Detection and clearance of *RET* variants in plasma cell free DNA (cfDNA) from patients treated with LOXO-292

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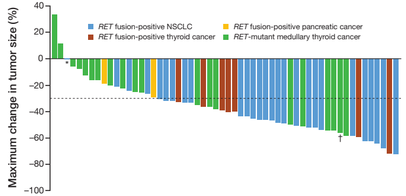
Background

- LOXO-292[1] is a novel, highly selective, small molecule inhibitor of RET currently in clinical development for patients with advanced cancers harboring oncogenic *RET* gene alterations,[2] such as:
  - *RET* fusions (non-small cell lung cancer, papillary and other thyroid cancers, other solid tumors)
  - Activating *RET* mutations (medullary thyroid cancer)
- At a cutoff date of April 2, 2018, the antitumor activity of LOXO-292 in patients with *RET* altered cancers in a phase I study is shown below (**Figure 1**; see also abstract 102, ASCO 2018).[3]

Figure 1. Antitumor activity in *RET* altered cancers



Note: Five patients not displayed (four due to treatment discontinuation prior to first post-baseline response assessment, one due to non-measurable disease at baseline (uCR); †Denotes patient with 0% maximum change in tumor size; ‡Complete response. NSCLC, non-small cell lung cancer

- We studied the modulation of *RET* variant allele frequencies (AF) in plasma cfDNA of patients receiving LOXO-292 therapy.

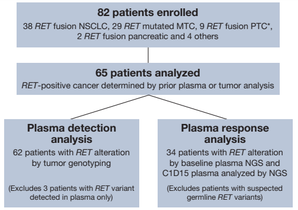
Methods

- This phase 1, open-label, dose-escalation, first-in-human study (NCT03157128) aims to evaluate the safety, tolerability, pharmacokinetics and preliminary antitumor activity of orally administered LOXO-292.
- The primary objective is to determine the maximum tolerated dose of LOXO-292 and/or the recommended dose for further study.
- One exploratory objective is the assessment and monitoring of *RET* gene alterations in plasma cfDNA.
- Blood samples were collected in Cell-Free DNA BCT® blood collection tubes (Streck) prior to treatment, after 15 days of treatment (cycle 1, day 15; C1D15), and at each restaging, and shipped to a central laboratory for plasma isolation within 72 hours.
- Gene alterations were assessed in *RET* and 72 other cancer-related genes, by next-generation sequencing (NGS) of cfDNA (Guardant360 assay; Guardant Health).[4]

Results

- As of April 2, 2018, 82 patients had been enrolled to 1 of 8 dose levels (20 mg QD–240 mg BID), and 342 plasma samples had been collected (**Figure 2**).

Figure 2. Analysis cohort



*Includes one patient with poorly differentiated thyroid cancer
C1D15, cycle 1, day 15; MTC, medullary thyroid cancer; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; PTC, papillary thyroid cancer

- Tumor *RET* gene alterations in the 65 patients analyzed are summarized in **Table 1**.

Table 1. *RET* alterations

	NSCLC	MTC	PTC*	Other
Gene fusions				
KIF5B-RET	19	0	0	0
CCDC6-RET	9	0	2	0
NCOA4-RET	1	0	3	0
CLIP1-RET	1	0	0	0
ERC1-RET	0	0	1	0
KTN1-RET	0	0	1	0
PRKAR1A-RET	0	0	0	1
RUFY3-RET	0	0	1	0
TFG-RET	0	0	0	1
RET fusion NOS	3	0	0	0
Mutations				
RET M918T	0	15	0	0
RET V804M	0	2	0	0
RET C618Y	0	1	0	0
RET C620R	0	1	0	0
RET C630R	0	1	0	0
RET A883F	0	1	0	0
RET C609Y	0	0	0	1

*Includes one patient with poorly differentiated thyroid cancer
MTC, medullary thyroid cancer; NOS, not otherwise specified; NSCLC, non-small cell lung cancer; PTC, papillary thyroid cancer.

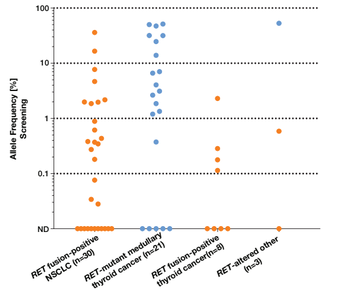
Plasma detection analysis

- The sensitivity of cfDNA analysis was studied in pretreatment plasma samples from 62 patients with *RET* alterations detected by tumor genotyping (**Figure 3**).
- The expected *RET* fusion was identified in cfDNA in 24 (60%) of 40 patients, with a median AF of 0.51%:
  - Positives included 19 lung, 4 thyroid and 1 other
- The expected *RET* point mutation was identified in cfDNA in 17 (77%) of 22 patients, with a median AF of 7.03%:
  - 4 had an AF in the range of 40–60%, suggesting a germline *RET* variant
  - Positives included 16 thyroid and 1 other
- The expected *RET* alteration was not found in 21 plasma samples:
  - 5 had no somatic mutations of any type detected; 2 of these samples had <5 ng DNA input (below the minimum required for the assay)
  - 12 may have had low tumor DNA content in plasma as the maximum AF of detected non-*RET* variants was <1%
  - 4 were negative despite a maximum AF of non-*RET* variants of >1%

Plasma response analysis

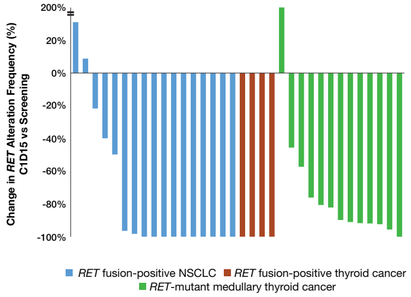
- Plasma response was studied in matched pretreatment and C1D15 plasma samples from 36 patients:
  - 2 of 36 samples were excluded from further analysis as a putative germline *RET* variant was identified (40–60% AF in baseline sample)
- Of the remaining 34, 21 had *RET* fusions and 13 had *RET* mutations detected in pretreatment cfDNA:
  - In 15 (44%) of 34 samples, the variant became undetectable at C1D15 (clearance)
  - In 27 (79%) of 34 samples, the AF decreased by at least 50%
  - The median AF decrease was 96% at C1D15
- Tumor type and starting dose were not major determinants of the magnitude of cfDNA response (**Figures 4** & **5**).

Figure 3. Plasma detection analysis



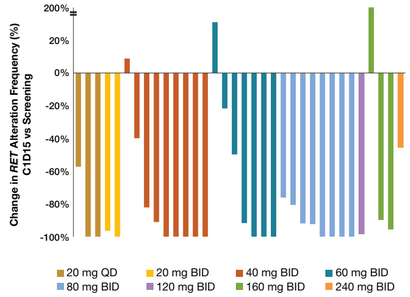
Variants not detected were arbitrarily assigned an AF of 0.01 for the purposes of plotting AF changes.
AF, allele frequency; ND, not detected

Figure 4. Plasma response analysis: by tumor type



C1D15, cycle 1, day 15; NSCLC, non-small cell lung cancer

Figure 5. Plasma response analysis: by starting dose

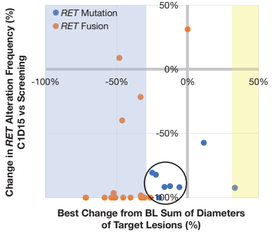


C1D15, cycle 1, day 15

Comparison of imaging- and cfDNA-based tumor changes

- Changes in tumor burden as measured by imaging (RECIST) and cfDNA analysis were compared for 27 patients where both measures were available.
- cfDNA analysis identified a subset of cases with radiographic stable disease but molecular evidence of a treatment effect (**Figure 6**, circled).
- The 3 cases with a RECIST partial response and a limited cfDNA response at C1D15 had a >90% AF decrease after longer follow-up.

Figure 6. Imaging (RECIST) vs cfDNA



One case outside of the plot range (8% tumor decrease, 191% plasma increase) is not shown.
BL, baseline; RECIST, Response Evaluation Criteria In Solid Tumors.

Conclusions

- The rapid clearance of *RET* variants from plasma cfDNA on LOXO-292 treatment supports the clinical activity of this agent across a range of doses, tumor types and *RET* alterations.
- NGS of plasma cfDNA can detect a range of targetable *RET* variants, though tumor genotyping remains critical if the initial plasma NGS is negative.
- Serial plasma genotyping warrants continued study as an early pharmacodynamic marker for novel targeted therapies.

References

1. Subbiah et al. *Ann Oncol*. 2018; Epub Apr 18.
2. Drilon et al. *Nat Rev Clin Oncol*. 2018; 15:151-167.
3. Drilon et al. *J Clin Oncol*. 2018; 36:(suppl; abstr 102).
4. Odegaard et al. *Clin Cancer Res*. 2018; Epub Apr 24.

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