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Developing a Manufacturing Process and Analyses for a Recombinant Protein Drug

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A recombinant Fab-superantigen fusion protein intended for cancer therapy will be used as a case study. The concept of using Fab-superantigens in cancer therapy is owned by Active Biotech, Lund, Sweden, and the company is today running clinical trials phase 2 with one of its constructs, while another will enter into phase 1 within short. The recombinant protein development from gene to active pharmaceutical ingredient has been performed in Stockholm by a group at Biovitrum (or earlier, by its predecessors within Pharmacia).

The protein is approx. 75 kDa and contains the Fab part of an antibody with one of the chains linked to a superantigen, Staphylococcal Enterotoxin A (SEA). The two parts of the protein excise targeting and extensive immunostimulation, respectively, thereby inducing tumor cell death. The protein is expressed in *E. coli* where it is secreted to the medium. A manufacturing process, including cultivation for approximately 80 hours with induction of protein expression after around 60 hours and a down-stream purification containing three chromatographic steps followed by a buffer exchange step, has been developed. The active pharmaceutical ingredient is thereafter collected as a purified bulk solution which can be stored at -70°C for several years.

The protein has been characterized thoroughly with a number of experimental techniques. Peptide map has been developed as the primary technique to characterize various protein modifications and also to include in the spec for identification. Biological activity has been monitored by SADCC (Superantigen mAb Dependent Cell-mediated Cytotoxicity). Aggregates and fragments have been studied by SDS-PAGE and size exclusion chromatography. The number and positions of the S-S bridges have e.g. been studied with the help of the peptide map. Free chains—where the S-S bridge keeping the Fab together is missing—have been monitored by reversed-phase HPLC. An extensive characterization regarding the presence of oxidized methionines has been undertaken. For this, protein structure investigations and molecular modeling have proven to

be forceful tools. Charge isomers have been studied with ion exchange chromatography and isoelectric focusing. This latter study resulted in the identification of one isoform which had a significantly decreased biological activity, but which was present to a too large extent. Accordingly, there was a need to improve the manufacturing process in order to diminish the amount of this protein variant. After this process improvement essentially no aggregates or hydrophobic variants were detected. Further, isoforms other than the main one were found to less than 5% and were equally as biologically active as the main component.

The characterization efforts performed illustrate the need to extensively characterize recombinant proteins. Many forms will always be found, which will result in specifications containing long lists of analyses to be executed. The knowledge about variants and their implications on the design of the manufacturing process is crucial.

Discussion

Chapman: I think this was a very useful presentation. Could you give us some idea of the time frame that all this work involved?

Florin-Robertsson: About 18 months were needed for phase I development and actually not very much more for phase II. It was a rather quick job because we had done the basic work rather extensively. And one of the reasons I briefly mentioned was that from the beginning we had the intention to make more than one construct with a similar design. So the others are actually genetically modified variants of this one.

Chapman: One of the things that has come up with the CREATE project is the use of affinity chromatography as part of the purification strategy. In most of our cases that involves using monoclonal antibodies. It was interesting to see that you use Protein G which essentially has the same problem. How do you handle that in terms of impurities?

Florin-Robertsson: Do you mean remaining human or animal protein in the Protein G, or remaining Protein G in the purified protein? Or both? To start with, the question whether you have contaminations of animal or human origin in your Protein G process was asked to us by the FDA this spring. So we have gone through that and I have presumed that Pharmacia, as the most common producer of Protein G affinity gels, uses human IgG for purification of the protein G. This is something we had to tell the FDA, but we have not got the reaction yet. I am very curious because Protein G is commonly used today, so if they say no, they actually say no to a number of products which they already have approved. If you look at the specification you see that we check the amount of Protein G after the process. We have found that there is no problem. The amount is really very, very, very low. One thing we learnt from the Protein G gel we have used is that when you have a very new gel and you change batches, you have not only to wash it with ordinary buffers, but you have to run a protein through it in order to remove excess Protein G. After that, it releases very, very little Protein G.

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Engineering a Vaccine for Allergen

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Current knowledge of allergic sensitization is mediated by the immune system when exposed to antigens under certain conditions. Based on the knowledge that allergens are often of natural source extract origin, it is assumed that vaccines should be based on major allergens in order to induce a specific allergic response.

Vaccines based on allergens can induce allergic responses in some individuals due to the inherent ability of the immune system to react mainly through the humoral immune system located on the allergen. The humoral immune system, T cell epitopes, can induce a reaction, without the need for adjuvants, in asthmatic subjects [2].

Genetic and protein engineering can improve the safety and efficacy of allergenicity, for a range of allergens by genetic engineering. The use of IgE-mediated antigenic epitopes of IgE-binding also

Specific IgE epitopes can be used to identify allergens and can be effective in the treatment of allergic diseases by denaturing and urea-denatured ragw