ALK Fusion Detection by RNA Next-Generation Sequencing (NGS) Compared to DNA in a Large, Real-World Non-Small Cell Lung Cancer (NSCLC) Dataset

Wade Iams[1], Rotem Ben-Shachar[2], Lisa Gai[2], Kyle Beauchamp[2], Nitya Sharma[2], Sumaiya Islam[2], Justin Guinney[2], Halla Nimeiri[2], Ryan Gentzler[3], and Ezra Cohen[1,4]

[1]Division of Hematology/Oncology, Vanderbilt University, TN, USA // [2]Tempus Labs, Inc., Chicago, IL // [3]Division of Hematology/Oncology, University of Virginia, Charlottesville, VA, USA // [4]Division of Hematology/Oncology, University of California San Diego, CA, USA // **Correspondence:** Dr. Wade Iams, wade.t.iams@vumc.org

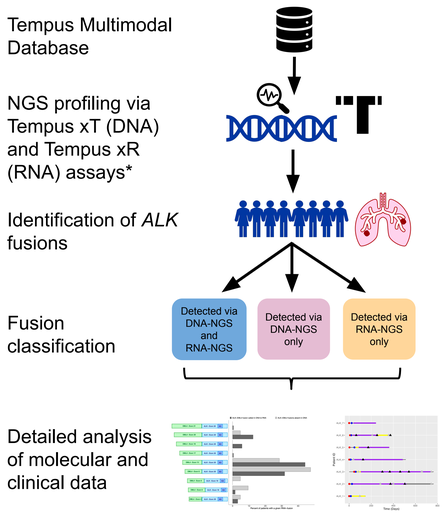
Publication #: 182P

INTRODUCTION

While DNA NGS can detect clinically actionable fusions in tumors, technical and biological limitations may lead to false negatives, preventing patients from receiving approved targeted matched therapies associated with outcome benefit. RNA-NGS is recommended by ESMO guidelines to maximize fusion detection but is not widely used clinically. In a large, real-world dataset, we quantify the benefit of concurrent RNA-NGS and DNA-NGS for *ALK* fusion detection in patients with advanced NSCLC.

METHODS

We retrospectively analyzed de-identified stage IIIB-C and IV NSCLC samples sequenced with the Tempus xT and xR assays (DNA-seq of 595-648 genes; enhanced whole-exome capture RNA-seq). *ALK* fusion prevalence in these samples was compared to public data from Dana Farber Cancer Institute (DFCI, N=4,497) and Memorial Sloan Kettering Cancer Center (MSKCC, N=5,317). Therapeutic adoption was analyzed for cases with ≥90 days of first-line medication data available.



*DNA-seq of 595-648 genes at 500x coverage; Enhanced whole-exome capture RNA-seq

Figure 1. Study overview

Acknowledgements: We thank Zach Rivers and Adam Hockenberry for data visualization support and poster review.

SUMMARY

- This large-scale, real-world analysis of 7,428 advanced NSCLC patients receiving NGS testing demonstrates that RNA-NGS increases the detection of *ALK* fusions by 18% compared to DNA-NGS alone.
- Increased *ALK* fusion detection is likely to translate to a larger number of patients matched to and receiving targeted approved therapies, motivating more widespread adoption of RNA-NGS into routine clinical care.

RESULTS

Patient demographics and clinical characteristics

	ALK fusion+ (n = 217)	*ALK* fusion- (n = 7,211)	p-value*
Gender			0.039
Female	54.8% (119)	46.2% (3333)	
Male	40.1% (87)	48.5% (3497)	
Unknown	5.1% (11)	5.3% (381)	
Age at Diagnosis			<0.0001
Min	28.0	18.5	
25%	49.6	61.4	
Median	60.0	68.0	
75%	69.1	75.2	
Max	>90	>90	
Unknown	5.0% (11)	5.2% (381)	
Smoking Status			<0.0001
Current or Former Smoker	22.6% (49)	72.5% (5228)	
Non-Smoker	60.8% (132)	12.1% (869)	
Unknown	16.6% (36)	15.4% (1114)	
Race			0.016
American Indian or Alaska Native	0.0% (0)	0.2% (18)	
Asian	6.0% (13)	2.8% (205)	
Black or African American	5.1% (11)	7.6% (551)	
Native Hawaiian or Other Pacific Islander	0.0% (0)	0.0% (1)	
White	44.7% (97)	50.0% (3608)	
Other Race	6.0% (13)	3.3% (237)	
Unknown	38.2% (83)	35.9% (2591)	
Histology			<0.0001
Adenocarcinoma	85.7% (186)	62.1% (4479)	
Adenosquamous	4.6% (10)	2.9% (209)	
Carcinoma, other or not specified	1.8% (4)	10.0% (720)	
Squamous	2.3% (5)	19.1% (1380)	
Unknown	5.5% (12)	5.9% (423)	
Stage			0.99
Stage 3B	6.9% (15)	6.9% (498)	
Stage 3C	1.8% (4)	1.9% (135)	
Stage 4	81.1% (176)	81.8% (5897)	
Other	1.8% (4)	1.8% (128)	
Unknown	8.3% (18)	7.7% (553)	

Table 1: Demographic and clinical information stratified according to *ALK* fusion status. *Mann-Whitney U test for age, Pearson's Chi-squared test for categorical values.

ALK fusion prevalence and breakdown by method-of-detection

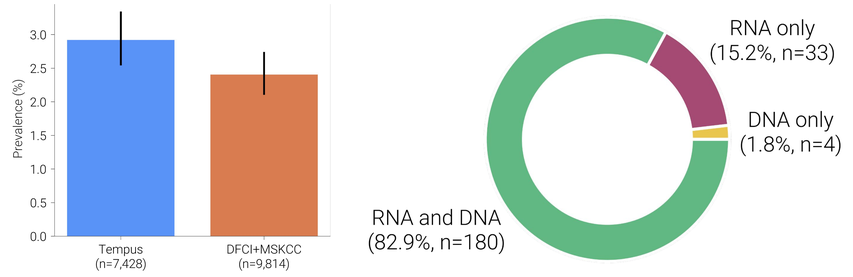


Figure 2. *ALK* prevalence in the Tempus dataset compared to combined DFCI and MSK external datasets (left, Error bar shows binomial 95% CI). Fraction of *ALK* fusions detected according to assay (right).

Fusion partner	Total patients	In both RNA and DNA	RNA only	DNA only
EML4	214	178	32	4
KIF5B	2	1	1	0
KLC1	1	1	0	0

Table 2: Breakdown of *ALK* fusion partners. When multiple partners were detected, the most canonical partner was assigned. *ALK* fusions were limited to known oncogenic partners detectable by FISH or IHC (*EML4, KIF5B, KLC1,* and *PICALM*).

Breakpoint locations for *EML4-ALK* fusions

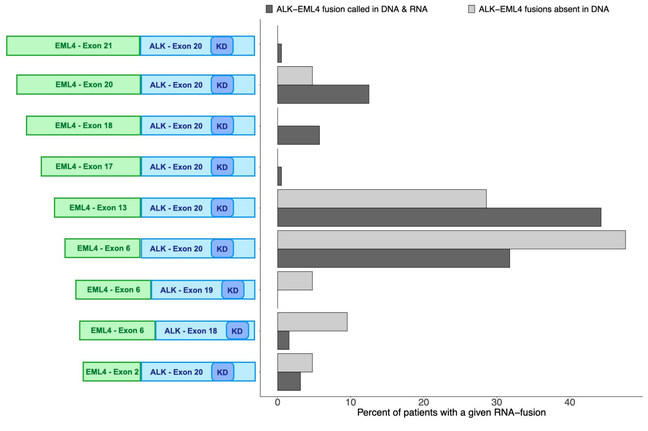


Figure 3. Schematic diagram of EML4-ALK fusion isoforms based off analysis of RNA-NGS reads (left) with their relative frequency among RNA-NGS only fusions (light gray) and fusions detected via both RNA-NGS and DNA-NGS (dark gray).

Treatment adherence for patients with *EML4-ALK* fusions detected via RNA-NGS only

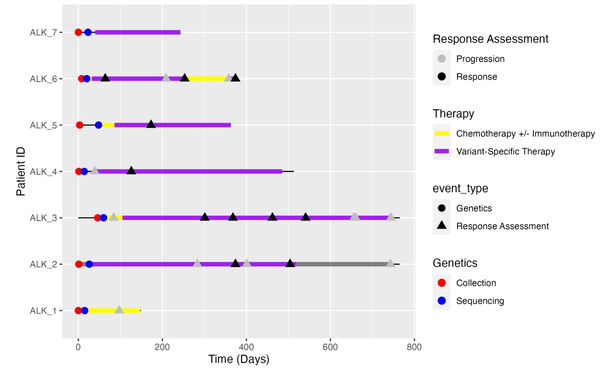


Figure 4. For a subset of patients with medication data who had an EML4-ALK fusion detected solely by RNA-NGS (n=7, depicted along the y-axis), 6 patients received approved targeted therapy post-testing ("ALK_2" through "ALK_7") and 5 remained on therapy for ≥100 days.

Presenting Author Declaration of Interest: Dr. Iams' travel and expenses have been compensated by Tempus. His full COI can be found in the abstract booklet.