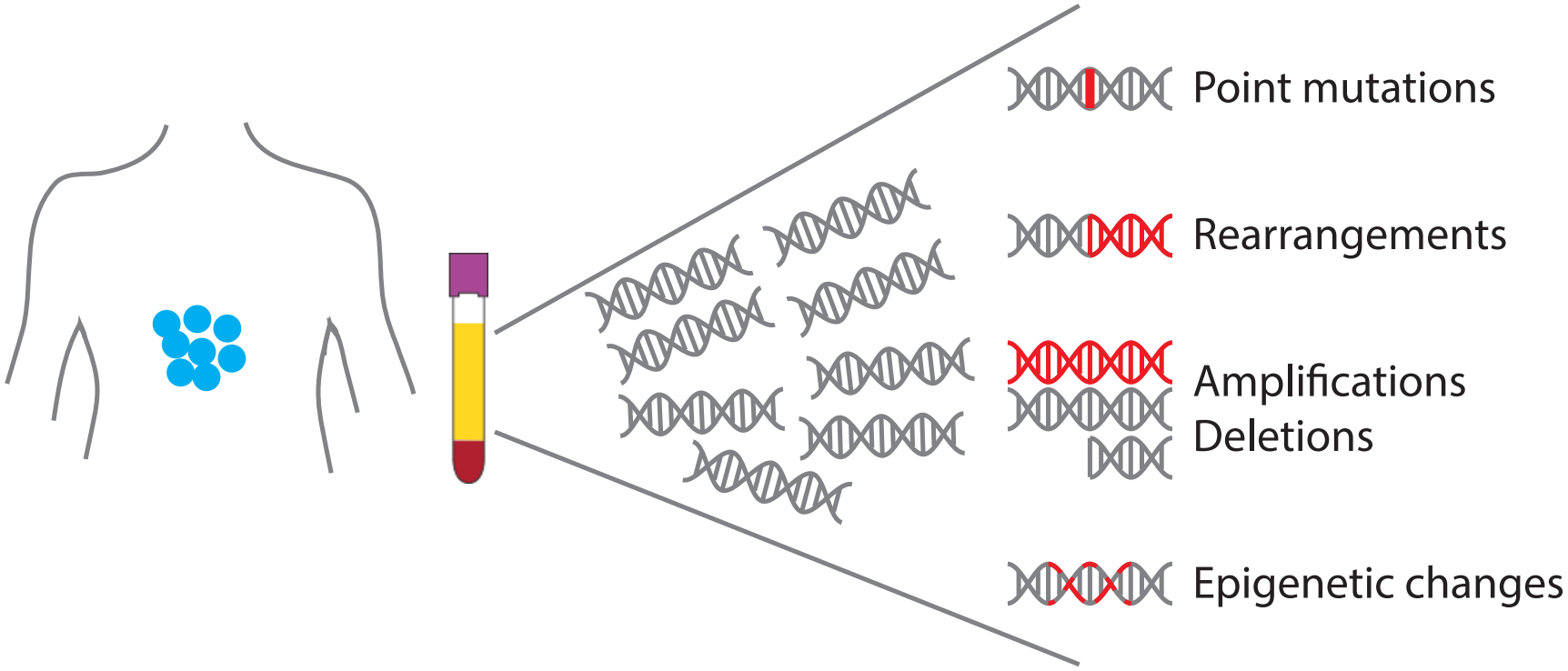
Capturing tumor heterogeneity and clonal evolution using ctDNA analysis

Muhammed Murtaza, MBBS, PhD
Assistant Professor and Co-Director, Center for Noninvasive Diagnostics, TGen
Assistant Professor of Medicine, Mayo Clinic

NCI Workshop on Circulating Tumor DNA assays in Clinical Cancer Research
September 29, 2016

Blood plasma has cell-free circulating DNA that carries somatic mutations



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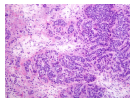
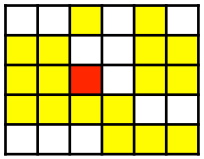
Tumor-guided analysis



TCCTCGGCGTTTCGCCAAGC
TTCGCCAAGCCTAATCAGCA
CTAATCAGCATCGGCGTTTC
ACAGAACGAGGTTTCGCCAA

Panel
Exome
WGS



Targeted
Analysis

Applications

- Monitoring tumor burden
- Treatment Response
- Residual disease
- Recurrence

Challenges

- Tumor tissue sequencing required
- Patient-specific assays needed
- Turnaround time

Tumor-independent analysis

Hotspot

TCCTCGGCGTTTCGCCAAGC
TTCGCCAAGCCTAATCAGCA
CTAATCAGCATCGGCGTTTC
ACAGAACGAGGTTTCGCCAA

Panel
Exome
WGS

Applications

- Noninvasive tumor genotyping
- Alternative to re-biopsy
- Clonal evolution
- Acquired therapeutic resistance

Challenges

- Variable mutation signal
- Sequencing noise
- Multiple testing
- Limited input material

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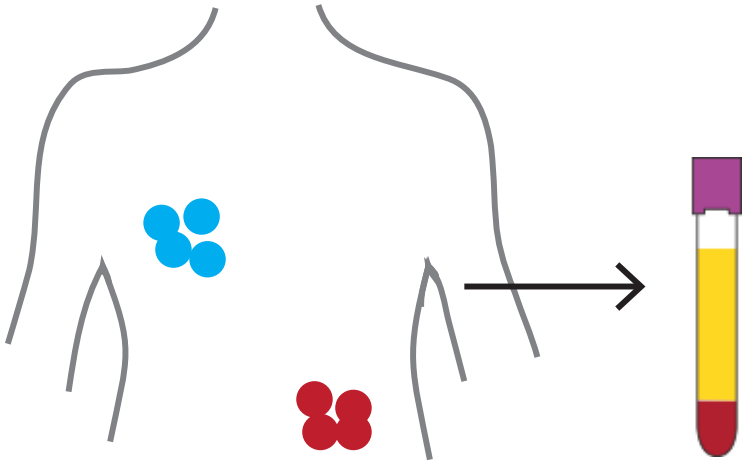
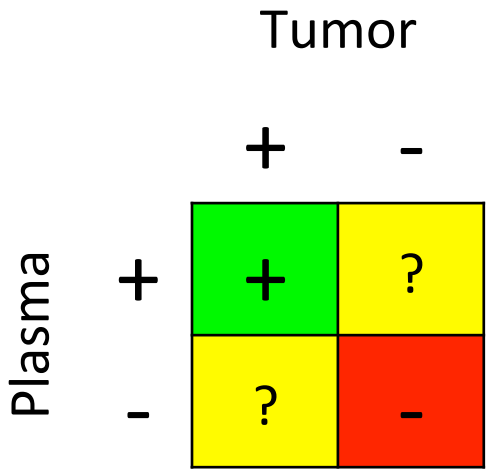
How well does ctDNA capture tumor heterogeneity?



- Treatment-sensitive genotype
- Treatment-resistance genotype

How concordant is plasma with tumor analysis?



- Technical factors affecting concordance
- Comparison of plasma with multi-regional sampling
- Clinical implications/opportunities and unanswered questions

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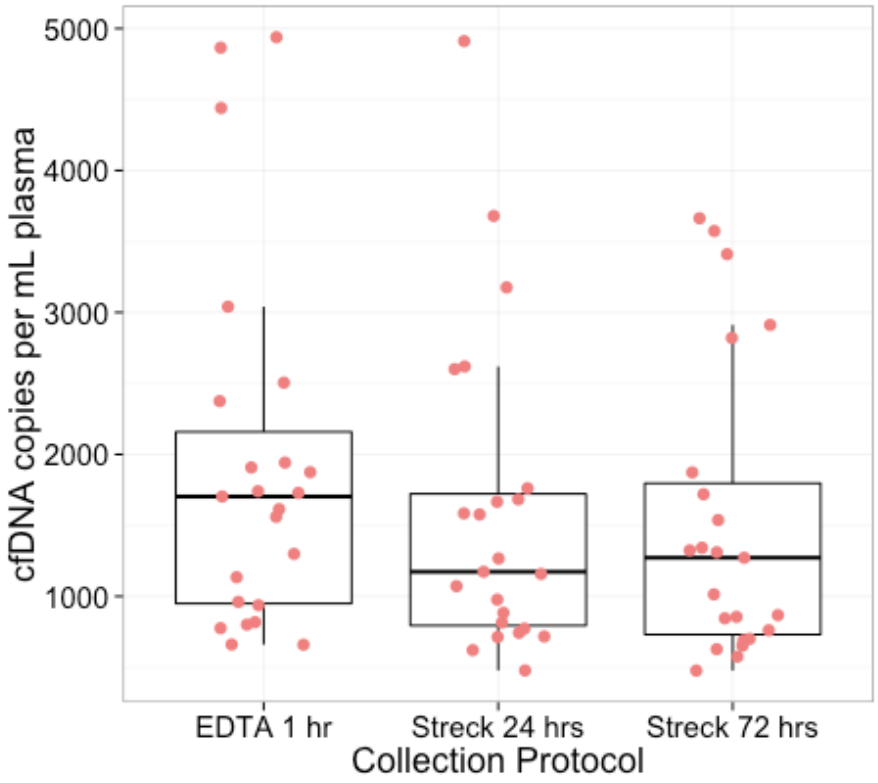
Technical factors affecting concordance: limited analyte

- In healthy controls, median of 1500-1900 GEs/mL plasma (5-7 ng/mL)



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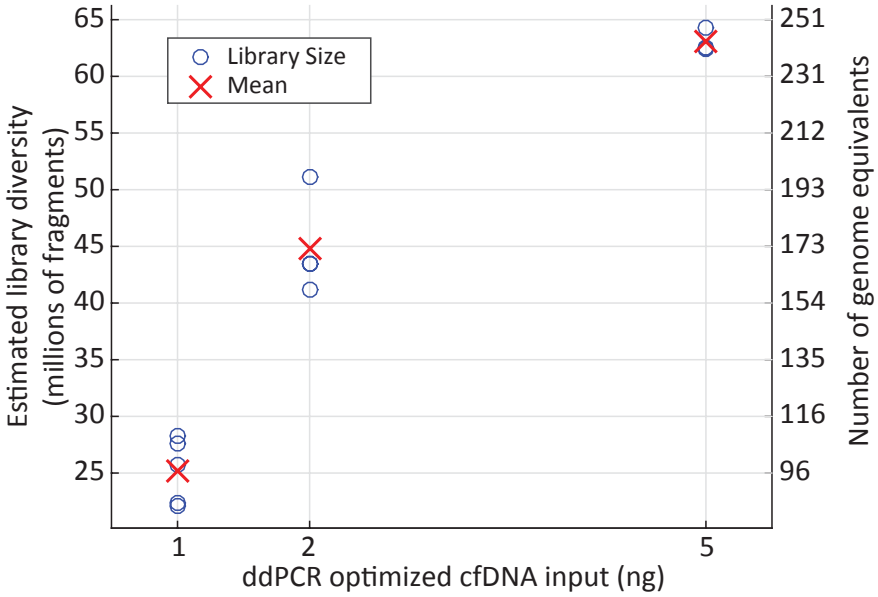
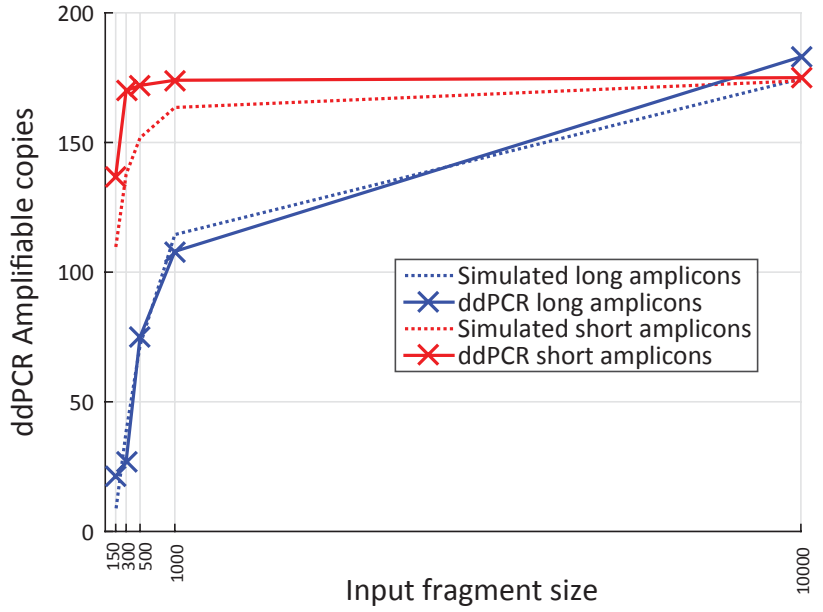
Technical factors affecting concordance: sampling inefficiency

- Ligation-based library preps: at best, 30% efficient at incorporating <10ng DNA input



- PCR based preps: efficiency is a function of amplicon size



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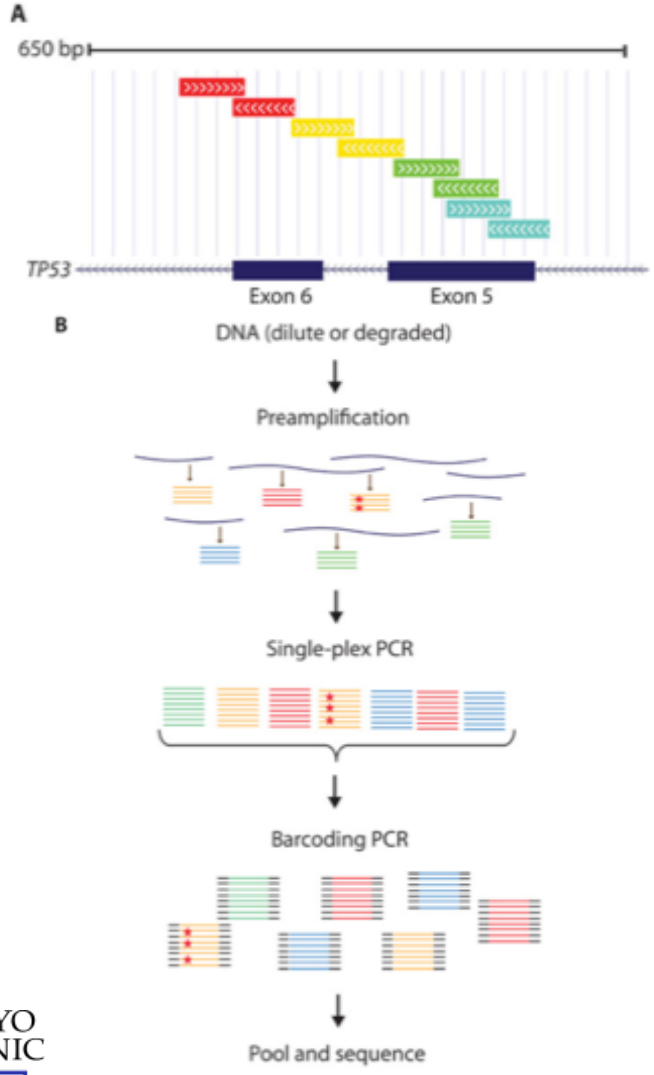
Technical factors affecting plasma-tumor concordance

- Pre-analytical processing
  - Delayed processing of blood leads to peripheral cell lysis, lowering measurable mutation fraction
- Background noise in the assay
  - Need to distinguish signal from PCR and library prep noise

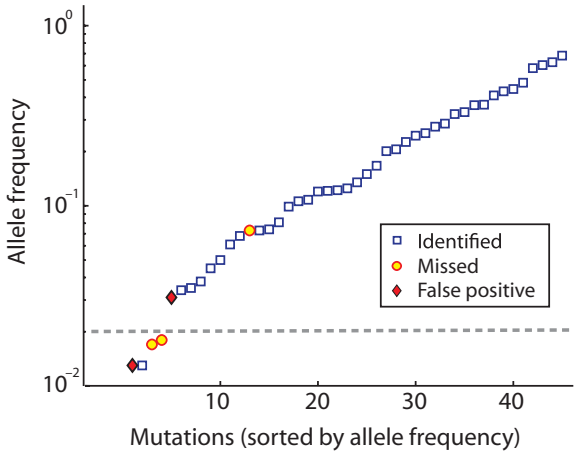
ctDNA and tumor biopsy concordance in ovarian cancer



n=38 patients

Summary of results	
Plasma samples analyzed	69
Mutations detected by digital PCR >2%	47
Mutations detected by TAm-Seq >2%	46
Missed by TAm-Seq due to sampling error	1
Sensitivity of TAm-Seq for mutations >2%	97.5%
Positive predictive value for mutations >2%	97.5%

Genes
TP53
PTEN
EGFR
PIK3CA
KRAS
BRAF

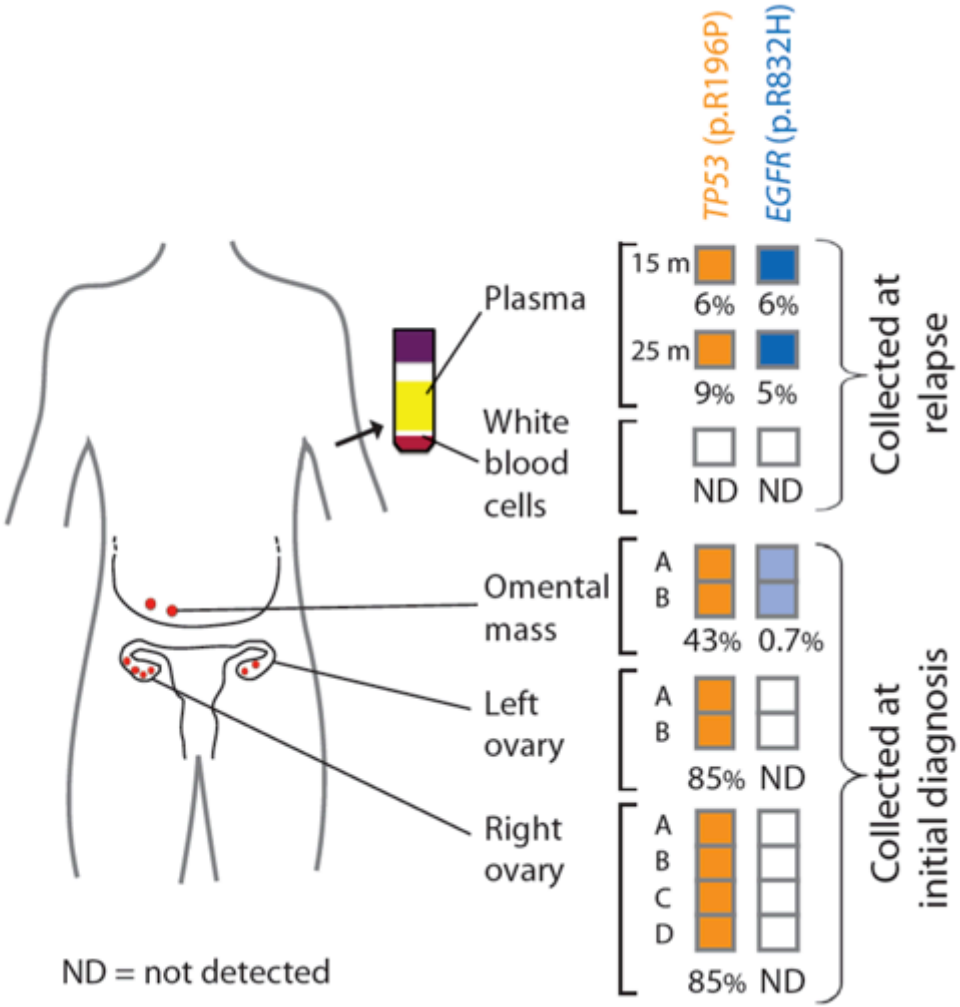


Tumor-independent: ~1% AF

Tumor-guided: ~0.1% AF

Forshew, Murtaza and Rosenfeld et al. Science Translational Medicine 2012

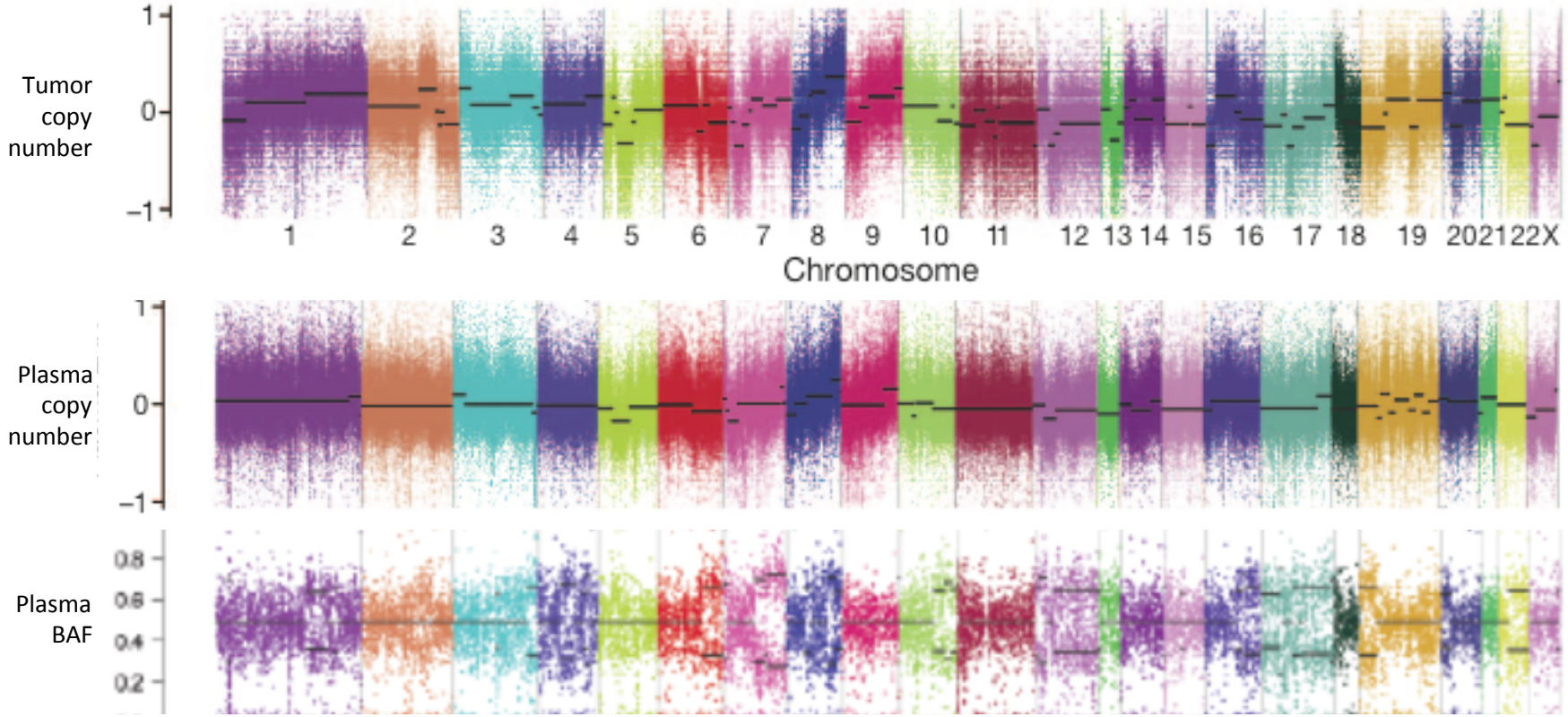
Intra-tumor heterogeneity in ovarian cancer



Forshew, Murtaza and Rosenfeld et al. Science Translational Medicine 2012

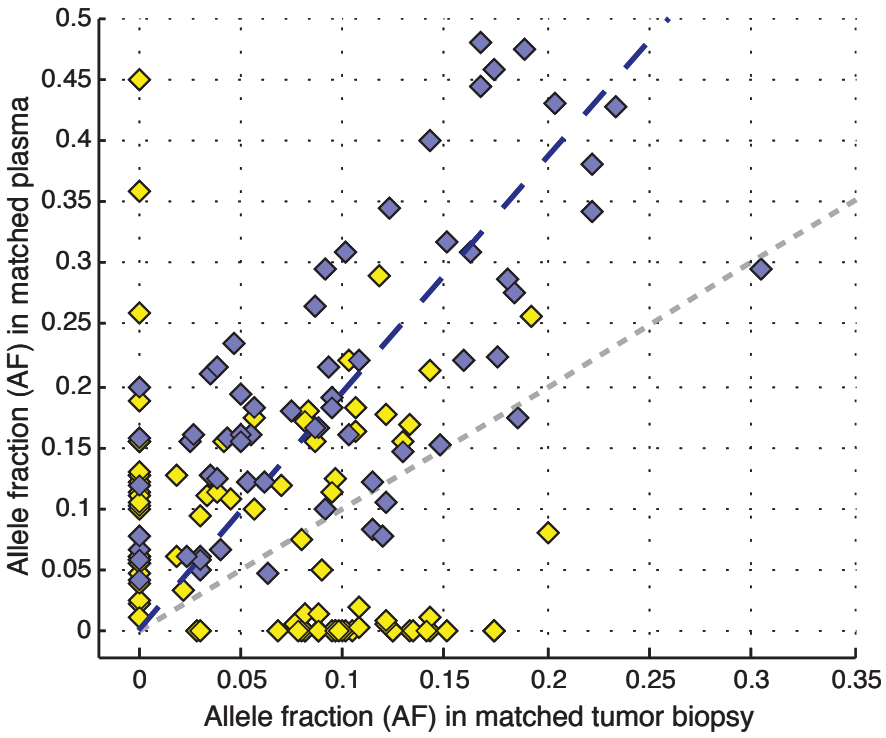
Exome-wide comparison between ctDNA and tumor biopsies

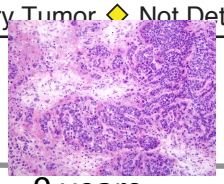
- n=6 patients (advanced breast, ovarian and lung cancers)
- 2-5 plasma sample each



Murtaza, Dawson, Caldas and Rosenfeld et al. Nature 2013

Comparison of tumor and plasma exomes





9 years

Primary tumor

Metastatic tumor and plasma

151 single nucleotide variants
- 62% in both sets
- 21% in plasma only
- 17% in mets only

64 SNVs shared with primary
- 100% detectable in plasma
- 89% detectable in both plasma and mets

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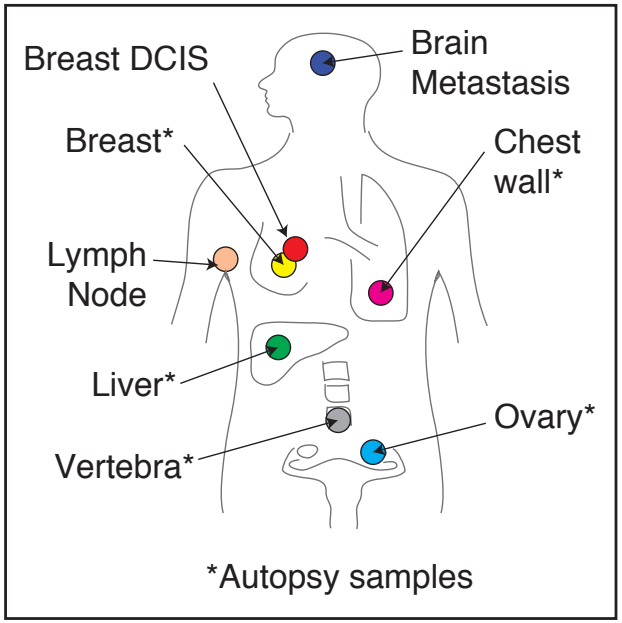
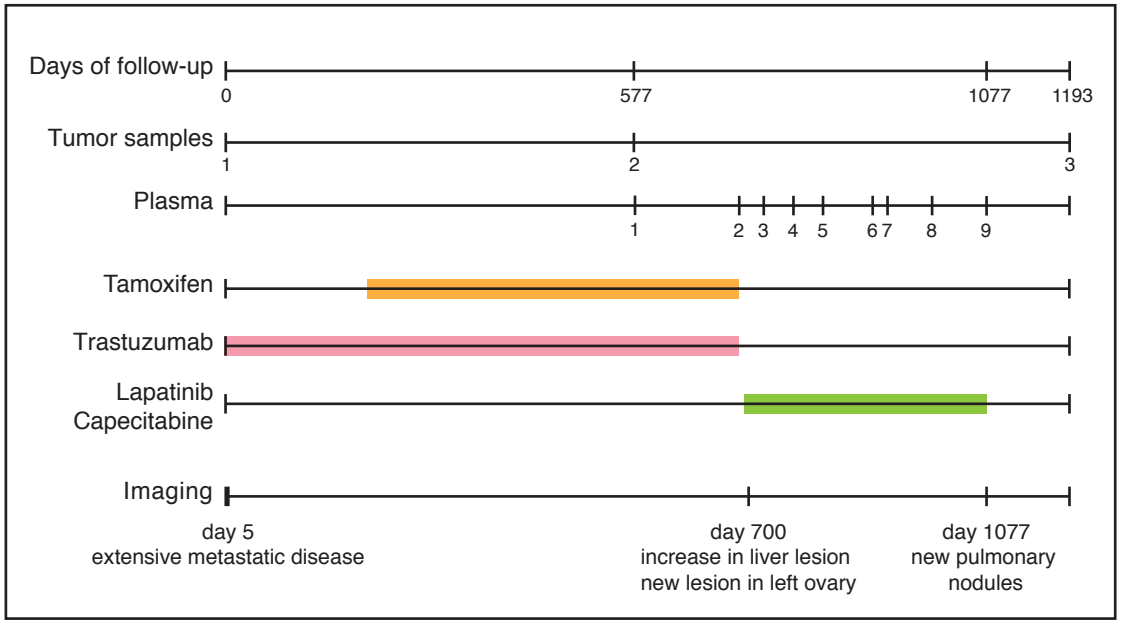
Murtaza, Dawson, Caldas and Rosenfeld et al. Nature 2013

Multi-regional and longitudinal sampling from a patient with breast cancer





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Murtaza, Dawson, Caldas and Rosenfeld et al. Nature Communications 2015

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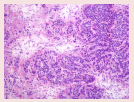
Exome and deep amplicon sequencing of tumor and plasma samples

Exome Analysis

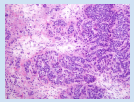
 x6

 x3

Germline: 26x
Tumor: 17x-74x
Plasma: 77x-140x

362
non-synonymous SNVs

Amplicon Sequencing

 x8

 x9

350 amplicons in multiplexed droplet PCR

310 successfully amplified

Germline: 4304x
Tumor: 965x-2777x
Plasma: 288x-8248x

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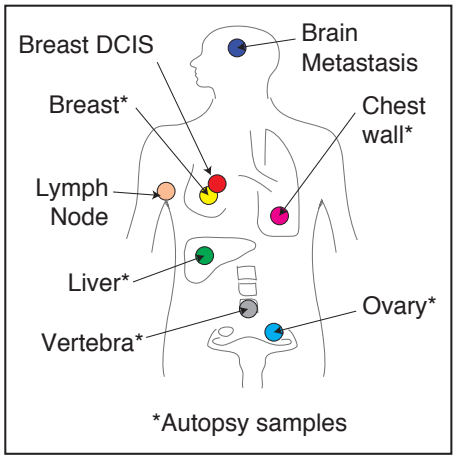
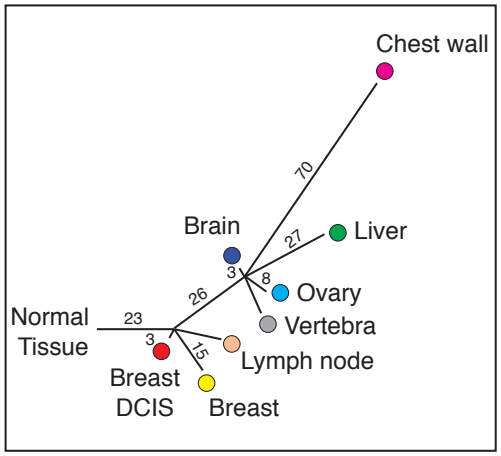
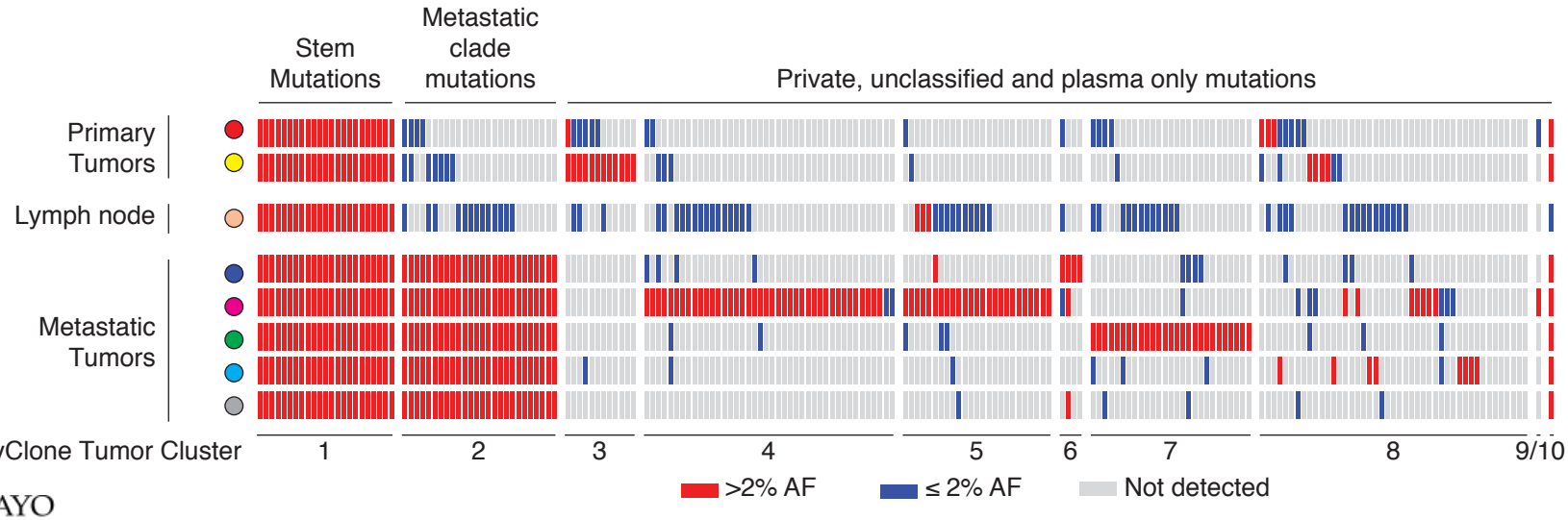
Murtaza, Dawson, Rosenfeld, Caldas et al. Nature Communications 2015 and Nature 2013

Multi-regional tumor heterogeneity







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Murtaza, Dawson, Caldas and Rosenfeld et al. Nature Communications 2015

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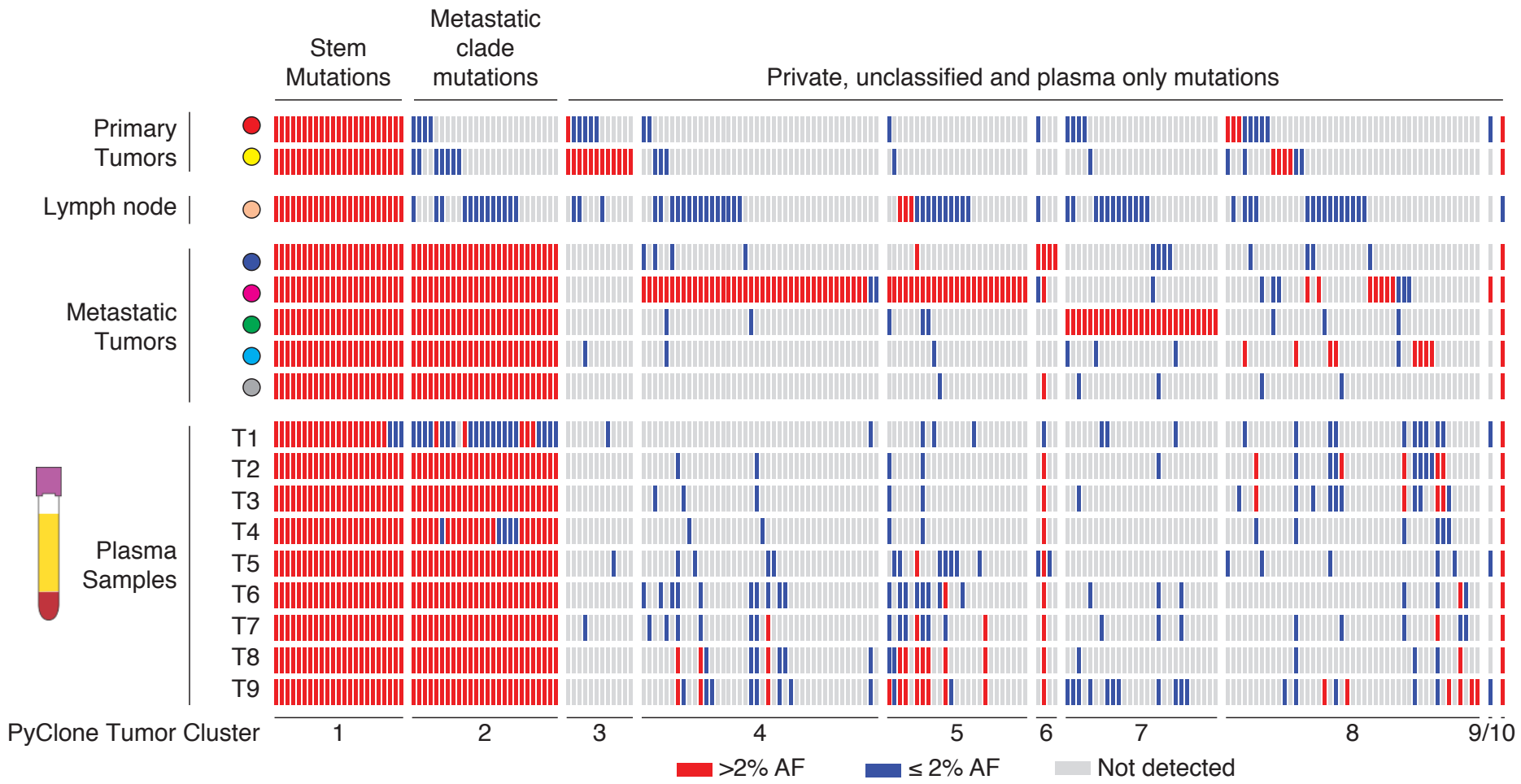
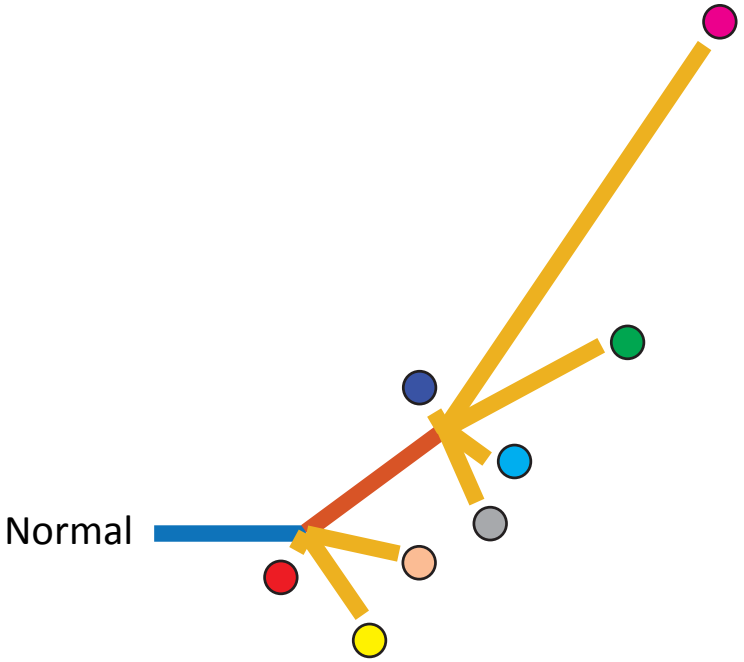
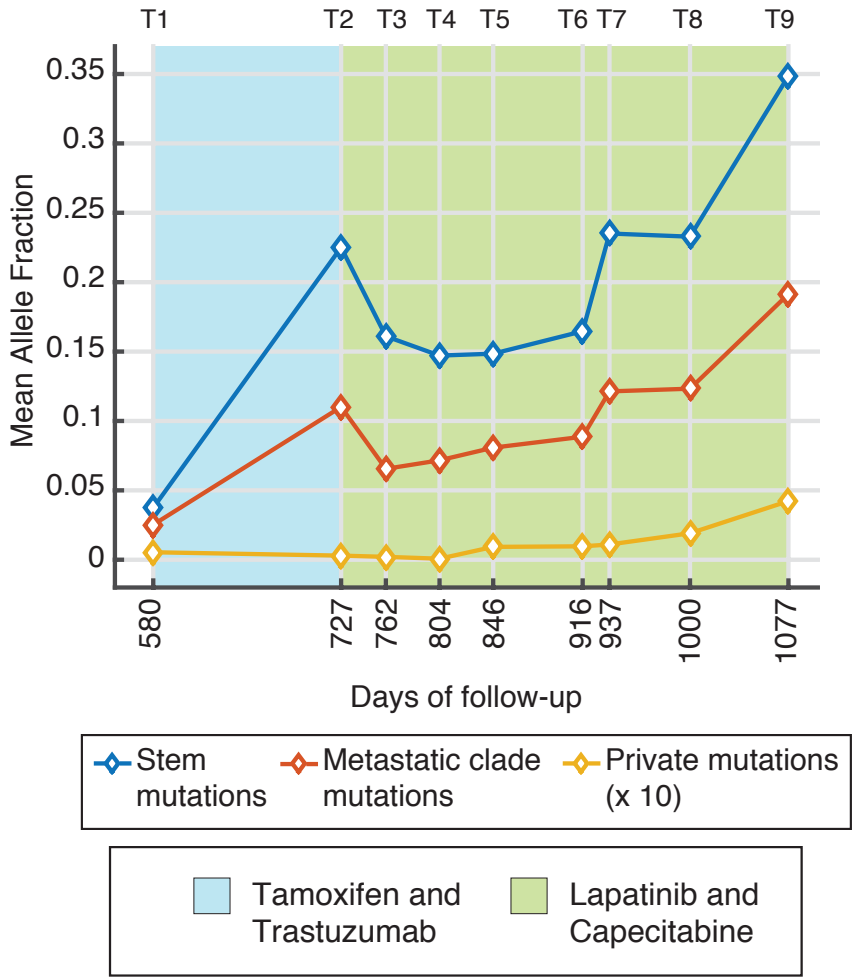
Is ctDNA concordant with tumor biopsies?



Murtaza, Dawson, Caldas and Rosenfeld et al. Nature Communications 2015

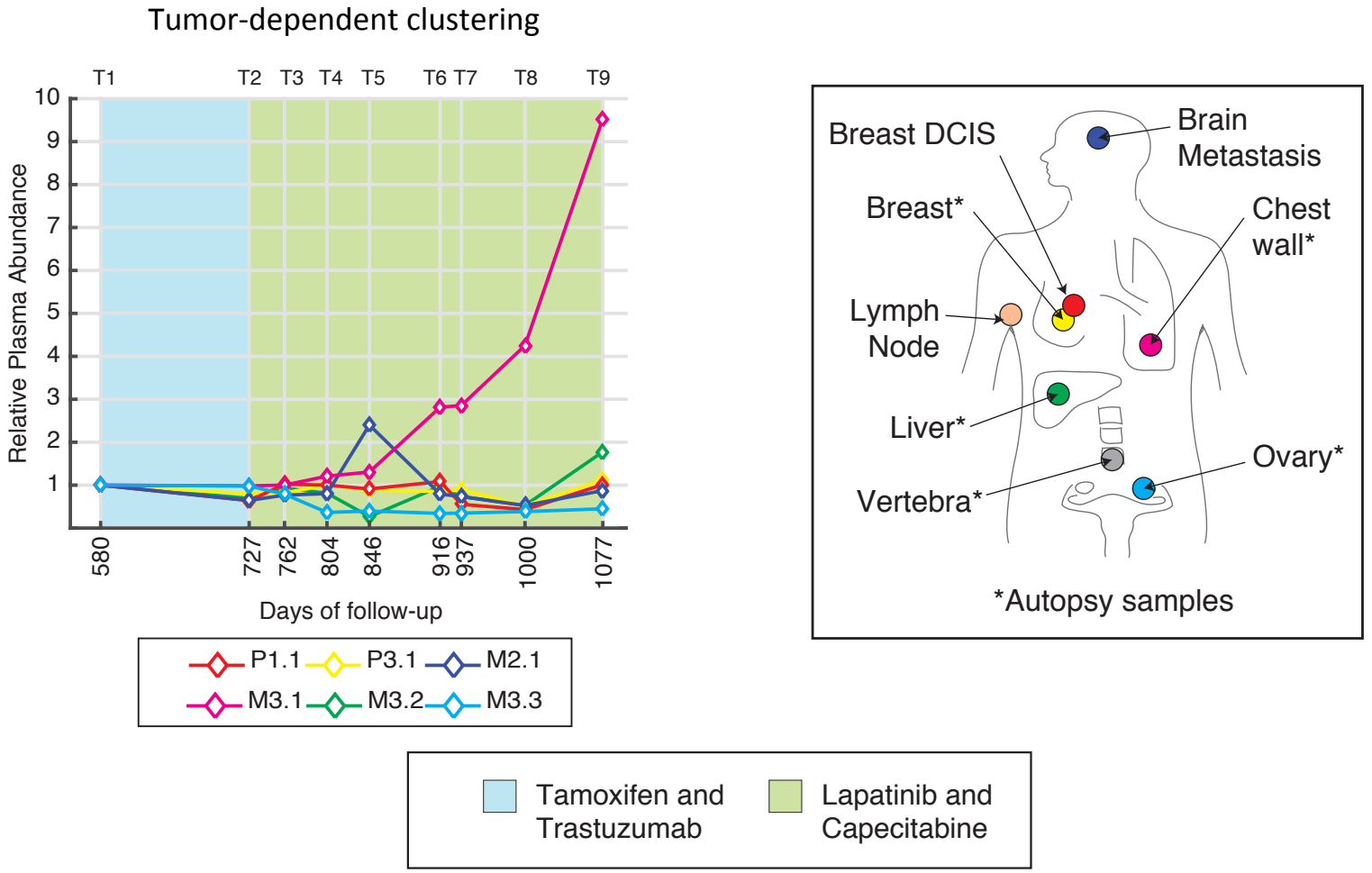
Circulating levels of somatic mutations reflect clonal hierarchy





Murtaza, Dawson, Caldas and Rosenfeld et al. Nature Communications 2015

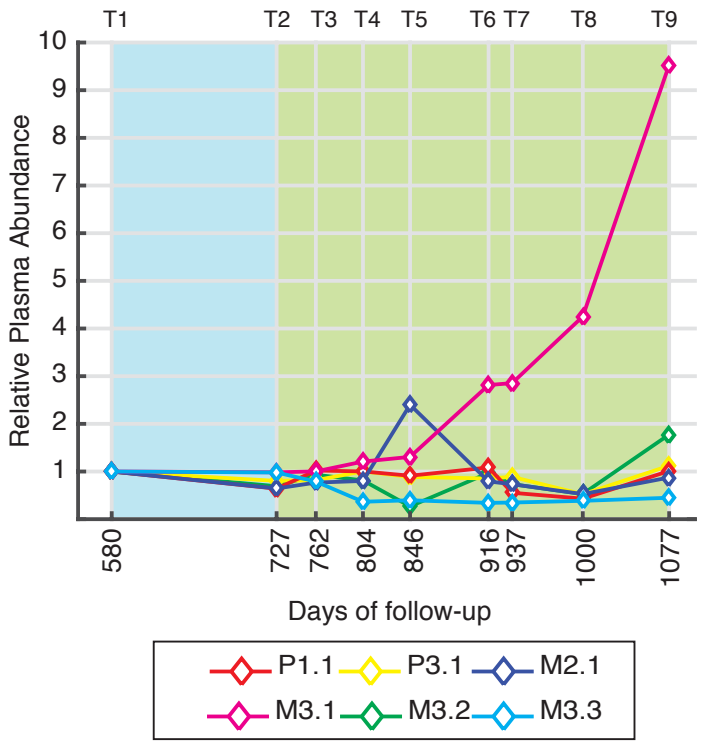
ctDNA tracks differential treatment response across metastatic deposits



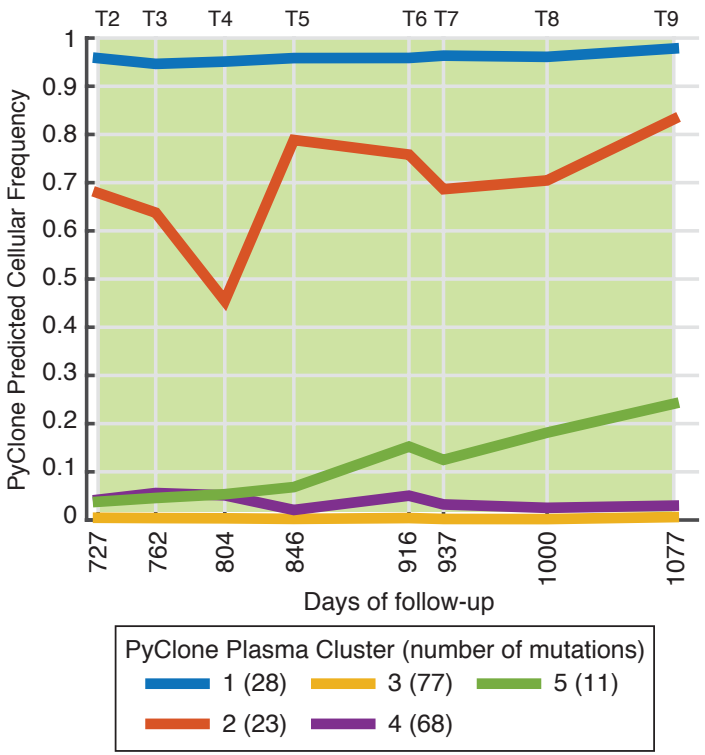
Murtaza, Dawson, Caldas and Rosenfeld et al. Nature Communications 2015

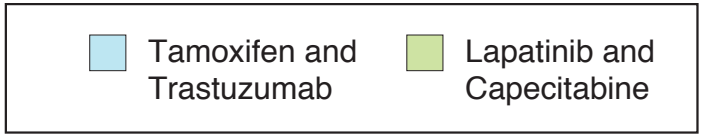
ctDNA tracks differential treatment response across metastatic deposits

Tumor-dependent clustering



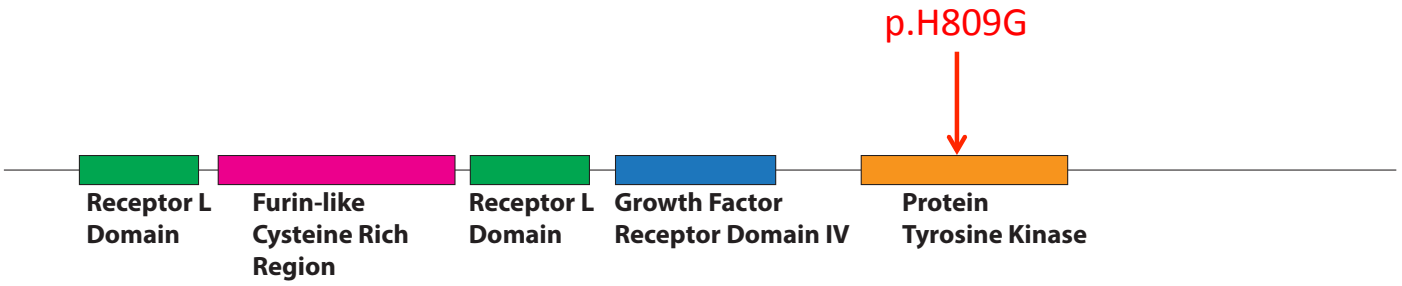
Tumor-independent clustering





Murtaza, Dawson, Caldas and Rosenfeld et al. Nature Communications 2015

ctDNA can identify potential drivers of treatment resistance



Cell Cycle

Receptor tyrosine kinase ERBB4 mediates acquired resistance to ERBB2 inhibitors in breast cancer cells

Kaleigh Canfield[a], Jiaqi Li[a], Owen M. Wilkins[a], Meghan M. Morrison[g], Matthew Ung[b], Wendy Wells[cd], Charlotte R. Williams[a], Karen T. Liby[ad], Detlef Vullhorst[e], Andres Buonanno[e], Huizhong Hu[f], Rachel Schiff[f], Rebecca S. Cook[g] & Manabu Kurokawa[ad]

Accepted author version posted online: 15 Jan 2015.

ERBB4 p.H809G allele fraction
Not signficantly detectable above background
Signficantly detectable above background

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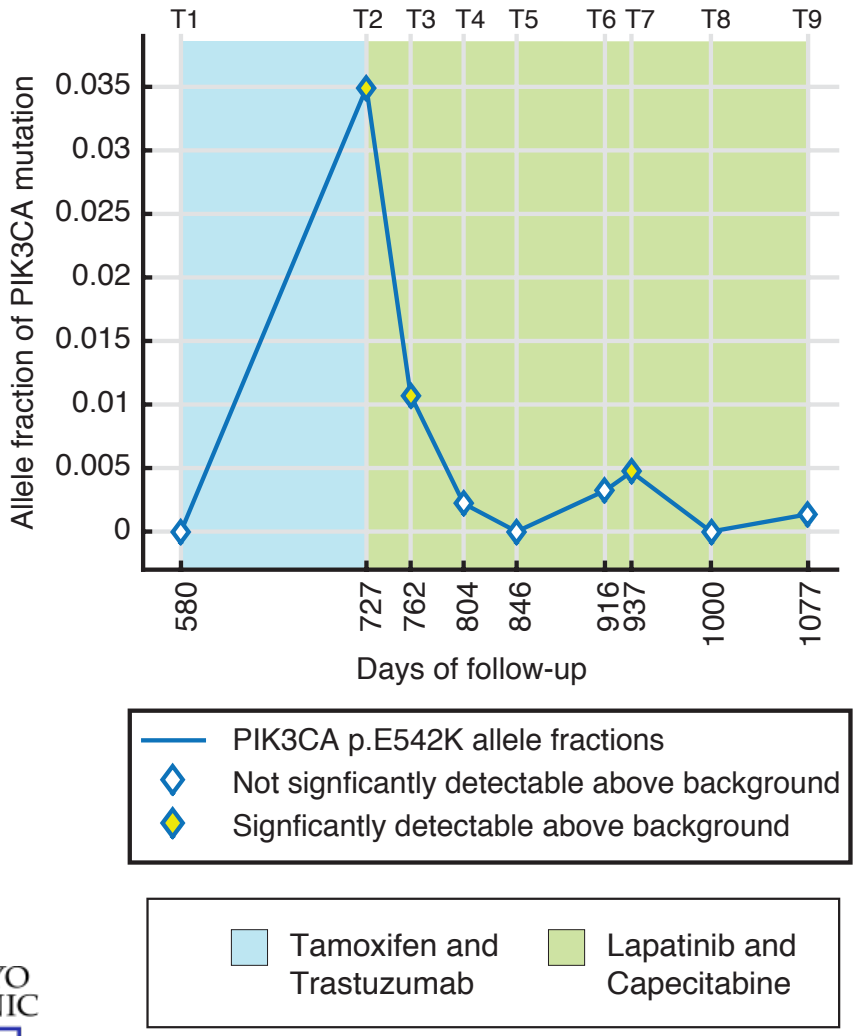
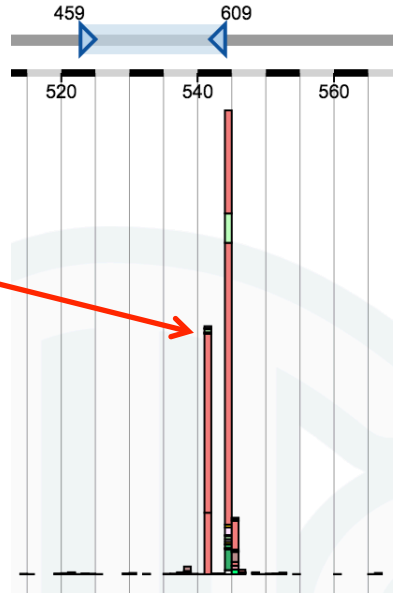
Murtaza, Dawson, Caldas and Rosenfeld et al. Nature Communications 2015

Heterogeneity for an actionable mutation



COSMIC



PIK3CA p.E542K

TCGA

Mutated Genes

Gene	# Mut	#	Freq
TP53 Ⓜ	354	346	31.3%
PIK3CA Ⓜ	378	340	30.8%
CDH1 Ⓜ	135	133	12.0%
GATA3 Ⓜ	127	119	10.8%
KMT2C Ⓜ	100	82	7.4%

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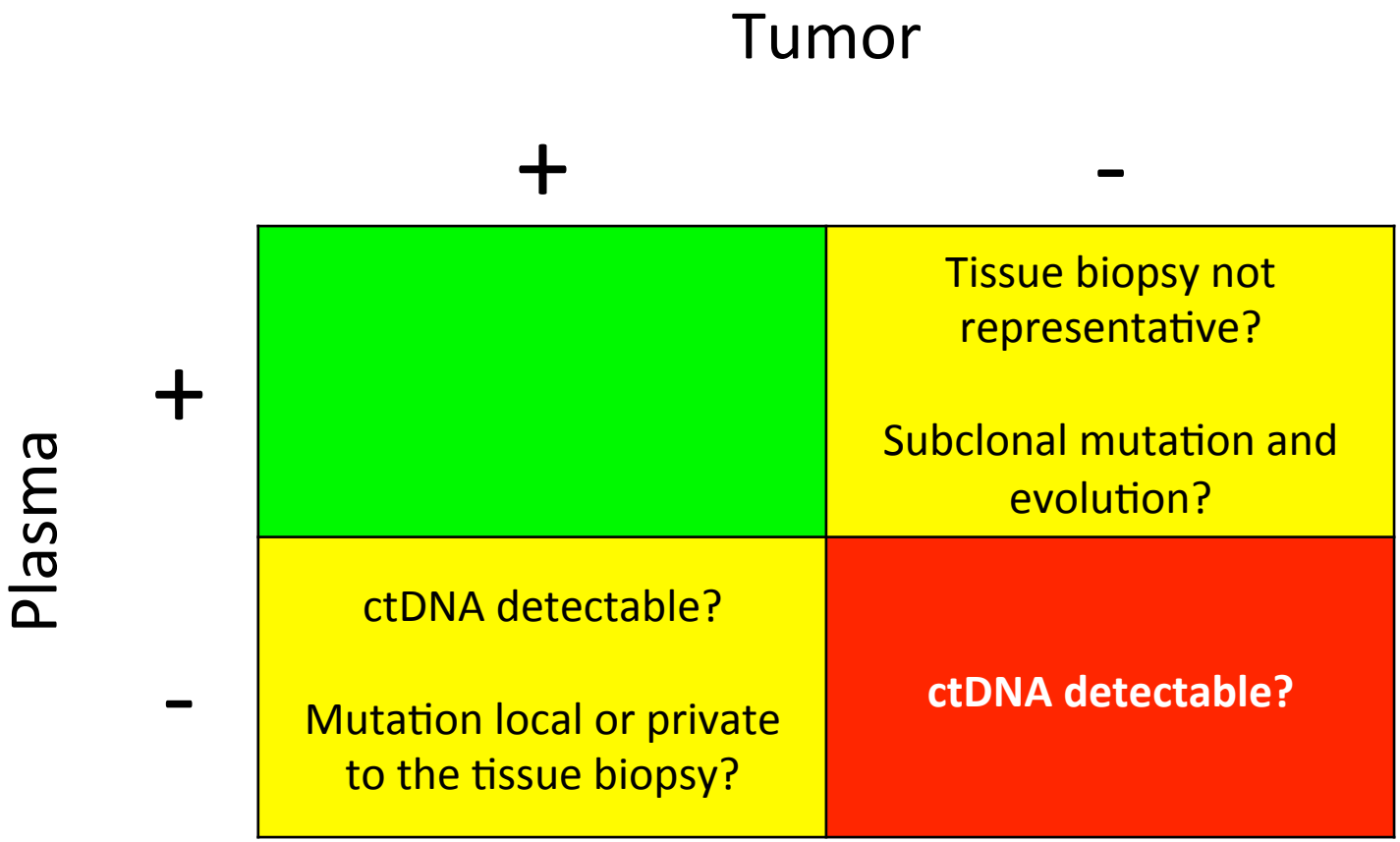
Murtaza, Dawson, Rosenfeld, Caldas et al. Nature Communications 2015; TCGA Cell 2015

How do we interpret plasma-tumor concordance for any given mutation?



Summary and Unanswered Questions

- Quantitative levels of somatic mutations in ctDNA represent phylogeny and fraction of systemic tumor each mutation represents
  - Can we use relative levels of multiple mutations to prioritize actionable mutations or further stratify patients?
- Founder mutations are more readily detectable in plasma compared with sub-clonal mutations
  - Can we use multiple founder mutations to achieve greater sensitivity for detecting ctDNA in low disease burden states?
- Longitudinal ctDNA analysis captures clonal evolution in real-time, as it happens
  - Can we leverage this, beyond re-genotyping, to maneuver tumor evolution towards improved outcomes?
  - Can we use this to understand treatment scheduling (intermittent dosing or ordering when multiple options are available)?

Thanks and happy to take questions.

mmurtaza@tgen.org

Current and former lab members


Tania Contente-Cuomo


Nieves Perdigones


Pankti Shah


Havell Markus


Ahuva Odenheimer


Alex Nazareno


Jun Zhao

Sridhar Srivatsa

Collaborators

Cambridge:
Nitzan Rosenfeld
Carlos Caldas
Dana Tsui
Sarah-Jane Dawson
Tim Forshew
Davina Gale
Francesco Marass
Oscar Rueda
H Raza Ali
Suet-Feung Chin

Patients

SCIENCE foundation ARIZONA INNOVATION AT WORK

IVY THE BEN AND CATHERINE FOUNDATION

STAND UP TO CANCER

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