A Novel Therapeutic Approach for Leptomeningeal Metastases


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Background

Leptomeningeal Metastases (LM) occurs when primary tumor cells metastasize, invade the subarachnoid space, and spread throughout the cerebrospinal fluid (CSF). As novel cancer therapies extend survival, LM has become increasingly prevalent, with approximately 110,000 diagnoses per year in the U.S. Despite advances in targeted radiation and chemotherapy, survival averages 3-6 months after LM diagnosis.

Neurapheresis

The Neurapheresis system is equipped with two 100 µL TFF filters. Tubing connected to the aspiration and return ports of the system are placed in the Erlenmeyer flask containing the aCSF and cells. Flow is initiated and slowly accelerated to 2 ml/min. Waste pressures are constantly monitored. Filtration continued for up to 10 hours.

Methods

Cell Harvest and Preparation

Initial stock of VX2 cells were thawed from liquid nitrogen and plated at passage 4. VX2 media Change was also changed every 48 hours until cells reach 80% confluence. Cells are passaged and harvested from 150 cm2 flasks (Corning). Appropriate volume of cells in aCSF is added to an Erlenmeyer flask to achieve 5x106 cells/mL concentration.

Filtration

The Neurapheresis system is equipped with two 100 µL TFF filters. Tubing connected to the aspiration and return ports of the system are placed in the Erlenmeyer flask containing the aCSF and cells. Flow is initiated and slowly accelerated to 2 ml/min. Waste pressures are constantly monitored. Filtration continued for up to 10 hours.

Cell Counting

100 µL samples were taken in triplicate to calculate cell concentration after each filtration cycle using a 1:1 dilution with Acridine Orange (AO) on the Cellometer Auto T4 Bright Field Cell Counter. The average of the three counts with the standard deviation was used for data presentation.

MTX Infusion and Viability Analysis

12mg of MTX was thawed and diluted in 2.76 ml of normal saline for experiments. A syringe pump infused 5 mL of MTX at 2.5 mL/min during Neurapheresis. A µL sample was taken in triplicate to calculate the average of the three counts with the standard deviation was used for data presentation.

Histopathological Analysis

Pilot studies were conducted to validate Leptomeningeal disease in vivo. New Zealand White Rabbits were inoculated using 10 expired VX2 media is added to an Erlenmeyer flask to achieve 5x106 cells/mL concentration.

Figure 1: Minnetronix Neurapheresis System overview

Figure 2: Tumor Cell Filtration

Average Concentration over Time

Filtration + MTX Infusion

Figure 3: MTX infusion in addition to filtration produced a 2.5-log reduction of VX2 cells less than half the time of filtration alone. Data in blue represents the filtration experiments with MTX infusion. In all filtration experiments, the VX2 cell concentration goes below the limit of detection after 3 cycles (2.5 hours) of filtration. Additionally, the percentage of viable cells is low. Data in black represents the MTX control, where VX2 cells were given a bolus dose of MTX, without filtration. The cell count was not reduced, nor was the viability to the same extent as with filtration. The x-axis lower limit represents the limit of detection for cell counting.

Figure 4: Tumor Cell Filtration

In vivo Studies

We will apply the results from our in vitro studies to our animal model. New Zealand White Rabbits. We will establish the disease model in a series of pilot studies, through which we will test 10-15 and 10-20 cells intracranially. Once confirmed, we aim to reduce tumor burden through filtration and simultaneous MTX infusion.

Enhanced Drug Distribution

Intrathecal Methotrexate (MTX) is commonly administered through an Omnipod which has a standard deviation was used for data presentation.

Tumor Cell Filtration

The x-axis lower limit represents the limit of detection for cell counting.

Validation of in vivo Leptomeningeal Disease model

Figure 5: VX2 cells present in the CSF.

Cytospin of VX2 cells grown in vitro and stained with Hemacolor (A). Cytospin and HNE stain of VX2 harvested from a New Zealand White Rabbit inoculated with 10 VX2 cells intracranially (B). Data in black represents the MTX control, where VX2 cells were given a bolus dose of MTX, without filtration. The cell count was not reduced, nor was the viability to the same extent as with filtration. The x-axis lower limit represents the limit of detection for cell counting.

Conclusions

• Neurapheresis is promising therapeutic approach for the treatment of LM, with the ability to significantly reduce the viable CSF tumor burden in vitro.

• The combination of filtration and administration of MTX allows for rapid reduction of tumor burden. MTX alone may allow for the accumulation of dead cells.

• The Neurapheresis procedure is an acute intervention that may potentially be used complementary to systemic chemotherapy, radiation and maintenance therapies to greatly reduce the viable CSF tumor burden in LM disease. In vivo preclinical and eventual clinical research is warranted.

References


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