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The DOI for this manuscript is doi: 10.5858/arpa.2020-0457-OA

The final published version of this manuscript will replace the Early Online Release version at the above DOI once it is available.
Assessment of Clonotypic Rearrangements and Minimal Residual Disease in Lymphoid Malignancies

A Large Cancer Center Experience Using clonoSEQ

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• Context.—Measurable (minimal) residual disease (MRD) is an independent prognostic factor for survival outcomes in patients with lymphoid and plasma cell malignancies and has been incorporated into consensus criteria regarding treatment response, strategy, and clinical trial endpoints. clonoSEQ (a next-generation sequencing [NGS]-MRD assay) uses multiplex polymerase chain reaction and NGS to identify clonotypic rearrangements at the immunoglobulin (Ig) H, IgK, IgL, T-cell receptor (TCR)-β, and TCR-γ loci, and translocated B-cell lymphoma 1/IgH and 2/IgH sequences for MRD assessment. Additionally, it can be used to confirm diagnoses of cutaneous T-cell lymphoma (CTCL).

Objective.—To review the technical aspects of our experience using the clonoSEQ Assay in routine clinical practice.

Design.—In this single-center experience, 390 patients with lymphoid and plasma cell malignancies were assessed with the NGS-MRD Assay at a central laboratory.

In the past decade, the clinical utility of assessing measurable (minimal) residual disease (MRD) in patients with plasma cell and lymphoid malignancies has been recognized increasingly.1 MRD has been identified as an independent prognostic factor for survival outcomes in multiple myeloma (MM), pediatric and adult acute lymphoblastic leukemia (ALL), mantle cell lymphoma (MCL), and chronic lymphocytic leukemia (CLL).2–5 Importantly, the findings of these individual studies have been confirmed by recent meta-analyses.2–9 MRD status is included in consensus criteria for assessing treatment response in patients with MM,10 is used to guide treatment strategy in patients with ALL,11,12 and is a recommended endpoint in clinical trials aimed at maximizing depth of remission in CLL.13 Use of MRD status to guide individualized treatment is under investigation and is likely to increase in the coming years in MM, CLL, other non-Hodgkin lymphomas (NHLs), and possibly other lymphoid malignancies.4,14–17 Various methods are currently used to assess MRD in patients with hematologic malignancies.1,18,19 Multiparametric flow cytometry is a cellular technology based on immunophenotyping.18 Allele-specific oligonucleotide polymerase chain reaction (ASO-PCR), digital PCR, and next-generation sequencing (NGS) are molecular technologies that capture disease-specific rearrangements of the immunoglobulin gene, T-cell receptor (TCR) gene, or some fusion genes.1,19 Typical sensitivity of NGS ranges from 1 in 10^5 cells to 1 in 10^6 cells, and standardized NGS methodologies can routinely achieve a sensitivity of 1 in 10^6 cells if at least 1

Results.—Median time from arrival of the shipment to initiation of the assay (defined as captured in Adaptive’s secure tracking system) was 2.1 hours. Overall, 317 patients had 1 or more samples submitted for sequence identification. Of these, 290 (91.5%) had trackable samples by malignancy (where n ≥ 10 samples, excluding CTCL samples) was 88.1%, across a variety of fresh and archived sample sources (177 of 201 samples). TCR-β and/or TCR-γ clonotypes were identified in 40 of 95 samples (42.1%) from 66 patients with suspected CTCL.

Conclusions.—This NGS-MRD Assay is a valuable and sensitive tool for monitoring MRD in patients with plasma cell and lymphoid malignancies and assisting in the diagnosis of CTCL.

(Arch Pathol Lab Med. doi: 10.5858/arpa.2020-0457-OA)

Accepted for publication April 9, 2021.
Supplemental digital content is available for this article. See text for hyperlink.
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This work was funded by Adaptive Biotechnologies Corp.
Hussaini declares participating on advisory councils or committees for, and receiving honoraria from, Adaptive Biotechnologies Corp and consulting fees from Amgen. Srivastava and Lee are employees of, and hold stocks/shares with, Adaptive Biotechnologies Corp. Sha declares participating on advisory councils or committees for Adaptive Biotechnologies Corp. Pinilla-Ibarz declares receipt of honoraria from Adaptive Biotechnologies Corp. Shain declares receiving honoraria from Amgen, Inc, and consulting fees from Adaptive Biotechnologies Corp. Alina has no relevant financial interest in the products or companies described in this article.
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million cells are analyzed. The suitability of an assay in a particular clinical setting may be affected by the requirements for different clinical samples (such as the ability to use both archived and fresh samples), the need for both baseline and follow-up samples, and the number of cells required for analysis to meet the specified assay sensitivity. High-quality specimens are essential to ensure success and accuracy in measuring MRD.

The clonoSEQ Assay (Adaptive Biotechnologies, Seattle, Washington; hereafter referred to as the NGS-MRD Assay) is an in vitro diagnostic assay cleared by the US Food and Drug Administration (FDA) that uses multiplex PCR and NGS to identify the frequency and distribution of clonotypic rearrangements consistent with the presence of a malignant lymphocyte population from bone marrow of patients with ALL or MM and from bone marrow or the peripheral blood of patients with CLL. It is also a laboratory-developed test used to assess B-cell receptor and TCR clonal rearrangements and subsequently monitor MRD in other B- and T-cell malignancies and specimen types, including peripheral blood. The vast immune receptor repertoire of B and T cells is primarily generated by somatic rearrangement between the variable (V), diversity (D), and joining (J) segments of either the B-cell receptor or TCR, which results in the creation of highly diverse sequences. The NGS-MRD Assay targets the complementarity-determining region 3 (CDR3) and assesses rearrangements present in the immunoglobulin (Ig) H, IgK, IgL, TCR-β, and TCR-γ loci, as well as translocated B-cell lymphoma (BCL) 1/IgH and BCL2/IgH sequences.

In the clinic, the NGS-MRD Assay (or its precursor, clonoSIGHT) has been investigated for the assessment of MRD in patients with MM, B-ALL (pediatric and adult), T-ALL, MCL, CLL, diffuse large B-cell lymphoma (DLBCL), and CTCL. Additionally, the NGS-MRD Assay can be used to assist with the diagnosis of CTCL by identifying clonotypic rearrangements consistent with malignancy. We report results of our experience in using the NGS-MRD Assay for 2 years at the H. Lee Moffitt Cancer Center & Research Institute (Tampa, Florida; herein referred to as the Moffitt Cancer Center).

MATERIALS AND METHODS

This was a retrospective, single-center study of the performance and application of the NGS-MRD Assay in patients with lymphoid and plasma cell malignancies. The study included patients with a diagnosis of MM, ALL, MCL, CLL, DLBCL, Other NHL, or Other Leukemia and patients with suspected CTCL who provided at least an initial diagnostic sample for NGS-MRD testing, and who were treated at the Moffitt Cancer Center between March 2017 and March 2019. Data were collected in the process of routine clinical care of patients seen at the Moffitt Cancer Center.

Sample Sources

Clinical samples for clonality assessment (ID) and tracking (MRD) were taken from patients with MM, ALL, MCL, CLL, DLBCL, Other NHL, and Other Leukemia. Only ID samples were taken from patients with suspected CTCL. Cases classified as Other NHL included follicular lymphoma, benign pseudolymphoma, and basal cell carcinoma (submitted as suspected CTCL). Other Leukemia cases included acute myeloid leukemia and reactive T-cell large granular lymphocyte (T-LGL) leukemia (secondary to a primary infection). Where an MRD test was ordered in the absence of a preceding ID sample, an archived tumor sample (either from in-house archives or external centers) was analyzed for adequacy for the ordering pathologist. To prevent self-referral patients who had not been reviewed by the Moffitt Cancer Center, the ordering clinician was contacted to request the ID sample. In cases where an archived sample was not immediately available (either from in-house archives or external centers), the treating clinician was notified, and tracing of diagnostic tissue initiated. In all cases, when a suitable ID sample was identified, 10 slides (3- to 4-μm tissue sections) or 4 slides (8-μm tissue sections) of fresh bone marrow or bone marrow clot sections, or more than 2 mL of either bone marrow aspirate or peripheral blood in lavender EDTA tubes, were shipped to Adaptive Biotechnologies at ambient temperature; the MRD sample was collected and shipped similarly. Note that decalcified bone marrow clot sections are not an acceptable sample type owing to possible genomic DNA degradation. The ID and MRD samples were not always sent concurrently; if the ID sample was not readily available, the MRD sample was sent to Adaptive Biotechnologies and extracted. Once obtained, the ID sample was sent to Adaptive Biotechnologies, as a separate shipment. The samples underwent genomic DNA extraction and testing using the NGS-MRD Assay. If a suitable ID sample could not be identified and diagnostic tracking was not possible, testing was cancelled.

NGS for MRD Monitoring

MRD monitoring requires both an ID sample and subsequent MRD samples. ID samples were required to contain at least 5% tumor cells (as a proportion of the total cellular content; estimated by morphology), and the immune repertoire of the sample is then checked for the presence of “dominant” DNA rearrangements or clonotypes consistent with the presence of a lymphoid malignancy and trackable for subsequent monitoring. Genomic DNA was extracted, and rearranged immune receptors were amplified by using a multiplex PCR. Sequencing libraries were then prepared and sequenced with the Illumina Sequencing System. Sequence data were processed by Adaptive Biotechnologies’ proprietary algorithm, which uses in-line controls to remove amplification bias. Specific DNA sequences met the criteria of the “dominant” as follows: rearrangements comprised at least 3% of all locus-specific sequences (for IgH, all IgH V J and D J rearrangements and for IgK or IgL, all light chain rearrangements); comprised at least 0.2% of the total nucleated cells in the sample; were discontinuously distributed (≥5 sequences in the next decade of sequences when ranked by frequency); and were carried by at least 40 estimated genome equivalents in the analyzed sample (See supplemental digital content for additional information on assessment of “dominant” sequences). Samples containing at least 1 dominant clonotype suitable for tracking were termed calibrated as opposed to polyclonal. When a sample fails to calibrate, the patient cannot be tracked for subsequent MRD. The calibration rate was used to define the proportion of ID samples for which dominant rearrangements were found and excludes patients with suspected CTCL, for whom the ID assessment was intended as a diagnostic test rather than to enable subsequent monitoring.

During and/or following treatment, tracking (MRD) samples were obtained and tested, and the dominant clonotypes identified before treatment were quantified to assess disease burden. The abundance of dominant clonotypes and the corresponding sample-level malignant cell count was calculated and was compared with the total nucleated cell count, in order to provide an estimate of the MRD frequency per sample.

NGS as a Diagnostic Tool for CTCL

ID samples taken from patients with suspected CTCL were analyzed for the presence of TCR clonal populations, using the NGS-MRD Assay. Identification of dominant clonotypes was used to confirm diagnosis of CTCL. Samples of multiple source types were analyzed for individual patients, to determine the extent, if any, of CTCL spread.
**RESULTS**

**Patients and Clinical Samples**

A total of 383 patients provided at least 1 ID sample from the Moffitt Cancer Center; diagnoses included MM (n = 192), ALL (n = 69), MCL (n = 23), CLL (n = 13), DLBCL (n = 10), suspected CTCL (n = 66), Other NHL (n = 6), and Other Leukemia (n = 4). We included another 7 patients with lymphoid malignancies and ID samples from different institutions, whose care subsequently moved to the Moffitt Cancer Center and who provided follow-up samples. In total, 766 ID and MRD samples were tested from these 390 patients. Of the 431 ID samples (including samples from patients with suspected CTCL), sample types included fresh bone marrow (n = 97, 22.5%), peripheral blood (n = 56, 13.0%), and formalin-fixed, paraffin-embedded (FFPE) slides of tissue from bone marrow (n = 168, 39.0%), skin (n = 54, 12.5%), lymph node (n = 14, 3.2%), and other sites (n = 41, 9.5%). Of the 335 MRD samples, 285 (85.1%) were fresh bone marrow, 48 (14.3%) were peripheral blood, and 2 (0.6%) were FFPE bone marrow slides.

**Calibration Rates**

A total of 317 patients provided an ID sample to facilitate MRD monitoring. Trackable sequences were identified in 290 of these patients (91.5%; Table 1). A total of 336 ID samples were analyzed for calibration rate determination (Table 1). Median cell count was highest for fresh bone marrow samples (Figure 1). Of 336 samples, a total of 292 (86.9%) calibrated. The median calibration rate for submitted samples across disease states was 88.1% (177 of 201 samples), ranging between 81.8% and 100.0%, for the malignancies with more than 10 samples (Table 1). Of the 292 samples that calibrated, 97 (33.2%) were found to contain 3 trackable sequences (Table 1). The current version of the clonoSEQ Assay assesses BCL1 and BCL2 translocations. In total, 220 patients were assessed with this version of the assay including 147 MM, 40 ALL, 12 MCL, 12 CLL, 8 DLBCL and 1 Other Leukemia cases, of which, 5 of 12 patients with MCL had a BCL1 rearrangement and 3 of 8 patients with DLBCL had a BCL2 rearrangement. All patients except for one with DLBCL had other immunoglobulin rearrangements within the CDR3. This frequency likely underestimates the incidence of BCL1/2 translocation, as the current version of the B-cell assay is not designed to be comprehensive with regard to all possible BCL1/2-IgH translocations.

**MRD Orders**

In total, 335 MRD samples were evaluated from 198 patients (Table 3). Median cell count was highest for fresh bone marrow samples (Figure 2). The numbers of MRD samples and the proportion positive for MRD are presented by sample source in Table 4. The median MRD positivity level was 257, 102, 108, and 207 residual cells (MRD) per million total nucleated cells in MM, ALL, MCL, and CLL, respectively. The per million count is calculated by dividing the total number of residual sequences identified and comparing that to the total number of nucleated cells assessed within the assay. This number is then expressed per 1 million nucleated cells to facilitate comparison of serial MRD assessments. For example, if 15 residual sequences were seen (MRD) and 1.5 million cells were assessed in a sample, the MRD count would be expressed as 10 residual sequences per million cells. The median number of cells

| Table 1. Calibration Rates by Type of Plasma Cell and Lymphoid Malignancy |
|-------------------------------------------------|
| **Patients with an ID sample, n** | 192 | 69 | 23 | 13 | 10 | 6 | 4 | 317 |
| **Patients with trackable sequence(s), n (%)** | 177 (92.2) | 64 (92.8) | 23 (100.0) | 13 (100.0) | 8 (80.0) | 3 (50.0) | 2 (50.0) | 290 (91.5) |
| **Total ID samples ordered, n** | 201 | 76 | 25 | 13 | 11 | 6 | 4 | 336 |
| **Samples with trackable sequence(s), n (%)** | 177 (88.1) | 65 (85.5) | 23 (92.0) | 13 (100.0) | 9 (81.8) | 3 (50.0) | 2 (50.0) | 292 (86.9) |
| **Mean trackable sequences across evaluable ID samples, n (%)** | 2.78 | 2.78 | 3.26 | 3.62 | 2.33 | 3.33 | 3.50 | NA |

Abbreviations: ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; ID, diagnostic; MCL, mantle cell lymphoma; MM, multiple myeloma; NA, not available; NHL, non-Hodgkin lymphoma.

| Table 2. Calibration Rates by Sample Type (Excluding Cutaneous T-Cell Lymphoma) |
|-------------------------------------------------|
| **Peripheral Blood** | Fresh Bone Marrow | FFPE Slide (Bone Marrow) | FFPE Slide (Lymph Node) | FFPE Slide (Skin) | FFPE (Other) | Bone Marrow Aspirate Slide | Total |
| ID samples tested, n | 15 | 96 | 168 | 14 | 1 | 41 | 1 | 336 |
| ID samples with trackable sequence(s), n (%) | 9 (60.0) | 81 (84.3) | 149 (88.7) | 13 (92.9) | 1 (100.0) | 38 (92.7) | 1 (100.0) | 292 (86.9) |

Abbreviations: FFPE, formalin-fixed, paraffin-embedded; ID, diagnostic.
assessed for MRD was approximately 1.6 million, 1.2 million, 1 million, 1.2 million, and 2.2 million for MM, ALL, MCL, CLL, and DLBCL, respectively. Overall, the median number of cells assessed for all disease states was 1,244,532, resulting in a median assay sensitivity of $1.24 \times 10^3$ for all MRD samples assessed.

**Assay Turnaround Time**

Of the 766 orders with evaluable data for turnaround time, 457 (59.7%) initiated (captured in Adaptive’s secure tracking system) in less than a day; the median time from arrival of the shipment of ID and MRD samples to initiation of the assay was 2.1 hours. The data were highly skewed toward the lower time durations. Among all analyzed orders, median time from assay activation to report delivery was 9.52 days for archived samples and 6.22 days for fresh specimens (overall, median, 7.14 days; range, <1 to 251 days); 957 of 1044 orders (91.7%) were reported within 2 weeks. Overall, median turnaround time from sample receipt to report delivery was 11.07 days for archived specimens and 8.01 days for fresh specimens (overall, median, 9.45 days).

**Identification of T-Cell Clones in CTCL**

A total of 95 ID samples were analyzed from 66 patients with suspected CTCL. Of these samples, dominant TCR-β

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**Table 3. Number of Measurable (Minimal) Residual Disease (MRD) Orders by Type of Lymphoid and Plasma Cell Malignancy**

| Patients with MRD test, n | MM | ALL | MCL | CLL | DLBCL | Other NHL | Total |
|--------------------------|----|-----|-----|-----|-------|-----------|-------|
| Total MRD tests ordered, n | 124 | 46  | 13  | 8   | 6     | 1         | 198   |
| Average No. of MRD orders per patient, n | 1.36 | 2.76 | 1.31 | 1.5  | 1.33  | 2.0       | 1.69  |

Patients, n, with:

| 1 order | 83 | 12  | 10  | 6   | 4    | 0         | 115   |
| 2 orders | 37 | 14  | 2   | 1   | 2    | 1         | 57    |
| 3 orders | 4  | 8   | 1   | 0   | 0    | 0         | 13    |
| ≥4 orders | 0  | 12  | 0   | 1   | 0    | 0         | 13    |

Abbreviations: ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; MCL, mantle cell lymphoma; MM, multiple myeloma; NHL, non-Hodgkin lymphoma.

* MRD tests were not ordered for patients with other types of leukemia.
and/or TCR-γ clonotypes were identified in 40 (42.1%) (Table 5).

**DISCUSSION**

In our experience at the Moffitt Cancer Center, the NGS-MRD Assay was successfully used to monitor MRD in a range of plasma cell and lymphoid malignancies. Excluding those malignancies with 10 samples or fewer (ie, those ordered as Other NHL [n = 6] and Other Leukemia [n = 4]), MRD sequences were identified in 287 of 326 ID samples (88%), corresponding to 92.8% (285 of 307) of patients. Trackable sequences were identified in 3 of the 6 patients with Other NHL (50.0%) and 2 of 4 patients with Other Leukemia (50.0%). For the cases in which trackable sequences were not identified, 1 of the 3 Other NHL cases was found to be a benign pseudolymphoma; a second case (suspected CTCL) was determined, following T-cell NGS assessment, to be a basal cell carcinoma with concomitant involvement by CLL/small lymphocytic leukemia. One of the 2 Other Leukemia cases was diagnosed as acute myeloid leukemia, and the other as a reactive proliferation of T-LGL secondary to infection, lacking B- and T-cell rearrangement. In subsequent MRD assessments, 250 of 335 samples (74.6%) were MRD-positive.

Calibration rates were also assessed according to sample source. The 2 most common sources of ID samples were FFPE bone marrow slides (n = 168) and fresh bone marrow (n = 96), which had similarly high calibration rates (149 of 168 samples [88.7%] and 81 of 96 samples [83.5%], respectively). The most common sources of MRD samples were fresh bone marrow and peripheral blood, of which, 77.2% (220 of 285) and 58.3% (28 of 48) were MRD-positive, respectively. Calibration rates (86.8% or 290 of 334 patients, excluding sample sources with ≤10 samples) and rates of MRD positivity (58.3% for peripheral blood and 77.2% for fresh bone marrow, excluding samples sources with ≤2 samples) were high for all sources, indicating that NGS-MRD can be used to monitor MRD across a range of sample sources. The calibration rate by sample was 100.0% (n = 13) for CLL samples and 81.8% (9 of 11) for DLBCL samples. For patients with ALL, including B-ALL, T-ALL, and precursor B-ALL, the calibration rate by patient was 92.8% (64 of 69 patients; Table 1). Overall, there was a high rate of calibration (92.7% excluding sample sources with ≤10 samples), indicating that most patients can be subsequently tracked for MRD with this method.

Several factors influence the ID calibration rate, including sample quantity and quality, and cellular biology. First, the sample must contain a high enough disease burden to ensure a clonal population can be identified. Practically speaking, we have defined this as 5% at the Moffitt Cancer Center. Second, the sample must be of high quality, meaning the DNA present is suitable for downstream PCR applications. For example, decalcified samples, which degrade DNA, are not accepted. Additionally, older samples in which DNA may have been degraded over time may not be suitable. Lastly, immature cells that have not undergone V(D)J rearrangement cannot be tracked by NGS as there is no rearrangement to track.
These data are consistent with previous studies, supporting the presence of at least 1 clonal TCR or immunoglobulin rearrangement in most patients with B- and T-ALL. The incorporation of primers designed to detect incomplete IgH DJ rearrangements, in addition to complete V(D)J rearrangements, enables detection of both complete and incomplete rearrangements, using the NGS-MRD Assay. This enables identification of clonal sequences in malignancies that are associated with precursor B- or T-cell lymphoblasts. Moreover, optimization of primer design allows for the identification of trackable sequences in the presence of alterations within V(D)J, such as somatic hypermutation. Together, these enable utilization of the NGS-MRD Assay in a number of lymphoid malignancies, including MM and CLL.

Traditionally, our center has used either flow cytometry or B- or T-cell gene rearrangements for assessment of residual disease. For assessment of flow cytometry, we collect information on 2,000,000 and 750,000 events for MM and B-ALL, respectively; the limit of detection being 1 tumor cell per 10^5 healthy cells. The implementation of flow cytometry–based MRD assays is technically challenging, since high-performance computer hardware is required to process the large amounts of data generated. Moreover, interpretation of the results is subjective, leading to interobserver variability; this necessitates time-consuming calibration of the technical equipment, and generates intensive discussions around interpretation of the results during weekly consensus conferences. For this reason, we do not routinely screen samples by flow cytometry before sending for NGS-MRD assessment.

In our experience, B- and T-cell gene rearrangement electrophoretic assays (such as those using BIOMED-2 primer sets; Invivoscribe Technologies, San Diego, California) are also highly sensitive methods for detection of MRD, the limit of detection being 1 tumor cell per 10^5 healthy cells. Limitations of this assay type include pseudoclonality (due to restricted B- or T-cell repertoires) and subjectivity in the interpretation of differentiation between clonal and nonclonal peaks. Given the high rate of positivity during TCR testing with gene rearrangement assays, our pathologists exercise caution when using the results to aid clonality determination in cases of suspected Sezary syndrome or T-LGL leukemia.

At the Moffitt Cancer Center, we have identified several advantages of using the NGS-MRD Assay for the assessment of MRD, compared with our previous techniques. The NGS-MRD Assay offers a sensitivity of at least 1 log deeper than either conventional flow cytometry or B- and T-cell gene rearrangement assays, as it can routinely detect 1 log deeper than either conventional flow cytometry or B- and T-cell gene rearrangement assays. As a result, the NGS-MRD Assay is able to detect clonal sequences in a number of lymphoid malignancies, including MM and CLL.
tumor cell in $10^6$ healthy cells.\textsuperscript{41,42} This greater sensitivity allows more accurate MRD detection at a lower tumor burden. In addition, assessment and interpretation of the results by a central laboratory (together with the objective nature of sequence-based assessment) eliminates much of the subjectivity of MRD detection, thereby reducing the interlaboratory and interobserver variability that occurs with flow cytometry and gene rearrangement techniques.

Numerous studies in a variety of lymphoid malignancies have assessed the correlation between MRD results assessed by NGS and flow cytometry.\textsuperscript{3,30,35,43} Ferrot et al\textsuperscript{3} assessed 233 patients with MM by NGS-MRD who were initially determined to be MRD-negative by multiparametric flow cytometry. Of these samples, 120 (51.5\%) were determined to be NGS-MRD-negative and 113 (48.5\%) were NGS-MRD-positive. Martinez-Lopez et al\textsuperscript{35} assessed 99 patients with MM by NGS and flow cytometry. Overall, 82 samples were concordant positive or negative. Twelve patients were NGS-positive/flow-negative and 5 patients were NGS-negative/flow-positive. Patients who were NGS-positive/flow-negative had worse outcomes than patients who were MRD-negative by both methodologies (50 months versus not reached; $P = .05$). Of the 5 cases that were NGS-negative/flow-positive, only 1 patient had progression at time of publication. Of the 62 patients who achieved complete remission, 36 (58.1\%) were NGS-MRD–positive. These patients had significantly worse outcomes than the 26 patients who were in complete remission and NGS-MRD–negative (151 versus 35 months; $P < .001$).

In ALL, Wood et al\textsuperscript{35} assessed the correlation of NGS and flow cytometry in 568 patients by using an MRD threshold of $10^{-4}$ for both methodologies. When assessing MRD results at the same threshold, NGS identified 55 patients with MRD that flow cytometry called MRD-negative. Seventeen samples were identified as flow-positive and NGS-negative. These results indicate that even when assessing MRD using the same threshold, NGS identified more patients with residual disease. Additionally, the 55 patients who were identified as NGS-positive and flow-negative had worse outcomes than patients who were MRD-negative by both methods.

Thompson et al\textsuperscript{44} assessed 62 patients with CLL by NGS-MRD who were initially determined to be MRD-negative by multicolor flow cytometry (sensitivity $10^{-4}$). When assessing MRD in the bone marrow and peripheral blood by NGS in these patients, 43 of 57 (75.4\%) and 13 of 29 (44.8\%) were NGS-MRD–positive. These patients had significantly worse outcomes than the 26 patients who were in complete remission and NGS-MRD–negative (151 versus 35 months; $P < .001$).

Concordance has also been demonstrated between NGS-MRD testing and PCR. In CLL, Logan et al\textsuperscript{44} demonstrated good concordance between high-throughput pyrosequencing and ASO-PCR. Several studies have shown reliable detection of MRD down to $10^{-6}$ by NGS.\textsuperscript{45,46} In ALL, NGS has been cited to be more accurate than current universal methods, including PCR.\textsuperscript{47} In another study with 378 samples spanning ALL, MM, and MCL, there was good correlation between PCR and NGS MRD ($R = 0.8; P < .0001$); furthermore, NGS had at least the same degree of sensitivity as PCR without the need for patient-specific primers.\textsuperscript{40,48} Other studies have shown NGS to pick up cases of undetectable MRD by PCR.\textsuperscript{49}

Detection of MRD by NGS also offers an orthogonal method to assess for clonality in the setting of immunotherapy (such as chimeric antigen receptor T-cell therapy) and monoclonal antibody therapy (eg, blinatumomab) for lymphoid malignancies. Targeted therapies can create a bias for tumor cells that show downregulation or lack/loss of relevant cell-surface markers during tumor evolution, leading to evasion of the therapeutic effect. Such “immune escape”\textsuperscript{50} may affect the reliability of MRD detection by methods such as conventional flow cytometry, which rely on such cell-surface markers, particularly if the targeted marker is a so-called gating antigen that is pivotal for assay utility (eg, CD19).\textsuperscript{49} Consequently, NGS-MRD may also represent a more reliable technique for patients who are receiving targeted therapies against such antigens. Finally, the NGS-MRD Assay has received FDA clearance to detect MRD in bone marrow from patients with MM or B-ALL and, more recently, peripheral blood or bone marrow from patients with CLL.\textsuperscript{51} The FDA clearance of this assay contributed toward the decision to implement the assay at our center for a subset of patients.

At our institution, the NGS-MRD Assay has informed clinical decision making. In patients with MM, NGS-MRD negativity is regarded as an indicator of disease control and provides support to the decision to discontinue therapy in patients with significant toxicities. In B-ALL, the presence of MRD after induction or consolidation has influenced decisions regarding use of stem cell transplant, additional consolidation before transplant, and conditioning regimen intensity. In MCL, MRD status could be used to guide the duration of maintenance therapy.

Our experience also demonstrates the value of the NGS assay in the differential diagnosis of CTCL. Clonality testing for the purpose of diagnosis is less commonly needed for the diagnosis of T-cell malignancies. In B-cell disorders, samples are obtained from patients with a known malignancy to facilitate longitudinal MRD assessment, and the baseline clonality sample is necessary to identify the trackable malignant rearrangement. In contrast, in suspected CTCL, the assay is used to determine whether a dominant monoclonal T-cell population is present or not, a crucial finding for early, accurate diagnosis.\textsuperscript{50} Making a timely, accurate diagnosis of CTCL is challenging as initial presentation may mimic benign skin dyscrasias such as atopic dermatitis and psoriasis, and the most common clinical test, TCR-$\gamma$ PCR, has a high false-negative rate.\textsuperscript{52} The NGS assay is often used on multiple skin biopsies obtained from a patient with suspected CTCL to discriminate malignancy from benign processes and has been shown to be more sensitive and specific than TCR-$\gamma$ PCR.\textsuperscript{53} Among 39 patients with clinically confirmed CTCL, Kirsch et al\textsuperscript{44} demonstrated that NGS identified T-cell clones in the skin and blood in all cases versus TCR-$\gamma$ PCR, which was successful in only 27 of 39 (70\%). Moreover, NGS could discriminate CTCL from benign inflammatory conditions, detect relapse, and allow response assessment.\textsuperscript{54} Rea et al\textsuperscript{56} found that NGS was more specific than TCR-$\gamma$ PCR in skin samples from 25 patients (100\% versus 88\%, respectively); sensitivity (68\% and 72\%) and accuracy (84\% and 80\%) were similar in this small study. In the current study, dominant TCR-$\beta$- and/or TCR-$\gamma$-dominant clonotypes were identified in 40 of 95 samples (42.1\%) taken from 66 patients with suspected CTCL. The clonality (ID) assessment resulted in no “dominant” sequences being found in most samples, indicating that the sample was either not representative of the disease process or the disease process was inflammatory rather than malignant.\textsuperscript{56} In 31 samples, both TCR-$\beta$- and TCR-$\gamma$-dominant clonotypes were identified; clonotypic TCR-$\beta$ or TCR-$\gamma$ sequences only were identified in 2 and 6 samples, respectively. Combined with the results of
previous studies that demonstrated the use of the NGS assay to measure residual disease during the continuum of care,27 and as a predictor for patients who may be at higher risk for disease progression, these data show that NGS has wider applications than the evaluation of response to treatment in CTCL.

Implementation of the NGS-MRD Assay was not without challenges. Initially, calibration rates with the NGS-MRD Assay were low (50%–60%) but increased to 82% and up to 100%, following implementation of pathologist-driven quality control. Another obstacle to implementation was lack of awareness among pathologists of the value of MRD testing in lymphoid and plasma malignancies. Removing these barriers required educational training for both the medical faculty and the Specimen Processing Laboratory staff. Our staff was educated on the clinical importance of MRD testing, and workflow details were devised for various ordering scenarios. This included a process for identifying, retrieving, and quality-checking outside diagnostic materials before sending them for sequencing. In addition, we implemented morphologic pathology review of all materials and only processed samples for MRD testing in those patients who had more than 5% tumor cells in the diagnostic ID sample. This step allows for exclusion of aspicular/acellular specimens and those with insufficient tumor for testing due to sampling bias in the clot section versus the decalcified bone marrow specimens (which cannot be used for MRD detection). Finally, implementation of the NGS-MRD Assay necessitated the creation of standardized protocols for locating and analyzing the adequacy of ID specimens, and for creating electronic medical record orders to facilitate ID and MRD testing.

To conclude, our experience corroborates previous studies that showed NGS-MRD testing provides an accurate measure of MRD in lymphoid malignancies and has utility in clinical practice. Our data demonstrate proof of principle that high calibration rates can be achieved in clinical practice for ID determination by NGS, including from archival FFPE specimens. The NGS-MRD Assay can be used to aid diagnostic confirmation of early CTCL and to assess prognosis (eg, prediction of progression-free survival, overall survival, and time to relapse) in MM and ALL. Importantly, NGS-MRD Assays have great potential to inform therapeutic decision-making. The recommendations of the International Myeloma Working Group acknowledge the potential of NGS-MRD and encourage further evaluation of NGS and other MRD-assessment methods in well-designed, response-adapted clinical trials.10 The recent literature reflects these developments. A recent study indicated that the presence of MRD in patients with B-ALL before transplant may warrant additional consolidation therapy in order to achieve improvements in overall survival, leukemia-free survival, and toxicity. The development and implementation of this standardized, robust, and objective methodology to assess MRD status will aid the exploration of personalized therapeutic approaches and holds the potential of improving outcomes in patients with lymphoid malignancies.

The authors would like to thank the patients who participated in the studies and their families as well as the staff members who contributed to conducting the studies and development of this manuscript. Writing support during the development of the manuscript was provided by Audrey Demaree, PharmD, Adaptive Biotechnologies, Lori K. Pender, PharmD, MPH, BCOOP, Adaptive Biotechnologies, Emily Morton, BSc, and Leon Newman, PhD, of Cirrus Communications, an Ashfield Company, part of UDG Healthcare PLC, which was funded by Adaptive Biotechnologies, and complied with Good Publication Practice 3 guidelines (Balttisi WP, Wager E, Baltzer L, et al. Good publication practice for communicating company-sponsored medical research: GPP3. Ann Intern Med. 2015;163(6):461-464). Editorial assistance was provided by the Moffitt Cancer Center’s Scientific Editing Department by Paul Fletcher, PhD, and Daley Drucker, BA. No compensation was given beyond their regular salaries.

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