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Commentary

An evolving technology for an evolving disease: A commentary on NGS-based MRD evaluation in B-ALL

The ability to detect "minimal residual disease" (MRD) is profoundly impacting the treatment approach to acute lymphoblastic leukemia (ALL). While several MRD assays are available, including flow cytometry and targeted PCR, "high throughput sequencing" (HTS) or next generation sequencing (NGS) methods appear particularly promising. One of these approaches – the Adaptive Biotechnologies clonoSEQ® assay – was recently granted FDA approval. No published trial tells us what to do with knowledge of NGS-based MRD status, but clearly this is the next step. As clinical trials are designed and conducted, the field of sequencing-based MRD detection is also evolving. There are multiple very similar HTS approaches. The work by Theunissen et al. represents one of the important steps in this evolutionary process which will likely occupy the field for a substantial part of the next decade. Their key contribution includes data suggesting that the most reliable approaches to NGS MRD detection for precursor B cell ALL will cast a wide net across both immunoglobulin (IG) and T cell receptor (TCR) genes.

MRD is unlike other approaches to risk stratification. The first validated incorporation of risk stratification into the care of ALL involved consideration of clinical features known to correlate with prognosis, such as age and presenting WBC count, which still make up the NCI risk criteria for pediatric ALL today. This was rapidly followed by the development of genetic markers that have been serially refined. Today, the state of the art invokes numerous clinical and cytogenetic features which help to inform the intensity of therapeutic options in ALL; these markers are assessed at the time of diagnosis. Of course, what sets MRD apart from these biomarkers is that it assesses the disease's response to therapy. Readily treatment-responsive disease which demonstrates not only early morphologic but also complete molecular remission (i.e. negative MRD) has a uniformly better prognosis than that which does not. Numerous clinical trials in both adult and pediatric ALL have consistently shown an event free survival (EFS) advantage among patients with negative MRD status, independent of detection method or treatment approach. Although prognosis is substantially worse for adult patients with ALL than for children, the relative impact of MRD status on survival outcome is shared among these cohorts. Across treatment protocols, these observations have informed the uniform intensification of therapy implemented in patients with positive post-induction and/or post-consolidation MRD. However, MRD detection methods are varied and inconsistent. Exactly how to perform these critical assays and how rigorously they need to be standardized remains unclear.

The work of Theunissen et al. addresses one of the key issues in the development of HTS MRD assays: what genes are to be used. V-D-J recombination of the immunoglobulin heavy chain (IGH) gene provides a "signature" for each B ALL clone. While we have traditionally understood cancer as a monoclonal proliferation, newer data suggest that tumors constantly evolve and are therefore composed of related subclones. While all the cells in a tumor stem from a single progenitor, each generation's offspring may be subtly distinctive from its ancestors and cousins. In B cell ALL, this genetic evolution is particularly evident at sites of antigen receptor gene rearrangements, namely IG and TCR loci. This variability poses challenges for how to optimally determine appropriate PCR targets for MRD detection. Using a platform commercially available in 2015 (Sequenta), Theunissen et al. utilized 6 target genes (involving various aspects of the IG heavy and light chain and TCR loci) to determine a "signature" for each diagnostic specimen. They then assessed which parts of the gene signatures could be identified at relapse and discovered that the more obviously prevalent a gene signature at diagnosis, the more likely that signal was to be detectable at relapse. Despite their findings of multiple NGS-detected clonal gene rearrangements per patient, evaluation of diagnosis-relapse samples by NGS of numerous antigen receptor gene rearrangement sites showed that the most predominant clones – at read frequencies > 10,000 or as index clones of > 5% frequency – were *more* likely to be preserved between diagnosis and relapse than were smaller clonal rearrangements. The authors propose that smaller clonal populations were less stable, less likely to be preserved, and thus do not make adequate MRD detection targets. Using these findings, they propose an algorithm for selecting *at least one* MRD target with anticipated stability in > 97% of patients, thereby mitigating the detection challenges involved in assessing solely the traditionally-monitored but highly variable IGH locus.

The authors also detected new clonal signatures emerging at relapse. These subclone populations would not have been chosen as MRD targets by Theunissen et al.'s algorithm and suggest a limited ability to reliably and consistently detect *all* MRD by targeting known, prevalent rearrangement patterns alone. While the authors' strategy of targeting relevant, stable relapse markers as MRD targets optimizes target stability between disease time points, the population of patients whose relapse is composed of newly evolved subclones may be left inappropriately under-risk stratified.

Although the details of the MRD approach are in flux, the lessons we learn from Theunissen's data can be applied to all NGS platforms. Sequenta performed the sequencing for this study, which included IG heavy and light chain loci as well as several TCR loci. Sequenta was purchased by Adaptive Biotechnologies, whose related but distinct pipeline recently received FDA-approval for MRD detection in multiple myeloma and ALL. This latter pipeline does not incorporate information from TCR loci. Other groups, particularly the EuroClonality-NGS consortium, are independently developing NGS MRD assays. Although

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harmonization of results across platforms is always a challenge, the field is moving fast enough that we can expect several groups to develop and test reasonable solutions regardless of the details. Perhaps, like Theunissen, the field will discover that the platforms involving the broadest array of gene rearrangements will provide the greatest stability in MRD detection targets. Because the backbones of these assays are so similar, however, it is unlikely that any of these assays could ever be demonstrably better than others. Far more important will be the evaluation of these assays in clinical trials.

Clinical trials in development are starting to readily incorporate comparative MRD findings between NGS platforms and other traditional detection assays, namely flow cytometry. Particularly in the setting of therapies designed to target specific surface antigens, flow cytometry-based MRD methods may be impacted. The Children's Oncology Group (COG), for example, is investigating NGS MRD techniques in comparison with flow cytometry as a secondary, exploratory aim as part of their Phase 2 study investigating Inotuzumab ozogamicin for CD22+ relapsed or refractory B ALL. The Pediatric Blood & Marrow Transplant Consortium (PBMTC) is also proposing an evaluation of NGS-MRD status as a means of enhancing MRD detection sensitivity and relapse prediction to identify candidates for removal of total body irradiation from B ALL hematopoietic stem cell conditioning. These promising new trial designs will offer further insight about the utility of NGS-MRD assays in clinical practice and may help to further evolve the field's approach to B ALL detection.

Carol Fries, Richard Burack*
University of Rochester Hematopathology Pathology and Laboratory Medicine Rochester, NY, 14642, United States
E-mail address: richard_burack@urmc.rochester.edu (R. Burack).

* Corresponding author.