New assay identifies clinically relevant gene fusions in pediatric tumors more accurately and efficiently

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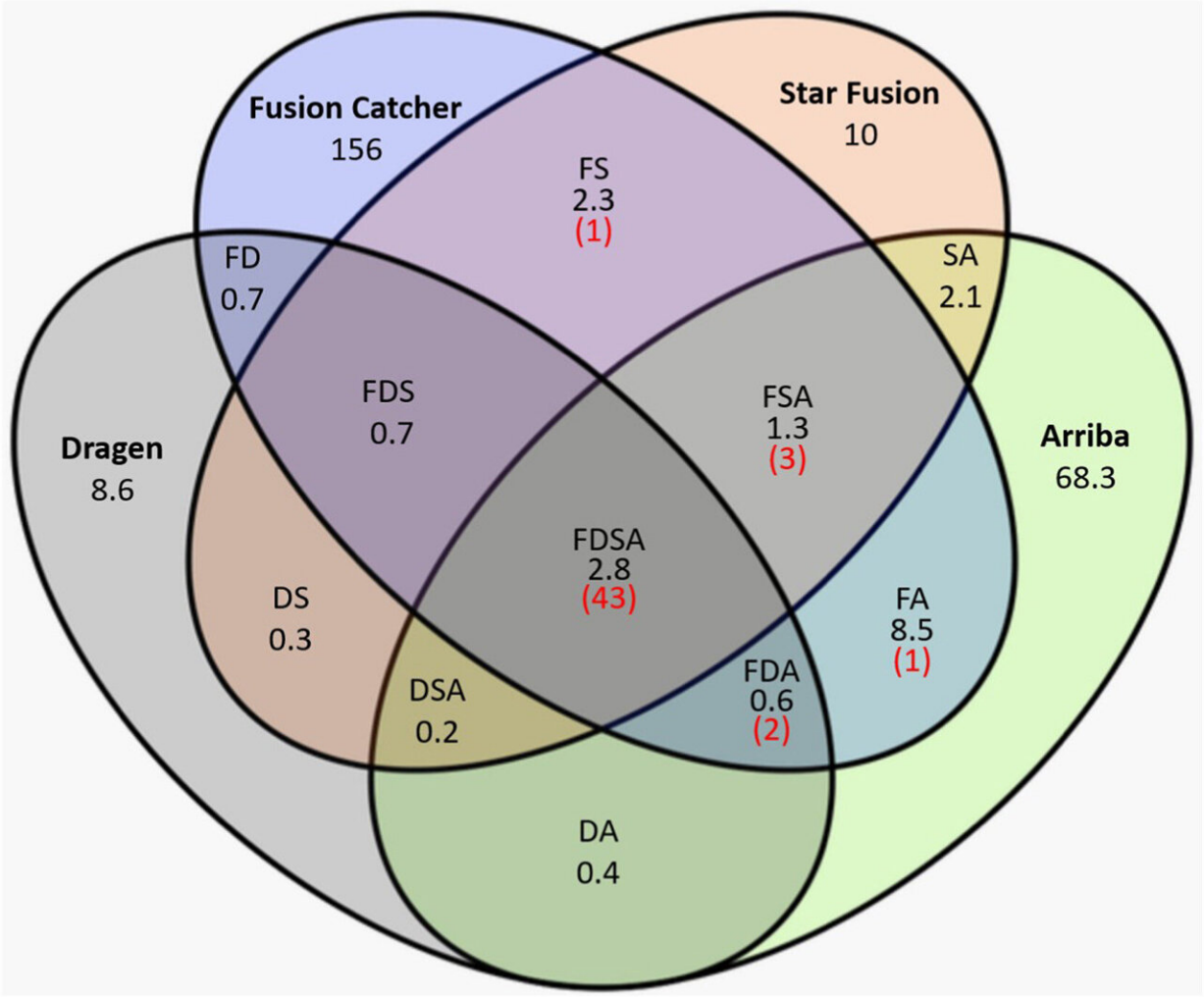

Venn diagram of mean number of all fusions (in black) called by the four callers, and the mean number of reportable positive fusion calls (in red). A, Arriba; D,

Dragen; F, FusionCatcher; S, STAR-Fusion. Credit: *The Journal of Molecular Diagnostics* (2023). DOI: 10.1016/j.jmoldx.2023.11.003

Identification of specific gene fusions is critical for the successful targeted treatment of pediatric cancer patients. Researchers at Children's Hospital Los Angeles have developed a novel assay that automatically integrates the data from multiple fusion identification tools (callers) and efficiently and accurately identifies clinically relevant gene fusions in pediatric tumors.

Their results are reported in *The Journal of Molecular Diagnostics*.

Gene fusions are an important class of driver alterations in cancer that occur when parts of two different genes join. Numerous gene fusions have been identified across different types of cancer, especially in pediatric malignant cancers. For example, the PAX3-FOXO1 fusion gene is found in alveolar rhabdomyosarcoma but not in embryonal rhabdomyosarcoma.

Targeted testing for gene fusions of interest at the DNA or RNA level is currently in clinical use as it has diagnostic, prognostic, and therapeutic significance.

Lead investigator Jonathan Buckley, MD, Center for Personalized Medicine, Department of Pathology and Laboratory Medicine, Children's Hospital Los Angeles; and Keck School of Medicine of USC, explains, "Accurate identification of gene fusions in pediatric tumors is critical for establishing a diagnosis and determining optimal treatment."

"Unfortunately, the leading software packages for calling fusions, based on genomic analysis of tumor tissue, yield many false positives and/or

fusions of no clinical importance, and they differ substantially in the sets of fusions called. Therefore, there is a compelling need for a tool to assist the clinical molecular geneticist to filter and prioritize the fusion calls to maximize efficiency and accuracy."

Dr. Buckley and his co-investigators developed, tested, and validated a new exome capture–based RNA-sequencing (RNAseq) assay. They used the Twist Bioscience Comprehensive Exome capture kit with RNA from fresh, frozen, or formalin-fixed samples from patients with pediatric hematologic malignancies and solid tumors and a gene fusion detection assay that employs four fusion callers (Arriba, FusionCatcher, STAR-Fusion, and Dragen).

The new bioinformatics platform detects fusions, prioritizes them, and custom curates downstream processes for consensus fusion calling with high accuracy and efficiency.

While the four fusion-caller software packages are in wide use, their results typically include a large number of false-positive calls and/or identification of fusions of no clinical value. This presents a significant challenge in clinical reporting, both in terms of efficiency and accuracy.

The investigators were initially surprised to note the differences among the sets of fusions identified by the four callers given identical RNAseq data processes, similar approaches, and the same end goal.

Dr. Buckley noted, "We were pleasantly surprised to determine that integration of data from the four callers, and application of ad hoc ranking methods based on both the genomic data and references sources (related to the reported fusions and their component genes), was highly effective in highlighting the key clinical fusion(s)."

The software proved highly effective in pinpointing known clinically

relevant fusions, ranking them first in 47 of 50 (94%) samples. To highlight the usefulness of the new assay and to show the importance of a genome-wide and nontargeted method for fusion detection in pediatric cancer, the researchers present findings from three diagnostically challenging cases.

The existing OncoKids DNA and RNA-based next-generation sequencing (NGS) assay used at the center did not identify driver gene fusions in these patients. However, the new method found disease-related gene fusions that were then confirmed by other established sequencing tests.

Dr. Buckley noted, "The full spectrum of gene fusions that function as drivers of tumorigenesis in pediatric tumors is still unknown. One gene can fuse with one or even hundreds of partner genes with the same net biologic effect. A genome-wide approach is therefore necessary when the expected or common gene fusions are not identified by routine methodologies."

Senior author Jaclyn A. Biegel, Ph.D., Children's Hospital Los Angeles and Keck School of Medicine of USC, concluded, "Our studies demonstrate that the combination of an exome capture–based approach for RNAseq and a bioinformatics platform to streamline the analysis is both robust and efficient to accurately identify pathogenic gene fusions in bone marrow samples as well as fresh, frozen, and formalin-fixed tissue specimens from pediatric cancer patients."

"Although we validated this for clinical use for childhood tumors, these approaches can easily be extended to adult patients with hematologic malignancies and solid tumors."

More information: Jonathan Buckley et al, An Exome Capture-Based RNA-Sequencing Assay for Genome-Wide Identification and

Prioritization of Clinically Important Fusions in Pediatric Tumors, *The Journal of Molecular Diagnostics* (2023). DOI: 10.1016/j.jmoldx.2023.11.003