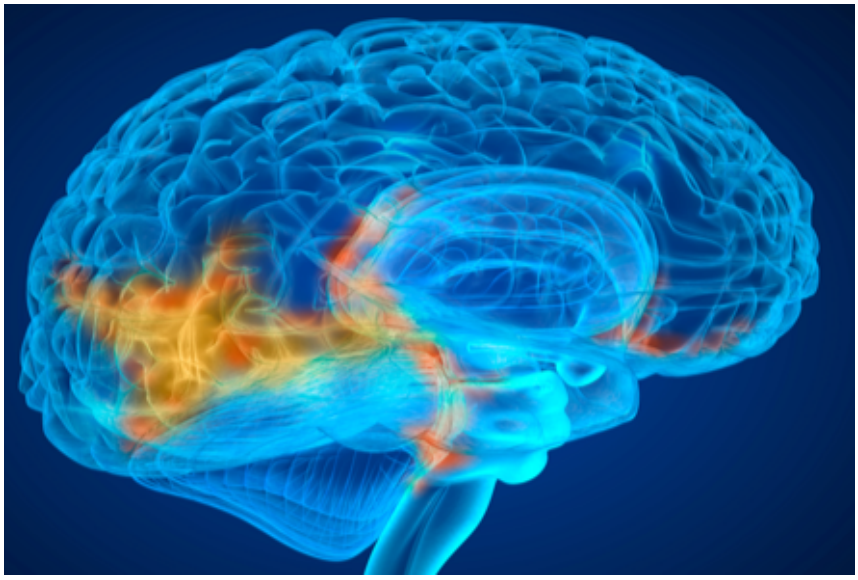
Damian McNamara
December 20, 2018

New Study Shows Promise of Combination Therapy in Fighting Deadly Brain Cancer

Employing advanced technology, innovative thinking, and some adaptive strategies, researchers at Sylvester Comprehensive Cancer Center at the University of Miami Miller School of Medicine have discovered a way to identify combinations of available medications that could optimally treat people diagnosed with glioblastoma, the most common – and deadliest – form of primary adult brain cancer.



Building on previous work, Nagi Ayad, Ph.D., Stephan Schürer, Ph.D., and their colleagues looked at important similarities and differences in gene expression between glioblastoma tumors and a large number of candidate medications. This helped them narrow down and identify treatment combinations more likely to help individuals with this specific type of brain cancer.

Their findings were published December 14 in *Nature Communications*.

Glioblastomas are particularly resistant to treatment because of the heterogeneity or wide variation in cell types within the tumor, the multiple number of pathways it uses to grow, and its overall aggressiveness.

Oncology researchers have been searching for better solutions for years, and Drs. Ayad and Schürer have devised a novel approach to treatment that holds the promise of improving patient outcomes through more targeted therapies.

They created a computational method to predict treatment efficacy based on genetic profiling – a strategy that could help patients avoid the time, expense, and potential side effects of treatments to which they might only partially respond or not respond at all.

To make their findings more practical, the researchers focused on drugs already approved by the FDA.

Nagi Ayad, Ph.D., principal investigator in the Ayad Research Lab at

Sylvester Comprehensive Cancer Center and associate professor of psychiatry and behavioral sciences at the Miller School of Medicine

“The question became how to search the very large space of drug combinations to pick the best candidates for a specific patient,” said Dr. Ayad, principal investigator in the Ayad Research Lab at Sylvester and associate professor of psychiatry and behavioral sciences at the Miller School. “Finding the right combination that provides a synergistic therapeutic boost beyond the benefits of the individual medications themselves was essential.”

Drs. Ayad and Schürer sent glioblastoma cells treated with different medication compounds to colleagues at the Broad Institute of MIT and Harvard, and also worked with researchers at Mayo Clinic. This collaboration helped to identify which of approximately 1,000 important genes changed their activity the most after exposure to a particular drug combination.

They then assigned a genetic “signature” to each drug.

Traditionally, researchers administer a drug combination and wait weeks or months to gauge the response in a particular patient. These genetic signatures now allow researchers to predict effective combinations in advance, giving them the ability to select medications most likely to work synergistically because their relevant genes act in the same direction. Compounds whose genes are all upregulated, or activated, or all downregulated and less active, are more likely to have a desired additive effect on glioblastomas.

Funded by the National Institutes of Health (NIH), the new findings and their translational promise build on the foundation of previous advances. For example, the results would not be possible without the SynergySeq algorithm created in the Schürer laboratory.

This algorithm computes a transcriptional consensus signature (TCS) for each compound using a large gene expression reference database, Connectivity Map. The database is generated at the Broad Institute as part of a national research consortium, the Library of Integrated Network-based Cellular Signatures (LINCS), an extensive, NIH-funded database with roughly 1,100 cells, 1,000 genes, 1,500 proteins, and 42,000 small molecules (potential therapies).

SynergySeq identifies drugs that target different sets of genes in different pathways with a combined gene expression signature that is maximally inverse to the disease. Most anti-cancer medications act on one specific mechanism, so combining two agents can deliver a more effective “one-two punch.”

Using SynergySeq, Drs. Ayad and Schürer and their colleagues screened 197 FDA-approved compounds against glioblastoma

signatures obtained from The Cancer Genome Atlas (TCGA). This effort helped them identify 83 compounds with perturbation profiles – those that showed specific genetic "pertubations" or changes to identify medications that may be beneficial to treat glioblastoma.

Gemcitabine, a medication used to treat pancreatic, breast, and other cancers emerged as the most promising compound. A mitoxantrone and imatinib combination also showed an ability to cut proliferation or growth of a specific subset of glioblastoma cells.

Stephan Schürer, Ph.D., program director, Drug Discovery, University of Miami Center for Computational Science, and associate professor of molecular and cellular pharmacology at

the Miller School of Medicine

“The availability of very large datasets, in particular LINCS, is essential for today’s data- driven research,” said Dr. Schürer, program director, Drug Discovery, University of Miami Center for Computational Science, associate professor of molecular and cellular pharmacology at the Miller School, and a principal investigator in the LINCS consortium.

The researchers then genetically profiled different glioblastoma tumor cells to generate patient-specific disease signatures.

“What was very exciting was that this signature changed for each cell line used,” Dr. Ayad said, noting that this discovery adds to the promise of an individualized approach to glioblastoma treatment.

“This is potentially transformative in patient care,” Dr. Ayad said. “Since a patient’s tumor can be rapidly analyzed with RNA-sequencing, by then using SynergySeq, a prediction could be made about which compound combinations to use clinically,” Dr. Ayad said.

This approach is also much faster than traditional analyses ¬– especially important with glioblastoma, which has a median two-year survival rate of 14% and a progression-free survival period of 6.9 months. This makes traditional patient-specific approaches for glioblastoma very difficult, according to Dr. Ayad.

“By the time the tumor is removed from a patient, propagated in mice, and tested for drug sensitivity, close to a year could pass,” he said. “During that time, the tumor would have likely recurred in the patient.”

The LINCS L1000 dataset contains transcriptional profiles of more than 30 cell lines treated with more than 1,700 small molecule medications. The research team had to improvise, however, because the data does not contain any glioblastoma cell lines.

“We therefore had to screen our own glioblastoma samples, using the L1000 platform,” Dr. Ayad said.

Combining Drug and Tumor Genetics

After assessing the genetic expression of different compound combinations and glioblastoma cell lines, Dr. Ayad’s team evaluated the results together. They identified genetic profiles for medications that were discordant, or as different as possible, from the glioblastoma cells.

“Disease discordance” increases the likelihood that the candidate compounds will attack the cancer cells through mechanisms that differ from the genetic strengths of the tumor cells. They also calculated genetic “drug concordance” among medications – a useful way to predict the most effective synergy among the different combinations.

To verify that their findings were specific to glioblastoma, they consulted a connectivity “heat map” created by colleagues at the Broad Institute. This visual display helped confirm the glioblastoma gene expression results were transcriptionally

similar to Mayo Clinic's glioblastoma reference cells, and dissimilar from other tumor cell types.

One of the most prominent clusters on the map, the bromodomain inhibitors, included the bromodomain and extra-terminal (BET) inhibitor JQ1. This agent proved important because it showed robust activity against glioblastoma. Once identified, researchers looked for other compounds with strong *in vitro* anti-tumor activity through a different mechanism. This strategy led them to an anti-cancer therapy now in development, alisertib, which displayed high concordance with JQ1 and therefore represents a highly synergistic combination.

The SynergySeq technology is only one pillar supporting the current work. The NIH's LINCS database, as well as the Big Data to Knowledge (BD2K) LINCS Data Coordination and Integration Center (DCIC) housed at the Miller School, were also instrumental in the study.

Genetic databases like LINCS contain large volumes of data, and the DCIC helps researchers locate the information most relevant to their project. The BD2K-LINCS DCIC also aims to ensure open access to the LINCS data consistent with the findable, accessible, interoperable, and reusable (FAIR) research principles.

The researchers also consulted The Cancer Genome Atlas (TCGA) and the Brain Tumor PDX databases to externally validate the relevance of their identified drug combinations. In addition, they internally validated their findings through a series of assays.

In the future, Drs. Ayad and Schürer plan to analyze whether SynergySeq can help identify drug combinations that target the cells responsible for tumor formation in specific glioblastoma subtypes. They are working closely with experts at Sylvester's Oncogenomics Facility to identify the cell types to target within each tumor by performing single cell RNA sequencing.

The team is already collaborating with colleagues Dr. Ricardo Komotar, Dr. Michael Ivan, and Dr. Macarena Ines de la Fuente, who treat glioblastoma patients at Sylvester, about how to use SynergySeq in current clinical trials.

"This could potentially provide targeted therapies and combinations that are less toxic and more effective for glioblastoma patients," Dr. Ayad said.