

Development of a Recombinant Trastuzumab Biosimilar Targeting HER-2 Receptor

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Abstract

Cancer incidence is rising rapidly, with over 18 million new cases and 10 million deaths reported in 2020. By 2040, global cases are projected to reach 29.9 million, with 15.3 million deaths. Among all cancers, breast cancer is the most prevalent, with 2.2 million new cases worldwide in 2020. In Uzbekistan, 5,022 breast cancer cases were diagnosed in 2022, accounting for 14% of all cancers and nearly a quarter of those in women. In many cancers, amplification of the HER-2 gene leads to an overexpression of its receptor, driving uncontrolled cell proliferation. HER-2 overexpression is observed in 20-25% of breast cancer cases, leading to excessive epidermal growth factor activity and aggressive tumor progression. Monoclonal antibodies targeting HER-2, such as trastuzumab, have significantly improved treatment outcomes. However, their high cost and increasing demand in Uzbekistan have led to shortages, limiting accessibility. To address this challenge, we aimed to develop a cost-effective local alternative to trastuzumab. To achieve this, trastuzumab analogs were analyzed, and the gene encoding the humanized trastuzumab protein sequence was codon-optimized for expression in CHO cells. A virtual construct was designed using the pVITRO1 plasmid, incorporating essential elements for the light and heavy chain coding sequences. The trastuzumab heavy chain gene was engineered with an IgE signal peptide and cloned under the mEF1 promoter, including the mEF1-1a intron, while the light chain was cloned under the rEF1 promoter with the same IgE signal peptide. The construct was introduced into *E. coli*, where positive clones were selected and verified for correct gene placement. After confirmation, the plasmid was transfected into CHO cells for transient expression. The molecular mass of the expressed protein was analyzed via native and denaturing gel electrophoresis, and initial purification was performed using anion and cation exchange chromatography. The target protein exhibited low synthesis levels during the first five days, followed by a significant increase from day six onward. Maximum protein production was observed between days 7-9. The molecular mass of the native protein was confirmed to be approximately 150 kDa, while under denaturing conditions, it separated into two fragments of 50 kDa and 25 kDa. Purification using anion and cation exchange chromatography achieved ~90% purity, with 75-80% of the total target protein successfully recovered. Future studies will focus on developing stable CHO cell lines expressing trastuzumab and evaluating the in vitro and in vivo efficacy of the recombinant protein.

Keywords

HER-2, Breast Cancer, Trastuzumab, Antibody