Analysis of Neurocognitive Gene Expression in Pediatric Cancer Patients Treated with Methotrexate

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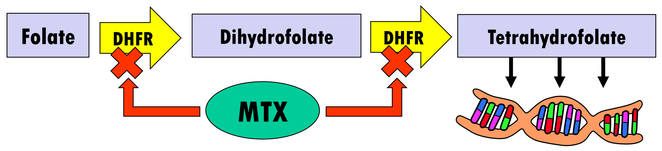
Background

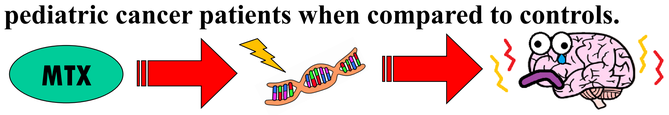
- New advances in pediatric cancer care have earned a record-high 5-year survival rate of 90% for patients afflicted with acute lymphoblastic leukemia (ALL).[1]
- The antimetabolite methotrexate (MTX) is commonly used successfully to treat Pediatric ALL.
  - MTX blocks the production of folic acid needed by cancer cells to create new nucleotides for DNA synthesis.



- However, prior studies have suggested that MTX can cause poor neurological late effects for survivors:
  - ❑ One review found that pediatric survivors initially treated with MTX performed worse than controls on tests of memory, attention, and intelligence.[2]
  - ❑ Another study found that white matter from MTX-treated rats exhibited a permanent decrease in oligodendrocytes, corpus callosum volumes, and myelin basic protein.[3]
  - ❑ Our lab has previously published a study in the American Journal of Audiology that discovered hearing loss in some MTX-treated survivors.[4]
- We seek to solidify this linkage between MTX and neurological upset by quantifying neurocognitive gene expression in pediatric cancer patients.

Hypothesis

Methotrexate chemotherapy deregulates the expression of multiple genes related to healthy cognitive function in pediatric cancer patients when compared to controls.

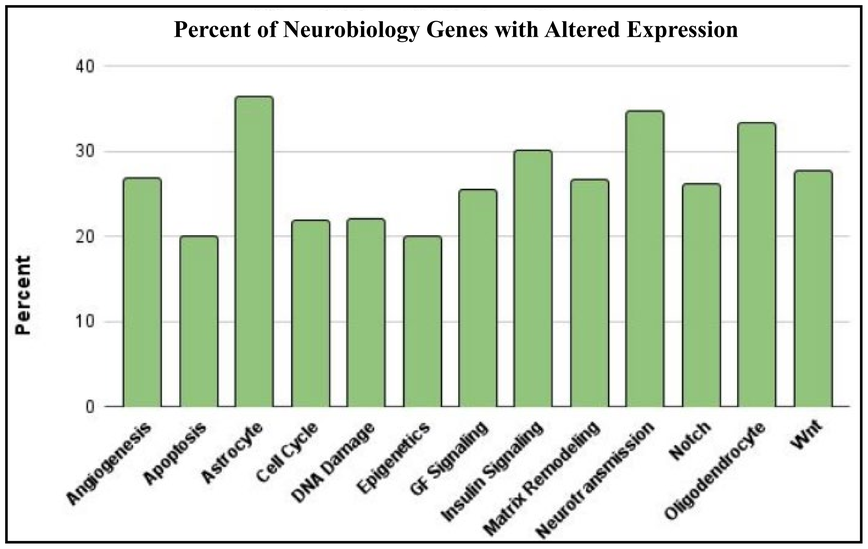


Preliminary Results

Table 1. Analysis of the preliminary Nanostring Neuroinflammation Panel yielded numerous findings of abnormal mRNA expression for several genes involved in neurological function. Notable expression results that can be linked to cognition are listed in the tables below.

Gene	Encoded Protein	Expression Change (Relative to Controls)	Neurocognitive Function
GJA1	Connexin 43	+8.17	➢ Plays a role in memory formation and neuron recovery.[5-6] ➢ Upregulation in memory disorders, such as Alzheimer's Disease.[7-8]
AGT	Angiotensinogen	+5.23	➢ Derived proteins angiotensin II and angiotensin-(1-7) enhance long-term potentiation in the hippocampus.[9]
FOS	Protein c-Fos	+3.38	➢ Expression correlates with learning and memory retrieval.[10-11]
SHANK3	Proline-Rich Synapse-Associated Protein 2	+2.18	➢ Overexpression has been linked to mania.[12] ➢ Involved in the development of language.[13]

Gene	Encoded Protein	Expression Change (Relative to Controls)	Neurocognitive Function
APOE	Apolipoprotein E	-1.88	➢ The less efficient E4 allele is heavily associated Alzheimer's Disease.[14-15]
GRM2	Metabotropic Glutamate Receptor 2	-1.97	➢ Knockout mice show an impaired spatial working memory and impulsitvity.[16-17]
CD24	Signal Transducer CD24	-8.26	➢ Expression might increase hippocampus neurogenesis following injury.[18] ➢ Inhibits neurite outgrowth.[19]

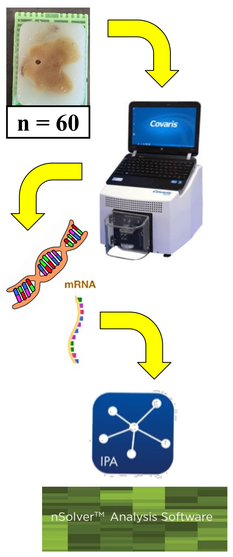


- As shown in the chart above, the Nanostring Panel demonstrated dysregulation in the expression of many different gene types. Major affected gene categories associated with cognition include astrocyte function, neurotransmission, and matrix remodeling.
- Several genes after MTX treatment possess an expression pattern that suggests a functional cognitive decline in memory, learning, and restraint.

Conclusion

- Numerous pediatric survivors of ALL have experienced adverse cognitive late effects into adulthood after being treated with MTX chemotherapy.
- Our preliminary results display an abnormal expression of several neuroinflammatory genes in MTX patients versus controls.
  - ❑ Notable dysregulated genes include *GJA1*, *AGT*, *FOS*, *APOE* and *GRM2*.
- A review of recent literature shows that many of these genes are involved in memory, learning, and self-control of movements and ideas.
- If our future confirmatory study supports the preliminary results, it will illuminate the importance of screening pediatric cancer survivors for potential neurological risks into adulthood. Abnormal genetic results from this study would also motivate future research into MTX late effects.

Methods



- ➢ 60 white matter specimens embedded 48 hours post-mortem were age-matched between MTX patients and controls who were 2 to 22 years of age.
- ➢ Tissue samples were punched, excess paraffin was dissolved in xylene, and the Covaris truXTRAC® FFPE total RNA Kit was utilized to isolate DNA and RNA.
- ➢ RNA expression was studied with the Nanostring Neuroinflammation Panel during a small preliminary experiment. DNA methylation is also currently being probed in a small preliminary study with the Infinium Human MethylationEPIC v2.0 microarray. Nanostring's nSolver Software and the Qiagen Ingenuity Pathway Analysis are being used to decipher related late effects.
- ➢ Follow-up experiments with larger samples sizes will confirm preliminary results.

Inclusion/exclusion criteria

Table 2. Demographics of Select Patients from Preliminary Analysis

- Patients who had brain tumors, head radiation, or degraded genetic material were excluded from the study.
- Autopsy samples were collected from Children's Hospital New Orleans.
- Causes of death among controls include blood clots, asphyxiation, and leukemia (no MTX).

Patient #	Age Sex	Analysis	Cause of Death
1	18 y/o Female	RNA/DNA	Cardiac Death during ALL in Remission
2	20 y/o Male	RNA/DNA	Congestive Heart Failure post Bone Marrow Transplant for T-cell ALL
3	21 y/o Female	RNA	Pulmonary Hemorrhage associated with Relapse ALL
4	10 y/o Male	DNA	Graft vs. Host Disease post Stem Cell Transplant for ALL
5	16 y/o Male	DNA	Candida Lung Infection & Pseudomonas Pericarditis post Bone Marrow Transplant for ALL
6	15 y/o Male	DNA	Complications from Burkitt Lymphoma

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