Rapid and sensitive detection of EGFR C797S mutations using a blood-based droplet digital PCR assay

Victoria Edwards, Leisa Jackson, Jordan M Reese PhD, Hestia Mellert PhD, and Gary A Pestano PhD

Biodesix Inc., 2970 Wilderness Place, Boulder CO 80301

Abstract # 411

Background

The adoption of third-generation tyrosine kinase inhibitors (TKIs) to treat non-small cell lung cancer (NSCLC) for EGFR positive patient cases has led to the emergence of acquired resistance pathways. Approximately 40% of $EGFR^{T790M}$-positive NSCLC cases display an acquired mutation of amino acid 797 (C797S) that mediates this resistance, which can develop after a median of 10 months following treatment. The effectiveness of treatment for patients with $EGFR^{C797S}$ may depend on whether the mutation is present in *cis* or *trans* with $EGFR^{T790M}$, the type of sensitizing mutation ($EGFR^{del19}$ or $EGFR^{L858R}$), and previous treatment(s). Patients with $EGFR^{del19}$ or $EGFR^{L858R}$ in conjunction with $EGFR^{T790M}$ and $EGFR^{C797S}$ remain resistant to osimertinib. We have developed a blood-based test that can detect two of the most common C797S nucleotide mutations (T>A and G>C) using droplet digital™ PCR (ddPCR™) technology and assays. Analytic sensitivity and specificity were assessed using synthetic DNA designed to mimic the $EGFR^{C797S}$ variants and their locus detected in the assay. Normal healthy donor samples as well as reference positive and negative NSCLC donor samples were assessed for clinical specificity and sensitivity. Finally, the precision of the assay was evaluated with both clinical and analytical samples. Specifically, we evaluated the assay at high, medium, and low mutation frequencies over three consecutive days, including repeat runs on one day, and with multiple operators. This assay is capable of accurately and precisely detecting multiple $EGFR^{C797S}$ variants from blood specimens in the clinical laboratory. Consistent with other ddPCR blood-based EGFR variant assays we have developed, the limit of detection was 0.02% for C797S variants. The EGFR C797S assay described here is approved by NYS-CLEP and may be of utility as part of the molecular diagnoses for physicians treating patients with osimertinib.

Methods

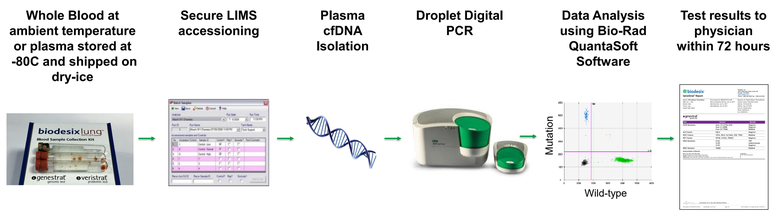

Figure 1. Overview of the GeneStrat® Test Sample Processing for cfDNA in the Biodesix Laboratory. Patient sample testing is initiated when whole blood is drawn and shipped to the Biodesix Laboratory. Patient samples for testing are accessioned into the Laboratory Information Management System (LIMS). Following nucleic acid extraction, samples are processed using the Bio-Rad QX200™ Droplet Digital PCR (ddPCR) system. Numbers of mutant and wild-type copies are assessed using QuantaSoft™ Software, and Test Result Reports (TRRs) are generated from the secure LIMS.

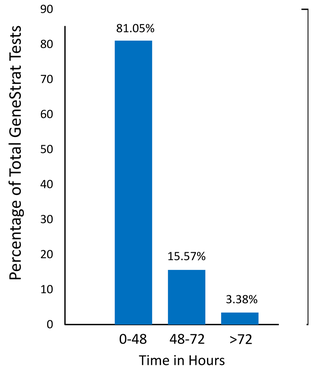

Figure 2. GeneStrat Test Turn Around Time (TAT) from sample receipt to result generation. TAT was compiled over a 34 month period (n=9,820). Data excludes weekends, holidays, and samples held for >24 hours due to incomplete clinical information on the Test Request Form.

Table 1. Validated variants within EGFR C797S assay.

Nucleotide Sequence	Amino Acid Sequence	COSMIC ID
c.2390G>C	p.C797S	COSM5945664
c.2389T>A	p.C797S	COSM6493937

Table 2. Validated variants within the EGFR C797S *cis/trans* assay.

Nucleotide Sequence	Amino Acid Sequence	COSMIC ID
c.2390G>C	p.C797S	COSM5945664
c.2389T>A	p.C797S	COSM6493937
c.2369C>T	p.T790M	COSM6240

Results

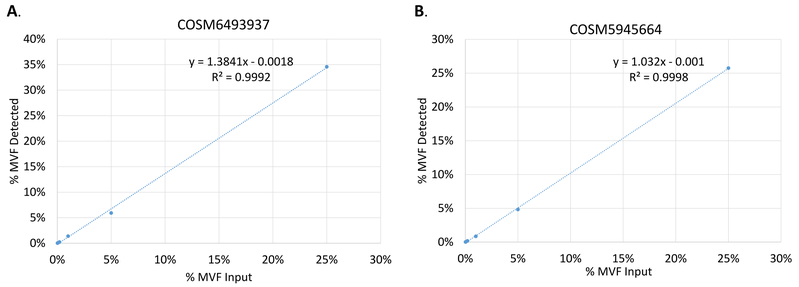

Figure 3. Analytic Limit of Detection. Analytic standards for EGFR C797S A. COSM6493937 and B. COSM5945664 variants were diluted to 0.02% in a background of EGFR wild-type human DNA. Each sample was called positive using the pre-defined 2 or more copy threshold. All samples above this threshold also contained at least 300 copies of wild-type. The specificity of each assay was also demonstrated by testing analytic standards for EGFR del19, T790M, and L858R.

Table 3. Summary Results from EGFR C797S assay Concordance Study.

		Reference Positive Samples	Refrence Negative Samples
Sample Number		3	10
Concordance*		100%	100%
Mean	Mutant Copies	172	0
	Wild-type Copies	4889	2775
Range	Mutant Copies	106-230	0-0
	Wild-type Copies	1586-7780	370-19520

* The GeneStrat KRAS G12C/D assays as well as NGS were used as the reference result for this study.

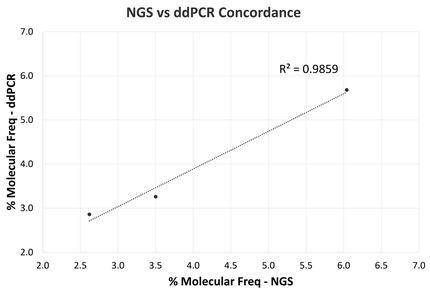

Figure 4. Concordance of ddPCR with NGS. Concordance of the molecular frequency of EGFR C797S positive clinical specimens expressing COSM5945664 detected by the EGFR C797S multiplex assay and the Oncomine™ Pan-Cancer Cell-Free Assay.

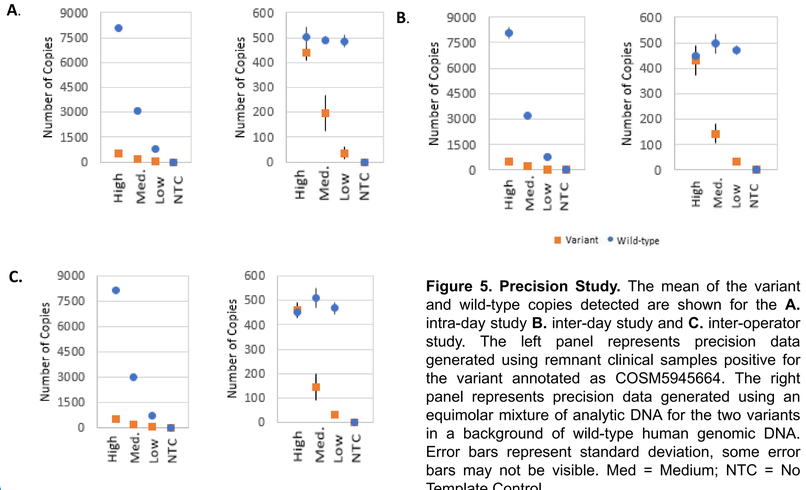

Figure 5. Precision Study. The mean of the variant and wild-type copies detected are shown for the **A.** intra-day study **B.** inter-day study and **C.** inter-operator study. The left panel represents precision data generated using remnant clinical samples positive for the variant annotated as COSM5945664. The right panel represents precision data generated using an equimolar mixture of analytic DNA for the two variants in a background of wild-type human genomic DNA. Error bars represent standard deviation, some error bars may not be visible. Med = Medium; NTC = No Template Control.

Results (continued)

Table 4. Normal Healthy Donor Specimen Evaluation

	EGFR C797S Multiplex Assay		
Sample	EGFR C797S Variant Copies	EGFR WT Copies	Result
NHD 1	0	482	Negative
NHD 2	0	728	Negative
NHD 3	0	390	Negative
NHD 4	1.8	832	Negative
NHD 5	0	736	Negative
NHD 6	0	674	Negative
NHD 8	0	654	Negative
NHD 9	0	688	Negative
NHD 10	0	450	Negative
Positive Control	88	528	Positive
NTC	0	0	Negative

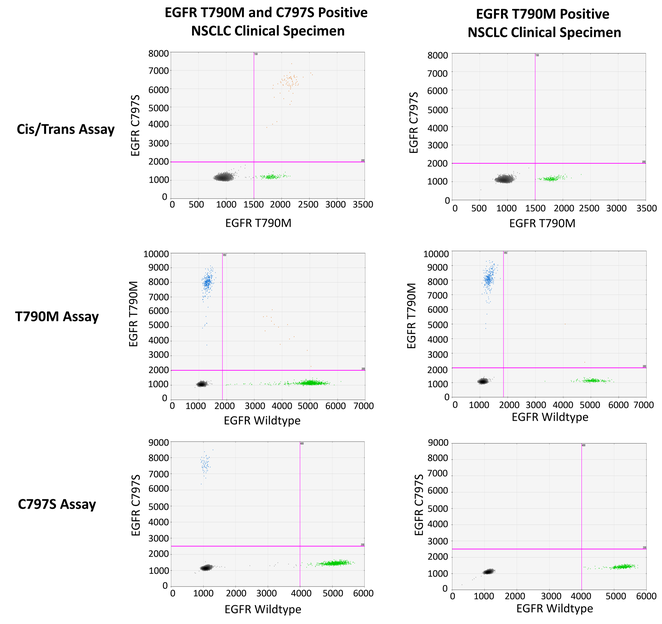

Figure 6. Representative Clinical Specimen Results. Comparative 2D plots generated using the EGFR C797S *cis/trans*, T790M, and C797S multiplex assays. A clinical specimen that is positive for COSM5945664 and COSM6240 and a clinical specimen positive only for COSM6240 are compared between the different assays. The specimen positive for EGFR C797S and EGFR T790M was confirmed using the Oncomine™ Pan-Cancer Cell-Free Assay, run on the Thermo Fisher Scientific Ion Torrent™ GeneStudio S5 system.

Summary

- NYS-CLEP approved assay for the detection of EGFR C797S in liquid biopsy samples
- Analytic Limit of Detection of 0.02% in a background of normal wild-type DNA was achieved
- Precision demonstrated with clinical samples and analytic standards at three concentrations over three runs, three days and two operators
- Normal Healthy Donor Specimen Study established that the assay was highly specific (n=10)
- Clinical concordance confirmed by orthogonal NGS methodology

Acknowledgement

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References

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- Mellert, *et al.* (2017) Development and Clinical Utility of a Blood-based Test Service for the Rapid Identification of Actionable Mutations in NSCLC. *JMD,* 19(3): 404-416.
- Thress, *et al.* (2015) Acquired EGFR C797S mutation mediates resistance to AZD9291 in non-small cell lung cancer harboring EGFR T790M. *Nature Medicine,* 21(6): 560-562.

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Contact: Hestia Mellert at hestia.mellert@biodesix.com