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SPECTRUM PHARMACEUTICALS

Redefining Cancer Care

Predictive ability of circulating tumor DNA by Guardant360 in poziotinib-treated patients with NSCLC harboring HER2 exon 20 insertion mutations.

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Abstract

Introduction: Detection of ctDNA in plasma samples permits temporal assessment of tumor mutation status during treatment. Poziotinib is an oral pan-ErbB TKI being studied in NSCLC patients harboring HER2 exon 20 insertion mutations. We assessed serial plasma samples for changes in tumor genotype in both treatment naive and second line patients to evaluate correlation with clinical response.

Methods: NSCLC patients harboring HER2 exon 20 insertion mutations identified by tumor tissue based NGS were required for entry into the ZENITH20 study. Plasma samples were collected prior to treatment and at C3D1 (8 weeks post treatment 16mg poziotinib QD). The Guardant360® 74-gene liquid biopsy assay to assess changes in ctDNA and subsequently, mean variant allele fraction (mVAF) was utilized for analysis of samples. The Guardant360 Response™ Molecular Response (MR) algorithm was calculated as a ratio of mVAF of oncogenic alterations at baseline compared to poziotinib treatment at C3D1.

Results: 23 patients with tumor tissue confirmed NSCLC harboring HER2 exon 20 insertion mutations were studied. 22 of 23 (96%) had baseline plasma samples with detectable ctDNA. 21 of 22 samples had detectable HER2 exon 20 insertion mutations resulting in a concordance of 95% versus tissue based NGS. The most prevalent HER2 exon 20 insertion alteration was the A775_G776ins YVMA variant found in 50% of the baseline blood and tumor samples using both methods (100% concordance). 16 of 17 patients had both baseline and C3D1 samples permitting assessment of temporal response. 15 of the 16 (94%) patients demonstrated a decrease in mVAF at C3D1 compared to the mVAF at baseline. 12 of 15 patients demonstrated a >50% reduction in mVAF at C3D1 with 7 of the 12 patients showing >95% reduction in mVAF at C3D1 with clinical outcomes of 5 PRs, 1 non-CR/non-PD and 1 SD. Interestingly, 3 of these patients showed complete clearance of ctDNA target HER2 exon 20 insertions at the C3D1 timepoint.

Conclusions: Baseline Plasma ctDNA genotyping correlated with tumor tissue based NGS in NSCLC patient population with HER2 mutations. Poziotinib treatment resulted in mVAF reduction which correlated with clinical response per RECIST1.1. Assessment of longitudinal changes in ctDNA during drug therapy may potentially be used to predict patient response and potentially tumor resistance. Further evaluation in larger cohorts and longer duration of treatment is required to help elucidate the impact of these findings.

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

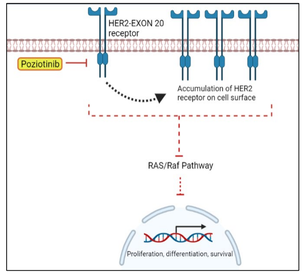
Poziotinib

Poziotinib is an oral pan-ErbB TKI with activity in patients with HER2 exon 20 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 mutations

Poziotinib MoA

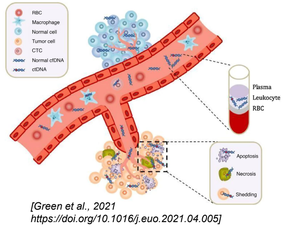
Poziotinib inhibits tyrosine kinase phosphorylation in patients harboring HER2 exon 20 mutations resulting in inhibition of the RAS/RAF pathway.

Poziotinib treatment also increases HER2 receptor expression on 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 primary methods of ctDNA release into circulation.
- ctDNA is altered in the plasma of cancer patients.
- 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 mean variant allele fraction (mVAF) of HER2 exon20ins somatic alterations at baseline and C3D1 were correlated to early 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 pilot study patients for inclusion meet the following inclusion criteria.

- *Plasma samples at baseline and C3D1 post treatment with poziotinib 16 mg QD*
- *Tumor size assessment at baseline and C3D1*
- *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]

Results

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

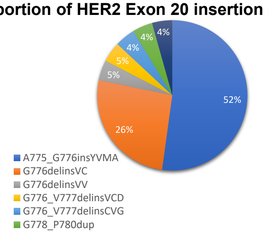
Patient Characteristics	HER2 exon 20 ins
Age, median (range)	62 (43-79)
Female/Male	10 /13
White/Asian/Black	16 / 5 / 2
Histological types	
Adenocarcinomas	23
Stage (n)	I (2) / IV (21)
ECOG status: 0/1	7 /16
Smoker/nonsmoker	3 / 20
Prior therapy ≥1	3
Tumor tissue genotype	23
Guardant360 ctDNA	21

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; cfDNA detected; x Discordant

Proportion of HER2 Exon 20 insertion mutation subtypes in the study



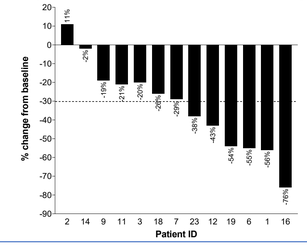
The most frequently occurring HER2 exon 20 insertion subtype was the duplication of 12-bp YVMA at codon 775 (Y772_A775dup (52%); followed by G776delinsVC, an in-frame 6-bp insertion (26%). Several other HER2 ex20ins were identified in this cohort. These results are consistent with the work of Robichaux et al, 2019 and Koga et al., 2018

Patients with HER2 Exon 20 insertion mutation in tumor at baseline NGS and ctDNA at screening and C3D1

Patient ID	ctDNA_Mut_aa	Tissue_Mut_aa	Concordance
2	A775_G776insYVMA	A775_G776insYVMA	✓
3	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	✓
23	A775_G776insYVMA	A775_G776insYVMA	✓
9	G776delinsVC	G776_delinsVC	✓
1	G776delinsVC	G776delinsVC	✓
7	G776delinsVC	G776delinsVC	✓
6	G776_V777delinsCVG	G776_V777delinsCVG	✓
11	G776_V777delinsVCD	G776_V777delinsVFD	x

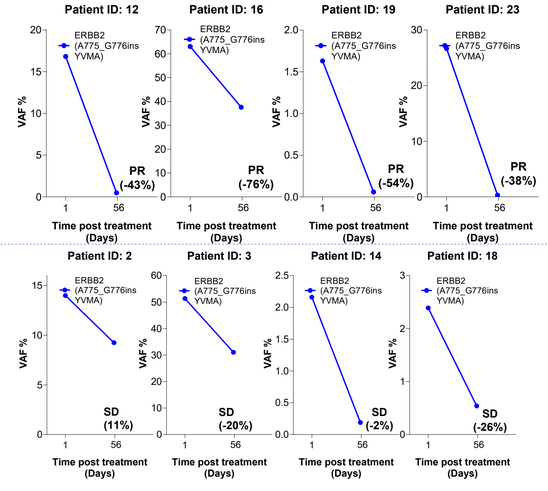
x Discordant

Tumor response to poziotinib treatment at C3D1 compared to baseline



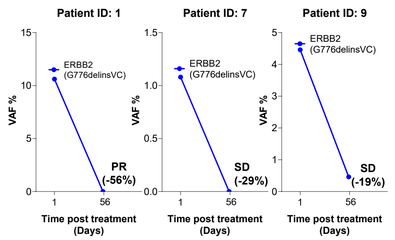
Change in ctDNA HER2 exon 20 insertion in C3D1 response to poziotinib treatment

A775_G776insYVMA

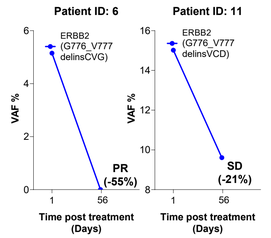


PR ctDNA reduction= 83±14%; SD ctDNA reduction = 61±14%

G776delinsVC



G776_V777delinsCVG/VCD



PR ctDNA reduction = 100%; SD ctDNA reduction = 75±19%

Conclusion

- Tumor tissue genotyping in patients with advanced NSCLC harboring HER2 exon20 insertion mutations correlated with plasma ctDNA with ~~a~~ concordance of 95%.
- Poziotinib treatment resulted in 88 ± 10 % reduction in HER2 exon 20 insertion mutation plasma ctDNA at C3D1 compared to baseline in patients with partial response (RECISTS1.1).
- Poziotinib treatment resulted in 66 ± 11% reduction in the target HER exon 20 insertion mutation plasma ctDNA at C3D1 compared to baseline in patients with stable disease (RECISTS1.1).
- Preliminary results suggest that decreases in plasma ctDNA s during poziotinib therapy correlate with clinical response in patients with tumor HER2 exon20 insertion mutations in NSCLC.
- Longitudinal assessment of changes in plasma ctDNA HER2 exon20 insertion mutations during poziotinib treatment is ongoing to assess the potential use of plasma ctDNA as a surrogate biomarker.

References

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