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Monoclonal Antibodies

A New Frontier in Treating Rheumatoid Arthritis.

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INTRODUCTION

The availability of monoclonal antibodies is nowadays an important success for pharmaceutical chemistry and other medical sciences. This achievement represents an extremely virtuous goal, continuously pursued by researchers and drug designers –that is, not only to develop a drug which might be as selective as possible in its pharmacological action on the receptor, but also to produce a deviation from the always detected typicality of a small biologically active molecule, capable of interacting with a large receptor molecule in order to either produce an effect or modulate a function.

Monoclonal antibodies represent the objectification of what Ehrlich –a German chemist active in the early 1900s– had forecast. He claimed, in this regard, that the ideal drug should be a sort of "magic bullet", able to head exclusively towards its molecular target, ignoring all the rest and thus maximally minimising its side effects. Ehrlich coined such a phrase during his studies on arsphenamine –an antibiotic active against syphilis– which can be doubtless considered, from a conceptual point of view, both the precursor of monoclonal antibodies and the antecedent of modern chemotherapy.

It would be excessively optimistic to state that the use of monoclonal antibodies in common clinical practice is completely devoid of side effects. However, what makes this procedure so extraordinary is not the fact that the search for such an extreme selectivity of action has moved too far, but rather that the extreme antigen specificity which our antibodies present when produced has been taken as a model for creating structurally specific drugs by means of genetic engineering techniques.

Laboratoristic, diagnostic and therapeutical applications of monoclonal antibodies (Mab) are currently numerous. Nevertheless, this brief treatise will mainly focus on their utilisation against Rheumatoid Arthritis, a chronic degenerative autoimmune disease.

1. Autoimmunity and autoimmune diseases

One of the classically accepted characteristics of the immune system is its capability of distinguishing between *self* and *non-self*. Due to this capacity, living organisms develop a response against a wide number of exogenous molecules, while, in normal conditions, they do not to generate any immune response against autoantigens, thus proving to be tolerant towards their *self* [2].

In most cases, when the immune system of an organism reacts against autologous antigens, organ damage is produced. This leads to a condition formerly known as "horror autotoxicus" and subsequently renamed 'autoimmunity'[43.]

Autoimmunity is the final result of a failure of one or more mechanisms regulating immunological tolerance. For this reason, 'autoimmune diseases' can be defined as those pathological forms related to a dysregulation of mechanisms regulating self-tolerance. What can typically be observed in autoimmune diseases is tissue damage caused by immunological reaction of the organism against its own tissues.

Normally, the lack of reactivity towards autoantigens depends on three main factors. The first is their sequestration, that is to say, they become inaccessible to the immune system (cryptic selves); the second is T- and B-cells tolerance; and the last one is the limitation of potential reactivity exerted by regulatory mechanisms. The alteration of such physiological processes may predispose to the development of autoimmunity[2].

Tolerance induction is due to the fact that autoantigens-reactive lymphocyte clones are eliminated during the development of the immune system (deletion) [43]. Actually, when a lymphocyte meets a specific antigen, the lymphocyte can be either activated or inactivated –i.e. eliminated. Lymphocyte activation determines a specific immune response, whereas lymphocyte inactivation induces tolerance towards the antigen. Another possibility is 'antigen ignorance' in which the antigen does not induce any response, neither positive nor negative[43.]

Immunological tolerance can be either central or peripheral. The former occurs at the level of the central lymphoid organs –i.e. the thymus for T-lymphocytes and the bone marrow for B-lymphocytes– and entails the encounter between a maturing lymphocyte and its respective antigen. In this case, lymphocytes are not activated and so become tolerant towards that antigen. Such antigens are just autoantigens. On the contrary, antigens from the external environment are captured by cells of the innate immune system and are carried to secondary lymphoid organs –i.e. spleen, lymph nodes. Besides, immature lymphocytes with high affinity receptors for autoantigens encountered at the level of primary lymphoid organs are eliminated (deletion). Central tolerance towards autoreactive T-lymphocytes is also known as 'negative selection'.

An analogous deletion occurs in the bone marrow with autoreactive B-lymphocytes. In fact, immature T-lymphocytes with high affinity receptors for autoantigenic peptides bound to major histocompatibility complex molecules (MHC) undergo a programmed death process, or apoptosis, known as clonal deletion.

On the other hand, peripheral tolerance is fundamental for determining the behaviour towards autoantigens expressed in peripheral tissues.

Peripheral tolerance towards B-lymphocytes essentially occurs when mature B-lymphocytes encounter specific autoantigens in peripheral tissues in the absence of specific T-Helper lymphocytes. In such conditions, B-lymphocytes become functionally unable to respond to the antigen.

Peripheral tolerance towards T-lymphocytes, instead, may occur through three main mechanisms: anergy, deletion and suppression.

Clonal anergy takes place when a mature T-lymphocyte encounters a specific peptide in the MHC niche of a presenting cell (APC, “Antigen Presenting Cell”) devoid of co-stimulatory molecules. It has been actually verified that lymphocytary activation occurs only in the presence of a double signal, represented both by the interaction between lymphocyte receptor and antigen (first signal) and the interaction between C28 molecules and co-stimulatory molecules (second signal). The sole reception of the first signal without the second drives lymphocytes into a state of functional non-responsiveness, instead of an activation state. Such observations suggest that the nature of APC is very important in the determinism of tolerance development and, conversely, of autoimmunity. When APCs are in resting phase and they do not express co-stimulatory molecules, antigen presentation by such cells determines clonal anergy of T-lymphocyte. Another mechanism of anergy is represented by the encounter between a mature T-lymphocyte and an APC endowed with co-stimulatory molecules, presenting, however, a peptide with altered amino acid residues in the contact zone with TCR (T Cell Receptor). In this case, the antigen mutates and the T-cell receives the second signal, which is normal, whereas the first signal is abnormal.

Clonal delection of mature T-lymphocytes occurs, instead, after a persistent T-cells stimulation by antigen, which results in a process known as ‘activation-induced cell death’. This latter is a form of apoptosis, induced by signals originating from receptors located on the membrane. The most important among them is Fas. Actually, the Fas/Fas Ligand interaction activates a series of proteases in the cell, called caspases, which determine apopoptic cell death. The final effect of this is a deletion of antigen-specific T-lymphocytes, whose repeated stimulation is triggered precisely by their specific antigen. This process is potentiated by the presence of high concentrations of IL-2, the principal growth factor for T-lymphocytes. A confirmation of what

stated above can be the fact that both Fas-deficient mice –or mice with mutation in Fas Ligand (FasL)– and IL-2-deficient mice spontaneously develop autoimmune diseases.

Some immune responses to autoantigens are inhibited by lymphocytes producing cytokines, which are able to block the activation of effector lymphocytes. Such inhibitor lymphocytes are also called T-suppressor lymphocytes. The most important suppressive cytokines are TGF-b, which inhibits T and B-lymphocytes proliferation, IL-10, which inhibits macrophage activation, and IL-4. IL-10 and IL-4 are principally produced by Th2 lymphocytes.

Clonal ignorance represents the third modality for maintaining tolerance and it is based on the capability of lymphocytes to respond to autoantigens without undergoing apoptotic death or anergy. Such a modality usually applies to autoantigens located in inaccessible anatomic sites, such as some eye tissues or the central nervous system. The reason for which some mature T-lymphocytes become anergic when they meet a specific antigen, whereas some others tend to ignore it, is still unknown. A possible explanation could be that, while high antigens concentration induces clonal anergy, antigens present at low concentration are ignored.

Based on the above considerations, it seems clear that both deficiency and alteration of the mechanisms which are normally responsible for the maintenance of tolerance might determine a response of the immune system to the “self”, thus determining the emergence of autoimmunisation phenomena. Such a potentiality exists in all the individuals since their lymphocytes express autoantigen-specific receptors. Besides that, many autoantigens are easily accessible to immune system cells.

The loss of tolerance to autoantigens can be a consequence both of an abnormal selection of autoreactive lymphocytes –that is a defect in central tolerance– and of alterations of autoantigen presentation to the immune system cells –that is an abnormality of peripheral tolerance.

Autoimmunity might obviously result from anomalies of B- lymphocytes, T-lymphocytes or both lymphocyte populations. However, T-lymphocytes anomalies definitely are the most important as they represent the central cells of all immune responses, with reference to both cell-mediated reactions and antibody production. Moreover, they are highly important since many autoimmune diseases are genetically linked to MHC, whose principal function is to present peptides to T-lymphocytes.

Deficits in central tolerance mechanisms, therefore, certainly constitute a valid model for explaining the emergence of an autoimmunisation process. In spite of this, it seems that the mechanisms responsible for peripheral tolerance are sufficient to evade the immune system response to autoantigens. In this respect, there are numerous experimental data which substantiate the possible role of peripheral tolerance defects in developing autoimmune diseases[43].

The rupture of peripheral tolerance might occur due to inflammatory processes capable of activating resting APCs present at tissue level, thus inducing on such cells the aberrant expression of co-stimulatory molecules, necessary to autoantigen presentation. This rupture might be linked to a defect in the expression or in the function of molecules which are normally destined for the inactivation of co-stimulation processes.

The loss of peripheral tolerance, and therefore the occurrence of an autoimmune disease, might also be a consequence of mutations which interfere with the apoptotic death of mature lymphocytes. In fact, genetically Fas and FasL deficient mice undergo a systemic autoimmune disease. A further cause –or contributory cause– might be a defect in suppression mechanisms mediated by T-lymphocytes, especially those capable of producing regulatory cytokines.

In the light of the above considerations, it seems clear that autoimmunity represents an occurrence linked to anomalies in the physiological processes of induction and maintenance of self-tolerance by the immune system. Such processes are manifold and they can be altered both by genetic anomalies and events occurring after a complex interplay of reactions between the immune system and numerous biologic agents counteracted and neutralised by the immune system in order to preserve homeostasis in the organism. Therefore, it is possible for immune responses against infectious agents to determine the rupture of immune tolerance to autoantigens –and consequently an autoimmunisation process– even if the presence of microorganisms cannot be found neither in lesion sites nor in the individuals in whom the autoimmune disease develops. However, infections can accordingly trigger an autoimmunisation process in different ways. In some cases, they might activate the expression of co-stimulatory molecules in tissue APCs, which otherwise would be resting. In some other cases, infections might favour the transformation of autoantigens into partially cross-reactive neoantigens. Finally, they might also cause the liberation of sequestered autoantigens, which are normally inaccessible to the immune system. This latter process might also occur after tissue damage resulting from traumas.

Cross-reaction can be explained by the fact that some infectious agents –such as bacteria and viruses– may share cross-reacting antigens with autoantigens, thus inducing an immune response also against “self” (mimicry). Therefore, in certain cases it is the stimulation of the immune system by exogenous agents what subverts the mechanisms regulating the response to autoantigens.

Molecular mimicry and cross-reactivity between bacterial products and autoantigens may induce the activation of auto-reactive lymphocytes. An example of molecular mimicry is rheumatic fever. In this disease antibodies against streptococcal M-protein cross-react with myosin, laminin and other matrix proteins. The reactivity of these antibodies at cardiac level triggers the

inflammatory response[2]. Molecular mimicry phenomena between bacterial proteins and body tissues have been also reported in other diseases, such as diabetes mellitus and multiple sclerosis.

Endogenous alterations of the immune system may as well induce the loss of immunological tolerance to autoantigens, thus favouring the development of autoimmunity. An example of endogenous alteration is represented by the loss of the so called 'immune privilege'. Many autoantigens, in fact, are located in immunologically privileged sites such as the brain or the anterior chamber of the eye –that is why in such sites transplanted tissues are incapable of developing an immune response.

This 'immune privilege' results from several events, among them are: (i) limited afflux of proteins from said sites to lymphoid tissue; (ii) local production of immunosuppressive cytokines such as Transforming Growth Factor β (TGF-β); (iii) local expression of molecules –such as Fas ligand– able to induce apoptosis of activated T-cells. As a result, lymphoid cells remain in state of 'immune ignorance' to proteins expressed only in immune-privileged sites. Hence, if privileged sites are damaged by trauma or inflammatory process, proteins expressed in such sites may become targets of an immune attack. An example of this can be multiple sclerosis.

Also physiological ageing phenomena are capable of causing alteration in the primary structure of proteins. This occurrence might result in an immune response to normal self-proteins.

Diffuse T-helper cells activation may induce autoimmunity phenomena. Besides, non-specific B-lymphocytes stimulation may equally provoke antibody production. Thus, alterations in the functionality of T and/or B-lymphocytes and in regulatory mechanisms of the immune system might also induce the development of autoimmunity.

Several mechanisms of autoimmunity have been evoked up to this point. However, none of them is individually able to explain all manifestations of autoimmunity. On the contrary, it seems that the overall picture is determined by a set of alterations. Besides, we must not forget that there are a number of important additional factors, such as age and genetic substratum, which contribute to the development of this phenomenon. Actually, it has been demonstrated that some genes are responsible for susceptibility to autoimmunity. Such evidences come from studies on twins which showed that illnesses like diabetes, rheumatoid arthritis, and systemic lupus erythematosus present a 15-30% disease concordance in monozygotic twin couples and a 5 % concordance in dizygotic twins.

Extensive research has been conducted on genes potentially capable of developing susceptibility to autoimmune diseases. The best known association for autoimmune susceptibility is that with the major histocompatibility complex (MHC). It has been hypothesised, in this regard, that the association between MHC genotype and autoimmune diseases might be due to differences in the

modality of presentation of autoantigenic peptides by different allelic variants of MHC molecules. In fact, there may be cross-reactivity between MHC autoantigenic peptides and peptides derived from proteins of common infectious agents due to molecular mimicry.

Tissue damage mechanisms in autoimmune diseases can be divided into antibody-mediated processes and cell-mediated processes.

The term autoimmunity only refers to the presence of antibodies or T-lymphocytes which are able to respond against autoantigens, without necessarily implying pathological consequences. Autoimmunity, therefore, may develop also as an isolated event and can be observed in healthy individuals as well. Besides, the expression of autoimmunity may be self-limited, i.e. it does not necessarily involve tissue damage. Hence, for classifying a disease as autoimmune it is necessary to demonstrate that the pathology observed derives from the immune response against an autoantigen.

Formerly, the demonstrated presence of antibodies against a diseased tissue in the serum of patients affected by a given disease was a sufficient evidence for considering the disease as autoimmune. However, it must be argued that such antibodies may be found even when tissue damage is secondary to trauma or infection and therefore the formation of antibodies is secondary to the damage itself. As a consequence, for classifying a disease as autoimmune it is necessary to demonstrate that the autoimmune process represents a true pathogenetic factor. Thus, it can be affirmed that there are two elements which may serve as presumptive evidence for immunological pathogenesis. The first is the existence of autoantibodies or lymphocytic infiltrates in tissue lesions. The second is the contextual demonstration of the capability of such autoantibodies or T-cells to cause tissue damage.

Provided that autoantibodies are pathogenic, it will be possible to transmit the disease to experimental animal models through the administration of such autoantibodies. This will lead to the development of a disease in the recipient which will present the same characteristics as the donor’s disease. Evidence of this is offered by autoimmune diseases transmitted from mother to foetus and observable in neonates born from affected mothers.

In many cases, critical factors converting autoreactivity into autoimmune diseases have not been identified yet. However, the relationship between autoimmunity and the development of autoimmune diseases might depend on factors such as antibody specificity, T-cells or effector functions of T-cells themselves.

The imbalance in cytokine production by T-helper cells plays a relevant role in the pathogenesis of some autoimmune diseases. As a matter of fact, T-cells differentiate into effector cells producing either interferon-γ (Th1) or IL-4 (Th2). The first ones stimulate both macrophage activation and the classical cell-mediated immune response, whereas the second ones play a

regulatory role, inhibiting the immune response. For this reason, in many autoimmune diseases – e.g. rheumatoid arthritis, multiple sclerosis, insulin-dependent diabetes mellitus and Crohn's disease– it seems that there is an imbalance towards Th1 which results in organ damage.

Autoimmune diseases might be either organ-specific –e.g. Hashimoto's thyroiditis, vulgar pemphigus, insulin-dependent diabetes mellitus– or systemic –e.g. systemic lupus erythematosus, rheumatoid arthritis, systemic necrotizing vasculitis. Organ-specific disorders include cell-mediated and/or humoral immune responses to antigens of individual organs. On the other hand, non-organ-specific types encompass responses against widely distributed self-components, determining anatomopathological lesions in different organs and systems. Systemic autoimmune diseases, hence, differ from organ-specific diseases precisely because of the presence of pathological lesions in several organs and tissues –LES, for example, involves kidneys, cutis, vessels, CNS.

This topic of study is definitely complex as there so are many variables at stake and, besides, its phenomenology has not been clearly outlined yet. Due to this, autoimmunity appears to be an extremely interesting clinical and scientific enigma.

2. Rheumatoid arthritis

Rheumatoid Arthritis is a systemic autoimmune disease of unknown aetiology, characterised by chronic phlogosis of synovial joints. It determines progressive anatomical damage, loss of functional capacities and disability onset[3]. Its typical pattern entails persistent synovitis involving peripheral joints in a symmetric distribution. Synovial inflammation causes cartilage destruction, bone erosions and, subsequently, joint deformity[2].

The incidence of RA is three times higher in women than in men. It affects all races from all over the world and its onset is most frequent during the fourth and fifth decades of life[2]. In US alone, over two million people are affected by the disease[36].

The aetiology of RA remains unknown. Nevertheless, a hypothesis might be the encounter with infectious agent such as Epstein-Barr virus, cytomegalovirus, parvovirus or rubella virus. However, there is still no sure evidence in this respect. What may probably happen is that structures belonging to an infectious agent are kept in the synovial tissue causing a chronic inflammatory reaction. Other scholars, instead, suggest that an organism exposed to a micro-organism might develop an immune response against its own joint constituents. This might be due to the infectious agent which induces its host to cross-react with its own articular structures because of molecular mimicry[2].

Progressive functional disability in RA is caused by two distinct yet correlated processes, i.e. synovial inflammation and joint damage.

Synovial inflammation is triggered and sustained by immune/inflammatory cells such as lymphocytes, macrophages and fibroblasts. Such cells induce excessive production of cytokines like TNFα. This latter promotes the inflammatory cascade which is responsible for both synovial thickening and pannus formation. Moreover, several inflammatory cytokines stimulate the production of osteoclasts, i.e. bone erosion and joint damage effector cells.

Microscopic examination shows hyperplasia, synovial cell hypertrophy and mononuclear cell infiltration. Hence, a large number of inflammatory cells can be found within the affected tissue[2]. The most common cell type in infiltrates is T-lymphocyte. More precisely, CD4+ lymphocytes (helper-inducer cells) prevail over CD8+ lymphocytes (cytotoxic-suppressor cells) [2]. In the specific case of RA, CD4+ T-cells are those which induce the immune response, most probably against an unknown endogenous or exogenous antigen[14].

Many cell types –including monocytes, macrophages and fibroblasts– are recruited at joint level. Here, important cytokines like TNF-α and IL-1 are produced. Such cytokines are basic to the

determinism of joint damage as well as to the production of matrix metalloproteinase and bone-destroying osteoclasts. Besides, we also find B-lymphocytes participation, demonstrated by the association of erosive disease with the presence of rheumatoid factor –an IgM antibody that is reactive with IgGs– which mediates the subsequent unexpected event, i.e. complement fixation. This latter phenomenon amplifies the destructive cascade process described so far, determining the attraction of more inflammatory cells to synovial sites and further production of cytokines. All this results in cartilage loss and bone erosion.

In addition to the accumulation of T-lymphocytes, rheumatoid synovitis is also characterised by B-lymphocyte infiltration and antibody-producing plasma cells.

Rheumatoid factor, which is very useful for diagnostic purposes, is also produced within the synovial tissue and this explains the local formation of immune complexes. Furthermore, synovial fibroblasts seem to be active in that they produce some enzymes such as collagenase and cathepsins which are able to degrade components of the articular matrix[2].

The rheumatoid synovium is also characterized by the presence of numerous secretory products of activated lymphocytes and macrophages. Cytokines produced by T-cells include: interleukin-2 (IL-2), interferon-γ (IFN-γ), IL-6, IL-10, TNF-β, IL-13, IL-16, and IL-17. Cytokines produced by activated macrophages, instead, are: IL-1, TNF-α, IL-6, IL-8, IL-10, PDGF and IGF[2]. These cytokines are responsible for synovium inflammation, cartilage and bone damage, as well as systemic manifestations of rheumatoid arthritis. In addition to the production of cytokines that propagate the inflammatory process, there is also the local production of slowing factors. Such specific inhibitors of cytokine action include an additional cytokine –i.e. transforming growth factor β (TGF-β)– which inhibits many of the manifestations of rheumatoid synovitis, including T-cell activation and proliferation, lymphocyte differentiation and, finally, migration of cells into the site of inflammation[2].

Within the rheumatoid synovium, CD4+ T cells differentiate into Th-1 effector cells producing the proinflammatory cytokine IFNγ and appear to be incapable of producing a sufficient number of Th2 effector cells, which would be able to generate the anti-inflammatory cytokine IL-4. The imbalance towards IFN-γ without the modulatory influence of IL-4 results in the activation of macrophages aimed at producing the proinflammatory cytokines IL-1 and TNF-α. Moreover, T-lymphocytes produce a variety of cytokines which promote proliferation and differentiation of cells into plasma cells[2].

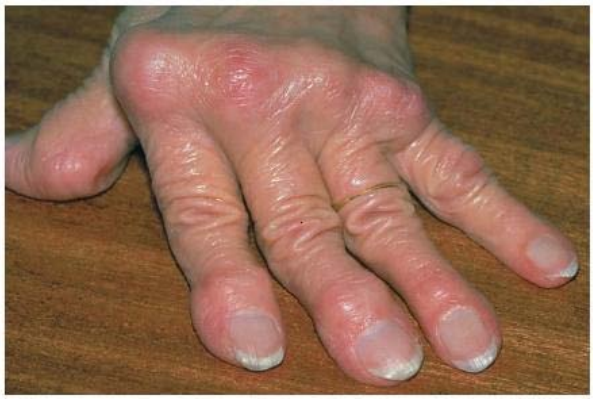
It is still unclear whether persistent T-cell activity represents a response to a persistent exogenous antigen or to an altered autoantigen such as collagen[2].

The final result is an increased influx of polymorphonuclear leukocytes into the synovium. In addition, vasoactive mediators such as histamine –produced by mast cells which infiltrate the synovium– facilitate the passage of inflammatory cells into the synovial fluid[2].

Vasodilation is also caused by locally produced PGE2 which facilitates the recruitment of inflammatory cells. For this reason, in the synovial fluid it is possible to find metabolites of arachidonic acid produced via cyclooxygenase and lipoxygenase pathways, which further accentuate the inflammatory process[2]. Besides, polymorphonuclear leukocytes reaching the synovial fluid can phagocytise immune complexes, thus releasing reactive oxygen metabolites and other inflammatory mediators, further adding to the pre-existing inflammatory substrate[2].

Synovial fluid contains a number of enzymes potentially able to erode the articular cartilage[2]. Over the course of synovitis, synovial hyperplasia takes place. This leads to the formation of the so called 'synovial pannus', which is typical of rheumatoid arthritis[4]. This pannus spreads so as to cover the articular cartilage. The cytokines IL-1 and TNF-α do stimulate the cells of the pannus since they favour the production of collagenase and proteases. These two cytokines also activate chondrocytes *in situ*, stimulating them to produce proteolytic enzymes that can locally degrade the cartilage. Besides, the above cytokines contribute to local bone demineralization by activating osteoclasts which are present in large number in the inflammatory site[2].

Neoangiogenesis is an essential event in the synovial proliferation process leading to pannus formation. Actually, vascular proliferation is present in the subsynovial connective tissue and provides nourishment for the hypertrophic synovial membrane, thus allowing cell migration in the articular environment[4].



RA, however, is a systemic disease and its systemic manifestations are determined by the release of phlogogenic molecules at synovial level, including IL-1, TNF-α, and IL-6. These molecules account for those generalised characteristic manifestations of active RA such as malaise, asthenia and elevated plasma levels of acute phase proteins.

Physiopathology and treatment of such a disease surely deserves further investigation. Nevertheless, it is fundamental to highlight the great causal importance of macrophage cytokines like TNF-α. A confirmation of this can be found in the marked amelioration of symptoms in patients affect by RA following the administration of a monoclonal antibody against TNF-α or, alternatively, of a synthetic molecule constituted by a soluble TNF-α receptor and an immunoglobulin fragment.

The process developing during the course of the disease is chronic and reiterative and is constituted by successive events which stimulate the progressive amplification of inflammation. In spite of this, it is not possible to predict time to disease progression –from the first initial non-specific inflammatory event to chronic inflammation with tissue lesion– in an individual patient. In fact, the time required to move from one stage to another varies from patient to patient.

Everything starts in genetically predisposed individuals with the exposure to a hypothetical etiologic agent and then follows with a progressive autoimmune synovial phlogosis characterised by synovial and lymphoid-cell hyperplasia. Subsequently, there is T-lymphocyte and macrophage activation followed by the liberation of a large number of qualitatively different proinflammatory cytokines such as interleukins, the fibroblast growth factor (FGF), the platelet-derived growth factor (PDGF), prostaglandins, nitric oxide and TNF.

The complexity of such events results in neoangiogenesis, synovitis, apoptotic processes alteration of various cell lines –lymphocytes and synoviocytes included– and, eventually, tissue destruction.

RA onset is insidious for more than half of patients as malaise and asthenia emerge before the appearance of synovitis. These prodromal symptoms may persist for weeks or months until the emergence of specific symptoms including the typical symmetrical involvement of joints, especially those of wrists, hands, knees, and feet. Only in 10% of patients, the onset is acute and presents polyarthritis accompanied by systemic symptoms such as fever, lymphadenopathy, and splenomegaly. Patients complain of pain, which is aggravated by movement, and show typical morning stiffness lasting over thirty minutes. What causes pain, swelling and limitation of movement is synovial inflammation. Pain results from stimulation of the joint capsule, which is extensively innervated and highly sensitive to stretching or distention. Joint swelling, instead, derives from accumulation of synovial fluid, hypertrophy of the synovium and thickening of the joint capsule.

Synovitis of wrist joints is an almost constant feature of RA and may determine median nerve entrapment (carpal tunnel syndrome). On the other hand, knee joints are involved with synovial hypertrophy, chronic effusion and frequently ligamentous laxity. Persistent inflammation

in such sites leads to (i) the manifestation of joint deformity caused by laxity of supporting soft tissue; (ii) destruction or weakening of ligaments, tendons or the joint capsule; (iii) cartilage degradation.

Extra-articular clinical manifestations of RA can be: (i) rheumatoid vasculitis, which in its most aggressive form might cause cutaneous ulceration, digital gangrene and visceral infarction; (ii) pleuropulmonary manifestations such as pleurisy, interstitial fibrosis and pneumonia; (iii) neurologic manifestations including polyneuropathies and neuropathies of median, ulnar and radial nerves, i.e. nerve lesions deriving from proliferative synovitis and joint deformity. Among extra-articular manifestations associated with RA, osteoporosis, which is aggravated by the typical cortisone therapy, should also be mentioned.

In patients affected by RA, mortality is increased by a coronary and cerebrovascular atherosclerotic process. In point of fact, myocardial infarction probability is doubled with respect to that of non-affected subjects. Such patients, therefore, present a shortened life expectancy and an increased cardiovascular mortality.

AR is also associated with proatherogenic lipid profiles. As a matter of fact, in affected patients serum lipoprotein (a) levels are significantly increased compared to those of healthy subjects, whereas high-density lipoprotein levels are reduced[6].

Regarding laboratory data, there are no specific tests for diagnosing RA. In spite of this, rheumatoid factor is a highly indicative element since it can be found in more than two-thirds of RA patients, although its presence is not disease-specific. In fact, besides RA, numerous pathological conditions are associated with the presence of rheumatoid factor, e.g. SLE, Sjogren's syndrome, visceral leishmaniasis, mononucleosis, etc.

If the presence of rheumatoid factor is not predictive of a RA diagnosis, it cannot be considered a screening test. Nevertheless, it certainly has prognostic significance because patients with high titres of such immune complex tend to present more severe and progressive disease. As a consequence, a test for detecting the presence of rheumatoid factor might be employed to confirm a presumed diagnosis based on a clinical presentation of the patient. Moreover, if present in high titre it can be useful for detecting patients who are at risk of severe systemic disease.

Besides, it is possible to find elevated levels of phlogosis indexes, that is, high titre of several acute-phase proteins –like C-reactive protein– ceruloplasmin and ESR.

The analysis of articular fluid confirms the presence of inflammatory arthritis, although none of the detected parameters are RA-specific. The fluid appears turbid, with reduced viscosity and increasing protein concentration. The number of leukocytes in the synovial fluid is superior to 2000/microlitre.

In the initial phase of the disease, radiography of the affected joints is not helpful in establishing a diagnosis. In fact, it only confirms what emerged from the physical examination of the patient, namely, soft tissue swelling and joint effusion[2]. With the progression of the disease, abnormalities become more evident. In spite of this, none of the radiographic findings is diagnostic of RA in absolute terms. The diagnosis, however, is supported by a characteristic set of abnormalities, such as symmetrical joint involvement, juxtaarticular osteoporosis, loss of articular cartilage and bone erosions.

The clinical course of the disease, despite being quite variable and unpredictable, generally shows that the majority of patients present a persistent disease with alternating phases of higher and lower activity accompanied by variable degrees of joint deformity.

Summing up, RA presents two distinctive yet interrelated processes causing progressive functional disability: synovial inflammation and joint damage. The former is triggered and sustained by immune/inflammatory cells such as lymphocytes, macrophages and fibroblasts, which determine excessive production of cytokines –like TNF-α– accounting for synovial thickening and pannus formation. On the other hand, joint damage results from the production of osteoclasts, i.e. bone erosion effector cells, stimulated by inflammatory cytokines. As a result, this pathology is far from being mild since over years it leads patients to disability, incapacity for work and inability to autonomously perform common everyday actions. Moreover, medial survival of RA patients is reduced by 50% compared to that of the corresponding healthy control population.

Such findings clearly point out that early diagnosis as well as timely and effective therapeutic intervention are an absolute necessity. In the following chapters we are going to deal precisely with the latest therapeutic advances focusing on two main aspects. Firstly, we are going to provide a pharmacological contextualisation of new molecules utilised in this field. Secondly we are going to discuss whether in a clinical setting such molecules may constitute an advance over traditional therapies, which are still widely used for treating RA.

As a consequence of what has been described until now, early diagnosis is not easy. Indeed, it has been estimated that the average time from the onset of symptoms to diagnosis is around nine months. This is strictly due to the non-specific nature of symptoms and it is only within 1-2 years after the onset that the disease assumes a characteristic clinical picture[2].

3. Traditional pharmacological treatment of rheumatoid arthritis

In the light of the above considerations, it becomes clear that RA treatment has always aimed at (i) relieving pain, (ii) reducing inflammation, (iii) protecting articular structures, (iv) maintaining functional capacity and, finally, (v) controlling systemic involvement. As a matter of fact, the pharmacological treatments employed are intended to ameliorate symptoms through a non-specific suppression of the inflammatory process and the prevention of progressive damage to articular structures[2].

Patients with RA should be managed with a multidisciplinary approach providing psychosocial and physiotherapical support. Other important aspects of RA therapeutic protocol are rest and physical exercise directed at maintaining muscle strength and joint mobility. Surgery might also help to decrease pain, correct articular deformities and improve joint functionality, especially in those patients with severely compromised articulations[2].

Traditional pharmacological treatments are based on the massive use of NSAIDs. Such drugs allow to control symptoms and signs related to the local inflammatory process. However, their effect on the progression of the disease is minimal[2]. Newer NSAIDs are specific inhibitors of the second cyclooxygenase isoform that is overexpressed at inflammatory sites. In the immediate post-marketing phases these molecules generated great enthusiasm within the scientific world. However, they were subsequently –and maybe unjustifiedly– condemned due to their severe cardiovascular side effects. Currently, their utilisation is fairly widespread since they present almost equal efficacy to that of classic NSAIDs combined with a lower risk of gastric injury and the possibility of long-term intake[2].

A second-line anti-inflammatory therapy involves glucocorticoids. New guidelines suggest using them as a temporary early therapy before resorting to DMARDs treatment. Their traditional indication, instead, involved their use in case of very high ESR or fever, until DMARDs treatment would not work[35]. Such substances still represent the most powerful and the most indicated treatment for inflammatory rheumatic diseases. However, very well-known are also their considerable side effects. For this reason, such drugs should be used with great caution, particularly during long-term therapy. They are currently being used in low-doses in mild course forms. Their antiphlogistic and immunomodulatory action influences cellular and humoral immune reactions and inhibits local accumulation of granulocytes and macrophages in the region where the inflammatory phenomenon occurs. Standard drugs in common clinical use are prednisolone and prednisone. In case of RA with visceral involvement medium doses of prednisolone are used initially (30-50

mg/day), whereas lower doses are more common (15-30 mg/day) in the presence of acute exacerbations of the disease, especially in case of rapid progression. Extremely low doses (3-6 mg/day) are instead sufficient for maintenance therapy. In this regard, it has been showed that such low doses delay radiological progression. Besides, maintenance dose should be kept as low as possible and individually adjusted. In case of malignant RA –i.e. a variant of the disease with significant joint involvement– bolus therapy with prednisolone or methylprednisolone (1 gram per day in physiological saline) is justified for several weeks. Such drugs, thus, are widely used in accordance with precise dosage schedules with the intention of suppressing signs and symptoms of the inflammation. However, they do not alter the course of the disease and present a considerable toxicity. In spite of this, recent evidence suggests that glucocorticoids play an important role in retarding the development and the progression of bone erosion. In those cases where systemic therapy might be ineffective against phlogosis, intra-articular infiltrations of corticosteroid constitute a possible alternative[2].

Third-line drugs include a variety of substances called DMARDs (Disease Modifying Anti-Rheumatic Drugs), i.e. medications that appear to have the capacity to alter the course of RA. These substances have no chemical affinity[35]. DMARDs reduce high levels of acute phase proteins, thus decreasing the inflammatory component of RA. However, they do not have immediate antiphlogistic and analgesic action. Methotrexate (MTX), gold salts, d-penicillamine, antimalarials, and sulfasalazine belong to this group. Despite having no chemical or pharmacologic similarities, these agents share a poor anti-inflammatory and analgesic action, so they can be used along with NSAIDs and glucocorticoids. DMARDs are also defined as ‘slow-acting anti-rheumatic drugs’ since it usually takes various weeks or months for their benefit to become evident[2]. DMARDs therapy allows clinical improvement in two-thirds of cases. There is frequently an improvement in serologic evidence of disease activity. As a matter of fact, titers of rheumatoid factor, C-reactive protein and the ESR do decline[2]. Moreover, recent evidence shows that DMARDs retard the development of bone erosions and promote healing if used in a timely and massive manner. In case of partial or complete long-term remission, discontinuation of DMARDs is always risky and, among other things, therapy resumption does not guarantee that the same substance will be successful again. Hence, dose reduction would represent a fair compromise[35].

There is no certainty about which should be the drug of first choice. Actually, controlled clinical studies have failed to demonstrate a consistent advantage of one over the other. Nevertheless, methotrexate has emerged as the DMARD of choice for what concerns rapidity of action, prolonged improvements and possible long-term treatment of patients. Obviously, each

DMARD has a specific toxicity, which is why thorough evaluation and careful patient monitoring are necessarily required[2].

MTX is used as a first treatment option for patients with diagnosed RA[35]. Besides, it is one of the most longly administrable DMARDs against rheumatoid arthritis and it is effective not only against early active and progressive cases, but also against long-term destructive RA which has proved to be resistant to other disease-modifying drugs[35]. MTX is an antimetabolite since it interferes with folic acid metabolism. Its action, however, is only partly due to folic acid antagonism. Actually, MTX is capable of inhibiting (i) leukocyte migration into the inflamed joint capsule; (ii) synovial fibroblasts proliferation; (iii) leukotriene synthesis; (iv) IL-1 and IL-6 activity[35]. This drug is administered in a dose of 15 mg a week orally or by parental route in case of inadequate response to oral therapy. Anyway, what is important is that its onset of action is 4-8 weeks. Besides, MTX is able to develop real remissions with cessation of radiological damage progression[35].

Indications for initiating a therapy involving such agents are not clearly defined. However, it is current tendency to start a DMARD in early stages, since only then it can be effective in delaying bone lesions, positively influencing the radiological course[2]. The present trend involves the use of two or more DMARDs combined together. Several patterns of combination therapy have been suggested, including, for example, MTX plus sulphasalazine plus hydroxychloroquine; MTX plus leflunomide; MTX plus Anakinra or cyclosporine A[35].

In RA treatment we should also consider immunosuppressive drugs such as azathioprine, cyclosporine and leflunomide. Such remedies are associated with numerous side effects and their use is typically reserved for patients who did not respond to anti-rheumatic drugs[2]. All medications used to treat RA do slow radiographic disease progression.

The use of azathioprine and cyclosporine is well established in clinical practice. On the other hand, leflunomide deserves further examination since it is the latest drug introduced in the field of RA therapy. Commercially known as Arava®, leflunomide is an isoxazole derivative exerting an immunomodulatory action. It is a competitive inhibitor of dihydroorotate dehydrogenase, i.e. an enzyme involved in pyrimidine nucleotides synthesis. Pyrimidine-synthesis inhibition has anti-proliferative effects as activated lymphocytes are those which carry out the synthesis.

Additive effects should be expected from leflunomide and MTX co-administration since the former inhibits protein synthesis, while the latter interferes with purine synthesis.

Leflunomide is an orally administered pro-drug and is rapidly converted into its active metabolite, that constitutes the 95% form in which the drug is found in the circulation. Such metabolites are highly bound to plasma proteins and present a half-life of between 15-18 days due

to the enterohepatic recirculation which they undergo. This latter process is so extensive that it takes two years for circulating drug levels to become undetectable. Leflunomide slows progression of radiographic damage. However, this drug presents hepatic adverse effects, which include raised levels of transaminases and general hepatotoxicity. This toxicity is reversible after discontinuation of therapy. Nonetheless, it should be carefully monitored as it could bring about evident hepatic failure and even the death of the patient. Naturally, subjects characterised by high levels of alcohol consumption or previously suffering from hepatitis, should not start such therapy under any circumstances. Furthermore, the combination of MTX and leflunomide associated with a higher risk of hepatotoxic effects than treatment with leflunomide alone. In clinical practice, during the treatment with leflunomide loss of 20% of body weight has been observed, but the determining mechanisms still remain unknown. This effect, however, rarely necessitates the discontinuation of therapy. Besides, hypertension occurs in the first two months of treatment and requires regular monitoring during therapy. Leflunomide is teratogenic, thus, women deciding to begin such therapy should not plan pregnancy in the short term. Indeed, she should simultaneously start contraceptive treatment, considering that no decrease in fertility has been reported in patients who have taken leflunomide.

MTX certainly is the most commonly employed DMARD; however, leflunomide might be considered a viable alternative to be used in case of adverse reactions to methotrexate. Despite this, leflunomide-methotrexate combination seems to be more effective than MTX-placebo. Nevertheless, this would entail some disadvantages such as hepatic adverse events and intensive monitoring of patients.

4. Clinical use of biologics for RA: etanercept

Our brief discussion on RA etiopathology has emphasised the pathognomonic role of several pro-inflammatory cytokines, among which TNF-α has proved to be fundamental[14]. Patients affected by RA have high levels of it in the synovial fluid. TNF-α is localized to the junction of the inflammatory pannus and healthy cartilage. Besides, it is involved in the bone erosion process. For this reason, TNF-α is considered a potential pharmacological target and its inhibition represents a valid therapeutic strategy.

In recent years, progress in understanding RA pathogenetic mechanisms has lead to the development of a new class of drug called 'biologics'. The advent of such drugs has certainly revolutionised the management of RA[36]. Biologics are produced through genetic and molecular engineering techniques and selectively interfere with specific humoral and cellular targets of immune response during RA[5]. Among them infliximab, etanercept, anakinra and adalimumab are already on the market with such indications, whereas others (tocilizumab, abatacept) are still being studied to test their effectiveness against the disease[5].

In analogy to DMARDs, biologics are also defined DCARTs (Disease Controlling Anti-Rheumatic Therapy) since they are able to arrest the progression of radiological damage. Numerous clinical studies demonstrate their efficacy in controlling signs and symptoms of the disease. Their efficacy is also confirmed in combination therapy with traditional DMARDs –mainly methotrexate, although, combinations with leflunomide and cyclosporine are also being tested[5].

Biologics, however, present high costs which greatly condition both the choice of whether to use them or not and, above all, the most suitable time to do so. The early and widespread use of biologic drugs in RA treatment raises questions about long-term tolerability profile and economic implications that providing structures should face. Identifying predictive factors for response and toxicity as well as monitoring patients receiving the treatment is therefore highly important.

It is no coincidence that biologics were initially indicated for RA in active phase with an inadequate response to traditional DMARDs[5]. Some believe, in this regard, that using biologics from the very beginning might result in the hyper-treatment of patients susceptible to traditional DMARDs and also in the exposure to infections or other side effects. According to this school of thought, such drugs should be reserved for subjects presenting aggressive RA, with negative prognostic factors and resistant to a combination therapy involving methotrexate. On the contrary, new therapeutic algorithms entail the use of TNF antagonists at earlier stages, provided that high-dose DMARDs has proved to be ineffective after two months of therapy[5].

It has been estimated that the current percentage of patients treated with biologics ranges from 5 to 30%, according to the urban centre or the nation considered.

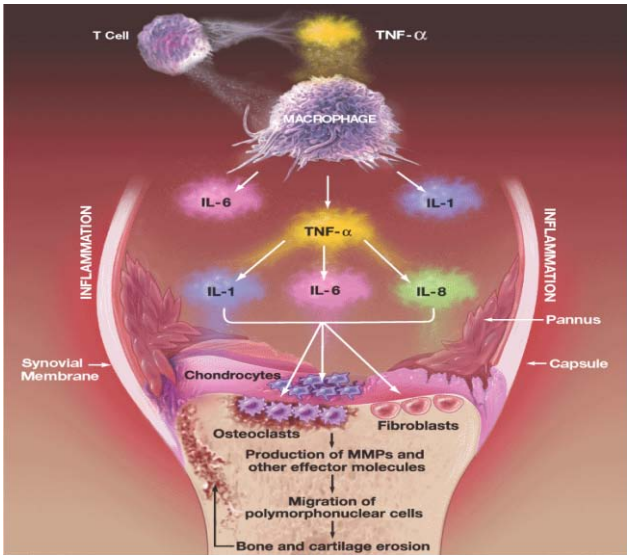
Among specialists and clinicians, there are discordant opinions. As mentioned above, some argue that an immediate use of biologic drugs might cause hyper-treatment of patients susceptible to traditional DMARDs, but also infection and other side effects typically detected in antibody treatments. In this respect, the purpose of our paper is not that of setting such controversy, but rather, of stimulating critical thinking by means of reliable data reported here and there in scientific treatises.

In accordance with the guidelines of SIR (Società Italiana di Reumatologia), biologics are currently reserved for those patients showing aggressive RA, that is, with negative prognostic factors and resistant to a combination therapy involving methotrexate[5].

The above mentioned biological agents are monoclonal antibodies (MAB) such as infliximab, which are able to bind and neutralize TNF-α. Only one of them is a TNF-α type II receptor fused to IgG1 (etanercept)[2].

Before embarking on a detailed discussion about biologics inhibiting TNF-α in RA treatment, let us just focus specifically on the role of this latter in the light of recent discoveries.

TNF-α is a soluble fragment of 17-kD protein, produced by enzymatic action of a metalloproteinase –i.e. TNF-α Converting Enzyme (TACE)– exerted on a 26kD precursor molecule represented by a transmembrane molecule present in a number of cells. TNF binds two distinct cell-surface receptors: a 55-kilodalton TNFR and a 75 kD TNFR. Both TNFRs exist either in membrane-bound and soluble form. Soluble TNFRs are thought to regulate TNF biological activity. TNF-α is mainly produced by macrophages and activated T cells and promotes other inflammatory cytokines synthesis (IL-1, IL-6, IL-8). It exists as homotrimer and its biological activity is due to the bond established with membrane TNFR. Besides, it (i) promotes the expression of adhesion molecules; (ii) stimulates metalloproteinases production by synovial macrophages, fibroblasts, osteoclasts and chondrocytes; (iii) inhibits cartilage proteoglycan synthesis[15].

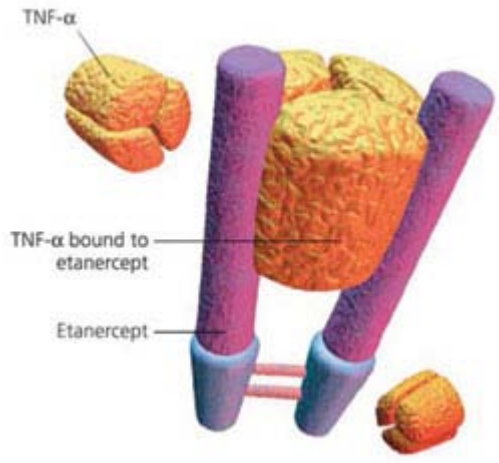


TNF-α (i) modulates cell growth and differentiation; (ii) stimulates lypolisis; (iii) activates cell apoptosis; (iv) induces the synthesis of cytokines like IL-2 and IL-18 –potent inducers of INFγ; (v) amplifies the response of Th-1s that are directly involved in RA pathogenesis. Last but not least, TNF-α induces nitric oxide (NO) synthesis, thus favouring arteriolar vasodilation and the expression of adhesion molecules –VCAM-1, E-selectin, ICAM-1– on the surface of endothelial cells. This results in the recruitment of an increasing number of lymphocytes and other potentially phlogogenic cells in the inflamed articular sites.

Both TNF-α transmembrane molecule and TNF-α soluble molecule show biological activity. As soon as it is produced, the soluble form tends to aggregate into homotrimers, which are capable of binding to specific receptors on the membrane of different cells such as fibroblasts, leukocytes and endothelial cells. P55 TNFR and p75 TNFR are both expressed on all the major inflammatory cells and work as components of signal transduction[15]. P75 TNFR originates T and B-cells that are activated by neutrophils and endothelial cells. On the other hand, p55 TNFR produces epithelial cells. Their average lifespan in the circulation is approximately four hours. TNFRs mediate a number of biologic functions and their expression and release into the circulation is modulated by several hormones and cytokines[8-9]. The function of TNFRs could be to regulate the bioavailability of TNFs in the body. TNF may act in several ways: (i) as TNF antagonists when present in excessive amounts; (ii) as a TNF-carrier protein; (iii) as a reserve for the release of TNF, thus prolonging its half-life[20].

TNF-α soluble form is present in variable concentrations according to disease activity. It can be found in the synovial fluid and in the serum of patients with rheumatoid arthritis, but it might be also expressed on the membrane of activated T-cells.

Having outlined the importance of the pro-inflammatory cytokine TNF-α in rheumatoid arthritis, we shall now specify that the first attempt to develop anti-TNF-α biological drugs dates few years. Actually, the first anti-TNF-α drug was Etanercept (Enbrel®), approved in 1998.



Etanercept is a fusion protein made up of a dimer of the extracellular portion of p75 TNF-α receptor fused with the Fc portion of human IgG1. It is obtained by recombinant DNA techniques and represents a soluble TNF-α receptor. In point of fact, it is engineered to be recognised in the body as a TNF-α receptor, thus his action is aimed at avoiding the cytokine fitting its physiological receptor[7]. Dimeric soluble receptors, such as etanercept, possess a higher affinity for TNF than monomeric receptors and therefore they are potent competitive inhibitors of TNF binding to its cellular receptors.

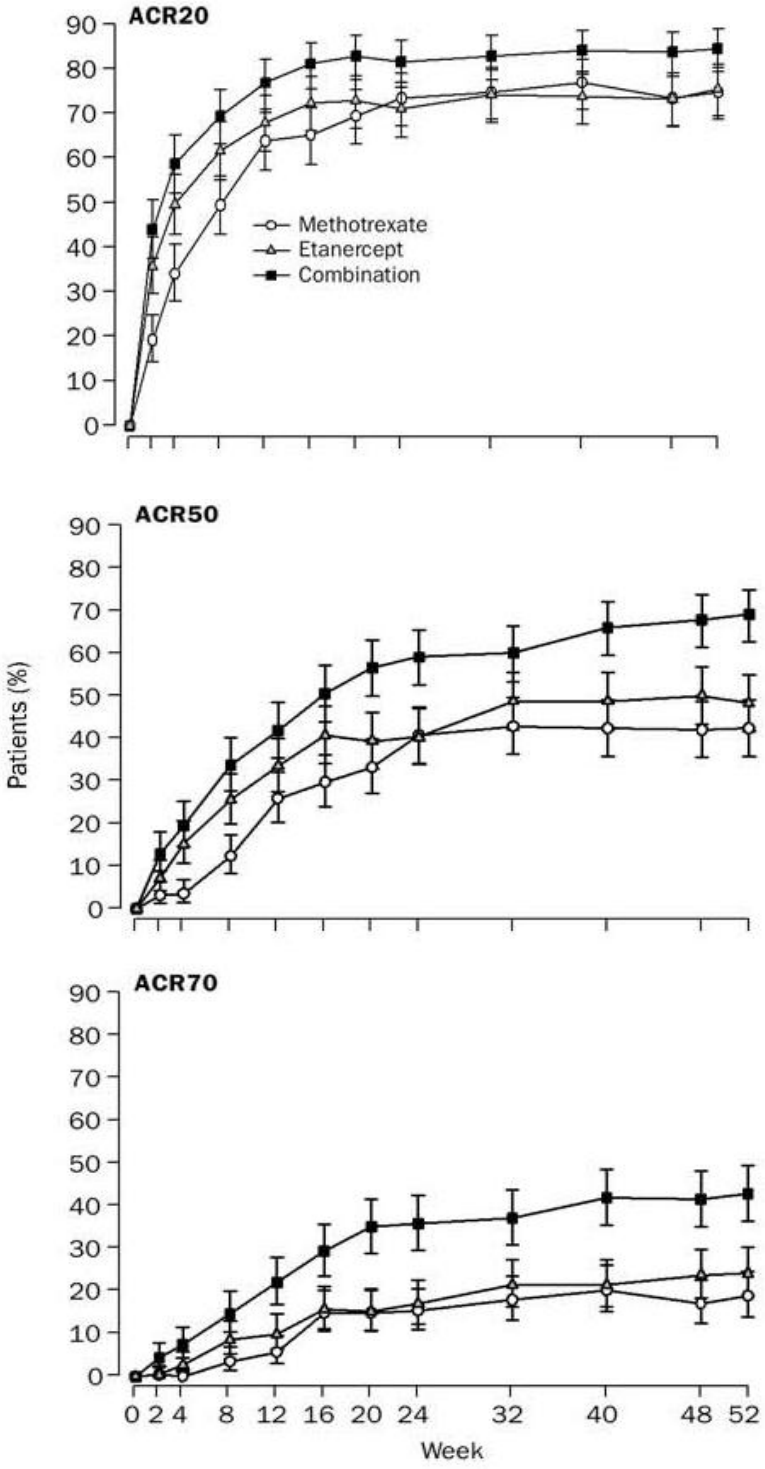
Etanercept is produced by introducing human DNA into Chinese hamster ovary cells. The result is a protein administered subcutaneously at a dose of 25 mg once or twice weekly under close medical supervision[7]. Absorption from the injection site is slow and peak plasma concentration is reached after 48 hours. Its half-life is no less than 70 hours. Besides, methotrexate does not alter its pharmacokinetics.

If administered to patients affected by readily diagnosed early rheumatoid arthritis, this drug reduces disease activity and slows joint destruction in a more rapid way compared to methotrexate[49].

In the active phase of the disease, Etanercept is superior in combination with MTX compared with methotrexate monotherapy.

Its approved therapeutic indications include the treatment of moderate to severe active rheumatoid arthritis in combination with methotrexate when the response to disease-modifying antirheumatic drugs, including methotrexate itself, has proved inadequate. Safety and efficacy of combination therapy are worth being evaluated in comparison with separate administration of both drugs.

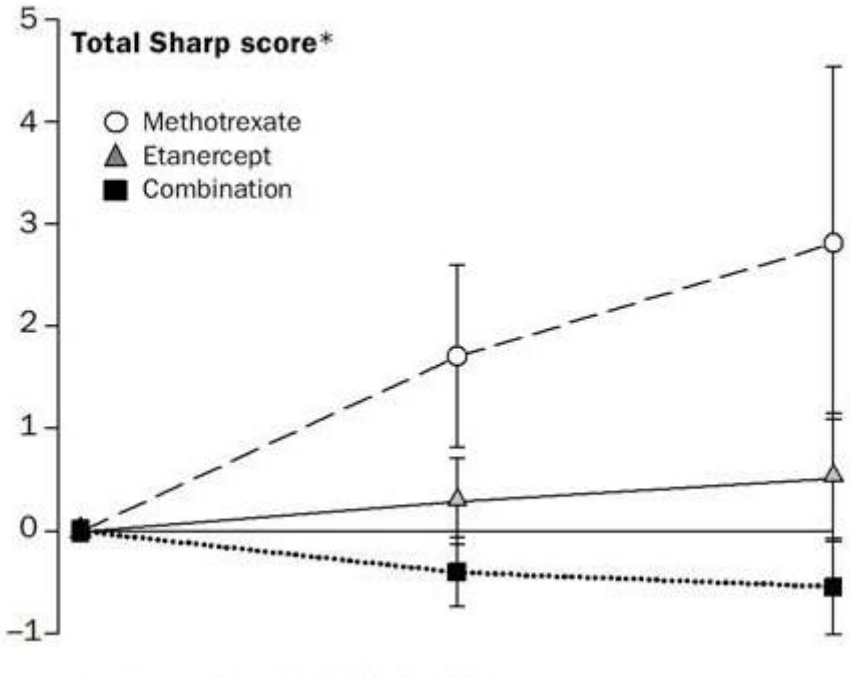
What follows is the description of an experimental study called TEMPO (Trial of Etanercept and Methotrexate with Radiographic Patient Outcomes). This was a randomized, double-blind, trial comparing precisely efficacy and safety of MTX alone, etanercept alone and combination therapy of both. A 25 mg dose of etanercept was administered subcutaneously twice a week plus an oral placebo or, alternatively, oral MTX (5-20mg) plus a subcutaneous placebo. The study concluded with observations made on week 24 and 52. The proportion of patients achieving ACR20 after fifty-two weeks was higher in the group treated with the combination therapy (85%) than in groups treated with MTX alone (75%) or etanercept alone (76%). Besides, the proportion of patients achieving ACR50 (69% versus 43% and 48%) and ACR70 (43% versus 19% and 24%) was markedly higher in the combination therapy group than in the other two[49].

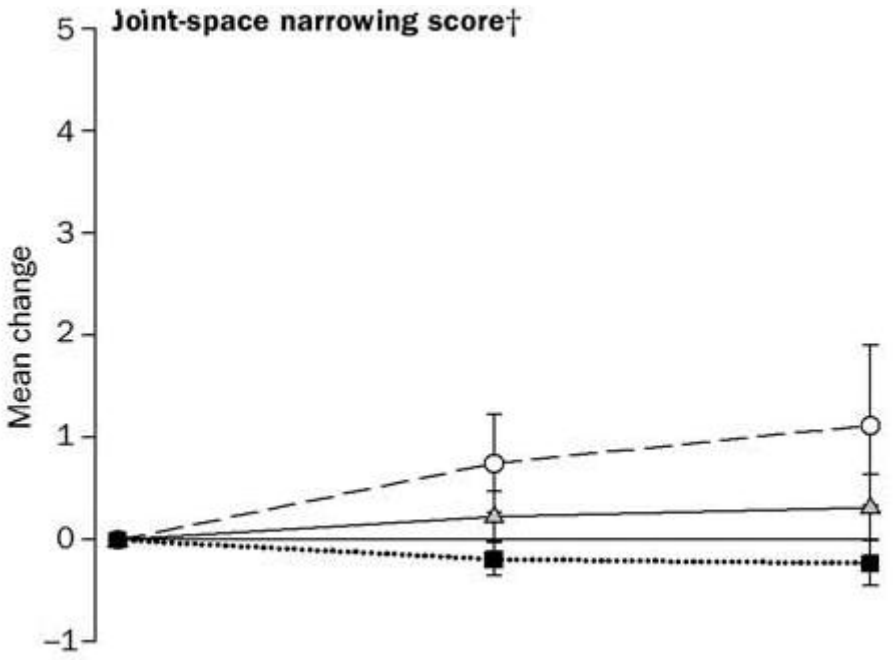


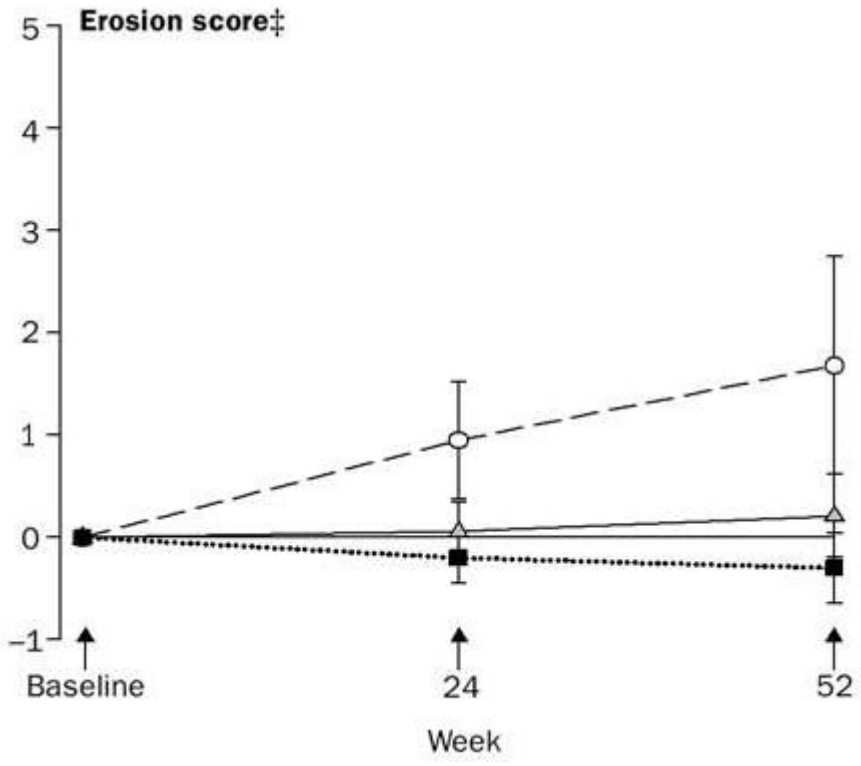
Numerically expressed disease activity after fifty-two weeks was lower in the combination therapy group than in the other two. On the other hand, the proportion of patients achieving remission with a disease activity inferior to 1.6 was significantly higher in the combination therapy group. Moreover, combination therapy showed higher scores also for what concerns physical disability of patients assessed in HAQ. Even Total Sharp Score (TSS) was in favour of the group treated with methotrexate plus etanercept. However, comparing the two monotherapy groups, TSS

turned out to be lower in patients receiving etanercept than in those receiving MTX at both time points. Disaggregating the value, we find out that etanercept had an advantage in slowing bone erosion, whereas there was no significant difference between the two monotherapy groups regarding joint space narrowing.

Combination therapy, however, is always to be preferred to both separate monotherapies.







Significantly enough, in addition to a slowdown in disease progression, the combination therapy group showed progression arrest at a TSS rate of < 0.5, which is a higher percentage compared to that of the other two groups. Actually, radiographic disease progression was slowed more in combination therapy group and etanercept group than in MTX group.

Thus, it is possible to state that a combination therapy with etanercept plus the DMARD gives more favourable clinical responses than the separate administration of the biologic. Moreover, a positive finding is that taking both drugs together does not result in a higher incidence of infections or other adverse events.

	Methotrexate (n=228)	Etanercept (n=223)	Etanercept and methotrexate (n=231)
Any adverse event	185 (81%)	192 (86%)	187 (81%)
Abdominal pain	40 (18%)	26 (12%)	42 (18%)
Accidental injury	25 (11%)	19 (9%)	21 (9%)
Asthenia	20 (9%)	23 (10%)	24 (10%)
Back pain	20 (9%)	28 (13%)	24 (10%)
Cough increased	17 (7%)	14 (6%)	25 (11%)
Diarrhoea	20 (9%)	23 (10%)	19 (8%)
Headache	32 (14%)	34 (15%)	34 (15%)
Injection site reaction*	4 (2%)	46 (21%)	23 (10%)
Nausea†	73 (32%)	22 (10%)	55 (24%)
Rash	21 (9%)	16 (7%)	23 (10%)
Vomiting‡	26 (11%)	7 (3%)	12 (5%)
Infections			
All	147 (64%)	131 (59%)	154 (67%)
Serious	10 (4%)	10 (4%)	10 (4%)

The potentiality of etanercept has been used to treat adults affected by several autoimmune pathologies; not only rheumatoid arthritis, but also psoriatic arthritis and ankylosing spondylitis. The drug is contraindicated for pregnant or nursing women, patients with active infections and decompensated diabetics. The use of Enbrel might entail serious infections such as sepsis, tuberculosis and other opportunistic infections which may be lethal, life-threatening or require hospitalization[50]. If a patient develops infection, the drug should be promptly discontinued. Reactivation of HBV has been reported in patients who are chronic carriers of this virus and are receiving TNF-antagonists, including Enbrel. This does not exclude treating such patients with the drug. However, they should be monitored for signs and symptoms of active HBV infection. Besides, there have been post-marketing reports of allergic reactions including angioedema, urticaria and worsening of congestive heart failure. Formation of antinuclear antibodies is also possible during the treatment as well as the development of other antibodies that are associated with cutaneous reactions compatible with a lupus from a clinical and bioptical point of view.

Etanercept is the only anti-TNF approved for use in children. As a consequence, paediatric Enbrel has been recently placed on the market.

The medicament is made up of a pre-filled syringe containing 50 mg/ml of etanercept. It is administered by subcutaneous injection and is very slowly absorbed. It reaches peak plasma concentration after more or less fifty hours and its half-life is four days. Comparative studies were conducted few years ago in order to define the dosage. A 10 mg dose of etanercept was compared to a 25 mg dose and a placebo. 234 patients were enrolled in a six-month randomized study. Both doses of etanercept appeared to be effective and resulted in ACR20 responses rating respectively 51 and 59 %, compared with 11% percent in the placebo group. The 25 mg dose was preferred as it resulted in a more rapid response and accounted for an ACR50 in 40% of cases, versus 24% in 10mg dose cases and 5 % placebo cases[14]. A 50 mg dose administered once a week appeared as effective as a 25mg dose taken twice weekly.

ACR is an acronym standing for American College of Rheumatology. ACR and EULAR (European League Against Rheumatism) have developed seven standard objective parameters for defining the response to the treatment during clinical trials. Said parameters include (i) the count of tender and (ii) swollen joints; (iii) the measurement of ESR or CPR levels; (iv) the degree of functional disability determined by means of HAQ (Health Assessment Questionnaire); (v) the degree of pain reported by the patient; (vi) physician's and (vii) patient's global assessment of disease activity. Hence, a statistically significant response to the treatment requires a 20% decrease of tender and swollen joints, as well as a 20% improvement in three of the five remaining parameters. All this determines ACR20 response[45].

ACR20 is a minimum response evaluation index as it represents the minimum differentiation value from placebo treatment. After ACR20, 50% and 70% response stratifications were introduced.

The efficacy of Enbrel was compared to the gold standard in RA treatment, i.e. methotrexate. 10 or 25 mg of etanercept were administered and tested in comparison with escalated doses of MTX. It was observed that patients receiving the highest dose of etanercept (25mg) showed a rapid response within the first two weeks, although the ACR20s of both groups were comparable after a year of treatment.

Afterwards, there was another study which turned out to be even more interesting. It was aimed at showing how etanercept might be used in addition to MTX in patients who were no more responsive to this latter. This combination resulted in significantly better scores than the association of MTX with a placebo.

As for side effects, increased susceptibility to infectious diseases –especially those affecting the respiratory tract– has been reported. Besides, reactions at injection site; dizziness and headaches are also possible[7]. The actual risk of infections during anti-TNF-α treatment is due to the fact that the cytokine TNF-α plays a fundamental role in synovial inflammation and host defense mechanisms, especially those concerning intracellular infections. Anyway, patients suffering from RA already have major risk of infections increasing with age. This results also from the steroid therapy to which they are subjected.

5. Infliximab

Etanercept was followed by infliximab (Remicade®) which was marketed in 1999. Infliximab is a monoclonal antibody, first approved for treating Crohn's disease, and is currently indicated also for ankylosing spondylitis, psoriatic arthritis and psoriasis[39].

It is a chimeric monoclonal IgG1 antibody against TNF-α and consists of human constant regions and murine variable regions. It binds to both soluble forms and membrane forms of TNF with high affinity, thus impairing the binding of TNF-α to its receptor. Infliximab also destroys cells expressing TNF-α through antibody-dependent and complement-dependent cytotoxicity

Its therapeutic indications include active rheumatoid arthritis when the response to disease-modifying anti-rheumatic drugs, including methotrexate, has been inadequate. Remicade is also indicated for patients with severe, active and progressive disease not previously treated with methotrexate or other DMARDs. In this patient population, a slowdown in joint damage progression has been demonstrated by radiographic evaluation[39]. In patients with rheumatoid arthritis, the drug markedly reduces signs and symptoms of the disease when used in combination with methotrexate.

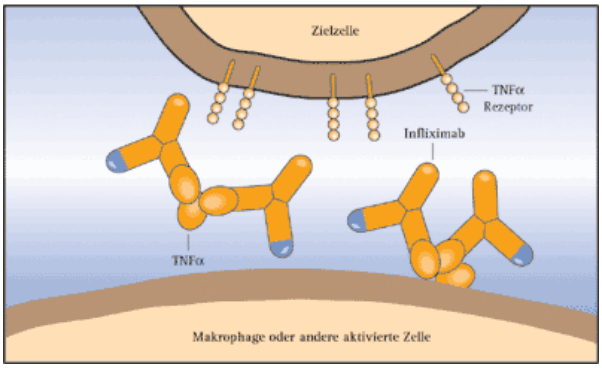
The efficacy of infliximab in RA was assessed in two multicentre, randomised, double-blind, pivotal clinical studies: ATTRACT and ASPIRE.

The ATTRACT (Anti-TNF Trial in Rheumatoid Arthritis with Concomitant Therapy) study showed that 50% of patients treated with infliximab plus methotrexate exhibited a 20% improvement according to ACR20 criteria. The therapy with methotrexate alone presented improvements only in 20% of patients and so did placebo therapy[8].

The ASPIRE (Active controlled Study of Patients receiving Infliximab for treatment of Rheumatoid arthritis of Early onset) study demonstrated that 59% of 722 patients using infliximab plus methotrexate did not show joint damage after a year, compared to 45% of 282 patients taking methotrexate alone. The same study, however, pointed out some disadvantages in the use of infliximab concerning the incidence of tuberculosis and other opportunistic infections. Tuberculosis was the most common granulomatous infection reported, but candidiasis, histoplasmosis, listeriosis and nocardiosis were also found. It was certainly for this reason that the U.S. FDA in 1999 approved the use of infliximab only in combination with methotrexate for those patients who were failing the treatment with methotrexate alone. Subsequently, in 2004 FDA itself extended the use of the antibody. As a result, Remicade was no longer considered just a complementary treatment to a therapeutic failure of the DMARD.

Regarding its mechanism of action, infliximab neutralises the biological action of the macrophage cytokine –i.e. TNF-α– by binding with high affinity to the soluble and transmembrane forms of the cytokine, thus preventing it from binding with its receptor.

The therapeutic utility of the drug is based on the neutralisation of TNF-α. This cytokine, in fact, accounts for the production of other pro-inflammatory cytokines –such as IL-1 and IL-6– as well as for the increase in leukocyte migration. This results in the augmentation of both endothelial permeability and expression of adhesion molecules by endothelial cells. Other consequences are the activation of neutrophils and eosinophils as well as fibroblast proliferation and prostaglandin synthesis. As a consequence, Remicade reduces (i) serum level of IL-6 IL-1 IL-8; (ii) lymphocyte migration into the joints; (iii) expression of adhesion molecules on the surface of endothelial cells. Moreover, the reduction of vascular endothelial growth factor (VEGF) can be observed after three weeks of treatment. Besides, those cells expressing transmembrane TNF-α are recognised and bound by infliximab. Afterwards, they are lysed by complement and effector cells.



Infliximab therapy, then, reduces infiltration of inflammatory cells into inflamed joint sites. Besides, it decreases the expression of (i) adhesion molecules such as E-selectin, ICAM 1 (Intercellular Adhesion Molecule 1) and VCAM 1 (Vascular Adhesion Molecule 1); (ii) monocyte chemotactic proteins (MCP-1 and IL-8); (iii) tissue-degrading metalloproteinases MMP1 and MMP3.

The drug is administered intravenously at doses ranging from 1 to 20 mg/kg of body weight every 4-8 weeks and its half-life is particularly long (between 8 and 9.5 days). This should be taken into account if a surgical intervention is planned. In this case, the patient should be closely monitored for the increased risk of infections. The infusion should be administered in a hospital environment by skilled health personnel under medical supervision.

Pharmaceutical formulation of Remicade entails 100mg vials which are to be diluted in 250 ml of physiological solution for intravenous infusion. The recommended infusion time is 2 hours[39]. All patients treated with infliximab are to be observed for at least 1-2 hours post-infusion in order to detect and promptly intervene in case of acute reactions. Emergency equipment, such as adrenaline, antihistamines, corticosteroids and an artificial airway must be available[39]. Actually, acute infusion-related reactions, including immediate anaphylactic shock, may occur in the immediate post-infusion period.

It takes two to four weeks after the first dose for Remicade to show its major benefits. Moreover, anti-TNF-α treatment is to be initiated by qualified physicians experienced in the diagnosis and treatment of rheumatoid arthritis.

Patients managed with biologics are to be carefully assessed before staring the treatment and closely monitored during the therapy and after. In particular, anamnestic information about the patient should be gathered once clinical and radiological features of the disease are detected[38]. Naturally, its clinical evolution is to be followed and monitored for the toxicity related to anti-TNF-α treatment. Such records shall be made by specialists on apposite computerisable sheets. The first step is to fill in a Treatment Initiation Form at the time of first prescription. Afterwards, a Monitoring Form is to be completed after 14, 22 and 54 weeks of therapy. This module shall be updated any time changes to treatment plan are demanded or serious adverse effects requiring the intervention of a specialist are reported. Patients whose follow-up exceeds 54 weeks are to be monitored at weeks 78 and 102[38]. The above mentioned Monitoring form allows periodic assessment of therapeutic response and tolerability. It also reports the results of laboratory tests and observations on treatment compliance and adverse events. However, completing this form does not substitute for normal adverse events reporting procedures. In Italy, such information will be sent electronically to SIR (Società Italiana di Reumatologia) database so as to be analysed.

Based on the foregoing, it seems clear that such a controlled and reported treatment regimen must be followed by accredited, specialised hospitals or universities with specific competences. Therefore, such medications should be dispensed by qualified centres with proven expertise in treating RA.

Again in Italy, such centres are classified either as *Strutture Complesse di Reumatologia* (complex centres of rheumatology) or *Strutture di Immunologia Clinica* (clinical immunology centres). Only in the case that there is an insufficient number of said places within the region of reference, other treatment centres will be spotted. These are *Strutture Semplici di Reumatologia* (simple centres of rheumatology), offering outpatient, day-hospital and longer hospital stay. As far as drugs for polyarticular juvenile rheumatoid arthritis are concerned, the dispensing centre should be instead a *Struttura Complessa di Pediatria* (complex centre of paediatrics) equipped with *Unità Semplici di Reumatologia* (simple units of rheumatology). Regions and autonomous provinces are the institutions which indentify specialised centres eligible to treat RA and communicate them to the Department of Health.

Clinical trials have showed that the combination anakinra plus etanercept resulted in severe infections without any additional clinical benefit compared to the use of etanercept alone. Given the nature of the events observed with this combination, similar adverse effects might occur with the combination anakinra plus infliximab, which, for this reason, is contraindicated[39]. Besides, it is also recommended not to administer live vaccines to patients receiving anti-TNF-α therapy.

During the treatment with infliximab, noticeable side effects are sings and symptoms of infection such as cold, cough and sore throat. This clearly shows how infliximab reduces the defence capabilities of the body against infections. Skin rashes, fever, blood in urine, muscle and joint pain are also detectable. Headaches, nausea and vomiting might also be included.

Normally, protocols involve Mantoux intradermoreaction for patients managed with infliximab therapy. This is aimed at testing a prior exposure to bacillus Calmette-Guérin. Said patients also undergo chest radiograph in two projections.

During the anamnesis interview, patients will be asked whether they have a history of heart failure. Actually, in cases showing pre-existing congestive heart failure, a higher incidence of mortality is detected. This is precisely due to the fact that infliximab worsens heart failure, especially in those patients treated with doses higher than 10 mg/kg, i.e. twice the maximum approved dose[39]. Infections, however, are the most common serious adverse event. Besides, it is worth mentioning malignant neoplasms and lymphoproliferative diseases, whose incidence is higher for patients treated with Remicade than for those treated with placebo. Moreover, in patients receiving the drug, mild or moderate elevations of ALT and AST have been observed without

progression to severe hepatic injury. These are transitional abnormalities observable both when the drug is given as a monotherapy and when it is used in combination with other immunosuppressive agents[39].

Studies have shown that, administering infliximab with MTX every 4 or 8 weeks determines, in radiologically-assessed joint damage prevention, more significant effects than the association of MTX with a placebo.

Reported indications for infliximab entail the administration of a 3mg/kg dose at weeks 0, 2 and 6, followed by further administrations every 8 weeks. Clinical experience suggests, instead, that dosage escalation would be useful for coping with incomplete responses or loss of responses. Several clinical trials show the efficacy of either an increase in the dose above 3mg/kg up to the limit of 7.5 mg/kg every 8 weeks, or a decrease in dosing interval below 8 weeks, involving, for example, the administration of 3mg/kg every 4 weeks.

Nowadays, clinicians are no more compelled to use this and other biologics according to standard posological schemes. This results in a greater number of patients identifiable as potential users of the therapy, for whom customised and highly personalised dosage cards might be created[40]. Moreover, over time antibodies against these drugs might develop thus reducing their efficacy. This would lead to a dosage escalation aimed at controlling disease activity, resulting, however, in the exposure to a greater risk of side effects. The most obvious consequence of this scenario is the increase in the consumption of the drug, which determines a rise in hospitals' procurement costs. Reliable hospital trusts are aware of the issues relating the optimisation of the use of biologic drugs to such an extent that there have been several attempts to reduce waste.

The solution fund by the Pini Institute in Milan was to summon all patients to receive infliximab on the same day, at the same time, so as to minimise the wastage of reconstituted drug which would have been thrown away for being superfluous. Instead of getting rid of the residual reconstituted vial, this latter could be used to produce the correct volume of drug to infuse to another patient on the same day. This allowed them to obtain a cost reduction of 1,000 €/year per patient. This figure is further rounded up if each centre benefiting from this solution takes into account the number of patients enrolled to start a treatment with biologics. The resources saved, then, might be used to treat other patients with such expensive, as well as innovative, drugs[40].

There has been a great commitment in making the medical community aware of expense problems, without depriving patients of proper treatments. Said issues are related to the enormous cost burden of biologics, which leads to the necessity of discussing the question in terms of pharmacoeconomics, for the massive impact exerted on the resources of centres deciding to stock them[40]. In this regard, escalating infliximab dosage has major effects and is very common among

new patients in the first year of treatment. Such practice was shown to entail considerable economic consequences on the expense.

Returning to technical characteristics of the drug, it must be specified that, although etanercept treatment is approved for children and adolescents aged 4 to 18 with polyarticular juvenile arthritis, infliximab is not authorised for such patients since its use in subjects aged 0 to 17 has not been studied. The two drugs differ also in their routes of administration. For this reason, infliximab is given at referral centres, whereas etanercept is delivered to the patient at time of check-ups in such quantity that the period of treatment between check-ups is covered.

6. Adalimumab

Adalimumab (Humira®) is a recombinant human monoclonal antibody expressed in hamster ovary cells, used in the treatment of moderate to severe, active rheumatoid arthritis in adult patients. It is structurally and functionally analogous to human IgG1, presenting the same half-life (14 days). Adalimumab selectively binds to TNF with high affinity and impairs TNF-α biological function by inhibiting its bond with p55 and p75 cell membrane receptors.

TNF-α is an osteoclastogenic cytokine which greatly promotes the inflammatory process in RA. Its inhibition determines a sustained control of both inflammation and joint damage which results from osteoclasts differentiation and activation. These latter differentiate from monocyte and macrophage precursor cells through a process that involves several cytokines such as macrophage colony-stimulating factor (M-CSF), Rank ligand, TNF, IL-1 and IL-6. Each of these cytokines binds to a specific receptor present on the surface of osteoclasts precursors, thus accelerating specific stages of differentiation. M-CSF promotes the initial stage of stem cells differentiation to monocytes. At this point, monocytes proliferate and then differentiate into osteoclasts precursor cells. Rank ligand, TNF, IL-1 and IL-6 control, instead, the subsequent stages of osteoclastogenesis. Rank and TNK act synergistically to promote osteoclast differentiation. During the late stage of the osteoclast differentiation process, precursor cells fuse to form mature multinucleated osteoclasts. After being differentiated, mature osteoclasts need be activated in order to erode bone tissue. TNF and Rank ligand are fundamental mediators in this activation phase.

During the course of RA, there an increase in TNF levels in the synovium, which results in abnormal osteoclast differentiation and activation, leading to joint damage.

Recently, osteoprotegerin (OPG) has been assessed for being an important regulator of osteoclasts differentiation process. It acts as an uncoupling protein, blocking and reducing the quantity of Rank ligand available for binding to its receptors. OPG and Rank ligand play a key role in osteoclastogenesis.

Pharmacological agents like adalimumab are TNF inhibitors as they bind to and neutralise it. As a consequence, the bound form of TNF cannot activate its respective receptor. The effect obtained is that of interfering with osteoclast differentiation and activation, thus inhibiting joint damage.

Adalimumab specifically binds to TNF-α and therefore controls inflammation and joint damage. Recent findings suggest that TNF inhibition entails increased OPG levels, which constitutes a further method to manage osteoclast-mediated joint damage.

Humira allows a rapid decrease in acute phase proteins such as CRP, ESR and cytokines like IL-6. Serum levels of matrix metalloproteinases (MMP-1 and MMP-3), involved in tissue remodelling that is responsible for cartilage destruction, also decrease.

Adalimumab, besides, modulates biological responses induced or regulated by TNF, including changes in the levels of adhesion molecules responsible for leukocyte migration.

The molecule can be used, besides, when the response to DMARDs is inadequate and is also indicated for treating severe, active and progressive RA in adults not previously treated with methotrexate. Moreover, it is used against ankylosing spondylitis, Crohn's disease and active psoriatic arthritis in case of inadequate response to DMARDS.

Adalimumab inhibits the progression of structural, radiographically assessed damage and might be used in monotherapy.

Its posology entails a single 40 mg dose administered every week via subcutaneous injection. During this therapy it is preferable to continue the treatment with methotrexate. Besides, concomitant use of glucocorticoids and NSAIDs is allowed.

In this regard, a study named PREMIER was conducted with patients presenting early, aggressive RA, combining adalimumab plus methotrexate. What was shown was that radiographic damage progression at two years is markedly higher in patients treated with methotrexate alone, whereas it decreases very significantly with the combination of both molecules. Adalimumab, then, offers radiographic control of the disease. As a matter of fact, the absence of radiographic progression could be observed in the combination arm, compared to the monotherapy arm. Moreover, 50% of patients reported clinical remission at 2 years. For this reason, biologics are considered a disease controlling anti-rheumatic therapy (DCART), being capable of arresting radiological damage progression.

After subcutaneous administration absorption is slow, with peak serum concentration being reached 5 days after administration. Its clearance is similar in both men and women and is not influenced by age or weight. However, using adalimumab in combination with MTX determines a 20% reduction of drug clearance after a single MTX dose and 44% decrease after several doses of MTX.

In several clinical studies confronting adalimumab and a placebo, or placebo/MTX with adalimumab/MTX, ACR responses significantly improve, compared to placebo, after 24-26 weeks. As expected, the combination with MTX determined more rapid and significantly superior ACR responses compared to monotherapy.

It is well-known that methotrexate alone slows the progression of joint damage and improves signs and symptoms of rheumatoid arthritis. However, it has been demonstrated that the

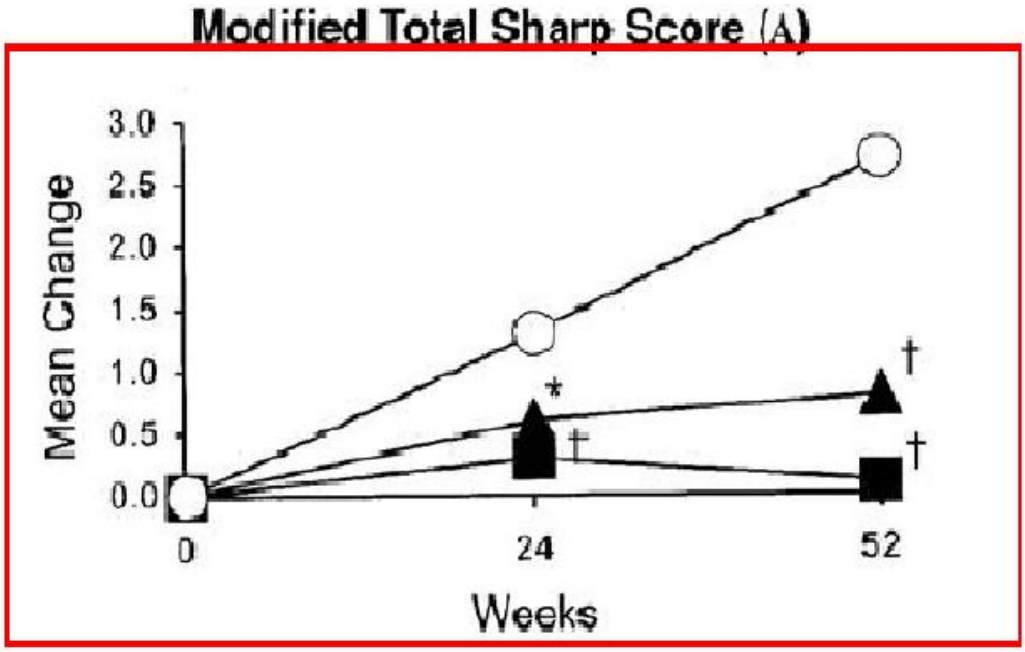
combination of adalimumab with a continuing regimen of MTX provides additional benefits in terms of radiographic progression and clinical, as well as functional, status of patients presenting active RA and an incomplete response to methotrexate[41].

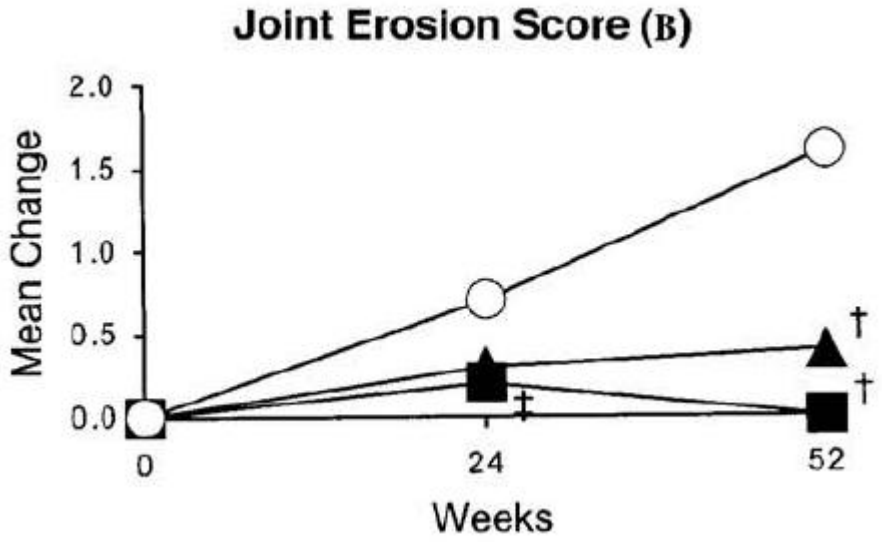
In a multicenter, 52-week, double-blind, placebo-controlled study, patients with active RA who had an inadequate response to MTX were asked to receive adalimumab subcutaneously at the dose of 40 mg every 2 weeks. Another group, instead, was administered 20 mg of adalimumab weekly. Finally, a third group received a placebo. All three groups underwent a therapy with concomitant methotrexate. The end point taken into account was total Sharp score at week 52 –i.e. the sum of bone erosion and joint space narrowing scores. The result is graphically illustrated in the table below, which shows the radiographic improvement obtained in the first two groups, compared to placebo.

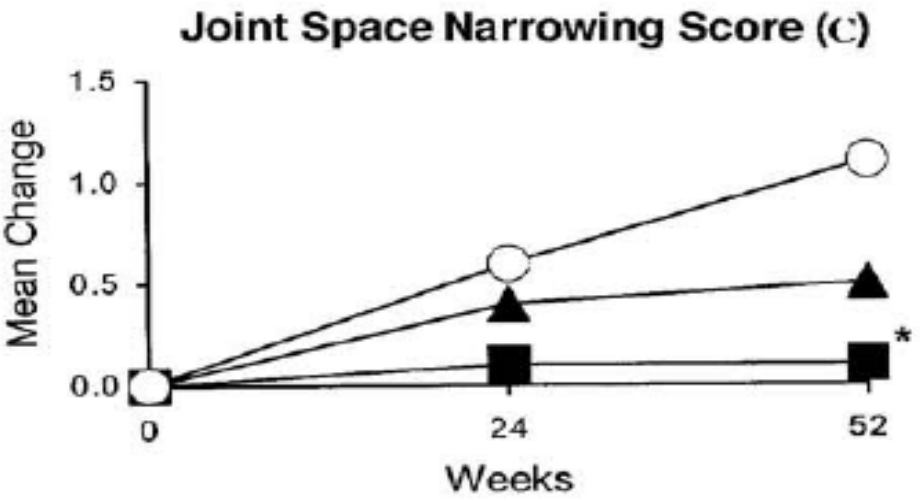
Table 2. Patients with at least 20%, 50%, and 70% improvement in the ACR response criteria*

ACR response*	Adalimumab 40 mg every other week plus MTX (n = 207)	Adalimumab 20 mg weekly plus MTX (n = 212)	Placebo plus MTX (n = 200)
ACR20 response			
Week 24	131 (63.3)†	129 (60.8)†	59 (29.5)
Week 52	122 (58.9)†	116 (54.7)†	48 (24.0)
ACR50 response			
Week 24	81 (39.1)†	87 (41.0)†	19 (9.5)
Week 52	86 (41.5)†	80 (37.7)†	19 (9.5)
ACR70 response			
Week 24	43 (20.8)†	37 (17.5)†	5 (2.5)
Week 52	48 (23.2)†	44 (20.8)†	9 (4.5)

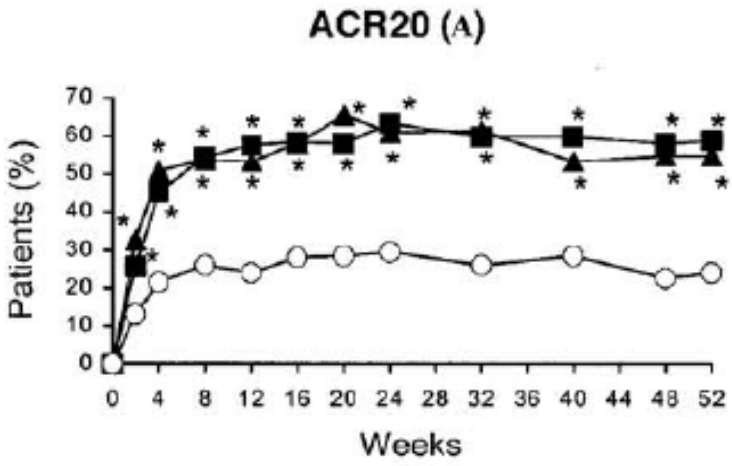
Note how differences in radiographic progression rates emerge already by week 24. However, differences between the first and the second group selected are not marked. Joint erosions were also lower in the groups treated with the biologic than in the third one, which, despite being the placebo group, still took the DMARD.

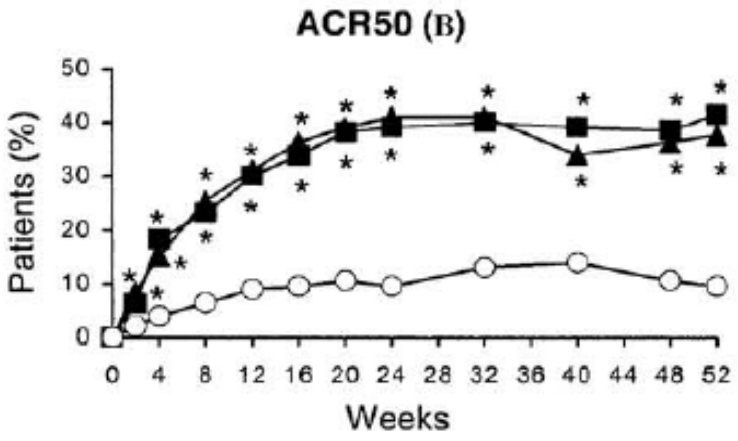

Modified Total Sharp Score (A)
Mean Change
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
24
52
Weeks
*
†
‡
†
†

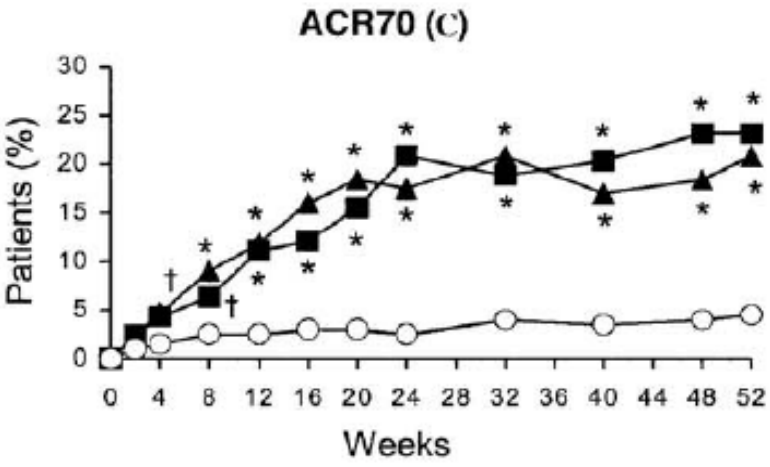

Joint Erosion Score (B)
Mean Change
2.0
1.5
1.0
0.5
0.0
0
24
52
Weeks
‡
†
†



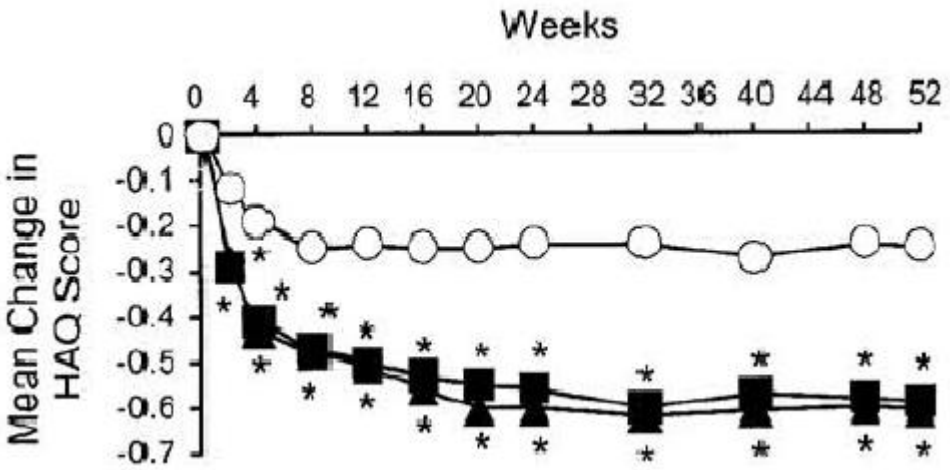
Patients treated with adalimumab also demonstrated very rapid clinical improvements compared with those treated with placebo, as measured by the ACR20, ACR50, and ACR70 responses registered at weeks 24 and 52. This effect could be observed already by week 2 and persisting through week 52,







With respect to the baseline, physical function of patients, measured by means of a questionnaire assessing their health status (HAQ), decreased at each measurement from week 1-2 in adalimumab group, compared to placebo group. This demonstrates that adalimumab has a positive impact on physical performance of patients as it allows them to improve their functional ability in common everyday actions.



Also in this case, contraindications to the use of the drug are active tuberculosis or other severe infections. Patients, therefore, must be monitored closely for infections, including tuberculosis, before, during and after the treatment with adalimumab.

Complete elimination of the drug might take up to five months; thus, monitoring should be continued throughout this period. If a patient develops a new serious infection during the treatment, administration should be discontinued until the infection is controlled by means of appropriate pharmacological therapy. Clinical trials have demonstrated an increased risk of other serious infections –such as pneumonia, pyelonephritis, and septicaemia– in patients treated with adalimumab.

Before starting the therapy, all patients must be evaluated for active or latent tuberculosis through tuberculin skin test and chest X-ray. If active tuberculosis is diagnosed, the therapy cannot be initiated. In case of latent tuberculosis, adequate antitubercular prophylaxis should be taken before adalimumab treatment. Patients will be warned to promptly notify whether during the therapy there is the occurrence of signs and symptoms suggestive of a tuberculosis infection, such persistent cough and low grade fever. Cases of invasive opportunistic infections, as for example pneumocystis carinii pneumonia, disseminated histoplasmosis, aspergillosis, have been reported in association with the therapy.

Reactivation of hepatitis B has occurred in patients chronic carriers of HBV when receiving a TNF-antagonist, including adalimumab. Some cases have been fatal. It will be necessary to assess carefully the opportunity to administer the treatment to such patients. If it is decided so, they are to be closely monitored for signs and symptoms of active HBV infection, even for several months following the termination of the therapy. In case of infection reactivation, administration should be stopped and effective anti-viral therapy should be initiated.

In the above described clinical study, among the arm treated with adalimumab 40 mg, the arm treated with adalimumab 20 mg and the placebo arm, the 40mg-adalimumab group was the one which presented the highest proportion of patients reporting infections –in some cases even requiring hospitalisation or intravenous antibiotic therapy, compared to placebo group. However, in the group treated with 20mg/week of adalimumab there were no significant differences compared to the placebo group.

The table below presents a comparison of adverse events reported in the three arms studied. It also shows that the most frequently reported side effects were injection-site reactions such as localized erythema, swelling, pain, and hemorrhage[41].

	Adalimumab 40 mg every other week plus MTX (179.2 patient-years) (n = 207)		Adalimumab 20 mg weekly plus MTX (186.7 patient-years) (n = 212)		Placebo plus MTX (161.3 patient-years) (n = 200)	
Adverse event	No. (%)	No./patient-year	No. (%)	No./patient-year	No. (%)	No./patient-year
Injection-site reaction†	54 (26.1)	0.30	47 (22.2)	0.25	48 (24.0)	0.30
Upper respiratory tract infection	41 (19.8)	0.23	41 (19.3)	0.22	27 (13.5)	0.17
Rhinitis	34 (16.4)	0.19	37 (17.5)	0.20	33 (16.5)	0.21
Sinusitis	33 (15.9)	0.18	31 (14.6)	0.17	26 (13.0)	0.16
Accidental injury	29 (14.0)	0.16	28 (13.2)	0.15	24 (12.0)	0.15
Headache	26 (12.6)	0.15	29 (13.7)	0.16	12 (6.0)	0.07
Infection	15 (7.2)	0.08	33 (15.6)	0.18	9 (4.5)	0.06
Nausea	19 (9.2)	0.11	26 (12.3)	0.14	25 (12.5)	0.16
Diarrhea	19 (9.2)	0.11	24 (11.3)	0.13	30 (15.0)	0.19
Arthralgia	14 (6.8)	0.08	29 (13.7)	0.16	24 (12.0)	0.15
Rash‡	22 (10.6)	0.12	20 (9.4)	0.11	15 (7.5)	0.09
Joint disorder	13 (6.3)	0.07	14 (6.6)	0.08	23 (11.5)	0.14
Clinical-flare reaction	12 (5.8)	0.07	8 (3.8)	0.04	29 (14.5)	0.18

The tolerability of Humira becomes evident considering percentages of occurrence of the most common adverse event –comparable in all tree arms. Apparently, the rate of serious infections is absolutely comparable with that associated to the disease itself as well as with the rate of infections reported for etanercept and infliximab[41]. Such results derive from the necessity of proving experimentally the non-inferiority of each new drug compared to those which currently represent the gold standard of care.

Another important difference, which emerged in the group treated with adalimumab, was the statistically significant decrease in white blood cell count and platelet count, accompanied by the increase in haemoglobin and lymphocyte concentration. This might be due to the anti-inflammatory activity of the drug[41].

The study showed, besides, an additional datum of general nature, confirmed also by later studies, i.e. that adalimumab treatment can be associated with the development of anti-nuclear antibodies (ANAs) and anti-double-stranded DNA antibodies. Such findings, however, are not univocally linked to adalimumab treatment, but they are also reported in therapies involving other biologics.

The study concluded that, in patients with moderate to severe rheumatoid arthritis and in patients partially responsive to methotrexate, additional use of adalimumab provides for rapid and valid results, which remain stable over the 52 observational weeks[41].

TNF-antagonists have been associated in rare instances with onset or exacerbation of clinical signs and symptoms of central nervous system demyelinating diseases including multiple sclerosis, transverse myelitis, aseptic meningitis, optic neuropathy, Parkinson's disease, Guillain-Barré syndrome, leukoencephalopathy. However, the size of the problem is unknown[30]. The most common neurological symptoms are paraesthesia, visual disturbances, confusion, and concentration problems usually occurring after 5 months of treatment and partially or completely resolved after treatment discontinuation. Rare instances of pancytopenia have been reported after the use of TNF-antagonists. All patients should promptly notify signs and symptoms of blood dyscrasias, i.e. persistent fever, ecchymosis, hemorrhages.Adalimumab might also worsen congestive heart failure. Several studies on anti-TNF treatment have reported a number of cases with neoplasms, compared to the control group.

In some instances adalimumab resulted in an immune response, determining the formation of anti-antibody antibodies. This is associated with increased clearance and reduced efficacy of the drug. However, there is no apparent correlation between the presence of anti-adalimumab antibodies and adverse events. Adalimumab immunogenicity has been reported in 5.5% of the patients treated. The incidence was higher, when the drug was not administered with concomitant MTX, compared to when adalimumab was used in combination. Actually, Humira can be given as monotherapy and in combination with methotrexate. However, without MTX reduced efficacy of adalimumab has been reported.

Administration is not recommended during pregnancy, therefore, women of childbearing potential are strongly recommended to use adequate contraception which should be continued for at least five months after the last adalimumab treatment. Regarding lactation, it is not known whether adalimumab is excreted in breast milk. Nevertheless, being it an immunoglobulin, such possibility is well-founded. Thus, women should not breast-feed for at least five months after the last treatment.

Finally, other common side effects are (i) gastrointestinal disorders (diarrhoea, abdominal pain and nausea); (ii) hepatobiliary disorders with elevated liver enzymes; (iii) skin rash, dermatitis and eczema; (iv) disorders of the nervous system (dizziness, headaches, paraesthesia), (v) musculoskeletal pain; (vi) asthenia and general malaise. Upper respiratory tract or urinary tract infections, herpes and candidiasis are also commonly observed[9, 27, 28].

7. Rituximab

Rituximab (Mabthera®) is another biologic medication. This anti-CD20 monoclonal antibody has achieved remarkable clinical responses in patients affected by RA. However, it is not considered a first-line drug and is indicated for patients with rheumatoid arthritis who had an inadequate response to TNF inhibitors. This is due to the fact that this drug determines B-cells depletion[10].

Rituximab was initially used as an antitumor drug particularly in the treatment of non-Hodgkin lymphoma.

Available data indicate that B-cells depletion does not influence the count of regulatory T-cells. Regarding T-lymphocyte subpopulations, in fact, no variation both in CD4 T-cells –i.e. T-helper lymphocytes– and in CD8 T-cells –i.e. cytotoxic T-lymphocytes– is observed. There is also no increase in the number of CD3+ cells, neither during the active phase of rituximab, nor during B-cells regeneration after 12 months.

B-cells play an important role in the mechanism leading to the alteration of articular structures. Nevertheless, they are not the only component involved in the auto-destructive reaction. Rituximab has immunomodulatory effects on the synovial B-cell infiltrate. This emerged from the immunohistochemical analysis of cellular infiltrate, performed on synovial biopsy specimens before and after treatment. All patients treated with rituximab showed an almost complete depletion of circulating B-cells. Serum IgM and IgM-FR decreased in nearly all patients. In spite of this, overall synovial architecture, synovial inflammation, synovial immunoglobulin levels, and cytokine synovial expression did not show significant changes from the baseline, neither in responsive nor in non-responsive patients. On the other hand, serum IgG remain unchanged.

Synovial B-lymphocytes depletion accounts only partially for rituximab effect, as it presents an inferior reduction of the number of B-cells, compared to peripheral blood depletion. Moreover, the response to the drug has proved to be independent from baseline antibody status. It might be thought that the action of rituximab derives from the suppression of B-cells –i.e. antibody-producing cells. However, the drug has shown to be effective also in patients that were seronegative for both rheumatoid factor and anti-cyclic citrullinated peptide. This confirms that other B-cells mechanisms –not mediated by pathogenic autoantibody suppression– contribute to rituximab effects.

Although rituximab effects are possibly not entirely due to synovial B-lymphocytes depletion, it has been observed that during and after the treatment B-lymphocytes persistence in said patients

is often associated with a less favorable prognosis, whereas in subjects with complete B-cell depletion more durable clinical responses can be found.

Rituximab treatment inhibits the progression of structural joint damage in patients with RA. As a result, the drug might be used for the treatment of patients with active rheumatoid arthritis who have had an inadequate response to one or more TNF-inhibitor therapies –thus with a particularly aggressive form of the disease– or provided that this latter therapy presented some contraindications.

Rituximab is suitable for intravenous administration and in combination with MTX improves disease activity. Such treatment is safe and well-tolerated. The recommended dosage is 1000 mg intravenous infusion every two weeks. The therapy might be repeated for keeping or further improving its efficacy, without increasing the number or the severity of adverse events. Indeed, rituximab treatment repetition prolongs the improvement of physical function, obtained already after the first treatment cycle; besides it improves physical and mental components scores of quality of life[10].

During rituximab treatment, mild to moderate adverse events have been reported. Their maximum frequency can be observed during the first treatment cycle. Actually, the incidence of acute infusion reactions decreases between first course therapy and subsequent treatments. More specifically, acute infusion reactions entail adverse events occurring during or within 24 hours after the infusion, such as pruritus, skin eruptions, hyperpyrexia, pharyngeal irritation, cough, bronchospasm, hypotension and hypertension.

Investigations have tried to find out if major adverse events might occur in patients with active RA previously receiving rituximab therapy and afterwards treated with one or more TNF-inhibitors. In other words, such studies have attempted to evaluate safety of TNF-inhibitors, specifically infliximab and adalimumab. Comfortingly enough, it has emerged that the incidence of severe infections seems to be similar before and after TNF-inhibitors treatment, even though patients obviously present a low level of peripheral B-cells due to rituximab treatment. Besides, the rate of serious infections is also similar to that in subjects treated for the first time with TNF-inhibitors from the beginning of the therapy[10]. Once rituximab treatment is discontinued, a gradual regeneration of peripheral B-cells can be seen. Such reconstruction shows a similar pattern after each cycle and is associated to a loss of response. However, temporal relationship between the two events is considerably variable, thus B-cell regeneration does not necessarily predict an imminent loss of drug response.

8. Anakinra

Another biologic drug taken into account for its use against RA is the IL-1 inhibitor anakinra (Kineret®) [13].

IL-1 is a crucial mediator of the inflammatory response. There are two different forms of IL-1, i.e. cytokines IL-1α and IL-1β. In turn, IL-1 acts on three types of receptors: IL-1R1, IL-1RII, and the accessory protein IL-1RAcP. IL-1 Ra, an endogenous ligand, is an IL-1 receptor antagonist; thus, it prevents the interaction of IL-1 with its receptors, acting as a natural IL-1 inhibitor. This is confirmed by the fact that, after the induction of arthritis in experimental models, the administration of both anti-IL1 and anti-IL-1Ra antibodies generates benefits[37].

Anakinra is a recombinant form of IL-1Ra, an endogenous antagonist binding IL-1 receptors, thus inhibiting IL-1 proinflammatory effects.

Randomized clinical trials against placebo have showed that anakinra administration has far superior efficacy compared to placebo. This is due to the fact that the drug has a protective effect on cartilage degradation and bone erosion progression. However, anakinra has a weaker action on disease activity than anti-TNF drugs since IL-1Ra is a weak IL-1 inhibitor.

Anakinra is used in combination with MTX in case of inadequate response to methotrexate alone. On the contrary, it cannot be associated with TNF-α inhibitors. The reasons for this are an increased risk of serious infections and neutropenia –resulting from the induction of marked immunosuppression– as well as the absence of added clinical benefits. Even so, the drug has a modest efficacy against severe rheumatoid arthritis –both in combination with MTX and alone.

Anakinra is administered subcutaneously once a day at the dose of 1-2 mg/Kg, showing high bioavailability –up to 95%.

Its way of administration determines low patients compliance. This is why technological research is trying to develop other pharmaceutical forms, such as parenteral depot preparations or subcutaneous implants[52].

Combination therapy with etanercept has proved to be inconclusive –no added clinical benefit compared to etanercept alone– and unsafe –increased risk of infection and neutropenia. For this reason, such combination therapy is not recommended[11].

242 patients with RA, not previously treated with biologics and taking methotrexate, were enrolled in a 24-week, randomised trial comparing the administration of etanercept alone (25 mg twice a week) with etanercept combined with anakinra (100 mg daily). Its results showed a higher

incidence of serious infections (7%) and neutropenia, compared to patients treated with etanercept alone. Such percentage is superior to that observed in previous studies on anakinra alone[12].

Anakinra has a modest efficacy also against ankylosing spondylitis and lupus.

Concerning side effects, the most frequently reported are injection site reactions. Such reactions –including not only pruritus, erythema and pain, but also ecchymosis and bleeding– tend to decrease in intensity over time. However, in some cases side effects compelled patients to discontinue the treatment[8]. Other adverse events with greater incidence than placebo are headache and abdominal pain[8].

In order to evaluate anakinra safety, a specific study was conducted. It involved 1,399 patients with other concomitant diseases and treated with NSAIDs, corticosteroids and/or DMARDs. Such patients were randomised to receive 100mg/day anakinra or placebo[13]. After a follow-up of 6 months, the incidence of major side effects between the two groups (7.7% against 7.8%) was very similar. Nonetheless, serious infections such as pneumonia, cellulitis, and osteoarticular infections, were more common with anakinra than with placebo (2.1% against 0.4%)[13]. The percentage of treatment discontinuation due to side effects was 13.4% in anakinra group and 9.2% in placebo[13].

Absolute treatment contraindications are, obviously, infective diseases in progress. Extreme caution is necessary also in case of neutropenia, immunosuppression, renal failure, and concomitant use of TNF-α- blockers[52].

9. TNF-alpha antagonists: a comparison

To date, the above described anti-TNF molecules have shown tremendous benefits in treating rheumatoid arthritis. In fact, they have proved to be effective in over 70% of patients, including those who did not respond to methotrexate, i.e. the gold standard of rheumatoid arthritis treatment. There are also differences in time to onset of effects –8 weeks or more in methotrexate therapy; 2 weeks with TNF-blockers. In addition, they have also proved to be very effective in relieving asthenia, that is, one of the most common RA symptoms. However, their mechanism of action is responsible for other major side effect reported during the treatment, e.g. an increased risk of infections, including unusual ones such as tuberculosis. In this regard, etanercept is less commonly associated with tuberculosis than the other two medications[7].

It still remains unclear how differences among the tree drugs are reflected in clinical practice. A good reference is the analysis conducted on rheumatic patients treated with the three TNF antagonists and reported in the Dutch register DREAM (Dutch Rheumatoid Arthritis Anti-TNF Monitoring). Here, patients treated with infliximab showed rates of good EULAR response inferior to that of patients treated with adalimumab or etanercept for 6-month. Besides, non-responder rate at months 3 and 6 were higher in infliximab patients than in adalimumab and etanercept patients. These two latter therapies also presented a higher disease activity reduction at months 3, 6 and 12, compared with subjects treated with infliximab. Etanercept and adalimumab patients also showed a higher reduction of functional disability at months 6 and 12, compared to those treated with infliximab.

The impossibility of distributing monoclonal antibodies in oral formulations represents a limit of such treatment. In fact, the three drugs are all administered parentally: subcutaneous injections for adalimumab and etanercept; intravenous administration for infliximab[7].

Available data also indicate that monoclonal antibodies such as infliximab and adalimumab are associated with a higher incidence of tubercular infections, compared to the soluble TNF-α receptor –i.e. etanercept[16].

Some articles claim that patients who do not respond to anti-TNF-α treatment, or develop serious side effects related to the therapy, may be treated with another TNF-α-blocker, obtaining a good therapeutic response[21].

With respect to local allergic reactions, it must be said that subcutaneously administered TNF antagonists-–such as etanercept and adalimumab– often cause itching and red rashes at injection site, which may last several days. On the other hand, 20% patients managed with

infliximab show infusion reactions such as headaches, fever, and rarely more serious reactions such as urticaria or anaphylactic shock.

Further differences among the three compounds concern the timeline of infectious complications onset, occurring within the first 12 weeks for infliximab patients, within the first 30 weeks for adalimumab patients, and within the first 11 months for etanercept patients. This is probably due to the difference in modalities and duration of the effect of cytokine blockade performed by such agents[22].

A careful evaluation of infection risk factors and patient monitoring are crucial for spotting the first warning signs. This is also true for minor processes such as upper respiratory tract infections. Even so, the risk of severe infections is *per se* increased during the course of rheumatoid arthritis because of the alteration of immune regulation. In addition, it has been shown that such risk is further increased by (i) advanced age; (ii) severity of RA; (iii) possible comorbidities (diabetes mellitus); (iv) use of other immunosuppressive drugs[23].

It has been hypothesised that RA is associated with an immune deficiency that predisposes patients to infections. This theory is supported by laboratory data showing that patients with RA present a reduced T-cell repertoire, whose diversity is fundamental for the recognition of a wide range of antigens[24].

It has also been demonstrated that RA profoundly alters T-cell functions, to the point of jeopardising patient's capacities to react against infections [26]. As a consequence, the use of said drugs in such circumstances demands careful patient monitoring in order to spot signs and symptoms of any infectious process.

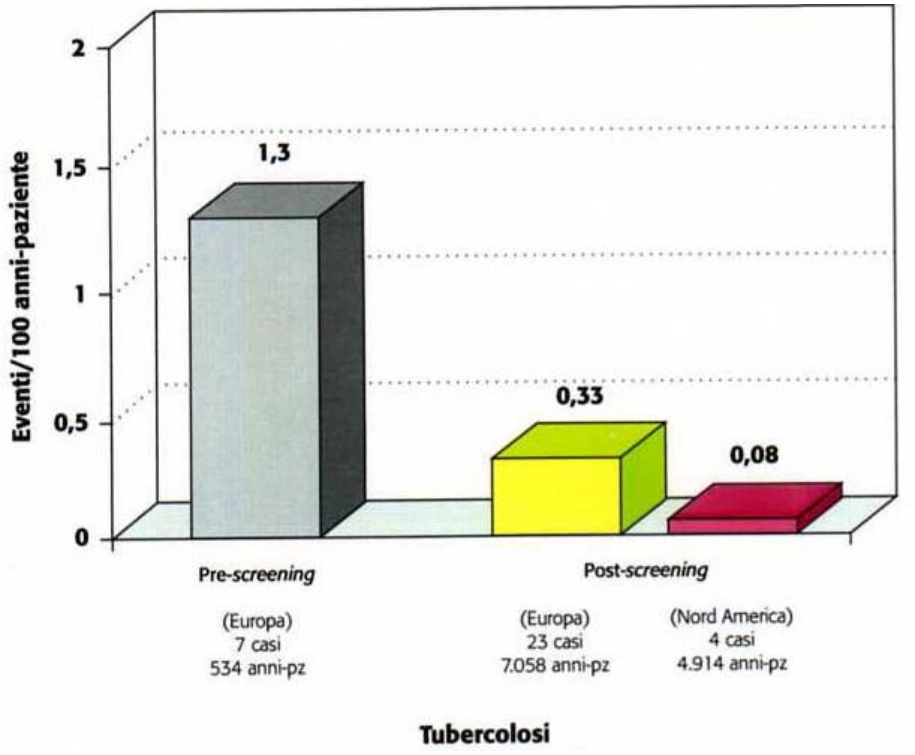
According to the British Society for Rheumatology Biologics Register, there is no significant difference in rates of serious infections among the three medicaments. The aforementioned study was conducted in UK with the aim of investigating long-term safety of biologic drugs used for treating rheumatic diseases. The following table reports rates of serious infections mediated by intracellular pathogens registered in the UK during anti-TNF treatment. The table was drawn on the bases of data on the safety of the three drugs provided by the pharmaceutical company releasing adalimumab.

NUMBER OF SERIOUS INFECTIONS IN THE UK			
	ETANERCEPT n = 2602	**INFLIXIMAB** n = 2871	**ADALIMUMAB** n = 915
Lower respiratory tract	42 16.7	85 21.6	12 18.7
Skin and soft tissue	26 10.4	37 9.4	8 12.5
Bones and joints	15 6.0	21 5.3	3 4.7
Urinary tract	12 4.8	13 3.3	6 9.4
Intracellular infections	7 2.8	10 2.5	0 0

Number of infections, rate/1000 PCs-year

The company producing adalimumab pointed out that the overall percentage of intracellular bacterial infections during adalimumab treatment was comparable to those of etanercept and infliximab. In spite of this, the number is slightly higher in these latter groups. Among the infections, TBC is greatly feared. Thus, before undergoing biologic treatment, patients are absolutely to be screened and subjected to Mantoux intradermoreaction. It has been showed that this procedure significantly reduces the incidence of infection.

Adalimumab was placed on the market precisely when the risk of tuberculosis infection became manifest. For this reason obligatory screening was introduced and this is why the incidence of tuberculosis infections is lower in patients treated with adalimumab. On the contrary, infliximab began to be used before the imposition of said prophylactic measures. Hence, this treatment is associated with a high incidence of tuberculosis infections. The picture below shows infection rates reported in Europe before and after the introduction of tuberculosis screening procedure.



The following table shows, instead, the incidence of serious adverse events (SAEs) reported in patients treated with TNF-blockers in Sweden.

OVERALL IINCIDENCE OF SERIOUS ADVERSE EVENTS IN PATIENTS TREATED WITH ANTI-TNF IN SWEDEN

	Infliximab with MTX n = 501	Infliximab without MTX n = 197	Etanercept with MTX n = 179	Etanercept without MTX n = 249	Adalimumab With MTX n = 48	Adalimumab without MTX n = 42
All SAEs	11.5	21.1	4.88	9.97	0	12.2
Reactions to administration	2.97	4.97	0	0.14	0	0
Infections	3.58	5.29	1.44	4.33	0	3.07
Malignancies	1.44	4.04	1.14	1.29	0	3.07
CVS	1.73	4.04	1.72	2.46	0	0
Other	1.78	2.76	0.58	1.75	0	6.06

It is worth noticing that such events increase for all three drugs alone, i.e. not in combination with MTX. Such findings might suggest that adalimumab provides a greater therapeutic improvement. However, this observation should be critically analysed since time of use is still relatively short to draw any definitive conclusion. Besides, total time of observation in patients

receiving adalimumab is inferior to that of the other groups. As always happens, knowledge of drugs is better and further defined by means of extensive clinical use. Indeed, passing the phases of clinical trials is not sufficient. Even so, the data listed in the above table clearly show that SAE incidence is higher in infliximab treatment, compared to etanercept and adalimumab treatments.

Incidentally, an adverse event is serious when requires patient's hospitalisation. Neoplasms are also classified as serious adverse events.

The above data were taken from a Swedish study which monitored patients treated with anti-TNF since 1999, assessing its efficacy in clinical practice and long-term safety.

Table 5. Rates of site-specific infections*

	DMARD		Anti-TNF		
	No.	Incidence rate/ 1,000 person-years	No.	Incidence rate/ 1,000 person-years	Adjusted IRR (95% CI)†
Lower respiratory tract	36	26.6 (18.7–36.7)	203	20.6 (17.9–23.6)	0.77 (0.46–1.31)
Skin and soft tissue	4	3.0 (0.8–7.6)	118	12.0 (9.9–14.3)	4.28 (1.06–17.17)
Bone and joint	4	3.0 (0.8–7.6)	68	6.9 (5.4–8.7)	1.12 (0.32–3.88)
Urinary tract	3	2.2 (0.5–6.5)	45	4.6 (3.3–6.1)	1.70 (0.32–9.03)

Another trial demonstrated that the risk of lower respiratory tract infections is reduced in patients treated with DMARDs, compared to the biologics group. The difference between the two groups, however, was not so marked. On the contrary, patients treated with anti-TNF drugs showed a significant increase in the risk of skin and soft tissues infections.

Table 6. Details of bacterial intracellular infections

Patient age/sex	Ethnicity	Organism	Site of infection	Treatment	Months from treatment start date
59/F	Caucasian	*Mycobacterium tuberculosis* (probable)	Cervical lymph node	Infliximab	7
74/F	Caucasian	*Mycobacterium tuberculosis*	Colon	Infliximab	3
47/M	Caucasian	*Mycobacterium tuberculosis*	Omentum	Infliximab	2
47/M	Caucasian	*Mycobacterium tuberculosis* (presumed)	Pleura	Infliximab	3
66/F	Caucasian	*Mycobacterium tuberculosis*	Lower respiratory tract	Infliximab	16
77/F	Caucasian	*Mycobacterium tuberculosis*	Posterior pharangeal wall	Adalimumab	11
50/F	Pakistani	*Mycobacterium tuberculosis*	Cervical lymph node	Infliximab	4
71/M	Caucasian	*Mycobacterium tuberculosis* (presumed)	Meninges	Etanercept	2
66/F	African Caribbean	*Mycobacterium tuberculosis*	Lower respiratory tract	Etanercept	9
63/F	Not known	*Mycobacterium tuberculosis* (probable)	Meninges	Infliximab	3
59/M	Caucasian	*Legionella pneumophila*	Lower respiratory tract	Infliximab	32
49/M	Caucasian	*Legionella pneumophila*	Lower respiratory tract	Infliximab	4
47/M	Caucasian	*Listeria monocytogenes*	Meninges	Infliximab	2
67/M	Caucasian	*Listeria monocytogenes*	Joint	Etanercept	0
60/F	Caucasian	*Listeria monocytogenes*	Joint	Adalimumab	14
63/F	Caucasian	*Mycobacterium fortuitum*	Lower respiratory tract	Etanercept	4
80/F	Caucasian	*Salmonella* sp.	Bowel and joint	Etanercept	9
57/F	Caucasian	*Salmonella* sp.	Joint	Infliximab	27
54/F	Caucasian	*Salmonella* sp.	Bowel	Etanercept	2

Intracellular infections were caused by bacteria such as Mycobacterium tuberculosis, Legionella pneumophila, Listeria Monocytogenes, Mycobacterium fortuitum and Salmonella. Listeria may result from foods made from unpasteurised milk, soft cheese, or meat. Salmonella, instead, may derive from undercooked meat and eggs. Hence, it would be a good practice to warn patients against high-risk foods before undergoing anti-TNF treatment. This primary prevention would help reduce the incidence of such infections, which was 0.5, 1.5, and 0.9 events/1,000 person-years with etanercept, infliximab, and adalimumab treatment, respectively[16].

TNF certainly plays a key role in both cutaneous inflammation and immune response. Actually this cytokine is responsible for cutaneous endothelial activation and thus recruitment of inflammatory cells to the skin. TNF is not the only cytokine in the skin, however, it is fundamental because many secondary cytokines and chemokines that are central to the defense against infection are released in its response. Consequently, TNF inhibition in the skin results in increased susceptibility to infection. Obviously, this occurs also with the use of all three biologics, the only authorised to date.

The study concluded that there is no difference in infection risk among the three drugs. Besides, the overall risk of severe infection did not increase in patients treated with biologics, compared to DMARD therapy. However, the risk of skin and soft tissue infections is four times greater[16].

The above study presents considerable references and it may be regarded as the largest prospective study on adverse events reported in patients treated with biologics. It also collects data from countries all over the world. In this regard, a remark should be made. Said data were processed taking into account the actual exposure period to the drugs. However, it cannot be excluded that patients may remain at risk of infection, attributable to the drug taken for the long period between cycles of treatment. Moreover, this period varies from one drug to another. Infliximab has a half-life of about 10 days; therefore, the interval between administrations lengthens and determines a longer risk window than etanercept, whose half-life is 3–4 days.

Apart from what we know about the risk of TBC and the absolute necessity of screening, there has been no great progress in finding out risk factors of other infections. Regarding adalimumab, return data obtained during the first twenty-four months of its marketing in the US are synthetically reported below[46].

SERIOUS ADVERSE EVENTS	
Serious adverse event	Rates of events / 100 patient-years reported with adalimumab (55,384 years-patient)
Tuberculosis	0.02
Lymphoma	0.04
Congestive heart failure	0.05
Demyelinating pathologies	0.01
Systemic lupus erythematosus	0.04
Opportunistic infections	0.05
Blood dyscrasias	0.07

Such data confirm adalimumab safety, already demonstrated in the above mentioned clinical studies.

It has been estimated that over 100,000 patients have been treated with adalimumab in 7 years. Infection rate detected is virtually comparable to that reported in the general population of patients affected by rheumatoid arthritis. Analysis of the incidence of lymphoma in adalimumab treatment taken from SEER (Surveillance, Epidemiology, and End Results cancer database) also shows an incidence comparable to that of RA patients naive to anti-TNF therapy[46].

Regarding viral infections, herpes zoster and herpes simplex are widely reported in patients treated with etanercept. This once again suggests subtle differences in both mechanism of action and clinical findings of the various compounds[29].

Long-term efficacy and safety of biologics still raise questions. This is due to the fact that data in this respect are still very few, although some case studies have reached 4-5 years. The study named STURE showed that after three years of treatment only 42% of patients kept taking infliximab at initial dosage and frequency of administration, whereas the other drugs required an increase[13]. Other studies suggest that in case of inefficacy or loss of efficacy of the medication, it is possible to switch to other biologics, also of the same therapeutic category. For example, it is possible to shift from a TNF-α antagonist to another with a good chance of success[21].

Open-label trials have demonstrated that the combination of biologics –e.g. anti-TNF plus anakinra– results in an increased risk of serious infections. *Ad hoc* studies are therefore needed for determining the efficacy and the tolerability of such drugs in combination therapy.

Initial studies aimed at defining side effects of TNF antagonists showed their substantial efficacy and safety, as mild adverse events were reported. However, it is well-known that actual drug toxicity can be spotted only by increasing the number of case studies and collecting post-marketing data.

The use of the above molecules was proved to determine higher susceptibility to (a) infections; (b) malignancies; (c) lupus-like autoimmune diseases; (c) demyelinating diseases like multiple sclerosis; (d) hepatic diseases; (e) hematologic abnormalities including aplastic anemia; (f) and severe allergic reactions.

As for demyelinating syndrome, exacerbation of quiescent multiple sclerosis and neo-occurrence of demyelinating neurological diseases have been observed. FDA Adverse Event Reporting System reports 18 cases of such events after etanercept therapy and 2 cases after infliximab treatment. Symptoms are variable and include paraesthesia, optic neuritis, and confusional state. Although a causal relationship has not been established yet, combination therapy is plausible enough.

The risk of opportunistic infections –especially tuberculosis– increases. For example, the rate of patients suffering from TBC and treated with infliximab was 24.4 per 100, 00, compared to 6.2 cases per 100,000 in patients with RA not taking the biologic. This is usually a reactivation of latent infection which occurs within the first 2-5 months of treatment.

Lymphoma has also been associated to the use of the principle anti-TNF drugs, namely infliximab, etanercept and adalimumab, even though the presence of causal relationship is still being discussed. This uncertainty is related to the fact that the incidence of lymphoma is higher in patients with RA, and increases as their clinical condition worsens. Thus, data showing that patients treated with anti-TNF present a 2.3 lymphoma incidence –6.4 times greater than in the general population– could be ascribed both to RA *per se* and the therapy.

Patients receiving etanercept and adalimumab both present persistent redness at the injection site. Although such local reactions are frequent, they rarely determine treatment discontinuation.

Headache and nausea are very common. Moreover, fever can be detected in 20% of patients during infliximab infusion. Such effects can be controlled by means of antihistamines, or infusion rate reduction.

Besides, symptoms such as hives –detected in only in 2% of patients– provide evidence of hypersensitivity response to the drug.

Adherence to appropriate protocol of administration has greatly reduced the incidence of infusional reactions, usually well controlled by symptomatic therapy[47]. Delayed reactions are rare and occur in less than 1% cases, especially in patients undergoing retreatment.

Immune response to the above mentioned chimeric antibodies is also possible. This was reported in 8.5% of patients treated with infliximab and it accelerates the clearance of this latter. On the other hand, anti-adalimumab antibodies were found in 12% of patients. However, this percentage was reduced to 1% if there was a concomitant MTX treatment. ACR20 response rate decreased in patients managed with adalimumab producing anti-antibody autoantibodies.

Another interesting finding is that in patients with heart failure high levels of TNF-α were discovered. Initially, this raised hopes that molecules like infliximab might be also used for this therapeutic indication. However, challenge data showed a lack of benefits and, besides, studies on infliximab reported increased cardiovascular mortality.

NYHA class III or IV congestive heart failure is an absolute contraindication to the use of infliximab and adalimumab, whereas precautions are required when using etanercept. Such drugs may, in fact, determine a substantial risk of aggravating the clinical picture and increase mortality[31, 32]. On the other hand, patients with NYHA class I or II congestive heart failure, candidate for anti-TNF-α therapy, should undergo an echocardiogram. In case ejection fraction is normal, they can be subjected to therapy under close monitoring. Even in the absence of obvious risk factors, new onset of congestive heart failure can be detected both with etanercept and infliximab[33].

10. New biologic drugs for rheumatoid arthritis

Infliximab, etanercept, adalimumab, and IL-1-receptor antagonist anakinra can be considered just the first generation of biologics. They are characterised by a quicker onset of action than traditional DMARDs, producing rapid and sustained therapeutic responses. However, it has been estimated that approximately 30–40% of patients treated with TNF-α antagonists fail to respond to them. Besides, while studies on short-term tolerability have provided reassuring responses, long-term tolerability still raises questions about possible positive outcomes. This has prompted researchers and drug companies to develop new biologics. Several agents with different mechanisms of action have been assessed in clinical trials during the last few years[36].

Pharmacological research is currently testing numerous molecules directed against selected targets. Some of them are (i) abatacept; (ii) MRA, that is an anti-L-6 receptor antibody, being IL-6 an acute phase cytokine in RA; and (iii) autovac, i.e. a genetically modified protein which consists in a TNF-α molecule in which an non-self epitope is inserted for inducing an immune response against TNF- α.

In the last 3 years a number of molecules have reached phase III or registration. Namely, they are: zanolimumab, keliximab, certolizumab, abatacept, rituximab and tocilizumab[36]. The development of zanolimumab and keliximab was discontinued due to negative results seen during trials.

Abatacept (Orencia®) is a biologic registered only in may 2007 and is administered intravenously at the dose of 500, 750 or 1,000 mg, depending patients' weight[12]. Its schedule entails one administration every two weeks for the first month, and afterwards every four weeks. Abatacept is a protein aimed at suppressing T-cell activity. Such cells account for joint inflammation and damage. However, in order to act they need previous activation. This latter occurs when some signaling molecules bind to surface receptors of T-cells. Abatacept was developed for binding to two of these molecules (CD 80 and CD 86). Thus, the interaction with C80 is blocked and T-cell activation is prevented. The drug is indicated in combination with MTX for the treatment of moderate to severe active rheumatoid arthritis in adult patients who had an inadequate response or intolerance to other disease-modifying anti-rheumatic drugs, including a tumour necrosis factor (TNF) inhibitor. Abatacept is regarded as a T-cell co-stimulation modulator.

Atlizumab is a humanised monoclonal antibody directed against IL-6, a cytokine involved in T-lymphocytes activation and synovial fibroblasts proliferation. This drug is effective in controlling rheumatoid arthritis and is administered intravenously every two weeks at the dose of 2-8 mg/Kg[52].

It is well-tolerated and no antinuclear, anti-dsDNA, or anti-drug antibodies have been observed. In spite of this, it entails the disadvantage of raising blood cholesterol levels after 24 weeks of treatment[52].

Tocilizumab (Actemra®), instead, is a humanised monoclonal antibody directed against the IL-6 receptor, and has been recently approved in Japan for the treatment of rheumatoid arthritis.

As already said, IL-6 cytokine plays a key role in chronic inflammatory process. Its function is that of stimulating antibody production by B-cells and T-lymphocyte differentiation into T- helper 17. These cells have proved fundamental in experimental models of autoimmune disease[36, 37]. IL-6, besides, binds to its receptor, namely gp80, involving also a co-receptor, i.e. gp130. IL-6 overproduction results in the pathogenesis of several autoimmune inflammatory diseases. This is why research aimed at developing an IL-6 receptor blocker, that is, tocilizumab. Its therapeutic potential becomes evident considering the high levels of IL-6 in the synovial fluid of patients with rheumatoid arthritis. Moreover, IL-6 circulating levels increase in parallel with joint damage progression. IL-6 plays an important role in synovial pannus formation as it attracts leukocytes to the inflammatory site and stimulates synovial fibroblasts and activated osteoclasts proliferation. Tocilizumab is well-tolerated. Actually, its safety profile is comparable to that of other biologic agents and immunosuppressive treatments. Its efficacy seems to increase with concomitant methotrexate treatment.

A study named SAMURAI was carried out in RA, entailing IL-6 inhibitor monotherapy. It showed that radiographic progression measured by Sharp score at one year decreased. In addition, the drug had positive effects on disease activity and physical functionality of the patients recruited. However, a disadvantage is that a higher incidence of serious infections was detected, compared to the group treated only with DMARDs[37].

Another interesting study is TOWARDS, which took into account tocilizumab combined with conventional DMARDs. The results showed an improved disease activity compared to DMARDs alone. This difference among the two therapies emerged already after two weeks of treatment. However, serious infections were still reported in the group treated with the biologic.

Other interesting phase III experimental trials still ongoing are RADIATE (Research on Actemra Determining Efficacy After anti TNF Failures) and AMBITION (Actemra versus Methotrexate Double Bind Investigative Trial in Monotherapy). The first assesses safety and efficacy of tocilizumab plus methotrexate in patients suffering from RA who have had an inadequate response to classic anti-TNF agents. The second shows that tocilizumab monotherapy is not inferior to methotrexate. Concomitant use of tocilizumab plus MTX is certainly preferable, unless risk of infectious episodes increases.

During tocilizumab treatment transient elevation of liver enzymes and increased cholesterol levels have been reported[37]. Clinical trials are being carried out to evaluate the potential use of tocilizumab in other chronic inflammatory diseases, such as Crohn's disease and systemic lupus erythematosus.

Attempts to block the IL-6 cytokine do not include only Tocilizumab. Other approaches are in fact being studied with this purpose. Among them there are (i) a fusion protein consisting of the extracellular region of gp130 and (ii) IL-6 co-receptor coupled with either the Fc fragment of a human IgG or a 7 amino acid oligopeptide capable of binding to the gp80 receptor of IL-6, thus inhibiting the interaction between the cytokine and its receptor.

Drugs such as tocilizumab, rituximab, and abatacept represent the newer generation of biologics in RA treatment. However, their utility, compared to that of previously existing biologics, needs further systematic examination.

Some trials evaluated the newer biologics –abatacept, rituximab, and tocilizumab– alone or in combination with DMARDs compared to placebo or DMARDs alone with a minimum 6-month follow-up. All trials reported ACR20, ACR50, and ACR70 response rates.

Table 1 Efficacy of newer biologics in the treatment of signs and symptoms of rheumatoid arthritis

		ACR 20 (%)		ACR 50 (%)		ACR 70 (%)	
Study	Treatment [n]	6 months	1 year	6 months	1 year	6 months	1 year
Combination therapy							
Long-standing (8–12 years),[a] active RA despite previous MTX (10–30 mg/week) therapy							
Edwards *et al.* [8]	RTX 1000mg [40]	65*	33	33	15	15	10
	RTX 1000mg + CYC [41]	76***	49*	41**	27*	15	10
	RTX 1000mg + MTX [40]	73**	65***	43**	35**	23*	15*
	MTX [40]	38	20	13	5	5	0
Emery *et al.* [9,10]	RTX 500mg + MTX [123]	55***	67	33***	42	13*	20
	RTX 1000mg + MTX [122]	54***	59	34***	38	20***	17
	PL + MTX [122]	28	45	13	20	5	8
Kremer *et al.* [11,12][b]	ABA 2 mg/kg + MTX [105]	42	42[c]	23*	23[c]	11*	12[c]
	ABA 10 mg/kg + MTX [115]	60***	63***	37***	42***	17***	21**
	PL + MTX [119]	35	36	12	20	2	8
Kremer *et al.* [13]	ABA 10 mg/kg + MTX [424]	68***	73***	40***	48***	20***	29***
	PL + MTX [214]	40	40	17	18	7	6
Long-standing (11–13 years),[a] active RA with inadequate response to anti-TNF-α therapy (infliximab, ETA, adalimumab)							
Cohen *et al.* [14,15][d]	RTX 1000mg + MTX [298]	51****	51	27****	34	12****	14
	PL + MTX [201]	18	33	5	8	1	4
Genovese *et al.* [16]	ABA 10 mg/kg + DMARD [258]	50***		20***		10**	
	PL + DMARD [133]	20		4		2	
Weinblatt *et al.* [17]	ABA 2 mg/kg + ETA [85]	48	48	26	28	11*	9
	PL + ETA [36]	31	31	19	17	0	6
Monotherapy							
Long-standing (9 years),[a] active RA despite previous MTX (8 mg/week) therapy							
Nishimoto *et al.* [18][d]	TOC 8 mg/kg [61]	80***		49***		30***	
	MTX [64]	25		11		6	
Early (2 years),[a] active RA							
Nishimoto *et al.* [19][d,e]	TOC 8 mg/kg [157]		89***		70***		47***
	DMARD [145]		35		14		6

Trials on combination therapy were conducted in patients with longstanding RA or with active disease despite previous MTX monotherapy. Abatacept administered as an intravenous

infusion at the dose of 10 mg /kg was effective in combination with MTX in the treatment of signs and symptoms of long-standing, active RA, refractory to MTX monotherapy.

Abatacept at the same dosage and combined with other DMARDs was also effective in the treatment of signs and symptoms of long-standing, active disease in patients who had shown an inadequate response to traditional anti-TNF therapy. Such dosage proved fundamental since addition of abatacept 2 mg/kg to etanercept in patients with inadequate response to etanercept alone provided no advantage in terms of efficacy. Moreover, abatacept 10 mg/kg slowed the radiographic progression of the disease in patients who had not responded adequately to MTX monotherapy.

As for rituximab, it was used at the dose of 500 mg / 1000 mg combined with methotrexate, or 1 gramme with cyclophosphamide. It was effective in long-standing RA that was previously refractory to methotrexate monotherapy[36]. 1 gramme rituximab with methotrexate also slowed radiographic progression. Tocilizumab monotherapy had the same effect. On the other hand, rituximab monotherapy showed moderate efficacy, which was not sustained beyond 6 months. In addition, rituximab in combination with methotrexate appeared to be highly effective in long-standing, active RA with inadequate response to anti-TNF-α therapy.

A Japanese trial assessed, instead, tocilizumab 8 mg/kg monotherapy in patients receiving MTX. It equally appeared to be effective in the treatment of long-standing, active RA refractory to MTX monotherapy[36].

All three new biologics were generally well tolerated in patients affected by RA followed for up to 1 year. The frequency of adverse events –including serious adverse events and discontinuation due to adverse events– was generally comparable to those reported in comparator groups. However, it must be pointed out that the combination of abatacept and etanercept was less well tolerated than etanercept monotherapy[36]. No serious withdrawal causes were reported. Actually, treatment discontinuation occurred for few patients and was mainly due to lack of efficacy. Infection incidence was also very low.

With respect to immune reactions, anti-antibody antibodies were observed in 4% of patients receiving rituximab and in 1% of the subjects treated with abatacept. However, this did not appear to be associated with toxicity or a decrease in efficacy.

In long-standing RA refractory to MTX, rituximab and abatacept can be used in combination with methotrexate as an alternative to anti-TNF-α biologics. Tocilizumab monotherapy appears to be equally effective in such patients.

Concerning radiographic progression, it is slowed by abatacept plus methotrexate combination, compared with the DMARD alone. Rituximab-methotrexate combination seems to be

also favourable to radiographic progression improvement in long-standing RA with an inadequate response to anti TNF-α therapy[36].

The above cited newer biologics appear to be well tolerated in the short to medium term (1 year maximum follow-up). However, larger studies are needed to draw a clearer pharmacological profile. Safe and effective experimented dosages of abatacept, rituximab, and tocilizumab are 10mg/kg, 500 and 1000mg, and 8 mg/kg, respectively.

11. Future therapeutic strategies

Current attempts to identify the safest and most effective remedy for the treatment of rheumatoid arthritis are manifold due to increasing awareness of the role of inflammatory mediators. For this reason, we will describe below some of the most interesting aspects in this regard.

Lately, an insightful and rational objective of research that is being pursued for the treatment of rheumatoid arthritis is selective modulation of T cells co-stimulation[34]. The basic assumption is that activated T-cells play a key role in managing the complex inflammatory response underlying the disease. Among other things, such cells are found in large quantities in rheumatoid synovium. Indeed, they promote the inflammatory cascade which causes inflammation and joint destruction. Macrophages, B-cells, and T-lymphocytes release cytokines, autoantibodies, and other inflammatory mediators. Such mediators send signals to downstream effector cells triggering a cascade of other mediators by chondrocytes and osteoclasts, whereas fibroblasts directly attack the joints. This complex set of processes is what promotes inflammation and destruction.

A very recent research work conducted by an important pharmaceutical company was based on the fact that such processes result from activated T-cells. For activating these latter, an antigen-presenting cell must present an antigen to them and the specific T-cell receptor must recognise it. At this point, the antigen is presented in the context of the histocompatibility complex by a dendritic cell, macrophage or B-lymphocyte. This represents the first signal for T-cell activation.

Moreover, it has been ascertained that there is a second signal, namely co-stimulation, which is needed for the complete activation of T-cells.

A specific antigen, however, has not been identified yet. In fact, the antigen-presenting cell may determine different co-stimulatory pathways aimed at reducing or intensifying T-cells activation. One of the best described co-stimulatory pathways is the binding of CD80/86 on antigen-presenting cells to CD28 on T-cells. This generates positive co-stimulatory signals and promotes T-cells activation, proliferation, and cytokine production by T-cells.

On the other hand, CTLA4 (Cytotoxic T-Lymphocyte Antigen 4) is a well-defined down-regulator of T-cell activation. It presents a more marked tendency to bind CD80/86 than CD28. The difference between them is that CD28 is constitutively expressed on T-cell-surface, whereas CTLA4 is expressed 24-48 hours after its activation.

Scholars have focused very much on CTLA4 pathway as it generates negative co-stimulatory signals. Actually, it represents one of the current objectives of research for the treatment

of rheumatoid arthritis. It might be possible, in fact, to intervene only in this pathway, leaving other co-stimulatory pathways intact. This would be an upstream intervention entailing the blockade of the activation of various cell types involved in the immune reaction, such as macrophages, B-lymphocytes, osteoclasts and chondrocytes.

In addition to such promising studies still ongoing, we should consider the potentiality that therapies interfering with immune cell migration might have. In fact, immune cells are activated in response to an antigen and therefore they migrate from secondary lymphoid organs to the tissues in order to mediate organ damage. Several protein families promote this cellular traffic. Among them there are adhesion molecules, complement components, and chemokines. Thus, striking these molecules might produce benefits in terms of target-organ-damage reduction. However, this approach does not appear to be totally immunosuppressive.

Other potential targets in RA are several anti-inflammatory cytokines such as IL-4, IL-10, IL-11[35]. Besides, molecules like mycophenolic acid, rapamycin, FTY 720, –which might inhibit lymphocyte migration and possible interventions on the osteoprotegerin-RANK-RANKL system– are being studied.

We should mention in this regard a fully human monoclonal antibody, i.e. denosumab. This binds to and inhibits RANKL, a ligand involved in osteoclast activation and thus in bone erosion that is characteristic of RA and other forms of erosive arthritis such as psoriatic arthritis[48]. This antibody is administered subcutaneously. It was assessed in a study involving 3 groups which were given respectively a 60 mg dose, a 180 mg dose, and a placebo. Injections were performed every 6 months. Radiographs of hands and feet were taken at baseline and surveys were made at months 6 and 12. The overall results were expressed as total Sharp score which takes into account bone erosion and joint space narrowing. Increasing scores reflected increased damage as we can see form the results reported in the table below:

Change in Score at 12 Months			
Measurement (Mean (SD))	Placebo	Denosumab 60 mg	Denosumab 180 mg
	n = 71	n = 69	n = 69
Total Sharp Score	1.87 (5.06)	0.85 (2.52)*	0.97 (2.70)†
Erosion Score	1.34 (4.40)	0.33 (1.22)#	0.19 (1.61)#
Joint Space Narrowing	0.53 (1.49)	0.51 (1.63)	0.78 (1.72)

Note how TSS increase is smaller in the groups treated with the two different doses of the drug than in the placebo group. The main contribution to the overall score was given by bone erosion score, since no detectable difference in JSN was observed compared to placebo group. What is comforting is that adverse events in the three groups were quite comparable[48].

Denosumab shows encouraging results in terms of its role in osteoclast inhibition. The collocation of this compound in the treatment armamentarium is still unclear as there is still no indication of action on disease signs and symptoms, nor on beneficial effects on cartilage. Probably, on the grounds of its action in bone erosion slowdown, this medication with its so far scarce side effects could accompany corticosteroid therapy in patients with rheumatoid arthritis, considering osteoporotic effects of glucocorticoids[48].

IL-15 is another potential pharmacological target in RA. Indeed, compounds capable of inhibiting IL-15 and IL-18 are being experimented. The IL-15 cytokine is strongly implicated in the pathogenesis of the disease as it is able to induce the production of several proinflammatory cytokines. This is why a newer fully humanised monoclonal antibody directed against IL-15, namely Hu-Max-IL-15, has been recently developed. It has been studied in a double-blind randomised placebo-controlled clinical trial in 30 patients affected by rheumatoid arthritis. Said trial has preliminarily suggested that Hu-Max-IL-15 administration is safe and affective[37].

Another potential target cytokine is IL-21, which equally plays a pathogenic role in rheumatoid arthritis as it may induce the differentiation of T Helper 17 cell line in the absence of IL-6. For this reason, preclinical studies on rats are being conducted. In such studies, said interleukin is blocked with a fusion protein consisting of the IL-21 receptor and the Fc fragment of a human immunoglobulin. What has emerged is that this strategy improves the course of arthritis in the animals.

Another viable strategy is to inhibit intracellular signal transduction pathways resulting from the interaction of IL-1, TNF-α, and other proinflammatory cytokines with their respective receptors. Being this what eventually determines cell activation, interfering with post-receptoral processes would be beneficial in rheumatoid arthritis treatment. Targets in this respect are NF-kB, and some protein kinases, such as cJun and p38, which are pathways known in inflammatory diseases[37].

With this purpose, clinical trials have been conducted using p38 inhibitors in patients with rheumatoid arthritis. However, no positive effects were detected. In addition, adverse events were reported due to the fact that this intracellular signaling exists in several cell types as a normal response to several homeostatic signals. Thus, the action of such inhibitors is toxic and little specific.

Currently clinical trials are being developed in California entailing the use of an anti-IL1R1 monoclonal antibody, which might inhibit all IL-1 biologic functions. This might allow a definitive judgment on how IL-1 may be an effective target in rheumatoid arthritis[37].

RA is a disease caused by cytokine dysfunction, thus it is no surprise that researchers have pointed at them as potential pharmacological targets. Actually, studies are being conducted on a recombinant monometric monoclonal antibody, i.e. pegsunercept, which seems to presents an antagonistic action of TNF P55 receptor[51]. Pegsunercept is a type 1 cytokine receptor and presents a pegylated structure. It can be defined as a second generation TNF-α inhibitor. To date, it has proved to be minimally immunogenic and highly effective. Besides, subjects enrolled in the experimental study showed a very low toxicity. This drug, then, might be soon placed on the market as a monotherapy or in combination with other anti-arthritic agents.

The last interesting intracellular pathway is that resulting from the interaction of cytokines, several hormones, and growth factors with cytokine receptors. These latter lack intrinsic tyrosine kinase activity and therefore require the participation of signal transduction proteins and transcriptional activators such as Janus Kinase and STAT[37].

REFERNCES

1. Goodman e Gilman di farmacologia;

2. Harrison. Principi di medicina interna. Quindicesima edizione. Vol III pg 2129:Autoimmunità e malattie autoimmuni; http://www.mhprofessional.com/downloads/products/0071741437/fauci_rheumatology_ch05_082-099.pdf

3. Reumatismo 2006 vol.58;

4. Atlante di Reumatologia.

5. Drugs of the future. 2003, 28:1182

6. Molecules of the millennium. A novel therapeutic approach in rheumatoid arthritis. Postgraduate department oh pharmacology and therapeutics, Govt medical college, Jammu,India.

7. www.arthritis.about.com

8. www.drugdigest.org

9. Humira: riassunto delle caratteristiche del prodotto.

10. Focus on Artrite reumatoide. Il ruolo del Rituximab definito dall'American College of rheumatology 69th annual scientific meeting.

11. Annual of Rheumatology Disease. Terzo supplemento anno 2005

12. Relazione EMEA sull'Orencia per il pubblico.

13. Bollettino di informazione sui farmaci 06/2003

14. New England Journal of Medicine 2004. New Drugs for Rheumatoid Arthritis.

15. New England Journal of Medicine 2001. 344: 907-916. Cytochine pathways and joint inflammation in Rheumatoid Arthritis.

16. Arthritis and Rheumatism august 2006. Vol. 54. Num.8 Rates of serius infections, including site specific and bacterial intracellular infection, in rheumatoid arthritis patients receiving anti tumor necrosis factor therapy.

17. Analisi dei farmaci e dei loro metaboliti nei liquidi biologici. Appunti lezione Prof.ssa De Tommasi su Anticorpi Monoclonali.

18. Aderka D. The potential biological and clinical significance of soluble tumor necrosis factor receptors. Cytokine and Growth Factor Reviews. 1996; 7: 231-240.

19. Robak T, Gladalska A, Stepien H. The Tumor necrosis factor family of receptors/ligands in the serum of patients with rheumatoid arthritis. Eur Cytokine Netw 1998; 9: 145-54.

20. Mohler KM, Torrance DS, Smith CA, Goodwin RG, Stremler KE, Fung VP, et al. Soluble tumor necrosis factor (TNF) receptors are effective therapeutic agents in lethal endotoxemia and function simultaneously as both TNF carriers and TNF antagonists. J Immunol 1993; 151: 1548-61.

21. Van Vollenhoven RF. Switching between biological agents. Clin Exp Rheumatol. 2004; 22 (Suppl 35): S115-21.

22. Ellerin T, Rubin RH, Weinblatt ME. Infections and anti- tumor necrosis factor alpha therapy. Arthritis Rheum 2003; 48: 3013-22.

23. Doran MF, Crowson CS, Pond GR, O' Fallon WM, Gabriel SE. Predictors of infection in rheumatoid arthritis. Arthritis Rheum 2002; 46:2294-2300

24. Wagner UG, Koetz K, Weyand CM, Goronzy JJ. Perturbation of the T cell repertoire in rheumatoid arthritis. Proc Natl Acad Sci USA 1998; 95: 14447-52.

25. Goodman and Gilman's. The Pharmacological Basis of Therapeutics. Thenth Edition. Chapter 53: immunomodulators: immunosuppressive agents, tolerogens, and immunostimulants.

26. Koetz K, Bryl E, Spickschen K, O' Fallon WM, Goronzy JJ, Weyand CM. T cell homeostasis in patients with rheumatoid arthritis. Proc Natl Acad Sci USA 2000; 97:9203-8

27. Rau R. Adalimumab (a fully human anti-tumour necrosis factor-alpha monoclonal antibody) in the treatment of active rheumatoid arthritis. Ann Rheumm Dis 2002; 61 (supp2): 1170-73.

28. Van de Putte LB, et al. Efficacy e safety of Adalimumab as monotherapy in patients with rheumatoid arthritis for whom previous disease modifying antirheumatic drug treatment has failed. Ann Rheum Dis 2004; 63 (5): 508-516.

29. Wallis RS, Broder MS, Wong JY, Hanson ME, Beenhouwer DO. Granulomatous infectious diseases associated with tumor necrosis factor antagonists. CID 2004; 38:1261-5.

30. Moreland LW, Schiff MH, Baumgartner SW, Tindall EA, Fleischmann RM, Bulpitt KJ, et al. Etanercept therapy in rheumatoid arthritis. A randomized, controlled trial. Ann Intern Med 1999; 130: 478-86.

31. Chung ES, Packer M, Lo KH, Fasanmade AA, Willerson JT. Randomized, double-blind, placebo-controlled, pilot trial of infliximab, a chimeric monoclonal antibody to tumor necrosis factor-alpha, in patients with moderate-to-severe heart failure: results of the anti-TNF Therapy Against Congestive Heart Failure (ATTACH) trial. Circulation 2003; 107: 3133-40

32. Mann DL, McMurray JJ. Presentation of results of the Reinassance, Recover and Renewal studies Heart failure 2002, Jun 8-11 Oslo, Norway.

33. Kwon HJ, Cote TR, Cuffe MS, Kramer JM, Braun MM. Case reports of heart failure after therapy with a tumor necrosis factor antagonist. Ann Intern Med 2003; 138: 807-11.

34. Brystol Mayers cd informativo per il farmacista ospedaliero. Gli inibitori della costimolazione.

35. Checklist Reumatologia. Terza edizione,2007.

36. The efficacy and tolerability of newer biologics in Rheumatoid Arthritis: best current evidence. New Current Opinion in Rheumatology. Maggio 2007

37. Finckh et Gabay. At the horizon of innovative therapy in Rheumatology: new biologic agents. Current Opinion in Rheumatology 2008. 20:269-75.

38. Proposal to establish a register for the long term surveillance of advers events in patients with rheumatic diseases exposed to biological agents: the EULAR Surveillance Register for Biological Compounds. Ann Rheum Dis 2000; 59:419-20 Silman A, Klareskog L, Breedeld F, Bresnihan B, Maini R, Van Riel P, Symmons D.

39. Remicade Infliximab. Riassunto delle caratteristiche del prodotto. Schering Plough s.p.a.

40. E.G.Favalli, A.Marchesoni, G.L.Colombo, L.Sinigaglia. Pattern of use, economic burden, and vial optimization of Infliximab for Rheumatoid Arthritis in Italy. Clinical and Experimental Rheumatology 2008 26:45-51

41. E. Keystone, A.F. Kavanaugh, J.T.Sharp, H.Tannebaum, Ye Hua, L.S.Teoh, S.A. Fishkoff, E.K.Chartash. Radiografic, Clinical and Functional Outcomes of Treatment with Adalimumab in Patients with Active Rheumatoid Arthritis receiving concomitant Methotrexate Therapy. Arthritis and Rheumatism Vol 50 N.8 May 2004 Pp 1400-1411.

42. Le malattie autoimmuni. Presentazione ad un convegno a cura del Prof. Manconi. Gennaio 2003.

43. Tolleranza ed autoimmunità. Aspetti biologici. Sergio Romagnani, Università degli Studi di Firenze, Dipartimento di Immunologia e malattie dell'apparato respiratorio.

44. Corso in Biotecnologie farmaceutiche. Dott.ssa Castellano, Università degli Studi di Salerno. Scuola di Specializzazione in Farmacia Ospedaliera.

45. Guidelines for the Management of Rheumatoid Arthritis. American College of Rheumatology Subcommittee on Rheumatoid Arthritis Guidelines Arthritis Rheum 2002, 46, 328–346.

46. Humira. Evidenze di sicurezza. Dati di pratica clinica. Abbott.

47. Padovan M, Massara A, Rizzo N, Lo Monaco A, Fotinidi M, La Corte R, et al. Dati di safety del trattamento con anti-TNFa in una coorte di 183 pazienti affetti da artrite reumatoide, artrite psoriasica e spondilite anchilosante. Reumatismo 2004; 56 (suppl 2): 388.

48. S.B. Cohen, P.A. Valen, C. Ritchlin, J. Schechtman, C.G. Peterfy, D. van der Heijde, L. Zhou, R. Newmark, W. Tsuji. Inhibiting RANKL with Denosumab reduces progression of

bone erosions in patients with Rheumatoid Arthritis. New Current Opinion in Rheumatology. 2006.

49. Klares et al.. Therapeutic effects of the combination of Etanercept and Methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double bind randomised controlled trial. Lancet 2004: 363:675-81

50. Enbrel. Riassunto delle caratteristiche del prodotto. Wyeth^{R}