Impact Of Sequence Uniqueness on MRD Monitoring in NGS Immunoglobulin Sequencing: An Analysis Of Ig Loci Among >1800 Diffuse Large B-Cell Lymphoma Patients Tested By clonoSEQ®

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INTRODUCTION

Diffuse Large B-Cell Lymphoma (DLBCL) is an aggressive form of non-Hodgkin lymphoma that most commonly affects older adults. While ~80% of patients achieve complete response to frontline therapy, roughly half of those are cured while the other half relapse.[1] Salvage treatment for relapse is typically CAR-T therapy or transplant, either of which may be curative. Additionally, significant efficacy in even later lines of therapy has been demonstrated with the recently approved bispecific therapies and therapies still under investigation.

Due to the aggressiveness of DLBCL and the potential for cure offered by approved and investigational therapies, there is a recognized need for improved disease monitoring by assessment of measurable (minimal) residual disease (MRD) burden during and post-therapy. Such improved monitoring techniques provide additional distinct information to imaging and can clarify in instances in which imaging is equivocal.[1] clonoSEQ®,[2] is a next-generation sequencing CLIA LDT which monitors MRD levels from ctDNA of DLBCL patients and may be performed on a routine basis to monitor for disease response and recurrence.

- The results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.
- clonoSEQ® is available as an FDA-cleared in vitro diagnostic (IVD) test service provided by Adaptive Biotechnologies to detect minimal residual disease (MRD) in bone marrow from patients with multiple myeloma or B-cell acute lymphoblastic leukemia (B-ALL) and blood or bone marrow from patients with chronic lymphocytic leukemia (CLL). clonoSEQ is also available for use in other lymphoid cancers and specimen types as a CLIA-validated laboratory developed test (LDT). For important information about the FDA-cleared uses of clonoSEQ including test limitations, please visit clonoSEQ.com/technical-summary.[2]

RESULTS

Across our clinical and RUO offerings, 2162 ID samples have been received and included several different sample tissues and preparation methods. 1827 (84.5%) of these samples calibrated (ie, clonotypic/dominant sequences were identified). Calibration was most successful in FFPE and gDNA preparations from lymphatic tissue or extranodal masses with 1018/1101 (92.5%) calibrated samples. Least successful sample types included whole blood (54/151 [35.8%] calibrated) and skin (22/43 [51.2%] calibrated). These lower calibration rates are likely a reflection of disease burden not being present in these tissues among a large proportion of the patients tested.

For samples run on the clinical offering where the sample requirements are more stringent, the most successfully calibrated sample type is FFPE lymph tissue with a calibration rate of 308/320 (96.3%).

Fig 1: ID Samples Processed on Clinical Offering

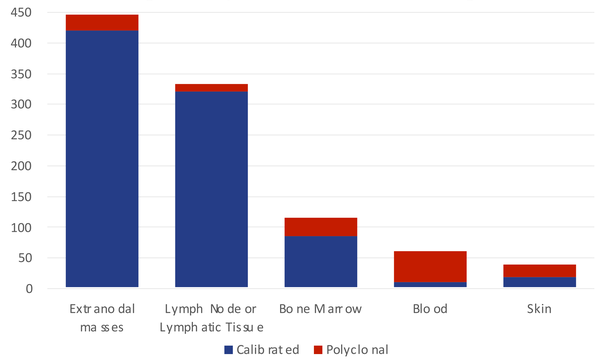

Fig 2: Distribution of Sequence Uniqueness by Ig Loci

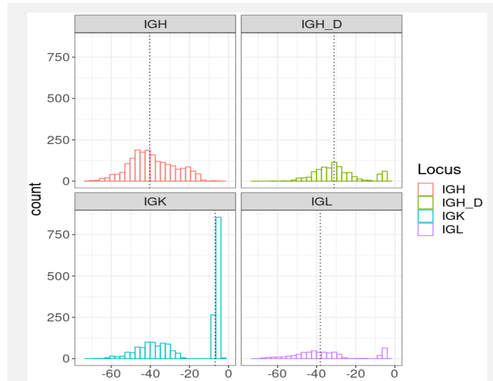

Figure 2: IgH (VJ), IgH (DJ), and IgL clonotypes have a higher median uniqueness relative to IgK clonotypes

Fig 3: Uniqueness Scores for Patients with Only 1 Dominant Sequence

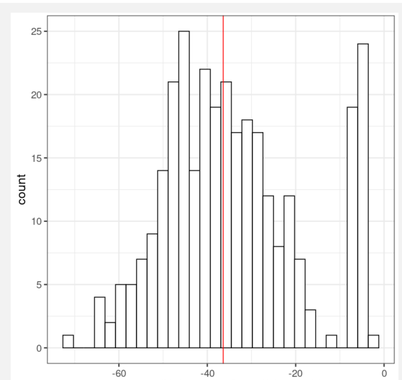

Figure 3: Only 3.0% of all patients have only low uniqueness sequences available for tracking

Across this population of 1805 DLBCL patients and 5168 clonotypic/dominant sequences, there was a range of 1-9 dominant sequences (avg 2.86; med 3) identified per patient. We assessed the distribution of uniqueness of these sequences across the Ig loci. As shown in Fig 2, IgH (VJ), IgH (DJ), and IgL clonotypes have higher median uniqueness scores relative to those from IgK. As has been well established, IgK sequences tend to be less unique than sequences from the other loci.

While the majority of patients have >1 dominant sequence, we investigated the fraction of patients with only one less unique sequence available for MRD assessment. Fig 3 indicates the uniqueness score for each patient with only one dominant sequence, with just 54 (3.0%) of all subjects having a dominant sequence with expected 'backgrounds' of > 1/100,000 of all Ig sequences.

METHOD

The clonoSEQ assay sequences the V(D)J regions (specifically, the CDR3) of Ig loci. A baseline (calibration) sample containing high tumor burden is sequenced to determine the clonotypic/dominant sequence(s). One aspect of the clonoSEQ algorithm assigns a uniqueness score to each dominant sequence based on analyses of Ig locus V, D, and J segments and non-templated nucleotides. This score reflects the probability that a sequence could be independently recreated in a non-malignant cell, including in another person, and is incorporated into the limit of detection (LOD). Consequently, sequences with a lower uniqueness must be observed at a higher rate to be above the LOD and reflect confidence they are tumor related in subsequent MRD samples. Previous reports have explored the uniqueness distribution of Ig loci but did not consider the impact of LOD on report interpretation.

Using data from our database, we assessed the characteristics of dominant sequences by sample type and Ig loci uniqueness among patients with orders from the clinical offering and from clinical trials. As of November 2023, our database contains 5168 dominant sequences from 1805 patients. 976 of these patients are from 28 clinical studies, and 829 are from use of the commercially available CLIA LDT assay.

CONCLUSIONS

Our analysis of >1800 DLBCL patients demonstrates that:

- Identification of dominant Ig sequences is most successful using lymph tissue
- The majority of patients rely on multiple dominant sequences for disease tracking
- In a small subset of patients (3.0%), disease is only trackable using one lower uniqueness sequence. In these cases, the LOD is particularly informative in providing guidance to contextualize the probability the sequence may not be associated with the disease being monitored
- MRD may be effectively tracked using clonoSEQ in all DLBCL patients with a calibrating ID sample.

REFERENCES

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