

Plasma genotyping of patients in the eXalt2 trial: ensartinib⁺ (X-396) in ALK+ non-small cell lung cancer (NSCLC)

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BACKGROUND

- Ensartinib is a novel, potent anaplastic lymphoma kinase (ALK) small molecule tyrosine kinase inhibitor (TKI).
- Additional activity against MET, ABL, Axl, EPHA2, TRKA, LTK, ROS1 and SLK.¹
- Acquired resistance to crizotinib can be mediated by ALK fusion amplification, point mutation in the ALK kinase domain, or upregulation of bypass signaling pathways.²
- Circulating free DNA (cfDNA) in plasma can be used to detect molecular alterations, including the presence of mutations which may mediate acquired resistance to drug therapy.

METHODS

Schema:

- Multicenter study
- Treatment with 225mg ensartinib QD with food or fasting for cycle 1
- 28-day schedule
- Assessed for response to therapy using RECIST 1.1
- Adverse events (AEs) using CTCAE version 4.03 were recorded
- Plasma samples were collected on the first day of each cycle

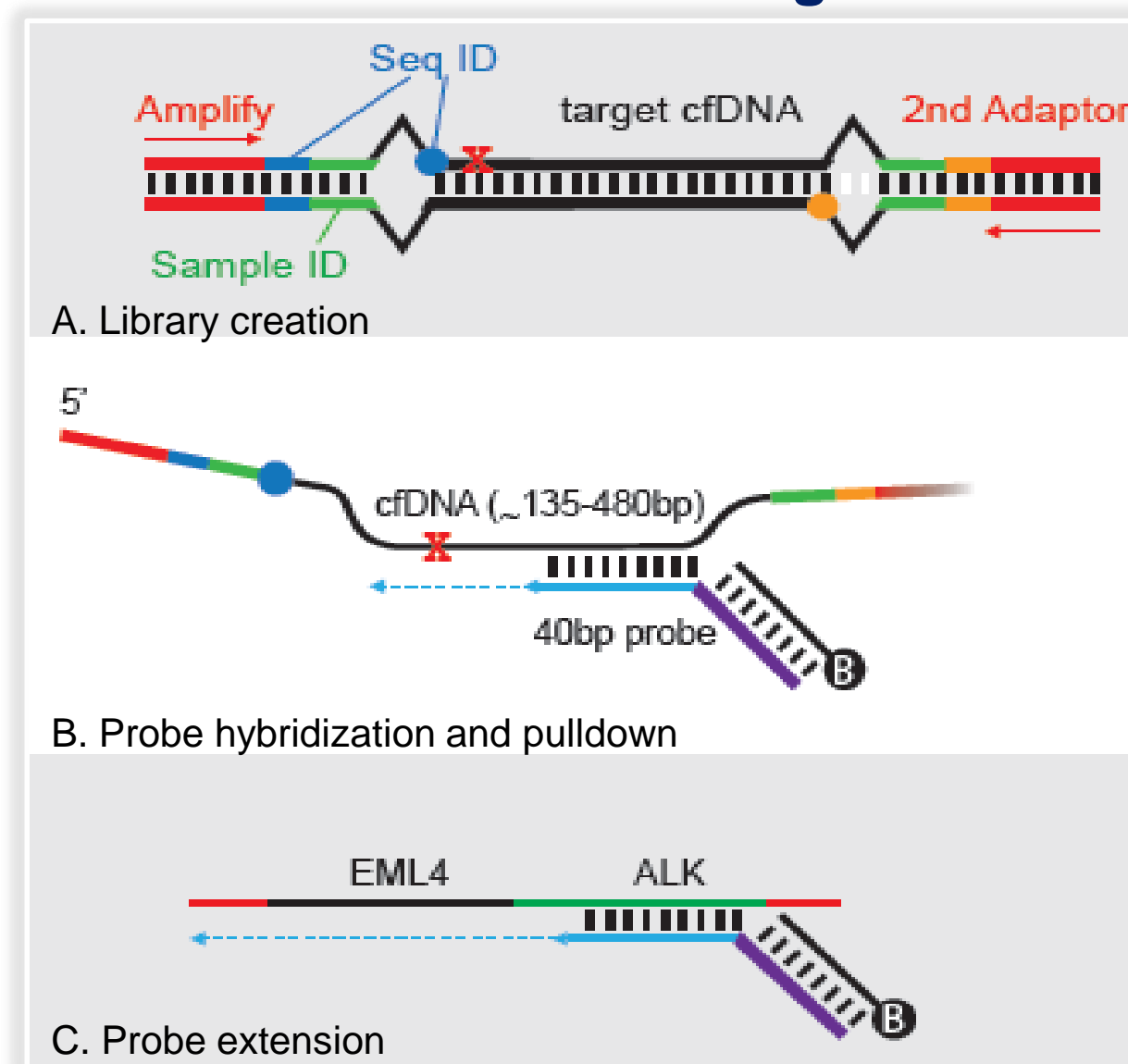
Expansion Cohort Major Inclusion Criteria:

- ALK+ (via FISH or IHC) advanced or recurrent NSCLC
- Patients must have measurable disease
- ECOG performance status (PS) 0-1
- Asymptomatic treated or untreated brain metastases (CNS) and leptomeningeal disease were allowed

Next Generation Sequencing:

- Next Generation Sequencing (NGS) on cfDNA from plasma samples was performed at Resolution Bioscience³ retrospectively on baseline and on study samples and compared with tissue FISH/IHC. The NGS panel targeted actionable mutations and rearrangements found in NSCLC (including ALK, RET, and ROS1 fusions and kinase domains).
- Isolated cfDNA was end repaired and cloned into libraries which were created by attaching multifunctional adaptors that help identify unique sequence clones (A).
- Amplified genomic libraries were denatured and hybridized with 40nt targeting probes (B).
- Primer extension of the probe is used to copy the captured genomic sequence information as well as the adaptor, creating on-target rates >90% and allowing detection of ALK (and other) fusion partners without a priori knowledge of partners or breakpoints (C).
- Following sequencing, bioinformatics analysis created a unique read consensus sequence for each family of PCR duplicates. Custom callers then detect single nucleotide variants (SNVs), indels, CNV, and fusion rearrangements.

Resolution Bioscience Targeted NGS



RESULTS

Note: Information in the database as of 13May2016

ALK+ Patients

Demographics – ALK+ Evaluable* Patients at ≥ 200 mg (n= 38)	
Median Age (Range)	53 (20-79)
Gender:	
Female	21 (55%)
Male	17 (45%)
Ethnicity:	
Caucasian	30 (79%)
Asian	7 (18%)
Unknown	1 (3%)
ECOG:	
0	14 (37%)
1	24 (63%)
Smoking Status:	
Never	25 (66%)
Former	12 (32%)
Current	1 (3%)
Lines of Prior Treatment:	
0	7 (18%)
1	7 (18%)
2	7 (18%)
3	6 (16%)
≥4	11 (29%)
Prior ALK TKI Treatment:	
ALK TKI Naive:	8 (21%)
Prior Crizotinib only	20 (53%)
Prior Crizotinib and Ceritinib	7 (18%)
Prior Crizotinib, Ceritinib, and Alectinib	2 (5%)
Prior Crizotinib, Ceritinib, and Brigatinib	1 (3%)

*Evaluable = Patient completed 1 cycle and had post baseline response assessment

ALK TKI Naïve Patients				
Best Response to Ensartinib (%)	Time on Ensartinib (mos)	NGS in Plasma Baseline (allele frequency)	NGS in Tissue Baseline (allele frequency)	NGS in Plasma End of Treatment (allele frequency)
PR (-88%)	11+	no variants detected	no variants detected	n/a
PR (-78%)	27	<i>EML4-ALK</i> (18%)	not available	not available
PR (-73%)	9+	no variants detected	not available	n/a
PR (-60%)	25	<i>EML4-ALK</i> (0.6%)	not available	not available
PR (-55%)	9+	<i>EML4-ALK</i> (2.4%)	not available	n/a
PR (-30%)	32+	no variants detected	no variants detected	n/a
PR (-30%)	12+	<i>EML4-ALK</i> (0.9%)	<i>EML4-ALK</i> (38.4%)*	n/a
PD (7%)	2	MET CNV (5 copies), no ALK alteration	not available	not available

* Archival tissue prior to pemetrexed

Prior Crizotinib Only Patients				
Best Response to Ensartinib (%)	Time on Ensartinib (mos)	NGS Plasma Baseline (allele frequency)	NGS in Tissue Baseline (allele frequency)	NGS in Plasma End of Treatment (allele frequency)
PR (-94%)	13+	<i>PRKAR1A-ALK</i> (0.8%)	<i>PRKAR1A-ALK</i> (18.18%)	n/a
PR (-65%)	9	no variants detected	<i>EML4-ALK</i> (41.5%)	not available
PR (-58%)	29	<i>EML4-ALK</i> (0.52%) <i>NA-ALK</i> (0.52%)	<i>EML4-ALK</i> (18.2%) <i>NA-ALK</i> (48.6%)	not available
PR (-57%)	5	<i>EML4-ALK</i> (21.3%) L1196M (0.9%)	not available	not available
PR (-54%)	13	<i>EML4-ALK</i> (1.35%) <i>ALK</i> -noncoding fusion (0.58%) G1269A (0.1%)	<i>EML4-ALK</i> (34%) <i>ALK</i> -noncoding fusion (20%) L1196M (0.04%)	<i>EML4-ALK</i> (4.35%) <i>ALK</i> -noncoding fusion (0.35%) L1196M (0.17%) G1269A (0.09%)
PR (-51%)	11	<i>EML4-ALK</i> (0.4%)	<i>EML4-ALK</i> (17.2%)	not available
PR (-49%)	4	<i>ALK</i> -noncoding fusion (23%) T1151M (1.4%)	not available	not available
PR (-46%)	5	<i>EML4-ALK</i> (10%)	<i>EML4-ALK</i> (5.6%)	<i>EML4-ALK</i> (4.8%)
PR (-42%)	18	<i>EML4-ALK</i> (31%)	not available	not available
PR (-30%)	23+	no variants detected	not available	n/a
SD (-5.6%)	2	<i>EML4-ALK</i> (1.8%)	not available	not available
SD (0%)	5+	<i>EML4-ALK</i> (0.43%)	not available	n/a
PD (response systemically, new brain lesion)	1	<i>EML4-ALK</i> (10.8%) QSLP1188P (0.4%) R1113Q (0.3%) S1206F (0.3%)	not available	not available

Prior Crizotinib and Ceritinib Patients				
Best Response to Ensartinib (%)	Time on Ensartinib (mos)	NGS Plasma Baseline (allele frequency)	NGS in Tissue Baseline (allele frequency)	NGS in Plasma End of Treatment (allele frequency)
PR (-94%)	9+	no variants detected	not available	n/a
PR (-36%)	5	<i>ALK</i> -noncoding fusion (3.69%), G1202R (0.7%), ERRBB2 splice mut (1.0%)	<i>ALK</i> -noncoding fusion (28.3%)	<i>ALK</i> -noncoding fusion (5.1%), G1202R (1.7%) V1149M (0.4%)
SD (-15%)	5	<i>EML4-ALK</i> (0.67%)	<i>EML4-ALK</i> (4.6%)	<i>EML4-ALK</i> (0.05%)
PD (-100% systemically, new brain lesion)	2	<i>EML4-ALK</i> (2.8%)	not available	not available
PD (13%)	1	<i>EML4-ALK</i> (1.3%) G1202R (2.1%)	not available	not available
PD (34%)	1	not available	not available	<i>EML4-ALK</i> (0.3%) G1202R (0.5%)

Fusion/Mutation Concordance of ALK Tissue (T) NGS and Plasma (P) NGS (n=13 patient samples)

	Total Fusion/Mutation	PR (%)	SD (%)	PD
T+P-	5	3 (75%)	1 (25%)	0
T- P+	5	3 (100%)	0	0
T+P+	17	7 (78%)	2 (22%)	0
T-P-	2	2 (100%)	0	0

Overall concordance of tissue NGS and plasma NGS is 70% (n=19 variants)

* Patient was ALK+ via FISH

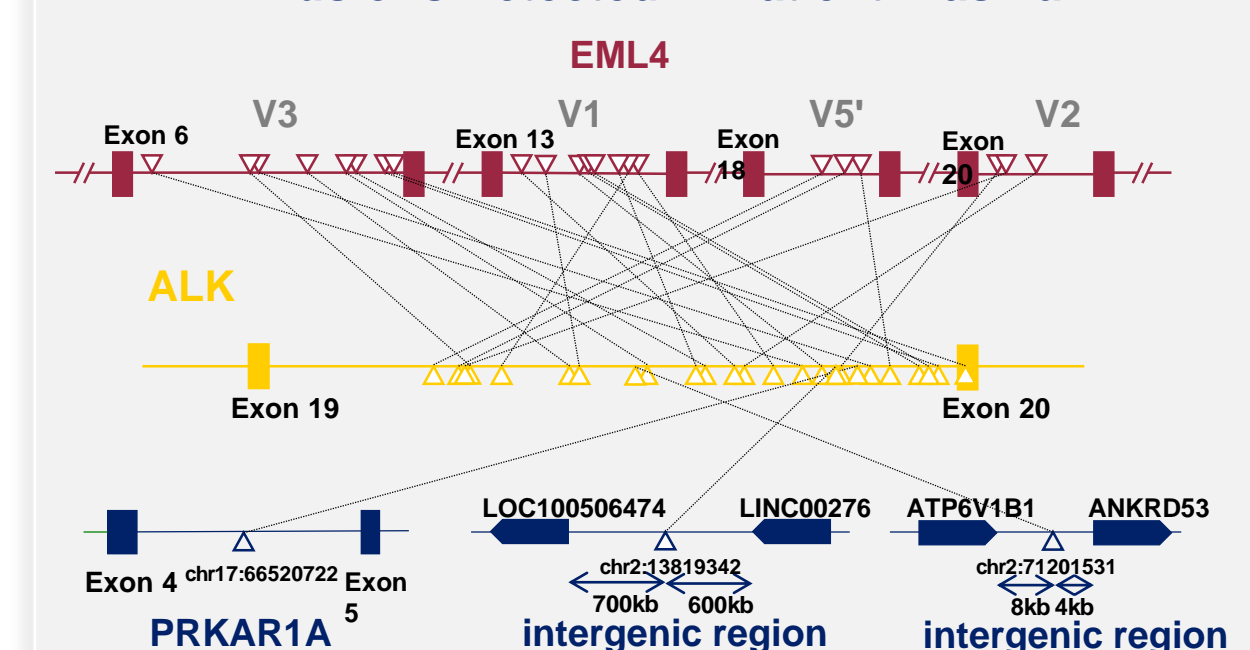
Fusion Concordance of ALK Tissue FISH (F) and Plasma (P) NGS (n=42 patient samples)

	Total	PR (%)	SD (%)	PD (%)	Inevaluable (%)
F+P-	11	6 (55%)	1 (9%)	1 (9%)	3 (27%)
F- P+	6	0	3 (50%)	2 (33%)	1 (17%)
F+P+	25	13 (52%)	2 (12%)	6 (24%)	3 (12%)

Overall concordance of ALK-fusion in tissue FISH and plasma NGS is 74% (n=31 variants)

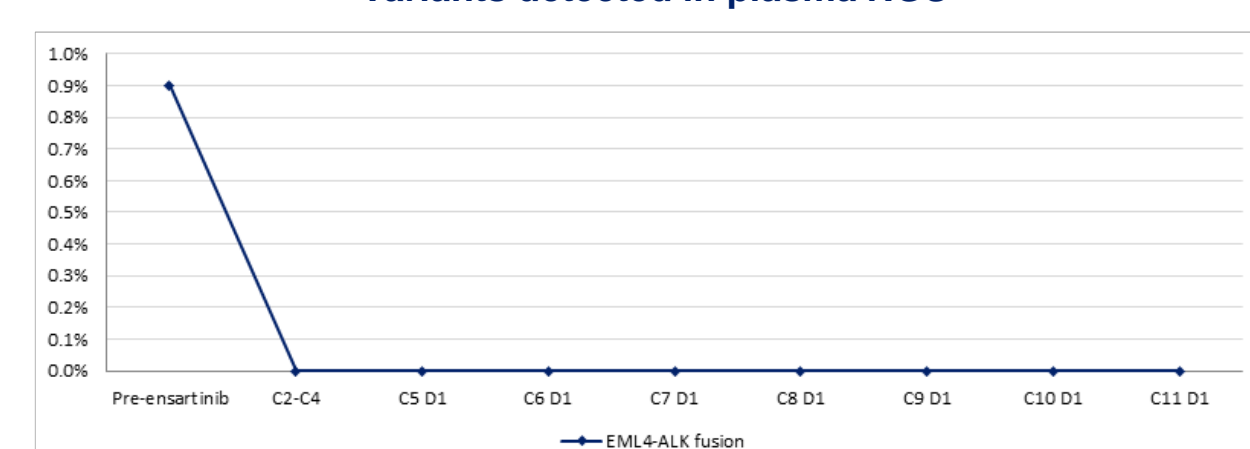
* Patients in the dose escalation with head and neck and ALK- NSCLC

Locations of Canonical and Non-Canonical ALK Fusions Detected in Patient Plasma



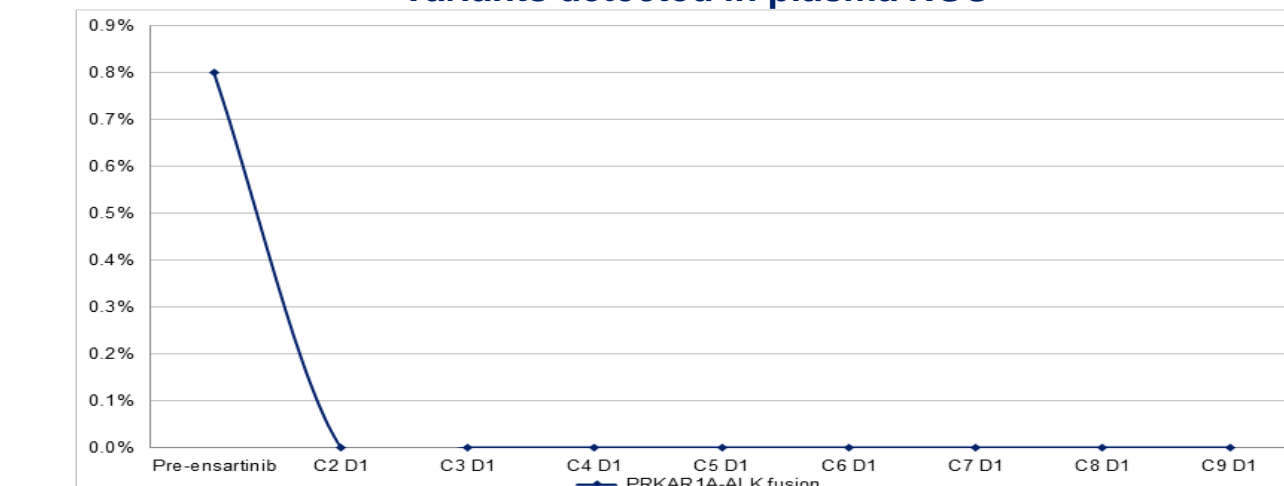
Schematic rendering of the genomic locations of ALK fusions detected in plasma in this study. The ALK intron 19 region is shown in yellow, and relevant regions of EML4 are shown in red with canonical variant classifications indicated in gray. Three examples of non-canonical fusion partners are shown in blue, including a predicted productive fusion to intron 4 of PRKAR1A and two fusions to intergenic regions on chr. 2 of unknown significance.

ALK TKI Naïve Patient – PR Variants detected in plasma NGS



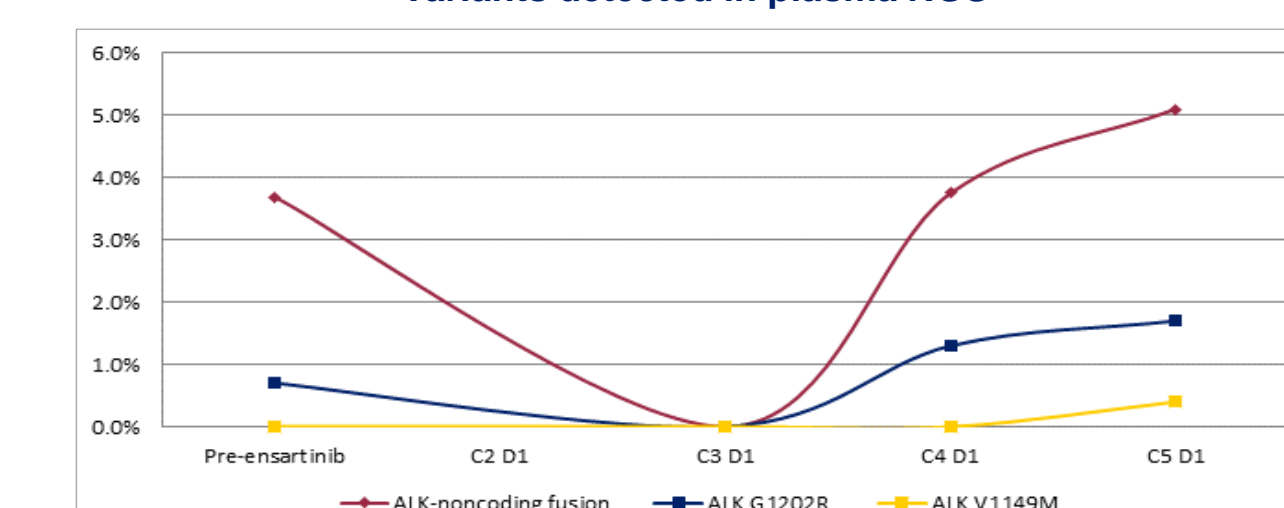
- 79 yr old female with ALK+ NSCLC
- Achieved PR after 2 cycles (30% reduction overall)
- Patient is still on treatment in cycle 14

Crizotinib Resistant Patient – PR Variants detected in plasma NGS



- 21 yr old male with ALK+ NSCLC
- Achieved PR after 2 cycles (70% reduction overall)
- Patient is still on treatment in cycle 13

Crizotinib & Ceritinib Resistant Patient – PR Variants detected in plasma NGS



- 40 yr old female with ALK+ NSCLC
- Achieved PR after 2 cycles (36% reduction overall)
- Patient progressed after 5 cycles

CONCLUSIONS

- Ensartinib has shown promising activity in ALK-positive NSCLC patients with durable responses observed in patients who are crizotinib naïve and patients with resistance to crizotinib and second generation ALK TKIs.
- Plasma NGS can be used to detect ALK kinase domain mutations and monitor changes in response to treatment in a non-invasive manner.
- In this study, ALK kinase domain mutations were detected in 4/11 patients who had prior crizotinib and 2/4 patients who had prior crizotinib and ceritinib. G1202R was found in both of the latter cases.
- 1/2 patients whose plasma detected the G1202R mutation prior to start of the trial responded to ensartinib.
- Further study of this methodology is ongoing to correlate the presence of ALK kinase domain mutations with response and resistance to ALK TKI therapy.
- A phase III trial is ongoing comparing ensartinib to crizotinib in TKI naïve ALK-positive NSCLC patients.

REFERENCES

1. Lovly et al., Cancer Research 2011 71:4920
2. Katayama et al., Clinical Cancer Research 2015
3. Paweletz et al., Clinical Cancer Research, 2016, 22(4):915

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 - Our colleagues at Xcovery Holding Company and Resolution Bio
- +ensartinib = proposed International Non-proprietary Name (INN), formerly referred to as X-396