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A recombinant antibody-enzyme fusion protein produced in *Pichia pastoris* in compliance with EU Clinical Trials Directive for Phase I/II cancer trials

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Recombinant proteins have great potential as new cancer treatments. To evaluate these molecules in Phase I/II clinical trials they must be manufactured to standards of Good Manufacturing Practice (GMP) in accordance with the 2004 EU Clinical Trials Directive¹. However, the prospect of GMP production for unique and complex recombinant molecules can deter clinical researchers from bringing these new treatments to the clinic.

To address this we have developed a GMP production facility and procedure for recombinant antibody-based bio-therapeutics using a yeast, *Pichia pastoris*, expression system. The procedure interfaces *P. pastoris* fermentation and soluble expression of recombinant proteins with capture of secreted hexa-histidine-tagged proteins directly from fermentation broth using expanded bed adsorption immobilized metal affinity chromatography. Clinical grade material is obtained after size exclusion chromatography and endotoxin removal.

The production procedure has been successful in generating a complex, dimeric, single chain Fv antibody-enzyme fusion protein for use in antibody directed enzyme prodrug therapy (ADEPT). The recombinant product has reproducible post translational mannosylation for favourable pharmacokinetics^{2,3} and is stable upon storage, enzymatically active and reactive with its target carcinoembryonic antigen. The product has been used safely in patients³.

The presented data demonstrate feasibility of small-scale production of new recombinant anti-cancer biologicals for testing in Phase I/II clinical trials.

References: ¹Directive 2005/28/EC, Official Journal L 91, 09/04/2005 P0013-19; ²Sharma et al 2005, Clin Cancer Res **11**:814 ; ³Mayer et al 2004, British J Cancer **91**:S8.

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