Validation of a platform to develop new dendritic cell vaccine cancer immunotherapies

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Introduction

- An autologous dendritic cell (DC) / allogeneic tumor lysate vaccine developed at the Mayo Clinic and licensed to Mill Creek Life Sciences (MCLS) to treat newly diagnosed glioblastoma (GBM), was used in a phase I clinical trial (ID# NCT01957956) performed at the Mayo Clinic. The clinical outcomes showed an increase in survival rate of patients that received the DC vaccine after the conventional therapy with surgery followed by radiation plus chemotherapy.
- The cell-based cancer immunotherapy/vaccine requires processes to obtain patient's dendritic cells, prepare antigenic proteins from cGMP tumor cell lines and deliver the primed dendritic cells to patients being treated for glioblastoma (Figure 1).
- Our unique protocol using the human platelet lysate (hPL) PLTMax® that was followed in the phase I trial at the Mayo Clinic, allows establishing tumor cell lines without genetically modifying cells and with minimal genetic drift. The use of PLTMax® and allogenic tumor cell cultures has proven to be a major advantage for the generation of large amounts of rich tumor antigen libraries for DC vaccines.
- Our technology differs from other clinically used DC vaccines in three primary ways:
  - a) Highly-efficient cell line establishment: The patented method for generating tumor cell lines using PLTMax®, which closely mimics the native microenvironment of tumors and is used to reduce genetic drift and senescence.
  - b) High genetic fidelity: GBM tumor cells expanded via PLTMax® maintain genetic fidelity and TAA expression—a feat that has not been previously achieved by any other tumor cell culture method.
  - c) Reduction of treatment delays and inclusion of all patients that qualify for treatment: Because of the use of allogeneic tumor cell lines, we will be able to avoid delays in treatment caused by the time required to culture an autologous tumor cell lines, and all patients may receive treatment earlier and without exclusion.
- Recently, we developed and established the protocols to expand GBM cells in bioreactors. Through our preliminary work, we transferred the technology from a labor-intensive, small-scale approach, to an automated scalable system. The work conducted established a platform technology that we hope to adapt and apply to other types of tumor cells. Therefore, we aim to be able to generate antigen libraries specific for tumors other than GBM expanding the portfolio of cancer vaccines that we will be able to offer. Our ultimate goal is to use this platform technology to develop DC vaccines for cancers that have a poor prognosis with conventional therapies, either because they are difficult to access or because they are highly aggressive.
- Within those, we started the preliminary studies on osteosarcoma, the most common malignant form of bone cancer. It generally causes patients to die of pulmonary metastasis. If detected before the tumor has metastasized, osteosarcoma has a 75% survival at 5 years after the conventional therapy with surgery and chemotherapy. However, most osteosarcomas are high-grade tumors that grow quickly and spread before being detected, lowering patient survival to 20%.

Results I

I. Compatibility of GBM cells with microcarriers for suspension culture

We obtained GMP grade GBM cells isolated from GBM patients and grown for few passages at the IMPACT laboratory at Mayo Clinic. These cells were evaluated at Mill Creek Life Sciences for compatibility with 6 different types of microcarriers in a 2D system (6 well plates) using media supplemented with PLTMax®. Cells were monitored and imaged for 7 days to determine which microcarriers offered a good substrate for GBM cells to grow. If cells would tend to grow in the bottom of the plate and overgrow rather than using the space available in the microcarriers, the last would be considered non compatible. On the contrary, if cells would grow equally as well or better in the microcarriers than in the bottom of the plate, the microcarriers would be labeled as compatible with GBM cells. Out of the 6 microcarriers used in the study, 2 (Plastic Plus and Fact III from Sartorius) were selected as the ones with the best compatibility (Figure 2).

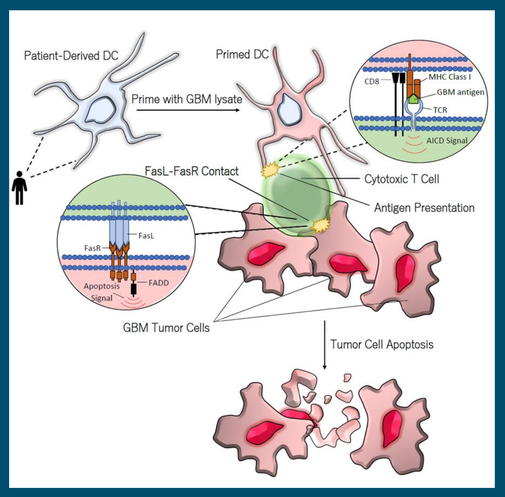

Figure 1: Therapeutic strategy of dendritic cell vaccines as a means for inducing apoptotic cell death in GBM cells.

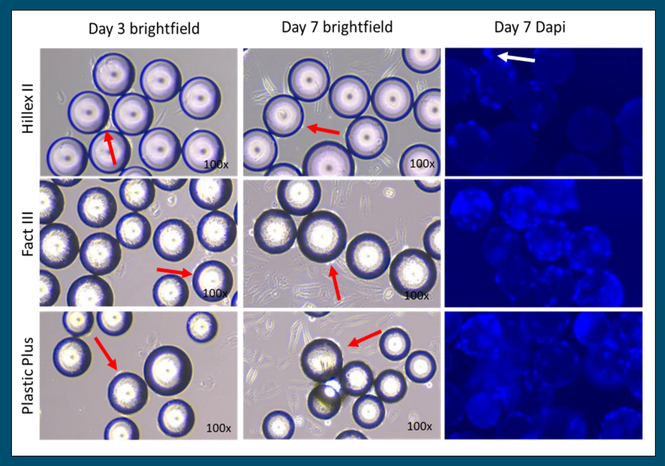

Figure 2: Imaging of cell growth using microcarriers in a 2D system. Left and middle panels: Representative brightfield images (100x magnification) of GBM cells at day 3 and day 7 of co-culture in 6 well plates with 3 types of microcarriers. Red arrows point examples of cells attached to the microcarriers. Right panels: Imaging of staining with Dapi (100x magnification) shows nuclei of live cells growing on the surface of microcarriers at day 7. White arrow shows an example of cells detaching from the microcarriers

II. Suspension culture of GBM cells using media supplemented with PLTMax®

Moving forward, and even though cell yield after growth with Fact III microcarriers was slightly better, Plastic Plus microcarriers were selected as the best choice for this technology, as they were the only ones free of xenogeneic components. We established a protocol that allowed us to obtain higher cell yields using a vertical wheel bioreactor from PBS Biotech (Figure 3A), than using the conventional cell culture in flasks used initially for the clinical trials.

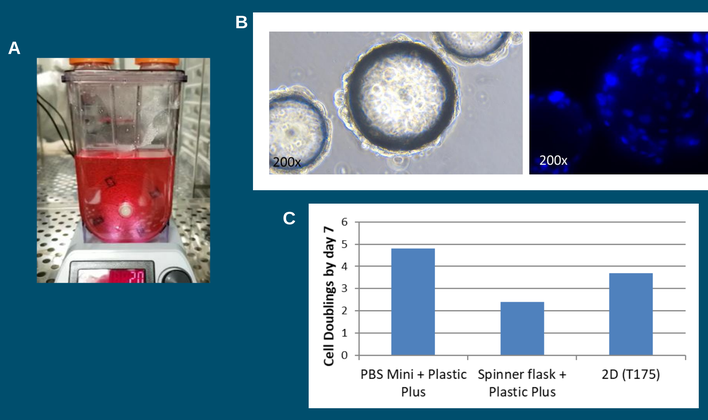

Figure 3: Expansion of GBM cells in suspension using the vertical wheel bioreactor PBS Mini. A) Image of PBS Mini with a 500mL vessel. B) Brightfield and corresponding Dapi image (200x magnification) of the same field at day 7 of culture of GBM cells in suspension in a vertical wheel bioreactor using Plastic Plus microcarriers. C) Comparison of GBM cell doublings obtained in a week using either T175 flasks, or suspension culture with Plastic Plus microcarriers in spinner flasks or PBS Mini vertical wheel bioreactors.

Results II

III. Long term expansion of GBM cells in suspension

To establish cell banks with cGMP glioblastoma cell lines obtained from cells isolated at Mayo Clinic from GBM patients, we needed to establish the best way to perform long term expansions using a bioreactor. If cells are not detached from the beads after day 7, they start overgrowing on the microcarriers and colonizing adjacent microcarriers generating clumps of beads completely surrounded by layers of cells (Figure 4 A). At this point and before day 9, if nothing is done to the culture other than replenishing medium, cells start going into senescence and dying.

To avoid overgrowth, we established a cell culture feeding protocol in which we collected half of the culture at day 6 and added new medium with microcarriers. The process was repeated at day 9. With this protocol, cells overgrowing on the microcarriers were able to contact and attach to new microcarriers to continue the expansion without forming big clumps of beads (Figure 4C) and therefore allowing for the long-term expansion, necessary for a large-scale production of cells (Figure 4 B). After the long-term culture is done, cells collected at each time point can be pooled together to generate a single batch of cells that will be used to generate antigen libraries.

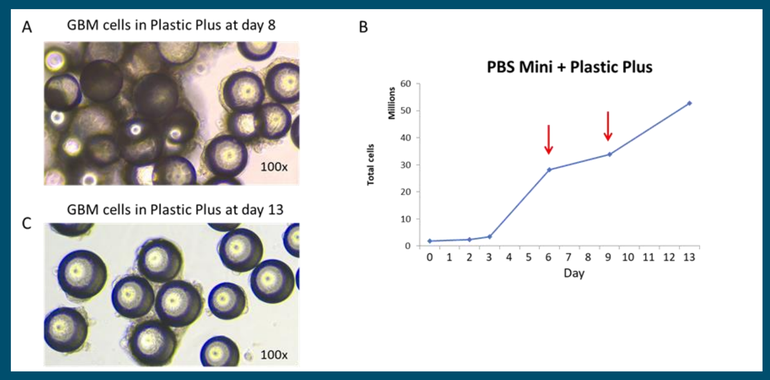

Figure 4: Long term expansion of GBM cells in suspension using the vertical wheel bioreactor PBS Mini and Plastic Plus microcarriers. A) Imaging of clumps of microcarriers with GBM cells (100x magnification) at day 8 of continuous culture in suspension without a feeding protocol. B) Total GBM cell number during the 2-week course of expansion in suspension with a feeding protocol. Red arrows mark the 2 time points in which half of the medium with microcarriers/cells was collected and the other half was kept in culture and supplemented with more media and microcarriers. C) Imaging of microcarriers with GBM cells (100x magnification) at day 13 of culture in suspension after implementing the feeding protocol.

IV. Validation of the platform technology with other tumor types

Cells from a primary malignant neoplasm of bone and articular cartilage Metastatic Ewing Sarcoma (Human Cancer Models Initiative, HCMI, ATCC) were expanded in media containing PLTMax® (Figure 4A). Cell yield after 1 week of culture was only 10% higher using PLTMax® than using fetal bovine serum (FBS). However, the difference in doubling times and cell yield (Figure 4B and C) observed in a short term experiment will exponentially increase resulting in a significant cell yield difference on the large scale expansion needed to generate antigen libraries for osteosarcoma. In addition, removing FBS and therefore all xenogeneic components from the process is necessary for the development of this technology for use in the clinic.

The use of alternative tumor cell types to validate the platform technology is in the early stages of development. Currently, we are developing the protocols for the expansion of primary osteosarcoma cells in suspension using the vertical wheel bioreactors to generate large amounts of cells. After generating osteosarcoma cell banks, we will ensure that we are able to grow and expand the tumor cells without reaching senescence and with a maximum of 10% drift in overall expression of characteristic TAAs.

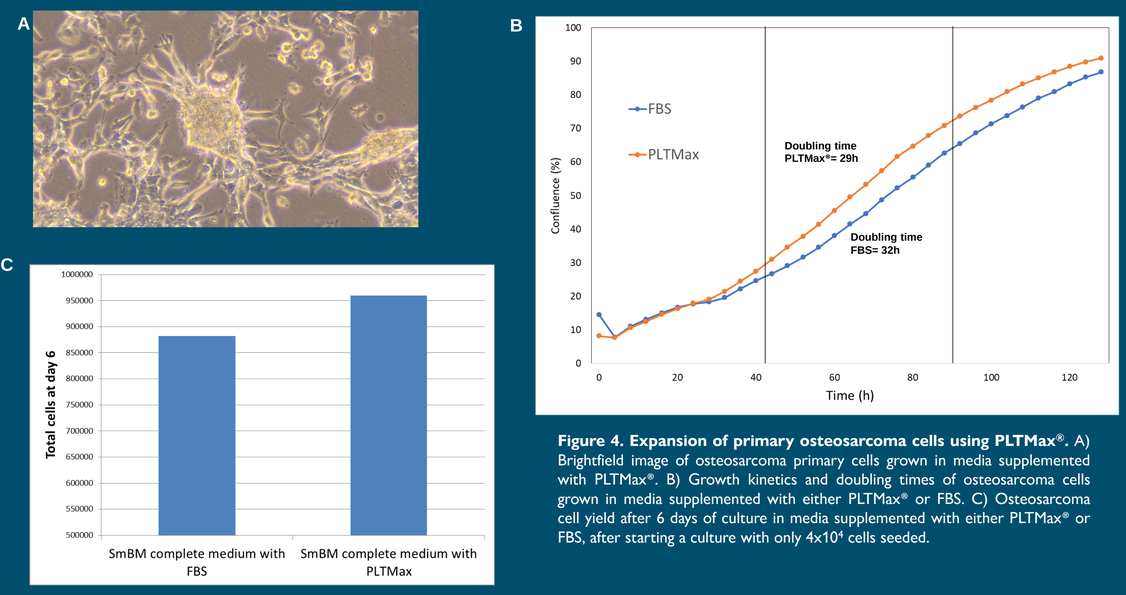

Figure 4. Expansion of primary osteosarcoma cells using PLTMax®. A) Brightfield image of osteosarcoma primary cells grown in media supplemented with PLTMax®. B) Growth kinetics and doubling times of osteosarcoma cells grown in media supplemented with either PLTMax® or FBS. C) Osteosarcoma cell yield after 6 days of culture in media supplemented with either PLTMax® or FBS, after starting a culture with only $4x10^4$ cells seeded.

Conclusions

- Using PLTMax® as a media supplement we were able to generate a protocol to expand GBM primary cells in suspension using vertical wheel bioreactors.
- Cell yield in suspension in vertical wheel bioreactors exceeded the cell yield obtained with the classical 2D system used for the clinical trials.
- Our protocol for suspension culture of GBM cells allowed us to obtain over 50 million cells from a single small scale bioreactor in less than 2 weeks, almost 50 times the original cell seeding.
- We are currently in the process of validating the use of PLTMax® for expanding cells from a variety of primary tumor types to generate a platform technology that could be used to generate antigen libraries for DC vaccines against an array of cancers that are currently refractory to conventional therapies.

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