Poster 1374

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Building an effective concordance study: Plasma Next Generation Sequencing (NGS) for oncogenic fusion detection in non-small cell lung carcinoma (NSCLC)

Background

- NGS of cell-free DNA (cfDNA) has shown promise in expanding access to precision medicine in patients with advanced NSCLC[1,2]
- Many clinicians use send-out liquid biopsy platforms, making technical evaluation between assays difficult
- Gene fusions are complex alterations that may be difficult to detect in plasma and may present technical challenges
- The FDA has approved several small molecule inhibitors of kinases involved in oncogenic fusions[3]
- Here we compare two commercial hybrid-capture plasma NGS assays in detecting fusion-positive NSCLC using tumor as a reference standard

Drug	Manufacturer	Fusion Target	Year Approved
Crizotinib	Pfizer	*ALK/ROS1*	2013
Ceritinib	Novartis	*ALK/ROS1*	2014
Alectinib	Roche	*ALK*	2017
Brigatinib	Takeda	*ALK*	2017
Lorlatinib	Pfizer	*ALK/ROS1*	2018
Larotrectinib	Loxo/Bayer	*NTRK*	2018
LOXO-292	Loxo/Lilly	*RET*	pending

Methods

- A database of advanced NSCLC patients at Dana-Farber Cancer Institute was queried to identify fusion-positive patients confirmed in tumor who had undergone plasma NGS by Guardant360
- A separate tube of plasma from consented patients was analyzed using ctDx-Lung
- Researchers involved in specimen and data handling were blinded until results were locked
- Unblinded cases were available for ad hoc analysis

	Guardant360[4]	ctDx-Lung[5]
Manufacturer	Guardant Health	Resolution Bioscience
Genes Covered	73	20
Performer	Provider	In-house kit
Run Time	Clinician ordered	Post-hoc

Results

Study Cohort

169 patients were found to have had clinical Guardant testing and to have consented to research

28 patients had tumor testing positive for an ALK, ROS1, or RET rearrangement

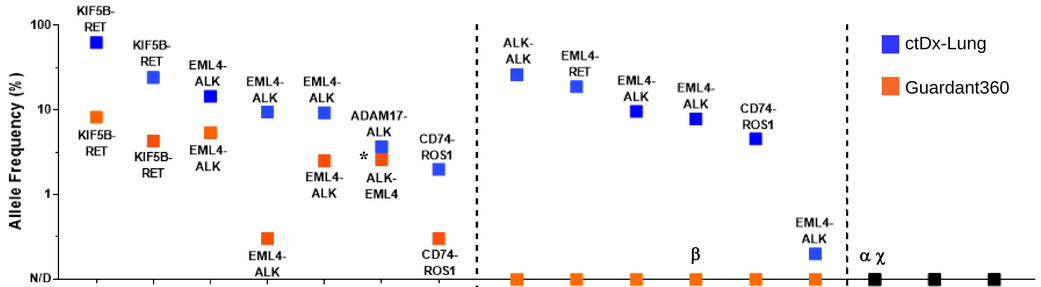
12 patients were excluded as there was no adequate plasma for analysis

16 patients were found to be eligible for plasma NGS on study, where **11** had ALK rearrangements, **2** had ROS1 rearrangements, and **3** had RET rearrangements

7 patients had fusions detected by Guardant

9 patients did not have fusions detected by Guardant

Reported Fusions

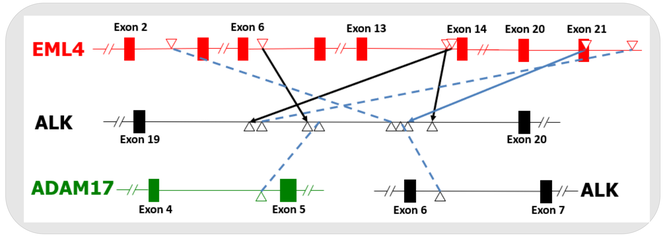


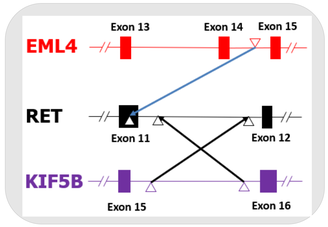
Days between Assays	238	0	0	13	0	0	14	90	0	279	0	6	176	16	0	0
Tissue Result	KIF5B-RET	KIF5B-RET	EML4-ALK	EML4-ALK	EML4-ALK	ALK FISH+	CD74-ROS1	ALK-NOS[1]	EML4-RET	EML4-ALK	ALK FISH+	CD74-ROS1	EML4-ALK	EML4-ALK	EML4-ALK	ALK-NOS[1]
TP53 Mutation AFs	2.86 1.2	N/D	0.99 1.0	N/D	N/D	N/D	N/D	0.70 0.4	N/D	16.7 4.6	6.88 7.0	3.76 7.5	N/D	N/D	N/D	N/D

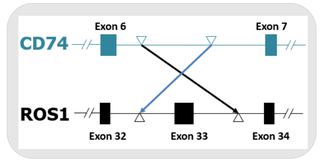
- ctDx-Lung detected 13 cases and tended to report higher AF % in fusions than Guardant360, which detected 7 cases
- Circulating tumor DNA (indicated by *TP53* SNVs) was detected in discordant cases

Breakpoint Schematics

- Fusion breakpoints detected are heterogeneous
- ctDx-Lung reports more out-of-frame/inverted fusion variants than Guardant360





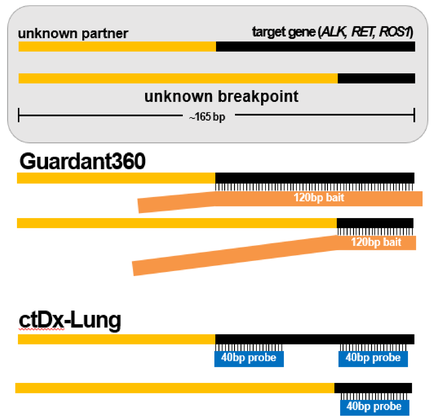


Detected by both assays
Detected by ctDx-Lung
Detected by ctDx-Lung, inverted

- In one case (*), ctDx-Lung reports ADAM17-ALK fusion, while Guardant360 suggests rare ALK-EML4 fusion
- In bioinformatic re-review after unblinding, ctDx-Lung reported 1 case (α) below threshold and Guardant360 reported 2 cases (β, χ) using updated pipeline

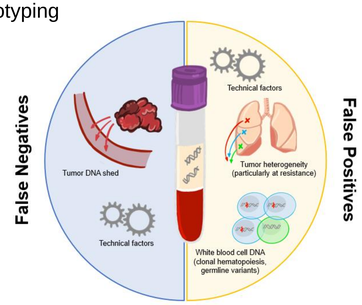
Technical Considerations

- Fusion partner and oncogenic breakpoints in NSCLC are often unpredictable
- Guardant360[4] and ctDx-Lung[5] cite different bait/probe lengths which may impact target fragment capture efficiency



Conclusions

- Benchmarking plasma genotyping assays should:
  1) Focus on actionable mutations
  2) Use tumor as a reference standard for establishing true/false positives/negatives
- Biochemical differences may affect probe capture efficiency
  - Higher AF % reported by ctDx-Lung
  - Capture efficiency may be affected by bait/probe length
- Bioinformatic differences may affect variant calling[6]
  - Increased reporting of unusual breakpoints by ctDx-Lung
  - Re-analysis by Guardant using latest data analysis pipeline produced 2 additional fusion calls
- Many factors impact tumor/plasma discordance,[7,8,9] with technical factors being one potentially underappreciated source of false negative plasma genotyping



- Rigorous evaluation of plasma NGS assays against a tumor standard is needed to effectively identify actionable mutations in more patients for improved treatment and clinical trial enrollment

References

1. Aggarwal C, et al. *JAMA Oncology* (2018).
2. Papadimitrakopoulou, V. Proceedings: AACR Annual Meeting 2019; Atlanta, GA.
3. Hematology/Oncology (Cancer) Approvals & Safety Notifications. *U.S. Food & Drug Administration* (2019).
4. Odegaard, J. I. *et al. Clin Cancer Res* **24**, 3539 (2018).
5. Paweletz, C. P. *et al. Clin Cancer Res* **22**, 915 (2016).
6. Stetson, D. *et al. JCO Precision Oncology* **3**, 1-9 (2019).
7. Hu, Y. *et al. Clin Cancer Res* **24**, 4437 (2018).
8. Slavin, T. P. *et al. JCO* **36**, 3459–3465 (2018).
9. Paweletz, C. P. *et al. JCO Precision Oncology* **3** 1-3 (2019).

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