Assessment of *EGFR* mutations in matched urine, plasma and tumor tissue in NSCLC patients treated with rociletinib (CO-1686)

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BACKGROUND

- Approximately 60% of patients who receive an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor develop the acquired resistance mutation T790M.[1]
- Acquisition of suitable tumor tissue is a challenge for a considerable fraction of advanced non-small cell lung cancer (NSCLC) patients who require *EGFR* testing.
- We examined the detection of *EGFR* T790M mutation in circulating tumor DNA (ctDNA) from urine, assessed urine sample requirements, and compared the results with contemporaneously matched tumor tissue and plasma in TIGER-X, a phase 1/2 clinical study of rociletinib in previously treated patients with advanced NSCLC and mutant *EGFR*.

Rociletinib

- Rociletinib (CO-1686) is a novel, oral, selective covalent inhibitor of *EGFR* mutations in NSCLC. Rociletinib inhibits key activating mutations along with the T790M mutation.[2,3]

METHODS

Patients

- Pretreatment urine or plasma was obtained from 68 patients with available tumor biopsy result in TIGER-X. Matched urine or plasma was available for 51 of these patients. Urine was available for 63 of these patients.
- Patients enrolled in TIGER-X were required to have documented evidence of an *EGFR*-activating mutation in their medical record.

T790M analysis

- Tissue: *therascreen*® EGFR RGQ polymerase chain reaction (PCR) test.
- Urine and plasma: Trovagene quantitative PCR next-generation sequencing (NGS) *EGFR* T790M assay.

Figure 1. Enrichment PCR-NGS assay design

- **PCR-I**: Gene-specific primers (**GSP1**, **GSP2**) with noncomplementary "common sequence tails" (**CS1**, **CS2**) amplify target.
- Wild-type (WT) blocker (**WTB**) limits WT amplification.
- Allele-specific cycling conditions (ASCC) limit WT amplification.
- **PCR-II**: Add flow cell adapters (**P5**, **P7**) and sample barcode (BC).

Table 1. Urine and plasma EGFR assay

Characteristic	Performance
ctDNA source	Urine (specimen kit, 90–100mL recommended volume, stable for 2 weeks at room temperature) Plasma (Streck whole blood tube, BD Vacutainer® K2 EDTA and CPT tubes)
Input DNA	Recommended DNA input 30–60ng for urinary test; 10ng for plasma test
Analytical sensitivity	EGFR T790M assay detects 2 copies of mutant DNA in a background of ≈20,000 copies of WT DNA (0.01%)
Reportable range	Verified linear range, 5–250 copies; 95% confidence intervals reported
Precision	CV=37% (average) and 4-fold discrimination within reportable range
Clinical specificity	96.4%

CPT=citratetrispyridossalphosphate; CV=coefficient of variation; EDTA=ethylenediaminetetraacetic acid.

RESULTS

Urine testing for T790M has high sensitivity with prespecified urine volume acceptance criteria and identifies some patients missed by tissue testing (**Table 2**)

- Recommended urine volumes for testing are 90–100mL (approximately half of normal void). 19 of 63 patients provided the recommended volume of 90–100mL; 14 of these patients had T790M-positive tissue.
- Total DNA yields tend to increase and T790M detection sensitivity increases with urine volume (**Figure 2**).
- Positive percent agreement (PPA) for urinary T790M when tumor tissue is used as a reference:
  - **93% (13/14)** for urine with recommended volume 90–100mL (**Table 2**).
  - When inadequate tissue specimens are factored in, urine testing identifies more T790M-positive patients than tissue testing.
    - 4 patients were identified as positive in urine but were negative (n=2) or inadequate (n=2) in formalin-fixed, paraffin-embedded (FFPE) tissue.
    - T790M tumor-negative/urine-positive results are likely not false positives.
      - ➢ 4 urine-positive/tumor-negative or inadequate cases had plasma available, and 4 were positive in plasma.
  - **72% (34/47)** for all urine samples examined (volumes ranged from 10–100mL) (**Table 3**).
  - When inadequate tissue specimens are factored in, urine testing identifies as many T790M-positive patients as tissue testing.
    - 9 patients were identified as positive in urine, but were negative by FFPE.
      - ➢ 7 urine-positive/tumor-negative cases had plasma available; 6 samples were positive for T790M in plasma.

Table 2. Urine/tissue concordance for T790M testing (urine 90–100mL)

T790M		FFPE Tumor, n			Total
		Positive	Negative	Inadequate	
Urine, n	Positive	13	2	2	17
	Negative	1	1	0	2
Total		14	3	2	19

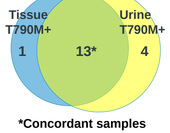


Table 3. Urine/tissue concordance for T790M testing (urine 10–100mL)

T790M		FFPE Tumor, n			Total
		Positive	Negative	Inadequate	
Urine, n	Positive	34	9	2	45
	Negative	13	4	1	18
Total		47	13	3	63

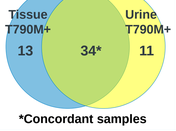
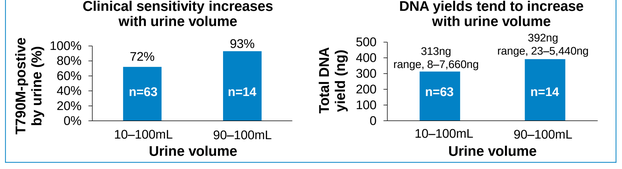


Figure 2. Clinical sensitivity: effect of urine volume



Urine testing has high concordance with plasma (**Tables 4** and **5**)

- PPA for urinary T790M when plasma is used as a reference:
  - **83% (10/12)** for urine with recommended volume 90–100mL and **73% (26/37)** for all urine samples.

Table 4. Urine/plasma concordance for T790M testing (urine 90–100mL)

T790M		Plasma, n		Total
		Positive	Negative	
Urine, n	Positive	10	2	12
	Negative	2	0	2
Total		12	2	14

Table 5. Urine/plasma concordance for T790M testing (urine 10–100mL)

T790M		Plasma, n			Total
		Positive	Negative	Inadequate	
Urine, n	Positive	26	3	3	32
	Negative	11	3[a]	0	14
Total		37	6	3	46

[a]3 patients were identified as negative in urine and plasma; 2 were also tumor negative and 1 was tumor inadequate.

Rates of T790M detection by M stage are similar in urine and plasma (**Table 6**)

- Mutations have been shown to be more readily identified in the plasma of patients with distant metastases (M1b) than in those with intrathoracic disease (M0/M1a).[4,5]

The number of T790M fragments in urine was higher for M1b patients than for M0/M1a patients

- M0/M1a: median 28 copies/10^5 genome equivalents (geq) (range, 11 to 118 copies/10^5 geq) (n=6, detectable patients only).
- M1b: median 75 copies/10^5 geq (range, 9 to >1,375 copies/10^5 geq) (n=36, detectable patients only).

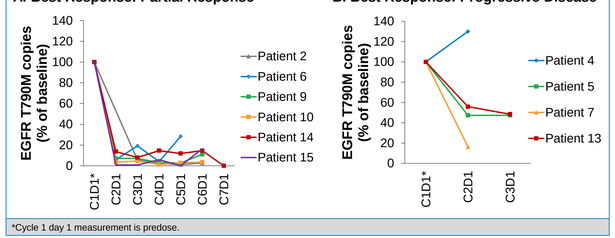
Table 6. Sensitivity of T790M detection in M1a vs M1b disease

T790M	Trovagene urine (10–100mL)	Trovagene urine (90–100mL)	Trovagene plasma (1–4mL)
M1a+M1b	73% (32/44)	92% (11/12)	89% (24/27)
M1a	50% (3/6)	100% (2/2)	100% (3/3)
M1b	76% (29/38)	90% (9/10)	88% (21/24)

Monitoring for treatment responses by urinary ctDNA (**Figure 3**)

- Urinary T790M levels were monitored in a preselected group of patients with varying clinical responses as determined by imaging (per Response Evaluation Criteria In Solid Tumors version 1.1 criteria) (**Figure 3**).
- More than 4-fold decrease in urinary T790M levels was observed by day 21 in 6/6 patients with partial response.
- Less than 4-fold decrease in urinary T790M levels was observed by day 21 in 3/4 patients with progressive disease.

Figure 3. Patients responding to rociletinib therapy have significant decrease in ctDNA *EGFR* T790M signal after first treatment cycle



*Cycle 1 day 1 measurement is predose.

CONCLUSIONS

- The analysis of ctDNA from urine identified a similar proportion of T790M-positive patients as tissue- or plasma-based testing, with the highest PPA among patients with approximately half of a normal void (90–100 mL, PPA=93%).
- Urine and tissue tests complement one another; each test identifies cases missed by the other. Discordant samples between urine and tissue that were not identified by the tumor test may be explained by tumor heterogeneity and/or inadequate biopsy.
- *EGFR* mutation detection from urine should be considered a viable approach, particularly when tumor tissue is not available.
- Monitoring urine ctDNA T790M mutation levels longitudinally is feasible and is being further explored as a means to inform choice of therapy.

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