Optimization of tumor cell line expansion for the production of tumor lysate-pulsed autologous dendritic cell immunotherapy

The thesis project is a collaboration between the Bioprocess Engineering (BPE) section at the Department of Biotechnology (TU Delft) and the Core Facility for Cell and Gene Therapy of the Erasmus MC.

Open per	February 2023
Duration	9 months
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1. General introduction

Within the Erasmus MC, the Core facility for Cell and Gene therapy focusses on the development, production, and quality control of Advanced Therapy Medicinal Products (ATMP). These are medicines for human use classified as either somatic cell therapy, gene therapy, or tissue engineered product. MesoPher is one of these ATMP products and is currently being evaluated as treatment option for mesothelioma (asbestos cancer) and pancreatic cancer (PDAC).

MesoPher is an autologous Dendritic Cell (DC) immunotherapeutic product with the intend to boost and re-invigorate the patient's own immune system to fight of the cancer cells. DC are the most potent professional antigen presenting cells within the human body and are experts in orchestrating (tumor) antigen specific immune responses. These responses are often suppressed and sub-optimal in cancer patients which causes tumor cells to escape the immune system and continue to grow and metastasize.

Within the MesoPher production process, the DC are loaded with tumor antigens obtained from an allogeneic whole mesothelioma tumor cell line lysate, named PheraLys. These antigens are taken-up, processed, and loaded onto the DC via which they can recognize and stimulate antigen specific lymphocytes *in vivo*.

PheraLys is produced from five different tumor cell lines derived from primary tumor material obtained from five individual mesothelioma patients. The cell lines are adherent meaning these need to adhere in order to optimally grow and proliferate. Currently, the cell lines are cultured in Cell Stack culture units. Although for the current scale this method is acceptable, these culture systems limit further scale-up of surface area. In addition, culture handlings are laborious and depend on open handling steps, increasing the risk for microbial contamination.

Therefore, we would like to optimize the tumor cell line culture expansion to allow for further scale-up and reduce labor intensity with the ultimate goal of scaling out MesoPher production (for which we need exponentially more PheraLys). Because the cell lines are adherent, we would propose to start investigating implementation of bioreactors in the culture with the addition of different kinds of carriers for the cells to grow on. Pilot experiments were previously performed and showed promising feasibility for these carriers.

Overall aim

The overall aim of this project is to identify bioreactor-based cell expansion approaches that are able to reduce the production time and efforts of the tumor-lysate material.

2. Work packages

WP 1: Project scoping (M1-M2)

In the project scoping phase you will perform a literature review on the product and production process. You will provide an overview of the found literature in the form of a literature review report (deadline: M1). In addition, you will be in the labs at Erasmus MC to understand and map out the current production process and provide a detailed overview of the current limitations. This will lead an overall experimental plan that forms the basis for the next work package WP2: Study design. This also includes making a planning until 9M.

Key deliverable

- Summary of literature review
- Overview of process data, including but not limited to: volumes, concentrations, equipment, equipment size, mode of operation, schedule
- Overview of experimental plan + 9M planning (Gantt chart)

WP 2: Study design (M3-M4)

This work package will focus on the development of an analytical workflow by identifying the required analytical techniques to evaluate the microcarrier study. This includes testing the required settings and the calculation methods to obtain comparative metrics. This work package will also include the selection of parameters that are evaluated in WP 3: Microcarrier study. This may include, but is not limited to, microcarrier type, microcarrier-to-cell ratio, static and dynamic binding conditions, and detachment protocols.

Key deliverable

- Analytical workflow
- Microcarrier study design

WP 3: Microcarrier study at EMC (M5-M6)

During the microcarrier study, the expansion of different tumor cell lines will be evaluated using the defined experimental plan from WP 2. It is envisioned that the expansion will evaluated using a scale-down model, such as 6-well plates.

Key deliverable

- Metrics on expansion performance
- Insight on impact of process parameters on tumor cell line expansion
- Proposal of expansion approach for (each individual) tumor cell line

WP 4: Alternative process design (M7)

This work package will focus on consolidating the insights obtained in WP 3 to formulate a protocol for tumor cell line expansion in a bioreactor. This may include comparing cultivation operations (batch, fed-batch, perfusion), harvesting techniques, and/or simulations to assess the impact of suspended cultivation in terms of shear stress and oxygen/nutrient requirements.

Key deliverable

- Expansion step design with scale-up potential

3. Proposed time line

Months / Activity	M1	M2	M3	M4	M5	M6	M7	M8
WP 1: Project scoping								
Literature review								
Training at Erasmus MC								
WP 2: Study design (at EMC)								
Analytical protocol								
Expansion protocol								
Parameter selection								
WP 3: Microcarrier study (at EMC)								
Carrier type								
Static versus dynamic								
Detachment								
WP 4: Alternative process design								
Thesis writing								

4. Overview of deliverables

MEP deliverables

- Literature report (M1)
- Experimental plan + 9M planning (M2)
- 3M Progress report (M3)
- Intermediate presentation at TU Delft (M5)
- 6M Progress report (M6)
- Written MEP thesis and final presentation (M8)

Project deliverables

- Schematic of current expansion protocol (M2)
- Experimental plan (M4)
- Insight on expansion performance (M6)
- Alternative expansions step design (M7)