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MEETING REPORT

# PEGS EUROPE - CHI'S THIRD ANNUAL PROTEIN & ANTIBODY ENGINEERING SUMMIT HANNOVER, GERMANY, OCTOBER 11-13, 2011

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## SUMMARY

*CHI's Third Annual Protein & Antibody Engineering Summit was held in Hannover in the framework of the BioTechnica exhibition and in parallel to two other CHI events: BioIT World Europe and the Molecular Diagnostics Summit Europe. The PEGS Europe comprised four tracks: "Enhancing Expression and Achieving Higher Throughput through Cell Line Development", "Therapeutic development with novel Antibody Products", "Solving Difficult Protein Problems from Expression through Purification" and 'Engineering of Novel Constructs & Alternative Scaffolds". This report focuses on the latter track, which included the following sessions: "Bispecific Antibody Engineering", "Advances from Academia", "Antibody–Drug Conjugates", "Measures to Extend Half-Life", "Enhanced Effector Functions" and "Expression and Selection". Two presentations from another track concerning the development of novel multispecific constructs are also included in this report. Most of the speakers focused on developing molecules with improved properties compared to standard monoclonal antibodies (MAbs). The majority of the presentations could be classified into three categories: bi- and multispecific binding proteins, increasing effector function/arming MAbs and extending pharmacokinetic properties of antibodies and alternative formats.*

**Key words:** Bispecific antibodies – Multispecific antibodies – Effector function/arming – Pharmacokinetics

## BISPECIFIC AND MULTISPECIFIC BINDING PROTEINS

An update on the development of bispecific T cell engagers (BiTEs) was given by Patrick Bauerle (Micromet). BiTEs are composed of two single-chain Fv (scFv) fragments connected by a short linker and therefore combine two specificities within a small protein. The most advanced product, blinatumomab (MT103), is an anti-CD3 x anti-CD19 BiTE. The mechanism of action of this molecule is to redirect cytotoxic T cells to $CD19^+$ cells for oncology indications. Blinatumomab has completed a phase II trial in adult minimal residual disease-positive (MRD+) acute lymphoblastic leukemia (ALL) and is currently in a pivotal trial for the same indication. Results from the phase II trial were presented. MRD (leukemic cells remaining during treatment) is predictive of hematological relapse and an adverse prognostic factor. Twenty-one patients having failed or relapsed after 3 cycles of chemotherapy were enrolled and treated with blinatumomab at 15 mg/$m^2$/day by continuous i.v. injections during a 4-week period followed by 2 weeks without treatment and 1 or multiple consolidation cycles. The primary endpoint was complete response by achieving MRD negativity, as assessed by quantitative polymerase chain reaction (qPCR). This endpoint was achieved in 80% of patients. The majority of adverse events were linked to flu-like symptoms due to the mechanism of action of the drug and CD3 engagement on T cells. Only very few grade 3/4 events were observed. Importantly, 14 of 20 patients presented disease-free survival. Blinatumomab is also currently being evaluated in adult relapsed/refractory acute lymphoblastic leukemia (ALL) and adult relapsed/refractory non-Hodgkin's lymphoma (NHL). Several other BiTEs are under development for solid and hematological diseases to further explore the potential of cytotoxic cell retargeting approaches for the treatment of other tumor types.

Another application of multispecific antibodies or antibody-like molecules was described by Prof. Dane Wittrup (MIT). Several trispecific molecules were generated by fusing engineered fibronectin type 3 (Fn3) domains at the *C*-terminus or *N*-terminus of the light chains and/or the heavy chains of an antibody. In one example, Fn3 domains that are specific for distinct epitopes on EGFR were selected using yeast display and fused to cetuximab (a chimeric anti-EGFR antibody). In this way, several molecules having two, three or four binding sites for distinct epitopes on EGFR could be studied for their biological effect of this receptor. Data were presented showing that expression of EGFR at the cell surface was reduced using bispecific cetuximab–Fn3 compared to cetuximab, and importantly, that trispecific (cetuximab–Fn3–Fn3) and tetraspecific molecules further

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reduced EGFR surface expression in a significant manner. The clustering of EGFR mediated by multispecific molecules leads to increased endocytosis. This effect was not observed using combinations of anti-EGFR monoclonal antibodies (MAbs) that target different epitopes on EGFR. The combination of antibodies also reduced EGFR expression, but by a different mechanism: inhibiting receptor recycling to the cell surface. Differences were observed depending on where the Fn3 domains were fused to the antibody. These receptor clustering events did not lead to agonist effects. Overall, the approach highlights the fact that triepitopic targeting of a receptor leads to superior EGFR inhibition in vitro and in vivo and enables mechanisms of action that are different from antibody combinations. Similar results with trispecific molecules targeting carcinoembryonic antigen (CEA; a GPI-anchored glycoprotein) suggest that increased receptor internalization via multiepitopic targeting might represent a general approach for receptor inhibition. At this stage, the toxicity and immunogenicity of these trispecific constructs have not been evaluated due to lack of crossreactivity towards the target in relevant laboratory species.

Paul Moore (Macrogenics) reported the development of dual-affinity re-targeting (DART) molecules. DARTs are based on a classical diabody format, stabilized by a disulfide bridge introduced at the *C*-terminus of the $V_H$ domains. Beyond retargeting approaches similar to BiTEs in terms of mechanism of action, as exemplified by CD19 x TCR or CD19 x CD3 DARTs, dual specific molecules targeting CD79B and CD32B have been described. In this modality, the inhibitory Fc receptor CD32B is cross-linked to the B cell receptor via binding to CD79B. This leads to a non-depleting inhibition of B cells that differs from B-cell-depleting strategies (such as treatment with rituximab) that are currently used for rheumatoid arthritis and has the potential for diminishing safety concerns. The CD79B x CD32B DART was shown to inhibit the proliferation of B cells derived from multiple healthy donors, as well as lupus patients, and was also effective in a mouse collagen-induced arthritis model. Due to their small size, DARTs are cleared very quickly from the circulation. Therefore, several half-life extension strategies have been presented, including PEGylation, fusion to albumin and Fc fusions. The Fc constructs include the "knob-into-hole" mutations described by Paul Carter in the Fc portion to force heterodimerization and proper pairing of the Fc–DART construct.

Manufacturing and stability issues of multispecific molecules have been addressed in several presentations and during roundtable discussions. Indeed, multiple bispecific formats have failed in the past due to lack of stability and drug-like properties. In this context, Tariq Ghayur (Abbott) presented a high-throughput approach for the parallel generation of multiple variants of dual-variable domain immunoglobulin (DVD-Ig), a tetravalent and bispecific Ig-like molecule. In this way, multiple arrangements of the $V_H$ and $V_L$ domains connected with different types of linkers can be tested for optimal target binding and drug-like properties. Extensive experience has been accumulated with this format, as a significant number of DVD-Igs have been expressed on a larger scale and characterized by using a battery of analytical methods in order to select candidates that have favorable characteristics for further development. The pharmacokinetics and immunogenicity of 10 anti-IL-1β x anti-IL-17 DVD-Igs were evaluated. Antidrug antibody (ADAs) responses were observed in mice and cynomolgus monkeys, but not in rats, and the response levels were dependent on the orientation of the binding domains. The half-life in cynomolgus monkeys was 8 days in males and 5.3 days in females.

In another presentation, the manufacturing and biological activity of a Cross-MAb, a bispecific antibody format developed by Roche, were described by Markus Thomas. This angiopoeitin-2 (ANG-2) x VEGF-A bispecific antibody targets two pathways involved in tumor neovascularization. The design of this molecule aims at resolving the incorrect pairing issues linked to the co-expression of two heavy chains and two light chains. First, the Cross-MAb contains the "knob-into-hole" mutations to force heterodimerization of two different heavy chains. In addition, the CH1 and CK domains in one heavy chain and one light chain have been swapped to force the correct pairing of the light chains with their respective heavy chains. Chemistry, Manufacturing and Controls (CMC) data were presented and large-scale manufacturing was achieved despite the expression of side products such has homodimers and various antibody forms lacking one or the other light chain. Aggregates could be reduced to acceptable levels and the pharmacokinetic properties of the Cross-MAb were evaluated in cynomolgus monkeys and shown to be comparable to IgG. Surprisingly, the biological activity of the ANG-2 x VEGF-A Cross-MAb appeared to be slightly superior to the combination of two parental monoclonal antibodies (anti-ANG-2 + anti-VEGF-A) in several tumor models. Although no explanation for this finding was provided, these results suggest that bispecific formats can lead to different biological effects compared to the use of antibody combinations.

These presentations highlighted that, although achievable, large-scale manufacturing of bispecific molecules is not trivial and still represents a significant hurdle for this class of molecules. With these manufacturing issues in mind, a novel bispecific format that is undistinguishable from a standard IgG was described by Nicolas Fischer (NovImmune). This molecule, called a κλ body, is a human IgG composed of two copies of a heavy chain and two different light chains (one κ and one λ) that drive the specificity of each antibody arm. A scalable purification process using affinity chromatography was presented.

## INCREASING EFFECTOR FUNCTION/ARMING MONOCLONAL ANTIBODIES

John Desjarlais (Xencor) presented different Fc engineering approaches. By site-directed mutagenesis, the affinity of the Fc for the inhibitory Fc receptor CD32B was increased 400-fold, leading to an increase in downstream signaling via the receptor's immunoreceptor tyrosine-based inhibitory motif (ITIM). An anti-CD19 antibody with this engineered Fc is currently undergoing a phase I clinical trial. The mechanism of action of this antibody is inhibition of B cell activation without depletion of B cells. Similarly, an anti-IgE with enhanced CD32B binding that can bind both soluble and cell-surface IgE was described. This antibody can inhibit soluble IgE binding to Fc receptors, but also inhibit the proliferation and activation of IgE-secreting B cells via activation of the CD32B receptor.

An update on benralizumab, a glycoengineered anti-IL5R MAb, was presented by David Lowe (MedImmune). This antibody was produced using the *FUT8*-deleted CHO cell line developed by BioWa

that leads to the absence of fucosylation and increased antibody-dependent cellular cytotoxicity (ADCC). The antibody mediates a dual mechanism of action by blocking IL-5 binding to its receptor and by killing eosinophils and basophils via ADCC. Eosinophils are key mediators in asthma, promoting inflammation, fibrosis and mucus secretion. Effective depletion of eosinophils was observed in cynomolgus monkeys. A phase I trial was conducted with single-dose injections ranging between 0.3 μg/kg and 3 mg/kg and a complete depletion of eosinophils was observed at doses above 30 μg/kg. The depletion lasted up to 84 days but was reversible. Benralizumab is currently in a phase IIb clinical trial for inadequately controlled eosinophilic asthma.

## EXTENDING PHARMACOKINETIC PROPERTIES OF ANTIBODIES AND ALTERNATIVE FORMATS

Beyond the introduction of mutations into the Fc region to increase binding to the neonatal Fc receptor (FcRn), an interesting alternative strategy for improving the half-life of antibodies was presented by John Desjarlais (Xencor). The concept is based on the observation that a lower pI correlates with a longer half-life for an antibody. Thus, mutations in the Fc that lower the pI were designed as a generic way to tune the pharmacokinetic properties of antibodies. The potential immunogenicity issues linked to such mutations are tentatively minimized by the use of amino acid replacements that are found in existing human immunoglobulin isotypes or by epitope filtering.

Fusion to albumin or using albumin-binding domains are strategies pursued by multiple groups and companies. However, the maximal half-life that can be achieved in this way is that of albumin. Chaity Chaudhury (MedImmune) presented the engineering of albumin to increase its binding to FcRn, while maintaining the pH-dependent binding properties that are crucial for an extended half-life via this receptor. Extensive mutagenesis of each amino acid of domain III of albumin was performed and tested for FcRn binding using a mammalian display system. Domain III of albumin was primarily targeted, as it mediates the binding of albumin to FcRn. Several hotspots were identified and combined to yield "superalbumins" with up to 40-fold increased affinity for FcRn while retaining 70% of pH binding dependency. Pharmacokinetic studies of these albumin variants are ongoing in cynomolgus monkeys.

## DISCLOSURES

The author is an employee of NovImmune.