Established and Novel ctDNA Methodologies

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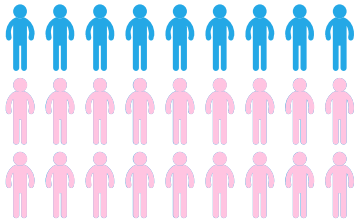
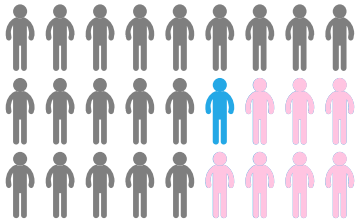
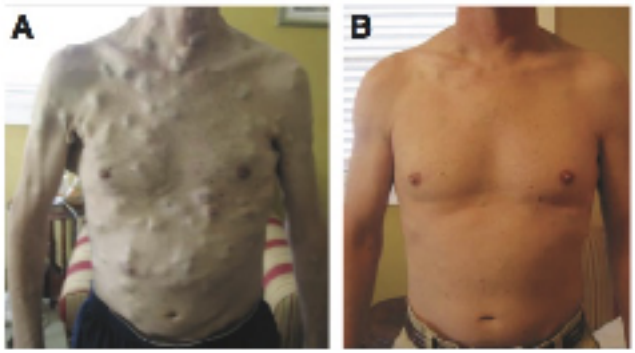
Disclaimer

- No disclosures

Evolution of precision medicine

	Chemotherapies	Targeted therapies
		
Target population	Large: unspecified	Medium: sub-group
Diagnostics	Histology, no specific biomarkers	Specific driver mutation e.g. *EGFR* or *BRAF*
Treatment	One treatment fits all, mixed responses	Targeted agents (e.g. BRAFi), improved response rates



Photographs were taken (A) before initiation of PLX4032, (B) after 15 weeks of therapy with PLX4032

Wagle N et al. J Clin Oncol 29:3085-3096 (2011).

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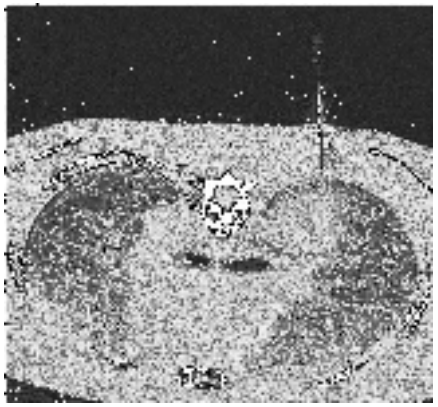
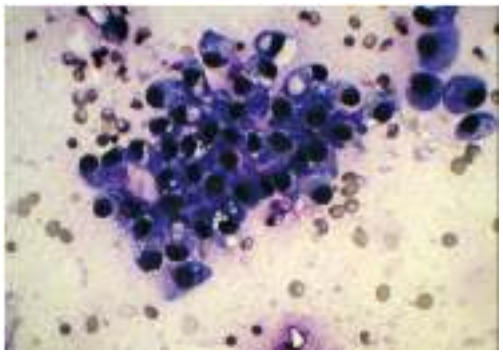
Conventional Tumour Biopsies

Tumour biopsy is current gold-standard for molecular analysis, but:

- Invasive medical procedure which can be difficult and expensive
- Can be obtained long time prior to analysis (at initial resection)





- Often limited amounts of material is obtained
- Which lesion to analyse in metastatic disease?
- Collection of serial biopsies for analysis are extremely rare

Liquid biopsies can add value …

when **monitoring** of disease progression / recurrence is essential

when tracking on-treatment **clonal evolution** of the tumour is needed

when tissue samples are **insufficient**, inadequate or exhausted

when solid biopsy is **difficult** or poses a high risk

due to lower cost, ease of collection

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Sources of genetic material in liquid biopsies

Circulating tumour cells (CTCs) shed from primary or metastatic foci

- Very rare: ~1-5 cells per millilitre of blood
- Scarcity currently limits analysis and clinical utility
- Expensive to detect

Exosomes and other extracellular vesicles

- Abundant: 10^{10} vesicles per millilitre of blood
- Source of lipids, proteins, RNA species and to some extent DNA
- Difficult to isolate and analyse, not proven clinically

Double-stranded **cell-free DNA (cfDNA)** released from all cells

- Higher cfDNA amounts in cancer patients
- Tumour-derived circulating DNA (ctDNA) is released following apoptotic and necrotic cell death
- Relatively simple to isolate and analyse

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Circulating Tumour DNA (ctDNA)

circulating tumour DNA (ctDNA) released into circulation by apoptotic and necrotic death of tumour cells

Advantages:

- Relatively simple to collect, isolate and analyse
- Provides real-time analysis of tumour (half-life <2hr)

- ctDNA generated from all disease sites, entire picture of disease

Disadvantages:

- Low concentration: ~5ng/mL plasma
- Highly fragmented (~170 bp)
- Background of 'normal' cfDNA dilutes out the tumour fraction of interest

Genetic information available from ctDNA

Somatic mutations:

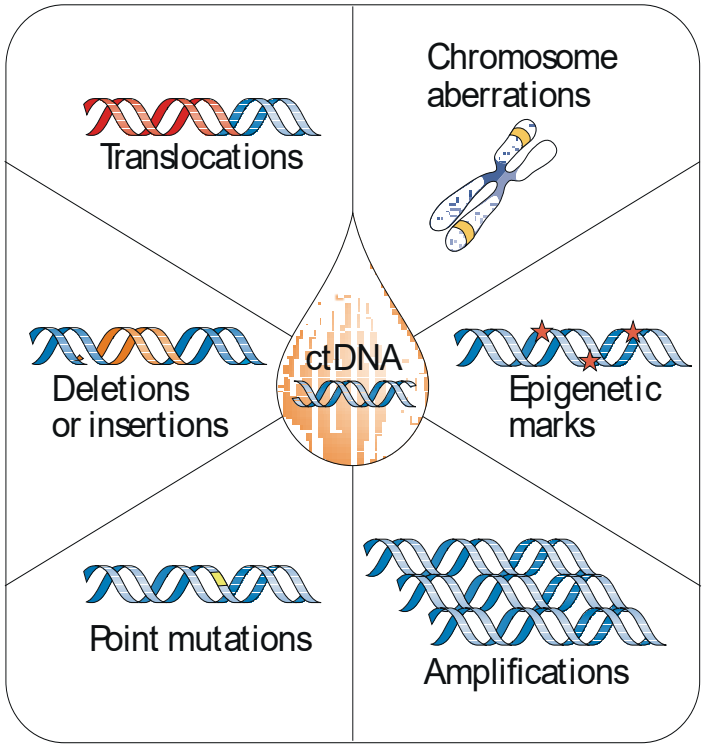
- Point mutations
- Insertion/deletions

Chromosomal aberrations:

- Amplifications/deletions
- Translocations

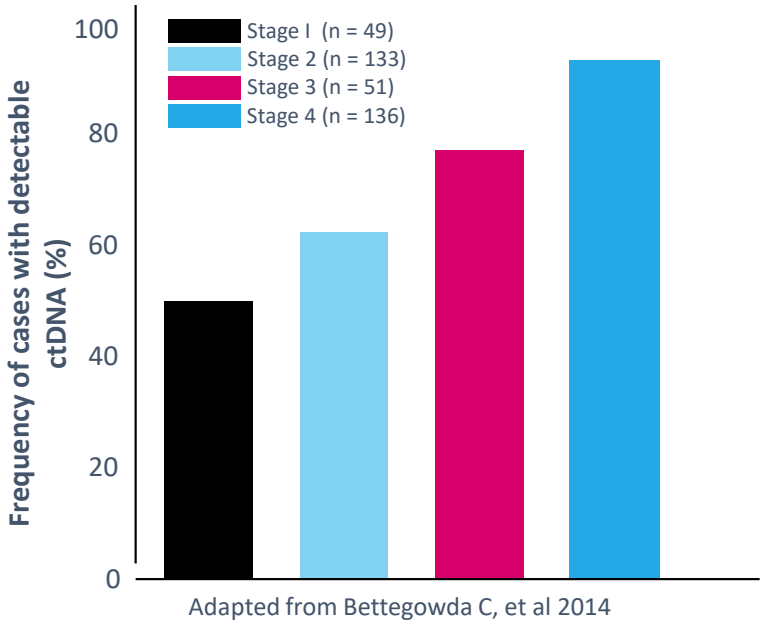
Epigenetic modifications:

- Hyper/hypo-methylation



Detection of ctDNA requires highly sensitive techniques

ctDNA increases with disease progression



Adapted from Bettegowda C, et al 2014

The amount of ctDNA released into the blood is dependent on several factors:

- **Tumour grade and histology**
- **Tumour vascularity**
- **Tumour proliferation and cell death rates**
- **Time of blood draw and therapy**

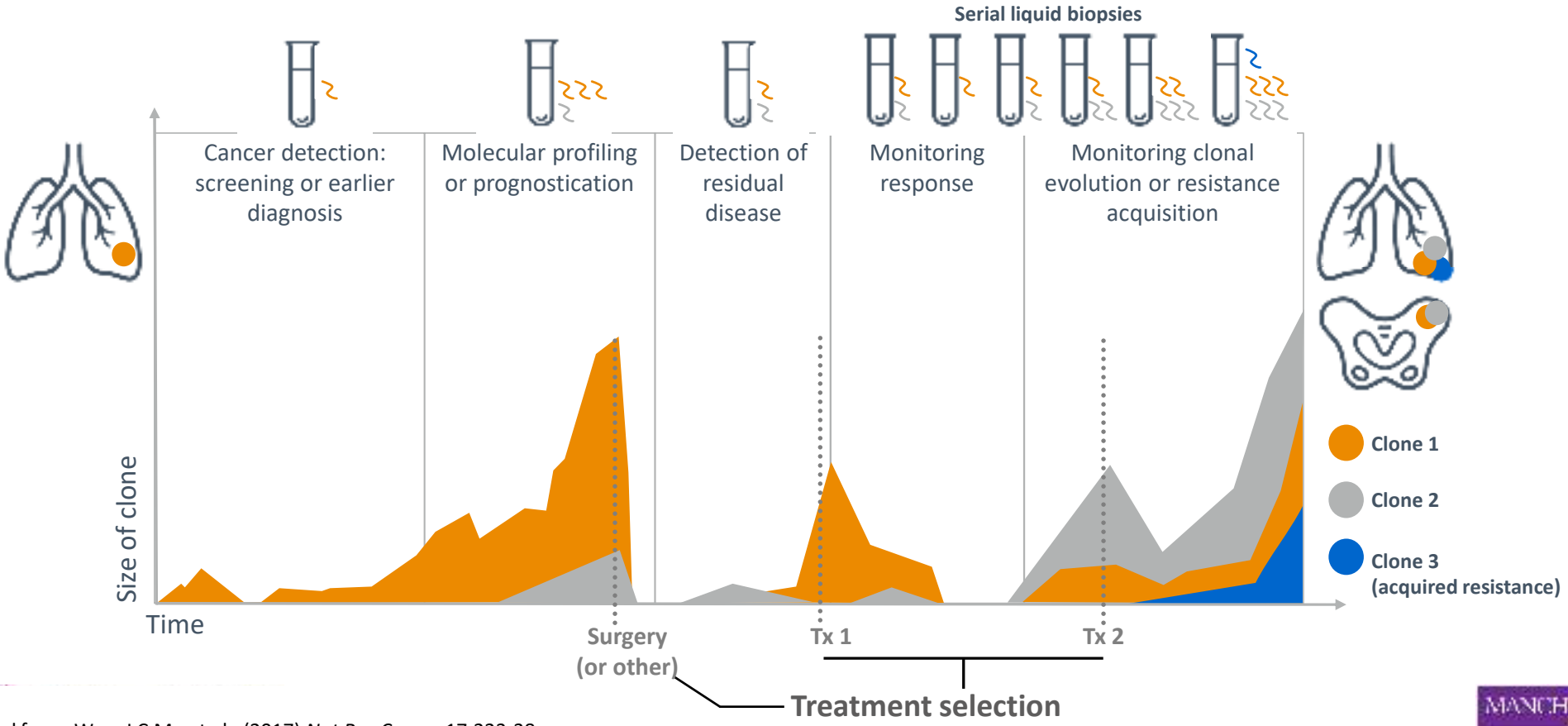
How reliable is Comprehensive Genomic Profiling (CGP) in liquid vs tissue?

Clinical utility of comprehensive cell-free DNA (cfDNA) analysis to identify genomic biomarkers in newly diagnosed metastatic non-small cell lung cancer (mNSCLC). *Leighl N.B. et al. AACR 2019.*

- 282 patients prospectively enrolled from 28 N American centers for standard tissue profiling and CGP in liquid (Guardant assay)
- cfDNA: 95% (268/282) completely genotyped
- Concordance of tissue and liquid was >98.2% for genes with FDA – approved targeted therapy (EGFR, ALK, ROS1, BRAF)
- Liquid results were returned faster than tissue results
- (median 9 vs 15 days; p<0.0001)

Liquid biopsies can be used to monitor disease progression

Time course of a hypothetical patient



Adapted from: Wan, J.C.M., et al., (2017) *Nat Rev Cancer* 17:223-38.

Liquid Biopsies in the Lung Cancer Clinic Today

- Molecular Diagnostic Test performed on circulating tumour DNA (ctDNA) detectable in cell free DNA (cfDNA) present in plasma from a single blood draw
- **2016:** FDA approved the 1st liquid biopsy test for detection of EGFR mutation in ctDNA (COBAS real-time PCR assay)
- **2020:** FDA approved the 1st liquid biopsy tests of comprehensive genomic profiling (CGP) - 'one stop shop' for multiple cancer related gene mutations, translocations, copy number changes
- Multiple laboratory developed / non-commercial assays being evaluated

cfDNA-based molecular analysis: Available approaches

Real-time/droplet digital Polymerase Chain Reaction (ddPCR)

- PCR-based platform allows for highly precise and sensitive detection of single known mutations in circulating tumour DNA
- Destructive test, limited information

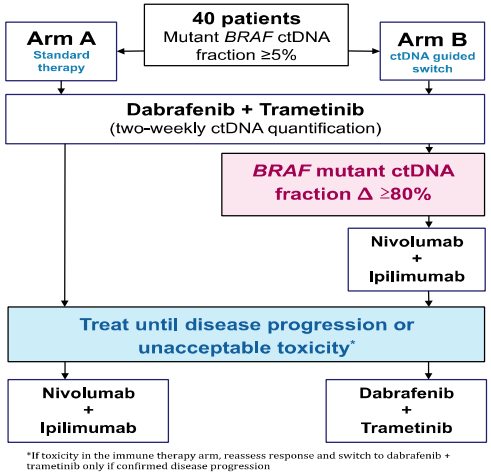
Targeted NGS (Next Generation Sequencing)

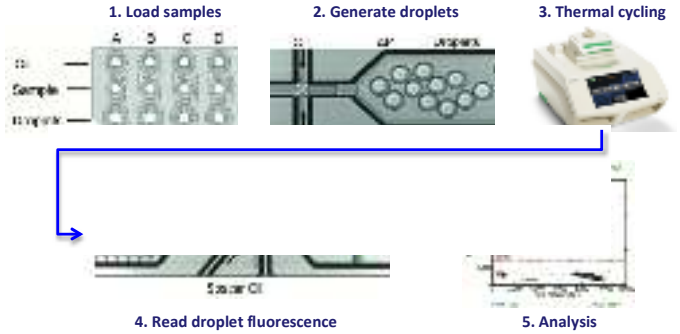
- **Comprehensive Genomic Profiling** identifies genomic alterations in many therapeutically relevant genes, allow clinicians to obtain information about genomic signatures, such as TMB and DDR
- Potential to re-analyse, highly informative

Rolfo et al., JTO 2021, 16(10).

ddPCR analysis of cfDNA (CAcTUS/DETECTION trials)

Circul**A**ting **T**umour DNA g**U**ided therapy **S**witch (CAcTUS)





ddPCR Assay	Control Cell Line / Mutation Status	A375 (BRAF V600E)	RM44 (BRAF V600K)	M381 (BRAF V600R)	RM59 (BRAF V600WT)
BRAF V600E	Determined %VAF	100.00	0.00	0.00	0.00
	SD %VAF	0.00	0.00	0.00	0.00
	Acceptance Range	>90%	<1%	<1%	<1%
	Outcome	**Pass**	**Pass**	**Pass**	**Pass**
BRAF V600K	Determined %VAF	0.00	78.77	0.00	0.00
	SD %VAF	0.00	0.54	0.00	0.00
	Acceptance Range	<1%	>75%, <85%	<1%	<1%
	Outcome	**Pass**	**Pass**	**Pass**	**Pass**
BRAF V600R	Determined %VAF	0.00	0.00	33.48	0.00
	SD %VAF	0.00	0.00	1.76	0.00
	Acceptance Range	<1%	<1%	>28%, <38%	<1%
	Outcome	**Pass**	**Pass**	**Pass**	**Pass**
BRAF V600plex (E + K +R)	Determined %VAF	100.00	78.39	32.15	0.00
	SD %VAF	0.00	0.85	1.78	0.00
	Acceptance Range	>90%	>75%, <85%	>28%, <38%	<2%
	Outcome	**Pass**	**Pass**	**Pass**	**Pass**

Hypothesis

- *In BRAF* mutant melanoma efficacy of immunotherapy is enhanced by **response** to pre-treatment with dabrafenib + trametinib
- Changes in ctDNA levels can be used to accurately inform when to switch from targeted to immunotherapy

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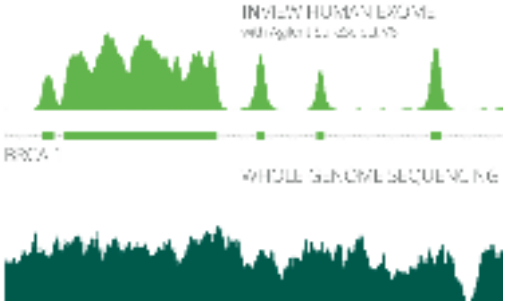
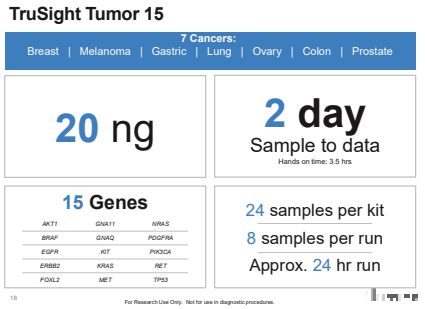
NGS Approaches – what do you need?





	WGS/WES	Targeted Panel	Focused Panel
Target Size	5-300x10^7	500kb-5x10^6	100bp-500kb
Samples per Run	<6	10-100	>100
Depth of Coverage	x30-40	x100-500	>x1000
Data Analysis time	12-48 hrs	<4 hrs	<1 hr

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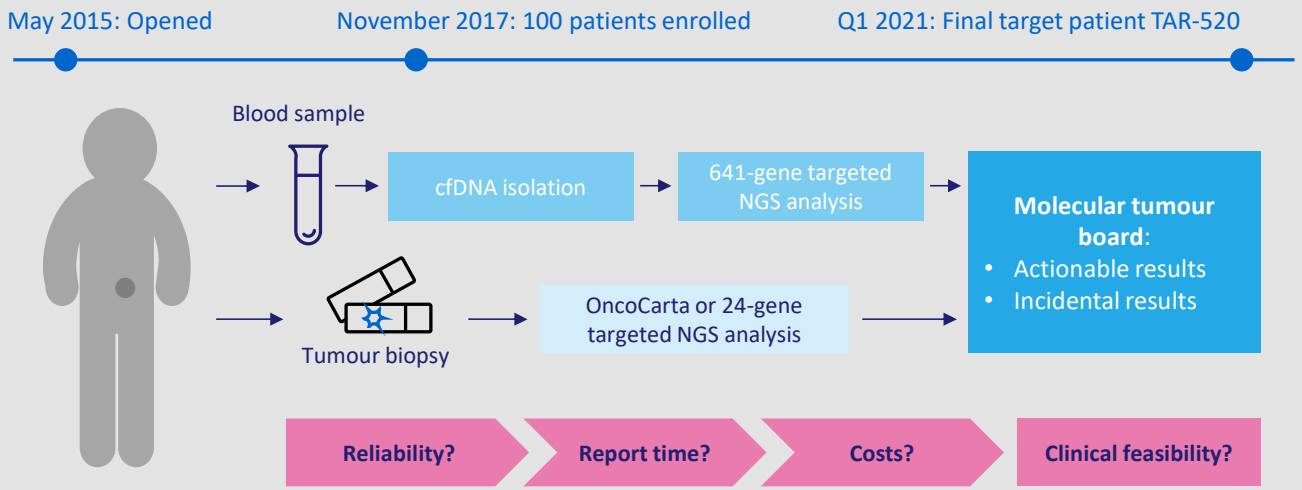
Clinical utility of ctDNA profiling: TARGET

TARGET: Tumour chARacterisation to Guide Experimental Targeted therapy

Develop a robust workflow supporting clinical decision-making that can be

- delivered on a **routine basis**
- with **data turnaround time** compatible with clinical practice
- at an **affordable cost**
- leads to **benefit** in a proportion of patients

Part A (100 patients): Establish analytical workflow and assess feasibility to support clinical decision-making



Part B (420 patients): Test clinical utility following selection of patients in real time to molecularly matched trials on the basis of their ctDNA and / or tumour genomic profile

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Clinical utility of ctDNA profiling: The TARGET trial

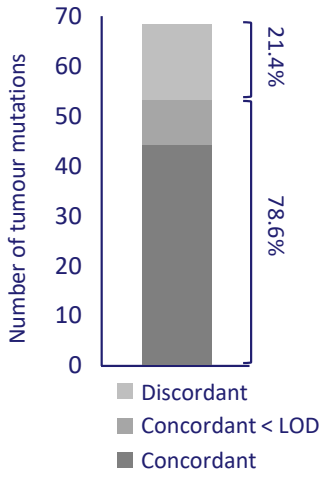
100
patients recruited

70
patients had ≥ 1 mutations (ctDNA)

54
patients had ≥ 1 mutations (tumour)

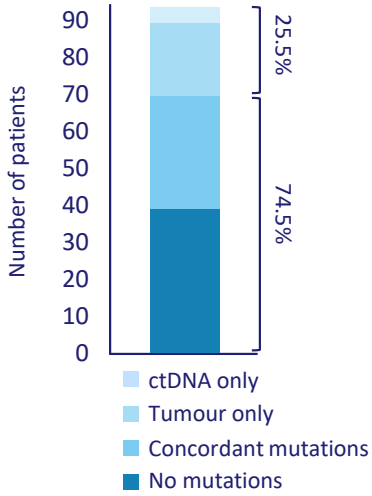
Concordance of detected mutations

78.3% (54/69) of **non-synonymous mutations** identified by tumour NGS were also identified by ctDNA NGS



Concordance within the patients

74.5% (70/94) of **patients** had mutational concordance between tissue and ctDNA



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Rothwell, D.G., et al. (2019) *Nat Med* 25:738-43.

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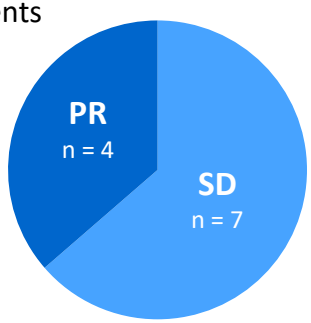
Clinical utility of ctDNA profiling: The TARGET trial

100
patients recruited

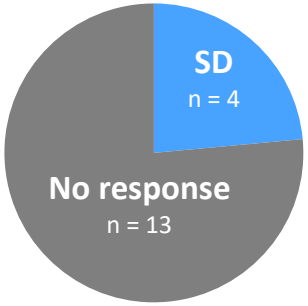
70
patients had ≥ 1 mutations (ctDNA)

41
patients had actionable mutations

Matched therapy
11 patients



Non-matched therapy
17 patients

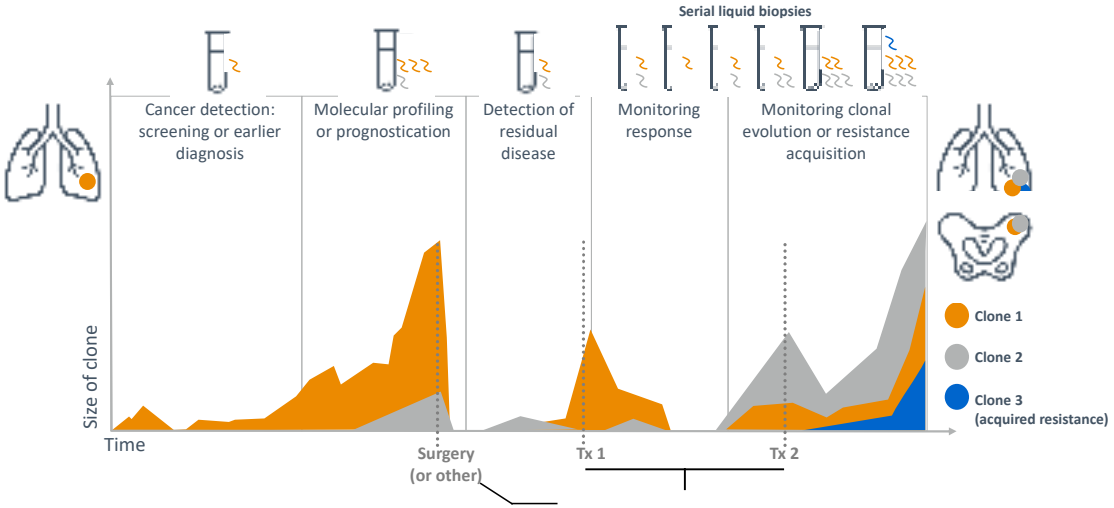


ORR	4/11	0/17
SD	7/11	4/17

Rothwell, D.G., et al. (2019) *Nat Med* 25:738-43.

Beyond molecular Diagnosis – detection of ctDNA after curative intent treatment

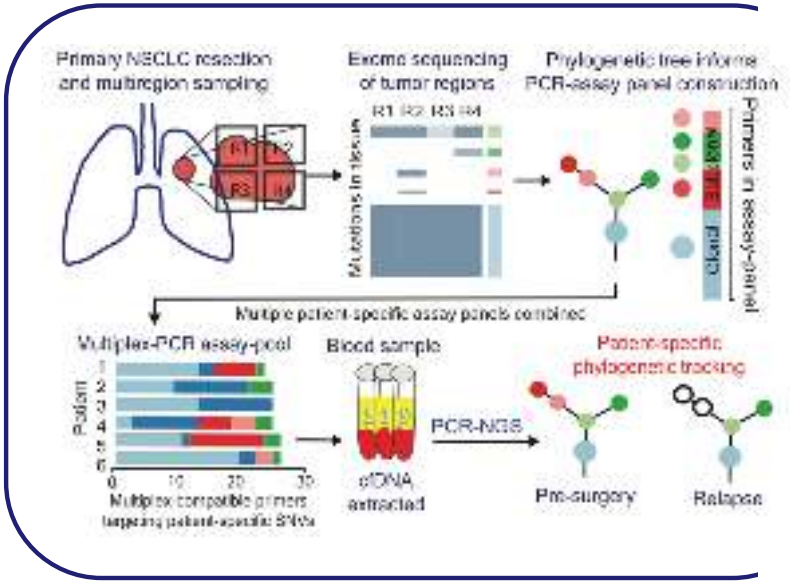
Time course of a hypothetical patient



- Numerous studies showing detection of ctDNA after treatment related to poor prognosis in patients
- ctDNA enables longitudinal analysis for MRD type studies

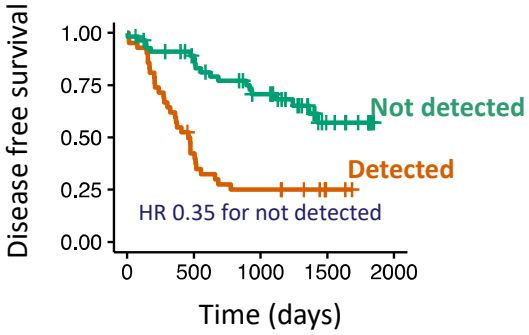
Pre-operative ctDNA - prognostic for LUAD & predicts utility for MRD monitoring

Tumour informed approach



Abbosh et al. *Nature* 2017

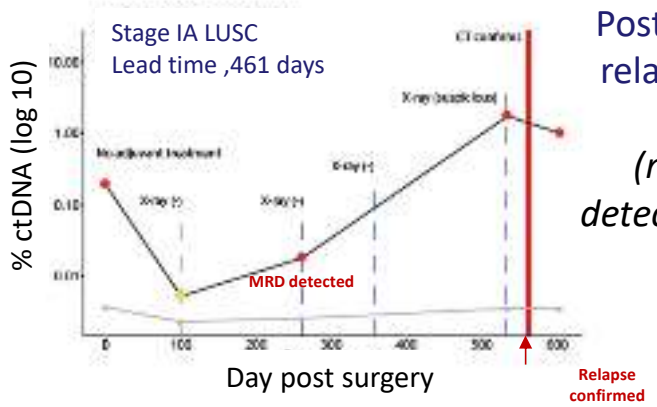
Pre-operative ctDNA detection



Patients with detected pre-op ctDNA have worse DFS independent of stage

Identifies subset of 'born to be bad' patients

Post-operative MRD monitoring



Post-op MRD monitoring predicts relapse prior to clinical detection

(median lead-time for ctDNA detection prior to relapse >70 days)

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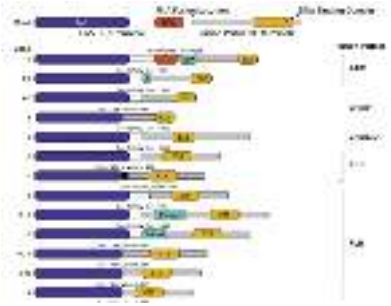
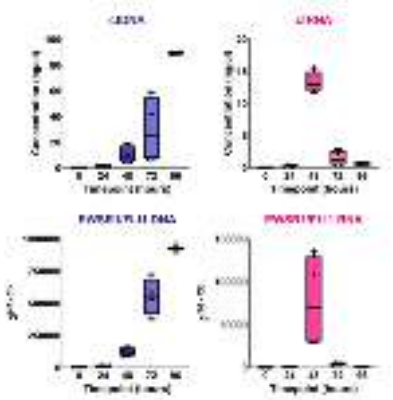
cfDNA-based molecular analysis: Novel approaches

Detection of cell-free RNA (cfRNA)

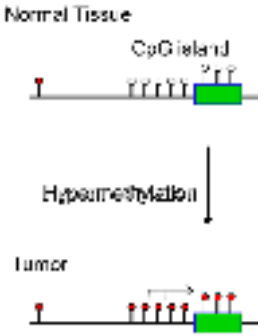
- Studies looking at presence of tumour specific RNA in plasma, potentially higher sensitivity and specificity
- Detection of cancer-specific RNA fusions in plasma of patients as sensitive marker of MRD



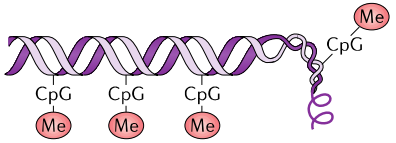


Methylation profiling of cfDNA

- Cancer-specific epigenetic changes used to detect circulating tumour DNA
- Allows low-cost and highly sensitive detection, classification and monitoring of cancer.



Adapted from Gazdar et al., Nat Rev Cancer 2017



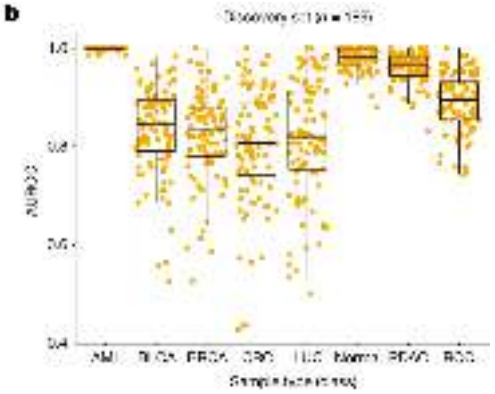
cfDNA methylation profiling for tumour detection

cfDNA methylation profiling shows clinical potential

- Changes in methylation profiles widely associated with cancer and thought to be an early event
- Aberrant DNA methylation a more broadly applicable marker of tumor DNA in blood than mutations
- Recent studies proved high sensitivity for early detection of various cancer types
- NHS piloting methylation based blood test that detects more than 50 cancers (*Garelli* blood test, GRAIL, 16% Stage I cancers)

Sensitive tumour detection and classification using plasma cell–free DNA methylomes

Shu Yi Shen[1,2], Rajat Singhania[1,2], Gordon Fehringer[1,2], Ankur Chakravarthy[1,2], Michael H. A. Roehrl[3,4], Dianne Chadwick[1], Philip C. Zuzarte[1], Avelet Borgida[1], Ting Ting Wang[1,4], Tiantian Li[1], Olena Kis[1], Zhen Zhao[1], Anna Spreafico[1], Tiago da Silva Medina[1], Yadon Wang[1], David Roulois[5,6], Ilias Ettayebi[5,6], Zhuo Chen[1], Signy Chow[1], Tracy Murphy[1], Andrea Arruda[1], Grainne M. O'Kane[1], Jessica Liu[4], Mark Mansour[4], John D. McPherson[7], Catherine O'Brien[1], Natasha Leighl[1], Phillippe L. Bedard[1], Neil Fleshner[1], Geoffrey Liu[1,4,8], Mark D. Minden[1], Steven Gallinger[9,10], Anna Goldenberg[11], Trevor J. Pugh[1,4], Michael M. Hoffman[1,4,11], Scott V. Bratman[1,4], Rayjean J. Hung[2,6] & Daniel D. De Carvalho[1,2]✉



ORIGINAL ARTICLE

Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA

M. C. Liu[1,!], G. R. Oxnard[2,!], E. A. Klein[3], C. Swanton[4,5], M. V. Seiden[6*] & on behalf of the CCGA Consortium[!]

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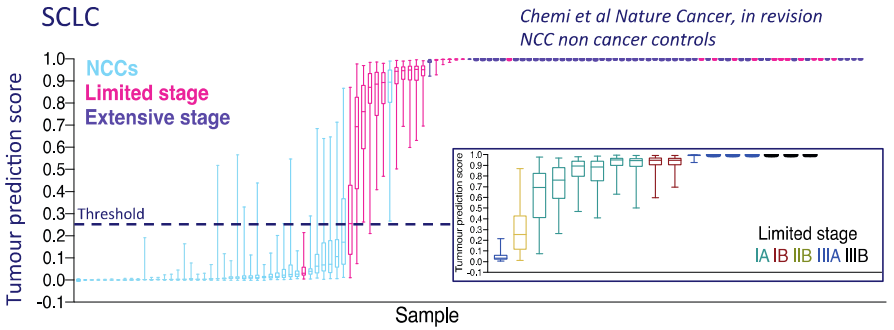
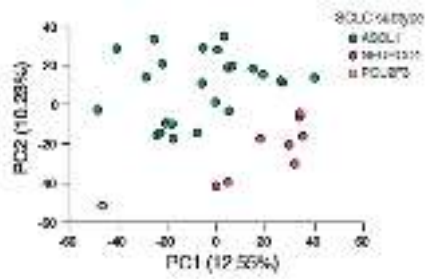
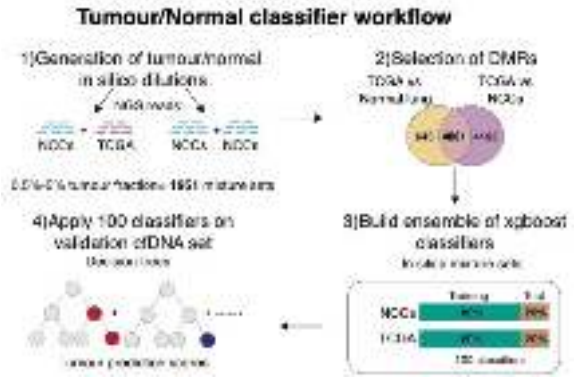
Lung cancer-specific methylation profiles in ctDNA

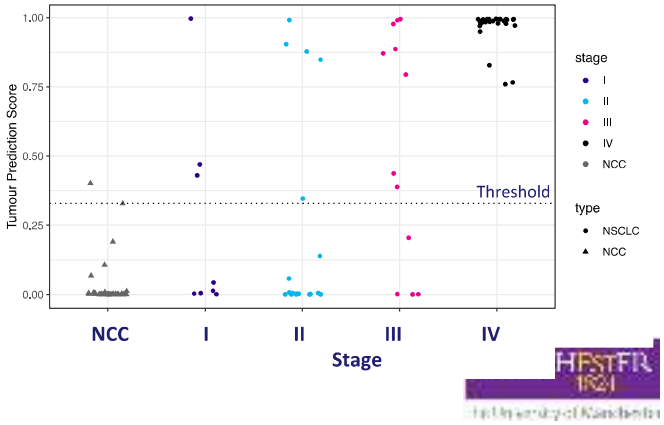
- T7-MBD-Seq assay for sensitive detection of cancer-specific global methylation profiles with low input cfDNA
- Detects stage IA SCLCs, SCLC subtypes and identifies CUPs





- NSCLC-specific classifiers under optimization, detect stage I-IV NSCLCs
- Targeted and fragmentomics workflow ongoing to improve sensitivity & specificity





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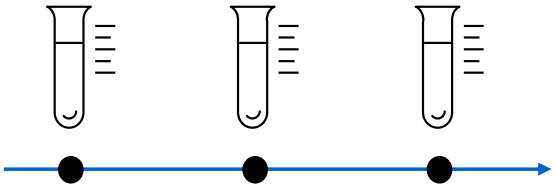
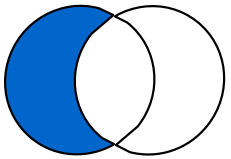
Conclusions: ctDNA in the clinic



ctDNA can simplify procedures for obtaining a sample, making a repeat biopsy feasible and allowing sensitive, real-time monitoring of tumour genomics



ctDNA genomic profiling provides a representative picture of the tumour genome and is complementary to tissue analysis

There is mounting evidence that encourages routine implementation of ctDNA testing as an adjunct to tumour testing

Acknowledgements

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Dan Slane-Tan
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Lynsey Priest

Histology Core Facility

Garry Ashton
David Millard

Patients and Families

National Institutes of Health
R01CA197936-05


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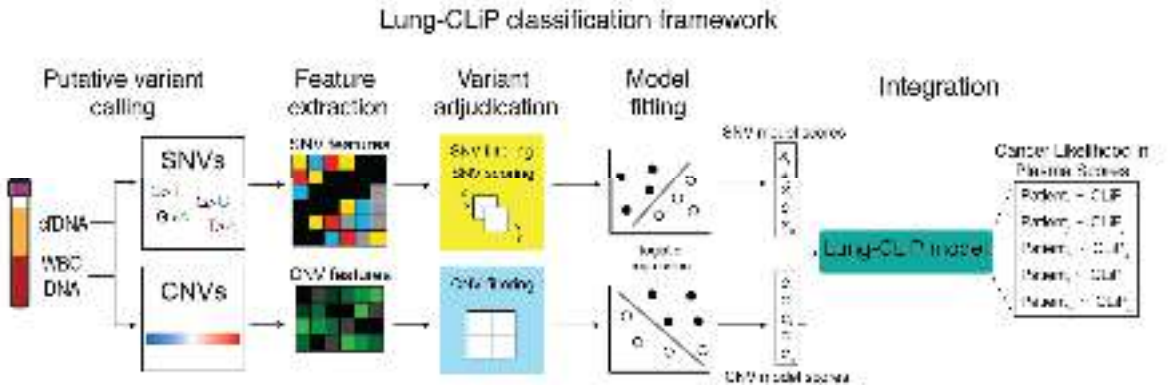
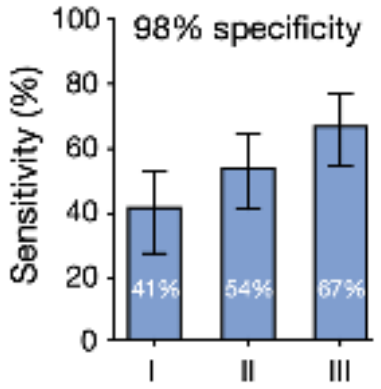
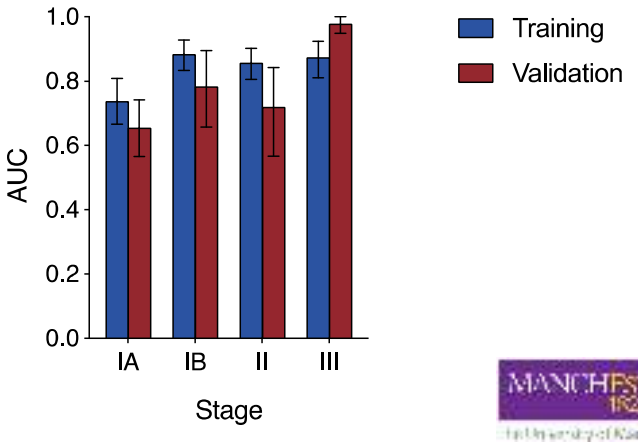
ctDNA in Early Detection: Lung cancer likelihood in plasma assay (Lung-CLiP)

- A somatic alteration-based cfDNA lung cancer early detection assay
- Combines sequencing of cfDNA and WBCs with multilayer machine learning framework
- Takes into account issue of CHiP variants present in cfDNA
- Validated in an independent, prospectively collected cohort
- Sensitivity for stage I NSCLC ~40% at 98% specificity

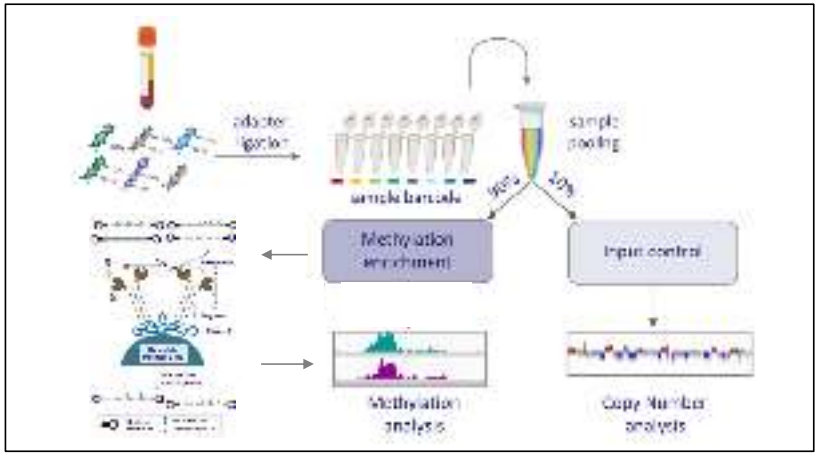
Chabon et al. Nature 2020







Clinical utility of cfDNA Methylation profiling



Technical development

- Pool multiple samples using unique sample barcode with NGS approach
- Pooling overcomes problem of limited DNA input from cfDNA

Bioinformatic development

- Whole genome NGS analysis to determine enriched DNA regions
- Identify tissue specific and cancer specific methylation changes (DMR)