

## Session 4: Advances in technology

Wednesday 12th November 2008. Moderator: Mark Glassy

[16.10–16.40]

### What is the potential for antibody fragments?

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Proteolytic and engineered Fab' fragments have been found to be invaluable as research tools in the laboratory and clinic for decades. This potential has now been extended to their successful therapeutic use, with ReoPro™ (Eli Lilly), Lucentis™ (Genentech) and Cimzia™ (UCB) approved for sale and Mycograb™ (Novartis) successfully completing phase III clinical trials. In addition to Fab', a whole spectrum of engineered antibody fragment derivatives and mimetics are being investigated as therapeutics, diagnostics and reagents due to their alternative biophysical and expression properties.

We will compare and contrast the various formats of antibody fragments with whole antibodies in terms of their current and potential end uses. This will be illustrated with special reference to UCB-Celltech's experience with production and clinical use of PEGylated Fab and F(ab)<sub>2</sub>.

[16.40–17.10]

### Capturing the natural humoral immune response by Symplex

John Haurum

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The Symplex technology captures the natural humoral immune response and enables the efficient discovery of target-specific fully human recombinant antibodies drug lead candidates.

The utility of Symplex has been verified by isolating large and diverse sets of unique, fully human antibodies from naturally immune or vaccinated donors with antibody responses against viruses and bacteria, e.g. vaccinia virus, respiratory syncytial virus (RSV), and tetanus toxoid.

The isolated antibodies are high affinity, demonstrate broad antigen and epitope recognition and have potent biological activity.

Molecular analysis of representative human Ab repertoires will be presented, as well as data demonstrating synergy of antibody mixtures against categories of antibody targets.

We present here a unique approach for the discovery of antibody-based therapeutics, which holds promise for a more efficacious therapeutic approach against infectious diseases.

[17.10–17.30]

### Anti-thrombopoietin receptor agonist antibodies improved by domain-subclass conversion

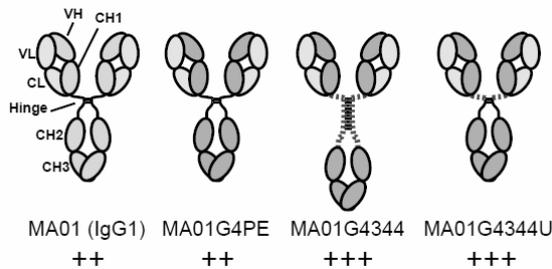
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Thrombopoietin (TPO) is a hematopoietic cytokine that regulates megakaryopoiesis and platelet production. Two types of recombinant TPO —full-length recombinant human TPO and pegylated recombinant human megakaryocyte growth and development factor (Peg-rhMGDF), a molecule consisting of the receptor-binding portion of human TPO conjugated to a polyethylene glycol moiety—have been clinically tested in a variety of thrombocytopenic disorders. However, the development was thwarted by the immunogenicity of Peg-rhMGDF. The problem induced attempts at clinical development of TPO-mimetic molecules. Here we report the generation of novel anti-c-Mpl agonist antibodies whose activity was enhanced by switching the subclass of their constant region. Our results suggest the importance of the hinge region in modulating agonist activity. The antibodies showed TPO-like activity *in vitro* and *in vivo*, as well as a good pharmacokinetic profile based on the whole-IgG structure, and thus may provide a new medical option for the treatment of thrombocytopenia.

## References

- [1] M. Kai et al., Switching constant domains enhances agonist activities of antibodies to a thrombopoietin receptor, *Nat Biotechnol* **26**(2) (Feb. 2008) 209–211.



[17.30–17.50]

### Plant farming of antifungal recombinant antibodies

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Infections by opportunistic fungal agents, in particular candidiasis and aspergillosis, cause a high morbidity and mortality in immunodepressed and hospitalized patients. In a recent work [1] a specific anti- $\beta$ -glucan monoclonal antibody was generated in vaccinated mice. This mAb, named 2G8, bound to the cell wall polysaccharide of *Candida albicans* hyphae inhibiting their growth *in vitro* and exerting *in vivo* a protective effect in models of systemic and vaginal infections.

The variable regions, VH and VL, genes of this murine antibody have been cloned from the cDNA of mAb2G8 expressing hybridoma and engineered to obtain novel anti- $\beta$ -glucan antibodies in different formats:

- i) a simple scFv antibody (fusing the variable domains through a linker peptide);
- ii) a scFv-Fc minibody (fusing the scFv 2G8 to a human IgG1 hinge, CH2, CH3 domains in a single protein able to dimerize in a complete antibody);
- iii) a human chimeric antibody (fusing murine VH and VL genes to CH and CL genes of a human IgG1).

In order to exploit the potential of plants as ideal cost-effective expression system, the two full-length

antibodies (human chimeric and scFv-Fc) have been transiently expressed in *Nicotiana benthamiana* plants by the vacuum-agroinfiltration technique. Western blot analysis of agroinfiltrated plant extracts revealed high expression levels of both chimeric light and heavy chains and of scFv-Fc. The full antibody and the scFv-Fc were purified from leaves by protein A affinity chromatography with very high yields (40 and 50 mg/kg of plant tissue, respectively). The correct assembly of both purified antibodies was evaluated by gel filtration and non reducing SDS-PAGE analysis. The binding specificity of purified human chimeric IgG and scFv-Fc was assayed by ELISA. Moreover, both antibodies bind *C. albicans* and *A. fumigatus* hyphae in immunofluorescence and ELISA assays and inhibit growth of *C. albicans* *in vitro*. In addition, the scFv format expressed only in *E. coli* periplasm and purified by IMAC, retained the binding specificity of 2G8 mAb in both immunofluorescence and ELISA assays.

In conclusion, we have generated a panel of recombinant antibodies, derived from a murine protective anti- $\beta$ -glucan monoclonal antibody. All recombinant antibody formats retain binding specificity of the original mAb therefore could represent valuable tools for both diagnostics and immunoprophylaxis/therapy of human fungal diseases. The high yield obtained in plants confirm the notion that plants represent ultimate bioreactors for the expression of fully functional monoclonal antibodies.

## References

- [1] Torosantucci et al., A novel glycoconjugate vaccine against fungal pathogens, *J Exp Med* **202** (2005), 597–606.

[17.50–18.10]

### The humanization of pharmaceutical glycoproteins using targeted expression or trapping in the plant secretory pathway

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While N-glycan synthesis in the endoplasmic reticulum (ER) is conserved in plants and mammals, N-glycan maturation in the Golgi apparatus is plant-specific and results in different oligosaccharide structures attached to glycoproteins in plants and mammals. In the prospect of using plants as alternative hosts to mammalian cell lines for production of therapeutic glycoproteins, significant progresses have been

made towards humanization of protein N-glycosylation in plants.

To date, most efforts to humanize the N-glycosylation pathway in plants have focused on the knock out of genes that code for plant-specific glycosyltransferases together with the knock in of genes catalyzing

the synthesis, transport and addition of human sugars.

We are currently taking advantage of a large panel of signals available for targeting of proteins in the membranes of the plant secretory pathway to engineer N-glycosylation of plant-made pharmaceuticals.