# Deep Sequencing of Circulating Tumor DNA for Cancer Detection and Monitoring

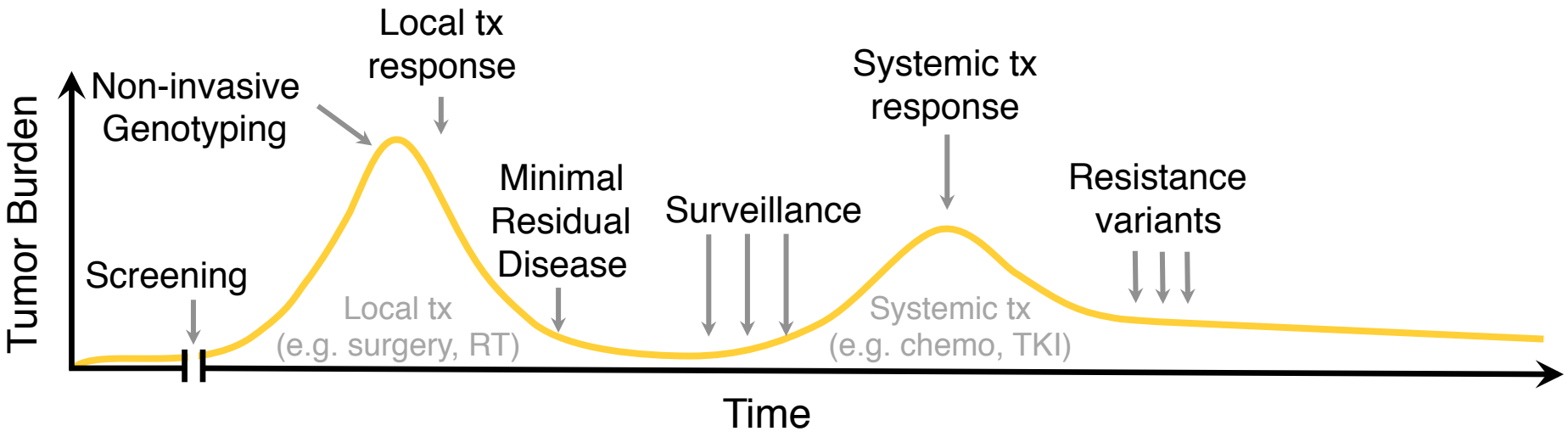

Max Diehn MD, PhD

Assistant Professor of Radiation Oncology
Stanford Cancer Institute
Institute for Stem Cell Biology and Regenerative Medicine
Stanford University School of Medicine

# Disclosure Information

- I have the following financial relationships to disclose:
  - Consultant for: Roche, Quanticel Pharmaceuticals, Novartis, CiberMed
  - Founder/stock holder: CiberMed
  - Grant/Research support from: Varian Medical Systems, Genzyme

# Potential Clinical Applications of ctDNA



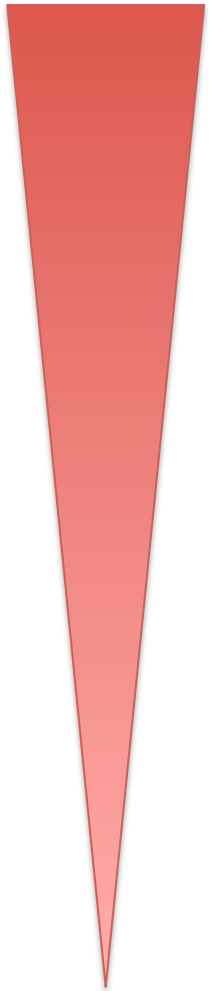
# Clinical Utility of ctDNA Detection

- Clinical utility of ctDNA is largely still being established
- Utility has been documented for non-invasive genotyping
  - EGFR activating mutations in NSCLC (FASTACT-2 trial - Mok et al. *CCR* 2015)
- Other potential applications are in early stages of being explored
  - Monitoring treatment resistance mechanisms
  - Minimal residual disease

# NGS-based ctDNA Detection

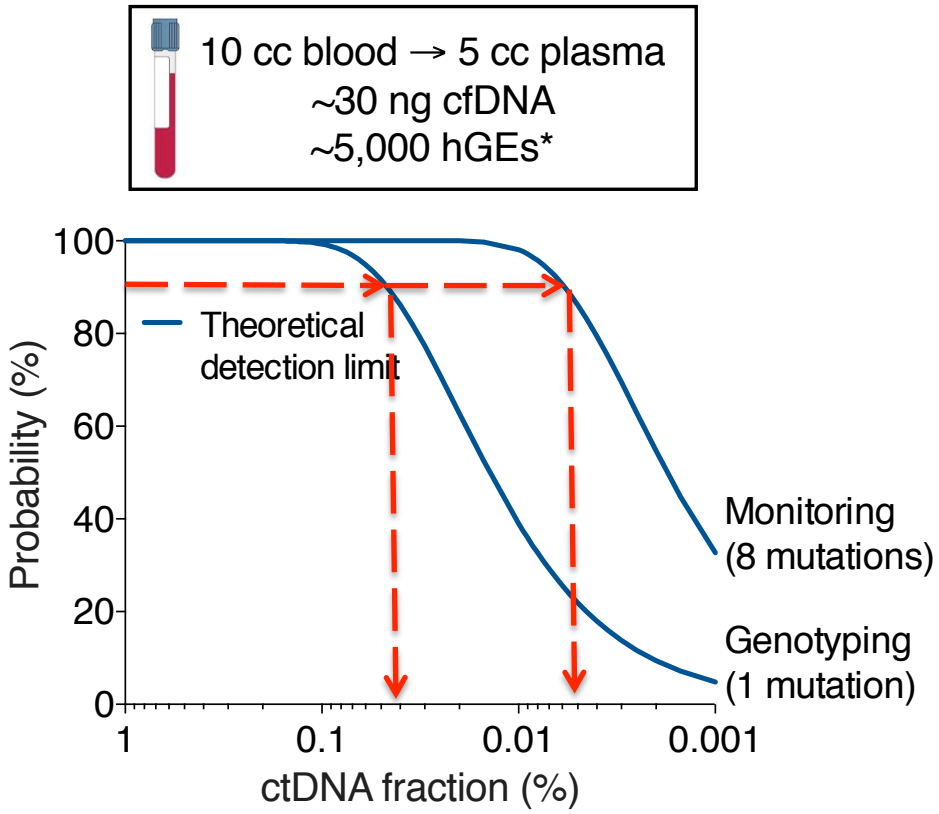
# Comparison of ctDNA Detection Limits in 10 mL Blood Draw

| ctDNA detection method | Detection limit* |
|---|---|
| Sanger sequencing | >10% |
| Pyrosequencing | ~10% |
| **Whole exome sequencing** | ~5% |
| **Whole genome sequencing** | ~1% |
| **WholeAmplicon NGS (e.g eTAm-Seq)** | ~0.3% |
| Allele-specific PCR (e.g. Intplex) | ~0.1-0.05% |
| **Barcoded Amplicon NGS (Safe-SeqS)** | |
| Digital PCR (e.g. ddPCR, BEAMing, etc.) | ~0.05-0.1% |
| **Capture-based NGS (CAPP-Seq)** | ~0.00025% |



*50% efficiency, 90% probability of detection

# Tracking Multiple Mutations Increases Maximizes Sensitivity

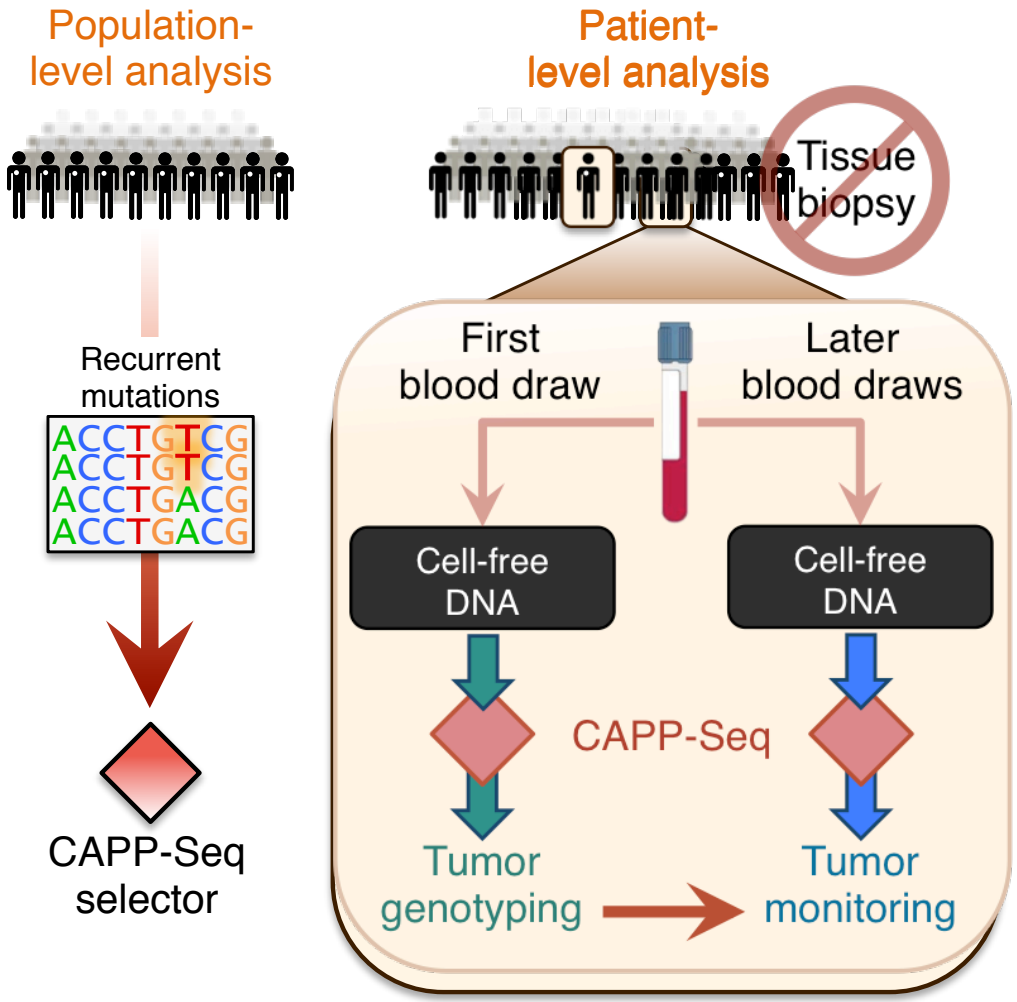



**50% recovery rate assumed*

M. Diehn / Stanford

Newman, Lovejoy, Klass, et al., *Nat Biotechnol.* 2016

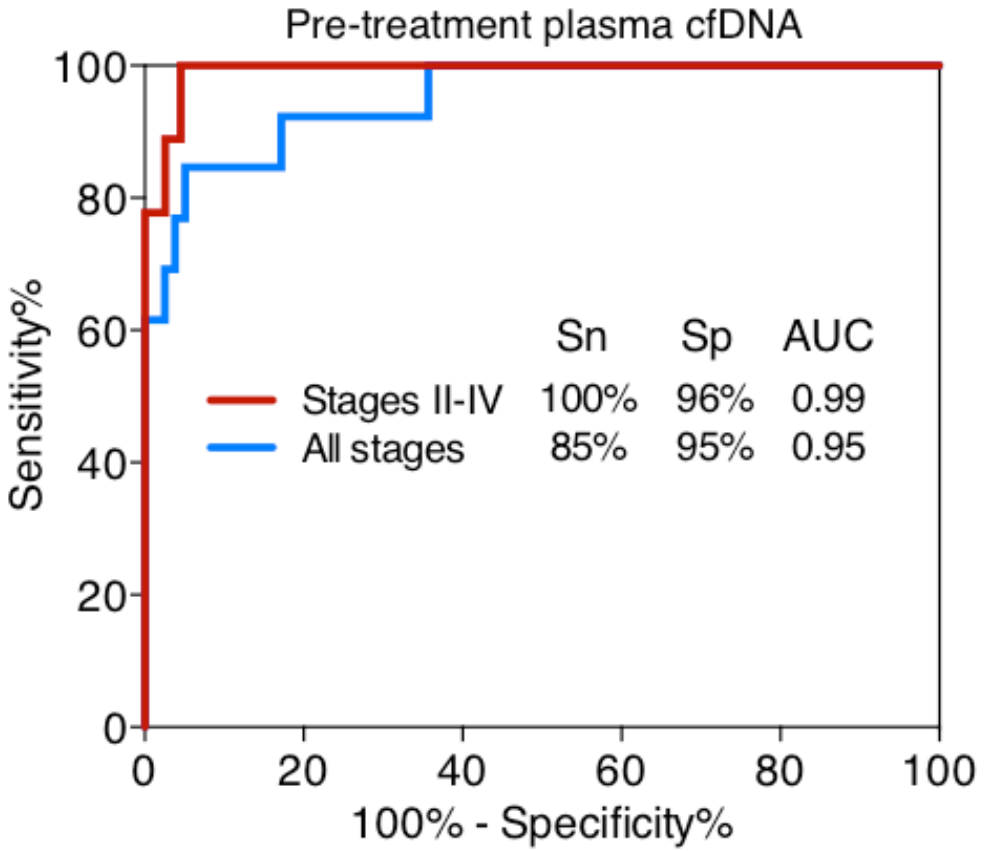
# Cancer Personalized Profiling by Deep Sequencing (CAPP-Seq)




**Personalized** biomarker for every patient

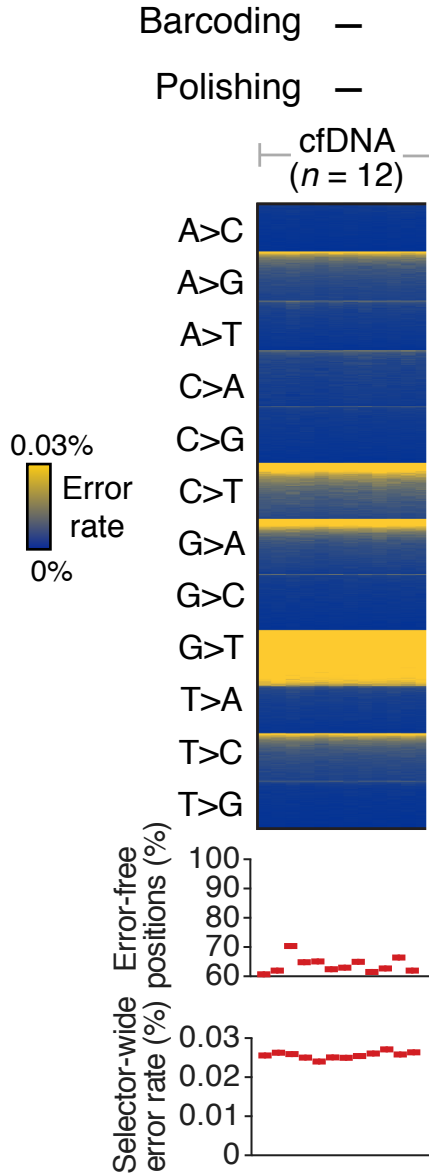
# Sensitive and Specific Detection of Circulating Tumor DNA

- Initial cohort:
  - Pre-treatment plasma samples from patients with Stage I-IV NSCLC
- % ctDNA:
  - 0.019 – 3.2%
- ctDNA concentrations:
  - 1.9 – 226 pg/mL




Newman & Bratman et al. *Nature Medicine* 2014

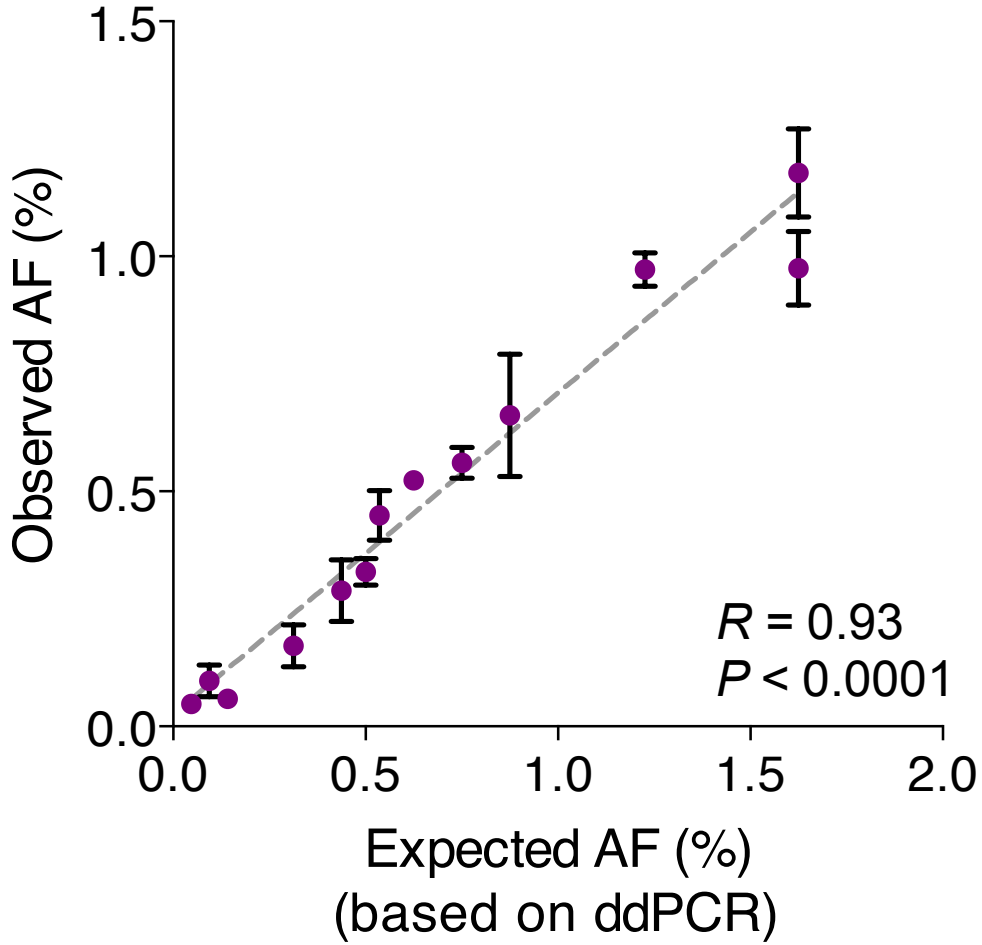
# Decreasing Sequencing Errors in Deep Sequencing-based cfDNA Analyses




= integrated Digital Error Suppression

Newman, Lovejoy, Klass, et al., *Nat Biotechnol.* 2016

# Comparison of iDES-enhanced CAPP-Seq to Digital PCR

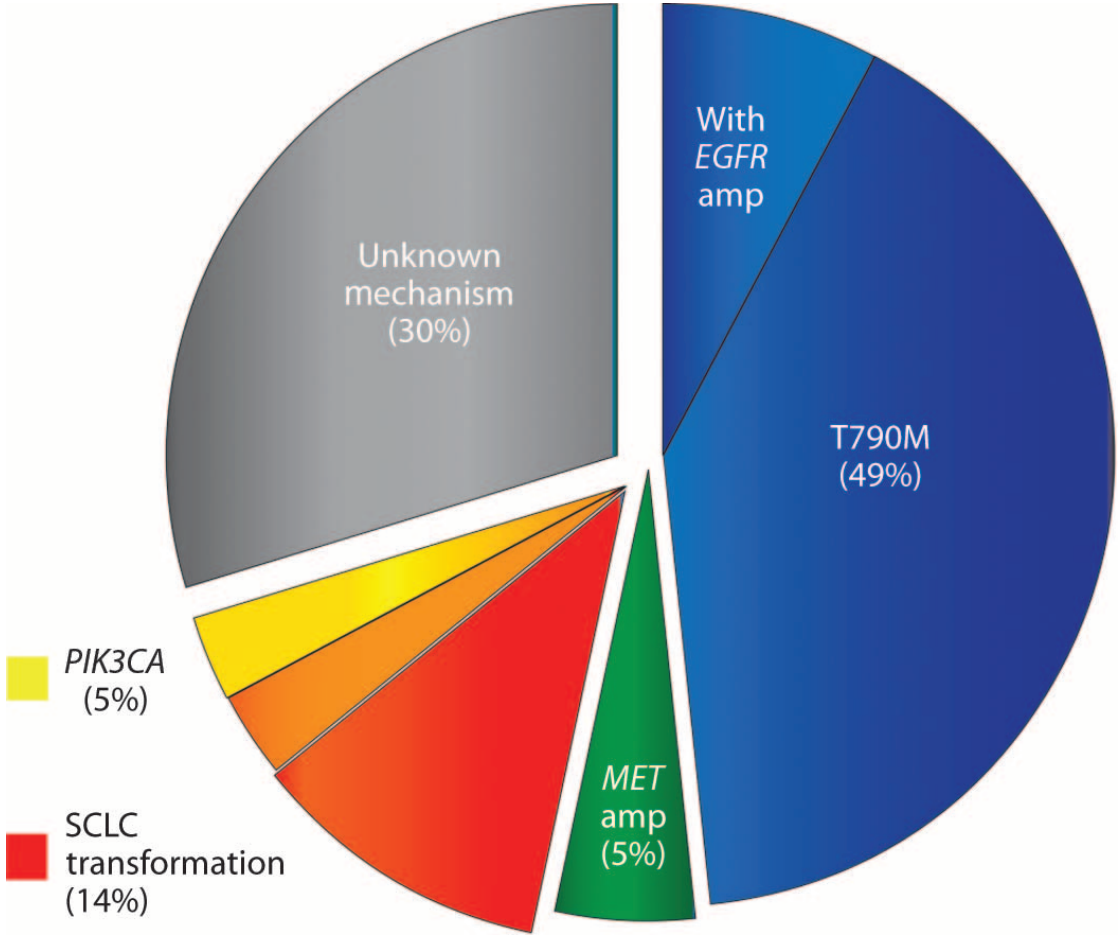


# Resistance Mechanisms in EGFR Mutant NSCLC

# Targeting EGFR Mutations in NSCLC

- Activating EGFR mutations occur in 15-50% of lung adenocarcinomas
- Sensitize tumors to EGFR tyrosine kinase inhibitors
  - First line: erlotinib, gefitinib, afatinib
- Resistance invariably develops
  - EGFR T790M is most frequent mechanism (~50-60%)
- "Third generation" EGFR TKIs target both activating and T790M mutations
  - Osimertinib, rociletinib, and others

# Frequency of First-line EGFR TKI Resistance Mutations in Tumor Biopsies




~5-15% of patients with more than one mechanism

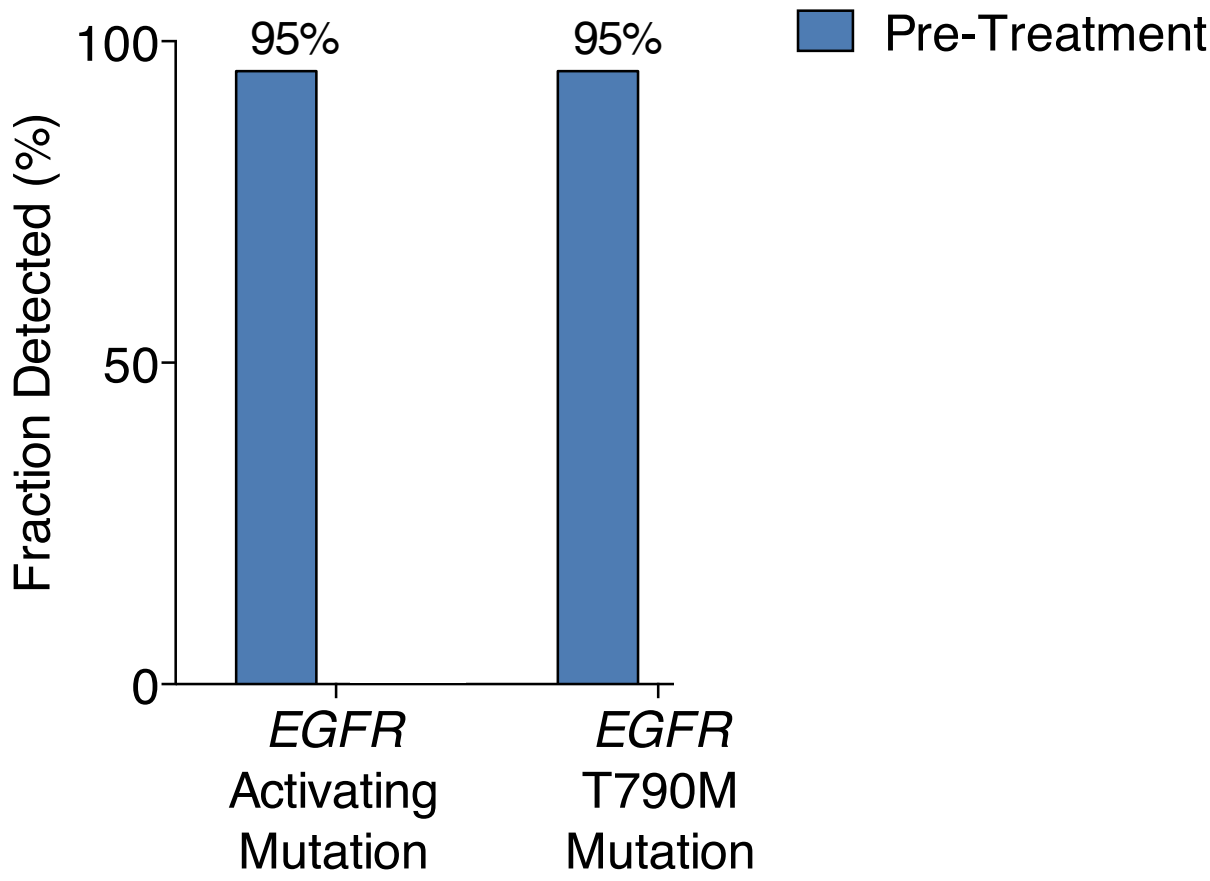


Sequist et al. *Sci Transl Med* 2011

# Heterogeneity of Resistance Mechanisms in Response to EGFR TKIs

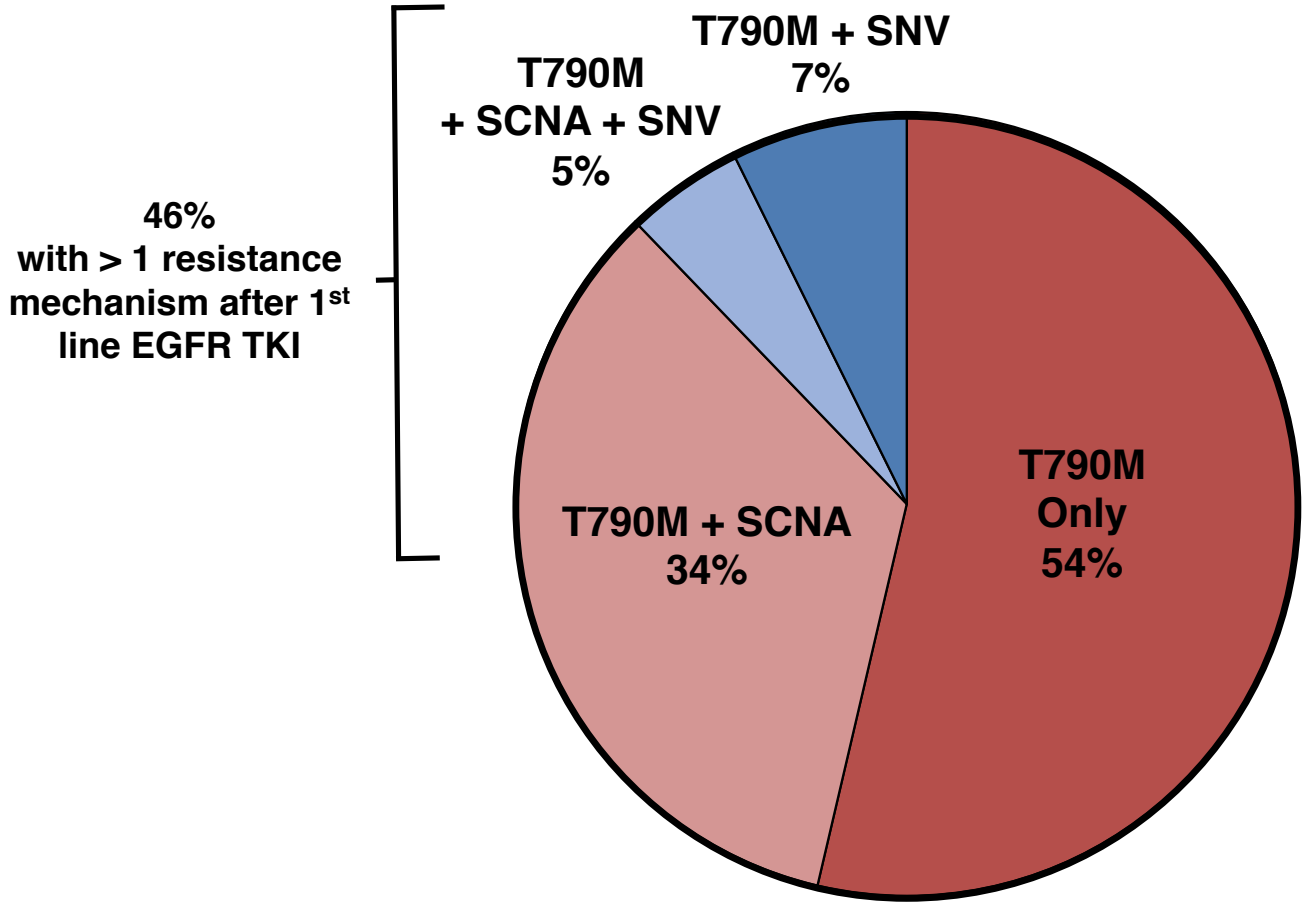
- Hypotheses
  - First-line EGFR TKI treatment frequently leads to intra-patient heterogeneity in resistance mechanisms
  - Patients with multiple resistance mechanisms respond less well to third generation EGFR TKIs
- Approach
  - Perform CAPP-Seq on plasma from 43 patients who progressed on first-line EGFR TKIs and were subsequently treated with rociletinib
  - Analyze baseline and emergent resistance-associated somatic alterations

# ctDNA Detection Summary



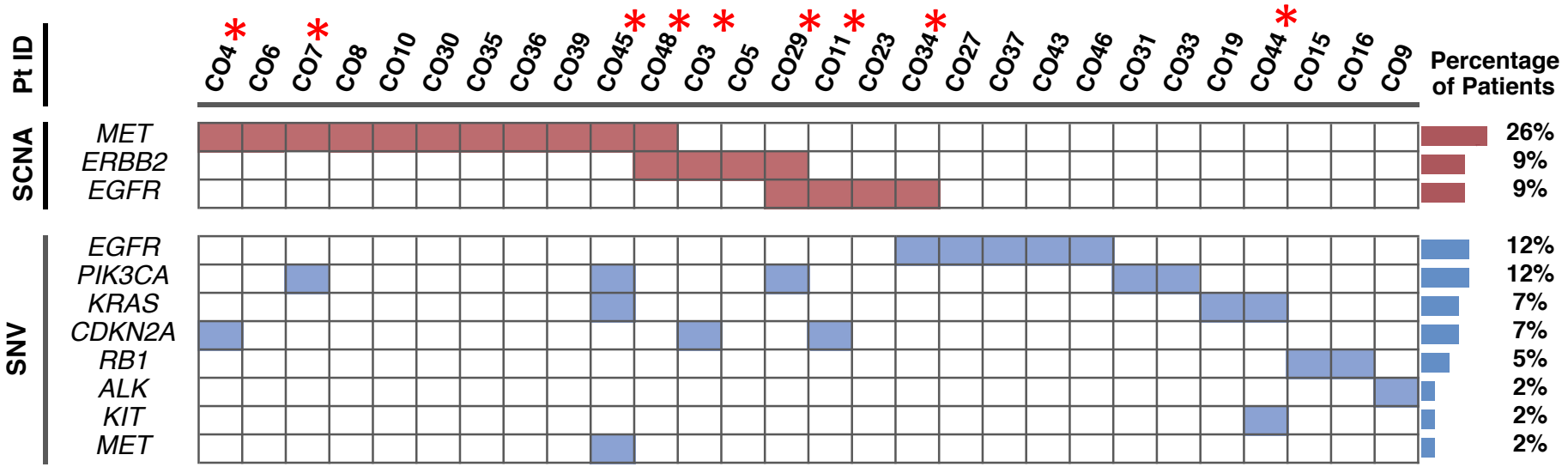

Chabon et al. *Nature Communications* 2016

# Intra-patient heterogeneity of resistance mechanisms to first-line EGFR TKIs

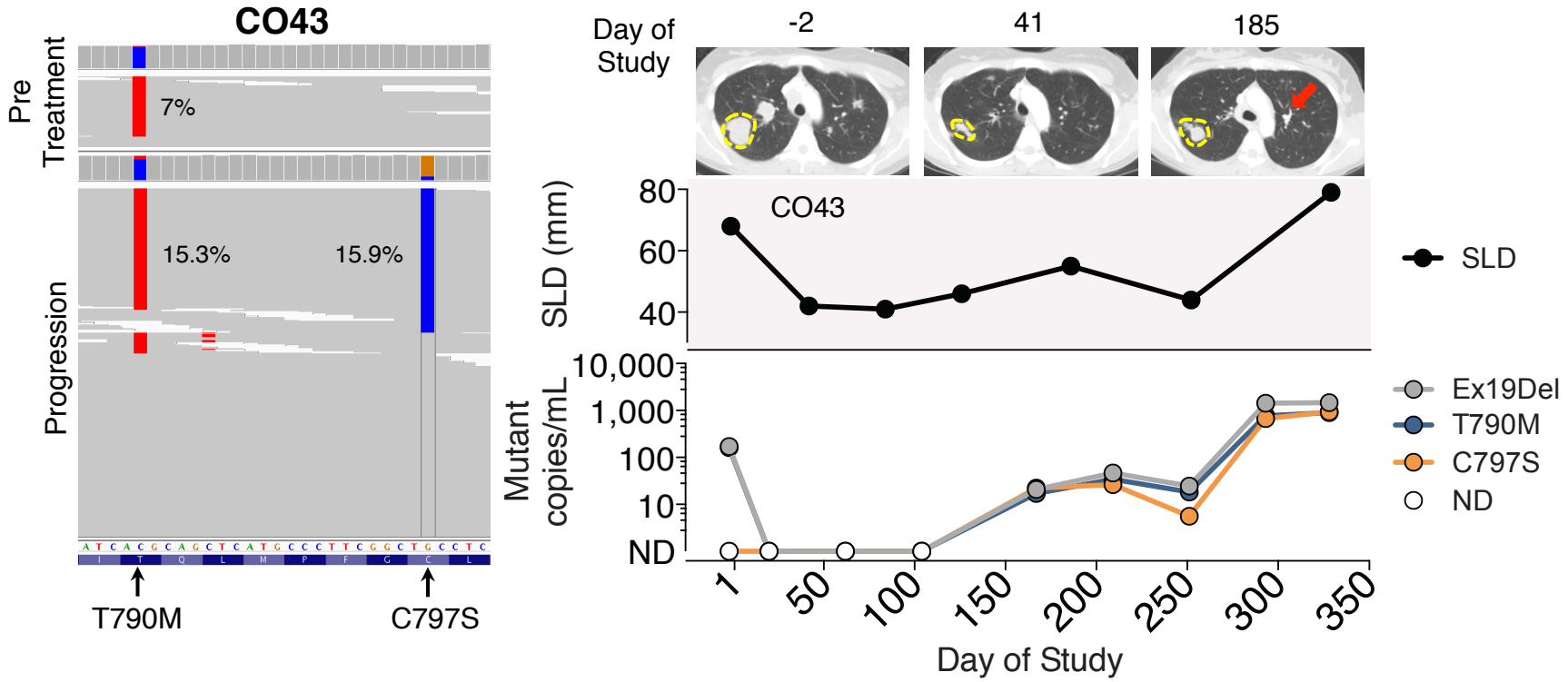


# Rociletinib Resistance Mechanisms

- Putative resistance mechanism definition
  - Absent before treatment and emerged at progression
  - Increased in relative abundance from baseline to progression
- Putative mechanism(s) identified in 65% of patients (72% of evaluable)
- Significant intra- and inter-patient heterogeneity
  - 9 genes involved
  - 21% of patients develop multiple resistance mechanisms (*)

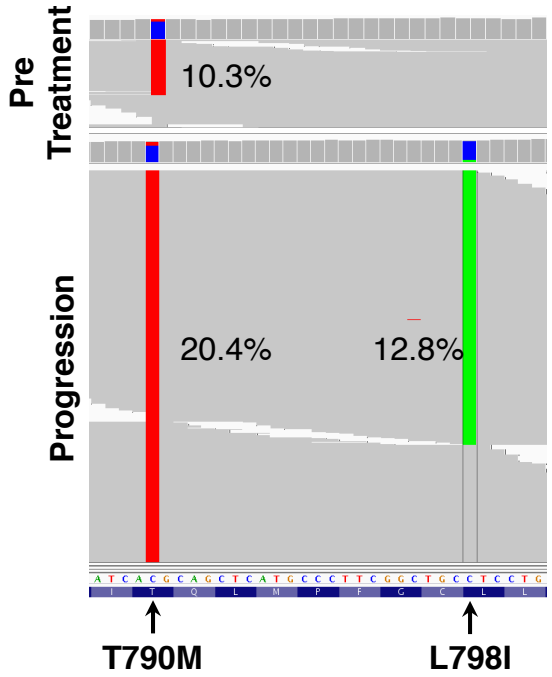


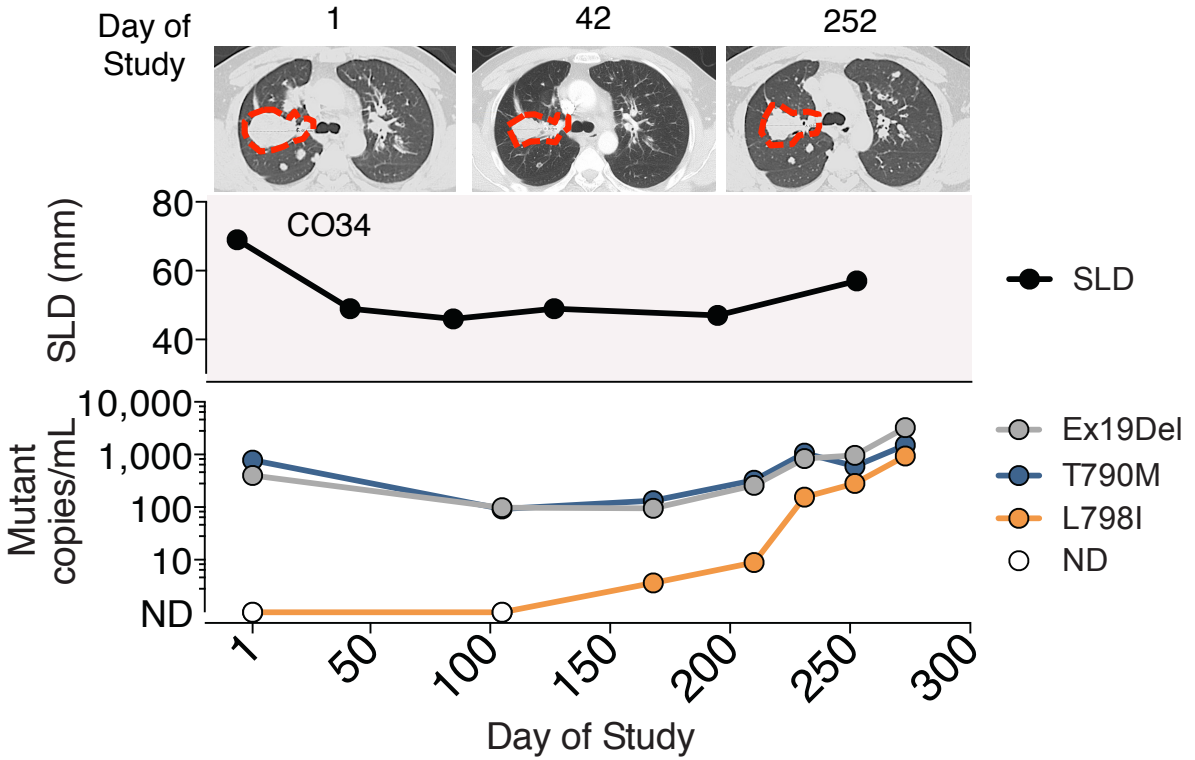
# Emergence of EGFR C797S in a Single Patient



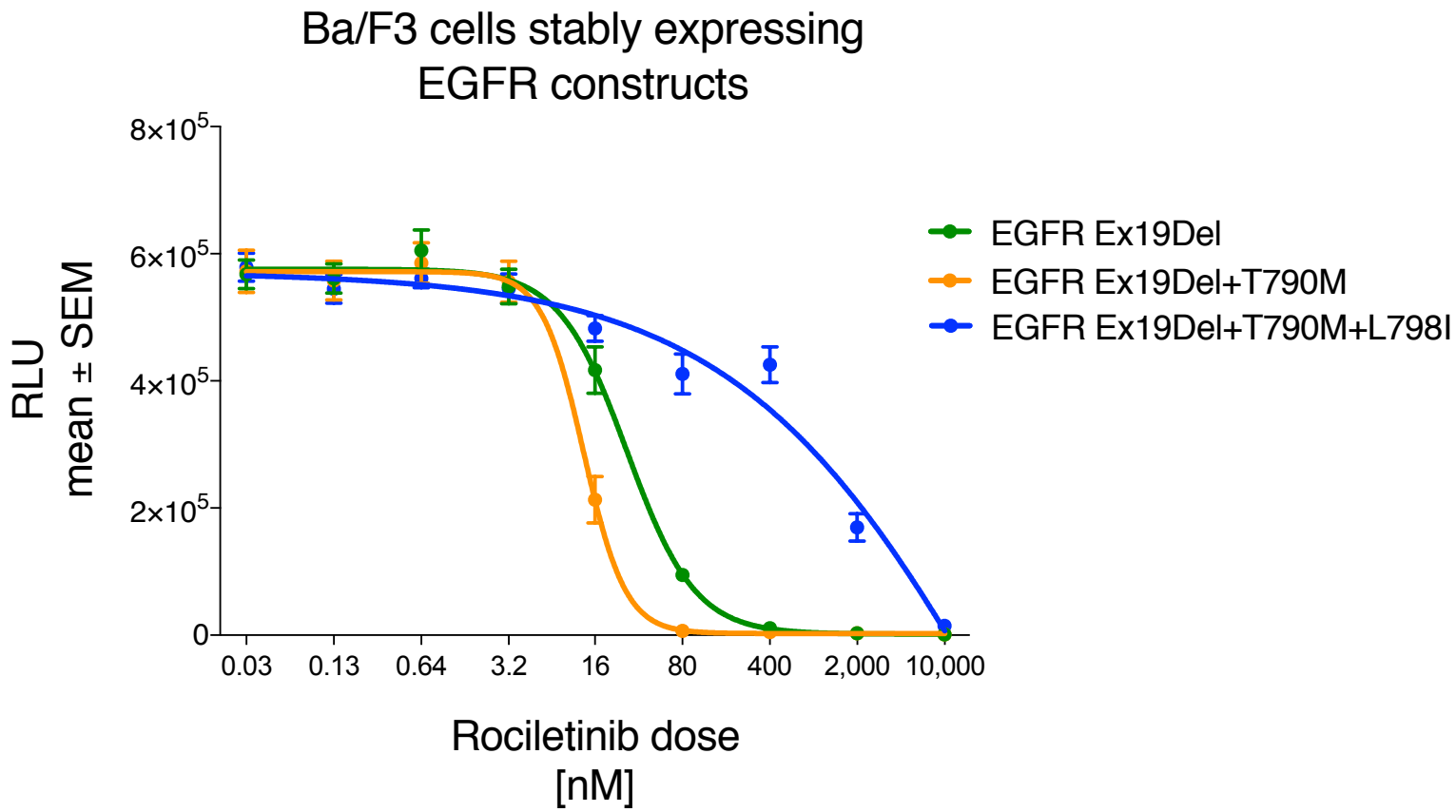

| Study | Compound | Evaluable Patients | C797S + | C797S Prevalence |
|---|---|---|---|---|
| Present Study | Rociletinib | 40 | 1 | 2% |
| Piotrowska *et al.* Cancer Discovery 2015 | | 9* | 0 | |

# Novel EGFR L798I Resistance Mutation



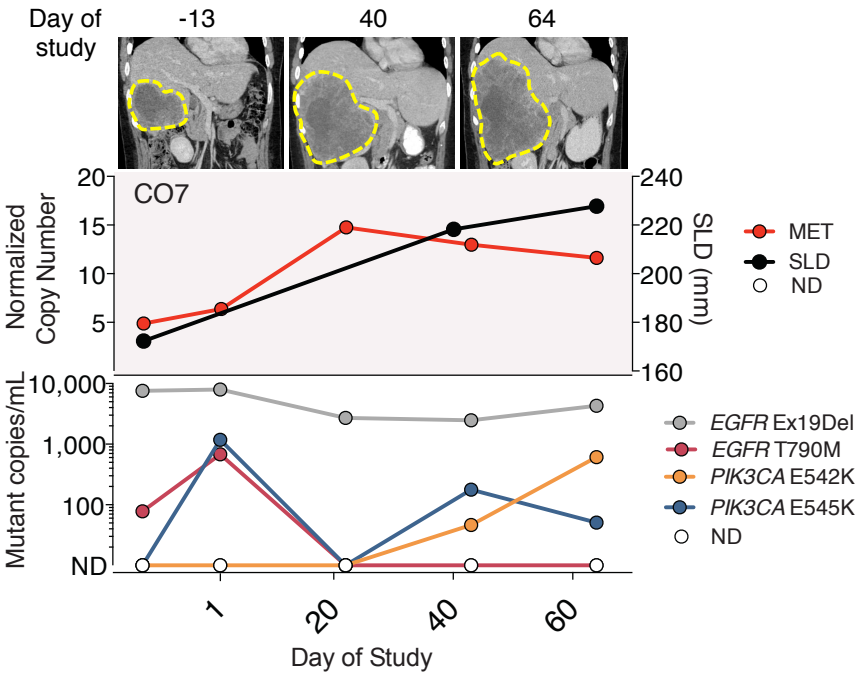



# EGFR L798I Mutation Causes Rociletinib Resistance

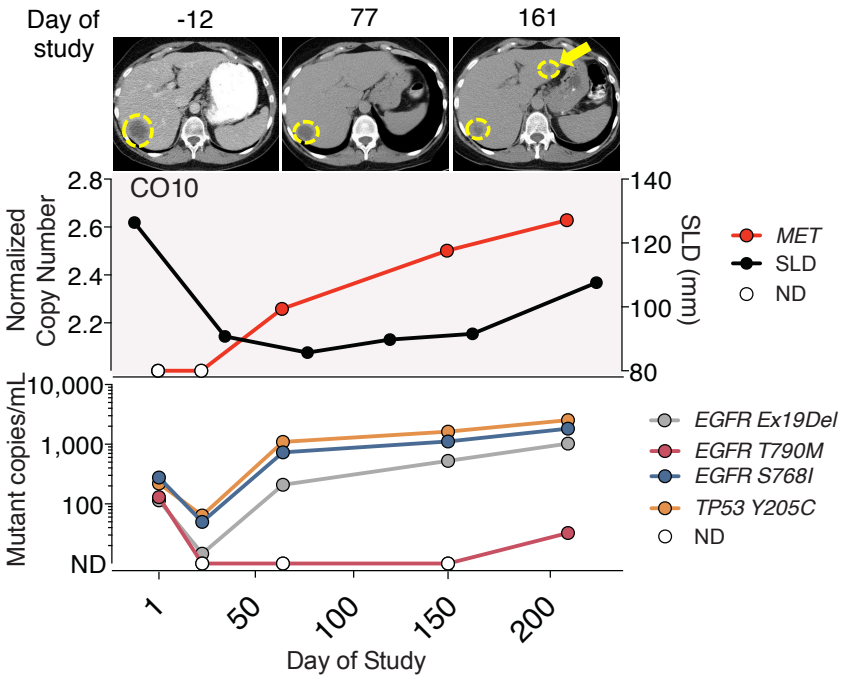


# *MET* amplification mediates innate & acquired resistance

## Innate Resistance



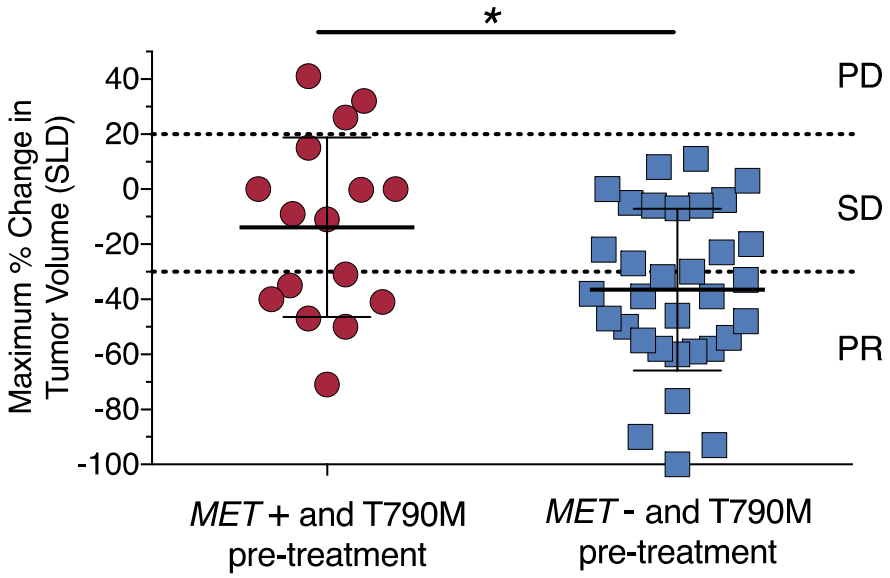

## Acquired Resistance

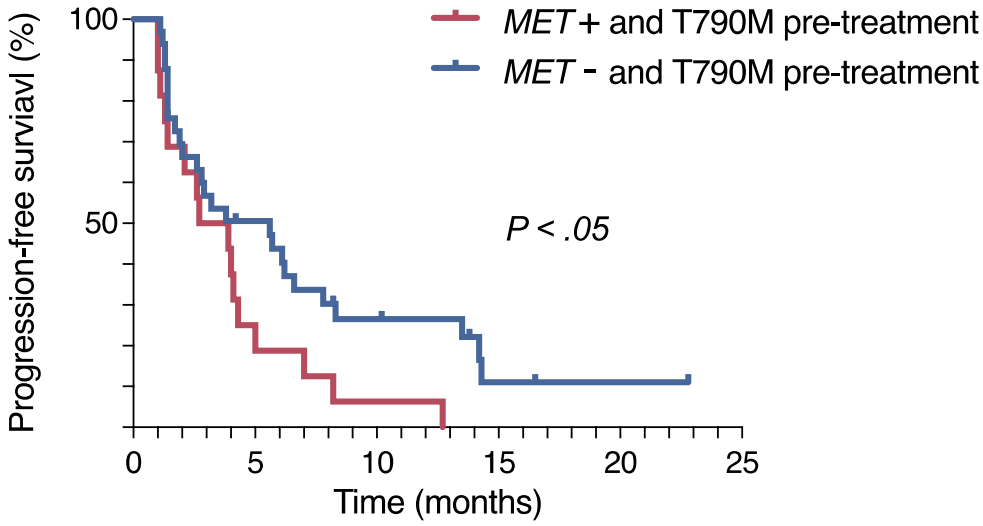


# Presence of Multiple Resistance Mechanisms predicts poor outcome

Expanded MET Cohort:

- 16 patients with T790M+/MET+
- 33 patients with T790M+/MET-



# Summary

- ctDNA analysis has many potential clinical applications
- NGS-based methods such as CAPP-Seq can achieve similar sensitivity as ddPCR and facilitate broad molecular profiling and monitoring
- Simultaneous monitoring of multiple resistance mechanisms may allow personalized targeting of emerging resistance mutations
- Detection of MRD requires ultrasensitive assays and may allow personalized therapy
  - Randomized trial in Stage II colorectal cancer ongoing (J. Tie et al.)
- More prospective clinical studies required to validate preliminary findings and to establish clinical utility

# Acknowledgements


**CAPP-Seq Team**

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Sharavani Sinha
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Other collaborators

Bill Loo
David Shultz
Michael Gensheimer
Heather Wakelee
Joel Neal
Joseph Shrager
Mark Berry
Robert Merritt
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Carmen Say
Justin Carter
Andy Simmons
Chris Karlovich

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