

UNlchip protein biochips from Protagen identify significant off-target binding for TNF-alpha inhibitors

Findings could explain side effects seen with these biological drugs for treating rheumatic diseases

Dortmund, Germany and New Jersey USA, 31 March 2008: Tumor Necrosis Factor (TNF)- α inhibitors used to treat rheumatic diseases may bind to a significant number of proteins other than their main target, according to the results of an *in vitro* study published in Analytical and Bioanalytical Chemistry. This off-target binding activity might explain some of the side effects reported for these commonly used biotherapeutics.

Researchers from University Children's Hospital Düsseldorf, Ruhr-University Bochum and Protagen AG used protein biochips to investigate the protein binding activity of three protein therapeutics for the treatment of Rheumatoid Arthritis and Juvenile Idiopathic Arthritis. Infliximab (Remicade[®], Centocor) is a chimeric human and mouse antibody; Adalimumab (Humira[®], Abbott) is a recombinant humanized monoclonal anti-TNF- α antibody; and Etanercept (Enbrel[®], Amgen and Wyeth) is a fusion protein consisting of a TNF- α receptor and the Fc portion from a human IgG1 antibody.

Side effects of Infliximab include hypersensitivity and other symptoms such as fever, itching, difficulty in breathing and chest pain, which could be caused by the patient experiencing an immune reaction to the murine component of the antibody. The side-effects of the other two drugs include an increased risk of contracting serious infections, as well as other problems that could be the result of a treatment-induced autoimmune response.

The researchers incubated UNlchip[®] AV-400 protein biochips with the TNF- α blocking agents to check their respective specificity. Each set of protein biochips contained a printed serial dilution of naturally folded TNF- α and a random selection of 384 different recombinant human proteins. Though the concentration of drugs used in the experiment ensured that these all had the same level of TNF- α binding activity *in vitro*, the products showed severe differences in specificity.

Infliximab was the most specific antibody with no off-target activity detected. Both Etanercept and Adalimumab showed off-target activity, with Etanercept binding to 10 proteins with affinities greater than 20% of its binding affinity for TNF- α . Adalimumab bound 19 proteins other than TNF- α , though its affinity for these was lower, at only 12-18% of its affinity for TNF- α . Several of these proteins are involved in the assembly of ribosomes and protein synthesis.

The authors said: "TNF- α inhibitors have been shown to be highly efficient and clinically useful drugs for the treatment of rheumatic diseases. At the same time there are reports about adverse side effects associated with TNF- α inhibitor treatment. Although there is no direct evidence that the off-target

activity we have seen leads to pathological consequences, this link cannot be excluded.”

Christoph Hüls, CEO of Protagen, the company that created the protein biochips used in this study said: “The results published today show how protein biochips, such as our UNlchip[®] products, could be highly useful in antibody development, as researchers can test the specificity of their therapeutic candidates. Those exhibiting the least off-target activity can then be taken forward to the next stage of development, as it is likely that these will cause fewer side effects when administered to patients. We believe that the power of quantitative analysis of off-target binding using biochips will mean that providing these data becomes a regulatory requirement for therapeutic antibody approval in the future.”

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About Protagen AG

Protagen (www.protagen.com) is a leading provider of products, services and software solutions for protein research. Operating internationally to provide customers with products and services of the highest quality, Protagen has offices in Dortmund, Germany and New Jersey, USA. The Company has a decade of experience in applying the most advanced protein research tools and techniques to improve the efficiency of the drug discovery and development process.

As an innovator in protein biochips, Protagen has developed UNlclone[®], a patent protected platform for protein expression libraries and protein arrays, and UNlchip[®], a versatile and highly efficient series of protein biochip products for antibody development. In addition, it has established UNlarray[™], a highly efficient technology platform for biomarker discovery on an industrial scale. Protagen has recently received a BioChance Plus grant from the German government to identify biomarkers for Multiple Sclerosis and Rheumatoid Arthritis.

Protagen provides customers with a flexible service program, including protein analysis and identification, differential protein display, antibody validation and specification, antigen identification, and full data management services.

Protagen AG is privately owned and has closed three financing rounds: at the end of 2004, in spring 2006 and in spring 2008. Shareholders of Protagen AG, besides the founders and the management team, are S-Venture Capital Dortmund GmbH, MIG AG & Co KG Beteiligungsfonds 1 and 3 and the Kreditanstalt für Wiederaufbau (KfW).

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