The Hard and Necessary Work of Biomarker Discovery and Validation

Biomarkers have the potential to accelerate the discovery of effective treatments for ALS, but finding, and just as importantly validating, biomarkers poses challenges that researchers recognize they must meet and overcome if this potential is to be realized.

"We know from other fields of medicine that biomarkers offer the possibility of accelerating therapy development," says Michael Benatar, MD, PhD, Professor of Neurology at the University of Miami, and Director of the CReATe consortium. A major aim of the consortium is to discover new biomarkers and to validate promising candidate biomarker for ALS and related diseases.

In multiple sclerosis, for example, the use of neuroimaging markers as a correlate of relapse and progression has rapidly advanced the development of ever more powerful drugs to combat the inflammatory changes that underlie the disease. The ability to rapidly and objectively quantify relapses has allowed the field to test and approve, or discard, potential therapies at a rate that is unprecedented in neurology.

The field of ALS treatment research needs that kind of progression biomarker, in order to make clinical trials faster and more informative. To date, trials have relied mainly on the ALS Functional Rating Scale, which is affected by multiple factors besides progression, including fatigue or illness, making it accurate and informative over many months, but not over the shorter intervals researchers would prefer.

While there is not yet any biomarker that can be taken into clinical trials as a surrogate for disease progression, significant progress has been made in identifying certain types of biomarkers, which in turn hold promise for accelerating therapy development.

Plasma and cerebrospinal fluid levels of **phosphorylated neurofilament heavy chain (pNfH)** and **neurofilament light chain (NfL)** are elevated in ALS patients, presumably as markers of neuronal damage. Researchers have found that higher levels of pNfH and NfL at the time of initial assessment are predictive of more rapidly progressive disease and shorter survival. Although levels of pNfH and NfL do not change, by and large, as disease progresses, both are potentially relevant to therapy development. If it could be shown, for example, that an experimental drug lowers the level of pNfH or NfL, this would provide strong evidence that the drug is having a useful effect by reducing neuronal damage. This in turn would provide hope that the drug would offer benefit to ALS patients.

Biomarkers that show a change in response to an experimental therapy are sometimes referred to as "pharmacodynamic" – 'pharmaco' referring to the drug, and 'dynamic' meaning change. One very promising pharmacodynamic biomarker is the level of **p75 neurotrophin receptor (p75NTR)** in the urine. Preliminary studies suggest that p75NTR is elevated in patients with ALS, that higher levels predict worse prognosis, and that levels continue to rise as disease progresses.

Progress has also been made in developing biomarkers that are relevant to specific genetic types of ALS. The major genetic cause of ALS is an expansion mutation in the C9orf72 gene. Among other effects, the expansion causes production of so-called **repeat-associated non-ATG (RAN) proteins**, which may themselves be harmful. Because they can be detected in the CSF using antibodies, they make an attractive candidate for a biomarker of disease activity and response to therapy. However, major challenges remain, including better understanding how their levels change over the course of the disease.

Whichever biomarker or suite of biomarkers emerge as the most promising for carrying into the ALS clinic, it will be critical to carry out exacting validation studies to allow their collection at multiple centers and, potentially, their measurement in multiple laboratories. Work must be done to standardize specimen collection procedures, including accounting for any effects of time of day, temperature variability, and patient health state (blood oxygen saturation, for example, may affect abundance or stability of serum proteins). Post-collection handling and lab protocols must also be standardized.

"As the field has evolved, we have begun to more fully recognize the challenges in developing a robust, highly informative set of markers that can accelerate treatment research," Dr. Benatar says. "But that recognition is a sign that the field is maturing. I believe that grappling with those challenges will lead us to discovery and development of the tools we need to make progress in finding new therapeutics for ALS."