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[11.00–11.20]

Molecular and functional characterization of ED-B fibronectin selective function blocking antibodies and the evaluation of phage angiomics for target identification and validation

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Fibronectin is an important component of the extracellular matrix that contributes to the maintainance of normal cell morphology and migration. Transformed cells however produce novel isoforms by alternative splicing processes. Here, the ED-B domain seems to be of special importance. ED-B co-localizes only with proliferative endothelium in various tumor samples, whreas normal tissue is not stained as demonstrated by immunohistochemistry using antibodies directed against ED-B. Despite these compelling results, hardly anything is known about the function of ED-B. The high degree of homology between mouse, chicken and human ED-B-fibronectin together with the selective expression pattern however point to an important function of this fibronectin splice variant. Our investigations demonstrate that recombinant ED-B triggers a receptor-mediated signalling cascade which is essential for both attachment, proliferation and matrix invasion/tube formation of human dermal microvascular endothelial cells *in vitro*. Based on these results, we hypothesize that the blockade of this ED-B-fibronectin – receptor interaction will be detrimental for proliferating endothelial cells.

To proof our concept, we have successfully identified several high-affinity ED-B-function blocking antibodies (30–80 pM in the monovalent Fab-format) in co-operation with MorphoSys. These Fab-fragments were tested in the ED-B-expressing murine F9-teratocarcinoma model, where we could clearly demonstrate first signs of efficacy after 5 days of daily i.v. treatment. Our data clearly demonstrate that 1) ED-B-fibronectin indeed has a biological function within the process of angiogenesis and 2) the specific blockade of this pathway leads to an inhibition of tumor growth.

Despite of this newly discovered signalling pathway there is still a need for new targets associated with the process of tumor-angiogenesis. For this purpose, we have developed Phage Angiomics as a complex, however efficacious antibody phage display strategy on freshly isolated tumor-derived endothelial cells. With this methodology, we have successfully identified several (tumor-) endothelium-specific antibodies as was demonstrated by PhageMab-immunohistochemisty. To further characterize the antibodies we successfully performed immunoprecipitation studies from whole cell lysates of early-passage human microvascular endothelial cells using antibody-expressing phage particles. This allowed us the rapid screening of a large number of immunohistochemically validated antibodies without the need for time consuming expression and purification of the soluble antibody fragment. Proof of concept for target identification was performed with antibody CS1, which precipitates a 90 KD cell surface antigen which is expressed on human dermal- as well as tumor-derived microvascular endothelial cells. This antigen was identified as the angiogenesis associated molecule endoglin (CD105) by mass spectrometry. In summary, our results demonstrate that Phage Angiomics is a valuable tool for angiogenesis-associated target identification.

[11.20–11.40]

Improving the immunogenicity profile of therapeutic antibodies and proteins

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Immunogenicity associated with therapeutic antibodies and proteins can limit the clinical effectiveness of otherwise excellent medicines and in some instances has proven to be a barrier to the clinical implemen-

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tation of novel therapeutic strategies. For therapeutic antibodies a number of engineering options exist, and clearly have in part been developed to address the issue of immunogenicity. Examples include "classic" humanisation by CDR engraftment, use of human Ig libraries such as phage display systems or similar, human Ig transgenic mice, xenotransplanted mice and others. However, in each case there should be an expectation that these can all result in antibodies still capable of eliciting an immune response in certain patients and clinical settings. This is connected to the process of affinity maturation during antibody generation that inevitably results in antibodies to which patients may not be naturally tolerant. Underlying this process is the presentation of T-helper peptide epitopes derived from the intracellular processing of the therapeutic antibody. T-cell epitopes can cause a sustained immune response and the generation of immunological memory concomitant with loss of efficacy or even more serious consequences for the patient.

Our approach at Biovation has been the development of what we have termed DeImmunisationTM technology. This involves the identification and removal of helper T-cell epitopes from therapeutic antibody and protein candidates. We use ex-vivo human T-cell assays in a variety of formats to identify the T-cell epitopes, to direct the substitution of residues within epitopes and also to validate successful removal of epitopes from therapeutic antibodies and proteins.

Our start point is to use naïve T-cell assays to provide an outline epitope map of the protein of interest. T-cell assays are performed using healthy human PBMC preparations in MHC selected donor panels and synthetic peptide antigens. The epitope map can be further refined using the enzyme-linked immunospot (ELISPOT) assay to measure the frequencies of activated T-cells according to their particular cytokine profile. We use a software tool termed Peptide Threading to model the effect of amino acid substitutions on the ability of a T-cell epitope sequence to interact with multiple different MHC class II allotypes. This can guide the design of variant sequences and in some studies, the design phase is augmented by fine scale mapping of individual epitopes using alanine scanning mutational analysis. Antibodies and proteins engineered using this approach are tested for an improved immunogenicity profile using time course T-cell assays and selected donor panels.

This approach is widely applicable and we have engineered a number of antibody candidates including two antibodies that are now in clinical trial and demonstrate no evidence of immunogenicity. We will illustrate this approach with examples from our therapeutic antibody programmes and new data from our Fc-linked cytokine projects.

[11.40–12.00]

Novel antibody hinge regions for efficient production of C_H2 domain-deleted antibodies

The Eleventh International Conference on Human Antibodies & Hybridomas
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C_H2 domain-deleted antibodies have a molecular mass of approximately 120 kDa and have been shown to penetrate and localize radioactivity to tumors more efficiently than full length IgG without the unfavorable pharmacodynamic profiles. The C_H2 domain-deleted antibody consists of a V_L C_K light chain and a V_H C_H1 heavy chain domain genetically fused to a C_H3 domain via a modified, flexible hinge. The hinge is composed of an IgG1 upper hinge, a portion of the IgG1 middle hinge, followed by a Gly/Ser connecting peptide. C_H2 domain-deleted humanized CC49 (dd huCC49) is an antibody that recognizes the tumor-associated TAG72 antigen expressed on a number of human carcinomas. dd huCC49 is currently in preclinical development as a radioimmuno-therapeutic. Cell culture biosynthesis of recombinant dd huCC49 produces two homodimeric isoforms of approximate 50:50 distribution. One isoform, referred to as Form A, contains a covalent interchain disulfide bond at heavy chain positions 239 and 242, Kabat numbering system. The second isoform, Form B, fails to develop an interchain disulfide bond as evidenced by the formation of a 60 kDa product following non-reducing denaturing gel electrophoresis. Both isoforms can be purified from crude cell culture supernatant by protein G chromatography. The two isoforms can be further purified and separated from each other using hydrophobic interaction chromatography. Compound stability and biodistribution studies support that Form A is the preferred molecule for therapeutic development. Current manufacturing processes discard at least 50% of the total antibody produced having a negative impact on the overall yield. To improve the homogeneity and yield of C_H2 domain-deleted antibodies we designed and tested antibodies containing modified hinges with the goal of identifying sequences that favor

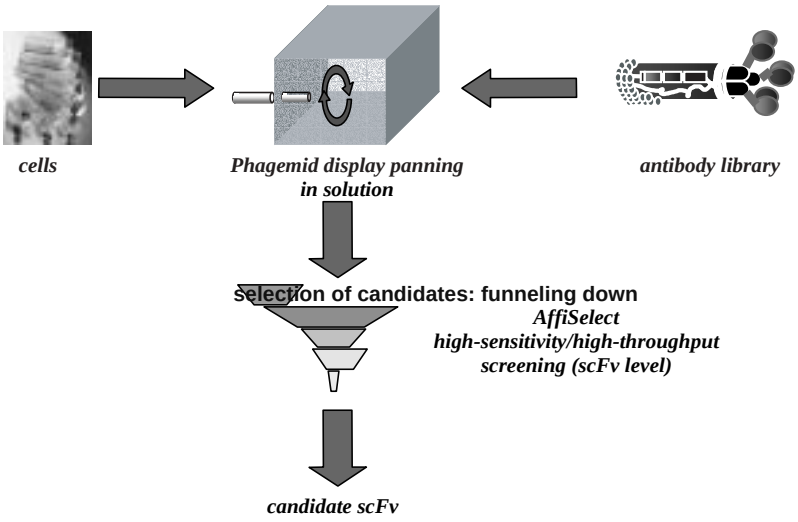

Fig. 1. The CBAS technology.

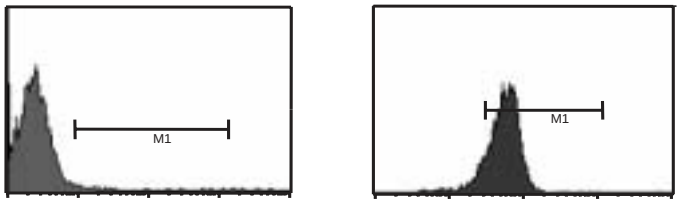

Fig. 2. FACS analysis of an exemplary scFv.

synthesis and secretion of the A isoform. Our results highlight the importance of hinge cysteine residues in producing Form A antibody. We also describe a novel synthetic hinge sequence that significantly favors the production of form A dd huCC49 with yields exceeding 90% following protein G chromatography being realized. Biodistribution studies in a human tumor mouse xenograft model unexpectedly showed lower accumulation of the hinge engineered dd huCC49 antibody in kidney while maintaining efficient tumor targeting.

[12.00–12.20]

CBAS: A new method for the identification of single chain antibodies specific for cell surface antigens

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Introduction: Endeavours to develop antibodies for diagnosis and/or therapy face the bottleneck of target shortage. Especially in the cancer area, the number of available (in terms of intellectual property) as well as suitable (in terms of clinical relevance) cell-surface antigens remains disturbingly low. In order to circumvent this humble stone, we developed a new technology that enables the identification of antibodies specifically binding to cell surface molecules, as well as the identification of the respective antigen. The CBAS (Cell Based Antibody Selection; Fig. 1) technology combines phagemid display on living cells with a high throughput screening method (AffiSelect).

The technology: In order to identify antibodies binding to native cell surface antigens in their natural appearance, CBAS combines phagemid display panning on living cells in solution with a high-sensitivity/high-throughput screening method (AffiSelect).

Results: Using the CBAS technology in a model of a certain human tumor type, we were able to identify a number of scFv candidates that are specifically binding to the respective tumor cells but not to normal reference

Table 1
Tissue-specificity of two candidates

Candidate scFv	Same tumor type	Other tumor types	Normal tissues
1	6/6	7/11	0/4
2	5/6	0/11	0/4

cell types (used for negative pre-panning). These candidates recognize the targets in their native form on the cell surface, as shown in flow cytometry (Fig. 2) and immunohistochemistry. Cross-reactivity studies were undertaken in order to examine the expression pattern of the respective target molecules (Fig. 3). A number of the identified candidates bind to target molecules that are clearly tumor-specifically expressed, thus offering the opportunity to develop powerful diagnostic and/or therapeutic antibody-based drug candidates. Ongoing activities concentrate on the identification of the respective targets as well as the further development of the leads.

[12.20–12.40]

Two next-generation systems for screening and characterization of antibody-antigen interactions

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Applied Biosystems has developed two systems for improving the workflow of hybridoma and phage display-derived antibodies. Utilizing FMAT® system technology, the Applied Biosystems 8200 Cellular Detection System performs homogeneous assays using fluorescent based confocal imaging to scan the bottom of each well in a clear-bottom 96-, 384-, or 1536-well microplate. By scanning only a 100 μm depth at the bottom of the wells, cells or beads can be clearly detected in relation to unbound fluorophores, eliminating the need for wash steps. This system combines the lower cost and higher throughput of ELISAs, to improve the productivity associated with the antibody screening process.

The Applied Biosystems 8500 Affinity Chip Analyzer uses SpotMatrix Surface Plasmon Resonance technology to provide simultaneous measurement of real-time binding kinetics between an unlabeled analyte with up to 400 biomolecular targets spotted onto an affinity chip. This higher chip capacity enables researchers to spot multiple target replicates and target concentrations in the same experimental run. Several antibody-based applications have been developed, including the characterization of antibodies, peptide epitope mapping, and the identification of matched antibody pairs. These three applications provide researchers with valuable tools for biomolecular interaction analysis and antibody characterization.

Together, the 8200 and 8500 systems provide a more complete workflow solution for antibody screening and characterization.

[12.40–13.00]

Clinical evaluation of a human monoclonal antibody against the envelope protein (E2) of HCV for prevention of HCV infection

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Background and aims: Hepatitis C virus infection after liver transplantation occurs in 100% of cases. Aiming at developing an effective therapy to prevent HCV after liver transplantation, human monoclonal antibodies directed against the envelope protein of the virus were generated. The antibodies having high affinity and broader activity against different viral genotypes were selected based on their neutralizing activity *in vitro* in a cell-based infection assay and *in vivo* in the HCV-Trimera mouse model.

Methods and results: In a phase 1a study, single doses ranging from 0.25 to 40 mg of HepeX-C were administered to a total of 15 chronic HCV patients. HepeX-C was well tolerated with no serious adverse events. In 8 out of 15 patients, HCV-RNA levels were reduced at least by half immediately following infusion and then returned to initial levels.

A multidose phase 1b study of 10, 20, 40, 80, and 120 mg of HepeX-C in 25 HCV chronic patients was conducted. Doses were administered weekly for 3 weeks and then 3 times a week during the fourth week. Multiple doses were well tolerated. 8 out of 25 (32%) patients had at least 1-log reduction and 18 out of 25 patients (72%) had at least 0.75-log reduction in HCV-

RNA from pre-treatment baseline at one or more time points following administration.

Conclusions: The good safety and efficacy data from this trial provided a basis for a phase 2a study of HepeX-C to prevent reinfection in liver transplant patients. A multicenter, blinded, placebo controlled study is currently underway.