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MEETING REPORT

THERAPEUTIC DEVELOPMENTS WITH NOVEL ANTIBODY PRODUCTS AND ENGINEERING OF NOVEL ANTIBODY CONSTRUCTS AND ALTERNATIVE SCAFFOLDS

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CONTENTS

SUMMARY

This meeting brought together scientists with a common interest in the discovery and development of novel biologic therapeutic agents. The 150 delegates were drawn mainly from pharmaceutical and biotechnology companies, with some academic representatives. The meeting was held in parallel with the protein expression tracks "Enhancing Expression and Achieving Higher Throughput through Cell Line Development" and "Solving Difficult Protein Problems from Expression through Purification", and the Biotechnica 2011 trade fair (www.biotechnica.de), which featured a number of innovative companies developing new products to aid drug discovery. The meeting was successful in making the audience aware that the next generation of improved biologic therapeutic agents is progressing rapidly from the laboratory bench to the clinic. This report summarizes the therapeutic presentations from the conference.

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BISPECIFIC ANTIBODIES

Bispecifics are now moving into the mainstream, with a total of USD 6.5 billion in deals signed so far. This includes companies such as: Micromet, with USD 1.9 billion in deals (signed with MedImmune, Bayer, Sanofi, Boehringer Ingelheim and Amgen); MacroGenics, with USD 2.1 billion in deals for their DART™ antibodies; F-star, with USD 2.4 billion for Fcab™ and MAb2 antibodies; and Zymeworks, with USD 178 million in signed deals for their Azymetric™ IgG_1 antibodies. Bispecific antibodies can operate at several different levels, including the neutralization of two different ligands (e.g., IL-12/IL-18; IL-1α/IL-1β; VEGF/ANG-2), the simultaneous blockade of a ligand and receptor (e.g., VEGF/HER2), a combination of two different modes of action (e.g., anti-angiogenesis plus growth factor receptor blockade [VEGF/HER2]), the blockade of two different receptors (e.g., HER2/HER3; EGFR/IGF-I receptor), natural killer (NK) cell engagement (e.g., CD16A/CD30) and T cell engagement. Patrick Baeuerle (Micromet) presented work on a T-cell-engaging bispecific antibody construct for cancer therapy. Micromet has developed BiTE (bispecific T cell engager) antibodies by fusion of two single-chain antibodies. Blinatumomab (MT103) is a CD19/CD3-bispecific BiTE antibody that targets T cells to $CD19^+$ lymphoma/leukemia cells to promote cell lysis. A phase I clinical trial has been performed with blinatumomab to test its safety, as has a phase II clinical trial for the treatment of patients with chemotherapy-resistant minimal residual disease. Results are encouraging, with high response rates in patients at low doses. Nicolas Fisher (NovImmune) described the κλ body format, which is a fully human IgG with a common heavy chain and two separate light chains (κ and λ) which give rise to two specificities. κ or λ phage display libraries are panned separately to iden-

tify specific variable fragments (Fv) which can be brought together to form the bispecific κλ body. A method was also developed to screen in a more efficient manner using next-generation sequencing (1). The κλ body can be expressed by transient transfection with a tricistronic vector to coexpress the heavy (H), light kappa (Lκ) and light lambda (Lλ) chains. This results in 50% expression of the desired bispecific κλ body, but also expression of the monospecific mono λ (25%) and mono κ formats. The κλ body can be purified in a three-step affinity purification (protein A, κ select, λ select). An example of a functional κλ body was demonstrated possessing dual affinity for interferon gamma (IFN-γ) and IL-6R.

Two key players involved in tumor angiogenesis and growth are VEGF-A, which is mainly produced by tumor cells, and angiopoietin-2 (ANG-2), which is expressed by endothelial cells. ANG-2 is a ligand for the TIE-2 receptor and is also implicated in sensitizing the endothelium for VEGF signaling. Targeting VEGF-A alone provides limited benefit, whereas a bispecific MAb targeting both VEGF-A and ANG-2 might be expected to be a more potent inhibitor of tumor angiogenesis. Markus Thomas (Roche Diagnostics) presented methods to produce a bispecific MAb combining bevacizumab and an anti-ANG-2 MAb. If one were simply to coexpress the two light and two heavy chains, only 12.5% of expressed antibody would be the required bispecific MAb. A method was described which expanded the "knobs-in-holes" methods, first described by Paul Carter (Genentech), and used to make one-armed anti-Met antibodies. This resulted in exclusive heterodimerization of the heavy chains, while also inducing a heavy chain to only associate with its own light chain by switching the constant light (C_L) and constant heavy (C_{H1}) domains (2). The resulting CrossMab displayed improved efficacy in mice bearing subcutaneous (s.c.) human colon adenocarcinoma COLO 205 xenografts compared to bevacizumab or anti-ANG-2 monotherapies in terms of reduced tumor volume and survival rate. Similar results were observed in an s.c. gastric cancer xenograft study. The CrossMab was also efficient in blocking angiogenesis in a cornea pocket assay.

TRISPECIFIC ANTIBODIES

The existing therapeutic anti-EGFR antibodies have modest efficacy as judged by EGFR downregulation. For example, cetuximab and panitumumab display 23% and 8% objective response rates, respectively. Some improvements have been reported by administering MAb cocktails such as Sym004 (Symphogen). However, K. Dane Wittrup (MIT) discussed the advantages of a single-agent cocktail with a simplified bioprocess, dosing, regulatory issues and possibly improved efficacy. He engineered a tri-fibronectin (Fn3) domain molecule where each domain bound to a separate epitope on EGFR. The anti-EGFR Fn3 molecule displayed far greater surface EGFR downregulation compared with a single MAb (cetuximab) or a dimeric fibronectin molecule in human colon adenocarcinoma HT-29, cervix epithelioid carcinoma HeLa or human mammary epithelial cells (HMECs). This was further improved for valency and enhanced clustering by construction of a *trans* antibody –a fibronectin fusion molecule where anti-EGFR fibronectin domains were fused to the *N*-terminus of the heavy chain and the *C*-terminus of the light chain. Here, the two different fibronectin domains and the antibody variable domains each bound to a separate epitope. The trispecifics resulted in EGFR clustering without agonist activity at the receptor, with no phosphorylation of tyrosine, serine or threonine residues in the tyrosine kinase domain over a 2-hour timespan. The triepitopics blocked the phosphorylation of ERK and Akt to a far greater extent than cetuximab and were far more effective in controlling *BRAF* and *KRAS* mutant tumor growth in vivo.

NOVEL SCAFFOLDS

Thomas Sandal (Pieris) discussed the generation of novel therapeutic molecules based on the anticalin scaffold. Anticalins naturally bind, store and transport a wide range of molecules in the serum. They possess a conserved β-barrel fold with four complement-determining region-like variable loops. Anticalin binding can either involve the "pocket" of the β-barrel or the surface-exposed loops, and so both regions can be randomized to generate large phage display libraries for target selection. They possess some advantages over MAbs, including no effector function, monovalent binding, ease of manufacture and their half-lives can be increased by PEGylation. There are several anticalins in various stages of clinical development, including the anti-VEGF PRS-050 for oncology, the anti-hepcidin PRS-080 to treat chronic kidney disease and collagen-induced arthritis, the anti-c-Met PRS-110 for oncology and the anti-IL-4R-alpha PRS-060 to treat asthma. The anti-VEGF PRS-050 is now in phase I clinical trials partnered with Sanofi. It binds VEGF-A with comparable affinity to bevacizumab and blocks the binding of VEGF to the receptors VEGFR-1 and VEGFR-2. It has been PEGylated (40-kDa branched) for half-life extension and displayed crossreactivity towards murine and cynomolgus monkey VEGF. The phase I results for pegylated PRS-050 were very encouraging, with a dose range of 0.1-10 mg/kg, a half-life of 6 days and no evidence of anti-PRS-050 antibodies at up to 10 mg/kg and five administrations. Early signs of efficacy included the disappearance of circulating VEGF-A upon dosing at 0.5 mg/kg and the observation of hypertension in a dose-dependent manner, which is a well-documented pharmacodynamic readout of anti-VEGF activity, as observed with bevacizumab. It was also demonstrated that anticalins can not only be administered by the traditional intravenous or subcutaneous injection route, but also to the lung by inhalation after nebulization. Also, anticalins can be linked to produce bispecific therapeutics.

John Tite (Bicycle Therapeutics) presented a new technology platform capable of generating constrained peptide binders to any given target molecule. Peptide phage display libraries are first generated containing three cysteine residues, which, prior to selection, are conjugated to tris-(bromomethyl)benzene (TBMB). This generates a novel peptide scaffold library containing two constrained peptide loops, which can then be used to select for specific binders to any given target. Loop lengths are typically 3-5 amino acid residues to enable full coverage of diversity for library sizes of up to 10^{10}. The utility of this approach was demonstrated by selecting for inhibitors of both urokinase-type plasminogen activator and the therapeutic target protease plasma kallikrein, a key target to inhibit the early steps of the blood coagulation pathway. There is a medical need for such inhibitors during cardiopulmonary bypass surgery, where contact of blood with artificial surfaces activates the blood coagulation intrinsic pathway, leading to severe inflammatory response syndrome. Initial selection resulted in chemically constrained peptide inhibitors with inhibition constants in the subnanomolar range. Interestingly, changes in the TBMB scaffold (for example, adding

methyl or ethyl groups to the benzene ring) resulted in altered inhibition constants, raising the possibility that increased diversity could be achieved by altering the nature of the chemical scaffold. The kallikrein inhibitors obtained were highly selective, with little inhibition of paralogous proteases (human coagulation factor XIIa or thrombin). Some inhibitors also displayed good inhibition of the orthologous rat and monkey kallikrein, useful for animal studies. High stability of the constrained peptides was observed in serum or whole blood and in hepatic microsome assays. No toxicity of the common core scaffold $AcCys_3TBMB$ was observed up to a concentration of 100 µg/mL in HEK-293 or Hep G2 cells. A problem with the constrained peptides is their very short half-life in vivo, but this can be lengthened to 5 hours by human serum albumin conjugation. Two constrained peptide motifs can also be linked together to give molecules with dual targeting ability, as demonstrated by a dual kallikrein and elastase inhibitor.

YEAST DISPLAY

Jonathan Belk (Adimab) described using yeast display of full IgG molecules to aid antibody selection. The advantage of screening libraries of surface-displayed IgG is that no reformatting is required and one has access to high-quality IgG for testing only 4 weeks after commencing a lead isolation project. Yeast strain engineering was required to give better MAb (not Fab) display because of issues with proteolysis. Adimab employed DNA 2.0 to make a synthetic human antibody library. Here, the total theoretical diversity ($20 \times V_H$ combined with $40 \times V_L$ equals 10^{18} possible combinations) is too great to represent in a single yeast display library, and so certain germline sequences were prioritized based on knowledge of the human repertoire. The library was prepared by transformation of the light-chain library (10^5) followed by transformation of the heavy-chain library. Sequencing revealed good diversity (average complementarity determining regions of heavy chain variable regions [CDRH3] length = 12 with Poisson distribution from 3 to 21). To allow a greater library selection, rounds 1 and 2 used a MACS (bead-based) selection and round 3 used a FACS selection. Typical affinities (K_Ds) in the 0.1 nM to 0.1 µM range were determined using the Fortebio system. A batch light chain shuffle was performed to achieve affinities (K_D) down to 5 pM. Also, thermal stability measurements were performed to select for antibodies with greater stability (e.g., melting temperature (T_m) of adalimumab [Humira®] ~ 73 °C, cetuximab [Erbitux®] ~ 69 °C). Yeast produced MAbs (minus glycosylation) with a T_m range of 85 °C to 60 °C. It is also important to screen for function. For example, it was found that only 8% of selected antibodies bound to the native receptor on the cell surface. However, it was found that FACS could sort yeast cells bound to mammalian cells, providing a useful secondary screen.

ALTERNATE ANTIBODY FORMATS

Paul Aldgate (Emergent Biosolutions) updated the conference on the progress of single-chain antibody (scFv)–Fc fusion therapeutic molecules. It was found that the length of the hinge between the scFv and Fc domains can influence signaling phenotype (receptor cross-linking and intracellular phosphorylation levels) and that this property can be engineered into the scFv–Fc molecules to either increase or decrease signaling phenotype. Nature already uses hinge length to alter the activity of antibodies: the hinge length of IgM CH2 > IgD > IgG_1 > IgA_2. As an example, it was found that an scFv–Fc molecule with a short hinge displayed greater antiproliferative activity than an equivalent MAb. SBI-087 is a monospecific protein therapeutic (SMIP™) anti-CD20 scFv–Fc fusion in phase I clinical trials, currently being codeveloped with Pfizer. Its mode of action is tumor cell killing by direct signaling together with antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). TRU-016 is an anti-CD37 scFv–Fc being codeveloped with Abbott. CD37 is an understudied 40- to 52-kDa glycosylated tetraspanin receptor that is expressed in B cell malignancies and may play a role in T cell/B cell interactions. It also has a possible role in T cell proliferation and participates in the regulation of IL-6 production by dendritic cells. CD37 is a validated target for non-Hodgkin's lymphoma (NHL), as previously a radiolabeled [^{125}I]-anti-CD37 MAb (MB-1; Biogen Idec) gave a 60-80% overall response rate and a 10-40% complete response rate. The mode of action of TRU-016 is tumor cell killing by direct signaling and ADCC. TRU-016 was found to induce higher levels of ADCC in chronic lymphocytic leukemia (CLL) cells compared with trastuzumab (Herceptin®; Genentech), alemtuzumab (Campath®; Genzyme) and rituximab (Biogen Idec). The in vivo efficacy of TRU-016 was demonstrated in a mouse tumor xenograft model employing the Bcl-2-overexpressing lymphoma cell line DoHH2. Both the survival rate and the percentage of mice that were tumor-free were significantly better for mice injected with TRU-016 compared with rituximab. Also, serum half-lives were similar to MAbs. A phase I trial with TRU-016 was commenced to determine the maximum tolerated dose from 0.03 to 20 mg/kg. Results indicate no apparent dose–response for severe adverse events and a clinical benefit in patients with CLL, as judged by a reduction in lymphocyte number and lymph nodes. Animal studies suggested a benefit in terms of survival rate and tumor clearance for combination therapy with bendamustine (Cephalon) and rituximab. Therefore, the planned phase II trial will involve combination therapy in CLL and NHL. There are also anti-CD3 and anti-IL-6 antagonist scFv–Fc molecules being developed in-house. The SCORPIUM™ molecule is a bispecific molecule with scFc or receptor ligands or receptor ectodomains at both the *N*- and *C*-termini of the dimeric Fc chains. This enables the targeting of multiple antigens, including cell-surface molecules and their secreted products. Sam Heywood (UCB New Medicines) highlighted that the Fab format has many advantages compared with the traditional IgG format. This includes high stability and expression yield, ability to be chemically modified (e.g., pegylated) for extended in vivo half-life, monovalency and low immunogenicity. Anti-albumin Fv can also be grafted onto existing Fabs for extended serum half-life.

ANTIBODY–DRUG CONJUGATES (ADCs)

Vincent de Groot (Synthon) focused on MS and oncology. The company has an antibody–drug conjugate (ADC) technology which includes a novel stable linker. The drug molecules are duocarmycins, which are DNA alkylating agents, where binding in the minor groove increases the drug's reactivity. Solid tumors are usually more responsive to DNA-directed drugs compared with tubulin-directed therapeutics. Synthon also has a unique linker chemistry

(SpaceLink), which is only cleaved as the antibody enters the cell, reducing premature release in the circulation, and thus toxicity. Duocarmycin is only released in active form after cleavage by lysosomal proteases. The technology was exemplified by construction of a trastuzumab–duocarmycin ADC, which was significantly more potent than trastuzumab alone in a murine xenograft model of human gastric carcinoma NCI-N87 measuring mean tumor burden and survival rate.

TARGET DISCOVERY

Dario Neri (ETH Zurich) presented work on a method for target discovery for surface-accessible protein upregulated in tumor tissue compared with healthy tissue by biotinylation in vivo in a mouse model, followed by tissue homogenization, enrichment of biotinylated proteins and on-resin digestion followed by a comparative proteomic analysis (Patent Application PCT/EP2007/001490). This revealed a number of targets upregulated in the neovasculature of a lymphoma, including BST-2 or CD317 (3). BST-2 was found to be a novel marker of the neovasculature in diffuse large B-cell lymphoma, Burkitt's lymphoma, mantle cell lymphoma, follicular lymphoma and CLL, as shown by immunohistochemistry. The proteomic study also revealed that specific splice isoforms of fibronectin and tenascin were excellent markers of angiogenesis. The human antibodies L19, F8 and F16 are currently in industrial development to exploit this. The angiogenesis markers have revealed that angiogenesis is a rare event in the adult, but is a common feature of aggressive tumors and other serious pathological conditions, including endometriosis, arthritis and atherosclerosis. The oncofetal splice variant of fibronectin was also found to be upregulated in many types of lung cancer, including small cell carcinoma and various non-small cell lung cancers, including squamous cell carcinoma, adenocarcinoma, bronchioloalveolar carcinoma and large cell carcinoma. In collaboration with Philogen, the anti-fibronectin antibody L19 (radioiodinated) has been used to image tumors in cancer patients. An L19 scFv–TNF bispecific fusion was prepared and is being codeveloped with Bayer as a tumor-targeting agent. The L19 scFv–IL-2 cytokine fusion (Darleukin) was shown to effectively reduce F9 tumor volumes in a mouse model. A phase II clinical trial employing Darleukin in combination with dacarbazine (DTIC) has been performed in patients with melanoma and showed a good safety and response profile, comparable to ipilimumab (Bristol-Myers Squibb). Other combination therapies, such as Teleukin plus cetuximab (Erbitux®; Imclone), Teleukin plus trastuzumab (Herceptin®) and Darleukin plus rituximab (Rituxan®), have strikingly superior antitumor activities in cancer models. Radretumab (L19-^{131}I) displayed promising results in phase I/II clinical trials for Hodgkin's lymphoma, although a relapse was observed for some patients, with a reappearance of tumors.

ENGINEERING HUMAN THERAPEUTIC MONOCLONAL ANTIBODIES

David Lowe (MedImmune) summarized various mutations to increase or decrease ADCC and CDC (e.g., IgG_1-TM triple mutant L234F, L235E and P331S has very low ADCC and low CDC). However, afucosylated IgG generated from the Potelligent® (BioWa) cell line (*FUT8*-deficient CHO cells) results in significantly increased ADCC by improved FcγRIIIa binding expressed on NK cells and macrophages. This was used to increase ADCC for benralizumab (anti-IL-5R antibody) now in phase II trials for asthma. Benralizumab potently kills human eosinophils and basophils with no eosinophil cationic protein or eosinophil-derived neurotoxin release. Eosinophils are key Th2 effector cells that also secrete HBGF-2, VEGF, TNF-α and angiogenin to activate angiogenesis. Fc engineering was performed to enhance half-life in serum by binding the neonatal Fc receptor (FcRn) and endosome recycling in endothelial cells. The FcRn epitope was mapped on the CH_2-CH_3 domains and it was found that the M252Y/S254T/T256E mutant increased the affinity for FcRn by 10-fold. Jonas Schaefer (University of Zurich) emphasized that the ideal IgG framework should not be prone to aggregation because this can lead to loss of potency and immunogenicity. Different variable chain classes are prone to aggregation to different extents. For example, V_H3 is considered to be "best in class" –capable of expression even without a V–; this is followed by V_H1a,b and V_H5 and then by V_H2, 4 and 6 with moderate folding ability. For the light chain V_K3 is best followed by V_K1,2,4 and next V_λ 1,2,3, which are expressed in low yield, although an enormous increase in expression yield is observed when co-expressed with V_H (4, 5). Jonas posed the question: Is it possible to use one superior antibody framework? The answer to this question is not if one wants to generate a library capable of binding proteins (here the CDR must present a flat surface) or peptides and haptens where the antibody must form a groove in its binding surface. In general, the frameworks binding haptens and peptides are less good at folding (6). It was also found that the peptide sequence EAEA at the *N*-terminus of V_H or V_L inhibited aggregation. This sequence is an artifact of pro-peptide cleavage in *Pichia pastoris*.

NEW TECHNOLOGY DEVELOPMENTS

Kristian Müller (Universität Potsdam) presented a method to construct viral particles engineered to display an antibody or other receptor binding molecule on the viral surface to target cancer cells with an upregulated cell-surface receptor, such as EGFR. Once the virus had entered the cell, a viral-encoded enzyme would be expressed to activate a prodrug, capable of killing the tumor cell when activated. This was demonstrated for human epidermoid carcinoma A-431 cells.

DISCLOSURES

The author states no conflicts of interest.

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The website for this meeting can be found at: http://www.pegsummit-europe.com/