ANALYSIS OF PRODUCTION BOTTLENECKS IN BITE® MOLECULES PRODUCING CHO CELLS

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Antibodies are very potent biopharmaceuticals used to treat severe diseases such as cancer. During the past decade, more complex antibody formats were developed including special bi-specific T cell-recruiting molecules developed by Amgen. These bispecific T-cell engager (BiTE®) molecules possess two different binding sites and function as adapter molecules between T- and tumor cells by binding both cell types (Figure 1). Through this linkage, T-cells are able to recognize tumor cells and destroy them. However, many complex and non-natural antibody formats oftentimes are difficult-to-express using mammalian cell culture, including Chinese Hamster Ovary (CHO) cells, the workhorse of the pharmaceutical industry. Even when using the same expression platform, titers of BiTE or dual BiTE (dBiTE™) molecules producing cell lines are drastically lower compared to a monoclonal antibody (mAb) producing cell line (Figure 2). The aim of the project was the analysis of CHO cell lines producing various difficult-to-express BiTE molecules with low titers and to identify potential organelle or molecular production bottlenecks.

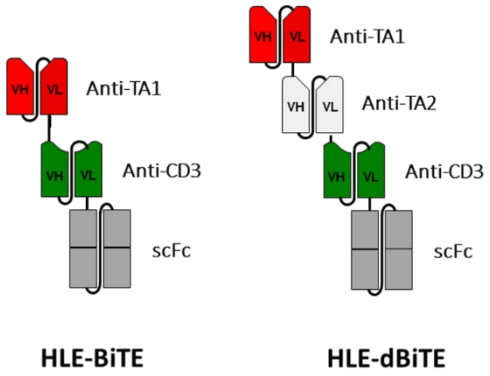

Figure 1: Visualization of different half-life extended (HLE) BiTE formats containing anti-tumor antigen (TA), anti-cluster of differentiation 3 (CD3) and single-chain variable (scFc) domains.

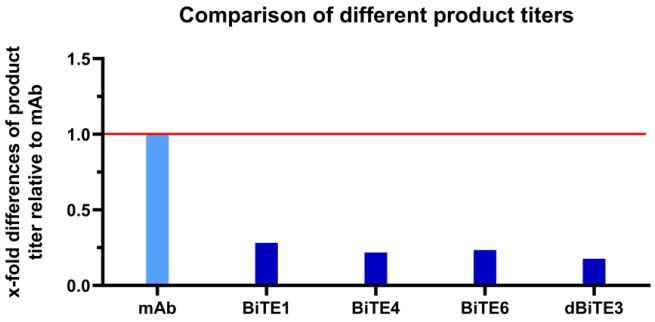

Figure 2: Comparison of product titers of CHO cells expressing different BiTE molecules in comparison to a mAb expressing cell line. Differences are visualized as fold changes relative to mAb.

Microscopic analysis of production organelles of BiTE and dBiTE molecules expressing CHO cell lines revealed consistent co-expression of recombinant protein and endoplasmatic reticulum as well as Golgi apparatus. In addition, no distinctive organelle morphologies such as e.g. Russell bodies were observed. Therefore, no cellular production bottleneck based on inhibiting organelles was identified. Assessing translation of BiTE and dBiTE molecules, Western blot experiments showed a largely reduced intracellular protein amount in BiTE and dBiTE molecules expressing cell lines when compared to mAb expressing cell lines. Since translation may be restricted by limited availability of mRNA transcripts, we performed qPCR analysis. Here, a significantly lower mRNA abundance for BiTE and dBiTE molecules expressing cell lines was observed. Focusing on identifying the root cause for this observed low mRNA abundance, current evaluations include assessment of transcription rate via *in vitro* transcription analysis, RNA stability via Actinomycin D assay and the potential influence of transcript length on transcription.

As conclusion, we suggest so far, that folding or intracellular aggregation of those artificial multispecific modalities are not necessarily the cause for reduced product titers. The rate limiting step might rather be located on mRNA-level for BiTE and dBiTE molecules production, which provides novel insight into potential bottlenecks of difficult-to-express multispecific formats. Our analyses may lead to further optimization strategies to overcome manufacturability challenges associated with various complex therapeutics.