Chapter 12

Pharmacological Properties of Monoclonal Antibodies Directed Against Interleukins

Kaloyan Georgiev and Marieta Georgieva

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/61774

Abstract

The road to individualized therapy goes through detecting specific targets (e.g., antigens), suitable for influence, and their selective targeting by using specially designed molecules (e.g., antibodies). A significant advance in this area is the development of therapeutic monoclonal antibodies. This approach enables maximizing the therapeutic effect on one hand, and reducing systemic toxicity on the other hand. In recent years, significant progress was made in improving their pharmacological performance – pharmacokinetics (longer half-life) and pharmacodynamics properties (better efficacy because of stronger affinity to human receptor), and safety profile (less antigenic and immunogenic reactions). Interleukins are a diverse, multifunctional group of proteins that carry out communication between various immune cells and control their gene expression. They manage the intensity and magnitude of an inflammatory response, and control differentiation, proliferation, and secretion of antibodies. Therefore, interleukin network represents an interesting pharmacological target, modulation of which using either biological or small chemical agents could contribute to suppression of excessive activated immune system and successfully treat the diseases that they are involved in.

Keywords: Monoclonal antibodies, pharmacological properties, pharmacokinetics, pharmacodynamics, cytokines, interleukins

1. Introduction

An effective immune response is possible only through the interaction of several cell types. To coordinate this process, there are a number of mechanisms for communication between immune cells, including a plurality of immunomodulating signaling molecules, such as cytokines. An officially recognized definition of the cytokines does not exist. Cytokines are a group of regulatory molecules with protein or glycoprotein structure (relatively small

molecular mass ~10-35 kDa) that carry intercellular signals between immune system cells. The name "cytokine" was coined to describe both – a cell, cyto, and a movement, kinos. Due to the immense structural and functional differences between cytokines their classification is very difficult. In natural immunity, the effector cytokines are mostly produced by mononuclear phagocytes and therefore often called monokines. Although monokines can be elicited directly by microbes, they can be also secreted by mononuclear phagocytes in response to antigen-stimulated T-cells, i.e., as part of specific immunity. Most cytokines in specific immunity are made by activated T-lymphocytes, and these molecules are often called lymphokines. T-cells produce several cytokines that function primarily to regulate the growth and differentiation of various lymphocytes and play important roles in the activation phase of immune response. The major group among cytokines is the group of interleukins, which are produced by a plurality of nuclear cells in response to various stimuli. The main functions of the interleukins are:

- Mediating the inflammatory response.
- Involved in the Th1- and Th2-immune response.
- Lymphocyte growth and differentiation.
- Chemoattractants for lymphocytes and polymorph nuclear leukocytes.
- Play role in hematopoiesis.
- Some of them could inhibit inflammatory process (e.g., IL-10, IL-13).

Therefore, the interleukins represent a huge interest for researchers. Their role in plenty of diseases with excessive activity of the immune system contributes to this as well. Antagonists of the interleukins or their receptors act immunosuppressive and are successfully applied in such disorders. The name "interleukins" was proposed by scientists in 1979 during the Second International Lymphokine Workshop in Switzerland and comes from the prefix inter, which means between (carrying out communication) and leukins, which determines their origin and their action (production from and influence on leukocytes). [1] By 1978, with the introduction of modern methods of purification of proteins, it became clear that interleukin (IL) can be separated into two proteins – IL-1 and IL-2 – depending on cell targets and functions. By that time, it was known that Interleukin-1, which is produced by monocytes/macrophages, was a lymphocyte-activating factor and Interleukin-2 was a T-cell growth factor, thymocyte-stimulating factor, and costimulator. Today it is clear that IL-2 supports the growth of natural-killer (NK) T-cells and especially the subpopulation of NK cells known as lymphokine activated killer (LAK) cells, which are involved in killing tumor cells. This discovery contributed to the approval of IL-2 as a cancer therapeutic drug that is able to stimulate the recruitment and expansion of natural killer cells in order to attack tumor cells.

Nowadays 37 different interleukins are known, the numbering of which is in order of increase in numbers from 1 to 37. [2] Approved monoclonal antibodies affect certain members of the family of interleukins, such as IL-1, IL-2, IL-6, IL12/23, or IL-17, the function of which is considerably studied and proven (Tables 1 and 2).

http://dx.doi.org/10.5772/61774

Interleukins	Origin	Biological function	References
IL-1	Macrophage, Monocytes, Fibroblast, and Dendritic cells	Inflammation, fever, activation of T- and B-cells	[2, 3, 4]
IL-2	Th type 1-cells	T-cell proliferation and expansion	[7, 8]
IL-6	Macrophage, Monocytes, Th type 2-cells, B-cells	Inflammation, fever, activation of B-cells	[14, 15]
IL-12	Macrophage, B and T-cells, Dendritic cells	Synergistic with IL-2, INF-γ, and TNF-α production in T-cells	[20, 21, 22, 23]
IL-17	Th type 17-cells	Inflammation, angiogenesis	[27]
IL-23	Antigen-presenting cells (APC)	INF-γ production, reduce CD8+ T-cell proliferation, angiogenesis	[20, 21, 22, 23]

Table 1. Some interleukins, their origin, and biological function.

MAb	Molecular type	Interleukin target(s)	Indication(s)
Canakinumab	Human	IL-1β	Cryopyrin-associated periodic syndromes (CAPS), active systemic juvenile idiopathic arthritis (SJIA)
Basiliximab	Chimeric	IL-2 (CD25)	Prevention of acute organ rejection
Daclizumab	Humanized	IL-2 (CD25)	Prevention of acute organ rejection
Tocilizumab	Humanized	IL-6	Rheumatoid arthritis (RA)
Ustekinumab	Human	IL-12/23	Plaque psoriasis, psoriatic arthritis
Secukinumab	Human	IL-17A	Plaque psoriasis

Table 2. Approved monoclonal antibodies directed against interleukins.

1.1. Therapeutic monoclonal antibodies

Therapeutic monoclonal antibodies have gained large attention over recent decades because of their desirable features, such as high potency and safety profile. Nowadays, they are used in almost all clinical fields, ranging from toxin and pathogen neutralization or clearance to influence endogenous cytokine, treating cancer, and in modulation of many other diseases. [3] The first monoclonal antibodies were generated in mice in 1975 using a hybridoma technique, first described by Kohler and Milstein [4]. They were rewarded ten years later, in 1984, with the Nobel Prize in Medicine for their discovery. The key feature of a monoclonal antibody is its unique specificity. Monoclonal antibodies are monovalent antibodies that bind to the same epitope and are produced from a single B-lymphocyte clone. This homogeneity will give rise to the same immunological effector functions. In principle, monoclonal antibodies can be produced in unlimited quantities, because the hybridoma cell itself survives after cryopreservation at least for decades. Monoclonal antibodies are classified according to an international terminology (Table 3).

Prefix	Target infix	Origin infix	Suffix
Variable	-o(s)-: Bone	-u-: Human	-mab
	-vi(r)-: Virus	-o-: Mouse	
	-ba(c)-: Bacteria	-a-: Rat	
	-li(m)-: Immune system	-e-: Hamster	
	-ci(r)-: Cardiovascular System	-xi-: Chimeric (e.g., mouse-human or hamster-human)	
	-tu(m)-: Tumor (general)	-zu-: Humanized	
	-neu(r)-: Nervous system	-axo-: Hybrid (rat-mouse	
	-ki(n)-: Interleukin		
	-mu(l)-: Musculoscelettal System		
	-tox(a)-: Toxine		

Table 3. Classification of monoclonal antibodies according to an international terminology.

Single syllables of the name suggest the origin and therapeutic area in which they are used. Examples: Cana-kin-u-mab – human monoclonal antibody directed against interleukins; Basi-li-xi-mab – chimeric immunotropic monoclonal antibody, etc.

The first licensed monoclonal antibody was Orthoclone OKT3 (muromonab-CD3), which was approved in 1986 for use in preventing kidney transplant rejection. [5] It is a monoclonal mouse IgG2a antibody whose cognate antigen is CD3. It works by binding to ε (epsilon) – chain of the CD3-proteins expressed on T-lymphocytes, inhibits CD3-associate effects and interrupts T-cell receptor mediated signal transduction. However, due to significant reported side effects, its use was limited to acute cases. [6] One of the most important adverse effects is a cascade of systemic cytokine release that has been termed cytokine release syndrome (CRS) or cytokine storm. CRS is associated with increased serum levels of cytokines (e.g., TNFα, IL-2, IL-6, INF-γ) that peak between 1 and 4 h after dose, duration 12–16 h, and severity could be mild to life-threatening. Signs and symptoms include fever, chilling, dyspnea, wheezing, chest pain and tightness, nausea, vomiting, and diarrhea. Hypervolemic pulmonary edema, nephrotoxicity, meningitis, and encephalopathy are possible [7].

1.2. Targeting IL-1

Although the family of interleukin-1 incorporates many members, two of them are studied best – IL1α and IL-1β, encoded by two related but distinct IL-1 genes – IL1A and IL1B, respectively. The both interleukins are strongly proinflammatory molecules that modulate the peripheral immune response during infection and inflammation [8]. These cytokines increase the expression and activity of adhesion molecules (e.g., VCAM-1, ICAM-1, L-selectine) that promote attraction of immunocompetent cells to the site of infection. The more potent inflammatory cytokine is IL-1β, which has been demonstrated in numerous in vitro and in vivo animal models [9]. Two types of IL-1 receptor are cloned with different physiological and

http://dx.doi.org/10.5772/61774

pharmacological characteristics: IL-1R1 and IL-1R2, respectively [10]. There is also a natural glycoprotein inhibitor of the receptors for IL-1, IL-1Ra (IL-1 receptor antagonist), which modulates the effects of both cytokines by competing with them for binding sites of the receptor, and a number of other molecules that directly regulate IL-1 activity, such as the IL-1 receptor type I (IL-1RI), the decoy receptor IL-1 receptor type II (IL-1RII), its coreceptor IL-1 receptor accessory protein (IL-1RAcP), and soluble receptor forms.

In humans, IL-1 plays a major role in bone resorption and cartilage destruction by inducing prostaglandin E2 and proteolytic enzymes, such as matrix metalloproteinase. [11] This led to the development of interleukin-1 inhibitors, as a possible therapeutic strategy in rheumatoid arthritis and other chronic inflammatory diseases.

IL-1β plays a crucial role in the pathogenesis of multiple myeloma (MM) [12]. This is based on the fact that IL-1β is the main cytokine in the bone marrow, which increases production of the paracrine IL-6, the primary growth factor, responsible for survival and expansion of the myeloma cells. Reducing IL-1β activity could be a possible way for slowing disease progression and induction of the chronic disease state in patients with smoldering or indolent multiple myeloma, as shown the results from phase II clinical trial with IL-1RA [13]. Since the receptor antagonist – anakinra – has a short plasma half-life and the need for frequent administration, the monoclonal antibodies would be superior options due to their longer plasma half-lives and less frequent administration.

The role of IL-1β was established in both types of diabetes. In very low concentrations (picomolar), IL-1β is able to destroy insulin-producing pancreatic β-cells [14]. High glucose concentrations stimulate IL-1β production from the β-cell itself [15], thus implicating a self-destructive role for IL-1β autoinflammation by the β-cell and the recruitment of immune cells via IL-1β-driven chemokines [16]. The IL-1β derives either from the β-cell itself or from the infiltrated blood monocytes in the islet. Caspase-1 dependent IL-1β production has been observed in macrophages available in human adipose tissues, which demonstrate the connection between obesity and type 2 diabetes [17, 18]. Improved insulin sensitivity and decreased insulin resistance have been shown in diabetic mice, administered with caspase-1 inhibitor [17].

IL-1 takes part in cardiovascular events, such as stroke, myocardial infarction, kidney failure, liver failure, acute lung injury, each one of them with rapid loss of function. The ischemic event in the myocardial infarction and thrombotic stroke starts with a sudden blockage of a blood vessel by a clot formatted after atherosclerotic plaque rupture. The result of the blockage is hypoxia (decreased oxygen supply) and death of the cells [19].

Pharmacological approaches affecting the function of IL-1 include:

- Using recombinant nonglycosylated molecule of human IL-1 receptor antagonist (IL-1RA) produced in E.coli – Anakinra, marketed as Kiniret® (Amgen). It was introduced to the practice in 1993 and it blocks the activity of both IL-1α and IL-1β. Anakinra currently dominates the field of IL-1 therapeutics and is the drug of choice in cryopyrin-associated periodic syndromes (CAPS), and it is also prescribed to treat rheumatoid arthritis in patients in whom one or more disease-modifying anti-rheumatic drugs (DMARDs) have failed.

- Using fusion protein, consisting of the ligand-binding domains of the extracellular portions of the human interleukin-1 receptor component (IL-1R1) and IL-1 receptor accessory protein (IL-1RAcP) linked to the Fc region of human IgG1 – Rilonacept (Arcalyst®), currently in use to treat CAPS.
- Using human monoclonal antibody against IL-1β – Canakinumab or Gevokizumab.
- Orally active small-molecule inhibitors of IL-1 production, such as caspase 1 inhibitors, which are under investigation.

1.3. Pharmacological properties of canakinumab

Canakinumab (ACZ885) is a high-affinity human monoclonal anti-interleukin-1β antibody, with molecular size ~150 kDa, designed to bind and neutralize the activity of human IL-1β. It is registered under trade name Ilaris® (Novartis) and is approved for treatment of cryopyrin-associated periodic syndromes (CAPS) and active systemic juvenile idiopathic arthritis (SJIA). The CAPS cover various progressive, hereditary inflammatory diseases caused by a mutation of the NALP3 gene. The NALP3 protein (cryopyrin) is a component of a protein complex, named inflammasome. The active inflammasome complex activates the enzyme caspase-1, which cleaves pro-IL-1β to the biologically active IL-1β. NALP3 regulates IL-1β levels. If there is a mutation, even a relatively weak stress agent such as cold is enough to stimulate the synthesis of IL-1β [20]. After administration, canakinumab bind with high affinity to IL-1β and form a complex – canakinumab-IL-1β. This complex is eliminated very slowly, due to its larger size, and this leads to increasing plasma concentrations of both unbound and bound IL-1β. Total IL-1β concentrations can therefore be used as a surrogate pharmacodynamic marker of "drug activity" (i.e., binding of IL-1β by the antibody), as it is easily detected following canakinumab administration [21]. From the following mechanism of action can be expected typical pharmacokinetic properties of human IgG-type immunoglobulins like slow serum clearance (0.174 L/day), low total volume of distribution at steady state (Vss ~ 6.0 L), and a long elimination half-life of 26 days. Bioavailability after subcutaneous administration is 70%. Canakinumab shows dose-dependent (linear) pharmacokinetics both given as an intravenous infusion and as a single subcutaneous administration [21]. In clinical trials, canakinumab achieved a complete and sustained remission in patients with CAPS and a mutation in the NALP3 gene, regardless of phenotype and severity. The effect occurs rapidly. After a single subcutaneous administration 97% of IL-1β-mediated symptoms (such as fever, joint and muscle pain, skin rashes, tissue damage and inflammation) had disappeared after eight weeks. A 90% remained symptom-free for up to one year if they received canakinumab injection in eight-week intervals [22]. In general, the adverse effects of canakinumab are mild to moderate. The most common adverse effects (>10% of treated patients) are reactions at the injection site, inflammation of the upper respiratory tract or sinuses, and headache [23].

1.4. Pharmacological properties of gevokizumab

Gevokizumab (XOMA 052, XOMA Corporation, Berkeley, CA, USA) is a recombinant, humanized IgG2 monoclonal antibody that binds to IL-1RI allosterically, reducing the

formation of the IL-1RI:IL1RAcP signaling complex [24], which distinguishes it from other monoclonal antibodies. The clinical significance of these differences is not known.

Gevokizumab is produced in Chinese hamster ovary cells and has shown activity on animal models against RA, gout, and Type 2 diabetes. Additionally, in mouse models of myocardial infarction and atherosclerosis, gevokizumab maintained left ventricular function [25] and decreased markers of atherosclerosis [26], respectively. Gevokizumab was investigated in a randomized, placebo-controlled, dose-escalation first-in-human study conducted in the United States (NCT00513214) and Switzerland (NCT00541983) on patients with Type 2 diabetes. For the purposes of the study, single and multiple doses of gevokizumab were given i.v. or s.c. to 98 patients with poor-controll diabetes, which are treated with standard anti-diabetes therapy [27]. In this study, gevokizumab was well-tolerated. The most serious adverse reactions were carcinomatous appendix and occlusion of carotid artery, which were not considered to be drug-related. One insulin-dependent patient experienced episodic symptoms of hypoglycemia, which was not considered to be related to treatment. The changes of pharmacokinetic parameters of gevokizumab were dose-related. The mean half-life was 22–23 days, which allows subcutaneous application once a month. After this study, XOMA started a IIb phase study of gevokizumab in 421 Type 2 diabetic patients at multiple sites in the United States during 2010 with metformin therapy on background (NCT01066715). The initial endpoint of the study (change in HbA1c levels from baseline compared to placebo) was not reached, but the development in cardiovascular diseases is ongoing. Another two studies are exploring the effects of gevokizumab on beta islet cell function in patients with Type 1 diabetes.

Gevokizumab is in phase III clinical trial in treatment of both acute and controlled noninfectious uveitis (NIU) and Behcet uveitis – EYEGUARD™ – A, B, C [28]. Previous results were quite encouraging. Seven patients received a single infusion of 0.3 mg kg^{-1} gevokizumab. Rapid and long-lasting clinical response was established in all patients after the treatment with gevokizumab. Intraocular inflammation was resolved completely in 4–21 days (median 14 days), with a median duration of response of 49 days (range 21–97 days). One of the patients during the study was exacerbation-free. No one needed rescue therapy during the study period [29].

There are recent reports of a novel fully human anti-IL-1β IgG1 – P2D7KK, with greater affinity for IL-1β than canakinumab, which potently neutralizes human, mouse, and monkey (rhesus macaque) IL-1β, and significantly reduces pathological signs of various models of animal diseases [30]. Authors of that publication argue that in animal model of MM the new monoclonal antibody showed significant protection from myeloma-induced lethality, as 70% of P2D7KK-treated mice survived compared with 20% in the isotype group, and are hopeful that it has potential as an anticancer therapeutic.

1.5. Targeting IL-2

Interleukin (IL)-2 is a small (15-kDa), α-helical cytokine produced primarily by recently activated T-cells. It binds to a receptor (IL-2R) that is composed of three subunits, an α-chain that functions only for IL-2 binding (IL-2Rα, CD 25) and the β (IL-2Rβ, CD 122) and γ (IL-2Rγ, CD 132) subunits (Figure 1), which function to augment ligand binding and induce cellular signaling.

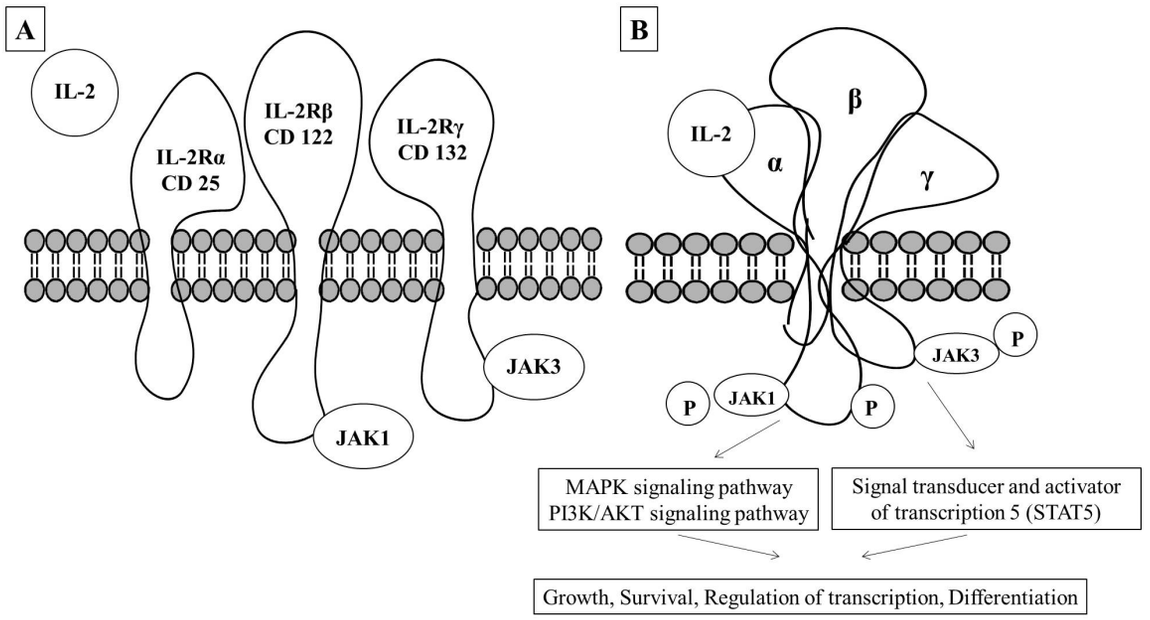

Figure 1. Structure of IL-2 receptor. **A.** In the absence of binding of IL-2 **B.** After binding to IL-2 and forming a stable heterotrimer, which then leads to the initiation of signal transduction.

IL-2 is an essential cytokine produced mainly by CD4+ T lymphocytes for promoting the clonal expansion of recently antigen-activated T cells [31]. Beside acting as T-lymphocyte growth factor, it has been found that IL-2 takes part in the activation of apoptosis and the development of regulatory T-cells and cytotoxic T lymphocytes [32]. This cytokine is of a great interest in pharmacology because, on one hand, the activation of interleukin-2 receptor results in stimulation of the immune system and can be used for inducing cytotoxicity on NK-resistant tumor cells; on the other hand, its inhibition could lead to suppression of unwanted immune responses, especially those that occur in autoimmune diseases or transplant rejection reactions. Recombinant human interleukin-2 (rhIL-2, Aldesleukin, Proleukine®, Chiron Corporation, Emeryville, CA) is used in clinical practice. It differs from the native IL-2 that lacks the first amino acid (alanine) and the cysteine at position 125 is replaced by serine. In contrast to the natural IL-2, aldesleukin is not glycosylated [33]. Aldesleukin stimulates the growth of activated T- and B-cells as well as NK (natural killer) cells by the IL-2 receptor. It can activate lymphokine-activated killer (LAK) cells and tumor-infiltrating lymphocytes (TIL) cells. It was approved in 1993 for treatment of metastatic renal cell cancer and later for metastatic melanoma. Aldesleukin displays biphasic pharmacokinetics, with an alpha (distribution) half-life of 13 min and a beta (terminal) half-life of 85 min. In cancer patients, the mean clearance rate of aldesleukin is 268 ml/min. The dose of aldesleukin in metastatic renal cell carcinoma and melanoma is 600,000 int. units/kg every 8 h for a maximum of 14 doses. Repeat after 9 days for a total of 28 doses per course. If needed, retreatment is made 7 weeks after previous course. The other product with IL-2 is denileukin diftitox (Ontac®, Seragen, Inc., Hopkinton, MA) consisting of diphtheria toxin fragments A and B fused to interleukin-2. In the recurrent or refractory cutaneous T-cell lymphoma, (CTCL), a rare slow-growing form of non-Hodgkin's lymphoma, in patients whose malignant cells express the CD25 component of the interleukin-2

http://dx.doi.org/10.5772/61774

receptor is indicated. It interacts with high-affinity interleukin-2 receptor on the surface of malignant cells and, via receptor-mediated endocytosis enters intracellular. Then enzymatically active fragment A portion of diphtheria toxin inhibits protein synthesis and leads to cell death. The pharmacokinetic parameters of Denileukin diftitox include terminal half-life of 70–80 min, Vd ~0.06 to 0.08 L/kg, and CL_{tot} ~1–2 ml/min/kg. Clearance could be significantly affected by the development of antibodies to denileukin diftitox, reducing mean systemic exposure by approximately 75%. In patients with CTCL, intravenous dose of denileukin diftitox is 9 or 18 mcg/kg/day; days 1 through 5 days every 21 days for 8 cycles.

1.6. Pharmacological properties of basiliximab and daclizumab

The chimeric monoclonal antibody basiliximab (Simulect®, Novartis, New York, NY, USA) and humanized monoclonal antibody daclizumab (Zenapax®, Roche Pharmaceuticals, Nutley, NJ, USA) have identical mechanisms of action, specifically bind the alpha subunit of the interleukin-2 (CD25) receptor on activated T-lymphocytes, thereby reducing IL-2-mediated T-cell proliferation. Daclizumab was approved by the FDA in 1997 as the first humanized therapeutic mAb, composed of 90% human and 10% murine antibody sequences with a binding affinity of 3 nM to IL-2Ra, about one-third that of its murine parental antibody. The both antibodies are indicated for prevention of acute organ rejection in adult and pediatric renal and liver transplant recipients in combination with other immunosuppressive agents [34], with no increase in opportunistic infections or adverse effects, proven to be a class of effective and specific immunosuppressive agents. Basiliximab requires only two doses. The first dose 20 mg should be given within 2 h prior to transplantation surgery. The recommended second dose 20 mg should be given 4 days after transplantation. Pharmacokinetic parameters of basiliximab are: total body clearance (Cltot ~0.075 L/h), volume of distribution at steady state (Vss ~8 L), and an elimination half-life of approximately 7,4 days [35]. Basiliximab was found to be safe and effective when used in a maintenance regimen consisting of cyclosporine, mycophenolate mofetil, and prednisone. [36] Although, daclizumab has a longer half-life (20 days), it is applied more frequently than basiliximab. The standard course of treatment with daclizumab requires administration of five doses. The first dose (1 mg/kg given intravenously over 15 min in 50–100 mL of normal saline) should be given no more than 24 h before transplantation. The four remaining doses should be given at intervals of 14 days [37]. Both drugs are well-tolerated, but anaphylactic reactions can occur. The risk of incidents of lymphoproliferative disorders and opportunistic infections is low [38]. There are many other diseases, with pathological immune response, which are a subject to the therapy with anti-IL-2 receptor antibodies, like multiple sclerosis, psoriasis, uveitis, etc. However, clinical trials are still ongoing.

1.7. Targeting IL-6

Interleukin-6 (IL-6) is a cytokine of approximately 26 kDa, which is synthesized by mononuclear phagocytes, vascular endothelial cells, fibroblasts, and other cells in response to IL-1 and, to a lesser extent, TNF. It exerts pleiotropic effects on many cells and plays a central role in diverse host defense mechanisms such as the immune response, hematopoiesis, and acute-phase reactions [39]. IL-6 is capable of stimulating the proliferation and activation of synovio-

cyte and osteoclasts, which leads to formation of synovial pannus. In combination with IL-1, they increase production of matrix metalloproteinases, which induces joint and cartilage destruction [40]. IL-6 induces the synthesis of the major mediators of the acute phase response, such as serum C-reactive protein (CRP) and amyloid A. IL-6 high levels correlate with disease activity and clinical manifestations of rheumatoid arthritis, systemic-onset juvenile idiopathic arthritis (sJIA), Castleman's disease, and systemic lupus erythematosus (SLE) [41, 42].

IL-6 transmits its signals in two pathways: binding to a membrane receptor (mIL6R or CD126) or to its soluble form (sIL6R) (Figure 2).

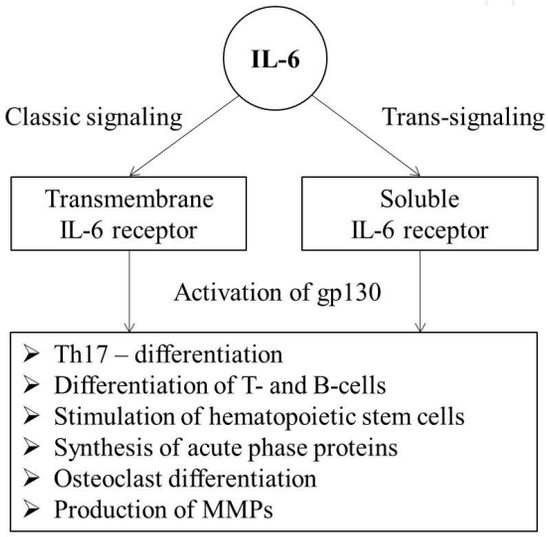

Figure 2. Pleiotropic effects of IL-6.

For both pathways, IL-6 stimulation activates Janus family tyrosine kinases (JAKs), which are associated with gp130 IL6 transducer (CD130), leading to the induction of two major signal transduction pathways, signal transducer and activator of transcription (STAT-3) pathway and mitogen-activated protein kinases (MAPKs) pathway [42]. Targeting and inhibiting IL-6R is a new promising pharmacological approach leading to a significant improvement of signs and symptoms in dysimmune diseases, such as rheumatoid arthritis (RA) or Castleman's disease, which was demonstrated in many clinical trials with a marked reduction in disease activity and the acute-phase response. Lately, modulating the function of IL-6 is used in treatment of cancers.

1.8. Pharmacological properties of tocilizumab and other newly developed monoclonal antibodies against IL-6

Tocilizumab (TCZ, Actemra®, Chugai Pharmaceutical Co Ltd, Tokyo, Japan; now a member of the Roche Group) is a humanized anti-IL-6 receptor antibody, licensed for the treatment of rheumatoid arthritis (RA), polyarticular and systemic juvenile idiopathic arthritis by intravenous administration of 8 mg/kg (and no less than 4.8 mg) in combination with methotrexate (MTX) or monotherapy [43]. The subcutaneous formulation (162 mg weekly) is now entering

phase III trials and the preliminary data have shown comparable efficacy and safety profiles to the established intravenous formulation [44]. Tocilizumab can simultaneously bind to both receptors – mIL6R and sIL6R – thereby interrupting the IL-6 signal pathway but does not affect signaling of other IL-6 family cytokines [45], and without increasing the IL-6 half-life [46]. Pharmacokinetic parameters of tocilizumab include – Vdss – 2.54–4.08 L (children) and 6.4 L (adults), the half-life is 6.3 days, but in steady state could be increased to 16–23 days (children) and 11–13 days (adults) [47]. Adverse events associated with the usage of tocilizumab include increased serum cholesterol and liver enzymes (ALT, AST), infusion-related reaction and infections (especially respiratory infection, and skin and soft tissue infections). Total cholesterol, low-density lipoproteins, triglycerides, and high-density lipoproteins are increased in 20–30% of patients treated with tocilizumab. The possible explanation of this effect is that IL-6 modulates lipoprotein receptor expression and lowers blood lipid levels via upregulation of the very-low-density lipoprotein (VLDL) receptor. If tocilizumab reduces the levels of IL-6, it will increase the levels of plasma proteins [48]. According to recent studies, this elevation of lipids does not show apparent increase in cardiac events in a follow-up of 5 years [49]. The most common adverse events of treatment with tocilizumab were infections (such as upper respiratory tract infection, nasopharyngitis, skin infections, pneumonia, gastroenteritis, and urinary tract infection, both viral and bacterial), nonsignificantly higher than the placebo group [50, 51]. Resolution of infections such as pneumonia, herpes zoster, limb abscess, osteomyelitis, sepsis, staphylococcal cellulitis, acute pyelonephritis, and staphylococcal polyarthritis have improved with appropriate treatment [52].

Siltuximab (CNTO 328, Sylvant®, Janssen Biotech) is a chimeric monoclonal antibody, an interleukin-6 (IL-6) antagonist, which is indicated for the treatment of patients with multicentric Castleman's disease (MCD) who are human immunodeficiency virus (HIV) negative and human herpesvirus-8 (HHV-8) negative. It is tested in clinical trials for multiple myeloma (MM), metastatic renal cell carcinoma (MRCC), and prostate cancer [53]. The clearance of siltuximab in patients is 0.23 L/day, according to population pharmacokinetic analysis and body weight is the only significant determinant for siltuximab clearance. The mean serum terminal half-life (t ½) for siltuximab in patients after the first i.v. infusion of 11 mg/kg is 21 days (range: 14–30 days). The CYP450 enzyme activity may reach its normal levels (previously downregulated by IL6), due to binding of bioactive IL6 by siltuximab. This may result in increased metabolism of CYP450 substrates compared with metabolism before treatment with siltuximab. If siltuximab is coadministered with CYP450 substrate drugs with a narrow therapeutic range, the dose of the concomitant medication may need to be adjusted. On the basis of the population pharmacokinetic analysis, no initial dosage adjustment is necessary for patients with baseline mild-to-severe renal impairment (CLCr ≥ 15 mL/minute) or for patients with baseline mild-to-moderate hepatic impairment (Child-Pugh Class A and B). Patients with baseline severe hepatic impairment (Child-Pugh Class C) were not included in clinical trials. Within the serum siltuximab exposure range observed following administration of 11 mg/kg i.v. every 3 weeks, no exposure–response relationships between serum CRP and siltuximab exposure or between durable tumor and symptomatic response rate and siltuximab exposure were identified. Following siltuximab dosing, 0.2% (1/411) of patients tested positive for anti-

siltuximab antibodies. Further immunogenicity analyses of the single positive sample revealed a low titer of anti-siltuximab antibodies with nonneutralizing capabilities [54].

Sarilumab (SAR153191/REGN88) is a fully human anti-IL-6Rα monoclonal antibody that binds membrane-bound and soluble human IL-6Rα with high affinity. It blocks cis and trans IL-6-mediated inflammatory signaling cascade. There was no reported evidence of complement-dependent or antibody-dependent cell-mediated cytotoxicity [55]. Sarilumab inhibits IL-6 signaling in a dose-dependent manner [56, 57]. Subcutaneous application in phase I studies in patients with RA has shown that sarilumab is generally well-tolerated, [58, 59] and reduces the acute-phase reactants such as C reactive protein (CRP) [60]. Sarilumab improves clinical signs and symptoms in RA patients with moderate-to-severe disease with tolerability similar to other IL-6 inhibitors. After completing the phase II clinical trial, the most favorable efficacy, safety, and dosing convenience were obtained by using 150 mg and 200 mg sarilumab q2w. Those doses will be further evaluated in phase III clinical trials [61].

Sirukumab (CNTO 136) is another human monoclonal antibody that targets only soluble IL-6 and is designed for the treatment of rheumatoid arthritis. It is currently in clinical phase III.

1.9. Modulating IL-11

Interleukin-11 is an ~20 kDa cytokine produced by bone marrow stromal cells. It stimulates megakaryopoiesis and may prove to be of therapeutic benefit in patients with platelet deficiencies. It also enhances the development of macrophages and perhaps other cells from marrow precursors. Neumega (oprelvekin) is a recombinant IL-11 protein product (MW ~19 kDa) approved for prevention of severe thrombocytopenia and reducing the need for platelet transfusions following myelosuppressive chemotherapy. The recommended dose for adults 50 mcg/kg/day once daily for ~10–21 days (until postnadir platelet count ≥50,000 cells/mcL) is administering subcutaneously. The most serious adverse effects reported are allergic or hypersensitivity reactions, including anaphylaxis. They may occur with the first or with subsequent doses. Patients who developed allergic reactions need to permanently discontinue the administration.

1.10. Targeting IL-12/23

Psoriasis is a chronic inflammatory disease of the skin, affecting 2–3% of the general population [62]. Plaque psoriasis, the most frequent form, has skin lesions in the form of scaly, red plaques. In psoriasis, destruction of normal immune-mediated signaling is caused by the overproduction and immature migration of keratinocytes to the surface of the skin [63]. Initially perceived as a skin disease, psoriasis can influence physical and mental condition of the body and lead to the development of joint disorders (psoriatic arthritis) and depression, thus significantly affecting the quality of life. Interleukins 12 and 23 play an important role in the formation of plaques of psoriasis. IL-12 stimulates production of IFN-gamma and tumour necrosis factor alpha (TNFα) and it is involved in differentiating naive T-cells into T-helper (Th)-1 cells, while IL-23 activates IL-17-producing T-cells (Th17) (Figure 3) [64, 65].

http://dx.doi.org/10.5772/61774

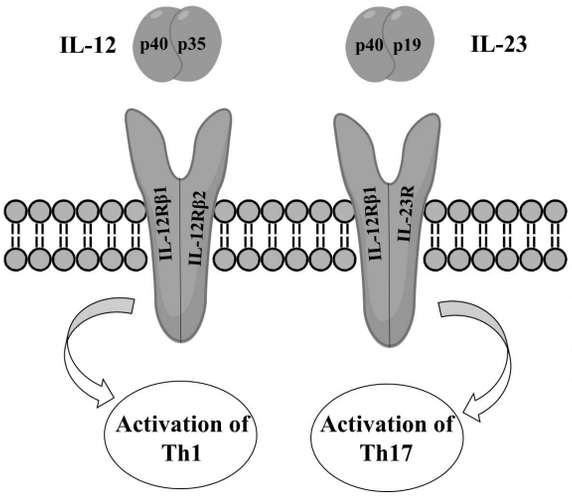

Figure 3. IL-12 family members.

1.11. Pharmacological properties of ustekimumab

Ustekinumab (UST, Stelara®) is an IgG1/kappa human monoclonal antibody composed of 1326 amino acid residues, with a molecular weight of approximately 148.6 kDa, directed against the shared p40 subunit of IL-12 and IL-23, thereby preventing IL-12 and IL-23 from binding to the receptor chain IL-12Rb1 to trigger downstream signaling pathways. It is currently approved for treatment of plaque psoriasis and psoriatic arthritis. The pharmacokinetic properties of ustekinumab in human population do not show any serious differences in patients with psoriatic arthritis and those with plaque psoriasis [66]. Absolute bioavailability after subcutaneous administration is approximately 57% and biological half-life is ~10–126 days. In clinical trials, ustekinumab has improved rapidly and sustainedly the symptoms of psoriasis. Even after one injection there was a significant improvement that persisted over the 1.5-year study period. It achieved the primary endpoint of 75% reduction in the Psoriasis Area and Severity Index (PASI) score in a large proportion of patients in the phase III PHOENIX trials (defined as 75% clinical improvement of affected skin areas determined by the size, severity of erythema, plaque thickness, and scaling) compared to 3% for placebo [67]. The effect lasted for 76 weeks after treatment with ustekinumab at more than 80% of the responders. In a randomized, multicenter study ustekinumab was compared with etanercept in the treatment of more than 900 patients with moderate-to-severe plaque psoriasis. After 12 weeks, 55% of patients receiving ustekinumab reached (45 mg at weeks 0 and 4) PASI-75; under etanercept (50 mg twice a week) it was 39% [68].

1.12. Targeting IL-17

Interleukin 17 (IL-17), similarly as interferon gamma, increases the production of chemokines in the various tissues that stimulate recruitment of monocytes and neutrophils to the site of inflammation. It was classified as a proinflammatory cytokine stimulating the production of IL-6 and IL-8, and the surface expression of the intracellular adhesion molecule-1 (ICAM-1) in

human fibroblasts [69]. IL-17 is produced by the recently identified T-cell subset Th17 and is under the influence of IL-23, a cytokine belonging to the IL-12 family [70]. IL-23 is proinflammatory mediator, which induces chronic inflammation through the activation of Th17 cells and the secretion of Th17 by non-T-cells, as mentioned earlier. This pathway is essential for production of many other mediators that are involved in chronic inflammatory responses in autoimmune diseases. Large amounts of Th17 cells are found in blood and skin lesions of people with psoriasis. Although many signaling pathways including that of Th17 are investigated in psoriasis, IL-17A inhibitors are able to relieve psoriatic symptoms in forms resistant to anti-TNF agents and have higher specificity than inhibitors of IL-12 and IL-23. The combination of IL-17A and TNF-α induces a proinflammatory signaling cascade; patients who are refractory to anti-TNF agents may respond to therapies that target IL-17A [71]. As opposed to the agents that target IL-12 and IL-23 (ustekinumab), inhibitors of IL-17A may induce a more specific response.

1.13. Pharmacological properties of secukinumab, izekizumab, and brodalumab

Secukinumab (AIN-457, Cosentyx®, Novartis Pharma AG) is a fully human IgG1κ monoclonal antibody that selectively binds and neutralizes the effects of IL-17A. It is indicated for the treatment of adult patients with moderate-to-severe plaque psoriasis who are candidates for phototherapy or systemic therapy. In the pivotal studies, 70% of the subjects achieved a clear or almost clear skin. For the subjects, the antibody was injected once a week for 16 weeks. The effect was greater than the treatment with the TNF-α inhibitor etanercept (Enbrel®) and it was held in the majority of patients on continued therapy at one year. First improvements of the skin image appeared after two weeks only. On average, the skin symptoms had reduced after three weeks to half of what was achieved with etanercept after seven weeks. In addition, fewer and milder side effects than the reference were registered. [72]

Ixekizumab is a humanized IgG4 monoclonal antibody (mAb) neutralizing IL-17A. The safety and efficacy of ixekizumab was assessed in a phase II, double-blind, placebo-controlled trial with 142 moderate-to-severe plaque-type psoriasis patients. Patients were randomized into five groups administering subcutaneously 150, 75, 25, 10 mg ixekizumab or placebo, at 0, 2, 4, 8, 12, and 16 weeks [73]. The achievement of 75% reduction of Psoriasis Area and Severity Index (PASI) after 12 weeks of treatment constituted the primary endpoint of the study occurring in 82.1, 82.8, 76.7, 29 and 7.7% of patients treated with 150, 75, 25, 10 mg ixekizumab or placebo, respectively. The amelioration of PASI score was significantly greater in all ixekizumab groups compared with placebo ($p < 0.001$ for each comparison), with the exception of the lowest dose (10 mg) [73]. Furthermore, a statistically significant reduction of PASI score by at least 90% was observed in the 150 mg (71.4%), 75 mg (58.6%), and 25 mg (50.0%) groups versus placebo 0% ($p < 0.001$). Regarding the safety profile, the occurrence of adverse events including nasopharyngitis, upper respiratory infection, injection site reaction, and headache was similar across all study groups, and no serious adverse events were reported. Phase III studies are currently ongoing; among them are a head-to-head trial with etanercept, another head-to-head trial with adalimumab on psoriatic arthritis, and a study evaluating ixekizumab efficacy and safety on psoriatic arthritis patients [74].

Brodalumab is a human mAb, which blocks IL-17RA, the receptor subunit shared by IL-17A, IL-17F, and IL-17A/F heterodimer ligands. The antagonism of IL-17 signaling by brodalumab was primary proven clinical, genomic, and histological in 10 patients with psoriasis after only 1 week in a phase I, proof-of-concept study [75]. Its efficacy is confirmed in recent phase II, randomized, double-blind, placebo-controlled, dose-ranging study involving 198 patients. They received subcutaneously brodalumab of 280 mg monthly, or 70, 140, 210 mg brodalumab or placebo at weeks 0, 1, 2, 4, 6, 8, and 10 [76]. The monthly administration of 280 mg improved PASI score, with 45%, while other dosages (210, 140, and 70 mg) achieved 85.9%, 86.3%, and 16% of PASI score, respectively, after 12-week study assessment. The greater frequency of adverse effects was observed in patients receiving the high-dose regimen of brodalumab. There were two cases of mild neutropenia (grade III) among the serious adverse events [76]. Presently, phase II trials are being conducted to assess brodalumab as a therapeutic option for psoriatic arthritis, while its ability to treat plaque-type psoriasis has already progressed to phase III studies. Brodalumab is currently being investigated for the treatment of psoriatic arthritis on phase II and III trials for the treatment of plaque-type psoriasis. Of relevance, one trial evaluates clinical to withdrawal-and-retreatment with brodalumab in psoriatic patients, and another study compares efficacy and safety of brodalumab compared with ustekinumab (anti-IL-12/IL-23p40 agent) and placebo [77].

1.14. Potential of pharmacokinetic and pharmacodynamics drug interactions using monoclonal antibodies

Most of the pharmacokinetic drug–drug interactions (DDIs) of small molecules occur at the level of modulation of activity/levels of the enzymes and/or transporters involved in their biodegradation/bioactivation and/or disposition. Because these enzymes and/or transporters are not involved in the elimination processes of mAbs and functional derivatives, it is believed that potential future interactions with concomitant administration of mAbs with small molecules are unlikely. Despite that, monoclonal antibodies are not metabolized by cytochrome enzymes (CYPs) and these are not suspected for drug interactions. However, it is known from in vitro studies [78-82] that the increased activity of certain cytokines is capable of reducing the activity of CYP enzymes:

- CYP1A2 levels by IFN-a, IFN-a-2b, IFN-b, IL-2 and IL-6
- CYP2C8 levels by IL-1
- CYP2C9 levels by IL-2 and IL-10
- CYP2C19 levels by IFN-a-2b, IFN-b, TNF-a, IL-2, and IL-6
- CYP2D6 levels by IFN-a-2b
- CYP2E1 levels by IFN-a-2b and IL2
- CYP3A levels by IL-1, IL-2, IL-6, and IL-10

In contrast to other cytokines, IL-4 was shown to increase the activity of CYP2E1, suggesting that another mechanism of enzyme activity regulation could be involved [78]. The molecular

mechanisms underlying downregulation of drug-metabolizing enzyme and transporter levels by cytokines are not fully defined but lower gene and protein expressions have been reported for some nuclear receptors involved in regulation of CYPs and transporters including pregnane X receptor (PXR), constitutive androstane receptor (CAR), and farnesoid X activated receptor (FXR) [83, 84]. Although in vitro systems (e.g., microsomes and hepatocytes) are routinely employed to predict in vivo DDIs of small molecules [85], the use of in vitro systems to predict in vivo interactions between small-molecule drugs and therapeutic proteins is still in development. Most of the problems are caused by poor correlation and extrapolation between in vitro and in vivo results.

Influencing the levels of expression of P-glycoprotein (P-gp), also known as multidrug resistance protein 1 (MDR1), can also contribute to DDIs. Several studies have shown that intestinal P-gp was inversely correlated with the inflammatory disease activity. Cytokine-mediated downregulation of P-gp in inflamed intestine of ulcerative colitis (UC) patients was presumably dependent on disease activity, with a possible contribution from IL-8 [86]. Presence of P-gp in the membranes of blood-brain-barrier (BBB) restricts passage of drug molecules into the brain. Some studies show that activity of P-gp was downregulated after short-term exposure to inflammatory mediators, whereas its activity was upregulated following more prolonged exposure [87, 88].

Modulation of activity of CYP enzymes by monoclonal antibodies, through interactions with ILs, can enter into interactions with drugs, which are metabolized by CYPs. Clinical studies have shown that IL-6 inhibits function of CYP1A2, 2C9, 2C19, and CYP3A4 and the function is normalized by anti-IL-6 receptor antibody – tocilizumab [89]. When patients taking drugs with a narrow therapeutic index that are metabolized by these CYP enzymes, for example, atorvastatin, calcium channel blockers, theophylline, warfarin, phenytoin, cyclosporine, or benzodiazepines, blood levels of these drugs should be monitored and clinicians should be alert for possible interactions. It should be taken into account that monoclonal antibodies have long elimination half-life and the effect on CYP enzymes activity may continue after treatment for several weeks.

Pharmacodynamic (PD) interactions using monoclonal antibodies are also possible. The basic mechanisms that are involved include reduction in target number or target-bearing cell number, thus affecting receptor-mediated clearance [90]. The use of pharmacodynamic interactions to improve the pharmacological effect of monoclonal antibodies is growing [91, 92]. In nonhuman primates, paclitaxel in combination with trastuzumab resulted in a 1.5-fold increase in trastuzumab serum concentration [93]. Similarly, paclitaxel enhances the therapeutic benefit of cetuximab, possibly through inhibition of angiogenesis and the induction of apoptosis [94]. Pharmacodynamic interactions have also been reported in other clinical and preclinical experiments [95-97]. A combination of cetuximab (mAb-targeting epidermal growth factor receptor, EGRF) with either gefitinib or erlotinib (EGRF tyrosine kinase inhibitors) was used to maximize EGRF signaling inhibition [98]. Synergism between these drugs has been reported in vivo in athymic nude mice and in vitro across a variety of human cancer cell models. Synergistic antitumor activity has been reported between a taxane compound (BMS-275183) and cetuximab using athymic nude mice [99].

http://dx.doi.org/10.5772/61774

In addition, a recently published study demonstrated in mice that coadministration of anti-VEGF antibodies reduced the delivery of a second mAb to tumor tissues. This pharmacokinetic–pharmacodynamic interaction was only observable when tumor tissue levels were analyzed as no change in plasma pharmacokinetics was observed [100].

2. Conclusions and future directions

Interleukins represent an attractive target for pharmacological interventions to fine-tune immune cell functions for treatment of human diseases. Discovery and development of therapeutic monoclonal antibodies is an option for achieving these objectives. However, the impact on these intracellular signaling molecules hides many pitfalls, as the border between useful and harmful influence is very thin. Examples of this can be the case with humanized monoclonal antibody directed against CD28 – TGN 1412. During the phase I clinical trial, all six volunteers receiving the drug were hospitalized, at least four of them developed multiple organ dysfunction, despite being administered at a supposed sub-clinical dose of 0.1 mg/kg; approximately 500 times lower than the dose found safe in animals. [101] Another case in this regard is the occurrence of progressive multifocal leukoencephalopathy (PML) in subjects treated with natalizumab. [102] Therefore, it is necessary to extrapolate properly the results obtained from preclinical studies to humans by knowing very well the pharmacological characteristics and evaluation of the risk benefits in order to prevent future tragedies.

Author details

Kaloyan Georgiev* and Marieta Georgieva

*Address all correspondence to: kalgeorgiev@hotmail.com

Department of Pharmacology, Toxicology and Pharmacotherapy, Faculty of Pharmacy, Medical University Varna, Varna, Bulgaria

References

[1] Mizel SB. The interleukins. *FASEB J.* 1989; 3(12): 2379–88.

[2] Akdis M, Burgler S, Crameri R, Eiwegger T, Fujita H, Gomez E, Klunker S, Meyer N, O'Mahony L, Palomares O, Rhyner C, Ouaked N, Schaffartzik A, Van De Veen W, Zeller S, Zimmermann M, Akdis CA. Interleukins, from 1 to 37, and interferon-γ: receptors, functions, and roles in diseases. *J Allergy Clin Immunol*. 2011 Mar; 127(3):701–21.e1-70.

[3] Liu JK. The history of monoclonal antibody development – Progress, remaining challenges and future innovations. *Ann Med Surg (Lond)*. 2014; Sep 11; 3(4):113–6.

[4] Köhler G., Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature*. 1975; 256(5517):495–97.

[5] Leavy O. Therapeutic antibodies: past, present and future. *Nat Rev Immunol*. 2010; 10(5):297.

[6] Sgro C. Side-effects of a monoclonal antibody, muromonab CD3/orthoclone OKT3: a bibliographic review. *Toxicology*. 1995; 105(1):23–29.

[7] Bugelski PJ, Achuthanandam R, Capocasale RJ, Treacy G, Bouman-Thio E. Monoclonal antibody-induced cytokine-release syndrome. *Expert Rev Clin Immunol*. 2009 Sep; 5(5):499–521.

[8] Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood*. 2011 Apr 7; 117(14):3720–32.

[9] Pascual V, Allantaz F, Arce E, Punaro M, Banchereau J. Role of interleukin-1 (IL-1) in the pathogenesis of systemic onset juvenile idiopathic arthritis and clinical response to IL-1 blockade. *J Exp Med*. 2005; 201:1479–86.

[10] Liu C, Hart RP, Liu XJ, Clevenger W, Maki RA, De Souza EB. Cloning and characterization of an alternatively processed human type II interleukin-1 receptor mRNA. *J Biol Chem*. 1996 Aug 23; 271(34):20965–72.

[11] Dayer JM, de Rochemonteix B, Burrus B, Demczuk S, and Dinarello CA. Human recombinant interleukin 1 stimulates collagenase and prostaglandin E2 production by human synovial cells. *J Clin Invest*. 1986; 77: 645–648.

[12] Lust JA, Donovan KA. The role of interleukin-1beta in the pathogenesis of multiple myeloma. *Hematol Oncol Clin North Am*. 1999; 13:1117–25.

[13] Lust JA, Lacy MQ, Zeldenrust SR, Dispenzieri A, Gertz MA, Witzig TE, Kumar S, Hayman SR,Russell SJ, Buadi FK, et al. Induction of a chronic disease state in patients with smoldering or indolent multiple myeloma by targeting interleukin1beta-induced interleukin 6 production and the myeloma proliferative component. *Mayo Clin Proc*. 2009; 84:114–22.

[14] Mandrup-Poulsen, T, Pickersgill L, Donath MY. Blockade of interleukin 1 in type 1 diabetes mellitus. *Natur Rev Endocrinol*. 6, 158–166 (2010).

[15] Maedler K, Sergeev P, Ris F, Oberholzer J, Joller-Jemelka HI, Spinas GA, Kaiser N, Halban PA, Donath MY. Glucose-induced β cell production of IL-1β contributes to glucotoxicity in human pancreatic islets. *J Clin Invest*. 110, 851–860 (2002).

[16] Donath, M. Y. & Shoelson, S. E. Type 2 diabetes as an inflammatory disease. *Natur Rev Immunol*. 2011; 11:98–107.

[17] Stienstra R, Joosten LA, Koenen T, van Tits B, van Diepen JA,. et al. The inflammasome-mediated caspase-1 activation controls adipocyte differentiation and insulin sensitivity. *Cell Metab*. 2011; 12:593–605. doi: 10.1016/j.cmet.2010.11.011.

[18] Dinarello CA, Simon A, van der Meer JW. Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nat Rev Drug Discov*. 2012 Aug; 11(8):633–52. doi: 10.1038/nrd3800.

[19] Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL, Ravussin E, Stephens JM, Dixit VD. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. *Natur Med*. 2011; 17, 179–188. doi: 10.1038/nm.2279

[20] Giat E, Lidar M. Cryopyrin-associated periodic syndrome. *Isr Med Assoc J*. 2014 Oct; 16(10):659–61.

[21] Chakraborty A, Tannenbaum S, Rordorf C, Lowe PJ, Floch D, Gram H, Roy S. Pharmacokinetic and pharmacodynamic properties of canakinumab, a human anti-interleukin-1β monoclonal antibody. *Clin Pharmacokinet*. 2012 Jun 1; 51(6):e1–18. doi: 10.2165/11599820-000000000-00000.

[22] Lachmann HJ, Kone-Paut I, Kuemmerle-Deschner JB, Leslie KS, Hachulla E, Quartier P, Gitton X, Widmer A, Patel N, Hawkins PN; Canakinumab in CAPS Study Group. Use of canakinumab in the cryopyrin-associated periodic syndrome. *N Engl J Med*. 2009 Jun 4; 360(23):2416–25.

[23] Dubois EA, Rissmann R, Cohen AF. Rilonacept and canakinumab. *Br J Clin Pharmacol*. 2011 May; 71(5):639–41. doi: 10.1111/j.1365-2125.2011.03958.x.

[24] Roell MK, Issafras H, Bauer RJ, Michelson KS, Mendoza N, Vanegas SI, Gross LM, Larsen PD, Bedinger DH, Bohmann DJ, et al. Kinetic approach to pathway attenuation using XOMA 052, a regulatory therapeutic antibody that modulates interleukin-1beta activity. *J Biol Chem*. 2010; 285:20607-14.

[25] Abbate A, Van Tassell BW, Seropian IM et al. Interleukin-1beta modulation using a genetically engineered antibody prevents adverse cardiac remodelling following acute myocardial infarction in the mouse. *Eur J Heart Fail*. 2010; 12, 319–22.

[26] Bhaskar V, Yin J, Mirza AM, Phan D, Vanegas S, Issafras H, Michelson K, Hunter JJ, Kantak SS. Monoclonal antibodies targeting IL-1 beta reduce biomarkers of atherosclerosis in vitro and inhibit atherosclerotic plaque formation in Apolipoprotein E-deficient mice. *Atherosclerosis*. 2011; 216, 313–20. doi: 10.1016/j.atherosclerosis.2011.02.026.

[27] Cavelti-Weder C, Babians-Brunner A, Keller C, Stahel MA, Kurz-Levin M, Zayed H, Solinger AM, Mandrup-Poulsen T, Dinarello CA, Donath MY. Effects of gevokizumab on glycemia and inflammatory markers in type 2 diabetes. *Diab Care*. 2012; 35:1654–62. doi: 10.2337/dc11-2219.

[28] Clinicaltrials.gov www.clinicaltrials.gov

[29] Gül A, Tugal-Tutkun I, Dinarello CA, Reznikov L, Esen BA, Mirza A, Scannon P, Solinger A. Interleukin-1-regulating antibody XOMA 052 (gevokizumab) in the treatment of acute exacerbations of resistant uveitis of Behcet's disease: an open-label pilot study. *Ann Rheum Dis*. 2012; 71:563–6. doi: 10.1136/annrheumdis-2011-155143.

[30] Goh AX, Bertin-Maghit S, Ping Yeo S, Ho AW, Derks H, Mortellaro A, Wang CI. A novel human anti-interleukin-1β neutralizing monoclonal antibody showing in vivo efficacy. *MAbs*. 2014 May-Jun; 6(3):765–73. doi: 10.4161/mabs.28614.

[31] Malek TR. The main function of IL-2 is to promote the development of T regulatory cells. *J Leukocyte Biol*. 2003, 74(6): 961–5.

[32] Liao W, Lin JX, Leonard WJ. IL-2 family cytokines: new insights into the complex roles of IL-2 as a broad regulator of T helper cell differentiation. *Curr Opin Immunol*. 2011; 23:598–604. doi: 10.1016/j.coi.2011.08.003.

[33] Whittington R, Faulds D. Interleukin-2. A review of its pharmacological properties and therapeutic use in patients with cancer. *Drugs*. 1993 Sep; 46(3):446–514.

[34] Chapman TM, Keating GM. Basiliximab: a review of its use as induction therapy in renal transplantation. *Drugs*. 2003; 63(24):2803–35.

[35] Kovarik JM, Kahan BD, Rajagopalan PR, Bennett W, Mulloy LL, Gerbeau C, Hall ML. Population pharmacokinetics and exposure-response relationships for basiliximab in kidney transplantation. The U.S. Simulect Renal Transplant Study Group. *Transplantation*. 1999 Nov 15;68(9):1288–94.

[36] Lawen J, Davies E, Morad F, et al. Basiliximab (Simulect) is safe and effective in combination with triple therapy of Neoral, steroids and CellCept in renal transplant patients. *Transplantation*. 2000: 69:S260.

[37] Vincenti F, Kirkman R, Light S, Bumgardner G, Pescovitz M, Halloran P, Neylan J, Wilkinson A, Ekberg H, Gaston R, Backman L, Burdick J. Interleukin-2-receptor blockade with daclizumab to prevent acute rejection in renal transplantation. Daclizumab Triple Therapy Study Group. *N Engl J Med*. 1998 Jan 15; 338(3):161–5.

[38] Hong JC, Kahan BD. Immunosuppressive agents in organ transplantation: past, present, and future. *Semin Nephrol*. 2000 Mar; 20(2):108–25.

[39] Ding, C., Cicuttini, F., Jones, G. Targeting IL-6 in the treatment of inflammatory and autoimmune diseases. *Expert Opin Investig Drugs*. 2009; 18:1457–66.

[40] Stannus O, Jones G, Cicuttini F, Parameswaran V, Quinn S, Burgess J, Ding C. Circulating levels of IL-6 and TNF-a are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults. *Osteoarthritis Cart*. 2010; 18:1441–47. doi: 10.1016/j.joca.2010.08.016.

[41] Ding C, Cicuttini F, Jones G. Targeting IL-6 in the treatment of inflammatory and autoimmune diseases. *Expert Opin Investig Drugs*. 2009; 18:1457–66.

[42] Cronstein BN. Interleukin-6–a key mediator of systemic and local symptoms in rheumatoid arthritis. *Bull NYU Hosp Jt Dis*. 2007; 65 (Suppl. 1):S11–S15.

[43] Oldfield V, Dhillon S, Plosker GL. Tocilizumab: a review of its use in the management of rheumatoid arthritis. *Drugs*. 2009; 69(5):609–32.

[44] Burmester GR, Rubbert-Roth A, Cantagrel A, Hall S, Leszczynski P, et al. A randomized, double-blind, parallel group study of the safety and efficacy of tocilizumab SC versus tocilizumab IV, in combination with traditional Dmards in patients with moderate to severe RA. *Arthritis Rheum*. 2012; 64(10):S1075. doi: 10.1136/annrheumdis-2013-203523.

[45] Mihara, M., Kasutani, K., Okazaki, M. et al. (2005) Tocilizumab inhibits signal transduction mediated by both mIL-6R and sIL-6R, but not by the receptors of other members of IL-6 cytokine family. *Int Immunopharmacol*. 5 (12): 1731–40.

[46] Kishimoto, T. Interleukin-6: discovery of a pleiotropic cytokine. Arthritis Res. Ther., (2006) 8 (Suppl. 2), S2.

[47] Zhang X, Chen YC, Fettner S, Rowell L, Gott T, Grimsey P, Unsworth A. Pharmacokinetics and pharmacodynamics of tocilizumab after subcutaneous administration in patients with rheumatoid arthritis. *Int J Clin Pharmacol Ther*. 2013 Aug; 51(8):620–30. doi: 10.5414/CP201904.

[48] Hashizume M., H. Yoshida, N. Koike, M. Suzuki, and M. Mihara. Overproduced interleukin 6 decreases blood lipid levels via upregulation of very-low-density lipoprotein receptor. *Ann Rheum Dis*. 2010; 69(4): 741–46.

[49] Genovese MC, Rubbert-Roth A, Smolen JS, Kremer J, Khraishi M, et al., Long-term safety and efficacy of tocilizumab in patients with rheumatoid arthritis: a cumulative analysis of up to 4.6 years of exposure. *J Rheumatol*. 2013; 40(6): 768–80. doi: 10.3899/jrheum.120687.

[50] Smolen JS, Beaulieu A, Rubbert-Roth A, Ramos-Remus C, Rovensky J et al. Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): a doubleblind, placebo-controlled, randomised trial. *Lancet*. 2008; 371 (9617), 987–97.

[51] Genovese MC, McKay JD, Nasonov EL, Mysler EF, da Silva NA, et al. Interleukin-6 receptor inhibition with tocilizumab reduces disease activity in rheumatoid arthritis with inadequate response to diseasemodifying antirheumatic drugs: the tocilizumab in combination with traditional disease-modifying antirheumatic drug therapy study. *Arthritis Rheum*. 2008; 58 (10), 2968–80.

[52] Nishimoto N, Hashimoto J, Miyasaka N, Yamamoto K, Kawai S, et al. Study of active controlled monotherapy used for rheumatoid arthritis, an IL-6 inhibitor (SAMURAI):

evidence of clinical and radiographic benefit from an X ray reader-blinded randomised controlled trial of tocilizumab. *Ann Rheum Dis*. 2007; 66 (9), 1162–67.

[53] Rossi JF, Lu ZY, Jourdan M, Klein B. Interleukin-6 as a Therapeutic Target. *Clin Cancer Res*. 2015 Mar 15; 21(6):1248–57.

[54] Deisseroth A, Ko CW, Nie L, Zirkelbach JF, Zhao L, Bullock J, Mehrotra N, Del Valle P, Saber H, Sheth C, Gehrke B, Justice R, Farrell A, Pazdur R. FDA approval: siltuximab for the treatment of patients with multicentric Castleman disease. *Clin Cancer Res*. 2015 Mar 1; 21(5):950–4. doi: 10.1158/1078-0432.CCR-14-1678.

[55] Genovese MC, Fleischmann RM, Fiore S, et al. Sarilumab, a subcutaneously-administered, fully-human monoclonal antibody inhibitor of the IL-6 receptor: Relationship between EULAR responses and change from baseline of selected clinical parameters. *Ann Rheum Dis*. 2013; 72(Suppl 3):620.

[56] Wang L-H, Xue Y, Liu X, et al. Preclinical development of sarilumab, the first fully human monoclonal antibody (mAb) against IL-6R alpha: utilization and value of double humanized animal model. *Ann Rheum Dis*. 2013; 72(Suppl 3):375.

[57] Rafique A, Martin J, Blome M, et al. Evaluation of the binding kinetics and functional bioassay activity of sarilumab and tocilizumab to the human IL-6 receptor (IL-6R) alpha. *Ann Rheum Dis*. 2013; 72(Suppl 3):797.

[58] Radin AR, Mellis SJ, Jasson M, et al. REGN88/SAR153191, a fully-human interleukin-6 receptor monoclonal antibody, reduces acute phase reactants in patients with rheumatoid arthritis: preliminary observations from Phase 1 studies. *Arthritis Rheum*. 2010; 62(Suppl 10):S470.

[59] Radin A, Mellis S, Jasson M, et al. Safety and effects on markers of inflammation of subcutaneously administered REGN88/SAR153191, an interleukin-6 receptor inhibitor, in patients with rheumatoid arthritis: findings from Phase 1 studies. *Ann Rheum Dis*. 2010; 69(Suppl 3):99.

[60] Huizinga TW, Kivitz AJ, Rell-Bakalarska M, et al. Sarilumab for the treatment of moderate-to-severe rheumatoid arthritis: results of a phase 2, randomized, double blind, Placebo-Controlled, International Study. *Ann Rheum Dis*. 2012; 71(Suppl 3):60.

[61] Huizinga TW, Fleischmann RM, Jasson M, Radin AR, van Adelsberg J, Fiore S, Huang X, Yancopoulos GD, Stahl N, Genovese MC. Sarilumab, a fully human monoclonal antibody against IL-6Rα in patients with rheumatoid arthritis and an inadequate response to methotrexate: efficacy and safety results from the randomised SARIL-RA-MOBILITY Part A trial. *Ann Rheum Dis*. 2014 Sep; 73(9):1626–34. doi: 10.1136/annrheumdis-2013-204405.

[62] Menter A, Gottlieb A, Feldman SR et al. Guidelines of care for the management of psoriasis and psoriatic arthritis: section 1. Overview of psoriasis and guidelines of

care for the treatment of psoriasis with biologics. *J Am Acad Dermatol.* 2008; 58:826–50.

[63] Nestle FO, Kaplan DH, Barker J. Mechanisms of disease: Psoriasis. *N Engl J Med*. 2009; 2652 361:496–509.

[64] Murphy KM, Reiner SL. The lineage decisions of helper T cells. *Nat Rev Immunol*. 2002;2(12):933–44.

[65] Langrish CL, Chen Y, Blumenschein WM, et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med*. 2005; 201(2):233–40.

[66] Zhu YW, Mendelsohn A, Pendley C, Davis HM, Zhou H. Population pharmacokinetics of ustekinumab in patients with active psoriatic arthritis. *Int J Clin Pharmacol Ther*. 2010 Dec; 48(12):830–46.

[67] Papp KA, Griffiths CE, Gordon K, Lebwohl M, Szapary PO, Wasfi Y, Chan D, Hsu MC, Ho V, Ghislain PD, Strober B, Reich K; PHOENIX 1 Investigators; PHOENIX 2 Investigators; ACCEPT Investigators. Long-term safety of ustekinumab in patients with moderate-to-severe psoriasis: final results from 5 years of follow-up. *Br J Dermatol*. 2013 Apr; 168(4):844–54.

[68] Yao Z, Painter SL, Fanslow WC, et al. Human IL-17: a novel cytokine derived from T cells. *J Immunol*. 1995; 155(12):5483–6

[69] Griffiths CE, Strober BE, van de Kerkhof P, Ho V, Fidelus-Gort R, Yeilding N, Guzzo C, Xia Y, Zhou B, Li S, Dooley LT, Goldstein NH, Menter A; ACCEPT Study Group. Comparison of ustekinumab and etanercept for moderate-to-severe psoriasis. *N Engl J Med*. 2010 Jan 14; 362(2):118–28.

[70] Gee K, Guzzo C, Che Mat NF, Ma W, Kumar A. The IL-12 family of cytokines in infection, inflammation and autoimmune disorders. Inflam *Allergy-Drug Targets*. 2009; 8:40–52.

[71] Bartlett HS and Million RP. Targeting the IL-17–TH17 pathway. *Natur Rev Drug Discov*. 2015; 14:11–12. doi:10.1038/nrd4518

[72] Langley RG, Elewski BE, Lebwohl M, Reich K, Griffiths CE, Papp K, Puig L, Nakagawa H, Spelman L, Sigurgeirsson B, Rivas E, Tsai TF, Wasel N, Tyring S, Salko T, Hampele I, Notter M, Karpov A, Helou S, Papavassilis C; ERASURE Study Group; FIXTURE Study Group. Secukinumab in plaque psoriasis-results of two phase 3 trials. *N Engl J Med*. 2014 Jul 24; 371(4):326–38. doi: 10.1056/NEJMoa1314258.

[73] Leonardi C1, Matheson R, Zachariae C, Cameron G, Li L, Edson-Heredia E, Braun D, Banerjee S. Anti-interleukin-17 monoclonal antibody ixekizumab in chronic plaque psoriasis. *N Engl J Med*. 2012; 366(13):1190-9. doi: 10.1056/NEJMoa1109997.

[74] Available from: http://www.clinicaltrials.gov/ct2/results?term=ixekizumab+AND+psoriasis&Search=Search

[75] Russell CB, Kerkof K, Bigler J, et al. Blockade of the IL-17R with AMG827 leads to rapid reversal of gene expression and histopathologic abnormalities in human psoriatic skin [abstract]. *J Invest Dermatol*. 2010; 130:S46

[76] Papp KA, Leonardi C, Menter A, Ortonne JP, Krueger JG, et al. Brodalumab, an anti-interleukin- 17-receptor antibody for psoriasis. *N Engl J Med*. 2012; 366(13):1181-9. doi: 10.1056/NEJMoa1109017.

[77] Available from: http://www.clinicaltrials.gov/ct2/results?term=brodalumab+AND+psoriasis&Search=Search

[78] Abdel-Razzak Z, Loyer P, Fautrel A, Gautier JC, Corcos L, Turlin B, et al. Cytokines down-regulate expression of major cytochrome P-450 enzymes in adult human hepatocytes in primary culture. *Mol Pharmacol*. 1993; 44(4):707–15.

[79] Huang SM, Zhao H, Lee JI, Reynolds K, Zhang L, Temple R, et al. Therapeutic protein-drug interactions and implications for drug development. *Clin Pharmacol Ther*. 2010; 87(4):497–503.

[80] Dickmann LJ, Patel SK, Wienkers LC, Slatter JG. Effects of Interleukin 1beta (IL-1beta) and IL-1beta/Interleukin 6 (IL-6) combinations on drug metabolizing enzymes in human hepatocyte culture. *Curr Drug Metab*. 2012; 13(7):930–7.

[81] Molanaei H, Stenvinkel P, Qureshi AR, Carrero JJ, Heimburger O, Lindholm B, et al. Metabolism of alprazolam (a marker of CYP3A4) in hemodialysis patients with persistent inflammation. *Eur J Clin Pharmacol*. 2012; 68(5):571–7. doi: 10.1007/s00228-011-1163-8.

[82] Yang Q, Doshi U, Li N, Li AP. Effects of culture duration on gene expression of p450 isoforms, uptake and efflux transporters in primary hepatocytes cultured in the absence and presence of interleukin-6: implications for experimental design for the evaluation of downregulatory effects of biotherapeutics. *Curr Drug Metab*. 2012; 13(7):938–46.

[83] Teng S, Piquette-Miller M. The involvement of the pregnane X receptor in hepatic gene regulation during inflammation in mice. *J Pharmacol Exp Ther*. 2005; 312(2):841–8.

[84] Beigneux AP, Moser AH, Shigenaga JK, Grunfeld C, Feingold KR. Reduction in cytochrome P-450 enzyme expression is associated with repression of CAR (constitutive androstane receptor) and PXR (pregnane X receptor) in mouse liver during the acute phase response. *Biochem Biophys Res Commun*. 2002; 293(1):145–9.

[85] Rostami-Hodjegan A, Tucker GT. Simulation and prediction of in vivo drug metabolism in human populations from in vitro data. *Nat Rev Drug Discov*. 2007; 6(2):140–8.

[86] Ufer M, Häsler R, Jacobs G, Haenisch S, Lächelt S, Faltraco F, Sina C, Rosenstiel P, Nikolaus S, Schreiber S, Cascorbi I. Decreased sigmoidal ABCB1 (P-glycoprotein) ex-

pression in ulcerative colitis is associated with disease activity. *Pharmacogenomics*, 2009; 10(12), 1941–53.

[87] Roberts DJ, Goralski KB. A critical overview of the influence of inflammation and infection on P-glycoprotein expression and activity in the brain. *Expert Opin Drug Metab Toxicol*., 2008; 4(10), 1245–64.

[88] Miller DS, Bauer B, Hartz AM. Modulation of P-glycoprotein at the blood-brain barrier: opportunities to improve central nervous system pharmacotherapy. *Pharmacol Rev*., 2008; 60(2), 196-209.

[89] Navarro-Millán I; Singh JA, Curtis JR. Systematic review of tocilizumab for rheumatoid arthritis: a new biologic agent targeting the interleukin-6 receptor. *Clin Ther*. 2012; 34(4), 788-802.e3. doi: 10.1016/j.clinthera.2012.02.014.

[90] Mould DR, Green B. Pharmacokinetics and pharmacodynamics of monoclonal antibodies: concepts and lessons for drug development. *BioDrugs*. 2010; 24(1):23–39.

[91] Sharpe AH, Abbas AK. T-cell costimulation–biology, therapeutic potential, and challenges. *N Engl J Med*. 2006; 355(10):973–5.

[92] Suntharalingam G, Perry MR, Ward S, Brett SJ, Castello-Cortes A, Brunner MD, et al. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *N Engl J Med*. 2006; 355(10):1018–28.

[93] Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med*. 2001; 344(11):783–92.

[94] Inoue K, Slaton JW, Perrotte P, Davis DW, Bruns CJ, Hicklin DJ, McConkey DJ, Sweeney P, Radinsky R, Dinney CP. Paclitaxel enhances the effects of the anti-epidermal growth factor receptor monoclonal antibody ImClone C225 in mice with metastatic human bladder transitional cell carcinoma. *Clin Cancer Res*. 2000; 6(12):4874–84.

[95] Xiong HQ, Rosenberg A, LoBuglio A, Schmidt W, Wolff RA, Deutsch J, et al. Cetuximab, a monoclonal antibody targeting the epidermal growth factor receptor, in combination with gemcitabine for advanced pancreatic cancer: a multicenter phase II trial. *JClin Oncol*. 2004; 22(13):2610–6.

[96] Dowlati A, Nethery D, Liu J. Combined inhibition of epidermal growth factor receptor (EGFR) and JAK/Stat signaling results in superior growth inhibition in A431 cell line as compared to single agent therapy [abstract]. *Proc Am Assoc Cancer Res*. 2003; 44(800):459.

[97] Finn RS, Wilson CA, Sanders J. Targeting the epidermal growth factor receptor (EGFR) and HER-2 with OSI-774 and trastuzumab, respectively, in HER-2 overexpressing human breast cancer cell lines results in a therapeutic advantage in vitro [abstract]. *Proc Am Assoc Cancer Res*. 2003; 44:235.

[98] Huang S, Armstrong EA, Benavente S, Chinnaiyan P, Harari PM. Dual-agent molecular targeting of the epidermal growth factor receptor (EGFR): combining anti-EGFR antibody with tyrosine kinase inhibitor. *Cancer Res*. 2004; 64(15):5355–62.

[99] Rose WC, Wild R. Therapeutic synergy of oral taxane BMS-275183 and cetuximab versus human tumor xenografts. *Clin Cancer Res*. 2004; 10(21):7413–7.

[100] Abuqayyas L, Balthasar JP. Investigation of the Role of Fc gamma R and FcRn in mAb distribution to the brain. *Mol Pharm*. 2012 Aug 23. doi: 10.1021/mp300214k.

[101] Suntharalingam G, Perry MR, Ward S, Brett SJ, Castello-Cortes A, Brunner MD, Panoskaltsis N. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *N Eng J Med.* 2006; 355:1018–28

[102] Stüve O, Gold R, Chan A, Mix E, Zettl U, Kieseier BC. Alpha4-Integrin antagonism with natalizumab: effects and adverse effects. *J Neurol.* 2008; 255 Suppl. 6: 58–65. doi: 10.1007/s00415-008-6011-0.