Use of *Pichia pastoris* to Analyze Protein Growth and Expression in Comparison To Standard BI21(DE3) E. coli

Julian Najera, Stephanie Medina, Dr. Charlotte M. Vines, Zachary Parada and Gisel Flores

The University of Texas at El Paso, El Paso TX 79968

Background

T-Cell Acute Lymphoblastic Leukemia (acute meaning that it is aggressive and progresses quite rapidly) is the overproduction of white blood cells, particularly immature T cells which are called T-Lymphoblasts. These leukemic cells which circulate in the blood system, are potentially fatal if not treated in a timely manner. In accordance to Silvia Buonamici's article (2009), these leukemic cells can use C-C Chemokine Receptor 7 (CCR7) to direct their migration through the blood brain barrier via a process called chemotaxis. In this case cells expressing CCR7 are attracted to CC-Chemokine Ligand 19 (CCL19) which is located on the cells inside of the barrier. By making their way across the barrier, these leukemic cells can remain inaccessible to chemotherapies that are given to children, since the chemotherapy normally circulates through the blood.

For physicians to be able to reach the cells that have traveled to sites in the body, behind the blood brain barrier, many children who have T-Cell acute lymphoblastic leukemia are being treated through the use of intrathecal chemotherapy. In this mode of treatment, the protocols involves the injection of a drug directly into the spinal fluid and into central nervous system (CNS). The problem with this type of treatment is that this can lead to CNS Prophylaxis Disease, which creates long-term neurotoxicity that can lead to cognitive impairment, endocrine disorders and a short stature. Because it is difficult to for the physicians to achieve high levels of chemotherapies in the brain without too much destruction of normal tissues, radiation therapy is used as well. In this case, the physician tries to "burn" the most rapidly dividing cells to death. In the brain the idea is that the leukemic cells are the most rapidly dividing, and therefore are most susceptible to the effects of chemotherapy.

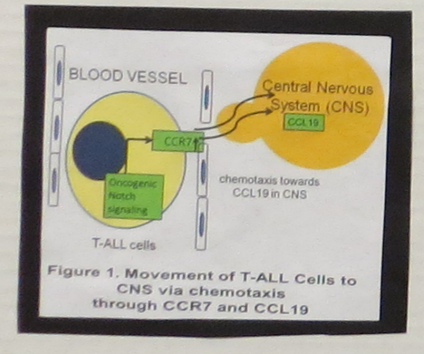

Figure 1. Movement of T-ALL Cells to CNS via chemotaxis through CCR7 and CCL19

Long term effects from this type of treatment can include developmental defects, behavior defects, headaches, cognitive impairment, stunted growth and even more devastating, new cancerous brain tumors. For many children under the age of 21, this type of therapy is very toxic as it has the potential to interfere with organ system function such as poor cardiac health. Overall, once the patients recover, these treatments can lead to morbid side effects, that interfere with maintaining a regular lifestyle for the rest of the patient's life.

Objective

Thus, it is critical that a different solution is discovered in order to treat T-ALL. Though these treatments may be considered successful, by developing a safer method, we can hopefully eliminate the need for such treatments such as radiation therapy or intrathecal injections. This can allow for a higher chance of a normal life post-treatment and even more so a reduced chance of recurrence of the leukemic T-Cells.

Materials

In order to successfully conduct our experiments there is certain materials that will be needed. One of the most important tools we will use is a micropipette which will be used to measure and transfer samples of solutions. We will use vials to hold and store any samples or solutions as well as flasks. A micro centrifuge will be used to spin down DNA, protein, or any other samples. In addition, Gel electrophoresis trays will be used to analyze DNA or RNA since they separate macromolecules based on size and protein expression will be examined with SDS-PAGE trays. A nutrient rich media will be necessary in order to grow and reproduce microorganisms. Finally, a spectrophotometer will be used to measure optimal density and a Nano drop to measure the concentration of DNA.

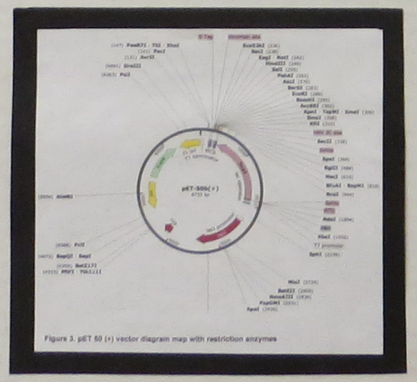

Figure 3. pET 50 (+) vector diagram map with restriction enzymes

Hypothesis

Dr. Vines' lab has worked intensely to develop a systemic antagonist to bind to CCR7. Currently, they are trying to use the protein 8-83 in order act as this antagonist. This would block CCR7's attraction to CCL9 and ultimately leave the T-Cell ALL to flow freely in the bloodstream rather than cross through the blood brain barrier to hide. However, Vines' lab has not been able to successfully induce consistent protein expression or purification.

Based on this, my partner and I hypothesize that different expression systems should be able to yield consistent protein expression leading to purification of 8-83. More specifically, we hypothesize that the yeast, *Pichia Pastoris*, will be a more appropriate host for the expression of 8-83. This is because in similar studies, *Pichia Pastoris* has been used to express 5P12-RANTES to act as an HIV inhibitor (Cerini, 2015). Furthermore, we hypothesize a nutrient rich media that best helps *Pichia Pastoris* to grow will improve protein expression.

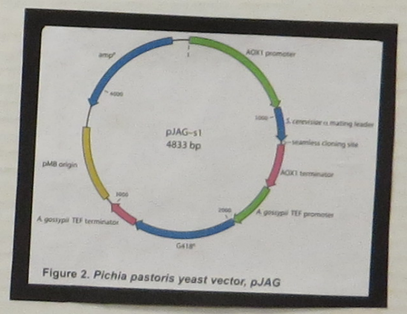

Figure 2. *Pichia pastoris* yeast vector, pJAG

Methods

In order to successfully sub-clone into the *Pichia Pastoris* vector we would have to isolate 8-83 from the pET-50 (+) vector by cutting with the NotI and BamHI restriction enzymes (digestion) then perform restriction digest mediated cloning for the yeast vector and ligate 8-83 cDNA into *Pichia Pastoris* (pJAZaMF) expression vector. Afterwards, the cultures will be grown to select our clones through the use of drug selection. Then we will isolate and purify the DNA from the E. coli and analyze for insertion of 8-83. We will run a diagnostic gel and quantify the DNA via nanodrop to verify the composition of the DNA.

Afterwards, we would transform the validated vector into the *Pichia* Pastoris, induce expression in the presence of methanol, and collect the cells. Following induction of the culture, we will use SDS-PAGE to verify that the 8-83 protein is being expressed by comparing the molecular weight (Daltons) in the ladder of plasmid with 8-83 and plasmid without 8-83. Finally, we will purify the protein.

Expected Results

Following completion of the experiment, we expect to see validation of *Pichia Pastoris* an ideal host cell. This host should be an appropriate expression system to consistently express 8-83. Moreover, when determining if Pichia is an ideal host we will, compare protein expression in E. coli to Pichia Pastoris. We expect that protein expression should be much higher in the yeast. Additionally, we anticipate that a higher concentration will be generated from the yeast vector as compared to pET vectors in E. coli. More importantly, we will not have the need to purify the yeast protein since it would be extracellular.

Future Directions

As we go through the experiment, we expect a better understanding of the mechanisms of yeast expressions and more specifically a better understanding of protein expression through yeast vectors. Moreover, we also expect to understand how to perform the process of seamless cloning for these vectors. Overall, we foresee a future in which 8-83 can be used as an antagonistic ligand in order to block the entry of T-Cell ALL into the blood brain barrier.

References

Biogrammatic. (n.d.). Pichia pastoris. Retrieved June 13, 2015 from http://www.biogrammatics.com/products/pichiapastoris-pjag-s1-expression-vector

Buonamici, S., Trimarchi, T., Ruocco, M. G., Reavie, L., Cathelin, S., Mar, B. G., ... Aifantis, I. (2009). CCR7 signaling as an essential regulator of CNS infiltration in T-cell leukaemia. *Nature*, *459*(7249), 1000–1004.

Cerini, F., Gaertner, H., Madden, K., Tolstorukov, I., Brown, S., Laukens, B., ... Hartley, O. (2016). A scalable low-cost cGMP process for clinical grade production of the HIV inhibitor 5P12-RANTES in *Pichia pastoris*. *Protein Expression and Purification*, *119*, 1–10.

Chiaretti, S., Foa, R. (2009, February). T-Cell Acute Lymphoblastic Leukemia. *Haematologica*, 160-161.

Leukemia CARE. (2014, July). T-Cell Acute Lymphoblastic Leukemia. Retrieved June 13, 2016 from http://www.leukaemiacare.org.uk/t-cell-acute-lymphoblastic-leukaemia

Waber, D. P. (1997, February 1). Toxicity of CNS Prophylaxis for Childhood Leukemia. *Oncology*, 34-36.