

Workshop on Circulating Tumor DNA assays in Clinical Cancer Research,

NCI Shady Grove • September 29th, 2016

Genomic analysis of circulating tumour DNA: pushing the limits for cancer applications



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University of Cambridge,
CRUK Cambridge Institute

Inivata Ltd.



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CAMBRIDGE



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INSTITUTE



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Disclosures:

Co-founder & CSO, Inivata Ltd.

Research funding, AstraZeneca



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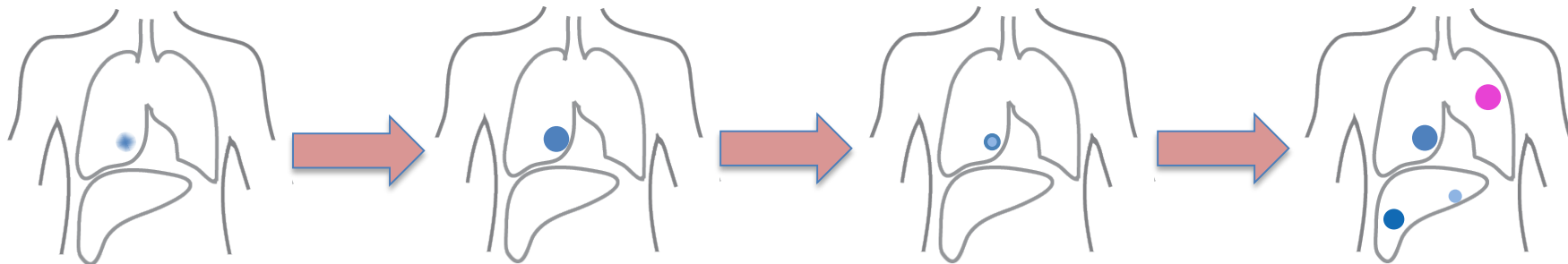


**Earlier
diagnosis**

**Treatment
selection**

**Disease
monitoring**

**Molecular
profiling**

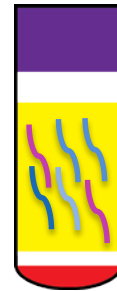
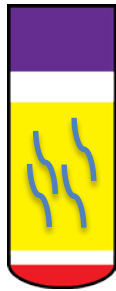
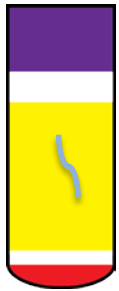
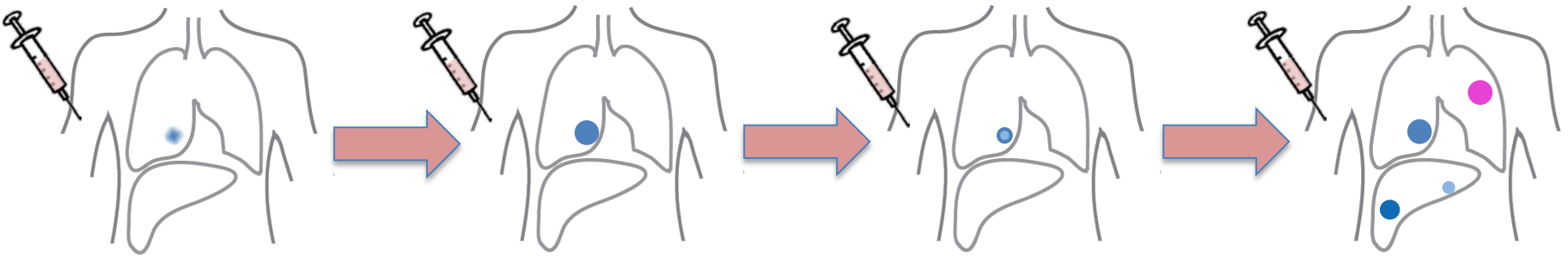


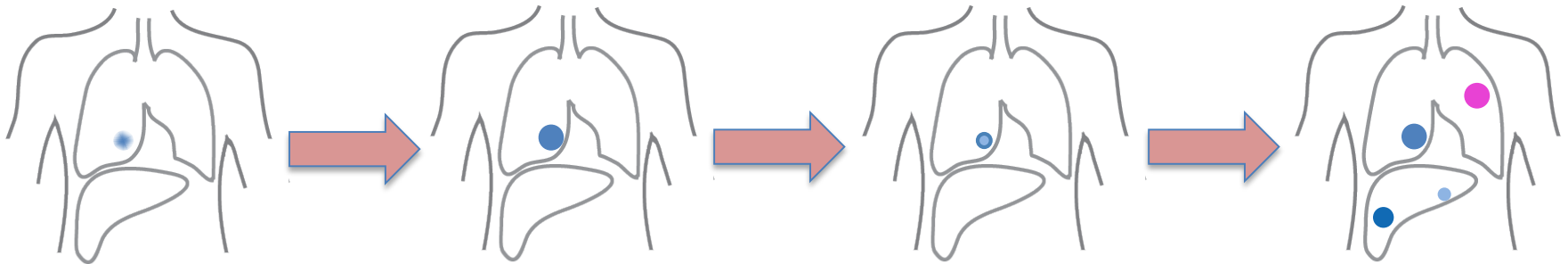
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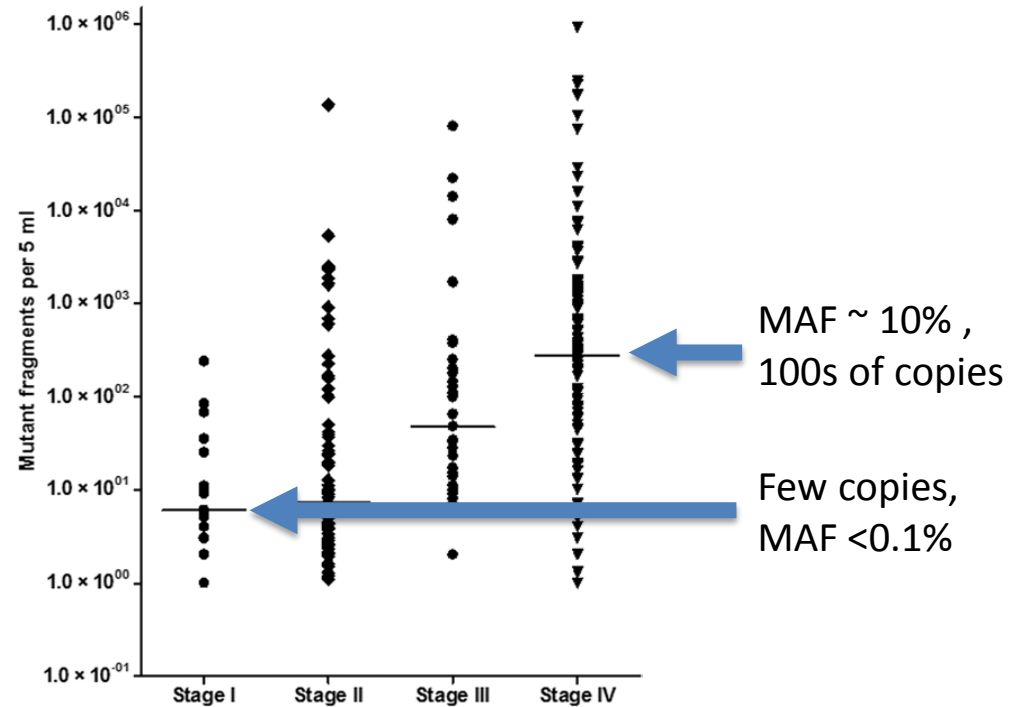
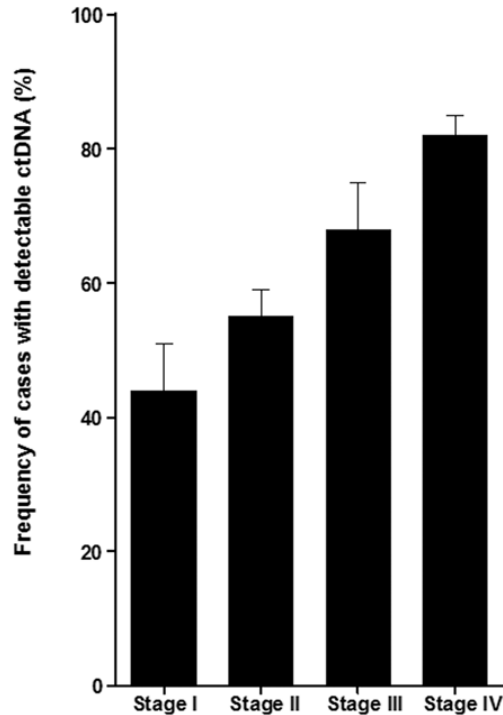


Cancers are unique, and evolve in response to selective pressure of therapy.

ctDNA can be used:

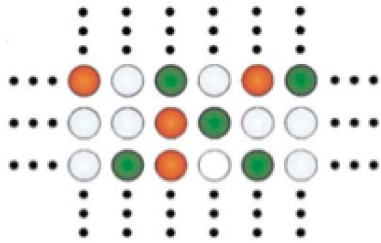
- As a **quantitative marker**, of tumour burden or residual disease
- As a **genomic tool** for molecular characterisation, to inform choice of therapy
- Integrated analysis to study cancer evolution and resistance to therapy

ctDNA levels span a wide range of values.
Applications need different types of information.
Methods need to be fit-for-purpose.

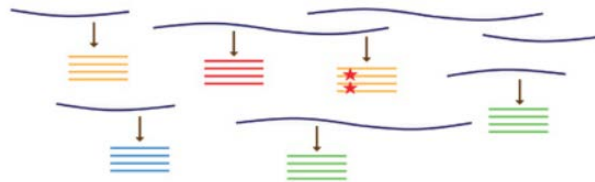


Cell-free DNA can be analysed at different scales of resolution

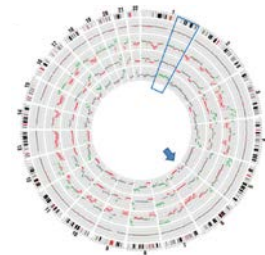
Increasing sensitivity for rare mutations



Single molecule analysis



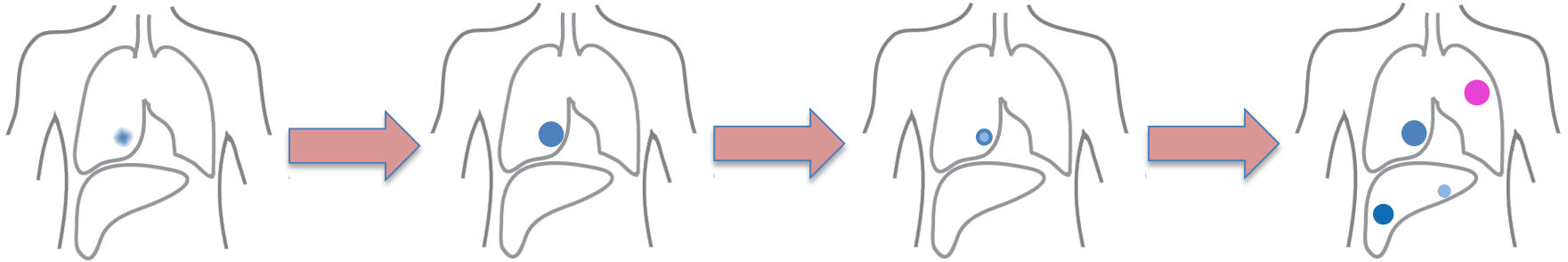
Targeted sequencing



Whole genome sequencing

Increasing genomic coverage (and cost)





Why use targeted sequencing:

Quantitative marker



- Standardised panel for monitoring, avoiding patient-specific assays
- Increase sensitivity for detection, look for multiple needles in a haystack

Genomic characterisation



- Non-invasive molecular profiling ('Liquid biopsy')
- Track multiple clones, monitor evolution and selection

Targeted sequencing provides a range of working-points

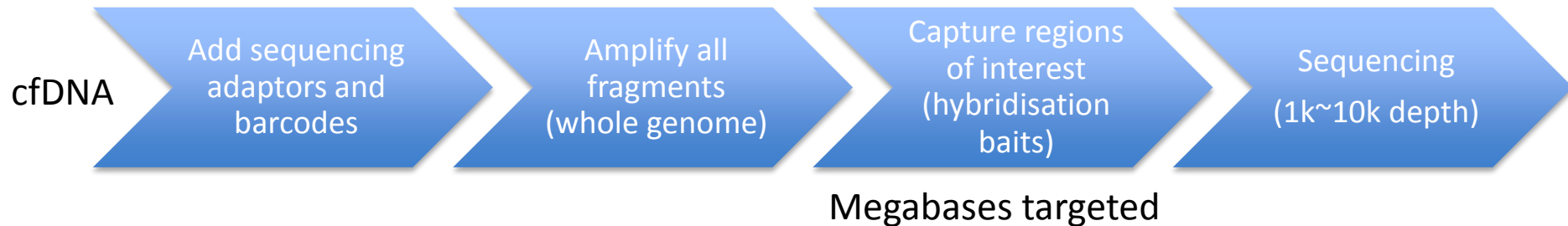
Tagged-Amplicon Sequencing of ctDNA

(Forshew, Murtaza et al., Sci TM 2012)

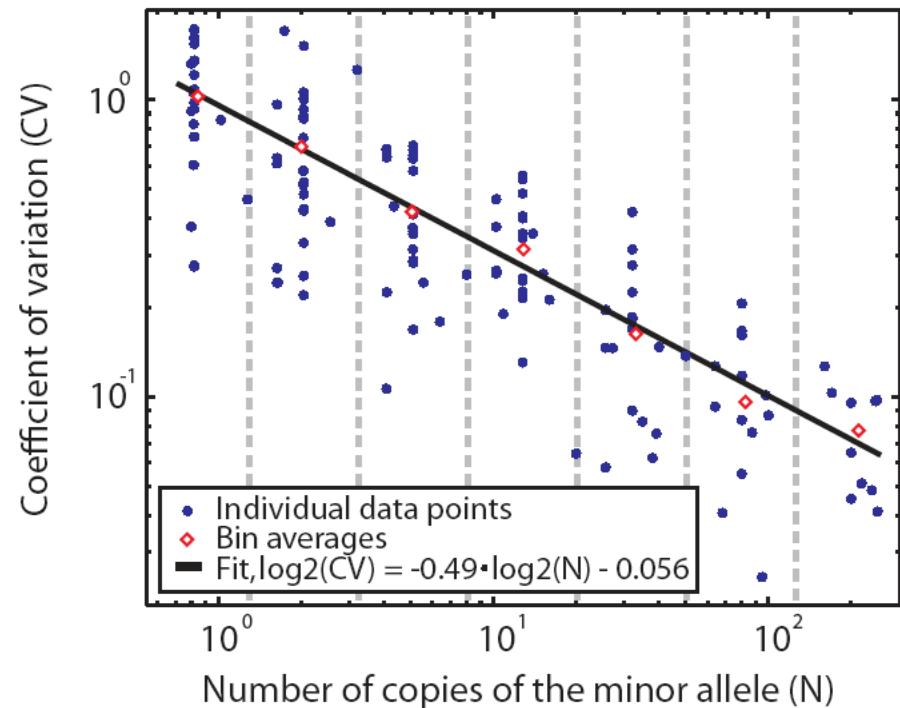
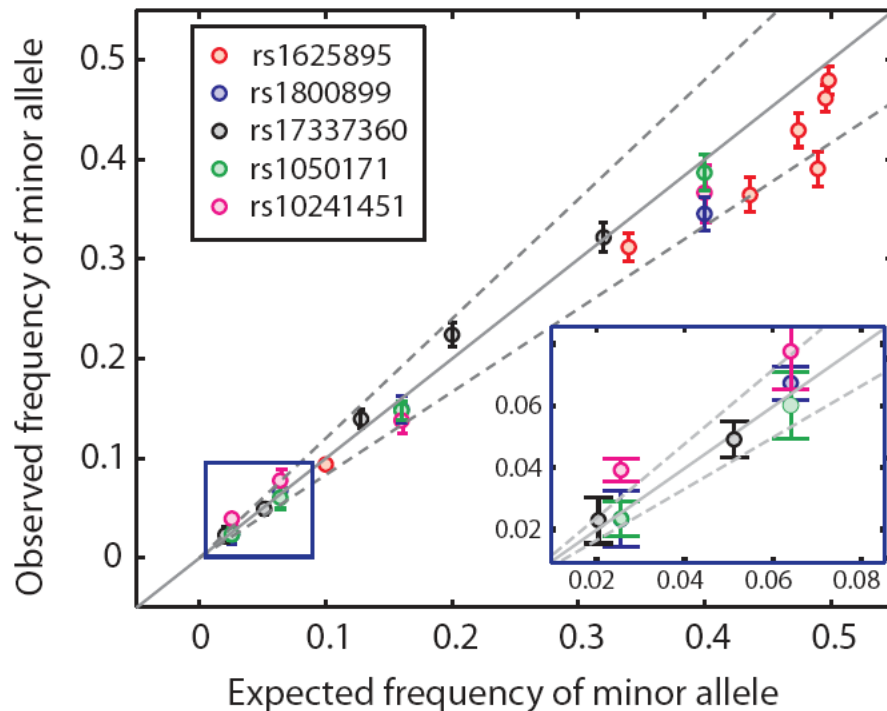


WGS/Hybrid-capture sequencing of ctDNA

(Murtaza, Dawson et al., Nature 2013)

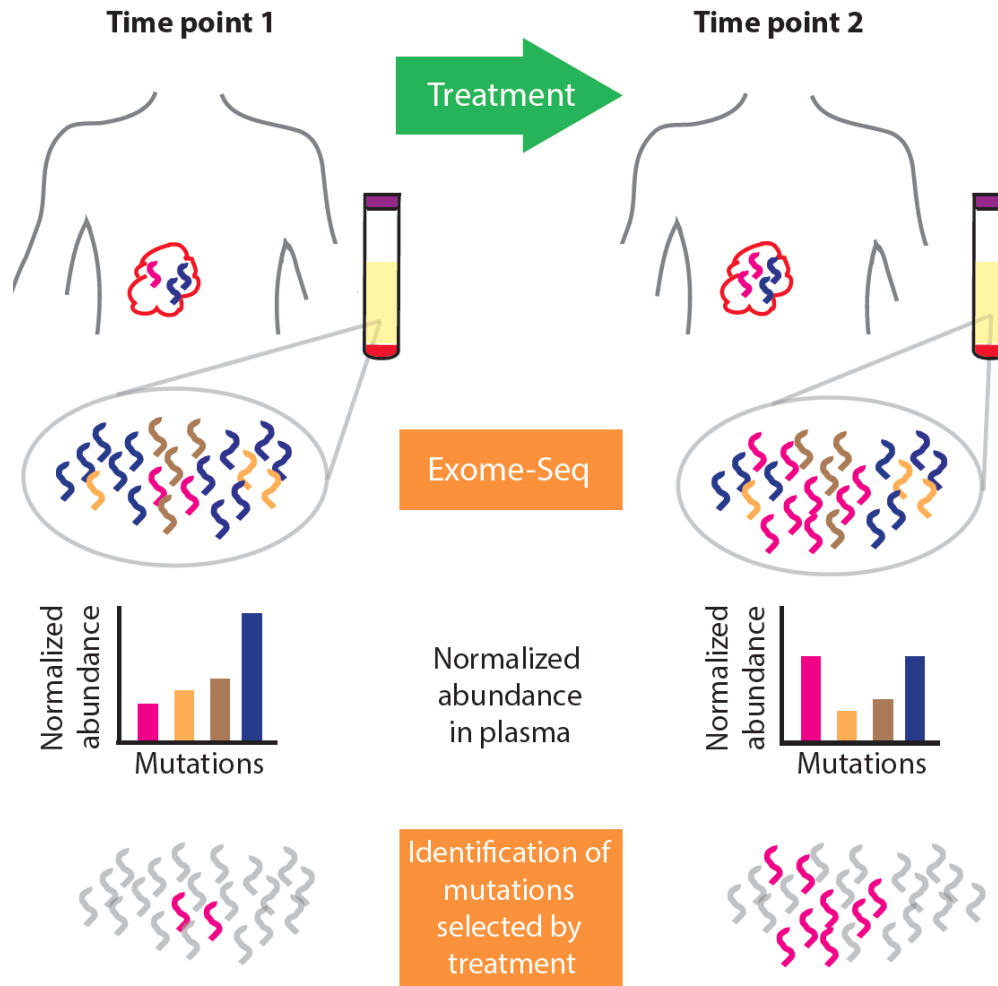


Tagged-Amplicon Sequencing: Accurate quantification down to individual mutant molecules; Mutation identification limited by PCR/sequencing noise

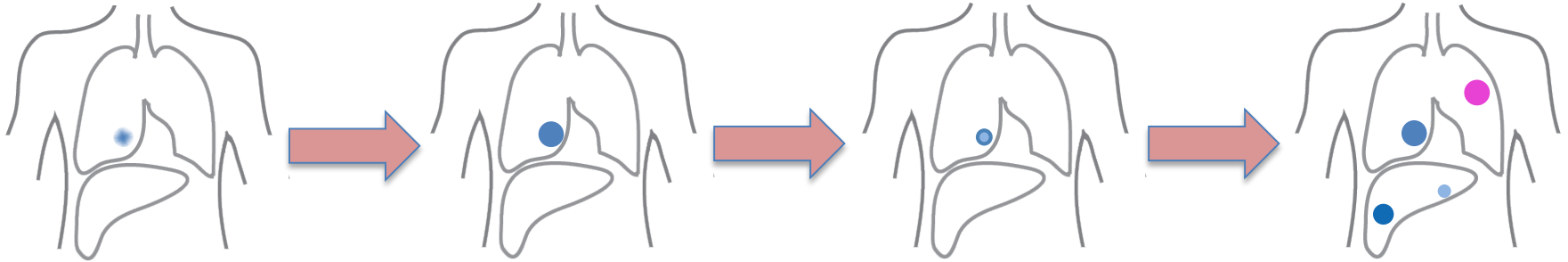


ForsheW, Murtaza, et al.
Science Transl. Med 2012

Exome sequencing of plasma DNA before therapy and at relapse can be used to discover novel resistance mechanisms



Muhammed Murtaza,
Sarah-Jane Dawson,
Dana Tsui, et al.,
Nature 2013



Why use targeted sequencing:

Quantitative marker



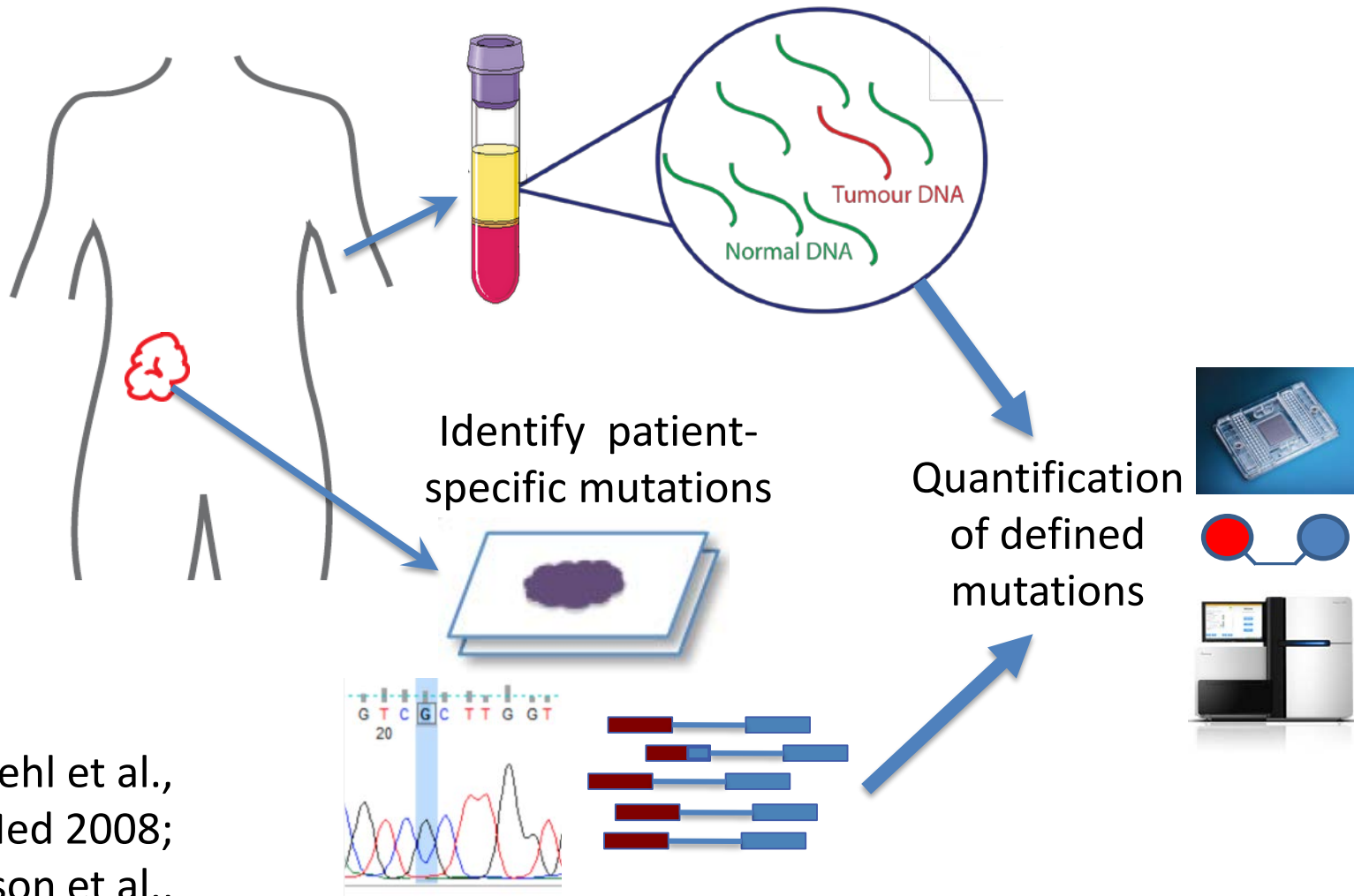
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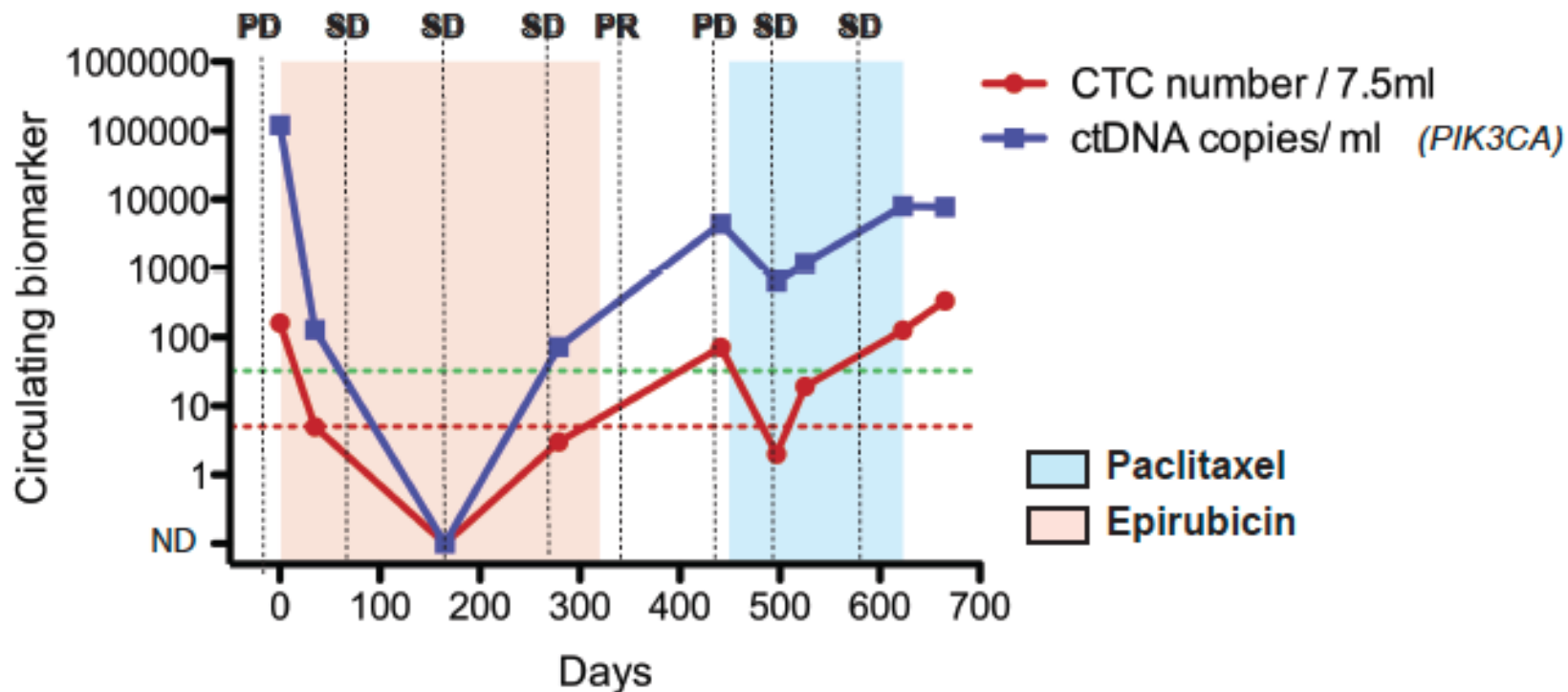
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Personalised monitoring of tumour burden: Quantification of patient-specific sequence alterations



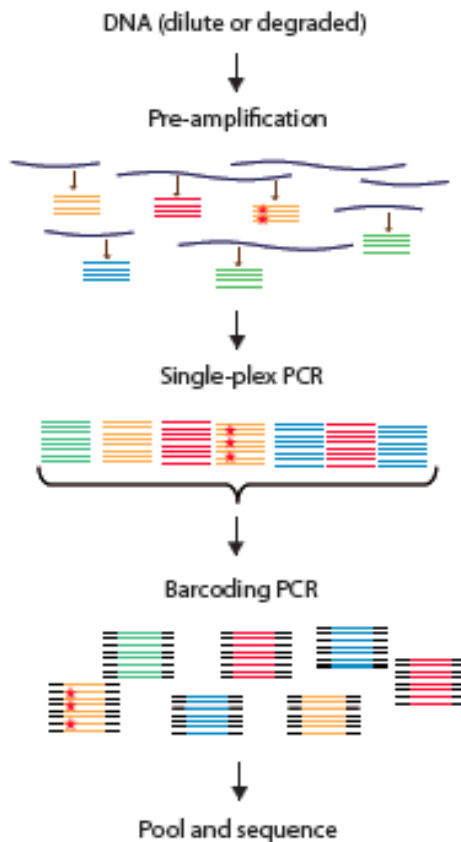
e.g. Diehl et al.,
Nat Med 2008;
Dawson et al.,
NEJM 2013

ctDNA levels are prognostic, and track dynamics of advanced cancer, identifying disease relapse ~6 months ahead of other markers/imaging



Metastatic breast cancer
Sarah-Jane Dawson, Dana Tsui,
Carlos Caldas et al., NEJM 2013

An expanded targeted sequencing panel for metastatic breast cancer: 175 amplicons covering regions in 17 genes

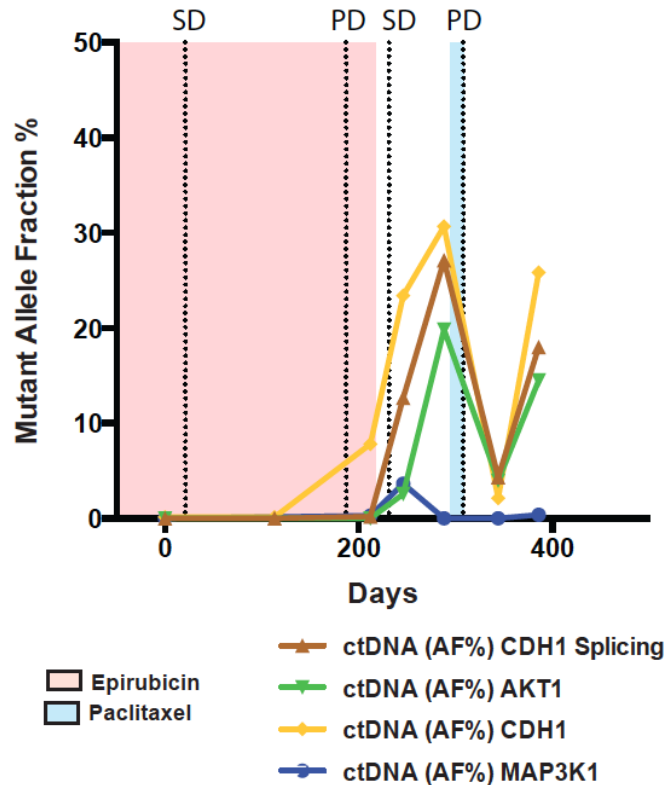


Gene	Amplicon #	Gene	Amplicon #	Gene	Amplicon #
AKT1	2	GATA3	18	CDKN1B	5
AKT2	1	CDH1	25	PTEN	14
CASP8	11	EGFR	13	KRAS	1
AR	7	MAP3K1	44	TBX3	2
TP53	16	MAP2K4	12	BRAF	1
PIK3CA	2	SF3B1	1		

Dana Tsui, Sarah-Jane Dawson,
Francesco Marass, Carlos Caldas
(unpublished)

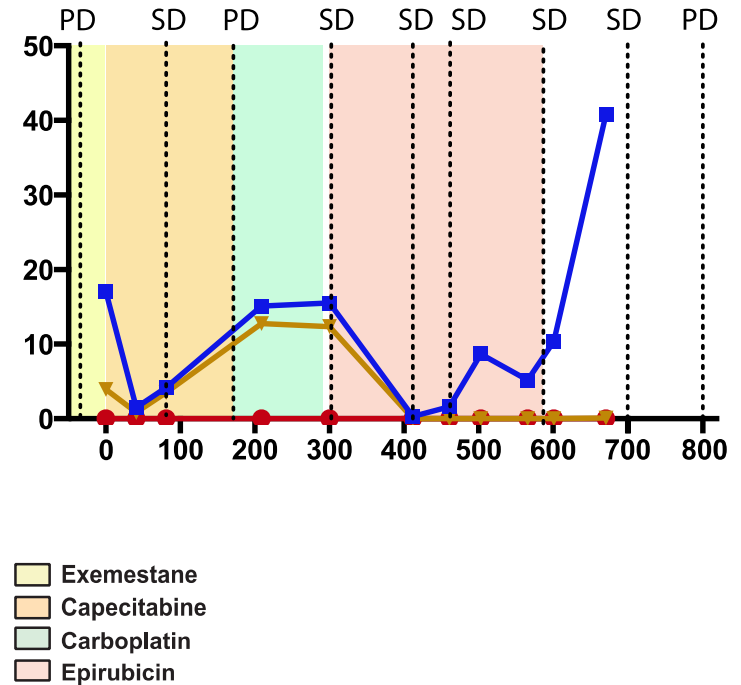
ForsheW, Murtaza, Brenton
(Sci Transl Med 2012)

Different mutations can show diverging patterns: ... a bug or a feature?



Mutant allele fractions
in primary breast tumour:

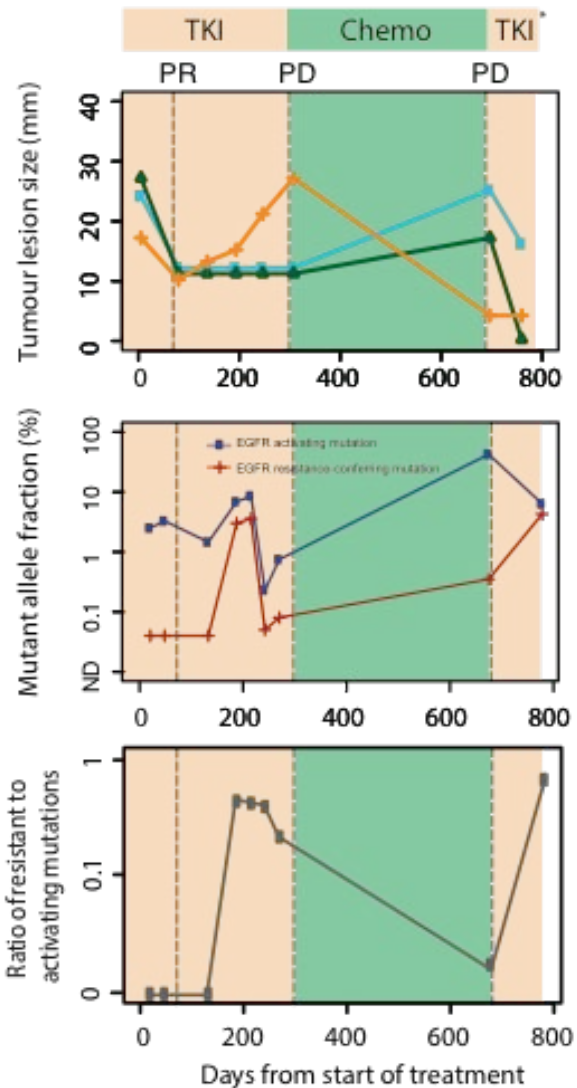
- CDH1 – 77%
- CDH1 – 74%
- AKT1 – 54%
- MAP3K1 – 14%



Mutant allele fractions
in primary breast tumour:
(9 years before T=0)

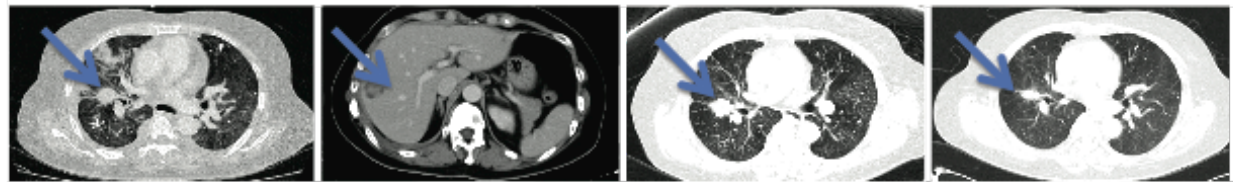
- TP53 splicing – 14%
- PIK3CA – 10%
- TP53 point mutation – 9%

Relative mutation levels in ctDNA demonstrate clonal dynamics in response to several lines of TKI/chemotherapy

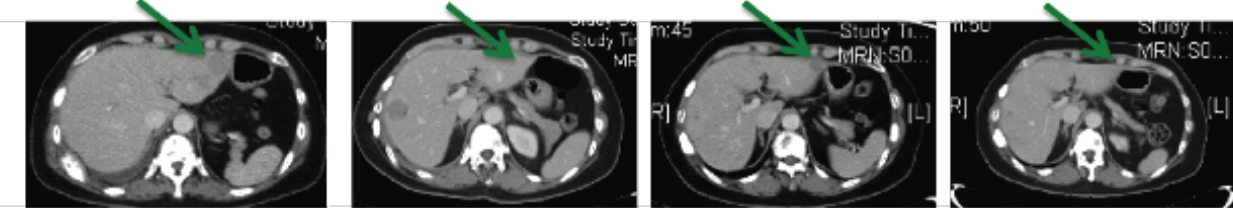


*Treatment discontinued and switched to supportive care.

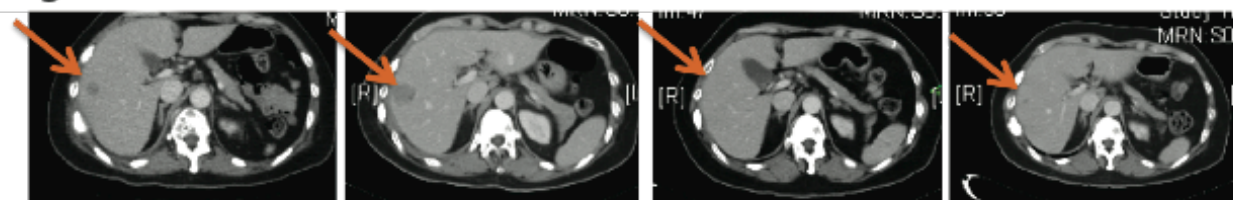
Lung lesions



Left lobe liver lesion



Right lobe liver lesion



Day 0

Day 297

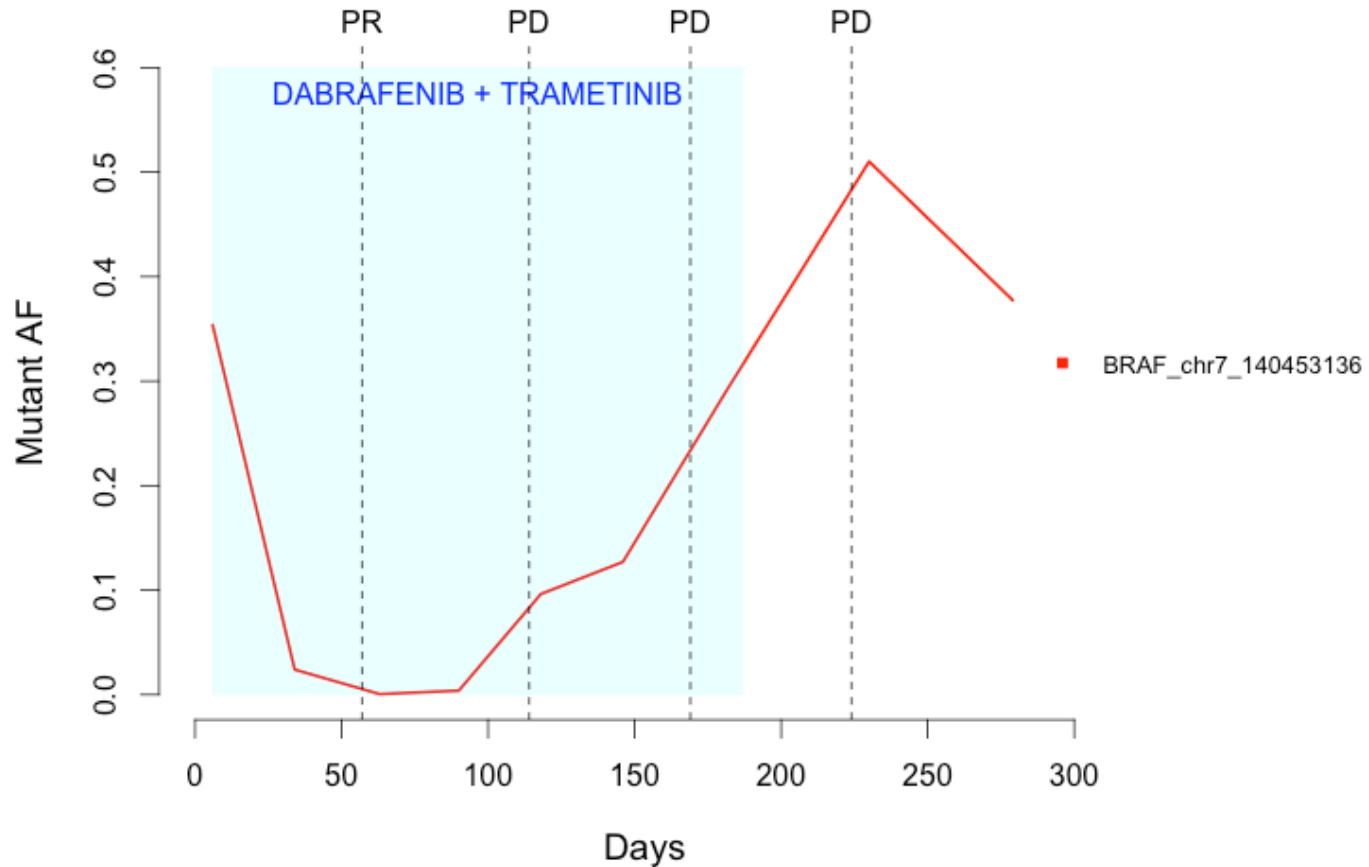
Day 679

Day 741

Dana Tsui, Tan Min Chin
(unpublished)



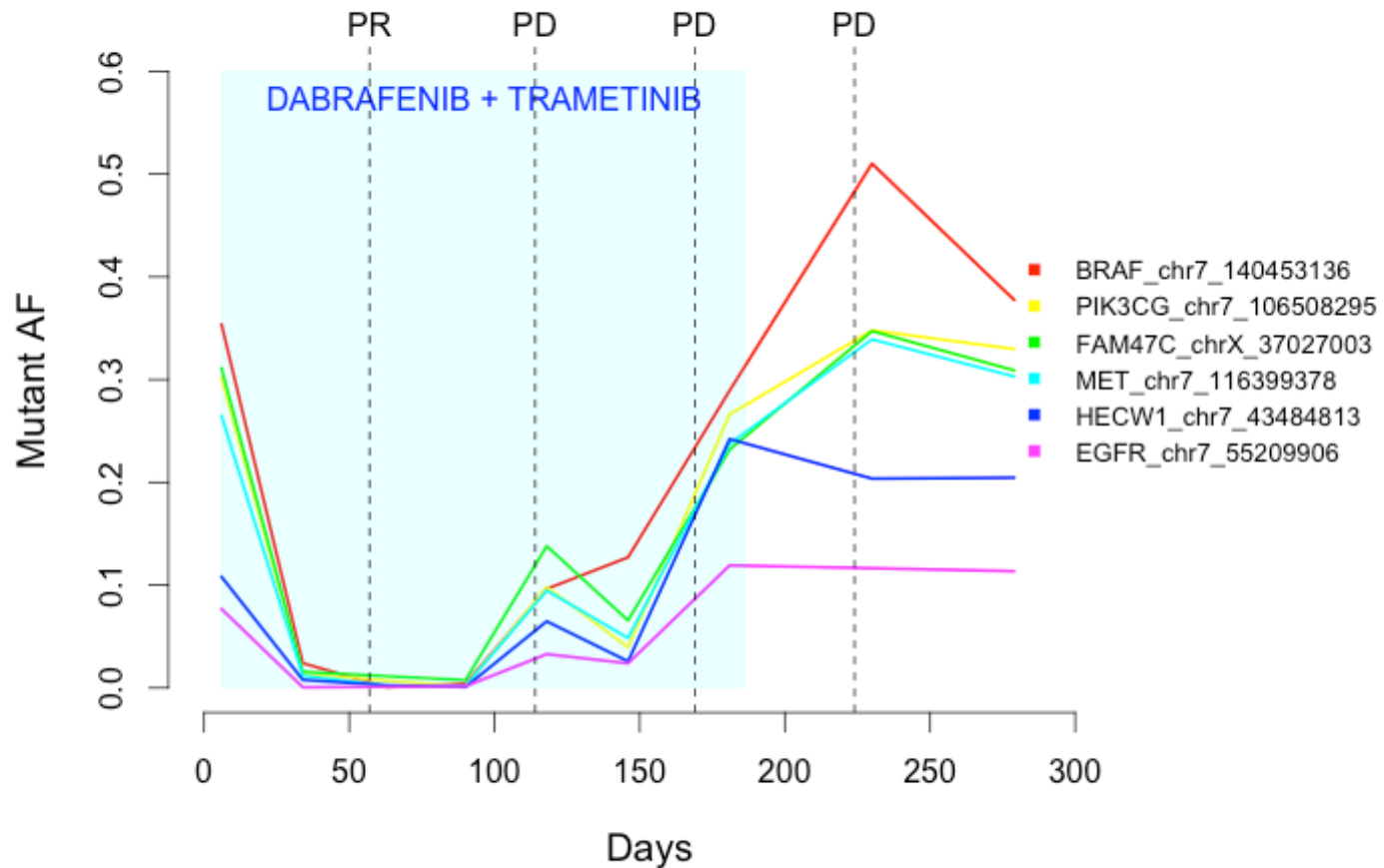
Why track 1-2 mutations ...



Jonathan Wan, Pippa Corrie
(unpublished)

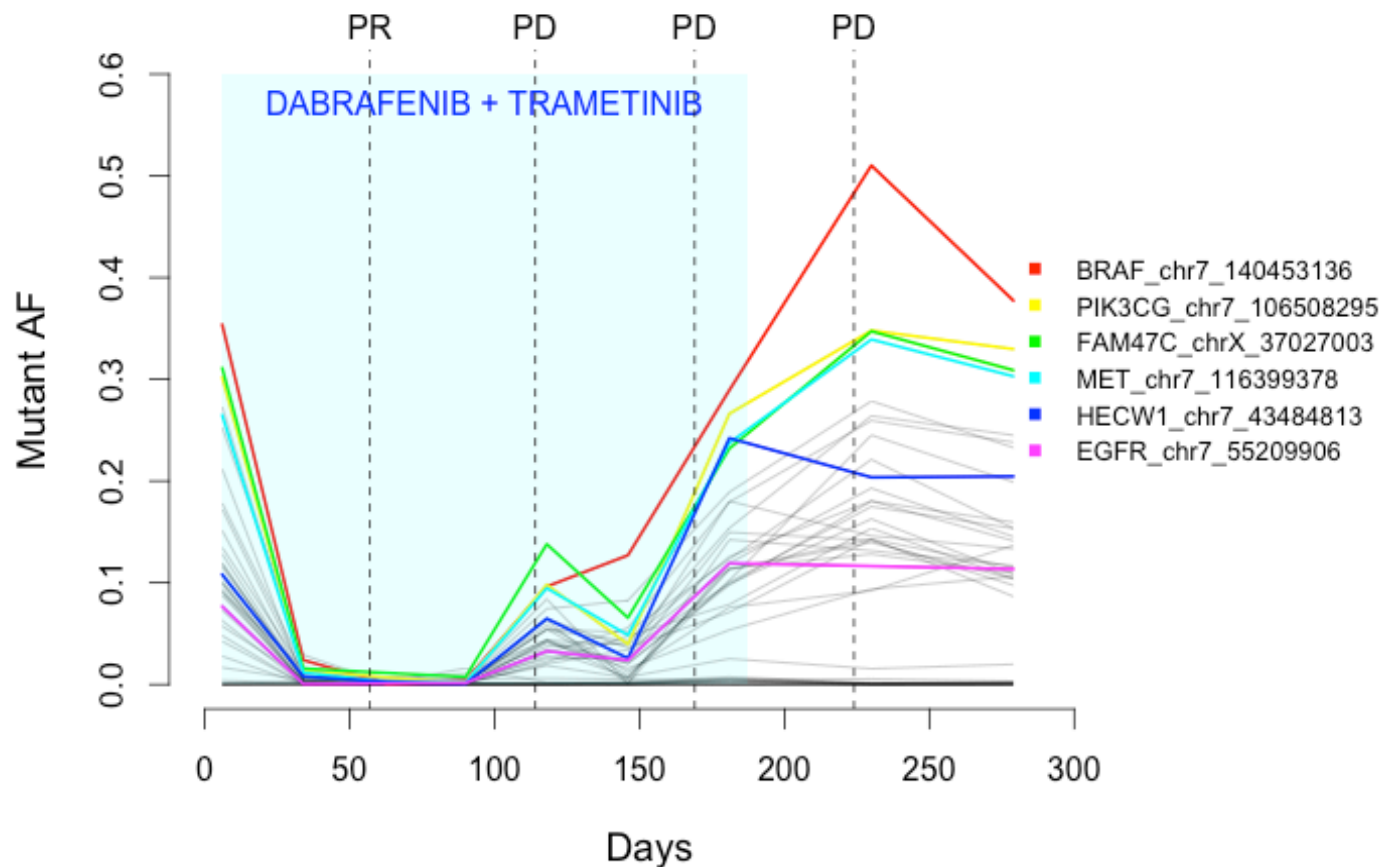


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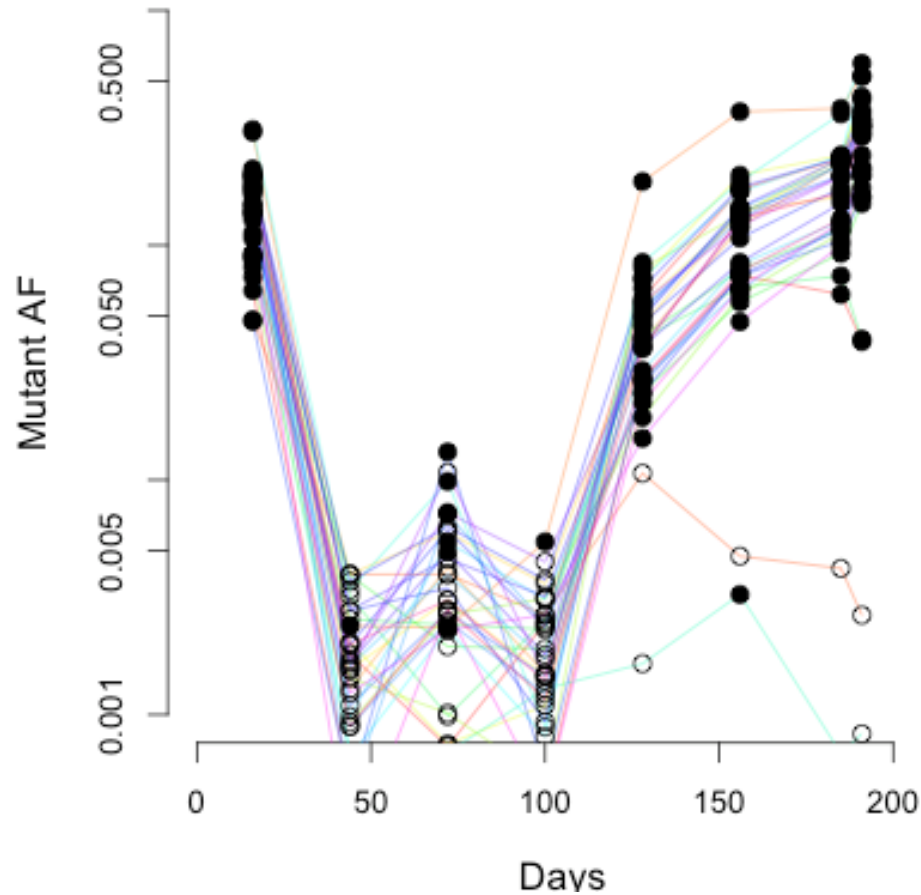
Jonathan Wan, Pippa Corrie
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Patient-specific targeted sequencing panels allow us to track dozens or hundreds of mutations



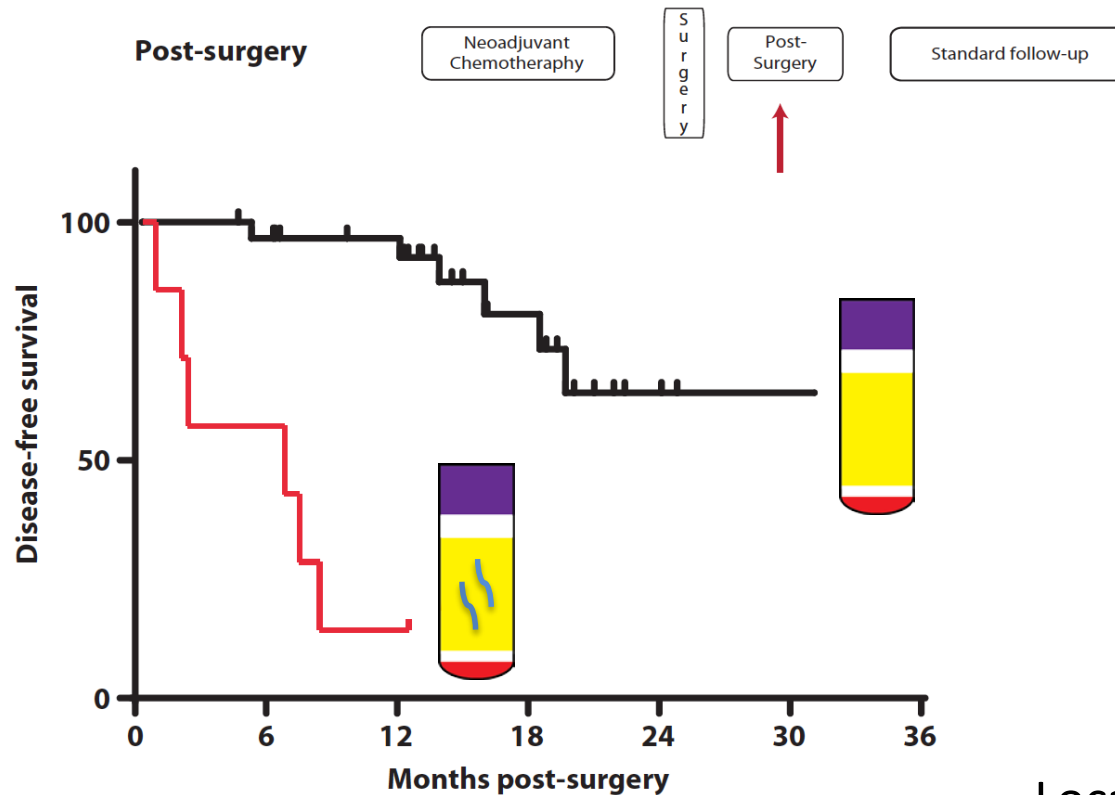
Jonathan Wan, Pippa Corrie
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Multiplexed detection of a large number of mutations can improve detection of low-burden disease



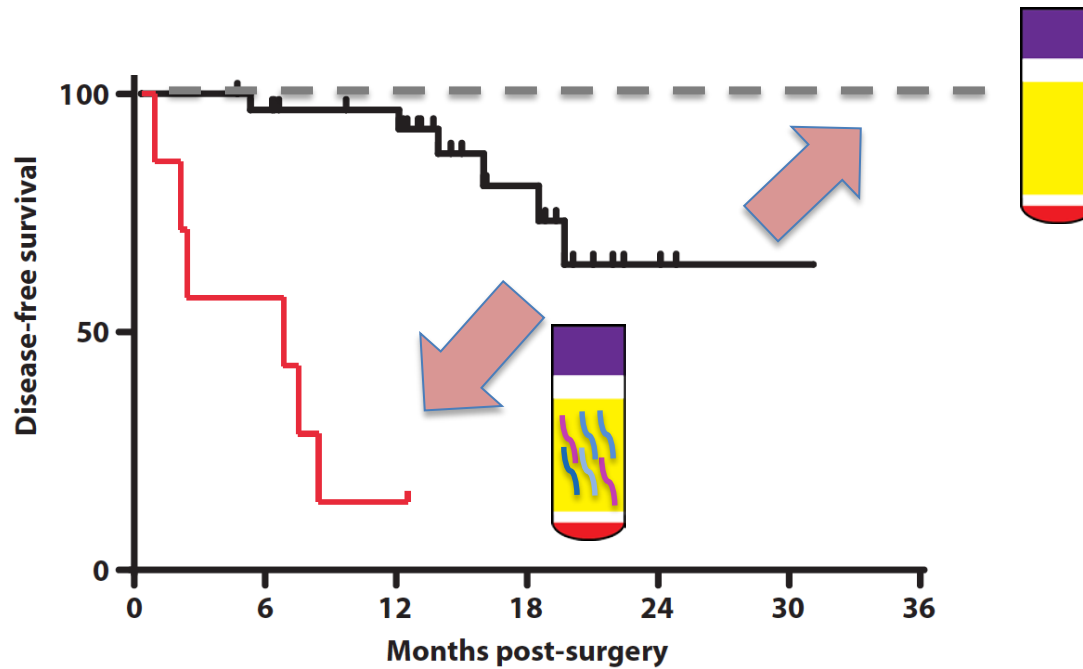
Jonathan Wan, Pippa Corrie
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Residual ctDNA post-surgery is prognostic, indicating risk of relapse. How to make this clinically actionable?



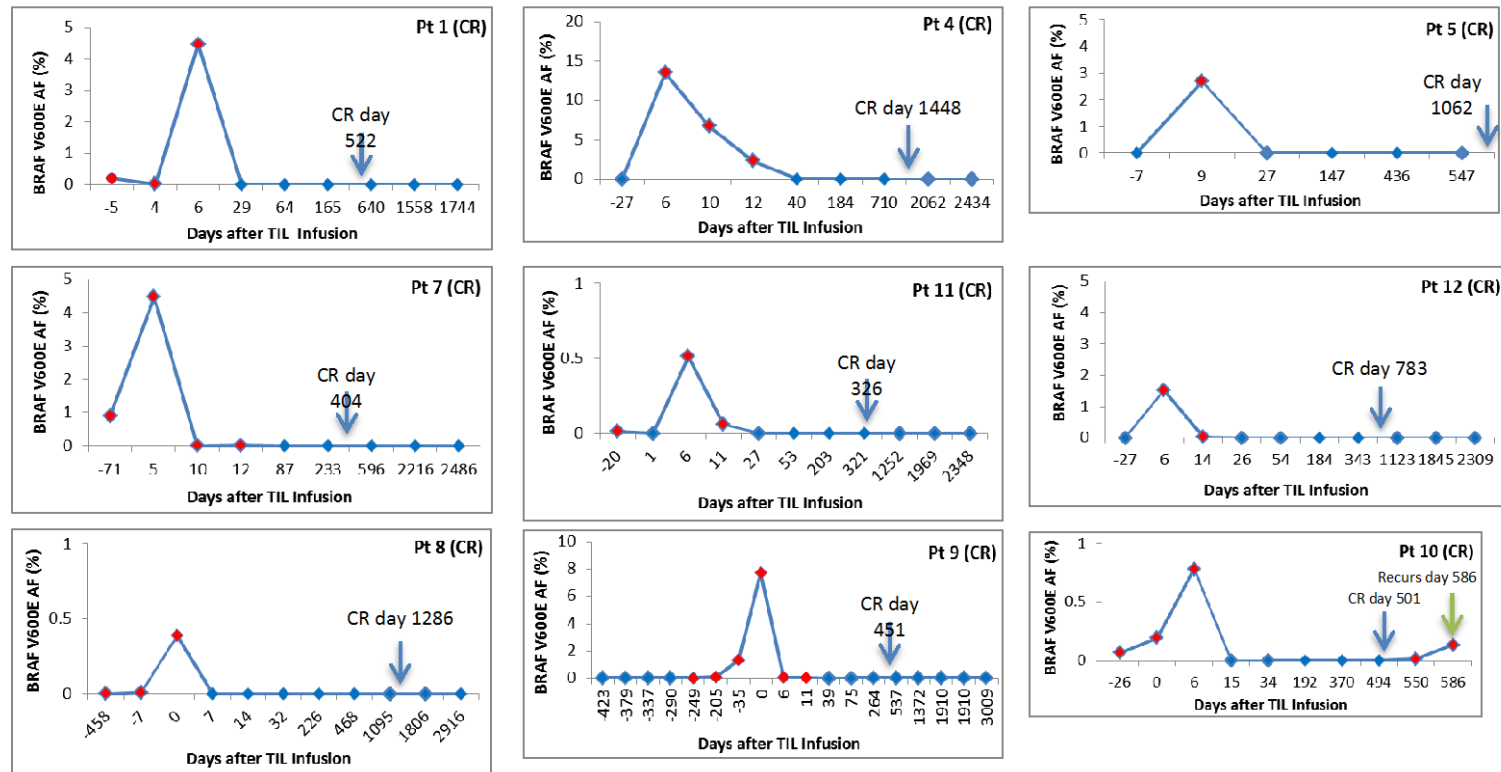
Localised breast cancer
Garcia-Murillas, Turner et al.
Sci Transl Med 2015
N=37

By enhancing sensitivity, can we more accurately identify those patients that have been cured?



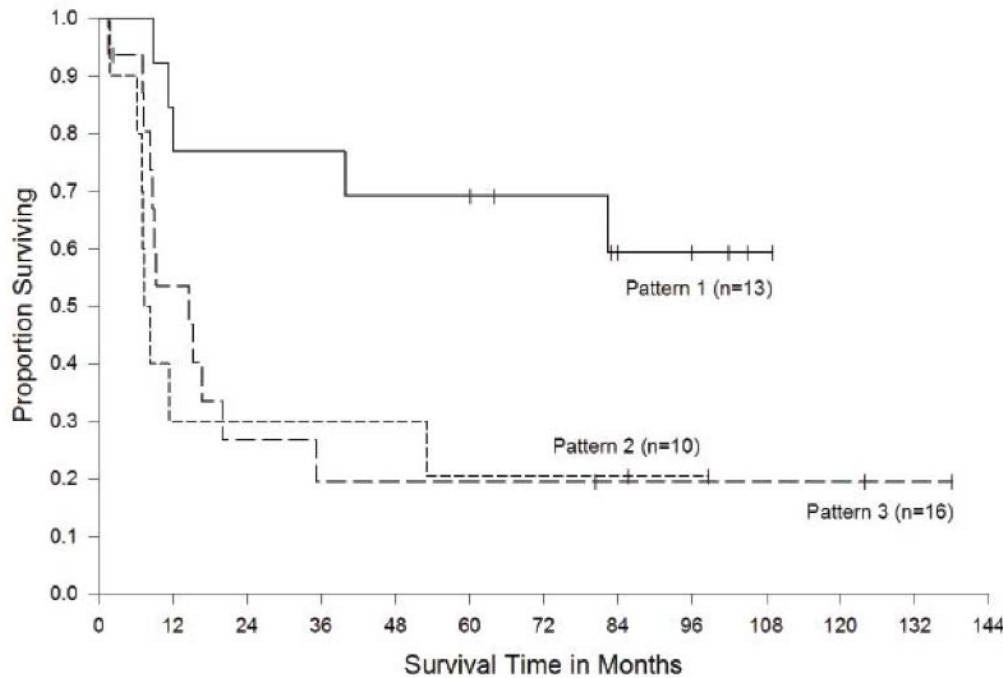
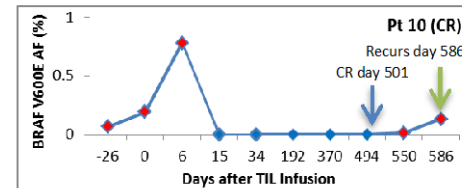
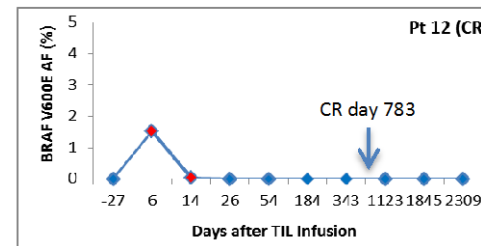
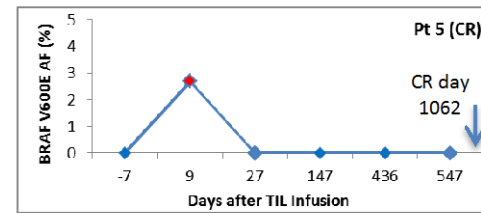
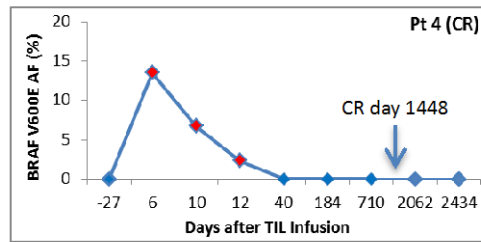
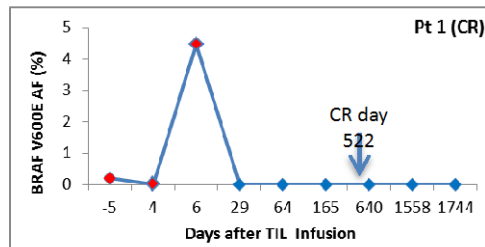
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Circulating Tumor DNA as an Early Indicator of Response to T-Cell Transfer Immunotherapy in Metastatic Melanoma



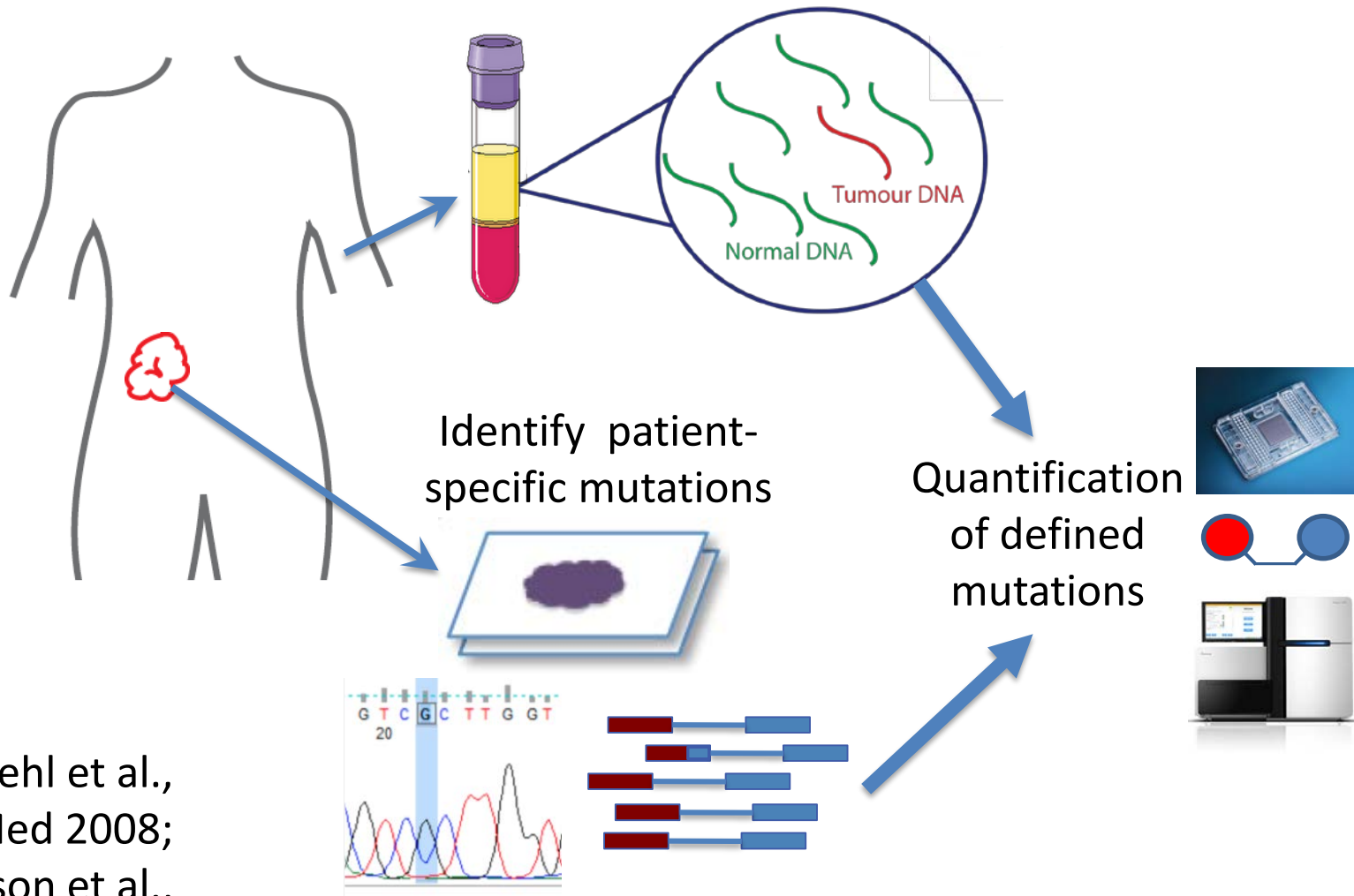
Dynamic patterns of BRAF-V600E in serum after initiating Tumor infiltrating lymphocyte (TIL) immunotherapy
Xi, Pham, Rosenberg, Raffeld et al., Clin Can Res 2016

Circulating Tumor DNA as an Early Indicator of Response to T-Cell Transfer Immunotherapy in Metastatic Melanoma

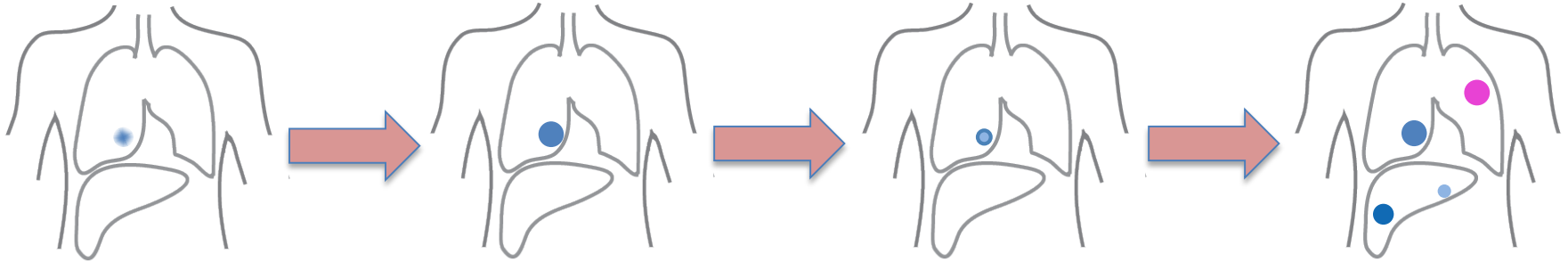


Xi, Pham Rosenberg, Raffeld et al., Clin Can Res 2016

Personalised monitoring of tumour burden: Quantification of patient-specific sequence alterations



e.g. Diehl et al.,
Nat Med 2008;
Dawson et al.,
NEJM 2013



Why use targeted sequencing:

Quantitative marker



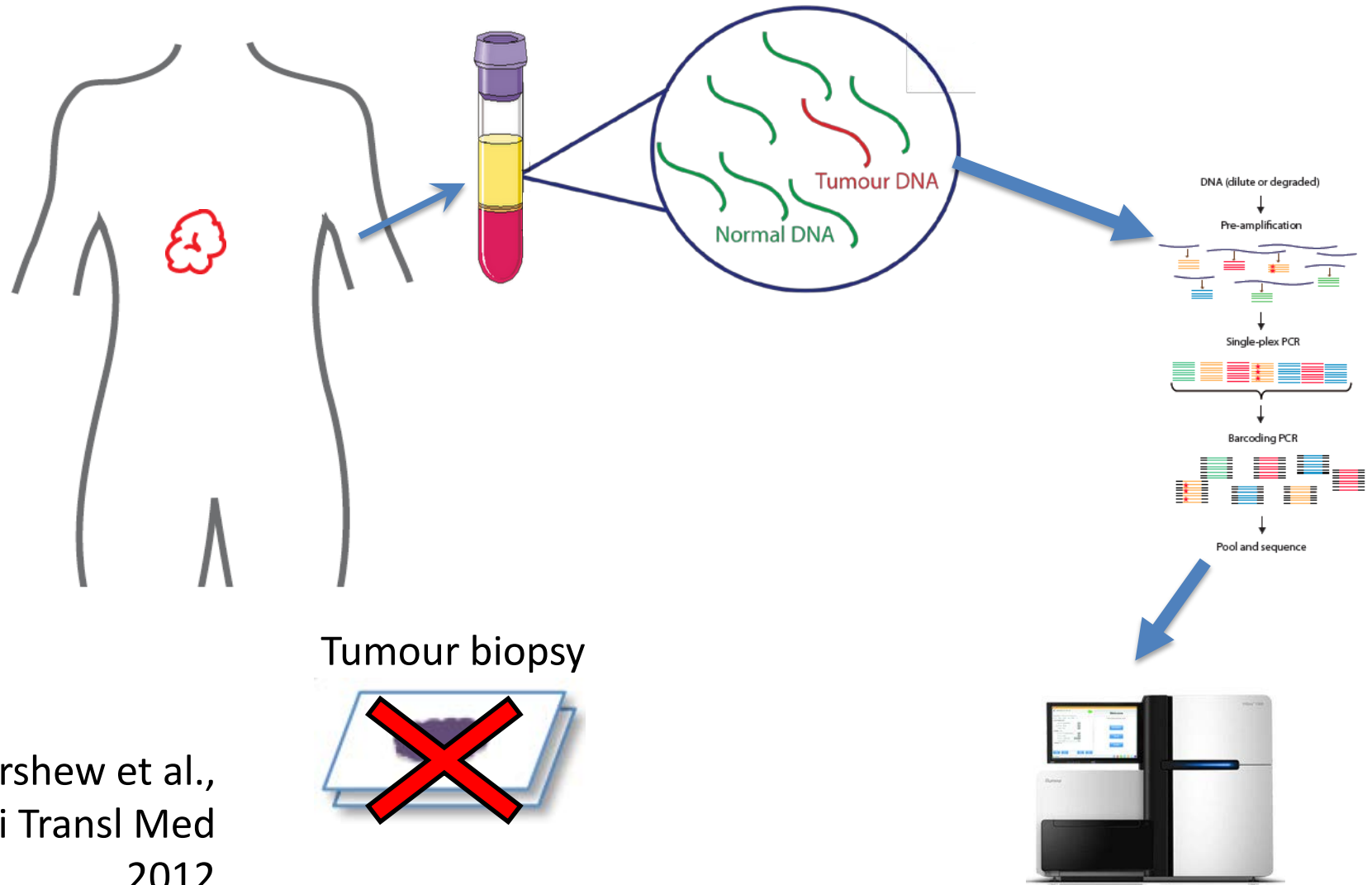
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Genomic characterisation



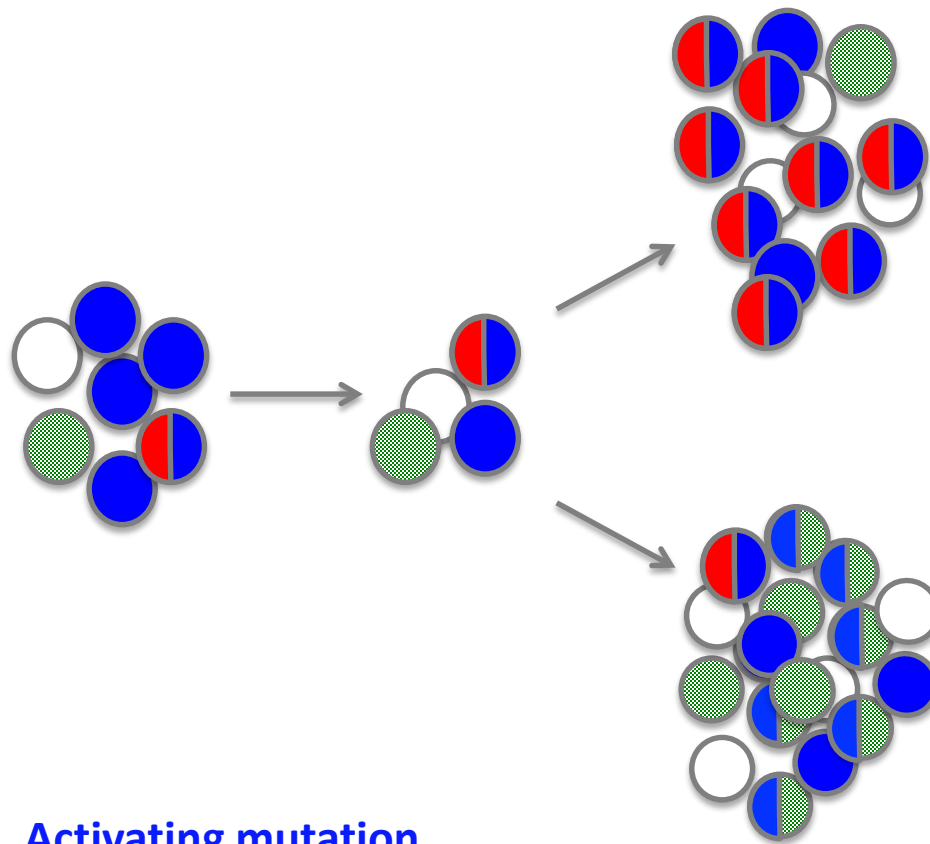
- Non-invasive molecular profiling ('Liquid biopsy')
- Track multiple clones, monitor evolution and selection

Next-generation sequencing panels to obtain molecular profiles of cancers directly from plasma, as a “liquid biopsy”



Forsheew et al.,
Sci Transl Med
2012

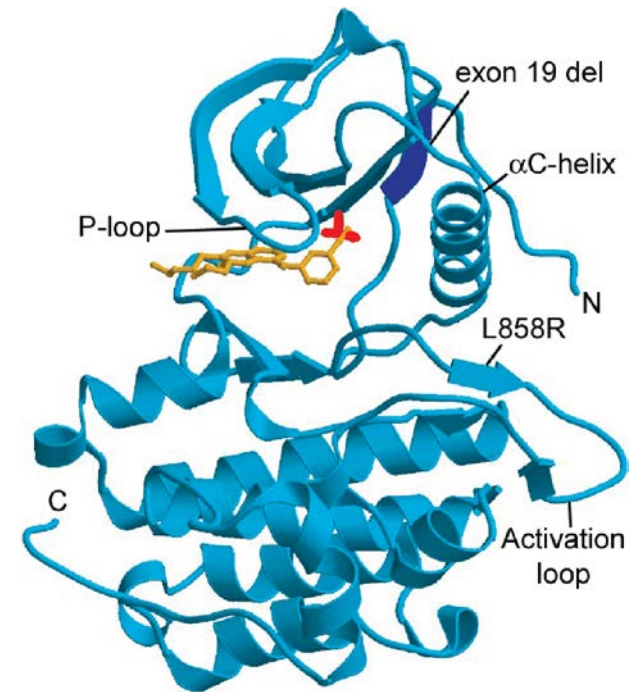
To select patients for targeted therapy, we need to identify and quantify multiple “actionable” mutations with high fidelity



Activating mutation

Resistance-conferring mutation

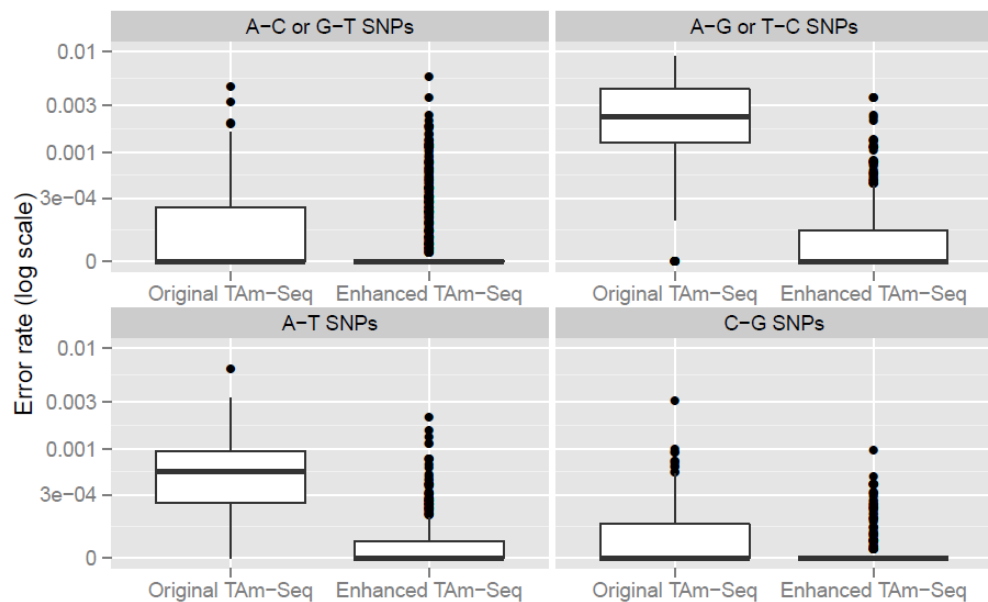
Other pathways



EGFR structure and mutations

(adapted from Clark et al. PLoS Med 2005)

Enhanced sensitivity for *'actionable'* mutations in cancer genes : noise-reduced sequencing across a panel of 100s of amplicons



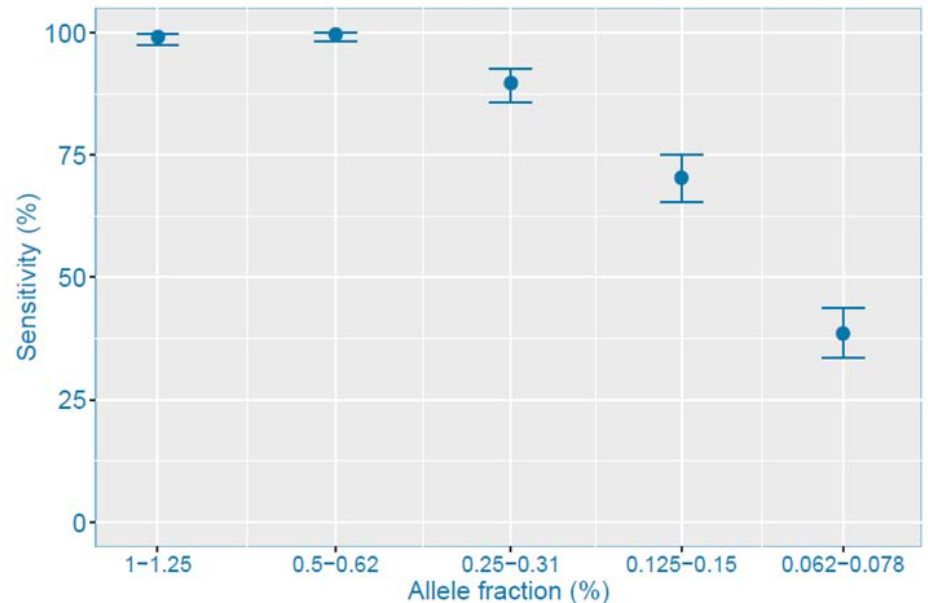
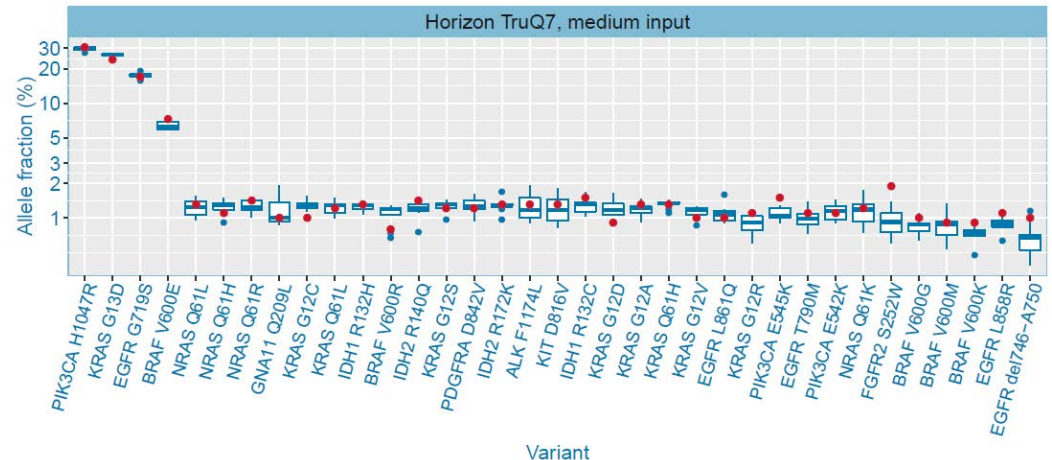
Lawson, Plagnol, Forshew, Gale, et al.
AACR 2015

AKT1	ESR1	HRAS	NRAS
ALK	FGFR1	IDH1	PDGFRA
BRAF	FGFR2	IDH2	PIK3CA
CCND1	FGFR3	KIT	PPP2R1A
CDKN2A	FOXL2	KRAS	PTEN
CHEK2	GATA3	MED12	RET
CTNNB1	GNA11	MET	STK11
EGFR	GNAQ	MYC	TP53
ERBB2	GNAS	NFE2L2	

- Exon tiling (88-100% covered)
- Hotspot regions
- CNVs (in development)

A fit-for-purpose gene panel for clinical plasma DNA sequencing

AKT1	CTNNB1	FOXL2	IDH1	MYC	PTEN
ALK	EGFR	GATA3	IDH2	NFE2L2	RET
BRAF	ERBB2	GNA11	KIT	NRAS	STK11
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Gale, Plagnol, Lawson, Forshew, et al.
AACR 2016

A fit-for-purpose gene panel for clinical plasma DNA sequencing

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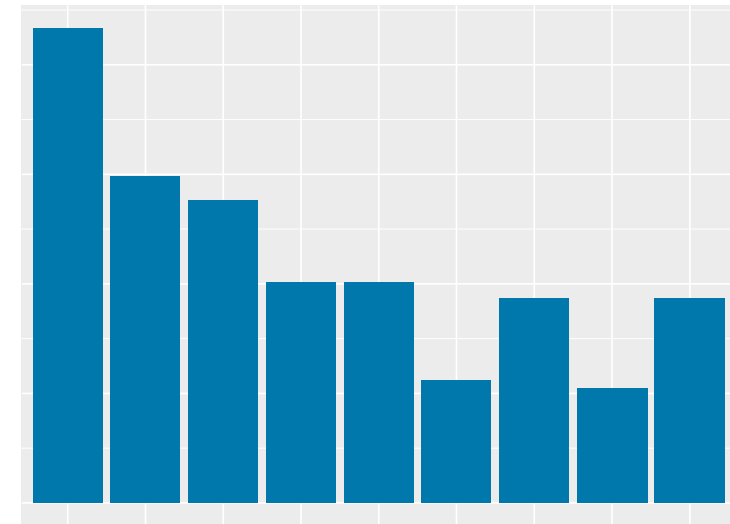
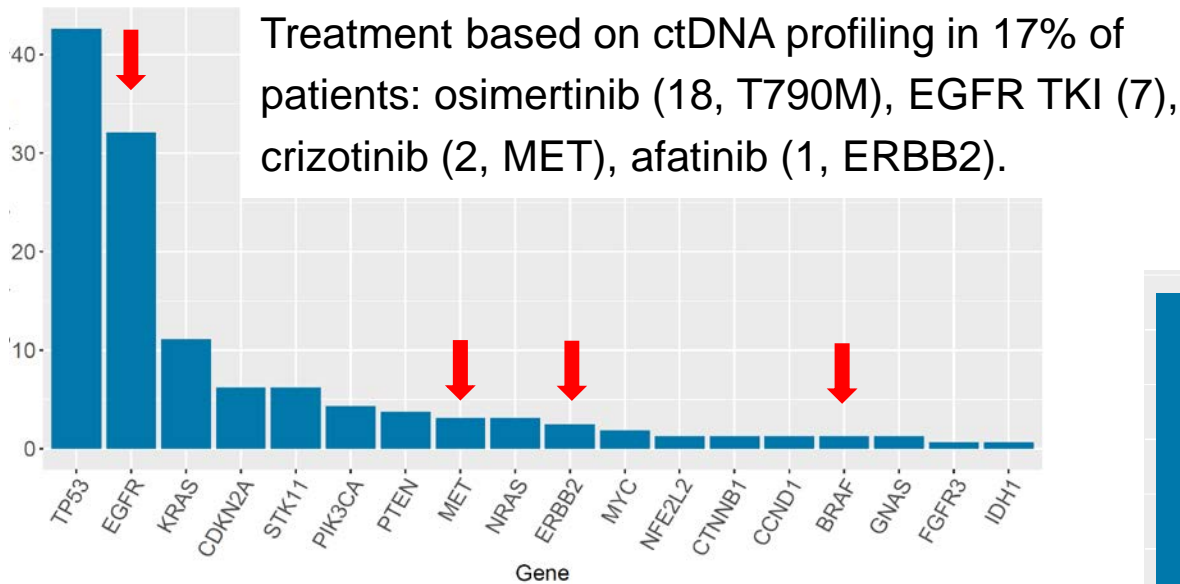
Exon tiling (88-100% coverage) Hotspot regions

1



Gale, Plagnol, Lawson, Forshew, et al.
AACR 2016

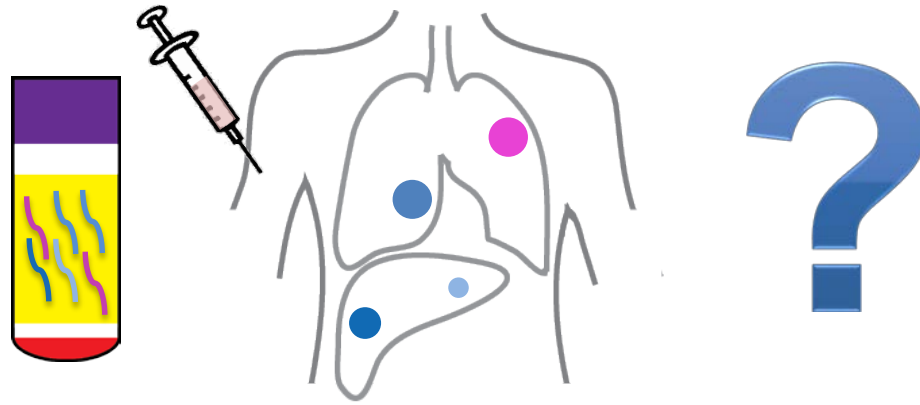
In 174 NSCLC patients: alterations detected in 79% of cases. Treatment given based on cfDNA report in 17% of cases



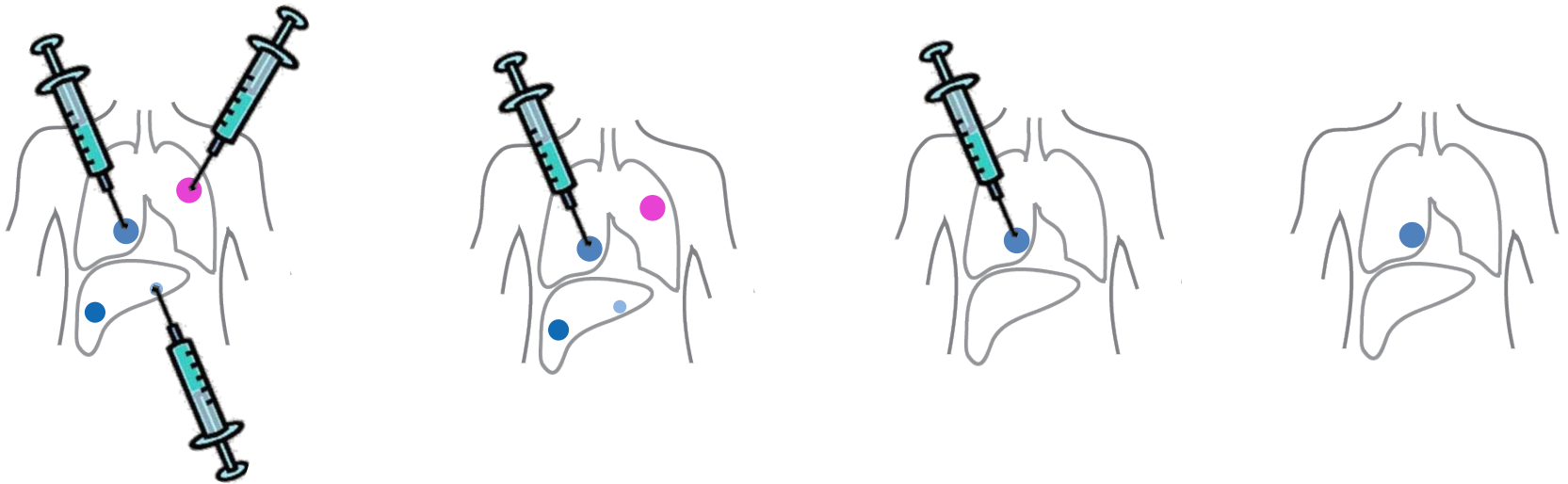
J Remon*, JC Soria*, D Planchard*, E Green@,
V Plagnol@, N Rosenfeld@, B Besse*, et al.
*Gustave Roussy; @Inivata Ltd

Molecular Analysis for Personalised therapy (MAP)
London, September 2016

MAF < 0.5% in 43% of cases.



How good is a liquid biopsy? Compared to ...



ctDNA assays have been approved for use by the EMA and FDA as companion diagnostics for detection of EGFR mutations in plasma

FDA approval: (June 1st, 2016)

<http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm504540.htm>

The agreement between the **cobas** EGFR Mutation Test v2 in plasma and the **cobas** EGFR Mutation Test v1 in tissue was evaluated for detection of EGFR mutations (Ex. 19del and L858R mutations) in NSCLC patients screened for participation in ENSURE. In 76.7% (70.5%, 81.9%) of tissue-positive specimens, plasma was also positive for an EGFR mutation.

The patients whose plasma results were positive for exon 19 deletion and/or an L858R mutations treated with erlotinib had improved progression-free survival (PFS) compared to those treated with chemotherapy.

The **cobas** EGFR Mutation Test v2 for use with plasma test is intended to be used to initially screen patients with metastatic NSCLC for EGFR mutations. Those patients in whom no exon 19 deletion and/or an L858R mutation is detected in their plasma specimens should then be reflexed to having their EGFR status determined from their FFPE tissue specimen.

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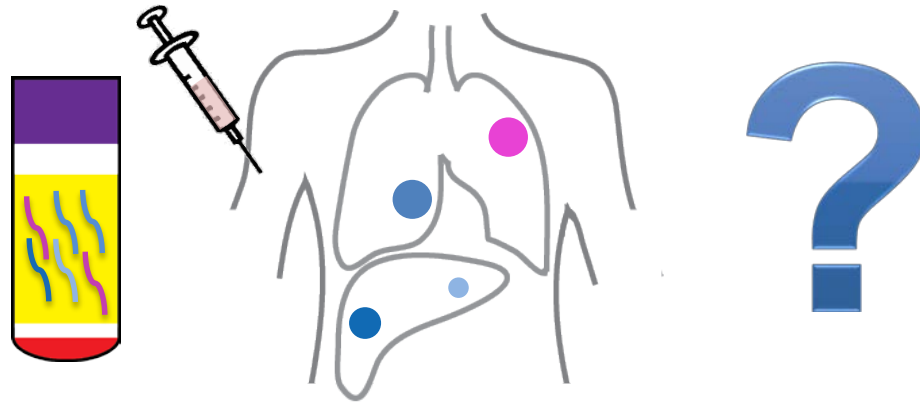
<http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm504540.htm>

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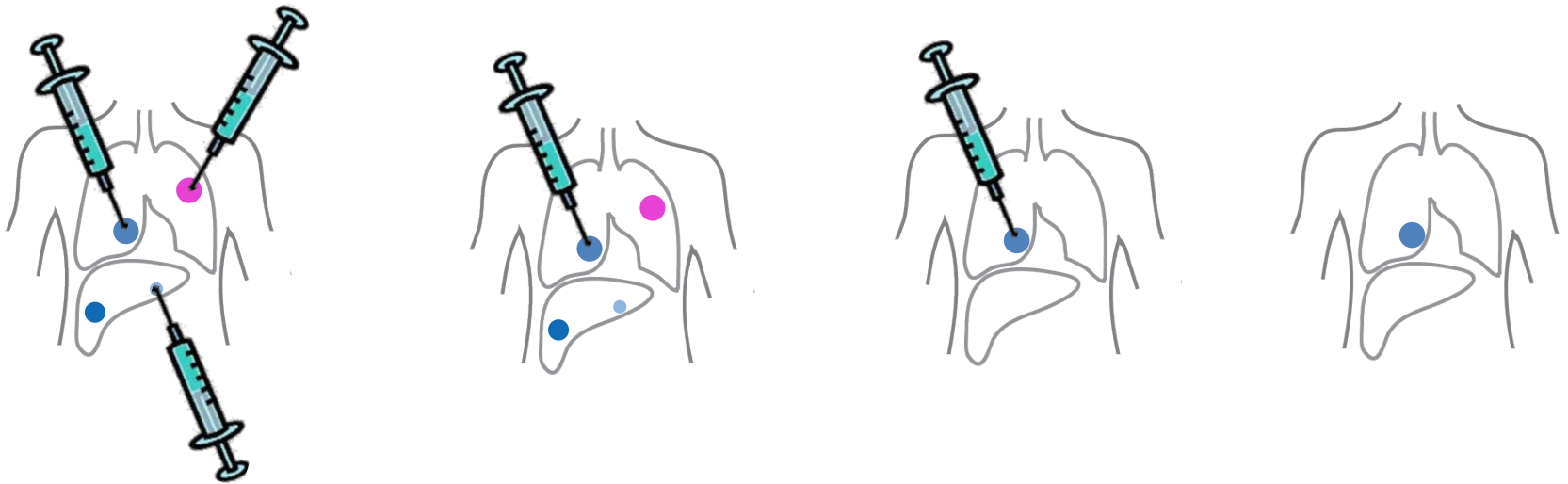
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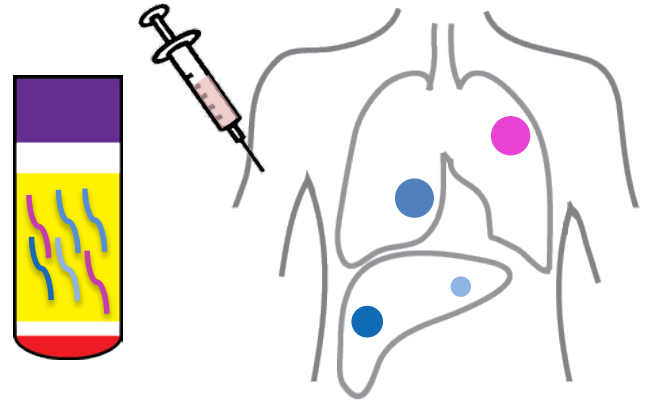
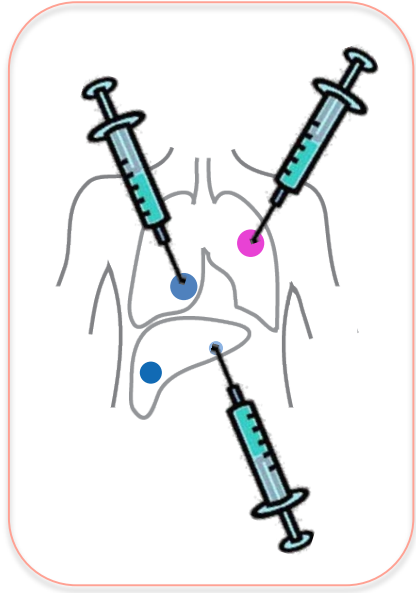
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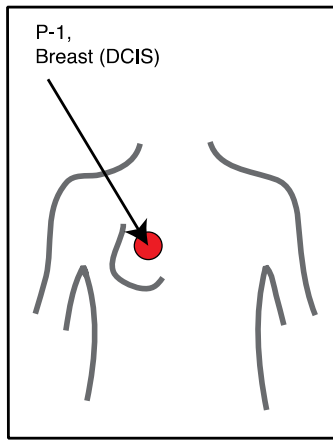
- **More sensitive assays**
- **Other mutations as "positive control" for EGFRnegative patients**
- **What is the expected level of concordance?**



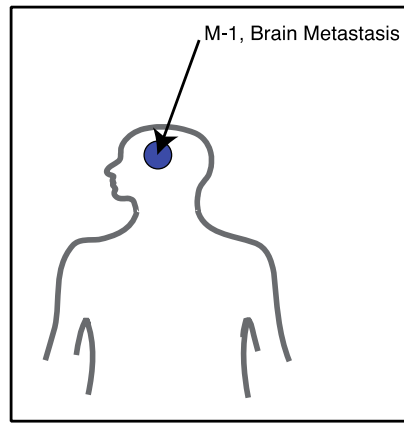
**How good is a liquid biopsy?
Compared to ...**



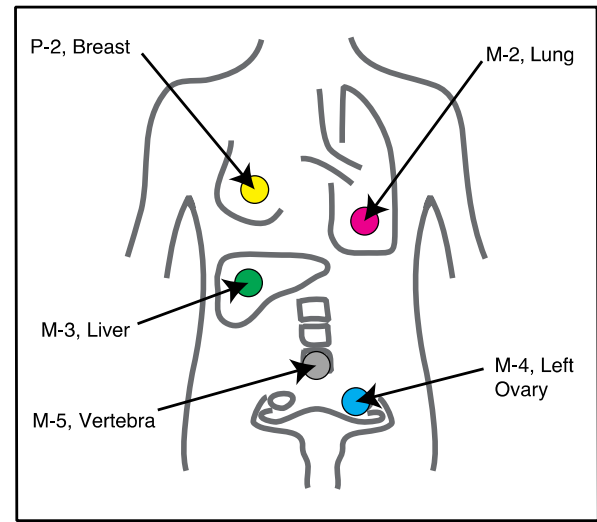




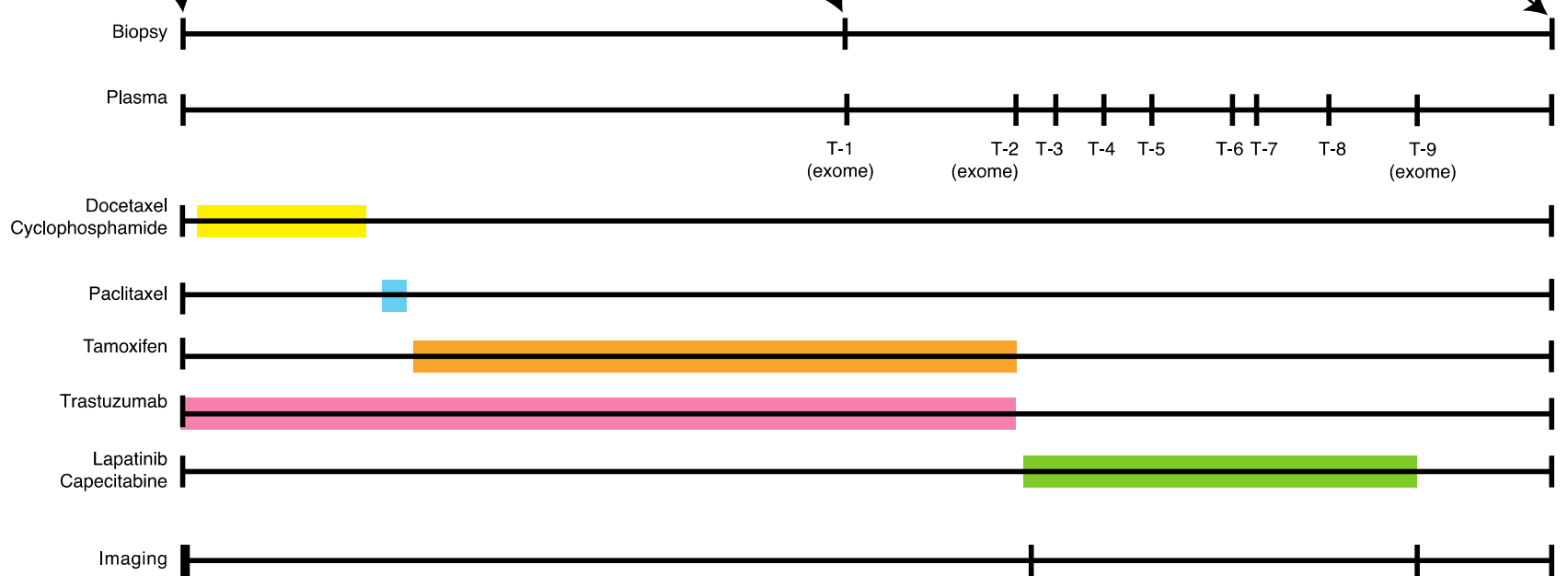
Day of diagnosis (day 0)



Symptomatic brain metastasis resected (day 577)



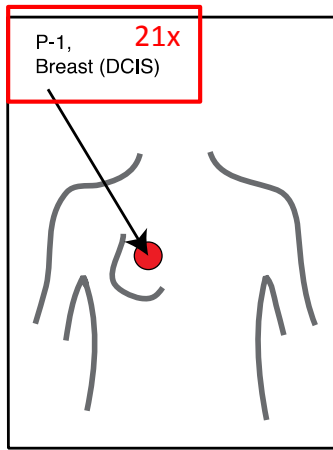
Autopsy (patient died on day 1193)



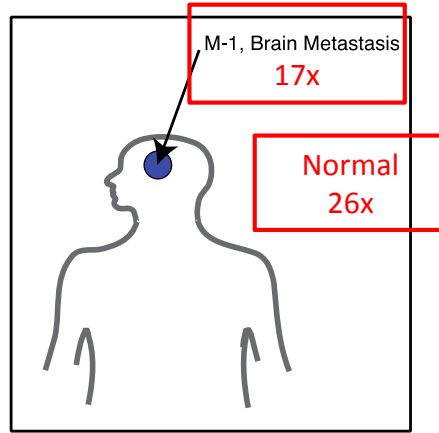
day 5: extensive metastatic disease (diffuse bone involvement, liver lesions, pleural effusion, axillary and retroperitoneal lymph nodes, Figure S1)

day 700: progressive disease (increase in size of liver lesion and new lesion within left ovary, Figure S4)

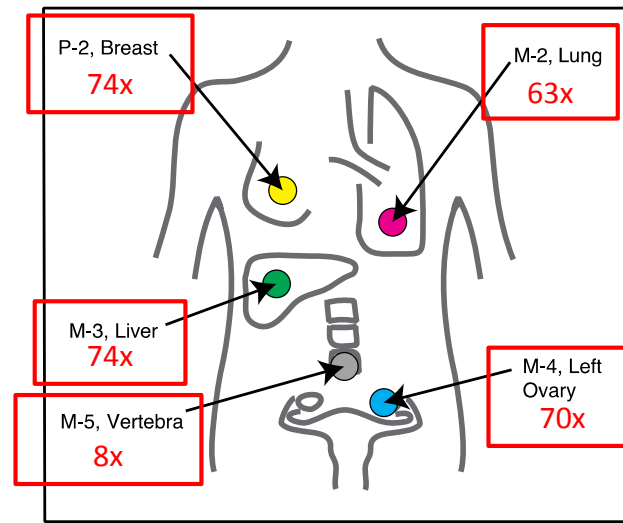
day 1077: progressive disease (no change in liver lesion, new pulmonary nodules, Figure S6)



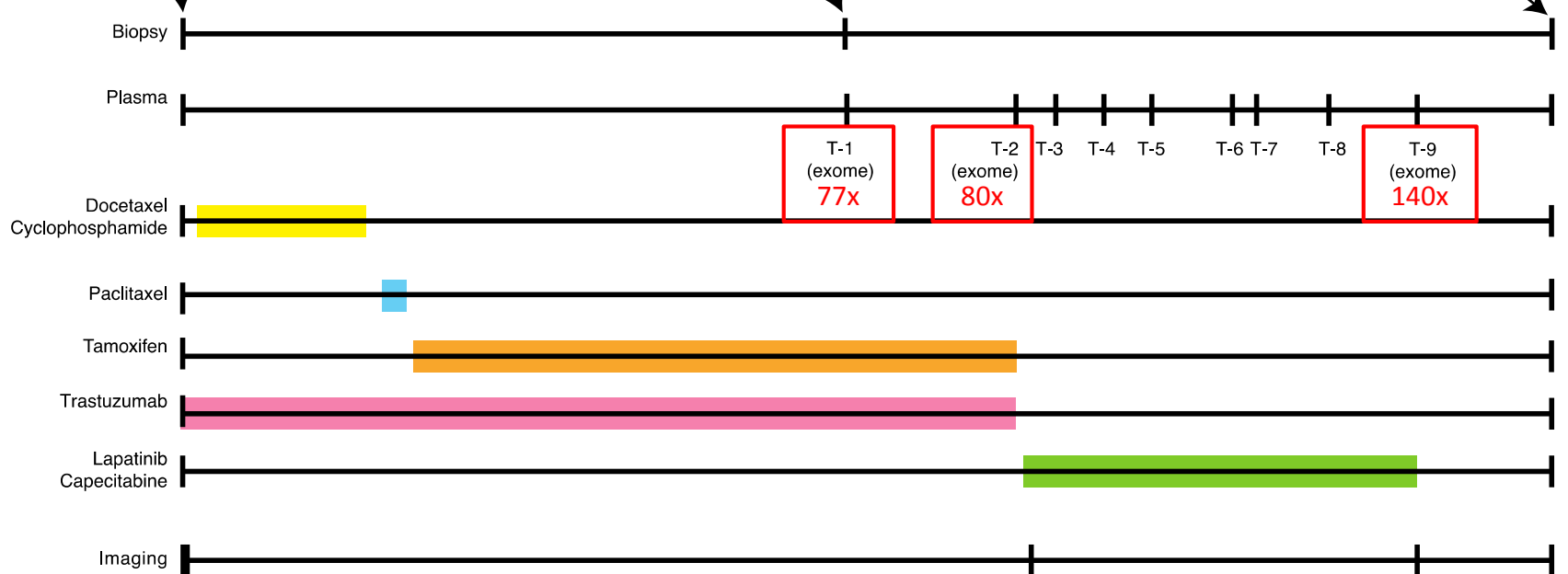
Day of diagnosis (day 0)



Symptomatic brain metastasis resected (day 577)



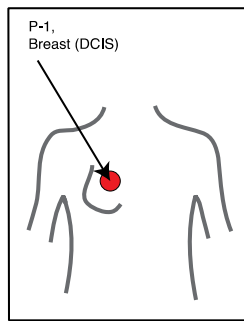
Autopsy (patient died on day 1193)



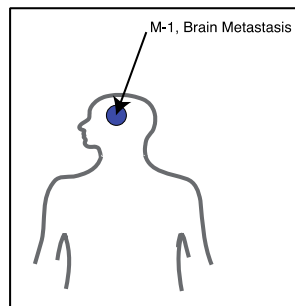
day 5: extensive metastatic disease (diffuse bone involvement, liver lesions, pleural effusion, axillary and retroperitoneal lymph nodes, Figure S1)

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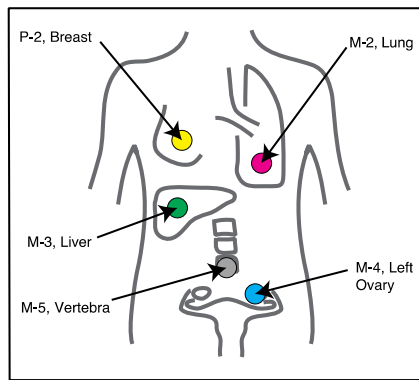
day 1077: progressive disease (no change in liver lesion, new pulmonary nodules, Figure S6)



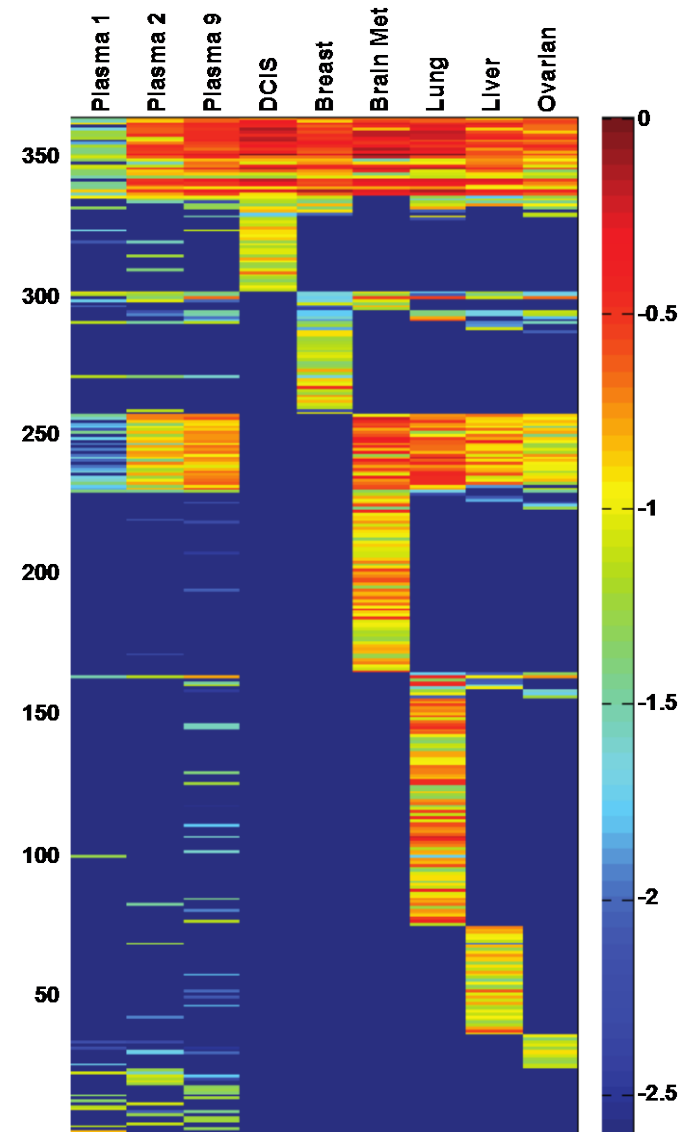
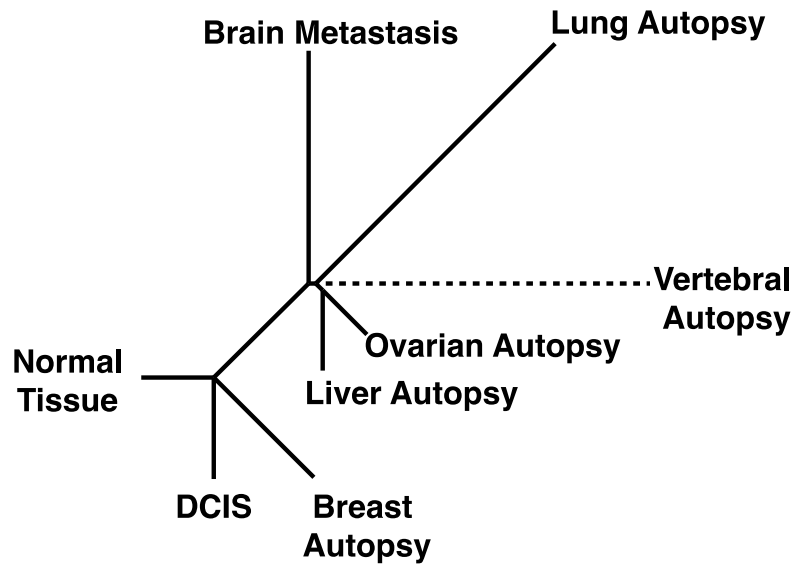
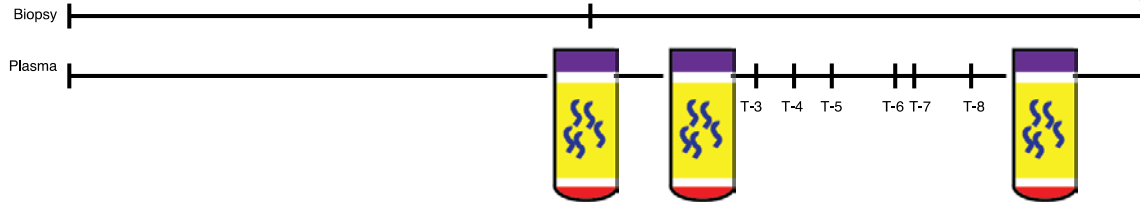
Day of diagnosis (day 0)



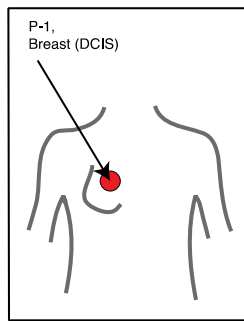
Symptomatic brain metastasis resected (day 577)



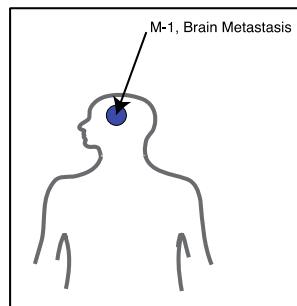
Autopsy (patient died on day 1193)



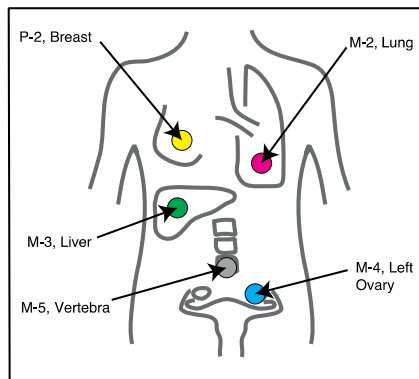
Murtaza,
Dawson,
Caldas et al.
(Nat Comm. 2015)



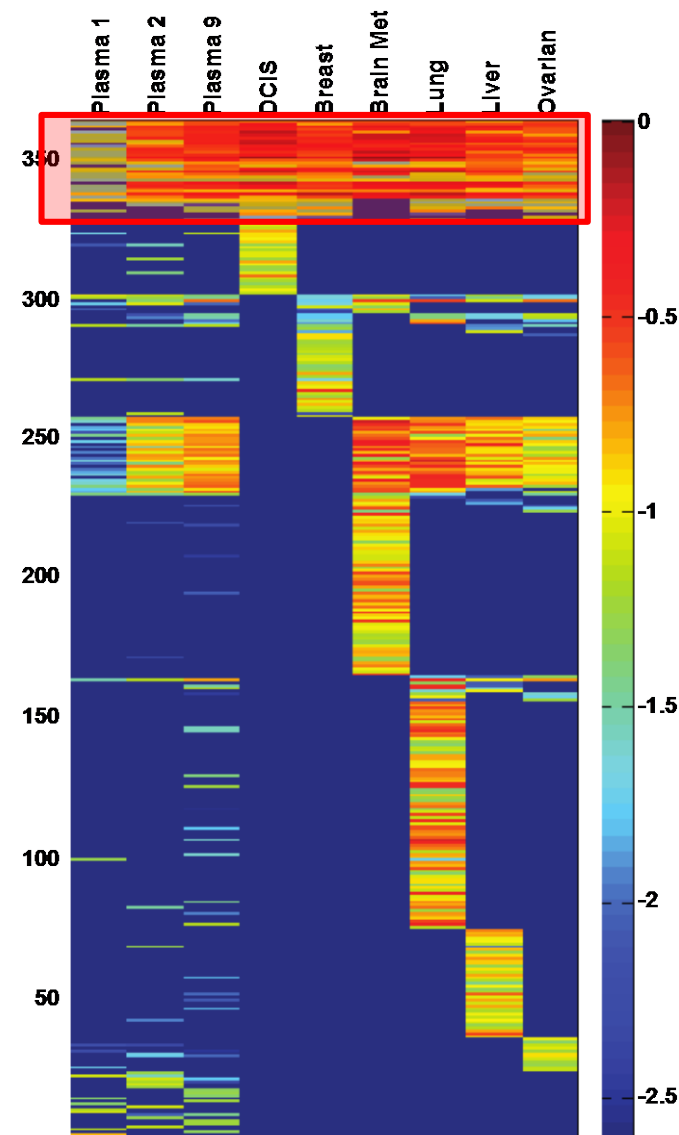
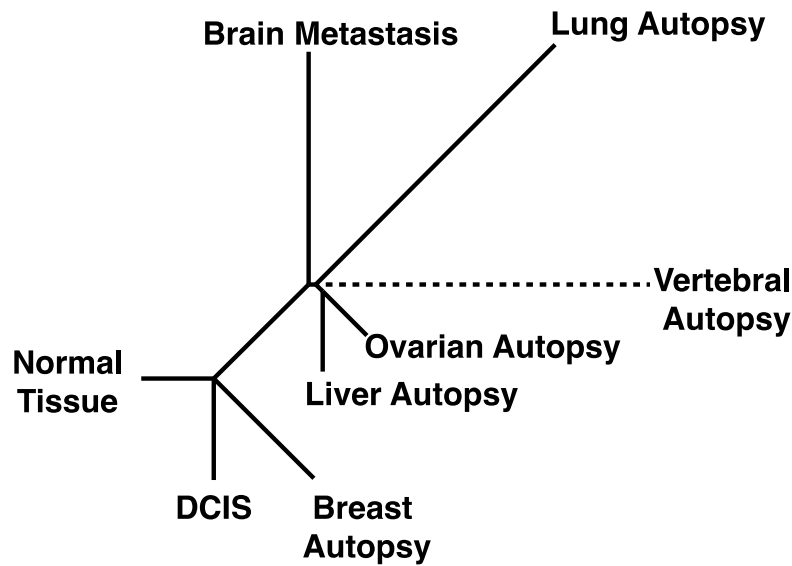
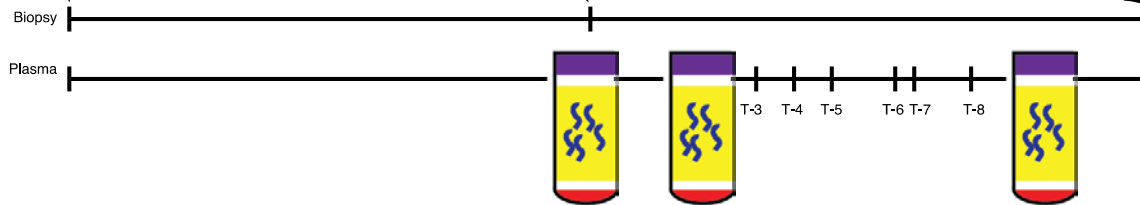
Day of diagnosis (day 0)



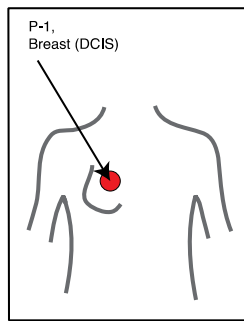
Symptomatic brain metastasis resected (day 577)



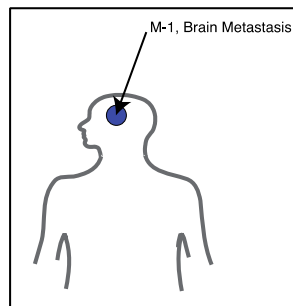
Autopsy (patient died on day 1193)



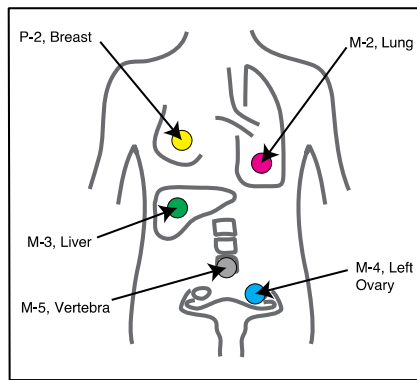
Murtaza,
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Day of diagnosis (day 0)



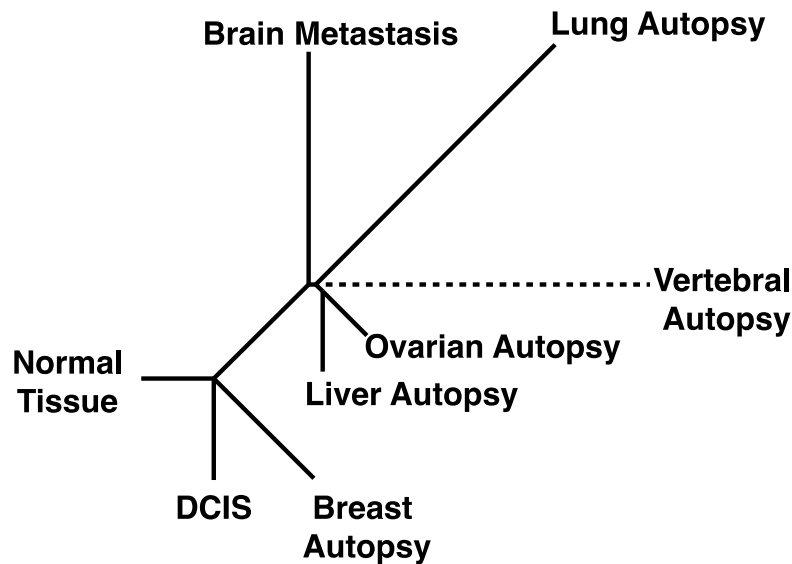
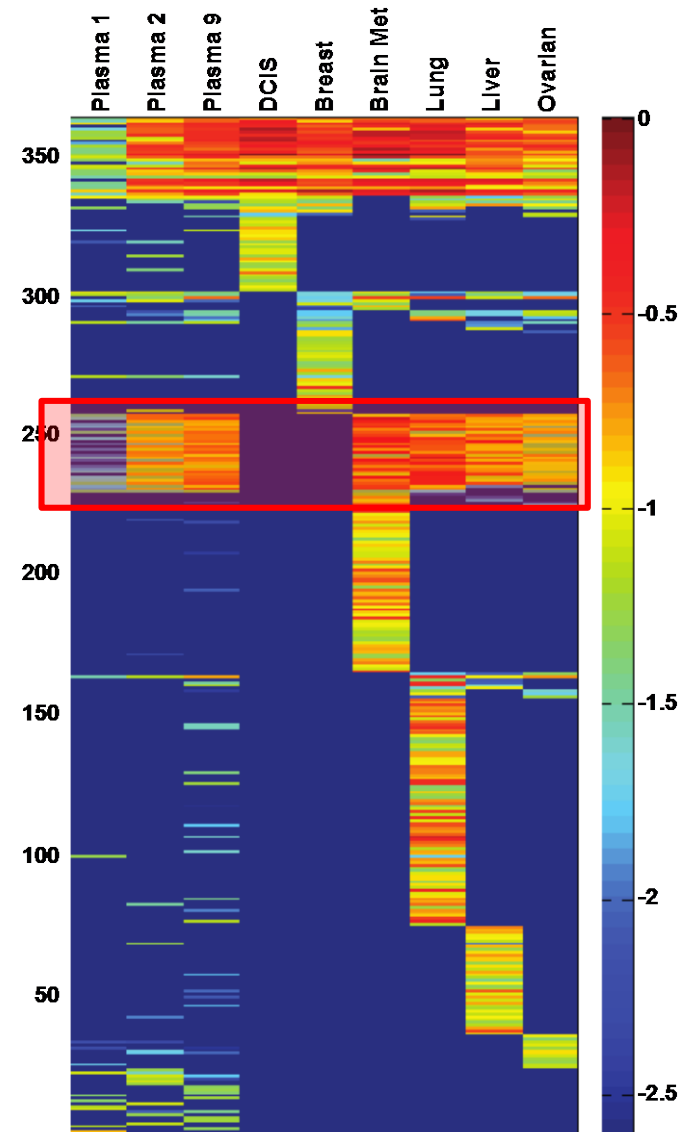
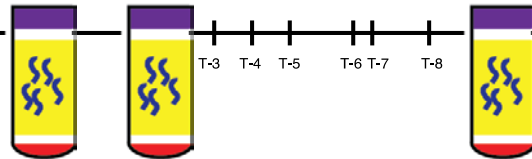
Symptomatic brain metastasis resected (day 577)



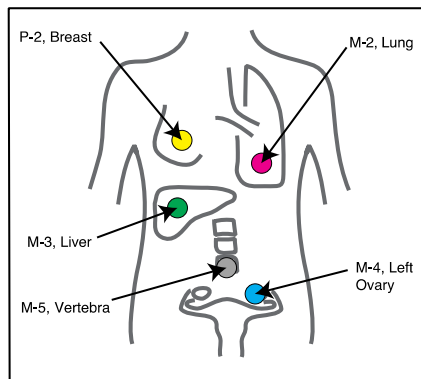
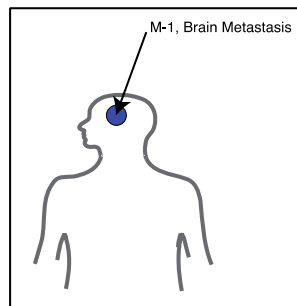
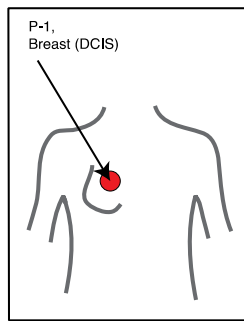
Autopsy (patient died on day 1193)

Biopsy

Plasma



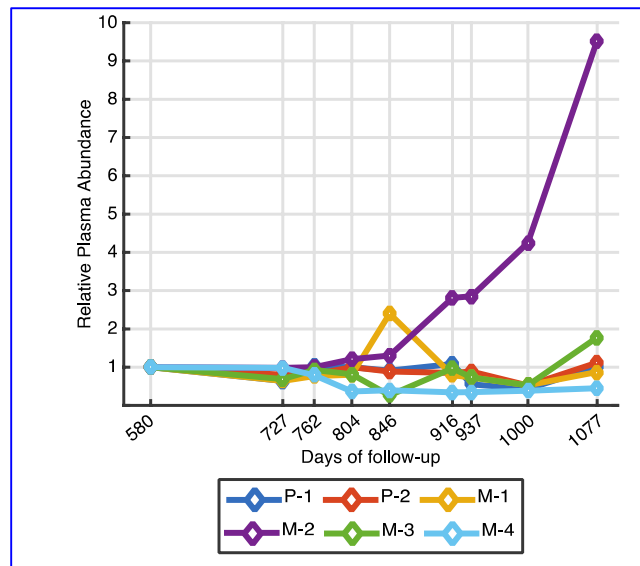
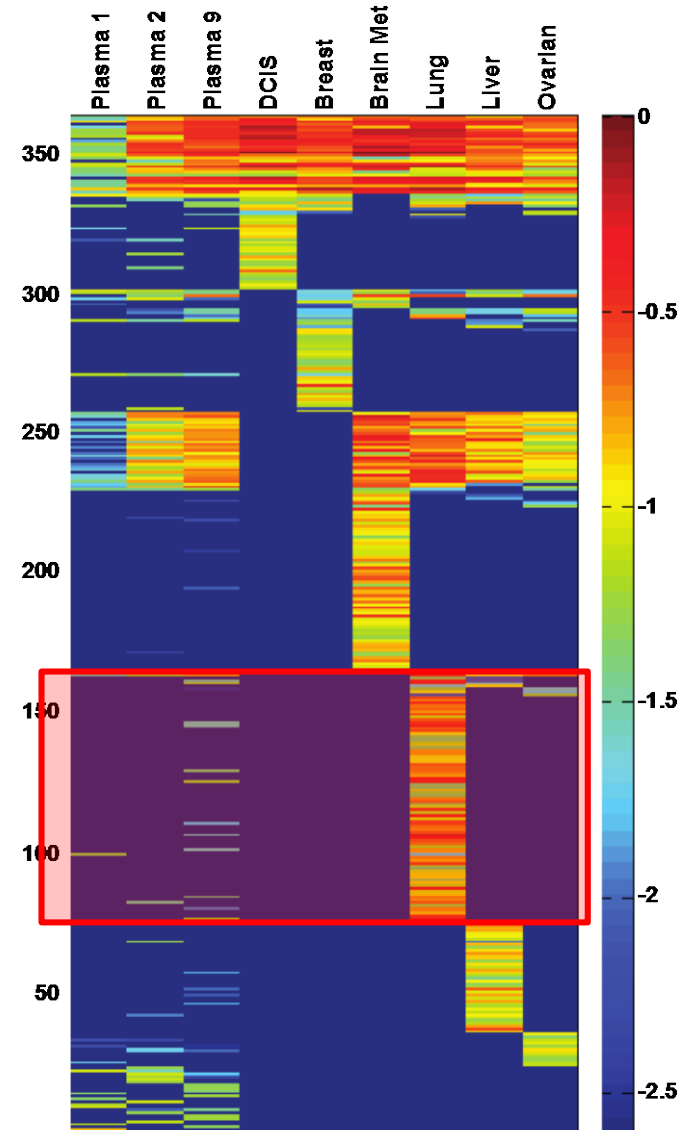
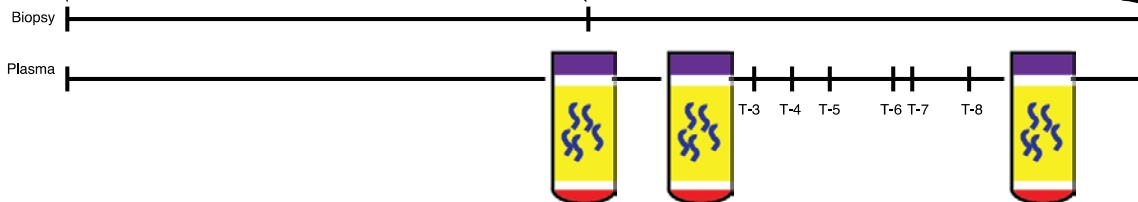
Murtaza,
Dawson,
Caldas et al.
(Nat Comm. 2015)



Day of diagnosis (day 0)

Symptomatic brain metastasis resected (day 577)

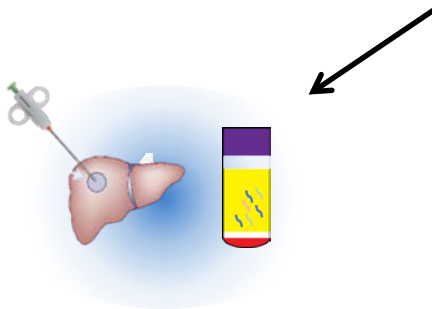
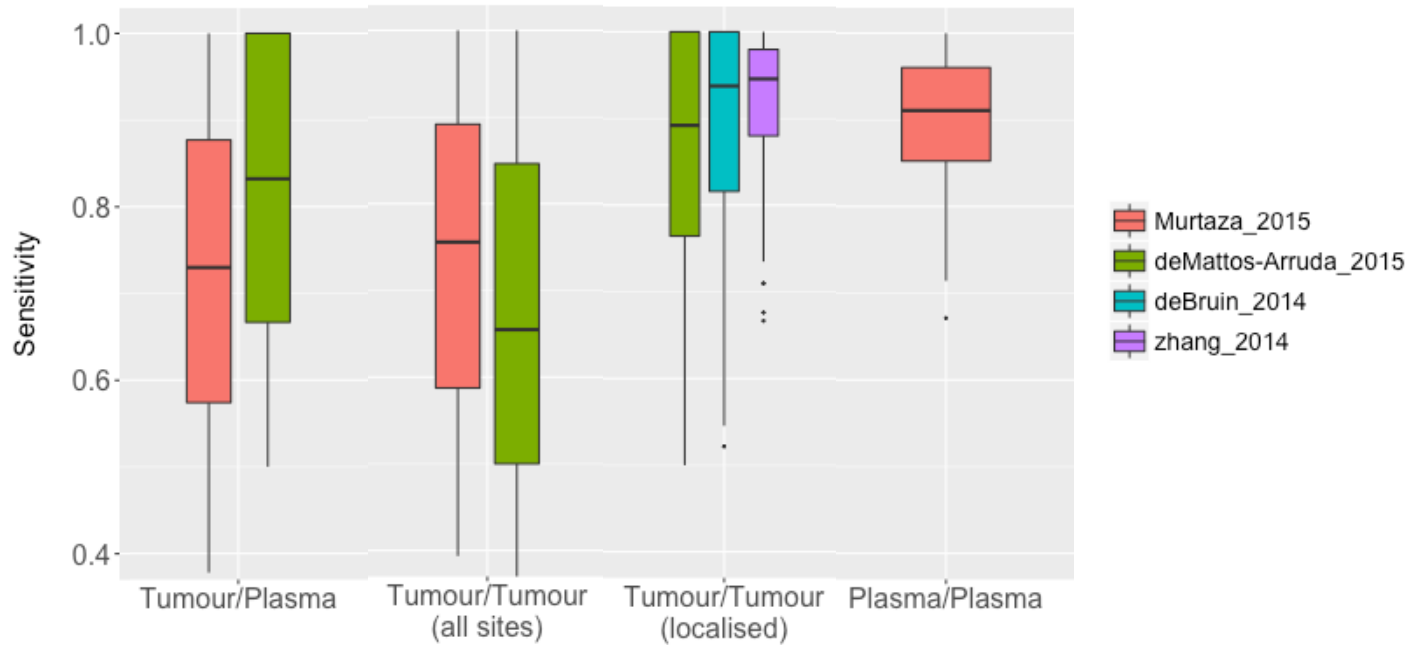
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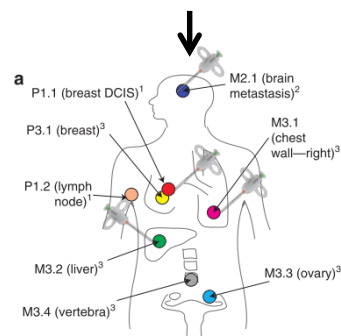
Murtaza,
Dawson,
Caldas et al.
(Nat Comm. 2015)

Tumour heterogeneity may confound measurements of ctDNA sensitivity

Pairwise comparison of sensitivity for all mutations



'ctDNA sensitivity'



Taken from Murtaza, Dawson et al. 2015

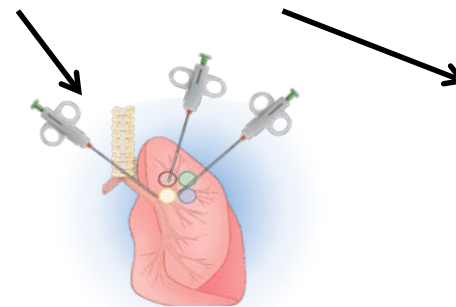
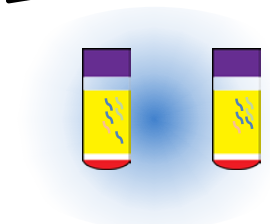
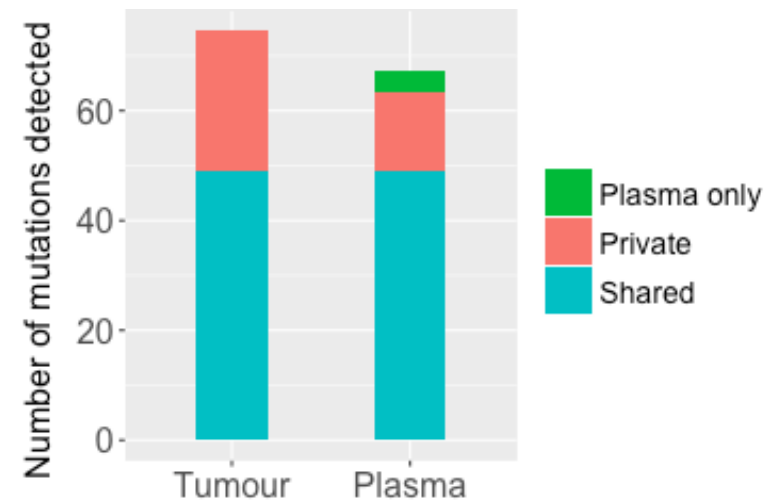
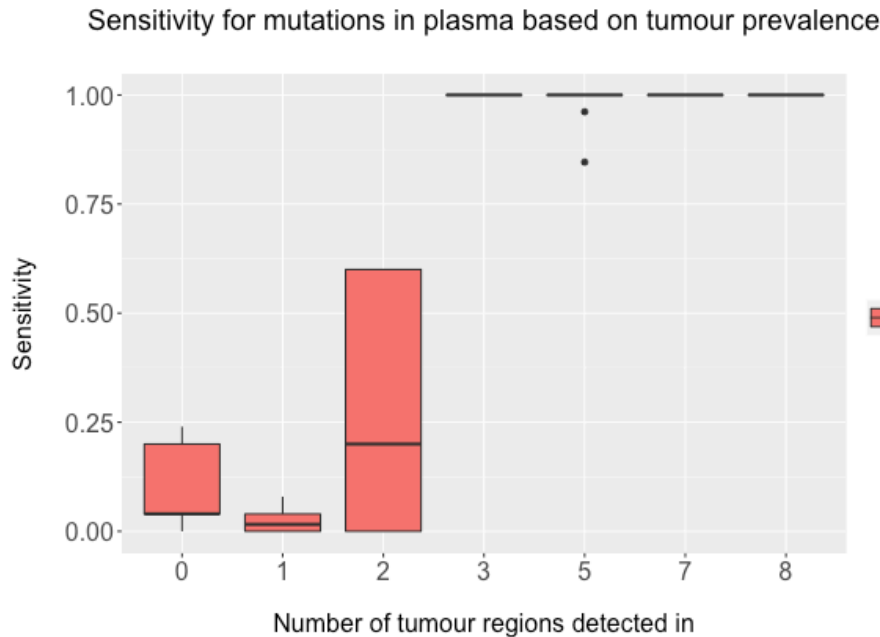


Figure adapted from Jamal-Hanjani et al. 2015



Longitudinal plasma samples

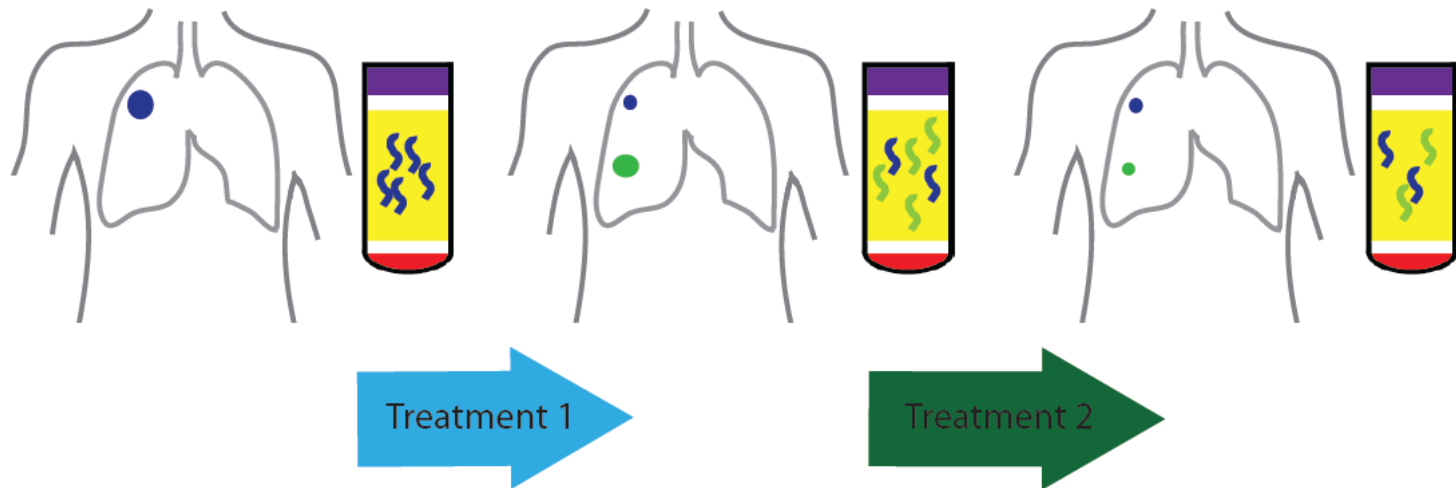
Plasma analysis tends to detect mutations shared between multiple tumour regions



Murtaza, Dawson et al.
2015

- Matched plasma analysis may aid interpretation of tumour mutation profiles from metastatic biopsies

**Stratify → profile → monitor → identify emerging resistance:
adaptive therapy targeting the most prominent clone(s) in real time**



What should we expect from ctDNA analysis going forward?

- Sensitive, quantitative, highly multiplexed
- Widely applied to a diverse range of applications in oncology
- Redefining the gold standard for oligo-metastatic disease?

Thanks to:



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Rosenfeld lab and alumni, CRUK-CI



• Charlie Massie • Davina Gale • James Morris
• Dineika Chandrananda • Florent Mouliere •
• Chris Smith • Keval Patel • Jonathan Wan

Alumni: • Tim Forshew • Muhammed Murtaza •
Dana Tsui • Suzanne Murphy • Francesco Marass

Inivata Ltd.: • Michael Stocum • Clive Morris
• James Clark • John Beeler • Amanda Bettison
• Davina Gale • Tim Forshew • James Brenton
• Vincent Plagnol • Emma Green • Greg Jones
• Andrew Lawson • Sarah Smalley • et al.

James Brenton's & Carlos Caldas' labs, CRUK-CI, University of Cambridge

Tan Min Chin, University Hospital Singapore • Benjamin Besse & team, IGR, France

Pippa Corrie, Christine Parkinson, Addenbrooke's Hospital, Cambridge

Ultan McDermott, Sanger Inst. • Lewis Family Charitable Trust • Cambridge CCTC

CRUK-CI Core Facilities: Genomics • Bioinformatics • Biorepository • Cambridge BRC, ECMC
Addenbrooke's Hospital, Cambridge: Research nurses & teams • Generous patients