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Assessment of Capture and Amplicon-Based Approaches for the Development of a Targeted Next-Generation Sequencing Pipeline to Personalize Lymphoma Management

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Targeted next-generation sequencing panels are increasingly used to assess the value of gene mutations for clinical diagnostic purposes. For assay development, amplicon-based methods have been preferentially used on the basis of short preparation time and small DNA input amounts. However, capture sequencing has emerged as an alternative approach because of high testing accuracy. We compared capture hybridization and amplicon sequencing approaches using fresh-frozen and formalin-fixed, paraffin-embedded tumor samples from eight lymphoma patients. Next, we developed a targeted sequencing pipeline using a 32-gene panel for accurate detection of actionable mutations in formalin-fixed, paraffin-embedded tumor samples of the most common lymphocytic malignancies: chronic lymphocytic leukemia, diffuse large B-cell lymphoma, and follicular lymphoma. We show that hybrid capture is superior to amplicon sequencing by providing deep more uniform coverage and yielding higher sensitivity for variant calling. Sanger sequencing of 588 variants identified specificity limits of thresholds for mutation calling, and orthogonal validation on 66 cases indicated 93% concordance with whole-genome sequencing. The developed pipeline and assay identified at least one actionable mutation in 91% of tumors from 219 lymphoma patients and revealed subtype-specific mutation patterns and frequencies consistent with the literature. This pipeline is an accurate and sensitive method for identifying actionable gene mutations in routinely acquired biopsy materials, suggesting further assessment of capture-based assays in the context of personalized lymphoma management. *(J Mol Diagn 2018, 20: 203—214; https://doi.org/10.1016/j.jmoldx.2017.11.010)*

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In aggregate, lymphoid cancers are the fourth most common cancer in Canada and the only common cancer with increasing incidence—a robust but unexplained trend that has been documented for >50 years. Lymphoid cancers disproportionately affect younger patients, exaggerating their impact in terms of productive years of life lost. Moreover, lymphoid cancers are the only common cancers that can regularly be cured in a subset of patients, even when widely disseminated, indicating the potential to improve outcomes if currently available treatments are optimized and strategically applied.

Modern diagnosis and molecular assessment of lymphoid cancers currently relies on techniques such as immunohistochemistry, cytogenetics, fluorescence *in situ* hybridization, flow cytometry, and molecular genetics to classify lymphoid neoplasms into >35 distinct entities, each with distinct treatment implications.[1] However, in recent years, these standard procedures and classification schemes have been challenged by the emergence of sequencing techniques that have the potential to add unique diagnostic, prognostic, and predictive value to standard assessment.[2,3]

Recent examples of clinically applicable sequencing-based assays include the development of the M7-FLIPI in follicular lymphoma (FL), clinical trials exploring potential differential efficacy of lenalidomide or ibrutinib to standard chemotherapy for diffuse large B-cell lymphoma (DLBCL) patients with ABC-subtype–specific mutations, and (sub) clonal *TP53* mutations and ibrutinib-resistance associated mutations in chronic lymphocytic leukemia (CLL).[4–6] Thus, genomic profiling has the potential to deliver clinically relevant information that is otherwise missed using current testing. In addition, genomic profiling will generate a rich platform that can be exploited for extensive additional discoveries.

Next-generation sequencing technologies have been instrumental in accelerating discovery in cancer genomics via whole-genome sequencing (WGS), whole-exome sequencing, whole-transcriptome sequencing (RNA sequencing), and deep targeted sequencing. These technologies have been extensively used in disease-specific contexts to identify somatic mutations, understand clonal evolution, and, most recently, advance personalized medicine.[7,8] In contrast to genome-wide applications, targeted cancer sequencing panels, which focus on a select set of genes or gene regions that have known associations with cancer, allow for the rapid detection of a variety of somatic mutations on a single platform.[9–11]

Two methods are commonly used for such targeted approaches: capture hybridization–based sequencing and amplicon-based sequencing, each having its own advantages and disadvantages. A recent study that compared these two types of methods head-to-head indicates that amplicon-based approaches may be preferable for their simplified workflow and smaller amounts of required DNA.[12] However, hybridization-based strategies are less likely to miss mutations and also perform better with respect to sequencing complexity and uniformity of coverage.[12–14]

Herein, to determine the ideal targeted sequencing platform in the context of detecting actionable mutations in lymphoid cancer, we performed a systematic comparison of capture hybridization against amplicon sequencing with the aim of accurately detecting the full spectrum of mutations in the lymphoid cancer gene panel. Specifically, we describe the development and application of a targeted sequencing assay to identify mutations in routinely obtained formalin-fixed, paraffin-embedded (FFPE) lymph node samples (FL and DLBCL) and enriched B lymphocytes of CLL specimens. Our findings demonstrate the feasibility and outline the clinical utility of integrating a lymphoma-specific pipeline into personalized cancer care.

Materials and Methods

Patients and Materials

A cohort of matched tumor and normal DNA specimens was assembled from 229 patients (Supplemental Figure S1), including 30 CLL, 80 DLBCL, and 119 FL cases. FFPE tissue blocks and peripheral blood DNA samples for all FL and DLBCL cases were acquired from the BC Cancer Agency (Vancouver, BC, Canada) lymphoma tumor bank. Tumor DNA was extracted from 1 × 10 μm FFPE sections using the AllPrep DNA/RNA FFPE Kit (Qiagen, Hilden, Germany) or from 15 × 20 μm snap frozen tissue (FF) sections using the AllPrep DNA/RNA Mini Kit (Qiagen). Peripheral blood DNA was extracted using the FlexiGene DNA Kit (Qiagen). Genomic DNA samples were quality checked on agarose gels and quantified using Qubit dsDNA BR Assay (Thermo Fisher Scientific Inc., Waltham, MA).

For CLL, fresh peripheral blood samples were collected and processed within 24 hours to obtain pure tumor (>98% purity) and germline cell fractions by separation methods [CD19-negative RosetteSep (tumor) and CD19 RosetteSep (germline); StemCell Technologies, Vancouver, BC, Canada]. The germline cell fraction was further purified by fluorescence-activated cell sorting [negative sort using CD19-PECy5 (BioLegend, San Diego, CA), CD20-PECy7 (Beckman Coulter, Brea, CA), and DAPI (Sigma, St. Louis, MO)] on a BD ARIA cell sorter (BD Biosciences, San Jose, CA; >97% purity). Purified cell fractions were extracted using the Qiagen AllPrep DNA/RNA Mini Kit standard protocol.

For WGS, specimens for 66 of 229 patients from the original cohort were frozen and embedded in OCT compound for DNA and RNA extractions, as well as frozen sections for histological correlation. Constitutional DNA was extracted from peripheral blood leukocytes. FF surgical tissue resections were divided into sections (50 μm thick), and four sections were added to each tube of 400 μL RLT Plus buffer (Qiagen) containing tris (2-carboxyethyl) phosphine. Simultaneous purification of genomic DNA and total RNA from 3 to 11 tubes, selected for tumor content and cellularity, was performed using the AllPrep DNA/RNA Mini Kit on the automated robotic workstation, QIAcube (Qiagen).