodes, the most commonly seen detrimental responses to the agent. Injection site reactions occur in up to 73 % of patients but cause cessation of treatment in less than 5 % of affected individuals. 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 sc 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.

Infections, particularly URTI, involving a wide variety of organisms have been reported, but it has been suggested that the risk of infection is associated with high doses of anakinra and in patients with comorbidities. Septicemia due to *S. aureus*, hemolytic streptococci, and *E. coli* occurred after anakinra was added to prednisolone for rheumatoid arthritis. Anakinra provoked reactivation of pulmonary tuberculosis, adenovirus, gastroenteritis, varicella pneumonitis, and visceral leishmaniasis and acute Epstein–Barr virus infection occurred in juvenile idiopathic arthritis patients treated with the cytokine. A patient with Still’s disease given anakinra developed systemic inflammatory response syndrome (SIRS) (Chap. 1, section “Systemic Inflammatory Response Syndrome”) together with ARDS and some other patients with this disease had the cytokine withdrawn because of infections or severe skin reactions. Other reported side effects of

anakinra include progression of rheumatoid arthritis, exacerbation of Crohn's disease, anaphylaxis with a positive skin test to the cytokine, cellulitis at injection sites, and an interstitial granulomatous reaction which resolved after withdrawal of anakinra and recurred on challenge.

Epoetins

Erythropoietin (EPO) is a heavily glycosylated cytokine with three *N*-linked and one *O*-linked oligosaccharide chains that are important for the protein's biological activity and stability. Activity is also dependent on two disulfide bonds between cysteines 7 and 160 and 29 and 32. In both native human EPO and rhEPO (epoetin alfa), the originally secreted molecule is a 166 amino acid peptide before the carboxy-terminal arginine is removed to give the final active protein of 165 amino acids.

In an early study of rhEPO in anemic patients with end-stage renal disease, the main observed adverse effects and their incidences were myalgias 5%, iron deficiency 43%, elevated blood pressure 35%, and seizures 5.4%. Hypertension is a common side effect with approximately one-third of dialysis patients affected. Hypertension and increased viscosity due to rhEPO may lead to encephalopathy, convulsions, cerebral edema, and seizures. Thromboembolism is said to be a potential outcome from EPO therapy, but controlled studies have not always provided support for this claim. Nevertheless, controlled studies on cancer patients revealed a 1.55-fold higher risk of thromboembolic events with rhEPO therapy than controls. Cerebral ischemia with increased metabolic rate and blood viscosity is a potentially severe side effect of EPO therapy and it has been pointed out that this could limit or halt the use of EPO for neurovascular diseases. 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 (Table 5.2).

Pure red cell aplasia (PRCA), caused by neutralizing antibodies to epoetin that cross-react with natural erythropoietin, produces a rapid decline in hemoglobin concentration, severe anemia, low reticulocyte count, and an almost total absence of red cell precursors. In cases of transfusion-dependent PRCA with neutralizing serum antibodies to EPO, patients should not be switched to another epoetin such as darbepoetin alfa. Development of wheals at former epoetin alfa subcutaneous injection sites on a patient with PRCA following intravenous injection with epoetin beta and darbepoetin alfa, provoked a systemic anaphylaxis/anaphylactoid response and anti-EPO antibodies cross-reactive with epoetin beta and darbepoetin alfa were detected in the serum. Other cases of anaphylaxis to epoetin alfa have occurred and a delayed hypersensitivity reaction in the form of acute exanthematous pustulosis following replacement of epoetin alfa with darbepoetin alfa was reported.

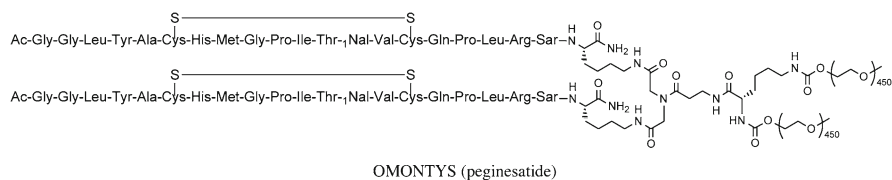


Fig. 5.11 The erythropoiesis-stimulating agent peginesatide (Omontys[®]) is a synthetic dimeric peptide of two identical 21 amino acid chains, pegylated via linkage of lysine residues to methoxypolyethylene glycol. Note the presence of the natural amino acid, sarcosine (*N*-methylglycine). *Ac* acetyl

In a large randomized, double-blind controlled trial comparing two different administration schedules of darbepoetin alfa for the treatment of chemotherapy-induced anemia, serious adverse events that were treatment related occurred in 3% of the 672 patients. Deep vein thrombosis was seen in 1.1–1.7% of patients, pulmonary embolism in 0.8% and hypertension in 0.3–1.1%. No antibodies to darbepoetin alfa were found in the serum of any patient. An investigation of the effect of darbepoetin alfa on exercise tolerance in anemic patients with symptomatic chronic heart failure revealed three events with a >5% difference in incidence between the treatment and placebo groups namely, neurological signs and symptoms, upper respiratory tract infections, and joint-related signs and symptoms.

Approved in 2012 and voluntarily recalled in February 2013, **peginesatide** (Omontys[®]) is an erythropoiesis-stimulating agent with no amino acid sequence homology to erythropoietin. Not a natural cytokine, peginesatide is a dimeric peptide of two identical chains of 21 amino acids, MW~4.9 kDa, covalently attached via lysine residues to a structure formed from β -alanine and iminodiacetic acid (Fig. 5.11). The peptide so formed is then pegylated by linking to methoxypolyethylene glycol to give a large molecule of MW~45 kDa. Approved indications and usage were as an erythropoiesis-stimulating agent for the treatment of anemia due to chronic kidney disease in adult patients on dialysis. Warnings and precautions were extensive with a black box warning of the risk of death, myocardial infarction, stroke, thromboembolism, thrombosis, and tumor progression or recurrence. Precautions included the risk of serious allergic reactions and the need for careful use of the drug in patients with existing cardiovascular disease and stroke. Uncontrolled hypertension was listed as a contraindication. Adverse reactions to peginesatide observed in controlled studies of dialysis patients were diarrhea, nausea, vomiting, dyspnea, cough, arteriovenous fistula, headache, muscle pains and spasms, arthralgia, pyrexia, hypotension, hypertension, hyperkalemia, and URTI. Seizures were seen in some patients. Antibodies to peginesatide were detected in 1.2% of patients (29 of 2357); 0.9% (21) were neutralizing antibodies and in approximately half of these patients hemoglobin levels declined. As a result of postmarketing reports of serious hypersensitivity reactions including potentially life-threatening anaphylaxis, the FDA announced a voluntary recall of Ormontys[®] Injection. Fatal reactions were reported in ~0.02% of patients usually within 30 min of the first intravenous dose. The overall rate of hypersensitivity reactions was estimated to be approximately 0.2% with about one third of these being serious reactions.

Bone Morphogenetic Proteins

BMPs are growth factors inducing the formation of bone and cartilage and important signaling proteins in some disease states such as adenocarcinoma and the progression of colon cancer. Of the 20 BMPs so far identified, six, numbers 2–7, belong to the TGF β cytokine family (Table 5.1). BMP-2 and BMP-7 promote the differentiation of osteoblasts and, on the basis of this action, recombinant forms of both cytokines (rhBMP-2 and rhBMP-7) are approved by the FDA for specific uses in orthopedic, oral, and maxillofacial surgery and implant dentistry although up to 85 % of their usage is said to be off-label.

Safety studies on BMPs have a curious and troubling history of discrepancies due to the possible involvement of inadequate peer review and editorial oversight. More recent results with rhBMP-2 indicate a much higher incidence of side effects and complications than reported in the original peer-reviewed and industry-sponsored work. No adverse events following rhBMP-2 administration were reported in 13 of the original studies involving analyses of 780 patients due, it seems, to methodological bias against the control group. Identification of previously unpublished adverse effects, study inconsistencies, and a comparative review of FDA material, revealed an adverse event frequency associated with rhBMP-2 in spine fusion of 10–50 %. In a retrospective review of adverse events associated with the use of rhBMP-2 in spinal surgery, a search of the Manufacturer and User Facility Device Experience Database for the period July 2002 to August 2011 was undertaken. Only 4 of 834 reports described procedures using rhBMP-2 in accordance with the approved indication while 370 reports (44.4 %) stated that the patient required revision surgery or other invasive interventions to deal with the adverse event. The adverse events reported were swelling; fluid collection; osteolysis; pain/radiculopathy; heterotopic bone; pseudarthrosis; surgical site infections and other wound complications; thromboembolic events; respiratory distress; cancer; and some others. In an examination of the prevalence and complications of BMP use in spinal fusion procedures, the following complications and incidences were identified: vertebral osteolysis 44 %; graft migration 31 %; graft subsidence 27 %; formation of neutralizing antibodies to BMP-2 26 %; ectopic/heterotopic bone formation 7 %; and hematoma 3 %.

In accordance with the classification followed in the recent review of rhBMP-2-associated complications by Tannoury and Howard, the main adverse events to this cytokine are considered under the headings of those occurring during lumbar spine surgery and those seen in or after cervical spine surgery. In posterior and transforaminal interbody fusions in particular, postoperative radiculitis may occur after BMP-2 use in lumbar spine surgery, occurring, it seems, without neural compression and possibly because of the formation of ectopic bone. Postoperative radiculitis was estimated to occur in 11.4 % of patients who underwent a minimally invasive transforaminal interbody fusion procedure. Postoperative nerve injury and ectopic bone formation with the use of rhBMP-2 have been reported, the latter with an incidence of 20.8 % compared to 8.3 % in the absence of the cytokine. Other major

adverse events seen following BMP-2 use in lumbar spine surgery include vertebral osteolysis, edema, and retrograde ejaculation. Although the formation of neutralizing antibodies to the bone growth protein is a theoretical concern, there is so far no clinical evidence that that has occurred. Reviews of complications after the use of rhBMP-2 in cervical spine surgery have revealed an incidence of 43 % for osteolysis and graft subsidence and 5.5–17 % for dysphagia and swelling (in particular, the neck) with respiratory difficulties. Other adverse events include hematoma with high doses of rhBMP-2; lucency and subsidence of fusion levels amongst allograft and demineralized bone matrix patients; and complications in posterior cervical fusion with BMP such as neurologic decline, wound complications, and asymptomatic heterotopic ossification.

Besides being a growth factor, BMP-2 receptors are expressed on some tumor cells and it is therefore not surprising that the cytokine has been investigated as a potential carcinogen in studies on breast cancer cells, malignant human gastric epithelial cells, oral cell carcinoma, and the risk of subsequent pancreatic cancer. Concern for the carcinogenic potential of rhBMP-2 was somewhat reinforced by a 2010 FDA Orthopedic and Rehabilitation Devices Advisory Panel report on the Amplify™ rhBMP-2 matrix of increased cancer rates among BMP-2-treated patients. At ≤ 24 months, cancer incidences were patients 5 % and controls 0.9 %; at 60 months, patients 5 %, controls 2.1 %. Other studies have reported tumor-enhancing, tumor-suppressing, or no dependence effects so, in this situation of uncertainty, it would be prudent to very carefully consider the question of the use of BMPs together with the risk-to-benefit ratio for cancer patients requiring spinal fusion.

Less widely used than rhBMP-2 which promotes better bone growth, rhBMP-7, also known as osteogenic protein 1 (OP-1), is a multifunctional growth factor thought to have other possible therapeutic applications besides bone and cartilage growth and development. These hoped-for potential applications include identification and treatment of cancer, and a beneficial role in Parkinson's disease, ankylosing spondylitis, diabetes, and asthma, as well as some diseases of the kidney, liver, intestine, brain, adipose tissue, and cardiovascular system. Apart from an FDA Public Health Notification of life-threatening complications associated with rhBMP (including rhBMP-7) in cervical spine fusion, and the reminder that rhBMPs are contraindicated in skeletally immature or pregnant patients and those with hypersensitivity to the protein, studies on, and reports of, adverse events to BMP-7 are not yet extensive. This is in contrast to the large and rapidly growing literature on rhBMP-2 induced adverse events. In an early clinical trial designed to evaluate rhBMP-7 in the treatment of tibial reunions, adverse events were reported to be mild or moderate and nonserious, for example, fever, nausea and vomiting, leg edema and discomfort, and hematoma at the operative site. Low levels of anti-BMP-7 antibodies were detected in 10 % of the treated patients but all titers were low with no related adverse events. With the possibility in mind that risks following the use of rhBMP-7 in anterior cervical fusion procedures might not be as high as seen with BMP-2, the safety of rhBMP-7 was examined in 123 patients undergoing anterior cervical discectomy and fusion using interbody cages. Assessed

over the first 30 days, there were no deaths or reoperations but 2.4% of patients experienced brachialgia and dysphagia. Although a slight increase on post-operative prevertebral swelling was seen on radiological evaluation, this was judged to be not clinically significant. The authors concluded that BMP-7 can be used safely in anterior cervical fusion surgery.

Pleomorphic sarcomas around heterotropic bone nodules were found in some animals during a study of rhBMP-7 in rats and five cancers, four nonosseous, and one recurrence of chondrosarcoma, occurred in 570 humans receiving OP-1. Note, however, published material on BMP-7 and carcinogenesis that is not related to manufacturers does not appear to be available.

Metreleptin

Leptin, a 167 amino acid protein of MW 16 kDa produced by a number of different cells in different organs but primarily adipocytes, 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. In February 2014, the FDA approved metreleptin, a recombinant analog of leptin (Table 5.2) as replacement therapy to treat leptin deficiency in patients with congenital or acquired generalized lipodystrophy. Metreleptin is not to be used in patients with general obesity, for HIV-related lipodystrophy or in patients with metabolic diseases (e.g., diabetes mellitus) without concurrent generalized lipodystrophy. Neutralizing antibodies may develop to metreleptin and because of this and the possibility of the occurrence of T-cell lymphoma in patients with acquired generalized lipodystrophy, the protein is only available under the Myalept Risk Evaluation and Mitigation Strategy Program.

Kinetic studies on metreleptin in relation to age, sex, production and clearance, demonstrated that the recombinant cytokine's half-life was 3.4 ± 1.5 h, older subjects show decreased production and clearance rates, and females have higher baseline levels which increase with increasing adiposity. In fact, an increased body mass is associated with higher endogenous leptin levels, a higher rate of production, and a longer half-life.

Common side effects observed in early clinical trials were headache, weight loss, hypoglycemia, and abdominal pain. In a randomized, double-blind study designed to evaluate the weight-lowering effect in human obesity of an amylin/leptin drug combination using pramlintide/metreleptin, adverse events specifically due to metreleptin occurring with an incidence of $\geq 5\%$ were 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. Other potentially 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

Ancestim (Table 5.2) is a recombinant human stem cell factor (SCF). Produced in *E. coli*, it is nonglycosylated but, after expression, retains an *N*-terminal methionine and is therefore also referred to as r-met-hSCF. SCF is produced by fibroblasts and endothelial cells in soluble and transmembrane forms, both of which bind to the c-Kit receptor and are biologically active. Sometimes referred to as a pluripotent growth factor, the cytokine is important for hematopoiesis, spermatogenesis, and melanogenesis; nonlethal point mutations in its receptor can cause anemia, impaired fertility, and pigmentation. The c-Kit receptor, also referred to as the stem (or mast) cell growth factor receptor, proto-oncogene c-Kit, tyrosine-protein kinase kit, and CD117, is a receptor tyrosine kinase type III expressed on a number of different cells including mast cells, melanocytes, and germ cells but, importantly for ancestim, on a range of early to mature hematopoietic progenitor cells. Although ancestim shows only weak colony-stimulating activity *in vitro*, it acts in synergy with some other hematopoietic growth factors such as G-CSF, GM-CSF, erythropoietins, and IL-2 to stimulate multiple hematopoietic lineages in humans and some other animals. It also activates mast cells and stimulates melanocyte development and the production of pigment.

Given in combination with filgrastim, ancestim produces increases in circulating peripheral blood progenitor cells (PBPCs) including CD 34⁺ cells compared to filgrastim alone although when given as a single agent, the effect appears to be minimal to weak. Ancestim is therefore used with filgrastim to effect a sustained mobilization of PBPCs and achieve a reduction in the number of apheresis 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. In the first place, for reasons of efficacy and the accumulated findings on combination therapy but not monotherapy, the cytokine should not be used alone. Together with filgrastim, ancestim should only be administered to patients who are at risk of inadequate PBPC mobilization. Care should also be exercised in the simultaneous use of the cytokine combination in patients given chemo/radiotherapy; administration should be avoided 24 h before and after the cytotoxic therapy.

Allergy may be a problem. Because SCF increases mast cell proliferation, adhesion, and survival 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.

The carcinogenic potential of aneastim has so far remained unstudied. Being a growth factor, it may stimulate the growth of a range of possible different tumors, particularly melanomas, mast cell or basophil leukemia, small cell lung cancers, and myeloid malignancies. Other precautions relate to the collection by apheresis of malignant cells, their stimulation and subsequent reinfusion into patients; and leukocytosis (the white cell count should be monitored frequently).

Bearing in mind that almost all reports of adverse events following administration of aneastim originate from treatments in which it is given in combination with myelopoietic-stimulating agents such as filgrastim, there is usually an element of doubt as to which of the two cytokines, or both, are responsible for the observed effects. Injection site reactions, occurring within 1–24 h, are the most commonly observed adverse events with up to 84 % of patients given aneastim showing mild-to-moderate reactions. Reported reactions consisted of erythema (59 %), pruritus (25 %), and urticaria (16 %) with occasional cases of hyperpigmentation and rash at the injection site. Erythema at a previous injection site has been seen in a few patients following an injection of aneastim at a different site. Rash, pruritus, and urticaria have occurred in 18 % of patients given aneastim/filgrastim and 5 % given aneastim alone. Other commonly seen reactions consisting of central/peripheral nervous system, gastrointestinal, cardiac, and respiratory events following aneastim are listed in Table 5.2. Respiratory problems, mainly cough, pharyngitis, and dyspnea, affected 25 % of recipients of combination therapy and 14 % of those on aneastim alone. Systemic allergic reactions, generally moderate to severe but not life threatening, occur more often at higher dosages; at <30 µg/kg/day of aneastim, 5 % of patients had such a reaction while 27 % of patients given 30–100 µg/kg/day had reactions. With regard to immunogenicity of aneastim, 23 of 258 (9 %) produced serum antibodies to the cytokine but no adverse clinical consequences, including a reduced therapeutic effect or serum sickness, have been recorded.

Concluding Remarks

Cytokines have already had a revolutionary impact on our understanding of cellular functions and extracellular messaging but although their biological effects seem to offer great potential for the treatment of a wide range of human conditions, their pleiotropism, potency, and complexity to produce cytokine “cocktails” with signaling cascades and accompanying side effects, demands caution in attempts to introduce individual members into the clinic. The range of biological events set in motion even by individual cytokines, warns of the possibility of unwanted side effects and the resultant caution is reflected by the relatively small number of cytokines currently approved by regulatory agencies and reviewed here. Good examples of the sort of doubts that exist and why clinical developments proceed so cautiously have been illustrated with interferons, aldesleukin, becaplermin, palifermin, and bone morphogenetic proteins. A glance at Table 5.2 shows that 16 of the 23 listed FDA-approved cytokine preparations (19 different cytokines with four also in

pegylated form) carry warnings with 10 of these being black box warnings. Having been used in human therapy for many years, interferon alfa preparations are well known for a number of often widely different, potentially serious side effects specified in boxed warnings. The diverse nature of these side effects including neuropsychiatric, autoimmune, ischemic, and infection adverse events, together with their therapeutic benefits, provides a good illustration of the two-edged nature of cytokine pleiotropism. Becaplermin, a growth factor, causes cell proliferation so the possibility of malignancy with its continued use needs to be kept in mind, especially in patients with known cancers. Likewise, palifermin, another growth factor and a valuable treatment for mucositis in cancer patients, has with it the potential for stimulation of tumor growth, especially since its complementary receptors occur widely on many different cell types in the body. Aldesleukin, the recombinant IL-2, is a potent activator of T lymphocytes and stimulates immune responses to cancer, producing regression of tumors in metastatic renal cell carcinoma and melanoma. However, this activity can also lead to a range of adverse cardiac and pulmonary events. Perhaps, the best example of the safety uncertainties and benefits-to-risk ratios associated with these heterogeneous, pleiotropic cell regulators, is seen with the bone morphogenetic proteins BMP-2 and BMP-7. Already with a troubled history of underreported adverse events, in the postmarketing period, these growth factors are currently a focus of attention and speculation as potential carcinogens. BMP-2 receptors are expressed on some tumor cells and increased rates of cancer following its use have been reported but, in keeping with the complexity of cytokine-induced responses, and the difficulty of ascribing many adverse effects to causes, tumor-suppressing effects, or no dependence, have also been reported.

In any consideration of adverse event profiles of approved biologics, two other potentially important contributing factors need to be recognized. Any drug brought to market under the Orphan Drug Designation program where development was mediated because of the rarity of a condition, may not reveal its full spectrum of adverse effects until well into its postmarketing period since a relatively smaller number of administrations results from a smaller pool of patients. The dose of a particular cytokine may also be of critical importance in avoiding dangerous side effects by narrowing the spectrum of activity of the pleiotropic agent and tipping the balance to a specific biological activity.

Lest the attention drawn in this review to the known and potential toxicities of cytokines obscures their often substantial benefits and the improved outcomes they can produce, readers are reminded that the focus here on adverse effects does not negate the clear clinical improvements each of the approved cytokines can bring. Cytokines may indeed sometimes provoke a wide range and number of toxicities and adverse events but, overall, the second edge of their pleiotropism often offsets the side effects profiles and this is reflected in their lists of indications and approved regulatory status. In fact, in some cases, toxicities correspond with improved outcomes. For example, in an assessment of the significance of autoimmunity in melanoma patients treated with interferon alfa-2b, interferon-induced autoimmunity was found to be a prognostic marker for improved relapse-free, and overall, survival.

Together with monoclonal antibodies, chimeric fusion proteins, vaccines, a range of recombinant enzymes, hormones, clotting factors, various receptor proteins, a few purified approved toxins and some cell-based and nonspecific adjuvant therapies, the pool of over 130 cytokines seems to offer, via genetically engineered or modifications of the natural proteins, the potential of a major expansion of biologic therapies, some revolutionary, over the forthcoming decade or less. Meanwhile, the relatively few currently approved recombinant cytokines are already revealing their true natures in relation to their efficacy and side effects, influenced above all by their pleiotropism, redundancies and potencies. The large range of activities displayed by the family of cytokine proteins, together with their potential for the treatment of many different diseases and our steadily accumulating knowledge and experience with the small number currently used clinically, may indeed end up helping to achieve the prediction that the future of therapy belongs to the emerging biologics.

Summary

- Cytokines, currently known to be more than 130 in number, are relatively small signaling proteins of MW < 30 kDa. They are usually glycosylated and produced by a variety of different cells including those of the immune system, epithelia, endothelia, and stroma. Cytokines are key modulators of the immune and inflammatory responses functioning in an autocrine, paracrine, or endocrine manner in infection, innate and adaptive immunity, autoimmunity, inflammation, and malignancy. Key to an understanding of these regulatory proteins is the recognition of their pleiotropism and sometimes overlapping activities, functional redundancies, and side effects.
- In the current genomic phase, cytokines are identified on the basis of homology with known, characterized cytokines. Many original names are still in use and many of the originally described “factors” share receptors with other cytokines, for example, some interleukins.
- For the 23 FDA-approved cytokine products from the CDER-approved Biologic Products list, the cytokine classification presented is based on the Kyoto Encyclopedia of Genes and Genomes.
- Nine main families are recognized with most of the cytokines of interest classified in the hematopoietic growth factor, interferon (IFN), platelet-derived growth factor (PDGF), and transforming growth factor β (TGF β) families. The approved cytokines are manufactured by recombinant DNA technology.
- Hematopoietin family: aldesleukin (rh-interleukin-2 [IL-2]), oprelvekin (rhIL-11), filgrastim and tbo-filgrastim (rh-granulocyte colony-stimulating factor [G-CSF]), sargramostim (rh-granulocyte macrophage [GM]-CSF), metreleptin (rh-leptin), rh-erythropoietins, epoetin and darbepoetin alfa, and stem cell factor (r-met-hSCF); IL-1 cytokine family: anakinra, a recombinant receptor antagonist for IL-1; interferon family: recombinant interferons alfa-1, alfa-2, beta-1, and

gamma-1; PDGF family: palifermin (rh-keratinocyte growth factor [KGF]) and becaplermin (rhPDGF-BB); and TGF β family: rh-bone morphogenetic protein [BMP]-2 and rhBMP-7.

- Interferons are a class of broad-spectrum antiviral cytokines with overlapping, but also some individual, activities. Of most interest for therapy are interferons alfa, beta, and gamma. Virtually all patients treated with interferon alfa experience some adverse effect(s) at some time during therapy.
- Interferon alfa preparations occasionally provoke an extensive range of adverse reactions including cardiovascular, respiratory, endocrine, hematologic, metabolic, urinary tract, and skin adverse events as well as adverse effects on the nervous and sensory systems.
- Peginterferon alfa-2a and peginterferon alfa-2b are covalent conjugates of the recombinant interferon with a single branched bis-monomethoxy polyethylene glycol (PEG) chain. Pegylation helps to protect the protein from immune recognition and increases the molecule's size thus extending protein half-life and circulatory time and reducing renal clearance.
- Peginterferon alfa-2a together with ribavirin is indicated for the treatment of chronic hepatitis C in adults who have compensated liver disease and for patients infected with hepatitis C and HIV. Peginterferon alfa-2a alone is approved for patients with chronic hepatitis B.
- Interferon alfa-2b is administered extensively for hepatitis B and C as well as several malignancies.
- Interferon alfa-induced neuropsychiatric disorders, particularly depression, cognitive dysfunction, and mania are well known and have been intensively studied. Of the patients who develop severe depressive symptoms, most occur within the first 3 months of treatment. The incidence of depressive disorders is estimated to be 23–41 %. Symptoms may be prolonged for 6 months or more after the cessation of therapy.
- Autoantibodies and development or exacerbation of autoimmune diseases including hypothyroidism, immune-mediated hemolysis, systemic lupus erythematosus, Raynaud's disease, and mixed connective tissue disease are known to occur in response to interferon alfa therapy.
- Pegylated interferon alfa-2b has been associated with acute myocardial infarction, pericarditis, pericardial effusion with tamponade, and sick sinus syndrome producing arrhythmias.
- Interstitial lung disease, reported for both interferon alfa-2a and 2b, is seen more frequently with the former agent and with high doses of the latter. Cases of fatal interstitial pneumonitis, adult respiratory distress syndrome, and bronchiolitis obliterans organizing pneumonia (BOOP) following pegylated interferon alfa-2b are known.
- Interferon alfa may have adverse effects on the nervous system in the form of seizures in patients with no history of epilepsy, involuntary facial movements and weakness, features resembling multiple sclerosis, restless legs syndrome, sensorimotor polyneuropathy, and Bell's palsy.

- Adverse effects on sensory systems, mainly not only the eyes but also the ears, occur particularly to interferon alfa-2b. Ocular complications include occlusive vasculitis, central retinal artery occlusion, anterior ischemic optic neuropathy, retinal hemorrhage, subconjunctival hemorrhage, and optic nerve edema. Other ocular complications described in patients treated with interferon alfa-2b include permanent loss of sight due to combined retinal artery and central retinal vein obstruction; development of an epiretinal membrane; and the T-cell-mediated autoimmune syndrome, Vogt–Koyanagi–Harada disease.
- Endocrine effects of interferon alfa, best illustrated by thyroid dysfunction, may have an autoimmune mechanism. It occurs with an incidence of 5–14% in patients treated for chronic hepatitis C. Hypothyroidism occurs more often than hyperthyroidism. Interferon alfa-2b can cause both conditions.
- Neutropenia induced by interferon alfa is fairly commonly seen. Other hematologic side effects include acute and autoimmune thrombocytopenia, pernicious anemia, bone marrow hypoplasia which may be immune mediated, and pure red cell aplasia.
- Renal complications to interferon alfa include renal thrombotic microangiopathy, acute nephrotic syndrome, hemolytic-uremic syndrome, renal insufficiency due to interstitial nephritis, tubular necrosis, and IgA nephropathy.
- The list of cutaneous reactions to interferon alfa is extensive and includes injection site reactions (erythema, necrosis, and vasculitis), pruritus, xerosis, urticaria, hyperpigmentation, psoriasis, alopecia, lichen planus, pityriasis rosea, sarcoid nodules, eosinophilic fasciitis, livedo reticularis, vitiligo, and fixed drug eruption. Interferon alfa is well known for exacerbating pre-existing psoriasis but cases of new onset psoriasis occur with both interferon alfa-2a and interferon alfa-2b.
- A flu-like illness is the most commonly occurring adverse event following administration of interferon beta and injection site reactions are also common.
- Neutralizing antibodies are found in about a quarter of patients treated with subcutaneously administered interferon beta-1b and the consensus is that they neutralize or reduce the cytokine's activity. Other immunologic effects observed to both beta interferons are some cases of a lupus-like syndrome and cutaneous lymphocytic vasculitis.
- Unlike interferon alfa, results from studies do not support an association of interferon beta with depression but the FDA mention depression, suicide, and psychotic disorders in their warnings and precautions for the cytokine.
- Interferon beta can induce thyroid disorders notably hyperthyroidism and a severe case of hypothyroidism resembling Hashimoto's encephalopathy has been reported.
- Skin reactions include urticaria to interferon beta-1a and an acneiform eruption to interferon beta-1b.
- In 2014, the FDA granted approval for Plegridy[®], a pegylated preparation of interferon beta-1a. Common adverse reactions to Plegridy[®] are similar to the nonpegylated form of the cytokine, viz., injection site reactions, an influenza-like illness, asthenia, arthralgia, and pruritus.

- Cardiovascular toxicity to interferon gamma, particularly at higher doses, include hypotension, arrhythmias, coronary vasospasm and ventricular tachycardia, and renal toxicity, namely acute renal failure, nephrotic syndrome, and tubular necrosis.
- The occurrence of fatal acute respiratory failure in some patients treated with interferon gamma-1b for advanced idiopathic pulmonary fibrosis prompted further investigation of the condition. No clear evidence for the involvement of interferon gamma-1b was found.
- Described as “the master regulators of granulocyte and macrophage populations,” the colony-stimulating factors (CSFs) are used to treat chemotherapy-induced neutropenia, mobilize stem cells for transplantation, and enhance the immune response to cancer. Currently, approved members of the CSF family are filgrastim and pegfilgrastim, both G-CSFs, sargramostim, a GM-CSF, and tbofilgrastim, a short acting biosimilar G-CSF.
- CSF-induced adverse events are usually mild and transient including headache, bone pain, myalgia, fever, flushing, and rash. More severe, and rare, events are adult respiratory distress syndrome, pulmonary toxicities, particularly pulmonary edema and interstitial pneumonitis, fluid retention, aortitis, capillary leak syndrome, thrombocytopenia, splenomegaly, and spleen rupture. G-CSF may be a risk for the progression of myelodysplastic syndrome. Other potentially life-threatening responses to CSFs, the subject of warnings, are anaphylactic/anaphylactoid reactions and severe adverse events such as acute chest syndrome, vaso-occlusive episodes, multiorgan failure, and death seen in patients with sickle cell disease.
- There is a long list of adverse skin reactions provoked by CSFs. The most commonly occurring cutaneous reaction is Sweet’s syndrome seen after therapy with sargramostim and filgrastim. Other adverse cutaneous events include psoriasis flare, pyogenic granulomas, pruritic erythematous maculopapular eruptions, palmoplantar pustulosis, erythema multiforme, and neutrophilic dermatoses.
- Recombinant human IL-11, or oprelvekin, is used to prevent chemotherapy-induced thrombocytopenia and reduce the need for platelet transfusions in patients with nonmyeloid malignancies. Fluid retention and an increase in plasma volume underlie many of the adverse events, for example, edema, dyspnea, pleural effusions, arrhythmia, dilutional anemia, and renal failure, and indicate that oprelvekin should be used with caution in patients with congestive heart failure.
- Becaplermin is a recombinant human platelet-derived growth factor (PDGF), a homodimer made up of two disulfide-bonded B chains and hence written as rhPDGF-BB.
- rhPDGF-BB promotes the growth of granulation tissue and wound healing via interaction with receptors on fibroblasts and endothelial cells. Becaplermin has therefore found use in gel form as a topical application for patients with difficult to heal diabetic neuropathic ulcers.
- The FDA has issued a boxed warning for becaplermin gel to the effect that becaplermin should be used with caution in patients with known malignancy and only used when the benefits can be expected to outweigh the risks.

- Palifermin, a recombinant human keratinocyte growth factor produced by mesenchymal cells and fibroblasts, stimulates differentiation, proliferation, and migration of epithelial cells via interaction with its complementary receptors on epithelial cells widely distributed in numerous tissues. Palifermin is an important 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 administration include rash, pruritus, erythema, paresthesia, edema, taste alteration, rhinitis, arthralgia, thickening of the tongue, and numbness. Numerous cutaneous reactions include acral erythema, a papulopustular eruption on the head and trunk, hyperpigmented papillomatous plaques in the axillae and inguinal areas, and a case of lichenoid papules. Being a growth factor, palifermin carries a warning of potential stimulation of tumor growth.
- Aldesleukin, a recombinant human IL-2, differs from the natural cytokine by absence of glycan residues and at position 125 and the end terminal amino acid. X-ray and NMR studies have shown that the IL-2 fold is similar to that seen in the myelopoietic-stimulatory factors G-CSF and GM-CSF.
- Cardiovascular adverse events are the main dose-limiting toxicities of aldesleukin with cases of hypotension, tachycardia, peripheral edema, pleural effusions, myocarditis, myocardial infarction, heart block, arrhythmias, cardiac eosinophilic infiltration, and coronary ischemic changes. Vascular leak syndrome causes hypovolemia, fluid accumulation in the extravascular spaces, oliguria, and pulmonary side effects. Hematologic adverse effects, particularly anemia, leukopenia, and thrombocytopenia occur but are rarely severe or dose limiting.
- Endocrine effects of aldesleukin usually manifest as hypothyroidism or, far less commonly, hyperthyroidism. Gastrointestinal perforation has been reported and IL-2-induced infectious toxicities, usually due to *Staphylococcus*, may occur at venous catheter sites. Neurological effects, especially to high doses during IL-2 therapy, include anxiety, depression, altered sleep patterns, somnolence, emotional fragility, vivid dreams, and confusion.
- The list of aldesleukin-induced cutaneous reactions is extensive, ranging from mild erythema, pruritus, injection site reactions and vitiligo, to urticaria, angioedema, reactivation of eczema, exacerbation of psoriasis, generalized erythema followed by desquamation, vasculitis, and severe manifestations like pemphigus, IgA bullous dermatosis, and toxic epidermal necrolysis.
- Anakinra 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. Anakinra acts as a biological response modifier in the treatment of diseases like rheumatoid arthritis and the spectrum of autoinflammatory syndromes collectively known as cryopyrin-associated periodic syndrome (CAPS).
- The most common adverse events to anakinra are injection site reactions which occur in up to 73 % of patients and infection episodes. Other reported side effects include progression of rheumatoid arthritis, exacerbation of Crohn's disease, anaphylaxis, systemic inflammatory response syndrome, ARDS, an interstitial granulomatous reaction, and some severe skin reactions.

- The main adverse effects of epoetin (rhEPO) in anemic patients with end-stage renal disease are myalgias, iron deficiency, elevated blood pressure, and seizures. Hypertension is a common side effect with approximately one-third of dialysis patients affected and hypertension and increased viscosity due to rhEPO may lead to encephalopathy, convulsions, cerebral edema, and seizures. Controlled studies on cancer patients revealed a higher risk of thromboembolic events with rhEPO therapy than controls.
- Cerebral ischemia with increased metabolic rate and blood viscosity is a potentially severe side effect of EPO. 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.
- Both epoetin alfa and darbepoetin alfa carry a boxed warning related to a possible increased risk of death, cardiac, and thromboembolic events and tumor progression or recurrence.
- In cases of transfusion-dependent pure red cell aplasia (PRCA) with neutralizing serum antibodies to EPO, patients should not be switched to another epoetin such as darbepoetin alfa since a systemic anaphylaxis/anaphylactoid response mediated by anti-EPO antibodies cross-reactive antibodies may result.
- An investigation of the effect of darbepoetin alfa in anemic patients with symptomatic chronic heart failure revealed three main adverse events: neurological signs and symptoms, upper respiratory tract infections, and joint-related signs and symptoms.
- Bone morphogenetic proteins (BMPs) are growth factors inducing the formation of bone and cartilage and important signaling proteins in some disease states such as adenocarcinoma and the progression of colon cancer. BMP-2 and BMP-7 promote the differentiation of osteoblasts and, on the basis of this action, recombinant forms of both cytokines (rhBMP-2 and rhBMP-7) are approved by the FDA for specific uses in orthopedic, oral, and maxillofacial surgery and implant dentistry although up to 85 % of their usage is said to be off-label.
- Recent results with rhBMP-2 indicate a much higher incidence of side effects and complications than originally reported. Identification of previously unpublished adverse effects, study inconsistencies, and a comparative review of FDA material has revealed an adverse events frequency associated with rhBMP-2 in spine fusion of 10–50 %. Reported adverse events were swelling, fluid collection, osteolysis, pain/radiculopathy, heterotopic bone, pseudarthrosis, surgical site infections and other wound complications, thromboembolic events, respiratory distress, and cancer.
- In an examination of the prevalence and complications of BMP use in spinal fusion procedures, the following complications were identified: vertebral osteolysis, graft migration, graft subsidence, formation of neutralizing antibodies to BMP-2, ectopic/heterotopic bone formation, and hematoma.
- Concern for the carcinogenic potential of rhBMP-2 was reinforced by a 2010 FDA Orthopedic and Rehabilitation Devices Advisory Panel report on the Amplify™ rhBMP-2 matrix of increased cancer rates among BMP-2-treated patients. At ≤ 60 months cancer incidences were patients 5 % and controls 2.1 %. Other studies have reported tumor-enhancing, tumor-suppressing, or no dependence effects.

- rhBMP-7, also known as osteogenic protein 1 (OP-1), is a multifunctional growth factor. Apart from an FDA Public Health Notification of life-threatening complications associated with rhBMP (including rhBMP-7, in cervical spine fusion), and the reminder that rhBMPs are contraindicated in skeletally immature or pregnant patients and those with hypersensitivity to the protein, studies on, and reports of, adverse events to BMP-7 are not yet extensive.
- In a clinical trial designed to evaluate rhBMP-7 in the treatment of tibial reunions, adverse events were mild or moderate and nonserious, for example, fever, nausea, vomiting, leg edema and discomfort, and hematoma at the operative site. Low levels of anti-BMP-7 antibodies were detected in 10% of the treated patients, but all titers were low with no related adverse events.
- Leptin, a 167 amino acid protein of MW 16 kDa produced primarily by adipocytes, 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. Metreleptin, a recombinant analog of leptin is used as replacement therapy to treat leptin deficiency in patients with congenital or acquired generalized lipodystrophy. It is not to be used in patients with general obesity, for HIV-related lipodystrophy or in patients with metabolic diseases (e.g., diabetes mellitus) without concurrent generalized lipodystrophy.
- Neutralizing antibodies may develop to metreleptin. Because of this, the possibility of worsening of renal disease, and the occurrence of T-cell lymphoma in patients with acquired generalized lipodystrophy, the protein is only available under the Myalept Risk Evaluation and Mitigation Strategy Program.
- Ancestim is a recombinant human stem cell factor (SCF) important for hematopoiesis, spermatogenesis, and melanogenesis. It acts in synergy with some other hematopoietic growth factors such as G-CSF, GM-CSF, erythropoietins, and IL-2 to stimulate multiple hematopoietic lineages.
- Together with filgrastim, ancestim should only be administered to patients who are at risk of inadequate peripheral blood progenitor cell (PBPC) mobilization. Ancestim is used with filgrastim to effect a sustained mobilization of PBPCs and achieve a reduction in the number of apheresis required. Care should be exercised in the simultaneous use of the cytokine combination in patients given chemo/radiotherapy; administration should be avoided 24 h before and after the cytotoxic therapy.
- Patients with a history of anaphylaxis, asthma, recurrent urticaria, angioedema, and mast cell diseases such as systemic mastocytosis who are to be given ancestim may be at particular risk and should be premedicated with H1 and H2 anti-histamines and a bronchodilator.
- The range of biological events set in motion even by individual cytokines, warns of the possibility of unwanted side effects and the resultant caution is reflected by the relatively small number of cytokines currently approved by regulatory agencies. Sixteen of the 23 listed FDA-approved cytokine preparations carry warnings with ten of these being black box warnings.
- The diverse nature of interferon side effects, including neuropsychiatric, autoimmune, ischemic, and infection adverse events, together with their therapeutic

benefits, provides a good illustration of the two-edged nature of cytokine pleiotropism. Despite this, cytokine adverse events profiles do not generally negate benefits and sometimes observed toxicities may even correspond with improved outcomes.

Further Reading

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