simAbs

White paper

The simAbs continuous production platform ensures high product quality and biological activity of biotherapeutics.

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Abstract

Alterations in the production process of biologicals can have a noticeable effect on their structure and activity. At simAbs, we designed a manufacturing process that consistently produces high-quality product, fulfilling the established quality target product profile. In this white paper, we demonstrate the comparability of a monoclonal antibody produced by continuous manufacturing with its originator molecule. This side-by-side similarity assessment focusses on product quality and biological activity.

Introduction

The manufacturing of biotherapeutics, including monoclonal antibodies (mAbs), is a very complex process and is associated with high production costs. Over the past decade, the pharmaceutical industry has been confronted with an increasing demand for the production of highly advanced biologicals to successfully treat the devastating diseases we are currently facing. These highly expensive treatments have not only become a burden on the patients, but also on the healthcare system. Finding a more cost efficient production platform is therefore needed to broaden current production capabilities but more importantly, to reduce the total production costs[1].

The structure and biological activity of mAbs, as well as other biologicals, is highly sensitive to subtle changes in the manufacturing conditions. Therefore, it is of utmost importance to prove the robustness and consistency of the manufactured product. In order to obtain this crucial information, we performed a case study for the commercially available Trastuzumab (Herceptin®) where we assessed the quality profile of the product manufactured using our continuous production platform and benchmarked this to the originator molecule.

Materials and Method

Continuous manufacturing

A stable CHO suspension cell line was developed for the production of Trastuzumab by making use of multiple transduction rounds in combination with single cell expansion. More specifically, a codon optimized vector was generated and used for the lentiviral transduction of a CHO suspension cell line. After passing internal quality control checks, a seed train using this stable cell line was initiated for the inoculation of a perfusion bioreactor. A constant viable cell density of $25x10^6$ cells/ml was maintained throughout the run at a perfusion rate of 1.5 vessel volume per day. The expression vector was designed in such a way that the produced mAbs were secreted in the culture media. To separate the mAbs from the cells, an ATF (Alternating Tangential Flow) cell retention system was used. Further purification was performed by protein A chromatography followed by viral inactivation, ion exchange chromatography, viral filtration, tangential flow filtration and a final sterile filtration (**Figure 1**).

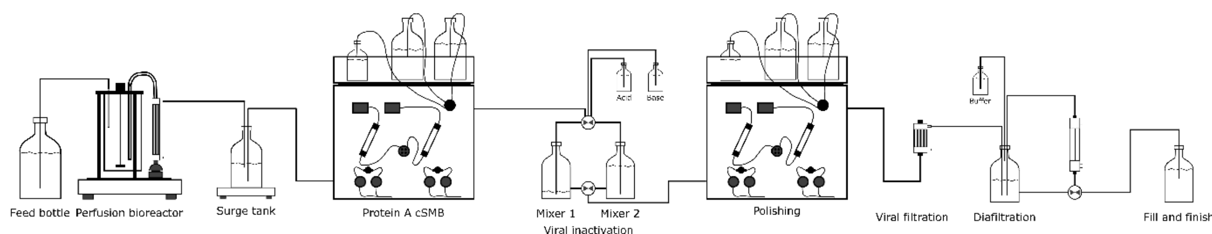

Figure 1. Overview of all unit operations composing simAbs continuous manufacturing platform.

Product quality assessment

Product purity and quality of the generated mAb were assessed using a panel of complementary tests and compared to the originator molecule Herceptin (Roche). Antibody integrity and purity was checked under reducing and non-reducing conditions using SDS-PAGE and SEC-HPLC respectively. Biological activity was determined by employing an antigen binding assay and a cell proliferation assay.

SDS-PAGE

Samples were prepared by the supplementation of a reducing agent that breaks the disulfide bridges, resulting in the separation of the light and heavy chains of the antibody. This way, shifts in molecular weight and product fragmentation can be assessed using SDS-PAGE. In addition, this assay can also detect sample impurities and elucidate their size.

SEC-HPLC

Sample purity, protein aggregation and protein fragmentation under native conditions were studied using size-exclusion chromatography (SEC)-HPLC. SEC is a chromatographic method that acts as a sieve to separate molecules in solution based on size. Especially for protein-based therapies, the quantitation of low- and high molecular weight side products is of particular concern, given their potential effect on efficacy and immunogenicity.

HER2 binding assay

The binding affinity of the antibody to its antigen was assessed using ELISA. Plates were coated with a recombinant HER2/ErbB2 protein, a receptor that is typically overexpressed in breast cancer tissue. After sample addition, binding affinity was determined by quantifying the amount of bound antibody using a detection IgG coupled to the Horseradish Peroxidase (HRP) enzyme. Read out and signal quantifications were done using a chromogenic substrate.

Cell proliferation assay

This assay has been developed to assess the antiproliferative effect of anti-HER2 monoclonal antibodies on human breast cancer cells. The extent of decelerated or blocked cell proliferation after antibody treatment is a direct indication of its biological activity. Breast cancer cells (BT474) were incubated for five days with an anti-HER2 antibody and the status of cell proliferation was determined using a tetrazolium salt to quantify the number of living cells.

Results

Perfusion bioreactor

A perfusion culture of five weeks was run in a single-use bioreactor with a working volume of one litre. During this time course, we were able to maintain a constant cell viability between 90 and 100% and targeted a viable cell concentration of around $25x10^6$ cells/ml. This was achieved by performing daily bioreactor bleedings, as can be appreciated by the jigsaw pattern in the VCD trend line (**Figure 2**). Product material was constantly harvested from the bioreactor using the ATF system that was equipped with 0.2µm hollow fibres. Such a pore size enables us to separate the produced mAbs and spent media from the producing cells, increasing cell longevity and mAb recovery. This way, cells are retained in the bioreactor and mAbs can be translocated to the first chromatography step.

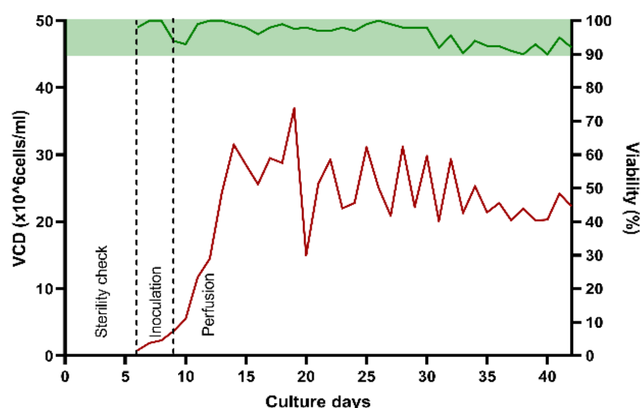

Figure 2. The continuous perfusion run where a constant VCD of approximately 20-30x10^6 cells/ml and a minimal viability of 90% (green highlighted box) was maintained along the run.

Continuous chromatography

As fresh feed media is constantly being added to the bioreactor, there is also a constant retrieval of spent media containing the mAb of interest. A first chromatography step was performed using protein A columns to capture and purify the antibody. By implementing the cSMB technique (continuous Simulated Moving Bed), a maximum utilization of the protein A resin capacity was achieved. This was done by coupling two identical columns in series, enabling a higher productivity (2- to 3-fold) and a reduced buffer consumption. Additional purification steps were positioned in-line of the continuous manufacturing platform such as viral inactivation (a two-vessel approach using a low-pH hold step), ion exchange chromatography, viral filtration, tangential flow filtration (TFF) and sterile filtration.

Evaluating Trastuzumab product quality

Purity

The performance of our continuous production platform was assessed by the manufacturing of the biosimilar Trastuzumab (Herceptin®), a commercially available monoclonal antibody used for the treatment of breast cancer. Along the 5-week-during production run, samples were collected weekly to assess both product purity and biological activity. For all five samples, the results obtained by SDS-PAGE show a comparable migration pattern compared to commercially available Herceptin, which operates here, but also in all our following analyses, as a benchmark molecule (**Figure 3**). This indicates that no aggregates nor fragments were generated during the cultivation of the CHO-S cells in the perfusion bioreactor. In addition, a high-level of product

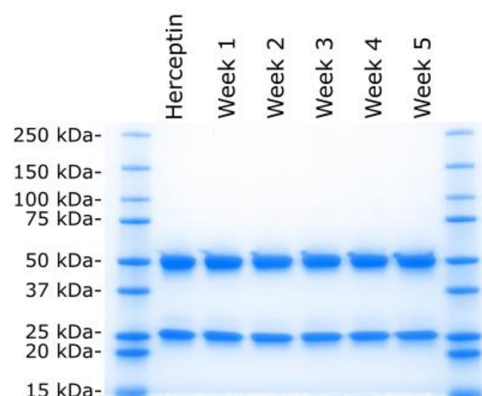

Figure 3. SDS-PAGE performed under reducing conditions showing the separation of the antibody light chain (25kDa) and heavy chain (50kDa).

purity was obtained when performing SEC-HPLC analyses on the final product (**Figure 4**). Sample purities ranged from 98.92 to 100% over the time course of the continuous manufacturing run, emphasizing the consistency in product quality during the production process. The biosimilar manufactured with our continuous platform even outperforms the benchmark molecule Herceptin that had a purity of 96.66%.

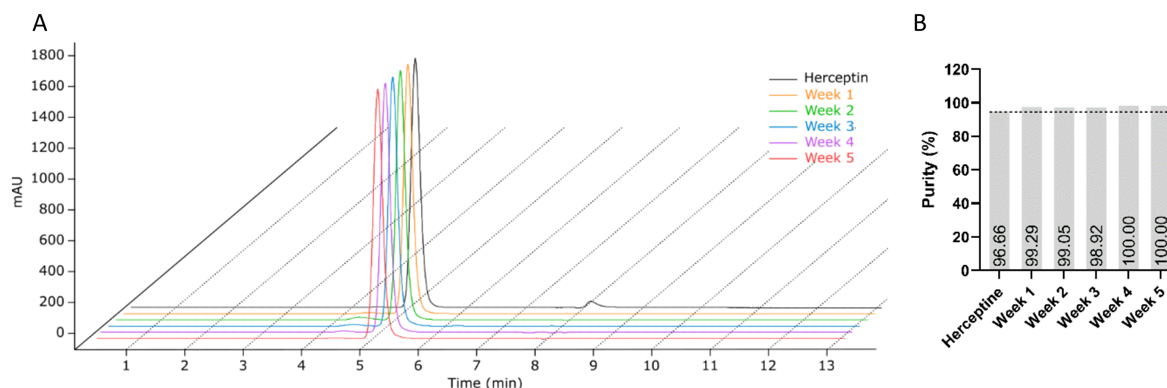

Figure 4. Product purity percentages determined by SEC-HPLC. Profiles for Herceptin and weekly samples are shown overlayed (**A**). Purity was calculated based on the area under the curve of each profile. The dotted line represents the purity of Herceptin (**B**).

Binding affinity

Besides sample purity, we also investigated the biological activity of the generated drug substance. Equally important to purity is the ability of the antibody to specifically bind the desired antigen or epitope. To determine the binding efficiency, samples were analysed using an ELISA-based antigen binding assay where the HER2/ErbB2 receptor protein was used as a target antigen. As can be seen from **Figure 5**, the binding of all five samples obtained during the continuous run showed an equal binding affinity to the HER2 antigen as compared to the originator molecule Herceptin. For all concentrations tested, no clear differences could be observed, indicating that the binding capabilities of the Herceptin originator molecule and the manufactured Trastuzumab are not different from each other.

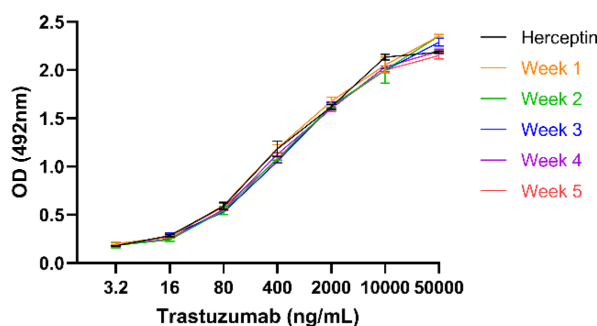

Figure 5. HER2 binding assay performed on a Herceptin sample and five Trastuzumab samples produced using a continuous production platform. Error bars represent the standard deviation from three technical replicates.

Biological activity

Herceptin has been developed as first in class HER2-targeted therapy for breast cancer. When Herceptin binds to the HER2 receptors on the surface of cancer cells, the proliferation of these cells is diminished and the cancer tissue is flagged for removal by the patient's immune system[2]. The ductal carcinoma cell line BT474 has been reported to overexpress the HER2/ErbB2 receptors and is therefore sensitive to HER2 therapeutic antibodies such as Trastuzumab[3]. Here, we performed a cell proliferation assay using BT474 cells treated with increasing concentrations of Trastuzumab. As depicted in the graphs below, the product generated using our continuous manufacturing platform showed similar responses to the originator molecule for all concentrations tested. No statistical differences could be detected when performing a paired student's t-test on the obtained dataset. All

together, these data show that there is no difference between the generated drug substance and the originator molecule Herceptin (**Figure 6**).

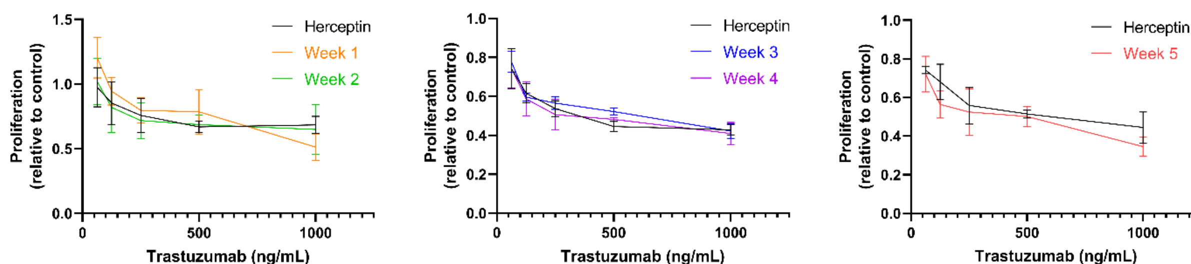

Figure 6. Proliferation assay performed using the breast cancer cell line BT474. Different concentrations were used for the originator molecule Herceptin and the different samples obtained during the continuous production process. Error bars represent the standard deviation from three technical replicates.

Conclusion

In this case study, we ran a continuous production process for five weeks during which the produced antibody was directly harvested and processed by a series of multiple purification steps. By applying a constant feed of fresh culture media, a stable CHO suspension culture was maintained, characterized by a high cell viability and stable cell density. This way, we were able to achieve a consistent product quality where purity and biological activity were indistinguishable from each other when comparing early and late product material. In all analyses performed, the antibody generated using the continuous platform had a comparable quality profile in terms of activity and purity during the entire production run. When looking at purity, there was even an increase compared to the originator molecule. We can conclude that our multi-step production line is able to meet the current standards in bioprocessing. We have created a continuous manufacturing platform that offers increased flexibility and consistent product quality while still maintaining very high yield and product purity.

About the simAbs team

The simAbs team consists of knowledgeable process engineers that are combining their complementary expertise in bioprocessing to develop a cost-efficient continuous manufacturing platform. We are bringing together a variety of expertise covering all aspects of the antibody production chain. Our ingenuity is constantly taken to the next level by collaborating with leading knowledge institutes in Flanders and seasoned product and software developers. Meet our experienced team at www.simabs.com.

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More information ?

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