Design and Engineering of Trastuzumab emtansine production plant

Part I: Introduction to T-DM1 production process and Upstream of monoclonal antibody Trastuzumab

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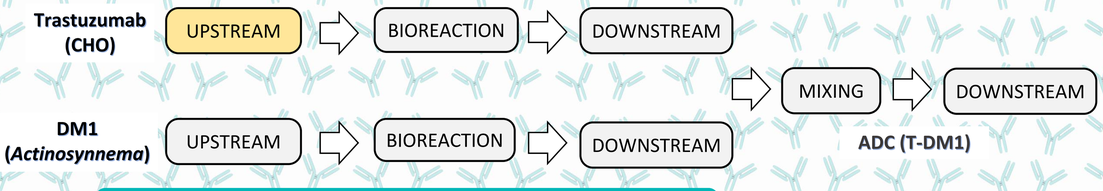
ABSTRACT

Breast cancer is the most common cancer among women following skin cancer. Specifically, HER2 overexpressing tumours reveal a particularly poor prognosis. For this reason, the aim of this project is to simulate an industrial plant for the production of the antibody drug conjugate Trastuzumab emtansine (T-DM1) which has resulted to be a successful therapy for this type of breast cancer. T-DM1 is composed by a monoclonal antibody (Trastuzumab) produced in CHO DG44 cells and a cytotoxic drug (DM1) produced in *Actinosynnema pretiosum*. Therefore, two parallel processes have been designed to produce these two components followed by a mixing step. All simulations have been done using SuperPro Designer®. This part is focused on the T-DM1's mechanism of action, the annual amount production of the plant, the block diagram and the upstream process of Trastuzumab which includes the genetic engineering as well as the clone selection and the scale-up.

INTRODUCTION

Antibody Drug Conjugates (ADC) are potent biopharmaceutical drugs that combine the selectivity of targeted treatments with the cytotoxic potency of chemotherapy drugs. In this case we design the production plant of Trastuzumab emtansine for the treatment of breast cancer HER2+ cells. This ADC is composed by:

- A highly selective monoclonal antibody (mAb) for a tumour-associated antigen → **TRASTUZUMAB**
- A potent cytotoxic agent designed to induce mitotic arrest→ **DM1**
- A **LINKER** stable in circulation that releases the cytotoxic agent in target cells



T-DM1 MECHANSIM OF ACTION

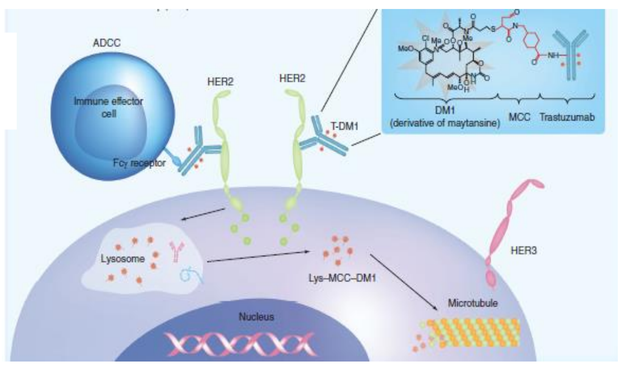
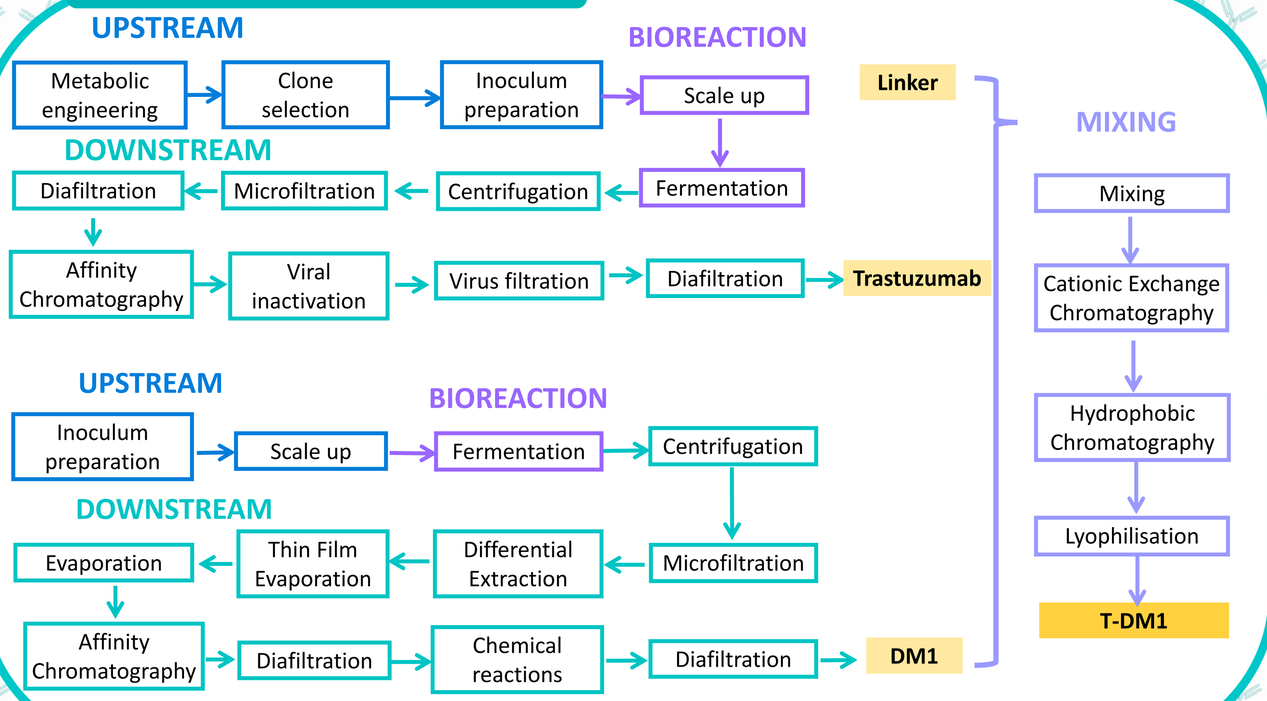


Figure 1. T-DM1 mechanism of action.

Human Epidermal Growth Factor Receptor (HER2) is overexpressed on the surface of cancer cells and receives signals that stimulate its **growth** and **proliferation.**

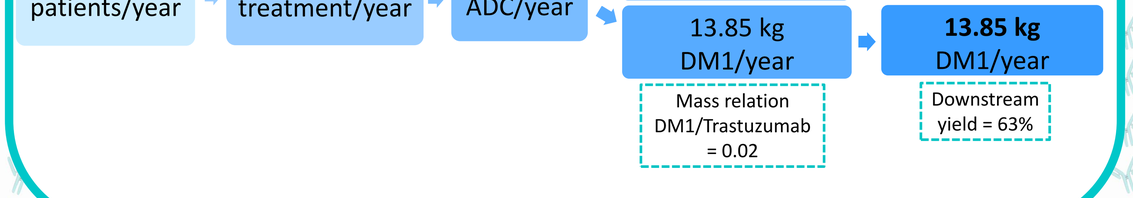
T-DM1 binds specifically to **HER2 receptor.** The complex is then endocyted into lysosome where the antibody is degraded to aminoacids level releasing the **Lys-Linker-DM1** complex.
This complex inhibits microtubule assembly, eventually causing **cell death**.
T-DM1 can be broken down inside tumour cells, but is stable enough not to release the drug into circulation.

BLOCK DIAGRAM



PRODUCTION

The objective is to treat the population from Europe and USA. Every year 6.25×10^5 new cases are diagnosed, from which the 25% that overexpress HER2 receptor can be treated with this therapy.
An average of 3.5 DM1 molecules are bound to 1 molecule of Trastuzumab.



UPSTREAM: GENETIC EGINEERING

BIOCATALYST: Why CHO cells?

1. Able to **fold** and **secrete** immunoglobulines.
2. **Glycosylation pattern** similar to humans.
3. Adaptable to growing in **suspension culture**.
4. Adaptable to growing in **chemically defined** and **serum-free** media.

CHO-DG44

Deficient in DHFR → used as selection marker

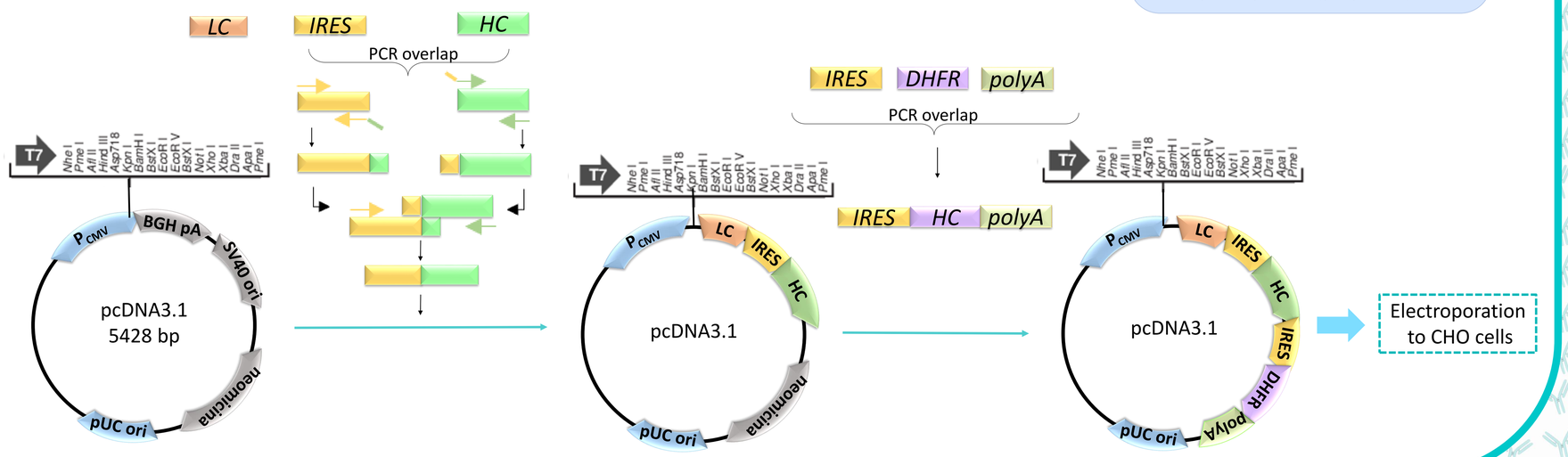
VECTOR CONSTRUCTION:

TRICISTRONIC VECTOR — LC | IRES | HC | IRES | DHFR | polyA

- Simultaneous expression of multiple genes from a single transcript.
- Same gene expression ratio.
- Reduces non-expressing clones.
- Allows faster and better stable clone selection.

Components:

- Light chain (LC)
- Heavy chain (HC)
- Internal Ribosome Entry Site (IRES)
- Dihydrofolate reductase (DHFR)
- Polyadenylation (polyA tail)



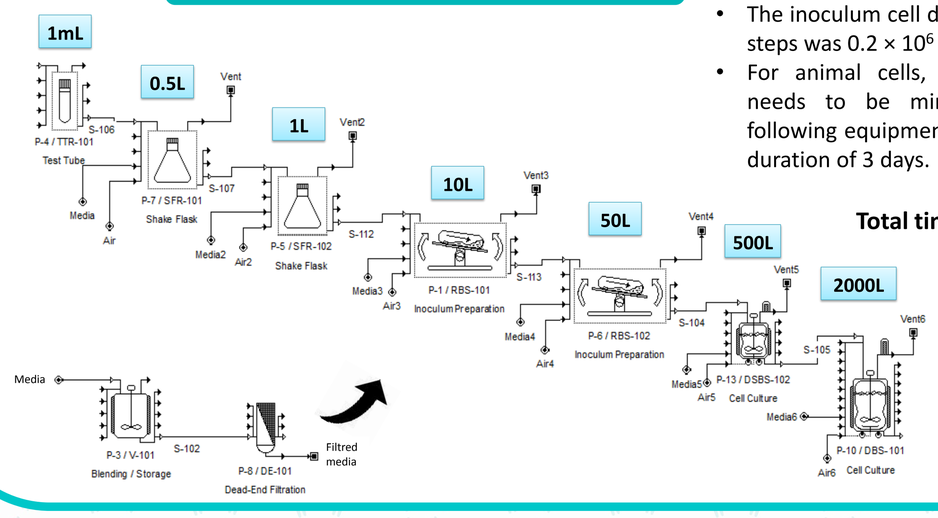
CLONE SELECTION

Maintaining consistent and comparable product quality is a challenge throughout all the production process.
In order to screen and select the stable clone with **higher productivity** and **best attributes**, different analytical assays and quality assessment criteria are employed to test antibody molecular properties.

1. ELISA and flux cytometry → 12-24 best candidates.
2. Fermentation in shake flasks → 4-6 best candidates.
3. Small-scale bioreactors → definitive clone.

Volumetric productivity
Molecule integrity
Aggregation
Glycosylation

SCALE-UP



- The inoculum cell density of all seed train steps was 0.2×10^6 cells/mL..
- For animal cells, the transfer volume needs to be minimum 10% of the following equipment and each step has a duration of 3 days.

Total time = 15 days

REFERENCES

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TRASTEM BIOTECH