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Trastuzumab: Antineoplastic agent; a recombinant DNA-derived humanized anti-HER2 monoclonal antibody

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Abstract

Herceptin contains the active substance trastuzumab (anti-p185, rhuMab HER2), which is a humanised monoclonal antibody that binds to the HER2 protein. Trastuzumab received marketing approval from the US Food and Drug Administration Sep. 25, 1998 for the treatment of patients with metastatic breast cancer whose tumors overexpress the HER2 protein. This review describes the overview of trastuzumab including mechanism of action, indications and adverse reactions.

Keywords: Herceptin, Monoclonal Antibody, Recombinant, Quality Control

1. Introduction

HERCEPTIN (trastuzumab) is a recombinant DNA-derived humanized monoclonal antibody [2] that selectively binds with high affinity in a cell-based assay ($K_d = 5 \text{ nM}$) to the extracellular domain of the human epidermal growth factor receptor 2 protein, HER2 [3, 4]. The antibody is an IgG1 kappa that contains human framework regions with the complementarity-determining regions of a murine antibody (4D5) that binds to HER2.

HERCEPTIN (trastuzumab) is a sterile, white to pale yellow, preservative-free lyophilized powder for intravenous (IV) administration. HERCEPTIN is supplied as a lyophilized, sterile powder containing 440 mg trastuzumab per vial under vacuum. Vials of HERCEPTIN (trastuzumab) are stable at 2 °C -8 °C prior to reconstitution. A vial of HERCEPTIN reconstituted with WFI, containing 1.1% benzyl alcohol, as supplied, is stable for 28 days after reconstitution when stored refrigerated at 2 °C -8 °C, and the solution is preserved for multiple use. Any remaining multi-dose reconstituted solution should be discarded after 28 days. As per the reports there is no experience with over dosage in human clinical trials.

The USAN for recombinant humanized anti-p185HER2 monoclonal antibody (rhuMab HER2) is trastuzumab (CAS Registry Number: 180288-69-1). Trastuzumab is a highly-purified 1328 amino acid humanized monoclonal IgG1 antibody with the following structural formula [1]

Light chain amino acid sequence

DIQMTQSPSSLSASVGDRTITCRASQDVNTAVAWYQQKPG
KAPKLLIYSASFLYSGVPSRFSRSGTDFTLTISLQPEDFA
TYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK
SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTK
SFNRGEC

Heavy chain amino acid sequence

EVQLVESGGGLVQPGGSLRLSCAASGFNKDTYIHWVRQAP
GKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQ
MNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGLVTVSSA
STKGPSVFLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS
GALTSGVHTFPAVLQSSGLYSLSSVTVPPSSSLGTQTYICNV
NHKPSNTKVDKKEPKSCDKTHTCPPCPAPELLGGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV

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NKALPAPIEKTISKAKGQPREPQVYTLPLPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKLSLSLSPGK.

2. Antibody Therapy

All cells have special proteins on their surface called 'antigens'. An antibody is also a protein, one that can match with an antigen, like two pieces of a jigsaw puzzle. Each antibody will match exactly with only one antigen. Antibodies are made by the body's immune system to target antigens on cells that don't belong in us, for example antigens on viruses or bacteria that have entered the body. They are an important part of our defence against infection. Antibodies travel round the body in the bloodstream. When they match with an antigen, they stick to it (or 'bind'). They then attract other cells of the immune system that can help to destroy the infection. Cancer cells also have antigens on their surface, just like bacteria and viruses. They also don't belong in us, and their antigens can be targeted by antibodies too.

Antibody therapy means giving antibodies that have been specially made to target an antigen on a cancer cell. When this man-made antibody sticks to a cancer cell, it helps the cell to die or tells other immune system cells to destroy it. The term 'monoclonal antibodies' used. 'Monoclonal' means that all the antibodies are exactly the same, so they will stick to exactly the same antigen. Antibody therapy can be used in a number of ways, firstly on its own – the antibodies work with the body's immune system to kill cancer cells in the same way as a virus would be destroyed, secondly along with chemotherapy-the antibodies 'sensitise' the lymphoma cells, making the chemotherapy work better, thirdly to deliver other therapies-the antibody is combined with a strong chemotherapy drug to make an antibody drug conjugate (ADC).

3. Indication and Usage

3.1 Early Breast Cancer (EBC)

HERCEPTIN (trastuzumab) is indicated for the treatment of patients with early stage breast cancer with ECOG 0-1 status, whose tumours overexpress HER2,

- following surgery and after chemotherapy
- following adjuvant chemotherapy consisting of doxorubicin and cyclophosphamide, in combination with paclitaxel or docetaxel
- In combination with adjuvant chemotherapy consisting of docetaxel and carboplatin.

3.2 Metastatic Breast Cancer (MBC)

HERCEPTIN is indicated for the treatment of patients with MBC whose tumours overexpress HER2.

4. Mechanism of action

Trastuzumab is a recombinant DNA-derived humanized monoclonal antibody [2] that selectively targets the extracellular domain of the human epidermal growth factor receptor 2 protein (HER2) [3, 4]. The antibody is an IgG1 isotype that contains human framework regions with complementarity-determining regions of a murine anti-p185 HER2 antibody that binds to human HER2.

The HER2 (or c-erbB2) proto-oncogene or c-erbB2 encodes for a single transmembrane spanning, receptor-like protein of 185 kDa, which is structurally related to the epidermal

growth factor receptor [3]. HER2 protein overexpression is observed in 25%-30% of primary breast cancers [3, 5]. Studies of HER2-positivity rates in gastric cancer (GC) using immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) or chromogenic in situ hybridization (CISH) have shown that there is a broad variation of HER2-positivity ranging from 6.8% to 34.0% for IHC and 7.1% to 42.6% for FISH. A consequence of HER2 gene amplification is an increase in HER2 protein expression on the surface of these tumour cells, which results in a constitutively activated HER2 protein [6]. Studies indicate that patients whose tumours overexpress HER2 have a shortened disease-free survival compared to patients whose tumours do not overexpress HER2. HER2 protein overexpression can be determined using an immunohistochemistry-based assessment of fixed tumour blocks, ELISA techniques on tissue or serum samples or Fluorescence in Situ Hybridisation (FISH) technology [7-9]. N.B., to date, only data derived from immunohistochemistry staining is relevant to treatment with trastuzumab.

Trastuzumab has been shown, in both in vitro assays and in animals, to inhibit the proliferation of human tumour cells that overexpress HER2 [10-12].

Trastuzumab is a mediator of antibody-dependent cell-mediated cytotoxicity (ADCC) [13, 14]. In vitro, ADCC mediated by HERCEPTIN has been shown to be preferentially exerted on HER2 overexpressing cancer cells compared with cancer cells that do not overexpress HER2.

5. Production and control of active substance

The active substance trastuzumab is produced in recombinant Chinese Hamster Ovary cells using a serum free medium. The Master Cell Bank (MCB), Working Cell Bank (WCB) and End of Production Cells need to be characterised sufficiently. MCB and WCB were adapted to growth in serum free medium.

Manufacturing process of the active ingredient starts with thawing and expansion of cells from the MCB or the WCB derived from the MCB. Cells are expanded using a seed train and fermenters from 80 liters up to 12000 liters.

After harvesting different chromatographic steps are used for purification. With affinity chromatography (Protein A) unwanted protein and potential endotoxin contaminants can be removed. Cation ion exchange chromatography removes antibody aggregates and fragments and CHO impurities. Anion ion exchange chromatography is intended to separate DNA, endotoxin, and retrovirus, if present. With hydrophobic interaction chromatography antibody aggregates, fragments and CHO proteins can be removed. After formulation and filtration into freeze/thaw stainless steel tanks the formulated bulk can be stored at 2-80 °C and/or frozen and stored at -20 °C or lower until further processing to finished product takes place

6. Finished product testing

A comprehensive assay control system was developed to ensure that the product meets rigorous standards of quality and batch-to-batch consistency. The quality control of recombinant proteins requires a careful selection of multiple assays that are complementary for the evaluation of identity, purity, potency, strength, and stability. In the case of a recombinant protein such as trastuzumab, the degradation pattern is complex and no single method can address all of the modes of degradation. Thus, a series of individual assays

are used to detect subtle molecular changes. Testing for purity and molecular consistency in production of trastuzumab is primarily performed on the Bulk for Storage. This step in the process was chosen because, at this point, all protein purification operations have been completed, and one bulk, or part of it, may be combined with other bulks, or parts of other bulks, prior to production of the Final Vial. Consideration has been given to molecular characterisation information, process validation results, compendial requirements, and assay validation results in devising the control systems.

7. Adverse reactions

The following adverse reactions are

- Cardiomyopathy
- Infusion reactions
- Exacerbation of chemotherapy-induced neutropenia
- Pulmonary toxicity

The most common adverse reactions in patients receiving Herceptin in the adjuvant and metastatic breast cancer setting are fever, nausea, vomiting, infusion reactions, diarrhea, infections, increased cough, headache, fatigue, dyspnea, rash, neutropenia, anemia, and myalgia. Adverse reactions requiring interruption or discontinuation of Herceptin treatment include CHF, significant decline in left ventricular cardiac function, severe infusion reactions, and pulmonary toxicity. In the metastatic gastric cancer setting, the most common adverse reactions (> 10%) that were increased (233 > 5% difference) in the Herceptin arm as compared to the chemotherapy alone arm were neutropenia, diarrhea, fatigue, anemia, stomatitis, weight loss, upper respiratory tract infections, fever, thrombocytopenia, mucosal inflammation, nasopharyngitis, and dysgeusia. The most common adverse reactions which resulted in discontinuation of treatment on the Herceptin containing arm in the absence of disease progression were infection, diarrhea, and febrile neutropenia.

8. Immunogenicity

As with all therapeutic proteins, there is a potential for immunogenicity. Studies indicate that among 903 women with metastatic breast cancer, human anti-human antibody (HAHA) to Herceptin was detected in one patient using an enzyme-linked immunosorbent assay (ELISA). This patient did not experience an allergic reaction.

The incidence of antibody formation is highly dependent on the sensitivity and the specificity of the assay. Additionally, the observed incidence of antibody (including neutralizing antibody) positivity in an assay may be influenced by several factors including assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of the incidence of antibodies to Herceptin with the incidence of antibodies to other products may be misleading.

9. Development of monoclonal antibody

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not

otherwise produce antibody protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells^[15].

10. Conclusions

The development and introduction of recombinant monoclonal antibodies creates several opportunities and challenges. Physicians, Biopharmaceutical companies, regulatory agencies and health authorities should collaborate closely to ensure equal efficacy and safety of recombinant monoclonal antibodies that will allow the accessibility of these powerful agents to a broader number of patients in less privileged areas of our planet.

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