Impact of E.Coli Strain Variation and Comparison in Protein Expression and Purification of the CCL19 Antagonist 8-83

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BACKGROUND

T-Cell acute lymphoblastic leukemia, or (T-ALL), is mainly characterized by the over production of white blood cells. It begins in the bone marrow, spreads to various organs, and may travel to the brain and spinal fluid. [9] This is especially dangerous once it's crossed the blood brain barrier. T-ALL most commonly affects children and young adolescents.

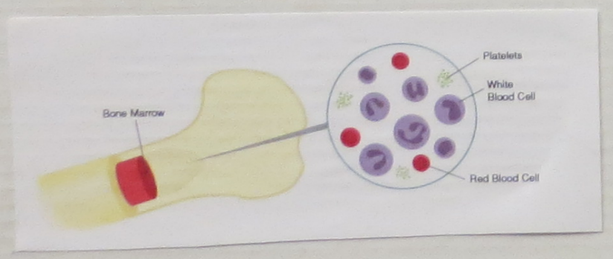
Treatment mainly consists of intravenous and intrathecal chemotherapy in three stages. These are induction, consolidation, and maintenance. Induction is the first, and patients typically go into remission after this stage, meaning that visible symptoms and t-cells are gone. Consolidation is the more intense phase, and it reduces the number of leukemia cells still in the body, making sure they don't grow a resistance to the treatments. The maintenance stage is last, in which the patient is still in remission and needs a few more months of treatment. However, leukemia may recur, in some cases called extramedullary relapse, which is a recurrence of t-cells in a specific part of the body. [1] This is treated with cranial radiation therapy, which can be especially dangerous.

Long term effects and risks of chemotherapy and radiation include: heightened risk for future cancer, respiratory problems, stunted growth, learning disabilities, fertility issues, brain damage, bone damage or osteoporosis, loss of endocrine function and even mental/emotional or psychological issues. [2]

Despite treatment, patients may still be in danger of remaining t-cells traveling to the brain, out of reach of radiation therapy. T-cells receive signals from a protein produced in the brain: CCL19 (chemokine ligand type 19). The signal causes them to produce a protein of their own called CCR7 (chemokine receptor type 7). This allows them to follow CCL19 beyond the blood brain barrier, making it near impossible to eradicate the cells from that point due to the delicate area. Radiation therapy is most likely out of the question due to the high damage risks.

To solve this, we will use an antagonist called 8-83, and have them attract the CCR7, destroying the opportunity of CCL19 being able to attach. This keeps the t-cells in the blood stream, ensuring that it will not pass the blood brain barrier, and it will be easier to destroy the t-cells. However, the proteins that are created become toxic to the cell, killing them.

These cells have been typically grown in a strain called BL21, but the proteins are being toxic to the cell. Based on the codons present in 8-83, we've found that the Rosetta strain expresses the same codons that are rare in e.coli. We've two strains, BL21 Codon Plus (DE3)-RP and Rosetta (DE3) as variants that may yield new results.[8]

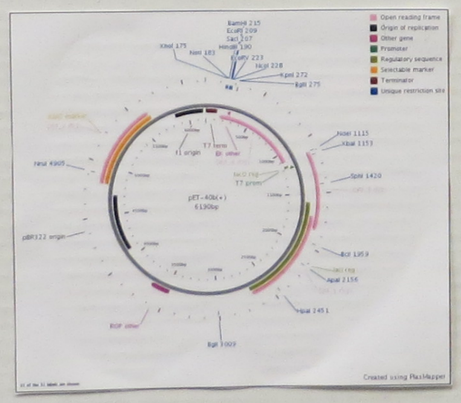


OBJECTIVE

Our goal is to induce protein production using different bacteria strains to determine if the host strain plays a significant role in facilitating 8-83. The BL21 Codon Plus (DE3)-RP will be our control, and Rosetta (DE3) will be our variant.

HYPOTHESIS

We hypothesize that using and comparing different host strains may facilitate better 8-83 yields. By using a different e.coli strain that expresses the same rare e.coli codons as 8-83, we will successfully express the protein without the cells dying. This will block migration of the t-cells into the brain, keeping them in the bloodstream instead.



EQUIPMENT

- SDS-Page
- Spectrophotometer
- IPTG
- Pet-40 Vector
- Rosetta (De3) Novagen
- BL21 (DE3) codon plus-RP

PROCEDURES

1. Transform Pet-40 with 8-83, insert with E. coli.
2. Grow by plating them on media and anti-biotics
3. Incubate at 37 degrees Celsius
4. Purify DNA, using mini/midi/maxi prep
5. Nanodrop measurement
6. Agarose gel for electrophoresis
7. Induction process begins when 0.8 is yielded
8. IPTG is involved to remove the oppressor from the lac operon
9. Run SDS-PAGE to check
10. After protein expression, purify until it's in a functional form.

TIMELINE

Our research will last an estimated five weeks with the variation of strains and their comparisons. As a result, the antagonist 8-83 protein should be purified and at its most functional state.

Starting June 20th we will start by doing our procedure for the transformation of our vector pET-40 with antagonist 8-83. The first step may take from a couple of hours to a whole day. The second step, growing the E.coli by plating them on media and antibiotics, will be done overnight. The next day we will get a colony, grow it, and then shake it in an incubator at 37 degrees Celsius 250RPM. In the fourth step we will purify the DNA using the mini/midi/maxi prep which will take 2-3 hours, and the fifth procedure will add be measuring the purity of the DNA using a nanodrop, if the sample has an absorbance 260/280 of 1.8, then we will know that the DNA is pure. The sixth procedure will involve running the agarose gel for electrophoresis comparison. An electrophotometer is then used to measure the optical density (OD) to determine how many cells grew. Induction may begin when an optimal OD is measured. This can all be completed in the same day if timing and circumstance permits. The ninth step check and run the SDS-Page. For the final step of the transformation we will try to purify the protein. Overall this first week it will take at least 3 days if not 4.

Within the second, third, and fourth weeks, we plan to do the same processes over several trials. The only difference will be the host strain, compared between BL21 and Rosetta.

By the fifth, final week, we anticipate to have our purified and functional protein.

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