Tumor exomes reveal new insights into cancer biology

Tumor exome sequencing from tissue biopsy samples enables a deeper understanding of the molecular landscape of cancers and guides biomarker discovery

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Studying the tumor mutational landscape provides valuable insights into mechanisms that drive tumor proliferation, survival, and response to therapy. Unlike conventional molecular profiling methods that only assess a limited set of commonly occurring mutations, whole-exome sequencing (WES) of tumors provides critical information about all the coding mutations that can influence tumor biology. Because the exome represents less than 2% of the genome, cancer exome sequencing using next-generation sequencing (NGS) technology is a cost-effective alternative to whole-genome sequencing (WGS).

Researchers at the IRCCS Istituto Romagnolo per lo Studio dei Tumori "Dino Amadori" (IRST) in Italy perform cancer WES from tumor biopsy samples to better understand the genetic underpinnings of tumor onset and progression, identify biomarkers, and predict response to therapeutic interventions. However, formalin-fixed paraffin-embedded (FFPE) biopsy samples can be challenging to process. After evaluating multiple library preparation and enrichment kits, Drs Paola Ulivi, Milena Urbini, and Gianluca Tedaldi at IRST recently implemented Illumina DNA Prep with Exome 2.0 Plus Enrichment* for their tumor WES projects. We spoke to Drs Ulivi, Urbini, and Tedaldi about their experience with implementing this kit and the potential of their research on the coding regions of solid tumors to transform cancer care in the future.

* Illumina DNA Prep with Exome 2.0 Plus Enrichment is now available as Illumina DNA Prep with Exome 2.5 Enrichment.

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Can you provide an overview of the IRST-IRCCS?

Milena Urbini (MU): IRST-IRCCS is part of the Italian Ministry of Health Network, which is a group of specialized institutes providing comprehensive health care and research across various disciplines in Italy. The Institute is an Italian center of excellence entirely dedicated to treatment, research, and training in the field of oncology. Our research group, the Translational Oncology Unit, is part of the IRST-IRCCS Bioscience Laboratory. Our mission is the study and discovery of molecular alterations and biomarkers characteristic of solid tumors to support personalized medicine efforts for cancer patients. Research in our laboratories focuses on thoracic, gastrointestinal, urogynecological, and breast tumors.

What techniques do you routinely perform in your laboratory?

Paola Ulivi (PU): Our primary research interests are tumor molecular characterization, defining therapy resistance or sensitivity, and delineating molecular changes that occur in tumors to identify prognostic biomarkers. Mainly, we work on tumor biopsy samples, FFPE tissue, in particular, for WGS or WES, or RNA sequencing (RNA-Seq) for transcriptomics analysis. We also work with liquid biopsy, cell-free circulating DNA, extracellular-vesicle (EV) microRNA, and circulating tumor cells. On these types of biological materials, we perform NGS analysis using both targeted gene panel assays and comprehensive genomic profiling approaches to detect DNA mutations and perform microRNA profiling, single-cell analysis, and transcriptomic analysis.

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Why is tumor WES important? How does it fit into your Institute's research goals?

MU: Our research goals are to identify prognostic or predictive biomarkers, define therapy resistance or sensitivity, and identify cancer-related genetic molecular alterations. We generally study biological samples collected from large patient cohorts. Frequently, we work with FFPE tissue or liquid biopsy for which the quality is variable and the quantity of DNA is limited. Tumor WES is an attractive approach, as it is a cost-effective method that provides a comprehensive picture of the tumor mutational landscape by focusing only on the coding regions of the genome. Though several large targeted panels have been developed, they do not provide a comprehensive view of tumor biology. WGS approaches are not practical for our research purposes either as they are more expensive and difficult to manage over a larger cohort of samples.

What challenges do you encounter with tumor WES?

Gianluca Tedaldi (GT): The first limiting step in WES experiments is obtaining the quality and quantity of input DNA necessary for library generation. The library preparation approach needs to be compatible with degraded DNA extracted from FFPE tissue samples or from limited amounts of DNA available from liquid biopsy samples. Secondly, though WES is a cost-effective method in terms of sequencing cost and data storage and analysis, it can be time consuming in hands-on time for library preparation. Fortunately, exome sequencing protocols can be automated on many liquid handlers, though optimization steps may need to be performed depending on the library kit and specific liquid handler model. We chose to implement Illumina DNA Prep with Exome 2.0 Plus Enrichment on our Hamilton STARlet, as minimal optimization was needed.

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Did you consider any other library preparation kits for WES? How did you evaluate them?

MU: FFPE biopsy samples typically contain degraded DNA and there is a risk of incurring low-quality sequencing. We require a protocol for library preparation with exome enrichment that would produce high-quality data from FFPE tissue. Also, this protocol must be easy to use and be automation-compatible. We decided to test the performance of four different commercially available library preparation kits for exome enrichment on eight FFPE tissue from lung cancer biopsies. Germline blood samples were sequenced in parallel for somatic variation calls. For each kit tested, we followed the manufacturer recommendations for library preparation. Libraries were sequenced together on the Illumina NovaSeq™ 6000 System. At least 100× coverage was obtained for FFPE tumor samples, while germline controls were sequenced at 50× coverage. Each library was aligned on the reference genome and sequencing statistics were evaluated using Picard and DRAGEN™ secondary analysis pipelines. We evaluated the percentage of reads aligned on target regions, uniformity of coverage, and insert size. The number of somatic mutations identified, their variant allele frequencies, and false positive/false negative call rates were compared between the four kits tested. We also assessed duplication rate, which can be an issue when working with FFPE samples.

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"...the Illumina DNA Prep with Exome 2.0 Plus Enrichment protocol is fast, user-friendly, and easy to implement on the Hamilton liquid-handling platform."

Why did you choose Illumina DNA Prep with Exome 2.0 Plus Enrichment for your tumor WES projects?

GT: Compared to other library preparation kits tested, the Illumina DNA Prep with Exome 2.0 Plus Enrichment protocol is fast, user-friendly, and easy to implement on the Hamilton liquid- handling platform. We have extensive experience with Illumina library preparation kits and so have good confidence with the Illumina exome enrichment protocol. Because the Illumina DNA Prep with Exome 2.0 Plus Enrichment kit is similar to other Illumina library preparation solutions we have used, we could implement it independently without the need for support. The high performance of the Illumina DNA Prep with Exome 2.0 Plus Enrichment kit compared to the other commercially available exome kits is what led us to choose this solution for our lung cancer WES project. We are testing this kit on other research projects as well. We are working on a project to determine if tumor WES can be applied to molecular characterization of colon cancer samples. The goal of this clinical research study is to compare the results obtained by WES with the currently used gene-panel–based workflow. WES analysis is performed in parallel for both germline and somatic DNA. Once completed this project will demonstrate the feasibility of tumor WES in a clinical setting.

How can tumor WES benefit patients in the future?

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PU: As a translational oncology unit we primarily work on clinical research samples, so our experiments do not directly impact clinical management in patients. However, we anticipate that the implementation of WES in clinical practice could have significant impacts on patient care in the future. As new biomarkers and targeted therapies are continuously developed, the routine application of WES for cancer patients could provide a complete picture of all molecular alterations present in their tumors. This knowledge can then be readily used for biomarker validation and clinical decision making. We expect that tumor WES will also play an important role in designing clinical trials in the near future.

How will NGS technology impact the future of cancer care?

MU: The IRST-IRCCS mission is to improve the quality of care and treatment for cancer patients. In the translational research space, our goal is to provide an increasingly complete analysis at the molecular level for all patients who access treatments at our institute. A single test that provides all information is the ideal goal, though it is not currently feasible. For example, it would be great to perform WGS for every patient seeking care at IRST-IRCCS.

However, currently WGS requires time for analysis, space for the vast amount of data generated, and expertise to interpret the results. With WGS, though we can get information for the entire genome, this can come with a loss of sensitivity leading to false negative results. Transcriptomics may also play an important role in the clinic by detecting gene fusions which are the targets of some drugs. If we can perform WGS and transcriptomics from a small amount of DNA or RNA, we will have all the information needed for clinical decision making. An approach that also incorporates liquid biopsy will enable clinicians to monitor the patient over time, which can be extremely useful because we know that cancers can change during treatment. Though there are many problems to solve, NGS technology truly has the potential to transform the future of cancer care.

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Learn more

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