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

Besse B,[1] Subbiah V,[2] Drilon A,[3] Shah M,[4] Wirth L,[5] Bauer TM,[6] Velcheti V,[7] Lakhani N,[8] Boni V,[9] Solomon BJ,[10] Johnson M,[6] Park K,[11] Patel J,[12] Cabanillas ME,[13] Sherman E,[3] Zhu E,[14] Gordon K,[14] Ebata K,[14] Tuch BB,[14] Oxnard G[15]

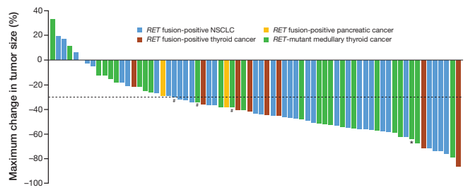
[1]Dept of Cancer Medicine, Gustave Roussy Institut de Cancérologie, Villejuif, FR; [2]Investigational Cancer Therapeutics (Phase 1 Program), The University of Texas, MD Anderson Cancer Center, Houston, TX, US; [3]Medical Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY, US; [4]Comprehensive Cancer Center, The Ohio State University, Columbus, OH, US; [5]Cancer Center, Massachuetts General Hospital, Boston, MA, US; [6]Sarah Cannon Research Institute/Tennessee Oncology, Nashville, TN, US; [7]Hematology and Oncology Department, Cleveland Clinic, Cleveland, US; [8]Developmental Therapeutics, START - Midwest, Grand Rapids, US; [9]Medical Oncology, START Madrid-CIOCC HM Hospital Sanchinarro, Madrid, ES; [10]Molecular Therapeutics and Biomarkers Laboratory, Peter MacCallum Cancer Center, Melbourne, VIC, AU; [11]Div. of Heamatology/Oncology, Medicine, Samsung Medical Center Sungkyunkwan University School of Medicine, Seoul, KR; [12]Thoracic Oncology, University of Chicago, Chicago, IL, US; [13]Department of Endocrine Neoplasia and Hormonal Disorders, The University of Texas MD Anderson Cancer Center, Houston, TX, US; [14]Translational Medicine, Loxo Oncology, Inc, South San Francisco, CA, US; [15]Medical Oncology, Dana-Faber Cancer Institute, Boston, MA, US

Abstract 3625

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)
- LIBRETTO-001 is a first-in-human clinical trial evaluating the safety and efficacy of LOXO-292 in patients with *RET* altered cancers. Best tumor response for the first 82 patients enrolled to the phase 1 portion of the study is shown in **Figure 1** (cutoff date July 19, 2018).[3,4]
- We studied the modulation of *RET* variant allele frequencies (AF) in plasma cfDNA of patients receiving LOXO-292 therapy.

Figure 1. Antitumor activity in *RET* altered cancers in the LIBRETTO-001 study



Note: 7 patients not displayed; 4 due to treatment discontinuation prior to first post-baseline response assessment, 3 due to non-measurable disease at baseline (2 stable disease, and 1 complete response). *Complete response; †Unconfirmed response awaiting confirmatory response assessment. NSCLC, non-small cell lung cancer.

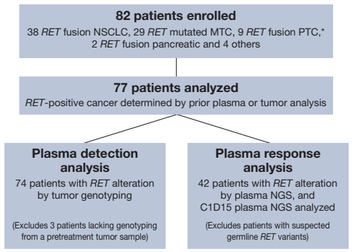
Methods

- This phase 1/2, 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 of phase 1 is to determine the maximum tolerated dose of LOXO-292 and/or the recommended phase 2 dose.
- 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 h.
- Gene alterations were assessed in *RET* and 72 other cancer-related genes, by next-generation sequencing (NGS) of cfDNA (Guardant360 assay; Guardant Health).[5]

Results

- As of April 2, 2018, 82 patients had been enrolled to 1 of 8 dose levels (20 mg QD–240 mg BID; **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 77 patients analyzed are summarized in **Table 1**.

Table 1. *RET* alterations

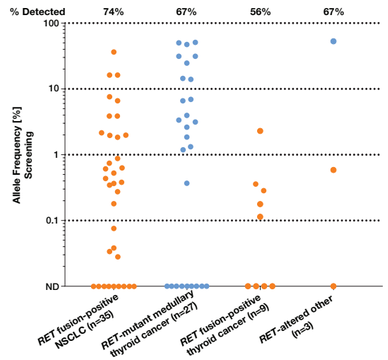
	NSCLC	MTC	PTC*	Other
Gene fusions				
KIF5B-RET	21	0	0	0
CCDC6-RET	10	0	2	0
NCOA4-RET	1	0	4	0
RET fusion NOS	4	0	0	0
CLIP1-RET	1	0	0	0
ERC1-RET	0	0	1	0
KTN1-RET	0	0	1	0
PRKAR1A-RET	0	0	0	1
RUFY3-RET	0	0	1	0
TFG-RET	0	0	0	1
Mutations				
RET M918T	0	17	0	0
RET V804M	0	2	0	0
RET C618Y	0	1	0	0
RET C620R	0	1	0	0
RET C630R	0	1	0	0
RET A883F	0	1	0	0
RET C609Y	0	0	0	1
RET E632_L633del	0	1	0	0
RET D898_E901del	0	1	0	0
RET D378_G385delinsE	0	1	0	0
RET D631_L633delinsV	0	1	0	0

Alteration not described for 1 patient, as not confirmed by independent pathology report. *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 74 patients with *RET* alterations detected by tumor genotyping (**Figure 3**).
- The expected *RET* fusion was identified in cfDNA in 32 (70%) of 46 tested patients, with a median AF of 0.60%:
  - Fusions were detected in 26 patients with lung cancer, 5 with thyroid cancer and 1 with pancreatic cancer
- The expected *RET* mutation (SNV or indel) was identified in cfDNA in 19 (68%) of 28 tested patients, with a median AF of 7.03%:
  - 4 had an AF in the range of 40–60%, suggesting a germline *RET* variant
  - Mutations were detected in 18 patients with thyroid cancer and 1 other
- The expected *RET* alteration was not found in 23 plasma samples:
  - 8 had no somatic mutations of any type detected; 3 of these samples had <5 ng DNA input (below the minimum required for the assay)
  - 11 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%

Figure 3. Plasma detection analysis

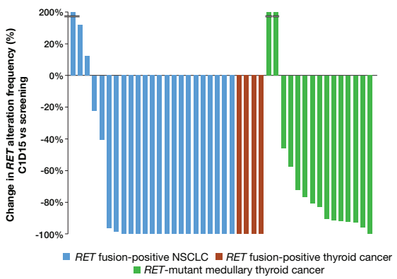


Variants not detected were arbitrarily assigned an AF of 0.01 for the purposes of plotting AF changes.
AF, allele frequency; ND, not detected; NSCLC, non-small cell lung cancer.

Plasma response analysis

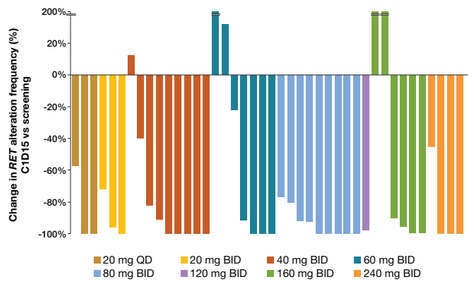
- Plasma response was studied in matched pretreatment and C1D15 plasma samples from 45 patients:
  - 3 of 45 samples were excluded from further analysis as a putative germline *RET* variant was identified (40–60% AF in baseline sample)
- Of the remaining 42, 27 had *RET* fusions and 15 had *RET* mutations detected in pretreatment cfDNA:
  - In 21 (50%) of 42 samples, the variant became undetectable at C1D15 (clearance)
  - In 34 (81%) of 42 samples, the AF decreased by at least 50%
  - The median AF decrease at C1D15 was 99%
- Tumor type and starting dose were not major determinants of the magnitude of cfDNA response (**Figures 4 & 5**).

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

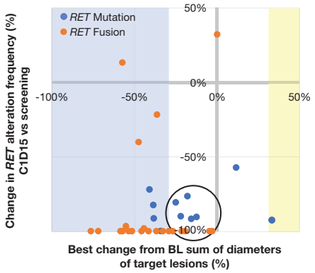


C1D15, cycle 1, day 15.

Comparison of imaging- and cfDNA-based tumor changes

- Changes in tumor burden as measured by imaging (RECIST) and cfDNA analysis were compared for 38 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



Three cases with large cfDNA increases are not shown to allow for a plot range that is easier to visualize. All three had not yet achieved a RECIST objective response.
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

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Acknowledgment

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Correspondence: Brian Tuch; brian@loxooncology.com