Antitumor Antibody Design: HER-2 as a target

Clara Pons Duran, Biomedical Sciences, Universitat Autònoma de Barcelona.

INTRODUCTION. Protein design is a broad field of molecular biology. It includes a very large set of techniques and protocols used with the same purpose: to create new and better proteins. The design and creation of new proteins rises from the need to understand the relationship between sequence, structure and function of proteins, as well as to take advantage of the many features that they can have. Antibodies are a specific type of protein on which modern medicine has keen interest. They can be used to target tumor antigens and treat cancer. Here, we will introduce a set of methodologies concerning its design and combination with other substances, using as example the tumor target HER-2.

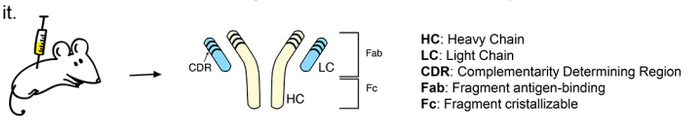
METHODS. To obtain the information, two different sources have been used:
- PubMed (NCBI): using the search criteria *protein design*, *antibody design*, *antibody HER2, HER2 breast cancer, humanized andibody, phage display, ADC, nanotechnology antibody* or *nanothechnology cancer.*
- Library of the Universitat Autònoma Barcelona: using the keywords *antibody engineering* and *antibody technology*.

From all the obtained results, the most important, useful and interesting references for our review were selected.

HER-2. The transmembrane receptor HER-2 is a proto-oncogene that belongs to the family of tyrosin-kinase receptors and subfamily of epithelial growth factor receptors (EGFR). It is an orphan receptor and therefore it has no high-affinity ligand known. However, different growth factors can activate it and, in some cases, it is constitutively activated.
HER-2 is overexpressed in different types of cancer, such as breast, ovary, endometrium, pancreas, liver and prostate cancers. It is particularly important in ductal carcinoma *in situ* breast cancer. Approximately 25% of breast cancers show overexpression of this receptor and they have bad prognosis.

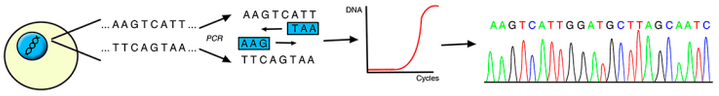
1 Antibody humanization

A. Inoculation of mice with the antigen of interest, in order to synthesize antibodies against it.

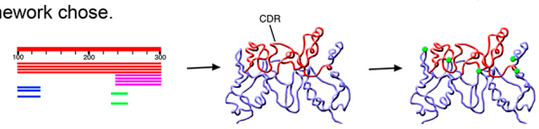


HC: Heavy Chain
LC: Light Chain
CDR: Complementarity Determining Region
Fab: Fragment antigen-binding
Fc: Fragment cristallizable

B. Cloning of the variable sequences from antibodies genes. Cloning is done in hybridoma murine cells. Using a PCR, cloned segments are amplified with the most common primers of these regions. Then, amplified fragments are sequenced.



C. Multiple sequence alignment to find those CDR with similar structures to the antibody of interest. Human frameworks with more homology are selected. Conservation of residues that maintain the structure of the CDR and exchange the others for the human framework chose.

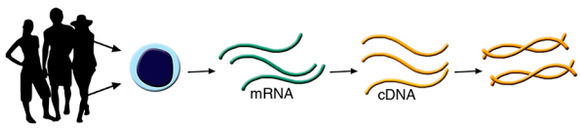


D. Isotype selection suitable for the type of therapy.

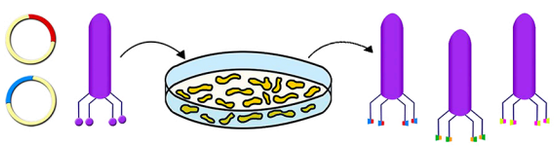
Trastuzumab was the first humanized monoclonal antibody targeting HER-2 developed. The murine antibody was humanized by *Genentech Investigators* and called Herceptin ©.
In cancers with metastatic over-expression of HER-2 and used as adjuvant of conventional chemotherapy, trastuzumab reduces recurrence in a 50% and mortality in a 30%.

2 Combinatorial libraries. Phage-display

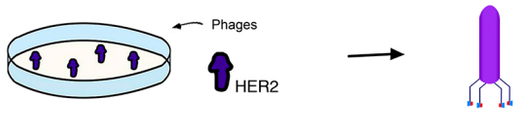
A. Collection of genetic material. Extraction of B lymphocytes from a large number of individuals and isolation of mRNA encoding variable regions of antibodies. With a reverse PCR, retrotranscription of mRNA to cDNA.



B. Creation of the phage library. Transfection of plasmids with sequences of those heavy and light chains obtained, using a helper virus. Cell culturing, obtaining multiple phages with different combinations of light and heavy chains.



C. Several rounds of selection, washing and phage elution to only keep those expressing the antigen of interest.

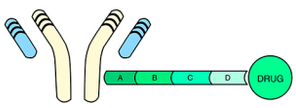


D. Purification of phage populations obtained.

E. Infection of a cell culture and production of antibodies.

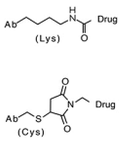
Because of the efficacy of trastuzumab, no antibody created by phage-display against HER-2 receptor has been developed. However, using this technique many groups are trying to improve the specificity of the existing antibodies and find new epitopes in order to create more efficient ones.

3 Antibody drug conjugation (ADC)

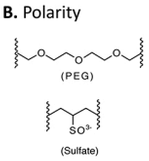


The design of the antibody follows the procedures above. First, the drug conjugate is chosen and then the linker is designed. This is the key point of the drug conjugation process. The linker is composed of four elements with a specific function:

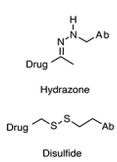
A. Connection to antibody



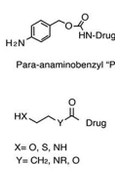
B. Polarity



C. Triger



D. Self-immolative spacer

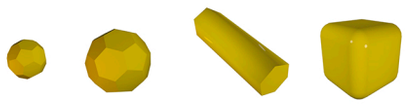


Trastuzumab emtansine (T-DM1) was the first antibody conjugated with a drug to target tumor cells with overexpression of HER-2. The chemotherapeutic drug is a derivative maytansinoid (DM1). Currently T-DM1 has passed phase I and II clinical trials and is still in study in phase II and III trials.

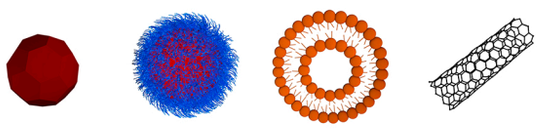
4 Conjugation with nanoparticles

Nanobiotechnology is a growing science. By conjugating antibodies with nanoparticles we can achieve effects that cannot be reached with other drugs, including better drug encapsulation, controlled release and hyperthermic effects. Some examples of nanoparticles are:

Gold nanoparticles, such as spherical nanoparticles, nanorods and nanocubes.

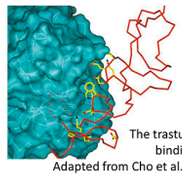


Superparamagnetic nanoparticles, polimersomes, liposomes and carbon nanotubes.



Carbon nanotubes are a type of nanoparticles with useful properties that may for the treatment of cancer. The designed antibody is bispecific for two regions of HER-2. Various anti-cancer drugs can be encapsulated inside the nanotube. Finally, the irradiation nanotubes with infrared waves or radiofrequencies may contribute to the elimination of the tumor by hyperthermia.

CONCLUSIONS.
- Little progress has been made in the techniques for design and synthesis of antibodies and the same techniques developed years ago are still in use due to their efficacy.
- The number of novel and more sophisticated conjugation procedures is increasing. Conjugation of antibodies with new drugs using different linkers combined with several types of nanoparticles, opens a wide range of possibilities.
- Especially, future groundbreaking advances in the field of nanobiotechnology will bring to a new level the design of conjugated antitumor antibodies.



The trastuzumab binding site.
Adapted from Cho et al. (2003)