Kinetic Monitoring of *EGFR* Exon 19 del, L858R, and T790M in Urinary Circulating Tumor DNA Predicts Radiographic Progression and Response in Patients with Metastatic Lung Adenocarcinoma

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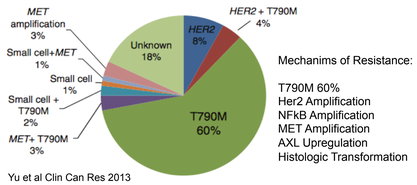
Background

Acquisition of the EGFR T790M resistance mutation is a mechanism of resistance among patients with metastatic EGFR mutant lung adenocarcinoma treated with first generation anti-EGFR inhibitors. Biopsies may be challenging in relapsed patients, and a non-invasive approach to detect T790M is desired.

We sought to monitor urinary circulating tumor (ct)DNA for the early acquisition of T790M and understand ctDNA kinetics in patients on second line anti-EGFR treatment.



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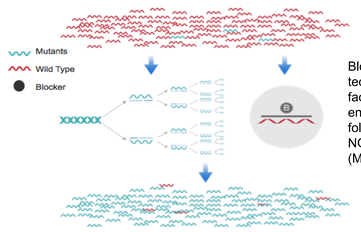
Clinical Study Design



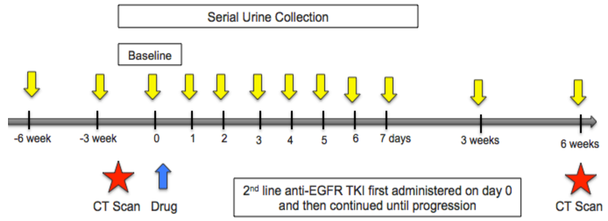
Specific Aim 1: Determine the concordance between tissue and urine: 14 patients with tissue confirmed T790M; 3 tissue T790M negative but high clinical suspicion for T790M

Specific Aim 2: Test the hypothesis that T790M could be identified prior to radiographic progression: 24 patients receiving treatment with erlotinib monitored longitudinally for acquisition of T790M mutation by urinary ctDNA; Collection frequency every 3-6 weeks

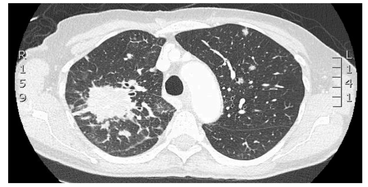
Specific Aim 3: Test the hypothesis that early dynamics of T790M mutational load in ctDNA within the first week will provide insight into tumor biology and predict radiographic response to therapy with second line anti-EGFR tyrosine kinase inhibitors: 13 T790M+ patients by tissue biopsy received treatment with second line anti-EGFR TKIs; Urine specimens were collected from patients prior to treatment, daily for 1 week, then weekly for 3 weeks, then monthly

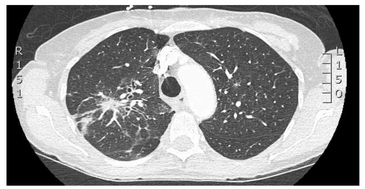
Results

UCSD IRB Approved Study Schema HRPP 130794

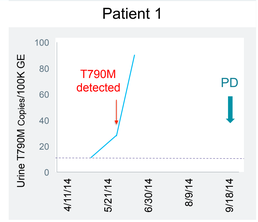


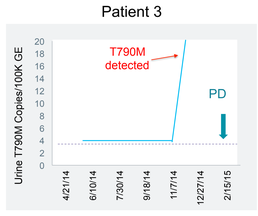
	Total Patients (N=35)	T790M Tissue Positive	T790M Tissue Negative	T790M Tissue not yet tested	Total
T790M Urine Positive	*14*	*3*	*8*	*25*
T790M Urine Negative	*0*	*0*	*10*	*10*
Total	*14*	*3*	*18*	*35*
Sensitivity of Urine ctDNA	***100% (14/14)** *at any time point*			

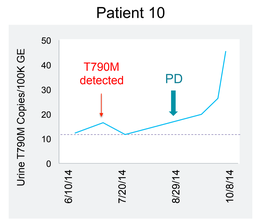


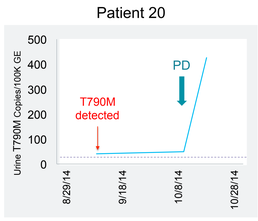


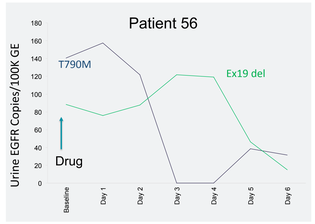
CT scan 2 weeks after initiation of second line anti EGFR treatment

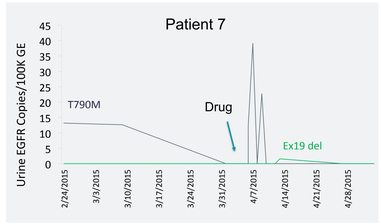




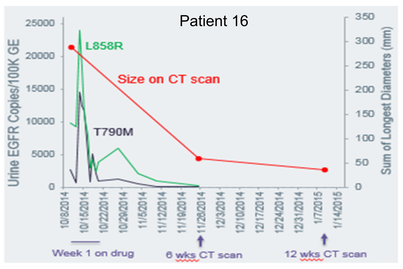


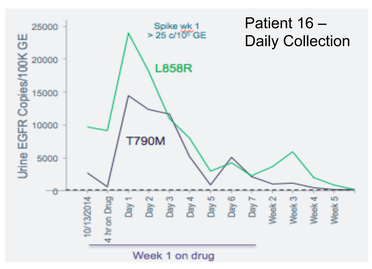


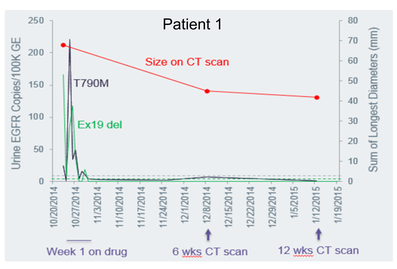


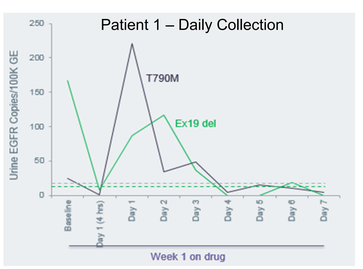


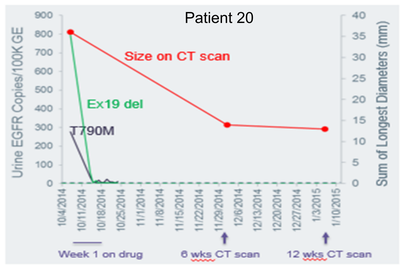
Patient 1		
Time on Drug	**T790M**	**Exon 19 del**
Baseline	24 (19-38)	167 (125-267)
Day 1 (4 h on drug)	< LOD	8 (6-13)
Day 1	221 (168-361)	87 (65-139)
Day 2	34 (28-55)	117 (88-187)
Day 3	48 (39-78)	36 (27-58)
Day 4	< LOD	< LOD
Day 5	15 (13-25)	< LOD
Day 6	< LOD	19 (14-30)
Day 7	< LOD	< LOD

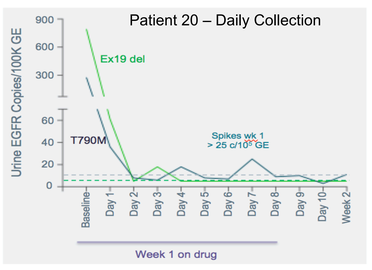


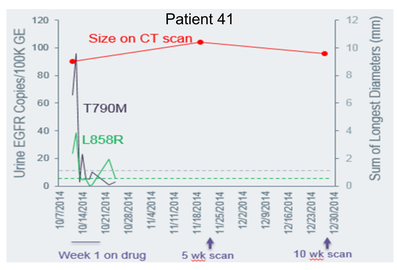


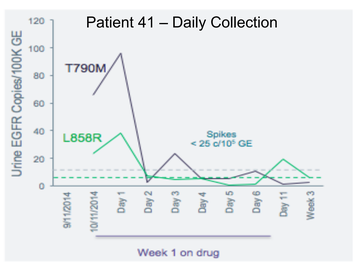












EGFR ctDNA Assay

Highly sensitive enrichment assays for the detection of *EGFR* mutations were developed: *E*xon 19 deletions, Exon 21 L858R, and Exon 20 T790M

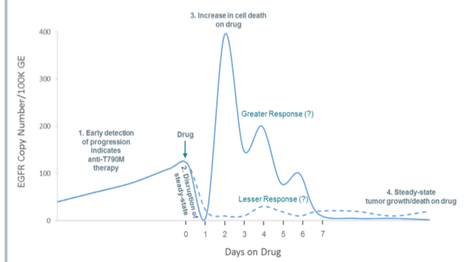
The assay is comprised of a mutant allele enrichment PCR step followed by massively parallel deep sequencing using MiSeq.

To achieve greater sensitivity in fragmented ctDNA, the enrichment PCR assay utilizes a 31bp footprint and selectively amplifies mutant DNA fragments while suppressing wild-type (WT) sequence amplification using kinetically-favorable binding conditions for a WT blocking oligonucleotide.

Barcoded adaptor primers are added for compatibility with massively parallel deep sequencing.

Following sequencing, a proprietary analysis algorithm allows accurate quantitation of input level of mutant DNA. Results are standardized by reporting number of copies detected per 10^5 genome equivalents (GE).

Conclusions



I. Concordance to urine: *EGFR* T790M mutation was detected in 24 of 35 (69%) patients receiving anti-EGFR treatment. Urine T790M detected in 14 out of 14 tissue-positive patients (100% sensitivity).

`II. Early acquisition of *EGFR* T790M: T790M mutation was detected as early as 3 months prior to radiological progression. Future studies are needed to characterize the therapeutic implications of earlier intervention with second line therapy.

III. Pharmacodynamics of early response seen in urine: Spikes in ctDNA after daily collection may correlate with early tumor lysis. Further studies are underway to monitor a diversity of allelic fractions within these time points and understand the relationship with radiographic response.

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