Fusion and rearrangement (RE) detection using DNA and RNA-based comprehensive genomic profiling (CGP) of sarcomas

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BACKGROUND

Actionability of a growing subset of gene fusions and rearrangement (RE) is well established, with several alterations linked to approved targeted therapies. While there are FDA-approved assays for DNA-based detection of key recurring actionable RE sarcomas can be enriched for rare RE not comprehensively covered on DNA panels.

METHODS

- DNA and RNA comprehensive genomic profiling (CGP; FoundationOneHeme®) was performed on 9,969 sarcoma tissue specimens
- DNA and RNA was co-extracted from up to1.2 mm³ of FFPE tissue.
- Adaptor ligation-based hybrid capture-based sequencing was performed for up to 406 cancer-related genes and select introns from 28 genes commonly rearranged in cancer, and 265 RNA fusion genes.
- Mean coverage depth was >600X
- Base substitutions, insertions and deletions (short variants; SV), rearrangements, and copy number changes were assessed
- Actionable genes: NTRK1/2/3, BRAF MET, ALK, ERBB2, EGFR, FGFR1/2/3, ROS1, RET, NRG1
- Diagnostic genes/fusions: EWSR1, STAT6-NAB2, BCOR-ZC3H7B, BCOR-CCNB3, FOX01-PAX3/7
- *ALK* fusions with breakpoints in intron 18, exon 19, or intron 19 were considered canonical
- Rearrangements where DNA detected a non-fusion and RNA detected a fusion were classified as "DNA resolved by RNA"
- DNA breakpoints detected outside of baited regions are due to incidental coverage from fully baited areas or baiting of the partner gene.

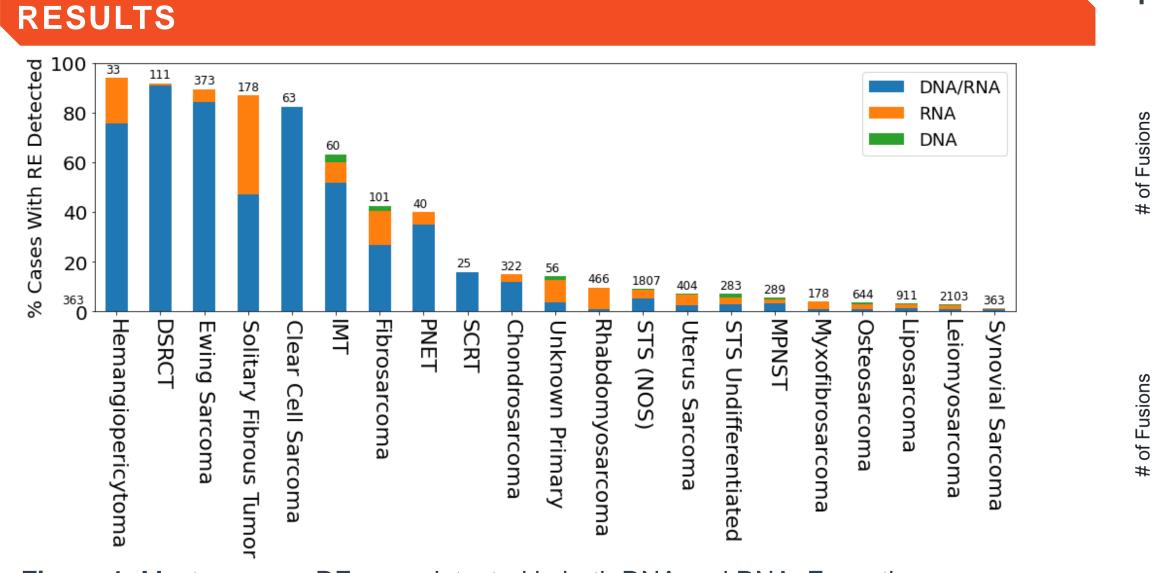


Figure 1: Most sarcoma RE were detected in both DNA and RNA. Exceptions are seen for fusions with rare breakpoints not covered by DNA baiting, particularly in hemangiopericytomas, solitary fibrous tumors, and rhabdomyosarcomas. Total cases indicated by bar label

LK Canonical-	0	0	1	0	0	31	2	0	0	0	0	0	7	1	1	0	0	0	12	0	Fusion Count	
lon-canonical-	0	0	0	0	0	1	0	0	0	0	0	0	2	2	2	0	0	0	27	0		
BCOR-	0	1	1	0	0	0	0	1	0	0	1	0	14	14	0	0	0	0	3	1		
BRAF	0	0	0	0	0	0	0	0	0	1	1	1	9	2	3	1	0	1	4	0		
EGFR-	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	1	0	0	0	0		
EWSR1-	1	101	331	0	52	0	26	14	4	43	2	8	58	2	3	2	1	13	3	2		
FGFR1-	0	0	0	0	0	0	0	0	0	2	0	1	4	2	1	2	2	4	0	0		
FGFR2-	0	0	0	0	0	0	1	0	0	0	1	0	6	1	0	0	1	0	0	1		
FOXO1-	0	0	0	0	0	0	0	0	0	0	0	33	1	0	0	0	0	0	0	0		
NTRK1-	0	0	0	0	0	0	4	1	0	0	1	0	18	1	4	4	0	2	2	0		
NTRK2-	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	0	0	5	0	0		
NTRK3-	0	0	0	1	0	2	9	0	0	0	1	0	10	1	0	5	1	1	3	0		
RET-	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	2	0		
ROS1-	0	0	0	0	0	4	0	0	0	0	0	0	5	1	2	1	1	2	0	0		
STAT6-	30	0	1	154	0	0	1	0	0	1	1	0	27	1	2	0	0	1	1	1		
	Hemangiopericytoma	DSRCT	Ewing Sarcoma	SFT	Clear Cell Sarcoma	IMT	Fibrosarcoma	PNET	SCRT	Chondrosarcoma	Unknown Primary	Rhabdomyosarcoma	STS (NOS)	Uterus Sarcoma	STS Undifferentiated	MPNST	Myxofibrosarcoma	Liposarcoma	Leiomyosarcoma	Synovial Sarcoma		
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Figu while non-canonical ALK fusions in LMS are frequently detected with RNA only. Breakpoints covered by DNA baiting are in red boxes.

SULTS NTRK1 intron intron exon 9 intron intron intron intron intron intron intron 10 12 9 8 11 NTRK3

exon 3 intron 3 intron 5 intron 10 intron 11 intron 13 intron 14 intron 17 RNA DNA Resolved by RNA DNA/RNA DNA

ure 4: Of 36 NTRK1/3 fusions detected in DNA (6 DNA only; 30 DNA + RNA), 79% were irmed in RNA. An additional 37 fusions were detected in RNA only, 100% were outside e DNA baited region (*NTRK1* intron 7, 8, & 9; *NTRK3* no intron baiting) Breakpoints ered by DNA baiting are in red boxes.

NCLUSIONS

most cases rearrangements were detected in both DNA/RNA. 6% (882/914) of all fusions detected in DNA were confirmed in RNA. 25% (316/1271) of fusions were only detected in RNA and 3% (41/1271) had complex DNA rearrangements resolved by RNA. RNA baiting increased the ensitivity for atypical fusions with noncanonical breakpoints which may not be overed by DNA baiting.

For ALK and NTRK1/3 non-fusion DNA rearrangements, RNA further resolved ne event: either an actionable RNA fusion was detected, or no RNA event was bserved to support the actionability of the DNA finding

sclosures: KG was a member of the Foundation Medicine, Inc. Advisory Board MR, BD, KT, JH, GO, JV, AW, and ABS are employees at Foundation Medicine, Inc., a wholly-owned subsidiary of Roche and report equity ownership in Roche.

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