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

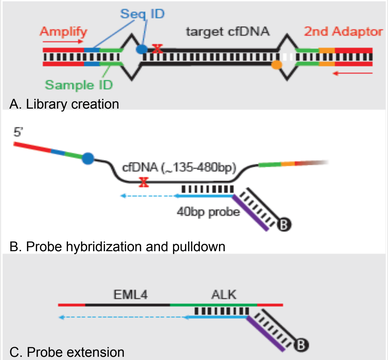
Expansion Cohort Major Inclusion Criteria:

- 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 Bioscience[3] 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	*EML4-ALK* (18%)	not available	not available
PR (-73%)	9+	no variants detected	not available	n/a
PR (-60%)	25	*EML4-ALK* (0.6%)	not available	not available
PR (-55%)	9+	*EML4-ALK* (2.4%)	not available	n/a
PR (-30%)	32+	no variants detected	no variants detected	n/a
PR (-30%)	12+	*EML4-ALK* (0.9%)	*EML4-ALK* (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+	*PRKAR1A-ALK* (0.8%)	*PRKAR1A-ALK* (18.18%)	n/a
PR (-65%)	9	no variants detected	*EML4-ALK* (41.5%)	not available
PR (-58%)	29	*EML4-ALK* (0.52%) *NA-ALK* (0.52%)	*EML4-ALK* (18.2%) *NA-ALK* (48.6%)	not available
PR (-57%)	5	*EML4-ALK* (21.3%) L1196M (0.9%)	not available	not available
PR (-54%)	13	*EML4-ALK* (1.35%) *ALK*-noncoding fusion (0.58%) G1269A (0.1%)	*EML4-ALK* (34%) *ALK*-noncoding fusion (20%) L1196M (0.04%)	*EML4-ALK* (4.35%) *ALK*-noncoding fusion (0.35%) L1196M (0.17%) G1269A (0.09%)
PR (-51%)	11	*EML4-ALK* (0.4%)	*EML4-ALK* (17.2%)	not available
PR (-49%)	4	*ALK*-noncoding fusion (23%) T1151M (1.4%)	not available	not available
PR (-46%)	5	*EML4-ALK* (10%)	*EML4-ALK* (5.6%)	*EML4-ALK* (4.8%)
PR (-42%)	18	*EML4-ALK* (31%)	not available	not available
PR (-30%)	23+	no variants detected	not available	n/a
SD (-5.6%)	2	*EML4-ALK* (1.8%)	not available	not available
SD (0%)	5+	*EML4-ALK* (0.43%)	not available	n/a
PD (response systemically, new brain lesion)	1	*EML4-ALK* (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	*ALK*-noncoding fusion (3.69%), G1202R (0.7%), ERRBB2 splice mut (1.0%)	*ALK*-noncoding fusion (28.3%)	*ALK*-noncoding fusion (5.1%), G1202R (1.7%) V1149M (0.4%)
SD (-15%)	5	*EML4-ALK* (0.67%)	*EML4-ALK* (4.6%)	*EML4-ALK* (0.05%)
PD (-100% systemically, new brain lesion)	2	*EML4-ALK* (2.8%)	not available	not available
PD (13%)	1	*EML4-ALK* (1.3%) G1202R (2.1%)	not available	not available
PD (34%)	1	not available	not available	*EML4-ALK* (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

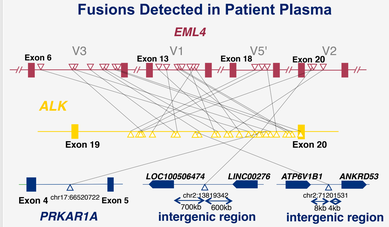
Fusion Concordance of ALK Tissue FISH (F) and Plasma (P) NGS (n=42 patient samples)

	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

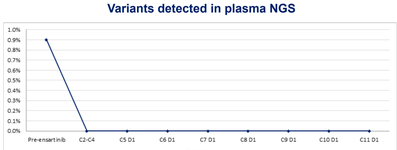
Locations of Canonical and Non-Canonical ALK Fusions Detected in Patient Plasma



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

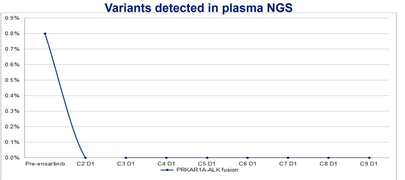
Variants detected in plasma NGS



- 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

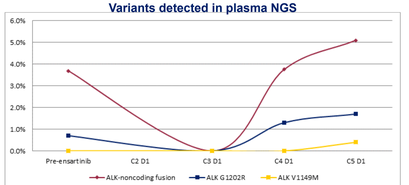
Variants detected in plasma NGS



- 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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+ensartinib = proposed International Non-proprietary Name (INN), formerly referred to as X-396