Uncovering Gene Fusions with 3D Genomics: From Clinical Validation to Actionable Insights for Undiagnosable Solid Tumors

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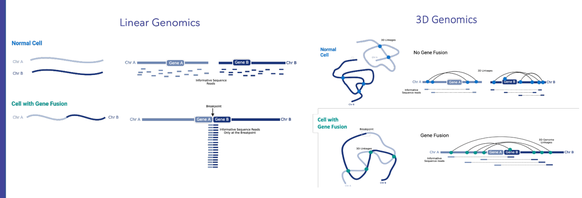
Introduction

Structural rearrangement biomarkers, such as translocations and gene fusions, have broad clinical utility for cancer patients including for accurate diagnosis, early detection, prognosis, and selection of optimal treatment regimens.

However, detecting translocations or gene fusions in tumor biopsies can be difficult for various reasons:

- Karyotyping is low resolution, and fluorescence in situ hybridization (FISH) assays are low throughput, biased, and often do not reveal the fusion partner.
- RNA-seq does not perform well in FFPE tissue blocks due to RNA degradation, low transcript abundance, and/or RNA panel design.
- Clinical NGS panels often fail to yield clear genetic drivers, in part because they are predominantly focused on coding regions of the genome and do not detect fusions outside of the targeted gene body such as those described in lymphoma, leukemia and other various solid tumor types[1-5].

3D genomics using Arima Hi-C technology offers a DNA-based partner-agnostic approach for detection of translocations and gene fusions in clinically relevant sample types, including FFPE tissue blocks[6-8].



Methods

To overcome these challenges, we developed a novel DNA-based partner-agnostic approach for identifying fusions from FFPE tumors using 3D genomics based on Arima-HiC technology, in some cases with target enrichment (Capture HiC), and NGS. Using this approach, we have profiled 164 tumors across tumor types.

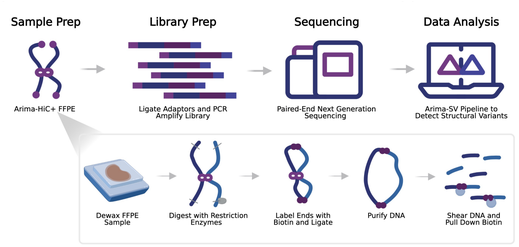


Figure 1. The Arima HiC+ FFPE Workflow[9]. Sample Prep: FFPE tissue scrolls were dewaxed, and the tissue rehydrated; then underwent chromatin digestion, end-labeling, and proximity ligation prior to DNA purification per the Arima-HiC FFPE protocol. Library Prep and Sequencing: Purified DNA was next prepared as a short-read sequencing library and sequenced on a NovaSeq.

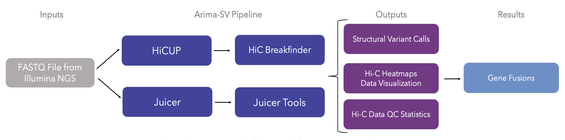


Figure 2. Data Analysis Workflow[10] FASTQ files input into the Arima-SV pipeline which enable the calling of variants, production of HiC heatmaps for identification of gene fusions.

For clinical concordance studies, we performed Arima Capture-HiC using a custom target enrichment panel for 884 cancer genes.

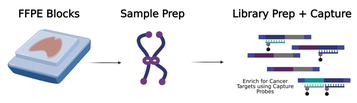


Figure 3. Detection of Gene Fusions in FFPE using Capture HiC. The workflow used on patient FFPE samples were subjected to Arima Capture HiC using a custom panel design for 884 known cancer-related genes.

Findings

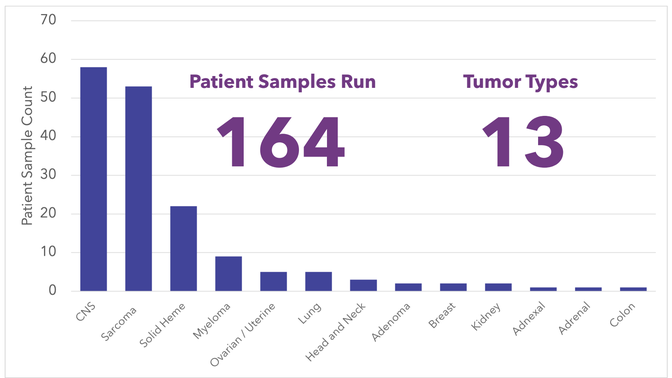


Figure 4. Patient samples profiled via 3D genomics using Arima technology. Bar plot showing the total number of patient samples profiled by tumor type.

Tumor Type	Fusion SEQ'er	Capture HiC
Adenoma	*CTNNB1-PLAG1*	*CTNNB1-PLAG1*
Adnexal	*WHSC1L1-NUTM1*	*WHSC1L1-NUTM1*
Breast	*ETV6-NTRK3*	*ETV6-NTRK3*
Colon	*EML4-NTRK3*	*EML4-NTRK3*
Lung	*CD47-MET*	*CD47-MET*
Lung	*KIF5B-NTRK1*	*KIF5B-NTRK1*
Lung	*MET-ZBTB20*	*MET-ZBTB20*
Lung	*SLC34A2-ROS1*	*SLC34A2-ROS1*
Lung	*KIF5B-RET*	*KIF5B-RET*
Lung	*KIF5B-RET*	*KIF5B-RET*
Lung	*ARHGEF18-INSR*	*ARHGEF18-INSR*
Sarcoma	*BCOR-ZC3H7B*	*BCOR-ZC3H7B*

Table 1. Subset of concordance study. Table showing concordance between RNA-based Fusion SEQ'er and 3D genomics-based Arima custom capture HiC for a subset of patient tumors profiled.

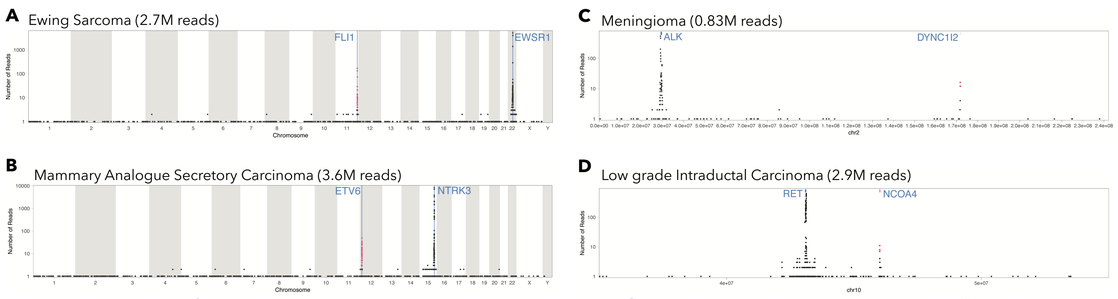


Figure 5. Examples of inter-chromosomal and intra-chromosomal gene fusions detected by custom capture HiC. A. Manhattan plot representation of an *EWSR1-FLI1* gene fusion detected with probes targeting *EWSR1*. **B.** Manhattan plot representation of an *ETV6-NTRK3* gene fusion detected with probes targeting *NTRK3*. **C.** Manhattan plot representation of a *DYCN1I2-ALK* gene fusion detected with probes targeting *ALK*. **D.** Manhattan plot representation of an *NCOA4-RET* gene fusion detected with probes targeting RET. For each tumor, the number of mapped reads are shown.

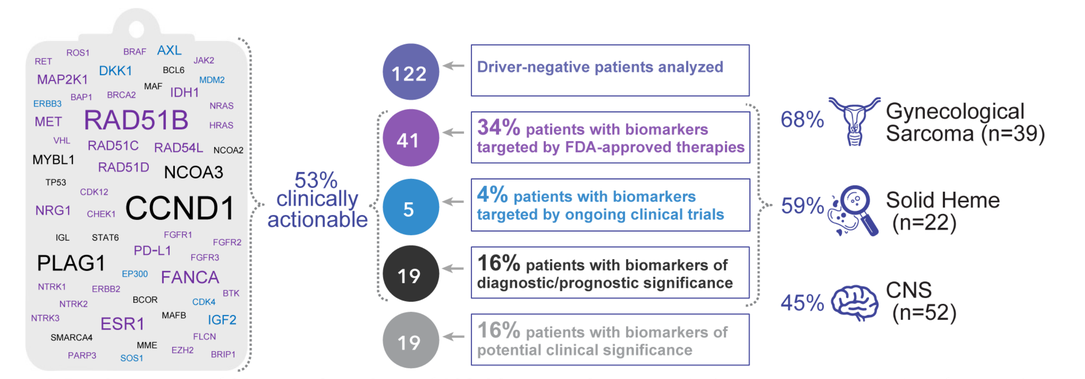


Figure 6. Result summary of 3D genomic analyses of 122 driver-negative tumors. In the center, a categorization of our results, including the total number of driver-negative patient tumors analyzed, and a binning of patients based on the clinical significance of their biomarkers according to the NCCN biomarker compendium, OncoKB, and World Health Organization (WHO) guidelines. On the left, a depiction of the clinically actionable biomarkers, color-coded by their tier of clinical significance. On the right, a depiction of the number of tumors from our top three most common indications, and the percentage of those with a clinically actionable biomarker.

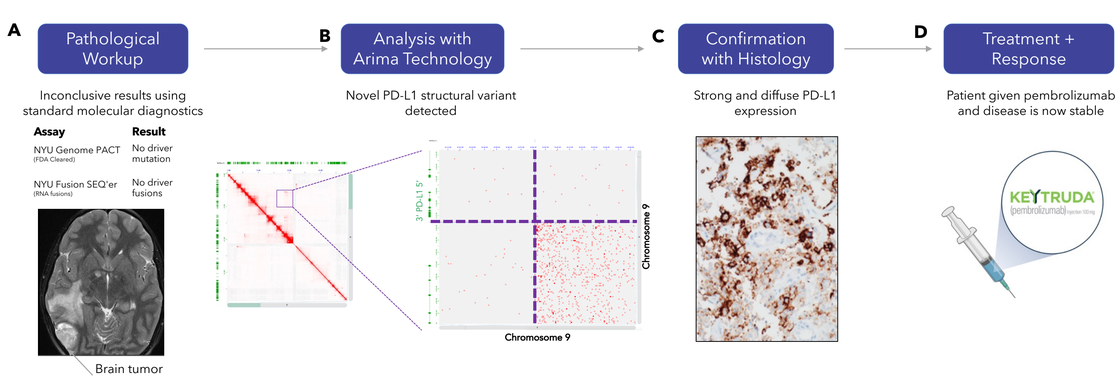


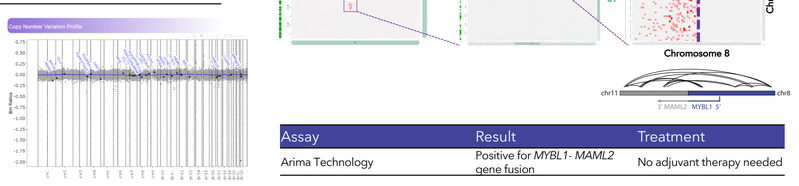
Figure 7. Case Study: 3D genome analysis alters the course of patient management in a pediatric glioma patient. A. A pediatric patient with Stage 2 glioma was initially treated with a subtotal resection of the tumor, and six months post-surgery experienced rapid progression. Comprehensive DNA and RNA sequencing of the primary and relapsed tumor was inconclusive, with no driver mutations identified. **B.** A subsequent analysis of the relapsed tumor by Arima revealed a novel PD-L1 translocation as shown in the Hi-C heat map. **C.** Immunohistochemical staining showed strong and diffuse PD-L1 expression. **D.** The patient was given pembrolizumab and her disease status has been stable for ~9 months.

A

Assay	Result	Treatment
DNA Next Generation Sequencing	Negative for IDH1/2 mutations	Total resection; Unclear if adjuvant therapy required
RNA Fusion required SEQ'er	Negative for gene fusions	

Brain Tumor Methylation Classifier

Class Score	Methylation Family	Interpretation
0.983	LGG, MYB	Positive
0.004	MTGF_GBM	
0.002	MTGF_IDH_GLM	
0.001	SUBEPN, SPINE	
0.001	LGG, RGNT	



Assay	Result	Treatment
Arima Technology	Positive for *MYBL1- MAML2* gene fusion	No adjuvant therapy needed

Figure 8. Case study: 3D genome analysis alters the course of patient management in a prospective glioma patient. A. Summary of patient presentation, initial treatment, and pathologic workup performed by NYU Langone, resulting in a brain tumor classification result of a probable *MYB/MYBL1* low grade glioma, but lacking any detectable diagnostic *MYB* or *MYBL1* gene fusion. **B.** 3D genome analysis identifies a *MYBL1-MAML2* gene fusion, supporting the *MYBL1* low grade glioma diagnosis, ultimately sparing the patient from adjuvant chemotherapy post-resection.

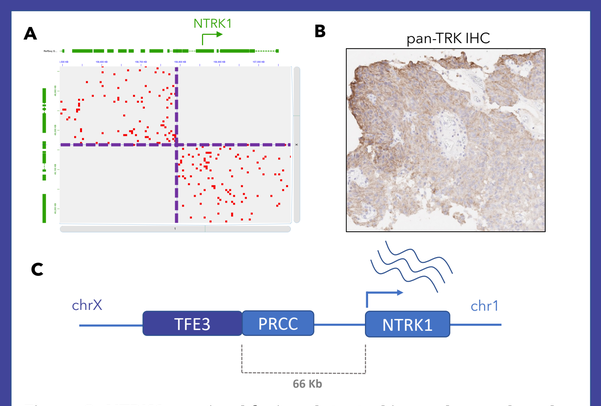


Figure 9. *NTRK1* proximal fusion detected in a subependymal giant cell astrocytoma with 3D genomics. A. Hi-C heat map showing *NTRK1* on chr1, 66kb downstream from a *TFE3-PRCC* gene fusion. **B.** pan-TRK Immunohistochemical staining, demonstrating NTRK protein expression in tumor cells (adjacent normal brain tissue with negative staining, not shown). **C.** Schematic depiction of *NTRK1* proximal fusion event.

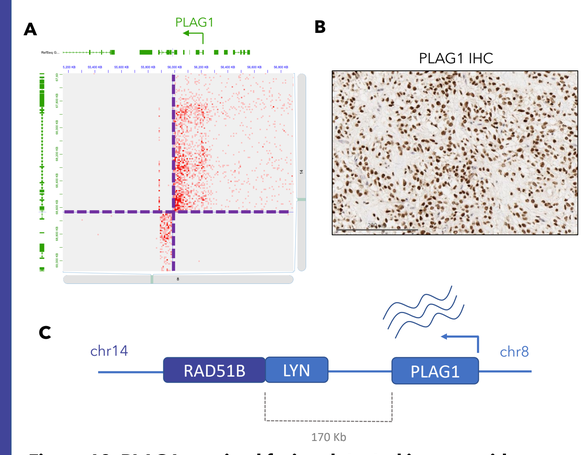


Figure 10. PLAG1 proximal fusion detected in a myxoid leiomyosarcoma with 3D genomics. A. Hi-C heat map showing PLAG1 on chr8, 170kb downstream from a RAD51B-LYN gene fusion. **B.** PLAG1 immunohistochemical staining, demonstrating PLAG1 protein expression in tumor cells. **C.** Schematic depiction of PLAG1 proximal fusion event.

Conclusions

- 3D genomics is concordant with NYU Fusion SEQ'er, a CLIA-validated RNA-based fusion panel
- 3D genomics identifies clinically actionable biomarkers in 53% of driver-negative tumors
- In a limited number of prospective cases, 3D genomics has identified previously undetected fusions, leading to changes in patient management
- 3D genomics readily identifies "proximal fusions" with breakpoints outside the cancer gene body, which may lead to activation of druggable targets or diagnostic biomarkers such as *NTRK1* and *PLAG1*, respectively.

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