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.

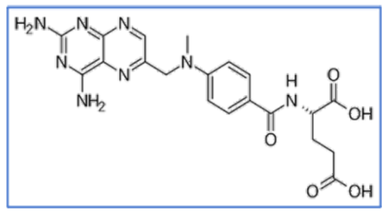
Incidence and survival:

- 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

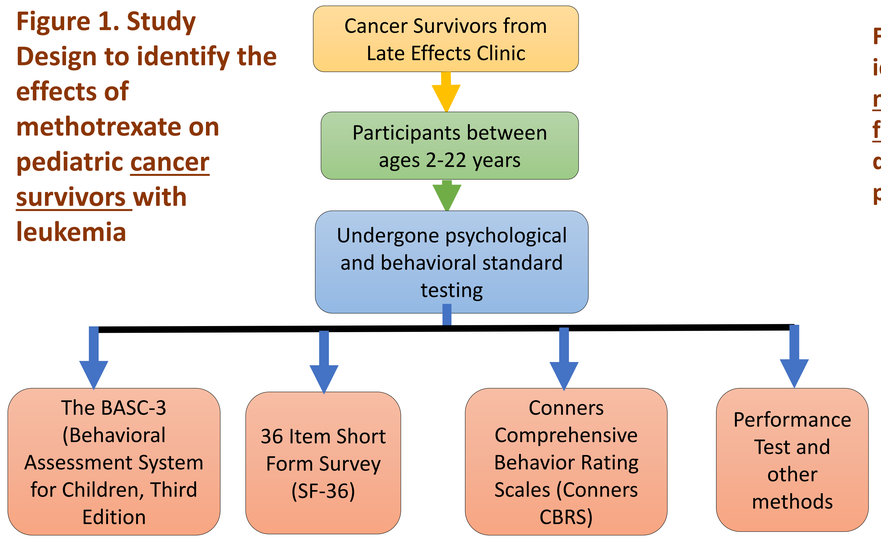
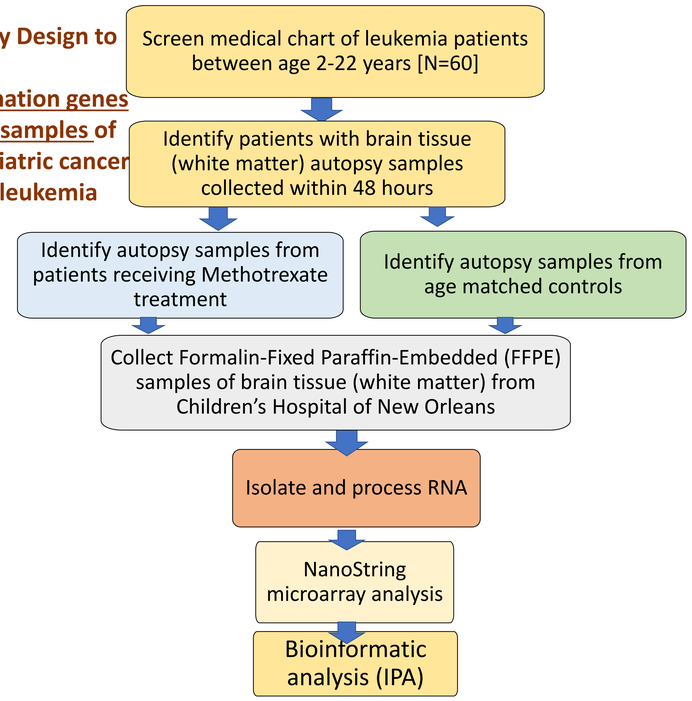
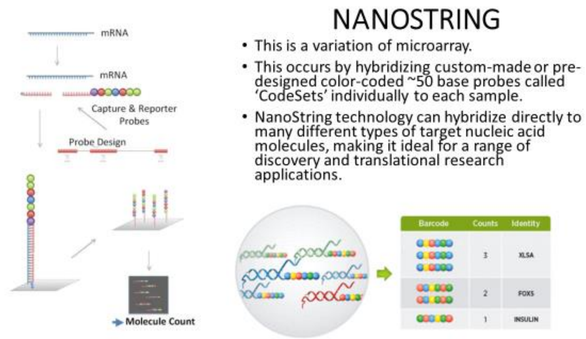


Figure 2. Study Design to identify neuroinflammation genes from autopsy samples of deceased pediatric cancer patients with leukemia



NanoString Microarray Analysis:



Bioinformatic Analysis:

- QIAGEN Ingenuity Pathway Analysis (IPA) will be performed. IPA will use the gene expression results to identify whether significant downstream biological processes are affected.

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.

Results from gene expression analysis

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

Genes	Location	Protein	Function	Clinical significance & health risks	Expression Ratio
GJA1	6q22.31	Connexin 43	Connexin 43 plays a role in cell-to-cell communication by forming channels or gap junctions, between cells.	**Nonsyndromic hearing loss Oculodentodigital dysplasia Coloboma Critical congenital heart disease**	**+ 8.17**
HSPB1	7q11.23	Heat shock protein beta-1	Protects cells under adverse conditions such as infection, inflammation, exposure to toxins, elevated temperature, injury, and disease	**Charcot-Marie-Tooth disease: hereditary sensory and motor neuropathies, muscle weakness**	**+ 5.72**
AGT	1q42.2	Angiotensinogen	Part of the renin-angiotensin system, which regulates blood pressure and the balance of fluids and salts in the body.	**Angiotensin blood vessels constriction, leading to increased blood pressure.**	**+ 5.23**
FOS	14q24.3	C-Fos protooncogene	Signal transduction, cell proliferation, differentiation, activates phospholipid synthesis	**Acts as an activated marker for neuron (fear and anxiety)**	**+ 3.38**
GPR34	Xp11.4	G-Protein coupled receptor	Expressed in macrophages and microglial cells, associated with immune cell function	**Alzheimer's disease, night blindness**	**- 3.1**
OLFML3	1p13.2	Olfactomedin like 3	Loss of OLFML3 expression suppresses microglia biological functions and leads to Amyotrophic lateral sclerosis (ALS). Also associated with glaucoma due to retinal ganglion cell (RGC) loss from insufficient blood supply.	**Alzheimer's disease**	**- 4.6**
P2RY12	3q25.1	P2Y purinoceptor 12	Bleeding disorders Hemorrhagic disease Thrombocytopenia: Low blood platelet count	**Bleeding disorders, mental health disorders, neurodevelopmental disorders**	**- 4.82**
CD24	6q21	Signal transducer CD24 or Small cell lung carcinoma cluster 4 antigen	-Damage to the myelin sheath or protective covering that surrounds nerve fibers in the brain, optic nerves and spinal cord.	**Bile duct cancer, multiple sclerosis, breast cancer, myelin sheath damage**	**- 8.26**

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