Circulating tumor DNA (ctDNA) in HER2 exon 20 insertion mutations predict responses in NSCLC HER2 exon 20 insertion treated with poziotinib

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Abstract

Introduction: ctDNA levels in plasma samples permits temporal assessment of tumor mutational status and tumor burden during therapy. Poziotinib is an oral pan-ErbB TKI in development for NSCLC patients harboring HER2 exon 20 insertion mutations. We assessed serial plasma samples for changes in HER2 exon 20 insertion mutations and other driver mutations in first- and second-line patients comparing to clinical response per RECIST1.1.

Methods: NSCLC patients harboring HER2 exon 20 insertion mutations were enrolled into the poziotinib ZENITH20 using tumor tissue based NGS. Serial plasma samples were collected at baseline, at C3D1, at Day 1 of every other cycle until disease progression. The Guardant360® 74-gene liquid biopsy assay was used to assess changes in tumor-associated somatic variants including the target variant HER2 exon20 insertion as well as other emergent driver mutations in ctDNA as expressed as percent variant allele frequency (%VAF).

Results: 23 first- and second-line NSCLC patients were evaluable with tumor tissue confirmation of HER2 exon 20 insertion mutations. 22 of 23 (96%) had baseline plasma samples with detectable ctDNA. 21 of 22 samples had detectable HER2 exon 20 insertion mutations (mean % VAF 20±5) resulting in a concordance of 95% versus tissue based NGS. 7 patients had serial testing through C7D1 permitting assessment of ctDNA dynamics and comparison to clinical responses. 5 of 7 (71%) serially tested patients treated with poziotinib at 16mg QD had undetectable HER2 exon 20 insertion at C3D1 which was associated with a tumor response PR. Tumor escape (PD) was observed in 2 of the 5 patients which correlated with increases in target HER2 exon 20 insertion VAF in the plasma with the remaining 3 patients ≥PR. Notably, the rise in HER2 exon 20 in ctDNA occurred prior to tumor escape. In one patient treated with poziotinib at 16 mg QD we observed undetectable levels of the HER2 exon 20 insertion in ctDNA at C3D1 which continued through C16. This patient's responses correlated with patient tumor response of SD at C2 which then became PR through C9 and CR through C17.

Conclusions: Poziotinib treatment resulted in reductions in HER2 exon 20 insertion mutations in ctDNA preceded and correlated with the clinical tumor response. Increases in ctDNA HER2 exon 20 insertion mutations were observed prior to confirmation of tumor escape. Serial monitoring of ctDNA is a potential predictive biomarker for treatment response and disease progression. Future evaluation in a larger population is required to confirm the impact of these findings.

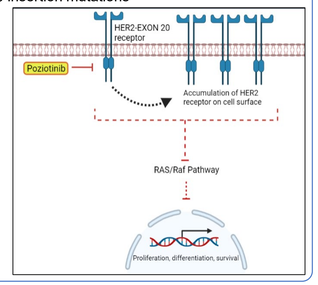
Poziotinib

Poziotinib is an oral pan-ErbB TKI with activity in patients with HER2 exon 20 insertion mutated NSCLC. HER2 exon 20 insertion mutations are a rare subset accounting for approximately 2-4% in NSCLC. There is no approved therapy for either treatment-naïve or previously treated NSCLC with HER2 exon 20 insertion mutations

Poziotinib MoA

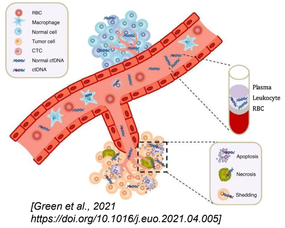
Poziotinib inhibits tyrosine kinase phosphorylation in patients harboring HER2 exon 20 mutations resulting in inhibition of the RAS/RAF pathway.

Poziotinib treatment also increases HER2 receptor expression on the surface of tumor cells harboring exon20 insertion mutations. (Cartoon)



Circulating Tumor DNA (ctDNA)

- Circulating tumor DNA, ctDNA, is tumor-derived fragmented DNA in plasma.
- Apoptosis and necrosis are the primary methods of ctDNA release into circulation.
- ctDNA is isolated and sequenced for mutational analysis and is known to reflect the mutational status of the tumor genome. (Cartoon)



[Green et al., 2021 https://doi.org/10.1016/j.euo.2021.04.005]

- In NSCLC patients treated with Poziotinib, we assessed changes in ctDNA.
- Changes in variant allele fraction (VAF%) of HER2 exon20ins somatic alterations at baseline and longitudinal plasma samples beyond C3D1 (Cycle 3 Day 1) in responders were associated with tumor response per RECIST1.1.

Methods

Patients from SPI-POZ-202 Cohorts 4 & 5 with tumors harboring HER exon20 insertion mutations received poziotinib 16mg QD were studied. In this sub analysis, 5/7 patients met the following inclusion criteria.

- *Plasma samples at baseline and longitudinal beyond C3D1*
- *Tumor size assessment at C3D1 was partial response (PR)*
- *Sufficient cfDNA and ctDNA to analyze samples*

Plasma isolation, cfDNA extraction, library construction and sequencing, and quality-control assessments were performed using Guardant360 as previously described. [Odegaard, 2018].

Variant calling was done by a validated custom bioinformatics pipeline that uses molecular barcoding and double-stranded consensus sequencing to achieve >99.99% analytic specificity per sequencing position (Mak (Talasaz), 2021)

- **Tumor tissue genotyping correlated with plasma ctDNA with a concordance of 95% at baseline.**
- **Poziotinib treatment resulted in reduction of the target HER2 exon 20 insertion mutation %VAF in plasma ctDNA at C3D1 compared to baseline and correlated to patient tumor response (RECISTS1.1).** (Thiagalingam A, et al., 2022; AACR, abstract #3400)

Summary of baseline demographics of 23 patients with HER2 exon 20 insertion mutations

Patient Characteristics	HER2 exon 20 ins
Age, median (range)	62 (43-79)
Female/Male	10 /13
White/Asian/Black	16 / 5 / 2
Histological types	
Adenocarcinomas	23
Stage (n)	I (2) / IV (21)
ECOG status: 0/1	7 /16
Smoker/nonsmoker	3 / 20
Prior therapy ≥1	3
Tumor tissue genotype	23
Guardant360 ctDNA	21

Baseline ERBB2 exon 20 insertion mutation subtypes in tissue biopsy NGS and plasma ctDNA demonstrating 95% concordance

Patient ID	ctDNA exon20 variant	Tissue NGS exon 20 variant	Concordance
2	A775_G776insYVMA	A775_G776insYVMA	✓
3	A775_G776insYVMA	A775_G776insYVMA	✓
10	A775_G776insYVMA	A775_G776insYVMA	✓
12	A775_G776insYVMA	A775_G776insYVMA	✓
14	A775_G776insYVMA	A775_G776insYVMA	✓
16	A775_G776insYVMA	A775_G776insYVMA	✓
18	A775_G776insYVMA	A775_G776insYVMA	✓
19	A775_G776insYVMA	A775_G776insYVMA	✓
20	A775_G776insYVMA	A775_G776insYVMA	✓
21	BLQ	A775_G776insYVMA	
22	A775_G776insYVMA	A775_G776insYVMA	✓
23	A775_G776insYVMA	A775_G776insYVMA	✓
9	G776delinsVC	G776_delinsVC	✓
1	G776delinsVC	G776delinsVC	✓
4	G776delinsVC	G776delinsVC	✓
5	BLQ	G776delinsVC	
7	G776delinsVC	G776delinsVC	✓
17	G776delinsVC	G776delinsVC	✓
15	G776delinsVV	G776delinsVV	✓
8	G778_P780dup	G778_P780dup	✓
13	G778_S779insCPG	G778_S779insCPG	✓
6	G776_V777delinsCVG	G776_V777delinsCVG	✓
11	G776_V777delinsVCD	G776_V777delinsVFD	x

BLQ= below limit of quantification; x Discordant.

5/7 patients had PR and sufficient plasma to perform longitudinal analysis

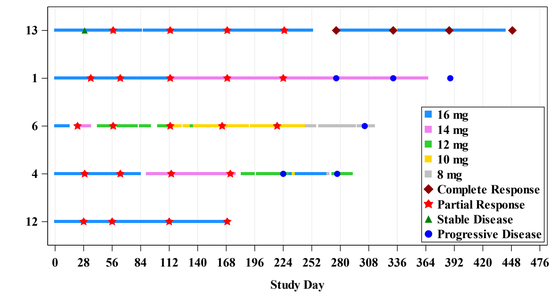
Here we report the longitudinal changes in ctDNA beyond C3D1during poziotinib treatment and changes in the target HER2 exon 20 insertion mutation, the %VAF of the variants at longitudinal timepoints and patient tumor response (RECISTS1.1).

Results

Summary of baseline demographics of the 5 responding patients

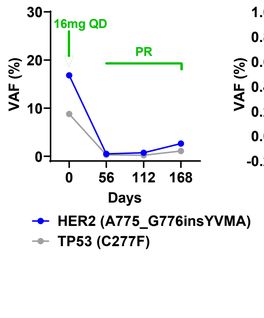
Patient Characteristics	HER2 exon 20 ins
Age, median (range)	62 (53-72)
Female/Male	4 / 1
White/Asian/Black	3 / 2 / 0
Histological types	
Adenocarcinomas	5
Stage (n)	IV (5)
ECOG status: 0/1	0 / 5
Smoker/nonsmoker	0 / 5
Prior therapy ≥1	1
Tumor tissue genotype	5
Guardant360 ctDNA	5

Tumor response to poziotinib treatment at longitudinal time points



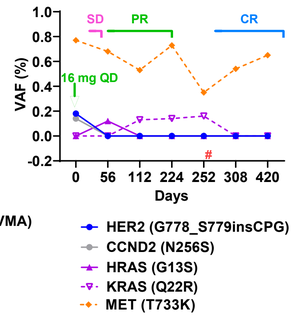
Longitudinal changes in ctDNA HER2 exon 20 insertion in responders to poziotinib treatment.

A775_G776insYVMA — **Patient ID: 12, 1L**



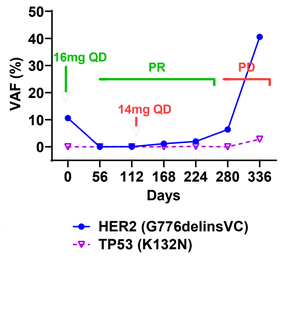
HER2 exon20 insertion mutation decreased >95% at Day 56 with low expression continuing through Day 168. This decrease in expression was associated with patient tumor response of PR through Day 168.

G778_S779insCPG — **Patient ID: 13, 1L**



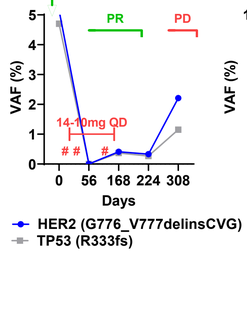
HER2 exon20 insertion mutation decreased >95% at Day 56 and persisted through Day 420 which was associated with tumor response of PR/CR. # denotes 23-day drug holiday.

G776delinsVC — **Patient ID: 1, IL**



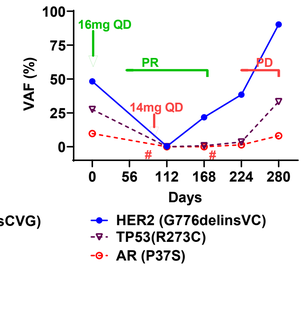
HER2 exon20 insertion mutation was decreased >95% between Day 56 -168 which was associated with patient tumor response of PR. At Day 113, patient was dose reduced to 14mg QD, and was associated with increased HER2 exon 20 mutation and PD later.

G776_V777delinsCVG — **Patient ID: 6, 1L**



HER2 exon20 insertion mutation decreased >95% at Day 56 which was associated with patient tumor response of PR. Multiple dose reductions beginning on Day 23 including 8mg QD on Day 247 was associated with increased HER2 exon 20 mutation and PD. # denotes 8-9 day drug holidays.

G776delinsVC — **Patient ID: 4, 2L**



HER2 exon20 insertion mutation decreased >95% on Day 112 which was associated with a PR. Dose was reduced and HER2 exon 20 insertion mutation rapidly increased was associated with PD. # denotes 7-day drug holidays.

Conclusion

- In poziotinib treated patients with advanced NSCLC harboring HER2 exon20 insertion mutations, baseline ctDNA presence was associated with the tumor tissue genotyping with a concordance of 95%.
- In responders, ctDNA reduced level were associated with tumor response.
- Serial monitoring of ctDNA is a potential predictive plasma biomarker for poziotinib treatment response and disease progression.
- Future evaluation in a larger population is required to confirm these findings.

References

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Poziotinib is an investigational drug that has not been approved by the Food and Drug Administration

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