Phase I/II trial of ensartinib+ (X-396) in patients with ALK+ non-small cell lung cancer (NSCLC): Correlation with plasma and tissue genotyping and response to therapy

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BACKGROUND

- Ensartinib is a novel, potent ALK small molecule tyrosine kinase inhibitor (TKI)
- Ensartinib has additional activity against MET, ABL, Axl, EPHA2, LTK, ROS1, and SLK
- It has demonstrated significant pre-clinical anti-tumor activity in both ALK TKI-naïve and crizotinib-resistant models of ALK rearranged NSCLC[1]
- Acquired resistance to crizotinib can be mediated by *ALK* fusion amplification, point mutation in the *ALK* kinase domain, or activation of bypass signaling pathways[2]
- Circulating tumor DNA (ctDNA) in plasma can be used to detect molecular alterations, including the presence of oncogenic fusions and also mutations which may mediate acquired resistance to drug therapy

METHODS

Study Design:

Eligibility
- Stage IIIB/IV NSCLC
- Tumor positive for ALK via IHC or FISH
- ECOG PS 0-1
- Measurable disease

Ensartinib
225 mg* daily with or without food for cycle 1
(cycle = 28 days)

Cohorts
- ALK TKI naive
- Prior crizotinib
- Prior 2nd generation ALK TKI
- CNS metastases (asymptomatic treated and untreated)
- Leptomeningeal disease

* Prior dose escalation cohorts established 225 mg as the dose for phase II

- Primary endpoint: Safety
- Secondary endpoints: response rate RECIST v1.1, PFS, CNS response, duration of response, and correlatives
- Optional plasma samples were collected on the first day of each cycle
- Targeted Next Generation Sequencing (NGS) of ctDNA was performed retrospectively at baseline and on study and compared with tissue results

Next Generation Sequencing:

- NGS on ctDNA 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 ctDNA was end repaired and cloned into libraries which were created by attaching multifunctional adaptors that help identify unique sequence clones.
- Amplified genomic libraries were denatured and hybridized with 40nt targeting probes.
- Primer extension of the probe was 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.
- Following sequencing, bioinformatics analysis created a unique read consensus sequence for each family of PCR duplicates. Custom callers then detect single nucleotide variants, indels, copy number variants, and fusion rearrangements.

RESULTS

Note: Information in the database as of 10August2016

Demographics	All Patients Dosed (n=86)	ALK+ Evaluable* Patients (n= 52)
Median Age (Range)	55 (21-80)	53 (21-80)
Gender:		
Female	44 (51%)	29 (56%)
Male	42 (49%)	23 (44%)
Ethnicity:		
Caucasian	67 (78%)	40 (77%)
Asian	10 (12%)	8 (15%)
Black/African American	5 (6%)	1 (2%)
Unknown/Other	4 (5%)	3 (6%)
ECOG:		
0	30 (35%)	23 (44%)
1	56 (65%)	29 (56%)
Smoking Status:		
Never	49 (57%)	33 (63%)
Former	33 (38%)	17 (33%)
Current	4 (5%)	2 (4%)
Lines of Prior Treatment:		
0	13 (15%)	10 (19%)
1	15 (17%)	10 (19%)
2	21 (24%)	12 (23%)
3	9 (10%)	5 (10%)
≥4	28 (33%)	15 (29%)
Prior ALK TKI Treatment:		
ALK TKI Naive		14 (27%)
Prior Crizotinib only		25 (48%)
Prior Crizotinib and Ceritinib		8 (15%)
Prior Crizotinib, Ceritinib, and Alectinib		4 (8%)
Prior Crizotinib, Ceritinib, and Brigatinib		1 (2%)

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

Most Common Drug- Related Adverse Events* (n=86)

AE	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)	Grade 4 (%)	All Grades (%)
At Least 1 AE	27 (31%)	29 (34%)	14 (16%)	2 (2%)**	72 (84%)
Rash (all)	28 (33%)	11 (13%)	9 (10%)	-	48 (56%)
Nausea	24 (28%)	6 (7%)	1 (1%)	-	31 (36%)
Pruritus	16 (19%)	5 (6%)	4 (5%)	-	25 (29%)
Vomiting	18 (21%)	5 (6%)	1 (1%)	-	24 (28%)
Fatigue	12 (14%)	5 (6%)	2 (2%)	-	19 (22%)
Decreased Appetite	15 (17%)	-	1 (1%)	-	16 (19%)
Edema (all)	7 (8%)	6 (7%)	1 (1%)	-	14 (16%)
Dry Skin	9 (10%)	1 (1%)	-	-	10 (12%)
AST Increased	9 (10%)	-	-	-	9 (10%)

*In ≥10% of patients
**Patient with thrombotic microangiopathy and patient with decreased platelet count and sepsis considered possibly related by the investigator; however, thought to be unlikely related by the sponsor

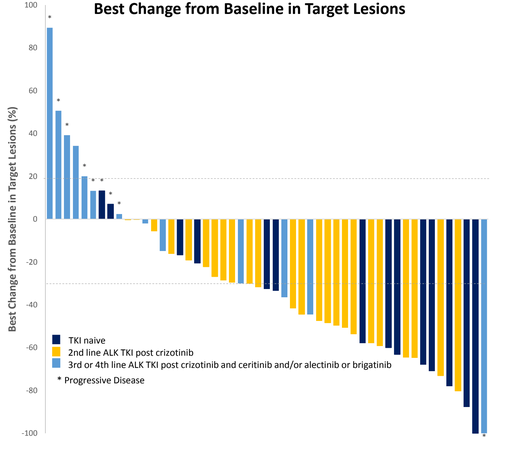
Treatment-Related Toxicities:

- Most common drug-related adverse events (AEs), mostly Grade 1-2, include rash, nausea, pruritus, vomiting, fatigue, decreased appetite, edema, dry skin, and increased aspartate aminotransferase
- Few drug-related AEs of diarrhea (9%, Grade 1), constipation (8%, Grade 1-2), abdominal pain (5%, Grade 1-2), myalgia/musculoskeletal pain (5%, Grade 1-3); alanine aminotransferase increased (9%, Grade 1), or QT prolongation (2%, Grade 1)
- Rash is most prominent AE: for Grade 1-2, treated topically, for Grade 3, hold dose until improvement, then resume at lower dose, use steroids if necessary

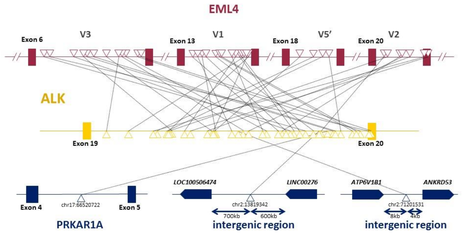
Overall Efficacy

Prior Therapy	PR	SD	PD
ALK TKI Naïve	10 / 14 (71%)	2 / 14 (14%)	2 / 14 (14%)
Prior Crizotinib	16 / 25 (64%)	8 / 25 (32%)	1 / 25 (4%)
Prior Crizotinib and Prior Second Generation ALK TKI	3 / 13 (23%)	3 / 13 (23%)	7 / 13 (54%)
CNS Target Lesion Response	7 / 12 (58%)	5 / 12 (42%)	-

- 29 patients had a PR (56%) at 200 mg or above
- Duration of treatment was from 1 to 35+ months
- Patients with measurable CNS had Intracranial DCR of 100%



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 exon 4 of PRKAR1A and two fusions to intergenic regions of unknown significance.

Fusion Concordance of ALK Tissue FISH (F) and Plasma (P) NGS and Tissue (T) NGS in Patients with at least one Prior ALK TKI
(n=33 patient samples with T and P and 17 patient samples with P and T)

	Total	PR (%)	SD (%)	PD (%)
F+P+	28	13 (46%)	9 (32%)	6 (21%)
F+P-	5	4 (80%)	1 (20%)	
P+T+	14	8 (47%)	4 (24%)	2 (12%)
P-T+	1	1 (100%)		
P+T-	1			1 (100%)
P-T-*	1	1 (100%)		

Overall concordance of ALK-fusion in tissue FISH and plasma NGS is 85%.
Overall concordance of ALK-fusion in plasma NGS and tissue NGS is 88%.

Activity Seen in Patients with ALK Kinase Domain Mutations

ALK Resistant Mutation	Best Response	Prior ALK TKI
L1196M	PR	crizotinib
L1196M G1296A	PR	crizotinib
T1151M	PR	crizotinib and ceritinib
V1194M G1202R	PR	crizotinib and ceritinib
G1202R	PR	crizotinib and ceritinib
S1206F	SD	crizotinib
E1154K	SD	crizotinib
D1203N C1156Y	PD	crizotinib and ceritinib

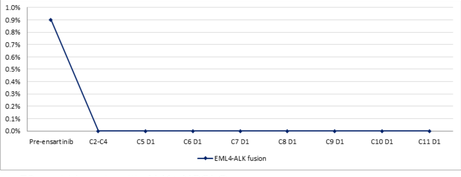
- ALK resistant mutations were found in 8 (17%) of 46 ALK+ evaluable patients analyzed by plasma NGS

Efficacy and FISH-NGS Concordance in ALK TKI Naïve Patients

	Total Fusion	Best Response
F+P-	2 (22%)	PD (100%)
F+P+	4 (44%)	PR (100%)
F+P no variants identified	3 (33%)	PR (100%)

- Two patients that were FISH positive but plasma NGS negative did not respond to ensartinib
- 4 patients had tissue available for NGS. Concordance was 100% with plasma NGS

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

CONCLUSIONS

- Ensartinib has shown promising activity in both ALK TKI naïve and crizotinib-resistant ALK+ NSCLC patients
- Responses are seen in patients with CNS disease and patients with prior 2nd generation ALK TKI
- Ensartinib is generally well tolerated with the most common toxicities being rash and nausea/vomiting, the latter often resolved with food
- Plasma sequencing appears to be promising to select patients for therapy and monitor for response and development of acquired resistance
- Two patients that were FISH positive, but plasma NGS negative did not respond to ensartinib
- A phase III trial is ongoing comparing ensartinib to crizotinib in TKI naïve ALK-positive NSCLC patients (NCT02767804)

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

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