

## Meeting report: “Antibodies-Europe. Engineering the Next Generation of Antibodies”

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During the past decade antibodies imposed themselves as one of the most promising therapeutic approaches, in particular in the field of cancer treatment. To answer to the need for human and highly specific antibodies, several display methods have been developed to select monoclonal antibodies (mAbs) from large collections of recombinant antibody fragments. These technologies are now widely exploited and recombinant antibodies currently represent over 30% of biopharmaceuticals in clinical trials. The meeting that took place in Vienna in November 2007 was the first European counterpart of the annual „Antibody“ meeting organized by Cambridge Healthtech Institute in Boston (USA). It covered the latest developments in antibody engineering, and in particular functional optimization and improvement of their clinical application. Some key presentations are highlighted here.

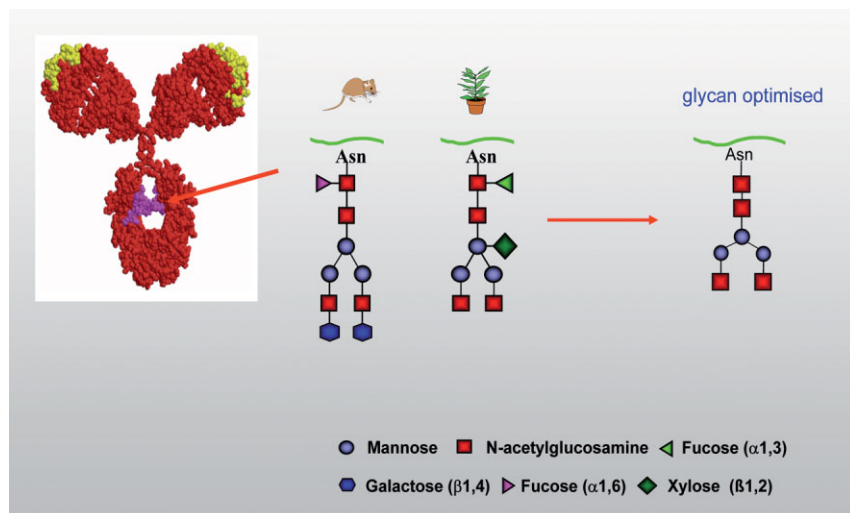


To improve library construction and means of selection, Philippe Mondon (MilleGen, member of the BTJ editorial board) reported a method to make hyperdiversified antibody fragment libraries using low-fidelity human polymerases to generate random mutagenesis mimicking the somatic hypermutation process [1]. Dr. Mondon also described a yeast two-hybrid system developed for the isolation of functional antibodies after intracellular expression. A poster from Dr. Pellis (Brussels University, Belgium) also reported a bacteri-

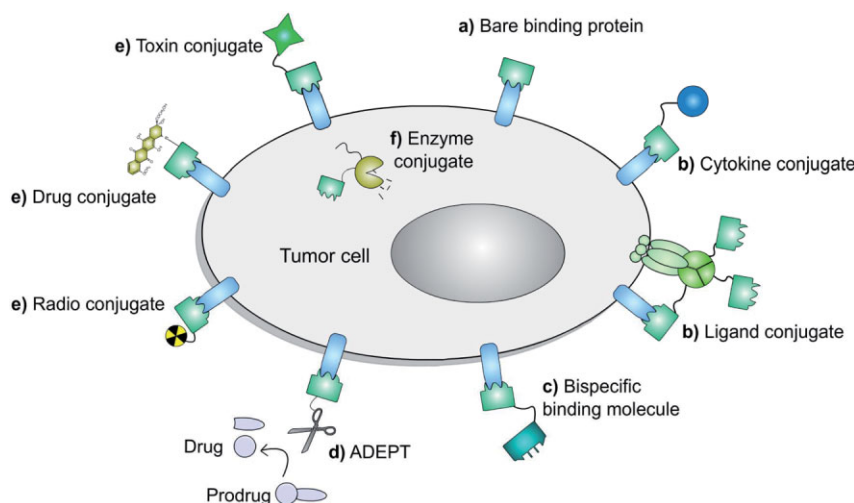
al two-hybrid system based on the camelid VHH library.

The generation of fully human antibodies is important for therapeutic applications both to reduce immunogenicity and to improve effector functions of the Fc part. Effector activity based on the human IgG1 isotype is the most widely used for therapeutic use. Robert Kelley (Genentech) presented a new generation of anti-CD20 that was optimized by affinity maturation and by phage panning to improve FcγR binding on both high- and low-affinity FcγRIII. As compared to Rituximab, this new anti-CD20 displays enhanced CD20 binding affinity, complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC).

Most therapeutic mAbs are produced using mammalian cells [Chinese hamster ovary (CHO), HEK, NSO], although it has been shown that the N-glycosylation obtained using these cell lines is not completely adequate. This may perturb the conformation of the mAb Fc region, altering binding to Fc receptors and the potency and efficacy of ADCC. To circumvent this problem, several groups have set-up production of mAbs in glyco-optimized plant-based expression system modi-



**Figure 1.** From H. Steinkellner (University of Agricultural Science, Vienna, Austria). Typical N-glycosylation pattern of IgG expressed in CHO cells and wild-type plants and after plant optimization.



**Figure 2.** From K. Binz and A. Plückthun (University of Zurich, Switzerland). DARPins: Therapeutic approaches.

fied to produce 'humanized' *N*-linked oligosaccharide structures (Greenovation and Vela Laboratories posters). In addition, fucosylation can inhibit ADCC by steric hindrance, and high mannose glycosylation strongly reduces CDC activity (which is correlated with side-effects). Herta Steinkellner [2] (Agricultura University Vienna, Pharma Planta) and Ann Depicker [3] (Ghent University) presented the production of human IgG in glyco-engineered tobacco plants with a knockout strategy on the b1,2 xylosyltransferase (XylT) and the core a,3 fucosyltransferase (FucT) (Fig. 1). Using plant seeds as a bioreactor seems to be an interesting option to solve the problem of yield and cost for the production of scFv-Fc antibodies (single chain Fv fused to Fc domain). About 6 kg/1 Ha, three times a year, can be reach with time cost of 6 months from the plasmid construct to the first seed generation. In the same way, John Gasdaska (Biolex Therapeutics) presented the LEX system™ developed for the expression of antibody-based on the aquatic higher plant *Lemna minor*. With this system, glycosylation of an mAb against human CD30 was opti-

mized by co-expressing the heavy and light chains of the mAb with an RNA interference construct knocking-down the expression of the endogenous FucT and XylT genes. The resultant mAbs contained a single major *N*-glycan species without detectable plant-specific *N*-glycans, had better ADCC, and displayed better effector cell receptor-binding activities than mAbs expressed in CHO cells [4].

Emerging technologies aimed at developing antibody alternatives were also disclosed during this meeting. Recombinant antibodies have been reduced in size, fused with many molecules, including toxins, enzymes, drugs and viruses, for pro-drug therapy, cancer treatment and gene delivery. Furthermore, innovative affinity maturation methods have been developed which enable rapid selection of extremely high-affinity reagents (pM). Due to their small size, typically less than 20 kDa, robust tertiary structure and composition of a single polypeptide chain provide several advantages compared with antibodies, because they consist of a single polypeptide chain, do not require disulfide bonds, and can easily be produced at low cost and

high yield in microbial host cells (about 200 mg/L). They offer stability during storage, faster pharmacokinetics and better tissue penetration. This may open efficient therapeutic applications. For example, these reagents might be used: (i) as antagonists by binding to cellular receptors and blocking them from interaction with natural signaling molecules, (ii) as tissue-targeting vehicles, by localizing toxic molecules, cytokines or enzymes to disease-related cell-surface receptors, or (iii) as antidotes, by rapidly scavenging toxic or otherwise irritant compounds from the body (Fig. 2).

Andreas Hohlbaum (Pieris) presented a human Anticalin® (a class of engineered ligand-binding proteins that are based on the lipocalin scaffold) directed against the VEGF that inhibits tumor growth in a xenograft model. Kaspar Binz (Molecular Partners) [5] and Thomas Huber (Zurich University) [6] demonstrated the use of designed ankyrin repeat-based proteins (DARPins) as good tools for immunohistochemistry diagnosis or co-crystallization. Another alternative to mAb was also represented by Stephen Quirk (Kimberly Clark), who developed a method (LUTI) for screening random peptides libraries and selecting high-affinity binders, usable for diagnosis or immunostaining applications. Paul Watts (Phylogica) developed a yeast two-hybrid screen of phylomer (peptides from parts of biodiverse proteins) libraries directed against intracellular targets. Anthony Keefe (Archemix) developed Aptamers as an alternative to antibodies. These oligonucleotides bind to molecular target with high affinity and are currently used in cardiovascular and oncology programs in the clinic [7].

Altogether, this meeting highlighted that the current challenges in developing the next generation of therapeutic anti-

bodies, or antibody alternatives, are: optimizing production and stability, improving effector functions and reducing immunogenicity.

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### References

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### Hyperlinks to referenced articles:

- ↗ <http://doi.wiley.com/10.1002/biot.200600205>
- ↗ <http://dx.doi.org/10.1111/j.1467-7652.2007.00273.x>
- ↗ <http://dx.doi.org/10.1073/pnas.0609997104>
- ↗ <http://dx.doi.org/10.1038/nbt1260>
- ↗ <http://dx.doi.org/10.1038/nbt0696-726>
- ↗ <http://dx.doi.org/10.1371/journal.pbio.0050007>
- ↗ <http://dx.doi.org/10.1038/nrd1955>