Genetic Evaluation of Methotrexate Treatment in Pediatric Cancer Patients

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Introduction

Childhood leukemia:
- Leukemia is the most common cancer in children and it starts in the bone marrow.
- Leukemias comprise approximately 30% of all pediatric cancers, with acute lymphocytic leukemia (ALL) and acute myeloid leukemia (AML) as the most common.
- Estimated number new cases of leukemia in Louisiana in 2021 is 850 and 330 will die from this disease (American Cancer Society)
- The 5-year survival rate for children with ALL has greatly increased and is now about 90% overall. The overall 5-year survival rate for children with AML is now in the range of 65% to 70%

Treatment:
- Methotrexate is used as a chemotherapy to treat childhood leukemia. Methotrexate works by slowing or stopping the growth of cells. It inhibits enzymes responsible for nucleotide synthesis leading to suppression of inflammation as well as prevention of cell division.

Chemical structure of Methotrexate:

Side effects of Methotrexate treatment:
- Methotrexate is associated with common neurological disorders like cognitive defects, abnormal behavior, brain impairment, and memory loss.
- Past literature revealed that Methotrexate has resulted histological changes in the white matter and not in the gray matter.

Objective and Hypothesis

- The objective of our project is to elucidate gene-environment interactions caused by methotrexate treatment.
- We hypothesize that gene expression changes occur in neuroinflammation genes due to methotrexate treatment.

Methods

Figure 1. Study Design to identify the effects of methotrexate on pediatric cancer survivors with leukemia

- Cancer Survivors from Late Effects Clinic
- Participants between ages 2-22 years
- Undergone psychological and behavioral standard testing
- The BASC-3 (Behavioral Assessment System for Children, Third Edition)
- 36 Item Short Form Survey (SF-36)
- Conners Comprehensive Behavior Rating Scales (Conners CBRS)
- Performance Test and other methods

Figure 2. Study Design to identify neuroinflammation genes from autopsy samples of deceased pediatric cancer patients with leukemia

- Screen medical chart of leukemia patients between age 2-22 years [N=60]
- Identify patients with brain tissue (white matter) autopsy samples collected within 48 hours
- Identify autopsy samples from patients receiving Methotrexate treatment
- Collect Formalin-Fixed Paraffin-Embedded (FFPE) samples of brain tissue (white matter) from Children’s Hospital of New Orleans
- Isolate and process RNA
- NanoString microarray analysis
- Bioinformatic analysis (IPA)

Results from gene expression analysis

Table 1. Genes identified by Nanostring analysis: (+) for overexpression, (-) for under expression

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Effect</th>
</tr>
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<tbody>
<tr>
<td>HSPB1</td>
<td>Heat shock protein beta 1</td>
<td>+ 3.26</td>
</tr>
<tr>
<td>GJA1</td>
<td>Gap junction protein alpha 1</td>
<td>+ 3.38</td>
</tr>
<tr>
<td>OLFML3</td>
<td>Olfactomedin like 3</td>
<td>- 3.24</td>
</tr>
<tr>
<td>CD24</td>
<td>Protein coupled receptor</td>
<td>+ 3.38</td>
</tr>
</tbody>
</table>

Conclusions

- Sample collection and analyses are ongoing.
- This project will reveal pathways contributing to neurocognitive and late effects secondary to methotrexate therapy.
- This project will help us to understand the concerns toward the long-term effects of Methotrexate on leukemia and improving the quality of life for pediatric cancer survivors.
- We will follow up with patients and regular check ins with neurologists, cardiologists, audiologists, and ophthalmologists.

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