

Longitudinal Monitoring Of ctDNA *EGFR* Mutation Burden From Urine Correlates With Patient Response To *EGFR* Tyrosine Kinase Inhibitors: A Case Series

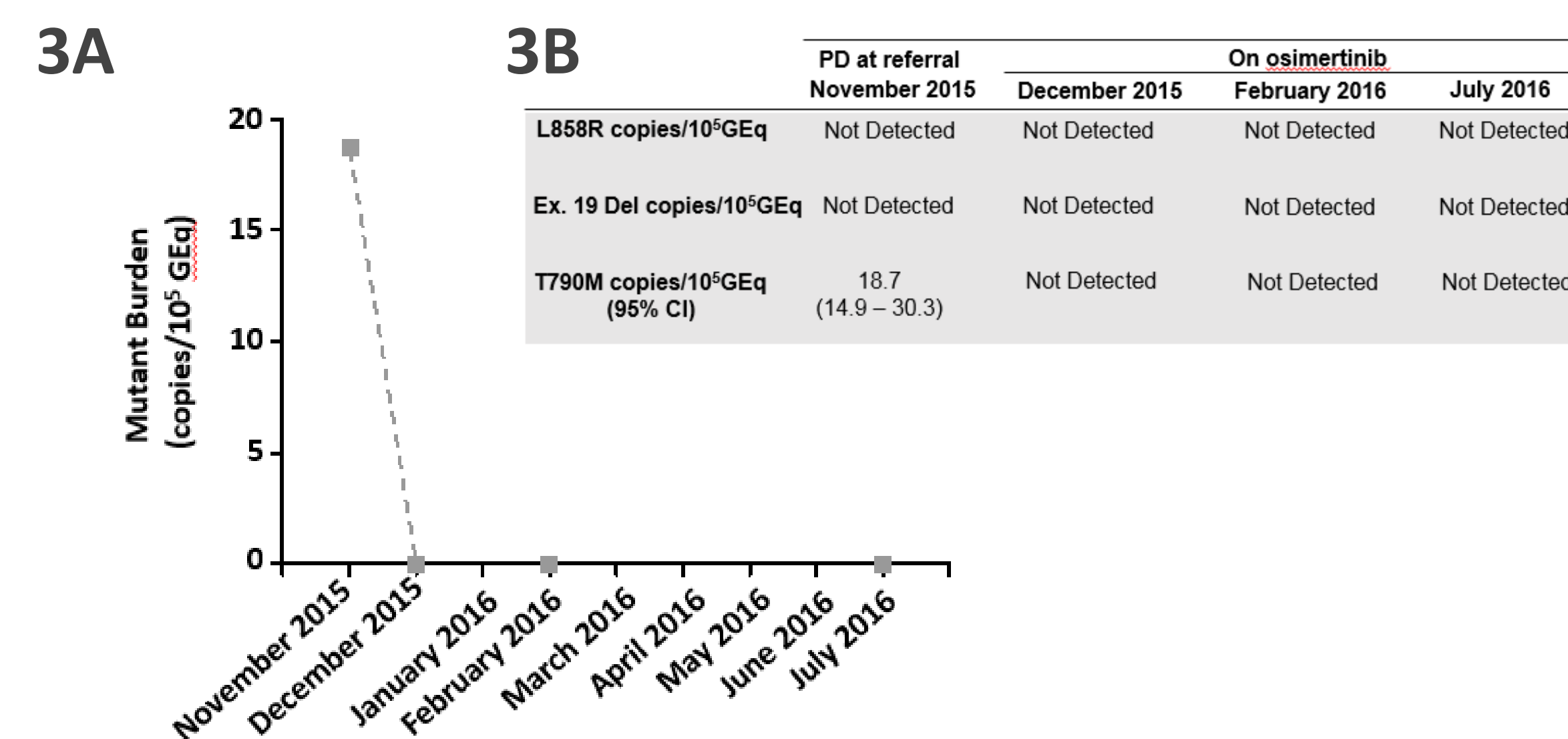
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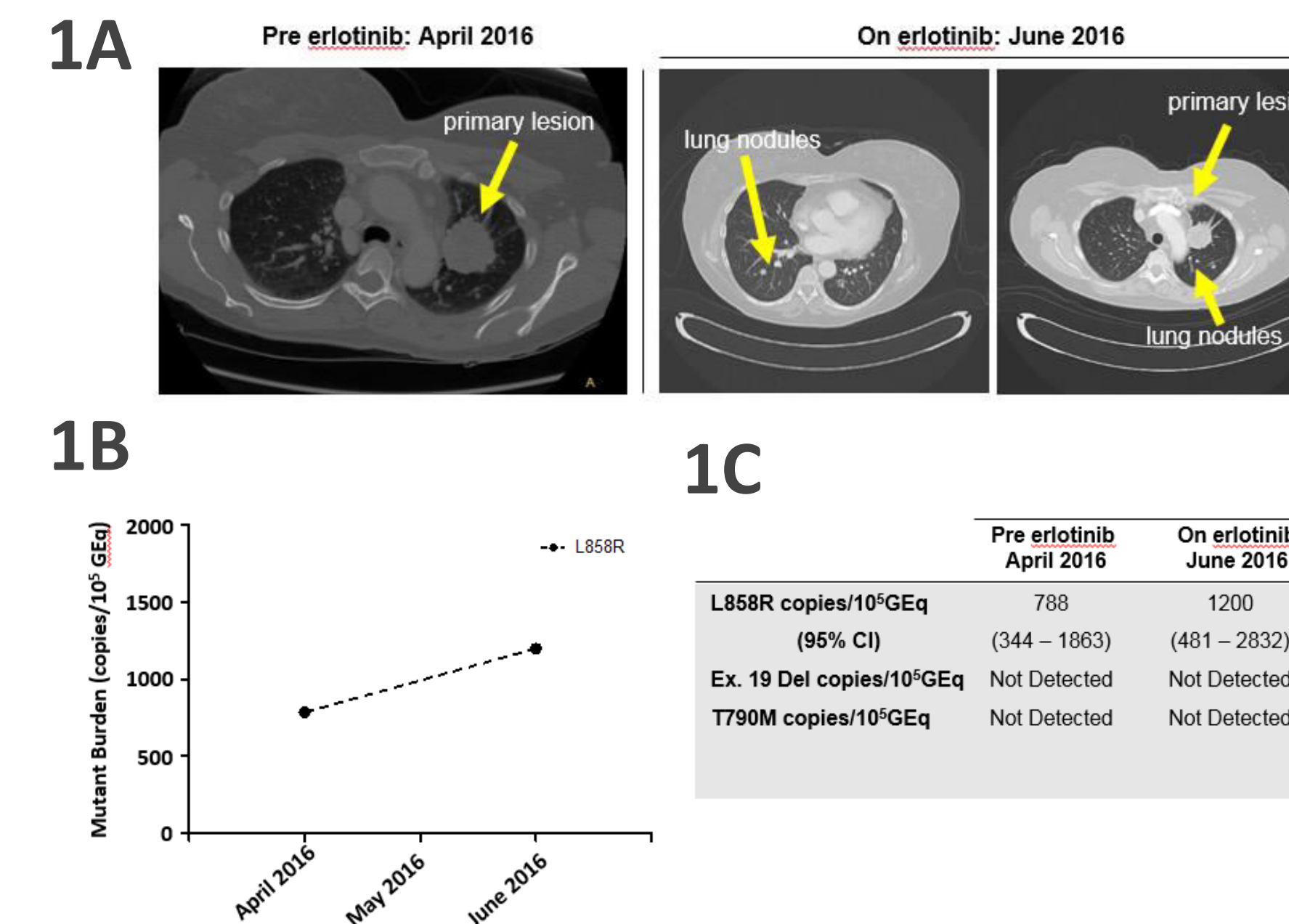
Introduction

- Tumor-associated *EGFR* mutations are prevalent in approximately 10-15% of Americans and 35-50% of Asians diagnosed with non-small cell lung cancer (NSCLC).
- Secondary *EGFR* T790M resistance mutations develop in more than half of treated patients leading to resistance to first and second generation TKI's and eligibility for third generation TKI's, specifically targeting this resistance mutation.
- A significant challenge in *EGFR* mutation assessment is the ability to obtain sufficient tumor tissue for molecular testing.
- Recent work demonstrated that circulating tumor DNA (ctDNA) can be used to identify actionable *EGFR* gene mutations. Quantitative assessment of systemic ctDNA burden has the potential to assess therapeutic response.
- We report on five patients, who were longitudinally monitored for *EGFR* mutations using an ultrasensitive and quantitative analytical platform from urine-derived systemic ctDNA in a CLIA-certified, CAP-accredited laboratory.

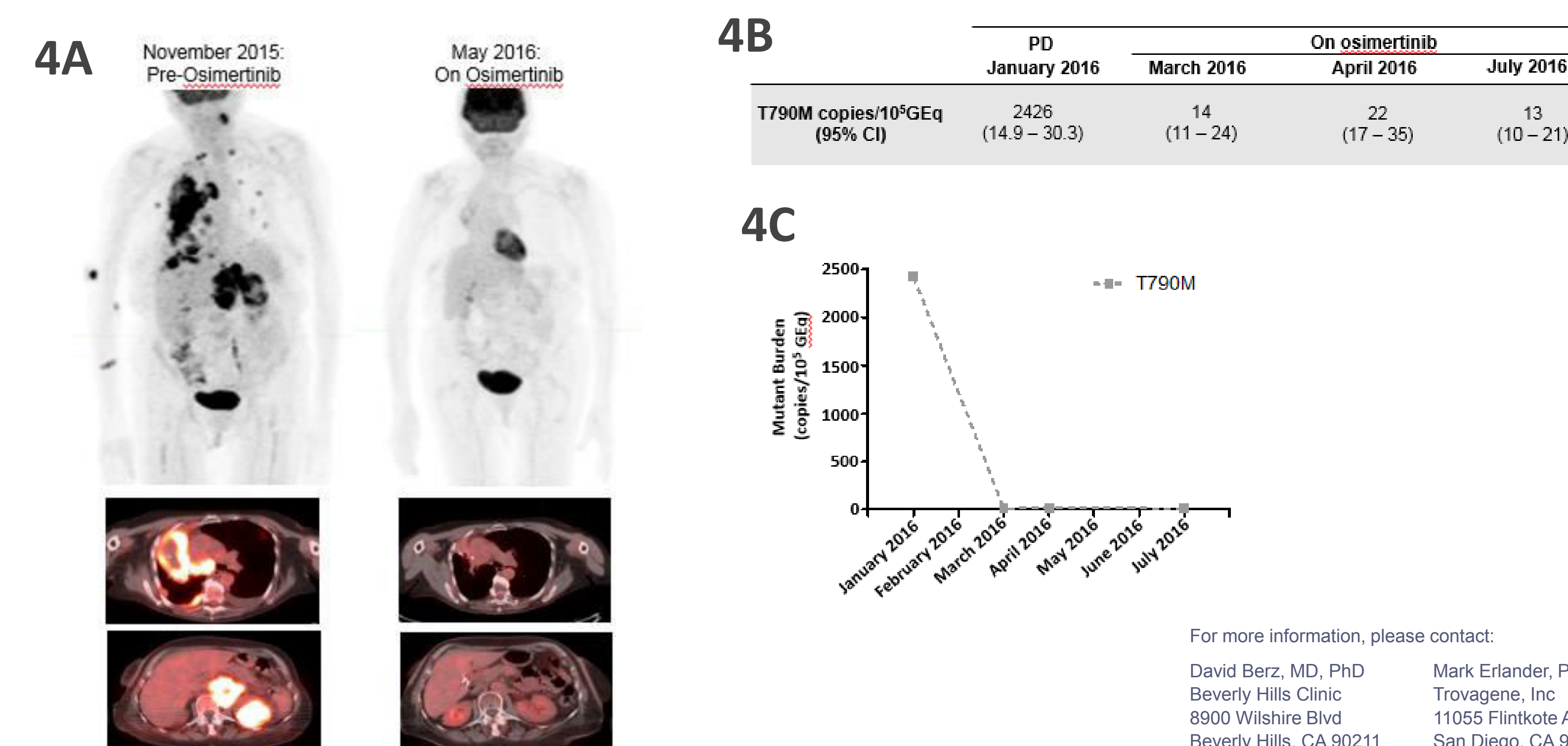
- Patient 3**
- 62-year-old Filipino female presented with dizziness and right-sided weakness.
 - Brain MRI revealed lesions that raised suspicion of metastatic disease. CT scan revealed a large left upper lobe mass compatible with metastatic carcinoma (images not available).
 - She underwent a craniotomy and excision of brain lesions. Tissue molecular analyses was positive for *EGFR* exon 19 deletion.
 - She underwent whole brain radiation followed by erlotinib with a response lasting 12 months.
 - CT confirmed progression. A tissue biopsy was positive for *EGFR* T790M.
 - Urine ctDNA analysis confirmed the T790M at 18.7 copies / 10⁵ GEq (Figure 3A, 3B).
 - She was enrolled on an expanded access trial for osimertinib.
 - One month after initiation of therapy, urine ctDNA T790M dropped below detection, which was predictive of, and consistent with, subsequent imaging studies revealing stable disease.
 - Eight months after therapy initiation, she remains on osimertinib and her T790M mutant allele burden remains undetected (Figure 3A, 3B).



- Patient 1:**
- 51-year-old Hispanic female presented with dizziness and headaches. Chest CT revealed a 4 cm left upper lung mass, enlarged mediastinal lymph nodes, left adrenal metastases, right renal metastases, and a right Iliac bone lesion (Figure 1A). Biopsy revealed adenocarcinoma. Urine ctDNA analysis identified *EGFR* L858R at 788 copies / 10⁵ GEq (Figure 1B, 1C).
 - She was started on erlotinib. After two months of therapy, a repeat chest CT revealed a decrease in the size of the left upper lobe lung mass, but interval progression in the lung nodules (Figure 1A).
 - Repeat urinary *EGFR* ctDNA analysis revealed an increase in L858R mutant allele burden (1,200 copies / 10⁵ GEq), without the presence of the T790M resistance mutation (Figure 1B, 1C).
 - A repeat CT scan of the chest performed 6 weeks later confirmed progression. She was taken off erlotinib and initiated on systemic chemotherapy.

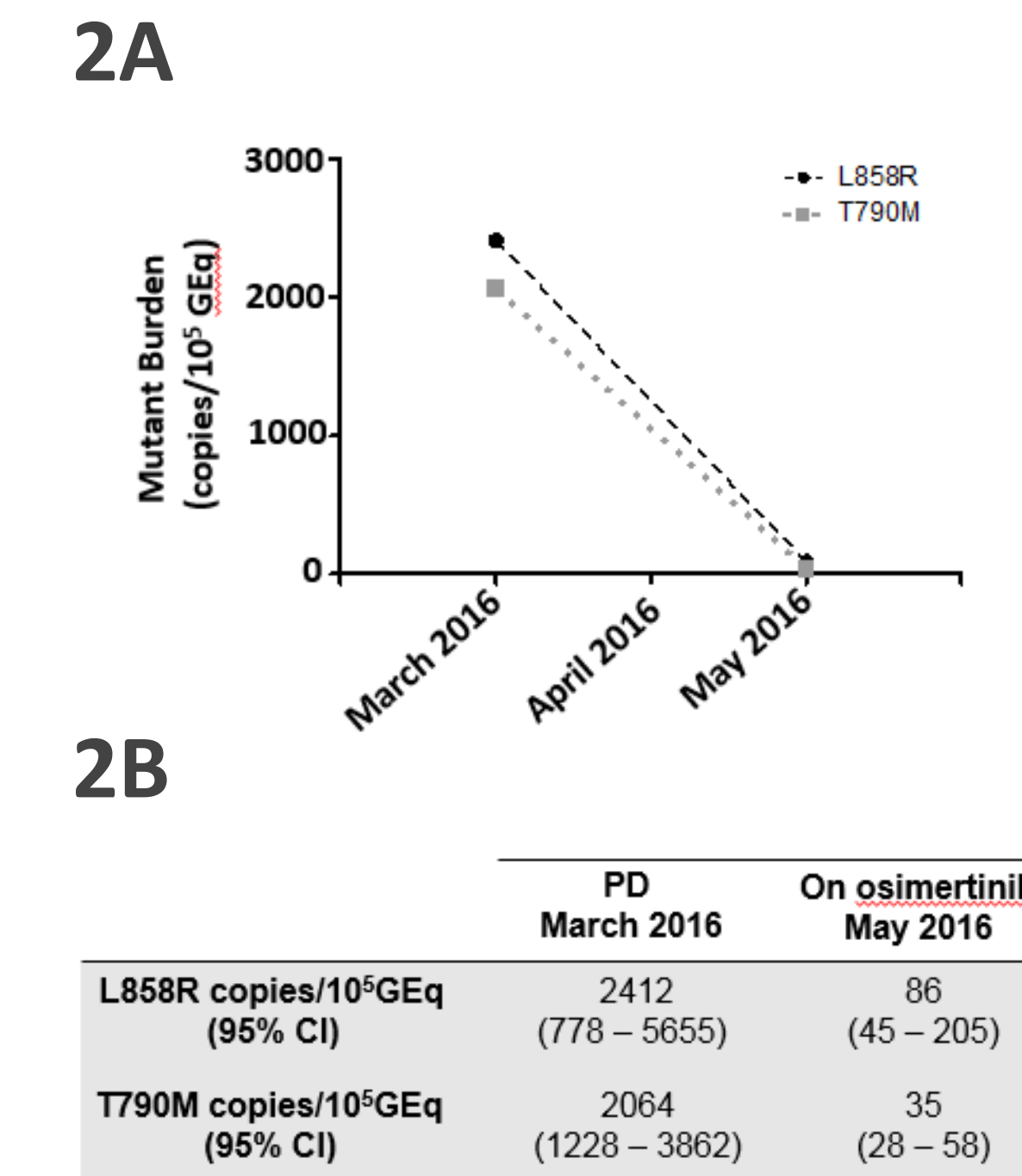


- Patient 4:**
- 74-year-old Caucasian woman was diagnosed with stage IB NSCLC and underwent complete resection with curative intent.
 - She was subsequently found to have recurrent, widely metastatic, *EGFR* exon 19 deleted NSCLC and was treated with erlotinib.
 - She presented with bulky intrathoracic and retroperitoneal progression (Figure 4A).
 - Given concern for progression, urinary ctDNA analysis was ordered and confirmed the presence of T790M at 2,426 copies / 10⁵ GEq. Based on these results, she was started on osimertinib.
 - She reported rapid clinical improvement at her first 14 day follow up visit.
 - This was paralleled by a decline of her urinary mutational ctDNA levels and imaging response at 2.5 months after initiation of osimertinib (Figure 3A).
 - Her urinary T790M ctDNA levels were found to decline to 14, 22 and 13 copies per 10⁵ GEq at week 14, 19 and 27, respectively (Figure 4B, 4C).
 - At her last follow up, she was without clear evidence of disease on CT scan.

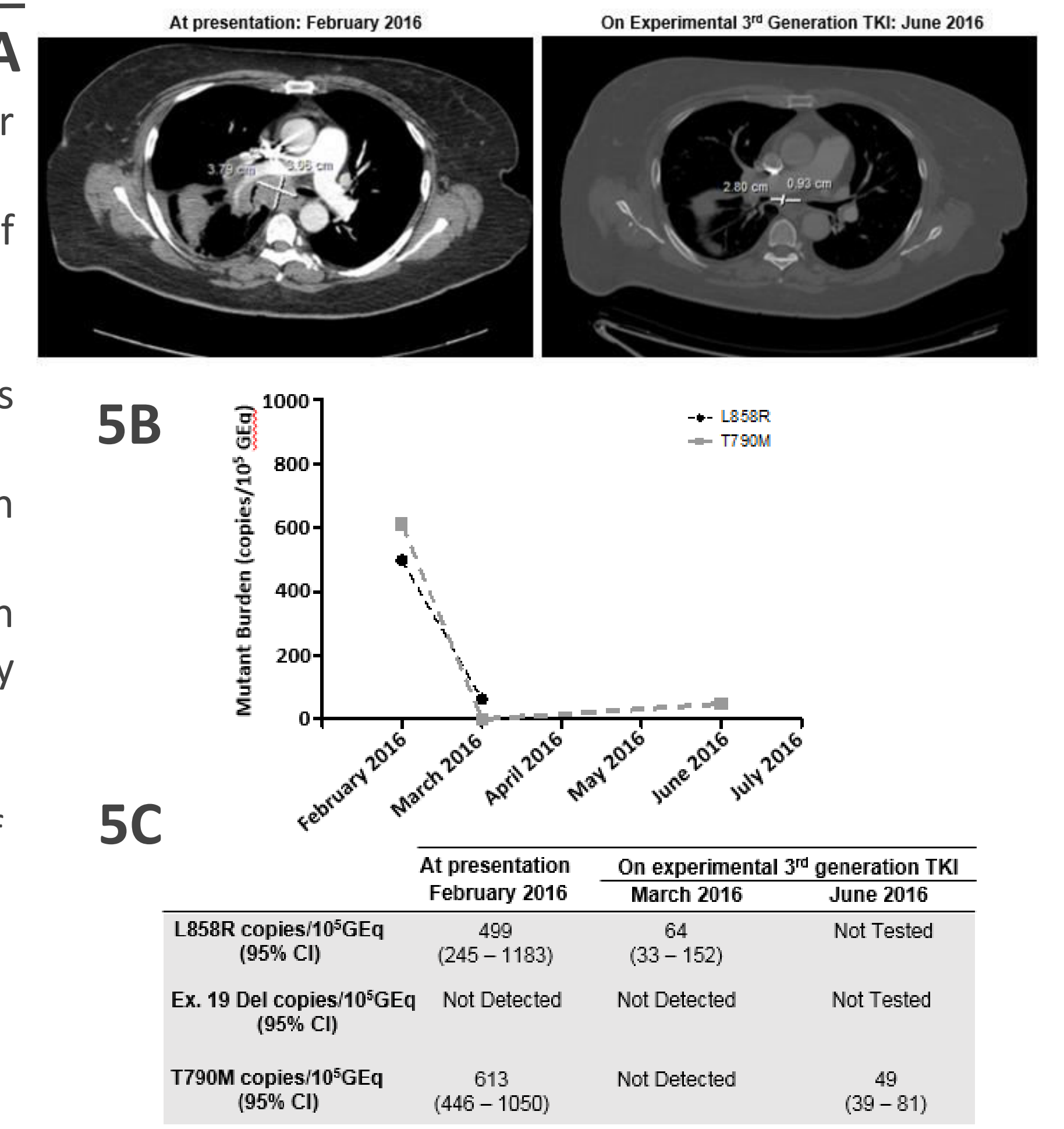


Patients

- Patient 2:**
- 49-year-old Caucasian female with tissue confirmed *EGFR* L858R NSCLC.
 - She was treated with whole brain radiation followed by erlotinib with a partial response lasting 17 months. A follow-up MRI showed probable disease progression.
 - Under suspicion of progressive disease, the patient underwent urinary ctDNA testing for L858R and T790M which revealed *EGFR* L858R (2,412 copies / 10⁵ GEq), and T790M (2,064 / 10⁵ GEq) (Figure 2A, 2B).
 - Molecular testing on pleural fluid was concordant with urine ctDNA results.
 - Based on the urinary ctDNA result, osimertinib was started. The patient had near complete resolution of her symptoms within one week.
 - Urinary ctDNA analysis was repeated 8 weeks after osimertinib initiation to assess response, revealing 86 copies and 35 copies per 10⁵ GEq for L858R and T790M (Figure 2A, 2B).
 - Repeat CT scan demonstrated a small pleural effusion but no other findings.



- Patient 5**
- 62-year old African American female presented with a right hilar / perihilar mass.
 - CT revealed multiple left lung pulmonary nodules along with indications of metastatic (Figure 5A).
 - Primary lesion biopsy was positive for *EGFR* L858R and T790M.
 - Urine *EGFR* ctDNA revealed 499 copies / 10⁵ GEq of L858R and 613 copies / 10⁵ GEq of T790M (Figure 5B, 5C).
 - She was randomized to a phase III clinical trial receiving a third-generation experimental TKI targeted against T790M.
 - After six weeks on therapy, her urinary *EGFR* ctDNA revealed a decrease in L858R (64 copies / 10⁵ GEq) and no detectable T790M, suggesting an early response to therapy (Figure 5B, 5C).
 - Four months after initiation of therapy CT showed a slight decrease in size of the dominant mass and lymph nodes, as well as complete resolution of a right lung nodule (Figure 5A).
 - Clinical assessment showed resolution of the majority of symptoms.
 - ctDNA *EGFR* mutation burden was concordant with radiographic and clinical assessments, revealing a low mutation burden of 49 copies / 10⁵ GEq of T790M *EGFR* ctDNA (Figure 5B, 5C)



Conclusions

- Here we present cases where urinary mutational *EGFR* assessment aided in the diagnostic workup and clinical management of patients with *EGFR* mutated NSCLC.
- Recent work demonstrated that *EGFR* ctDNA can serve as an early indicator of therapeutic response. This can provide valuable information particularly in the setting of late-stage patients treated with experimental therapeutics or in patients progressing on therapy. The non-invasive nature of urinary liquid biopsies allows for repeat testing to capture dynamic changes in systemic ctDNA load. The dynamic changes in ctDNA *EGFR* systemic load observed in this case series is consistent with previous findings.
- While further work is needed to characterize ctDNA as a formal tool of disease evaluation (e.g. RECIST), dynamic changes in ctDNA load have emerged as potentially viable biomarkers to monitor disease burden and early response to therapy.