The University of Manchester
Manchester Cancer Research Centre

MANCHESTER 1824

Precision Medicine: Panacea or False Dawn?

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ESMO
Monday 29th September 2014

AstraZeneca

Conflicts of Interest

The University of Manchester
Manchester Cancer Research Centre

MANCHESTER 1824

- Employment: Chair in Translational Medicine

AstraZeneca

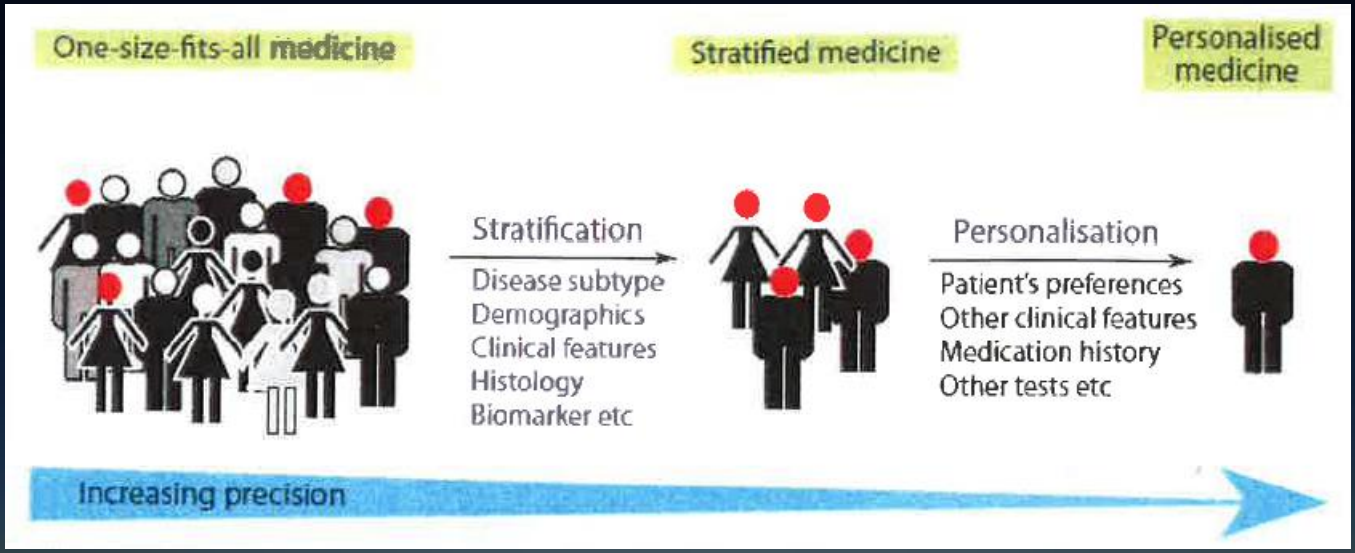
- Employment: VP Early Clinical Development
- Shareholder

CANCER RESEARCH UK

- Panel Member BMERP: Non-pecuniary

epistem

- Scientific advisor: Pecuniary



Source: Genomic Medicine 101 Keynotes and concepts, Centre for Evidence-Based Pharmacotherapy, 2014

Challenges to the dawn

1. "Precision Medicine" can be less efficient than "unselected"
2. The requirement for a contemporaneous molecular profile
3. Single molecular aberration trials can be *very* inefficient

1. Precision Medicine can be less efficient than “unselected”

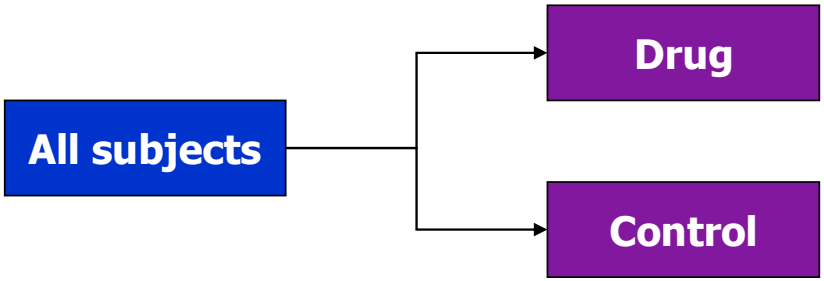
Assume you had a drug

•which doubled the time to progression (HR=0.5) in biomarker +ve subjects

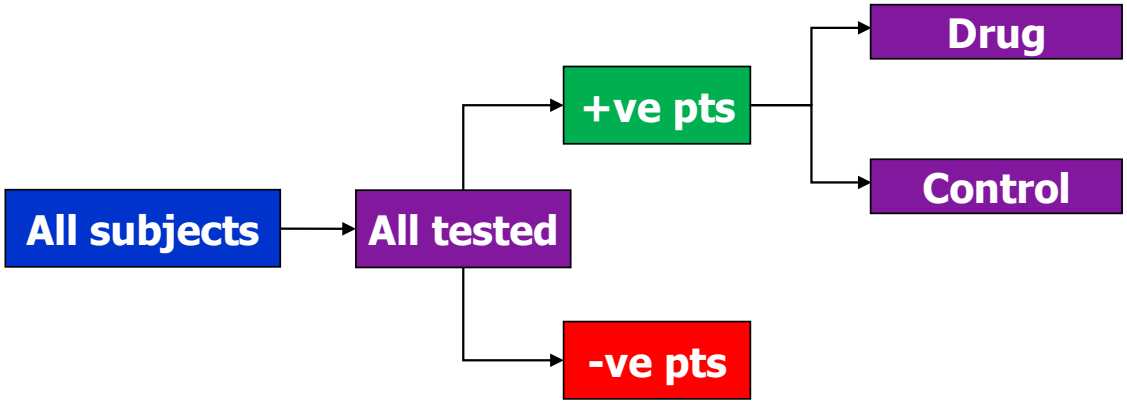
•had no effect in biomarker –ve subjects

•biomarker +ve subjects comprise 25% of the population

1. Unselected Design



2. Prospective selection (Precision Medicine)



In this scenario, a Precision Medicine approach is a more efficient development route than an unselected approach

	Control	Active	Effect (HR)
Biomarker +ve (25%)	6 mo	12 mo	0.50
Biomarker –ve (75%)	6 mo	6 mo	1.00
[illegible]			
Efficiency over unselected	**8.6 fold**		**2.1 fold**

But this assumes we have

-a perfect selection test (100% sensitive; 100% specific)

-there is no efficacy in the biomarker –ve population

What happens when this is not the case?

[1]median follow-up of 18 months assumed and no screen failures

An imperfect selection/stratification test lessens the efficiency of a Precision Medicine trial

Sens, Spec	PPV	Control	Active	Effect size	N req'd to enter	N req'd to screen
100%,100%	100%	6 mo	12 mo	0.50	117	468
95%, 75%	56%	6 mo	9.4 mo	0.64	260	613
75%, 95%	83%	6 mo	11 mo	0.55	149	663
75%, 75%	50%	6 mo	9 mo	0.68	317	845

NB : An Unselected trial required 1000 patients to be screened and entered

Even a small (one third*) effect in biomarker –ve patients erodes the apparent advantage of a targeted trial

	Control	Active	Effect (HR)
Biomarker +ve (25%)	6 mo	12 mo	0.50
Biomarker –ve (75%)	6 mo	7.5 mo*	0.80*
All patients	6 mo	8.7 mo	0.69

	Number required to enter	Number required to screen
All patients	384	
+ve (25%)	117	468
Efficiency over unselected	**3.3 fold**	**0.8 fold**

* Effect in –ve pts = 1/3 effect in +ve patients

Conclusion:

For Precision Medicine to be a more efficient drug development strategy over an unselected approach we would need to be very confident that (i) we had a very good stratification test and (ii) the untargeted population achieved minimal benefit from treatment

Understanding these two variables is a key deliverable of the pre-registrational clinical programme

2. The requirement for a contemporaneous molecular profile

nature medicine

An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage

Aaron M Newman[1,2,7], Scott V Bratman[1,3,7], Jacqueline To[3], Jacob F Wynne[3], Neville C W Eclov[3], Leslie A Modlin[3], Chih Long Liu[1,2], Joel W Neal[2], Heather A Wakelee[2], Robert E Merritt[4], Joseph B Shrager[4], Billy W Loo Jr[3], Ash A Alizadeh[1,2,5] & Maximilian Diehn[1,3,6]

LETTER

doi:10.1038/nature12065

Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA

Muhammed Murtaza[1]*, Sarah-Jane Dawson[1,2]*, Dana W. Y. Tsui[1]*, Davina Gale[1], Tim Forshew[1], Anna M. Piskorz[1], Christine Parkinson[1,2], Suet-Feung Chin[1], Zoya Kingsbury[3], Alvin S. C. Wong[4], Francesco Marass[1], Sean Humphray[3], James Hadfield[1], David Bentley[3], Tan Min Chin[4,5], James D. Brenton[1,2,6], Carlos Caldas[1,2,6] & Nitzan Rosenfeld[1]

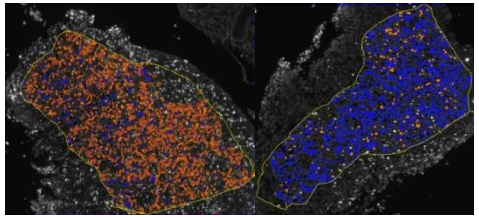
Bowman *et al. BMC Genomics* 2013, **14**:466
http://www.biomedcentral.com/1471-2164/14/466

BMC Genomics

METHODOLOGY ARTICLE Open Access

Multiplexed Illumina sequencing libraries from picogram quantities of DNA

Sarah K Bowman[1*], Matthew D Simon[1,4], Aimee M Deaton[1], Michael Tolstorukov[2,3,5], Mark L Borowsky[1,6] and Robert E Kingston[1]



With the advent of ultrasensitive genomic methods

Comes the Advantage of Repeat "Biopsy"

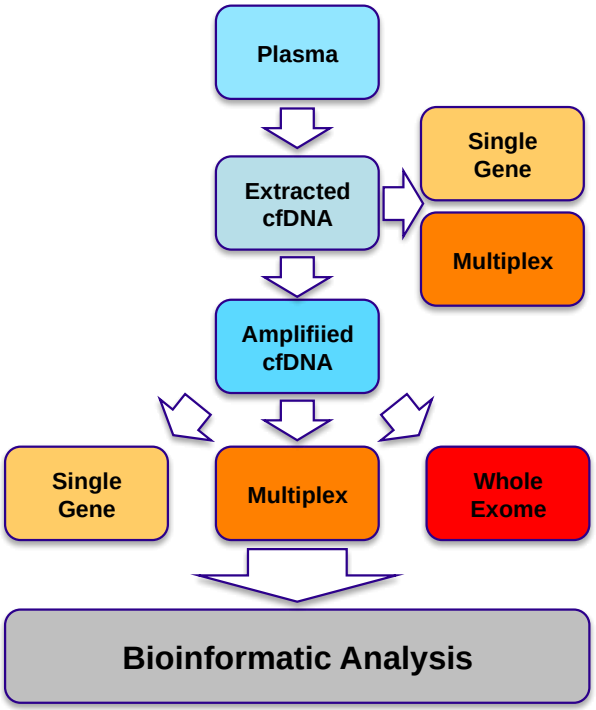
But the insight/challenge that repeat sampling show tumour molecular phenotype is dynamic

And the tumour heterogeneous

Circulating nucleic acid biomarkers: tumour, **cfDNA**, CTC, miRNA

cfDNA: Current Method Development in MCRC

- **Simple, robust, specific and sensitive**
- **Automatable**
- **Transferable to diagnostic laboratory**



(i). Can we work with preserved whole blood 96 hrs post draw?

- Avoids on site plasma preparation and variability
- Simple blood draw only

(ii). Can we analyse CTCs as well as cfDNA in the same sample?

- cfDNA from CellSave plasma - combine with GCLP CTC analysis
- Compare to Streck Cell-Free DNA BCT® -

(iii). Can we establish routine sensitive genome wide NGS from cfDNA

- Since levels of cfDNA are low – often at the level of 3 ng (~1000 genomes) need maximum capture efficiency to be representative
- Compare commercial kits and “home-grown”

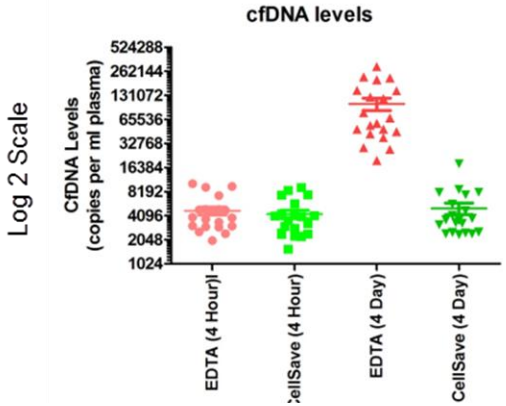
Courtesy Ged Brady, Dominic Rothwell, Caroline Dive

(i) Can we work with preserved whole blood 96h post draw?

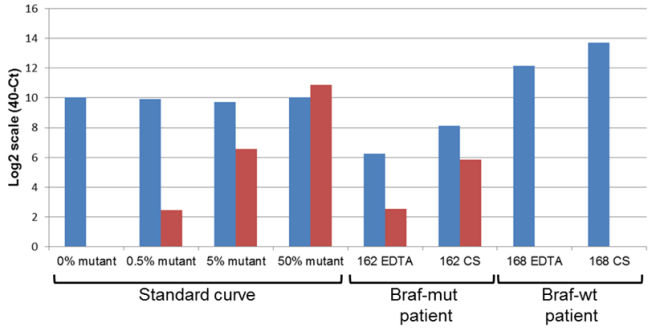
EDTA plasma samples require processing within 4 hrs of collection.
Can cfDNA be preserved in CellSave tubes?

- Maintenance of consistent cfDNA levels up to 96 hrs post-draw in CellSave tubes **(A)**
- Mutational status consistent in both EDTA and CellSave via real-time PCR analysis **(B)**
- Targeted NGS of EDTA and CellSave cfDNA showed good correlation in 4 SCLC patient samples **(C)**

(A)



(B)



(C)

SCLC Pts	Sample type	cfDNA Input (ng/tube)	TP53 Pos 7578212 G>A stopgain COSM99618			TP53 Pos 7577022 C>T stopgain COSM99947			TP53 Pos 7579312 G>T synonymous COSM45940		
			MUTATION DETECTED	Total coverage	% ALT	MUTATION DETECTED	Total coverage	% ALT	MUTATION DETECTED	Total coverage	% ALT
12068	WT gDNA	14.00	0	370.00	0.00	0	1038.00	0.00	0	4041.00	0.00
	EDTA cfDNA	1.35	0	309.00	0.00	0	854.00	0.35	0	1491.00	0.00
	CellSave cfDNA	2.30	0	430.00	0.70	0	1093.00	0.00	0	1733.00	0.06
12071	WT gDNA	20.00	0	580.00	0.34	0	1540.00	0.26	0	4224.00	0.05
	EDTA cfDNA	10.35	1	283.00	47.70	0	1300.00	0.15	0	1537.00	0.00
	CellSave cfDNA	14.65	1	358.00	47.77	0	1788.00	0.11	0	1803.00	0.06
12088	WT gDNA	20.00	0	533.00	0.19	0	1046.00	0.00	0	2699.00	0.04
	EDTA cfDNA	22.50	0	250.00	0.00	1	1122.00	66.31	0	1625.00	0.12
	CellSave cfDNA	19.45	0	549.00	0.36	1	1930.00	66.48	0	2569.00	0.04
12090	WT gDNA	20.00	0	289.00	0.00	0	748.00	0.00	0	2841.00	0.04
	EDTA cfDNA	1.50	0	205.00	0.00	0	497.00	0.00	1	708.00	36.16
	CellSave cfDNA	0.75	0	350.00	0.00	0	532.00	0.38	0	770.00	0.26

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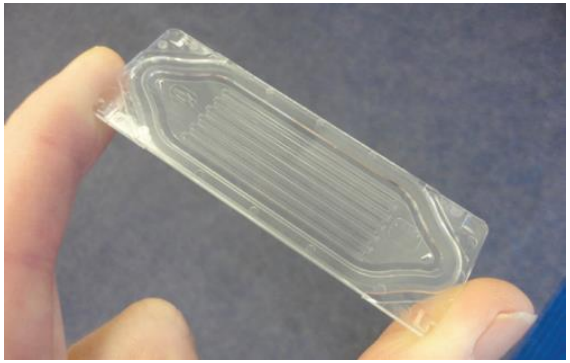
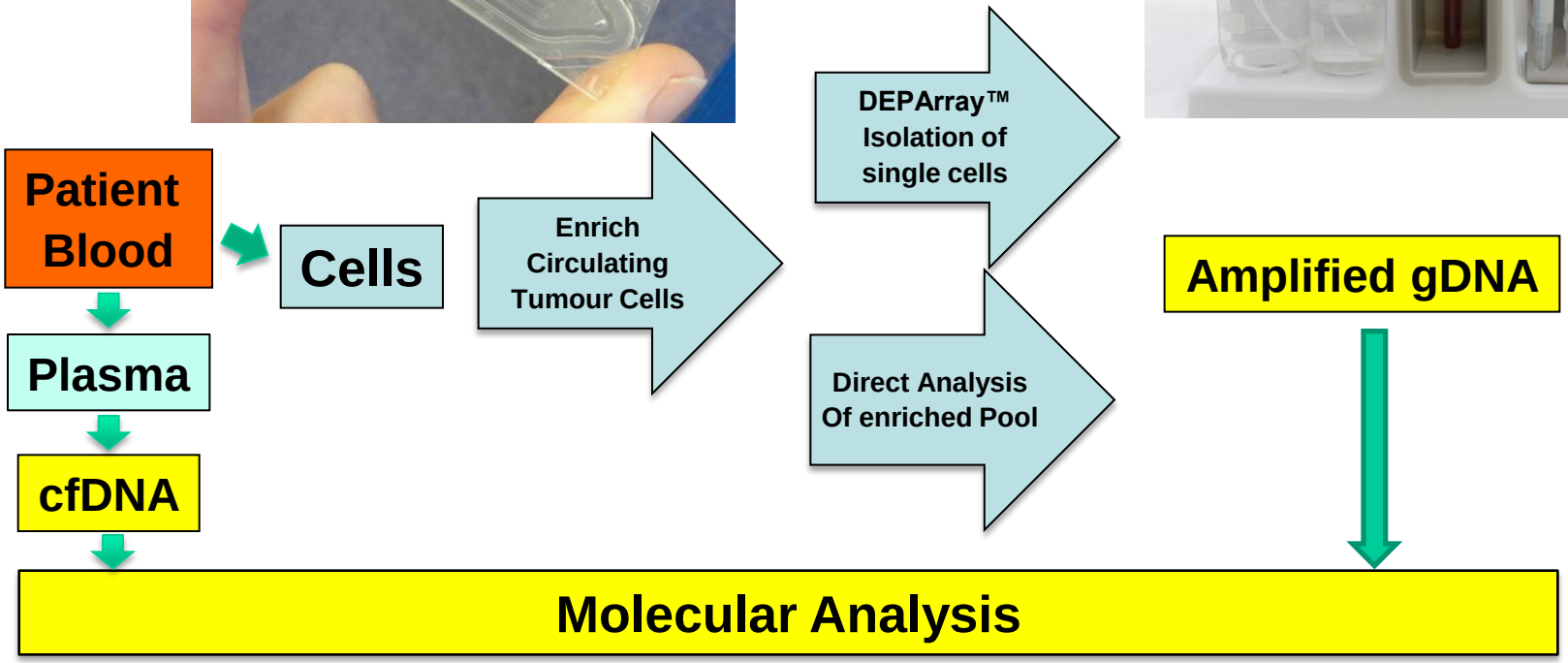
(ii) Can we analyse CTCs as well as cfDNA in the same sample?: PARSORTIX

- Chip utilising cell size and deformability – **epitope independent**
- Compatible with blood preservatives – CellSave, Streck Cell-Free DNA BCT®
- Plasma and cells obtained from a single blood sample
- Captured cells can be fixed and stained in the cassette
- Cells can be recovered for external staining and/ or genetic analysis





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(iii) Can we establish routine sensitive genome wide NGS from cfDNA

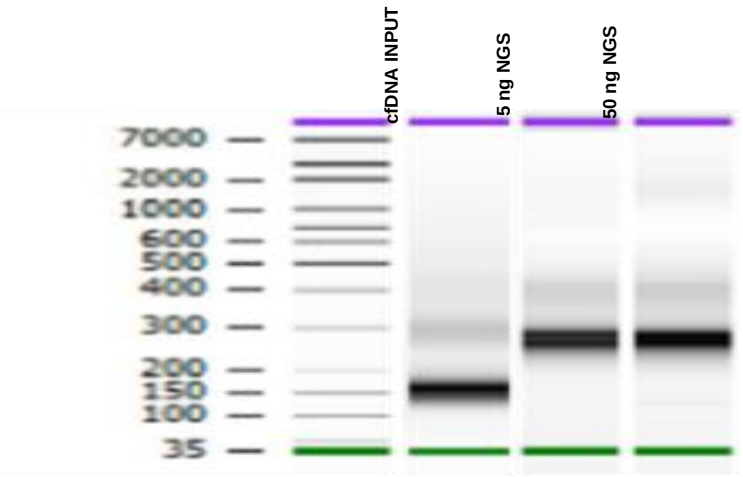
Approach

Reference Samples

- Replicates of SCLC cfDNA – contain known TP53 mutation
- 8 x 0.5 ng input = 165 genomes and 4 x 2.5 ng input = control samples
- Targeted pull-down of x 7 cancer associated genes – NGS analysis to determine pull-down efficiency

Commercial Kits

- NEB Ultra
- Microplex (Rubicon)
- KAPA
- HomeGrown *– in development*



Targeted pull-down NGS

	NEB	Microplex	KAPA
Total Reads (genomewide)	97,904	108,913	107,265
NRAS	28	38	43
PIK3CA	27	32	46
BRAF	37	72	84
EGFR T790M	27	31	26
KRAS 63bp	24	38	74
TP53 E	50	48	47
TP53 H	27	24	19
Total 'on target' reads	220	283	339
Ratio of 'non-target' to 'target' reads	445.02	384.85	316.42
Fold enrichment	44944	52083	63291

Developing NGS approaches to analyse cfDNA to determine copy number aberrations and Whole Exome Sequencing of patient samples.

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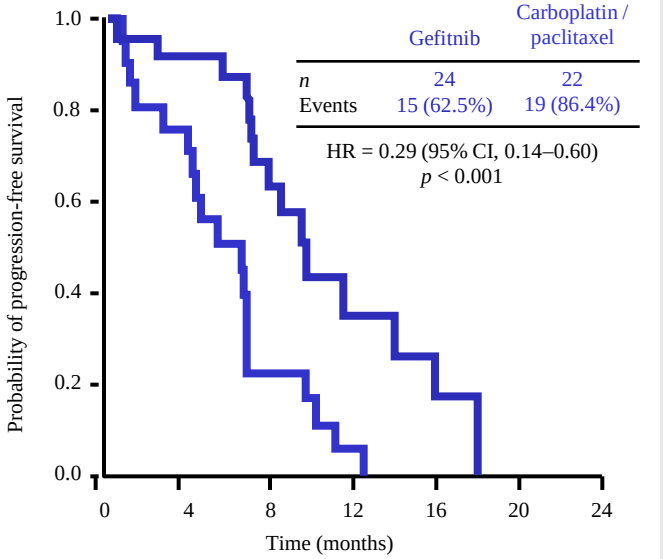
Beginning to become Practice Changing:

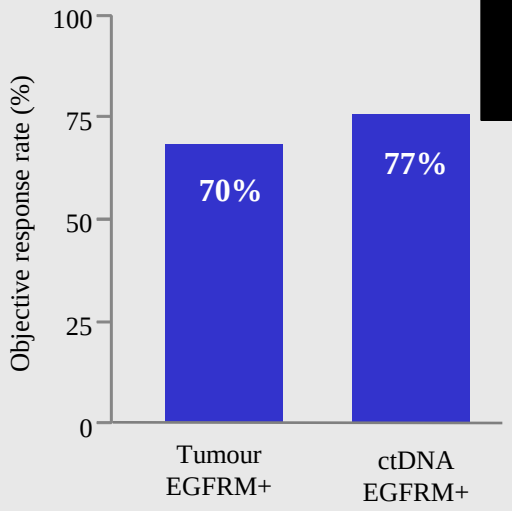
IRESSA: Application for update to EU label*

ON 16th May 2014, AZ submitted an application for a Type II Variation for IRESSA (gefinitib) regarding use of circulating tumour DNA (ctDNA) for the assessment of EGFR mutation status in advanced NSCLC patients for whom tumour samples are unavailable or unevaluable.

IPASS: PFS in patients is improved by IRESSA in patients where EGFR mutations are identified by ctDNA



IFUM: ORR in patients in response to IRESSA in patients where ctDNA is used to determine EGFR status



Diagnostic performance of ctDNA vs Tumour:

Specificity = 99.8%
Sensitivity = 65.7%
Turnaround = 3-4d

IPASS: Goto *et al.* (2012) Journal of Thoracic Oncology 7:115 –121

IFUM: Douillard *et al*. (2014) British Journal of Cancer 110: 55-62

*The type II variation application is subject to review by EMA/CHMP

...and now “Business as Usual” across AZ Oncology Portfolio

- Currently >80% of AZ oncology clinical projects have personalised healthcare strategies

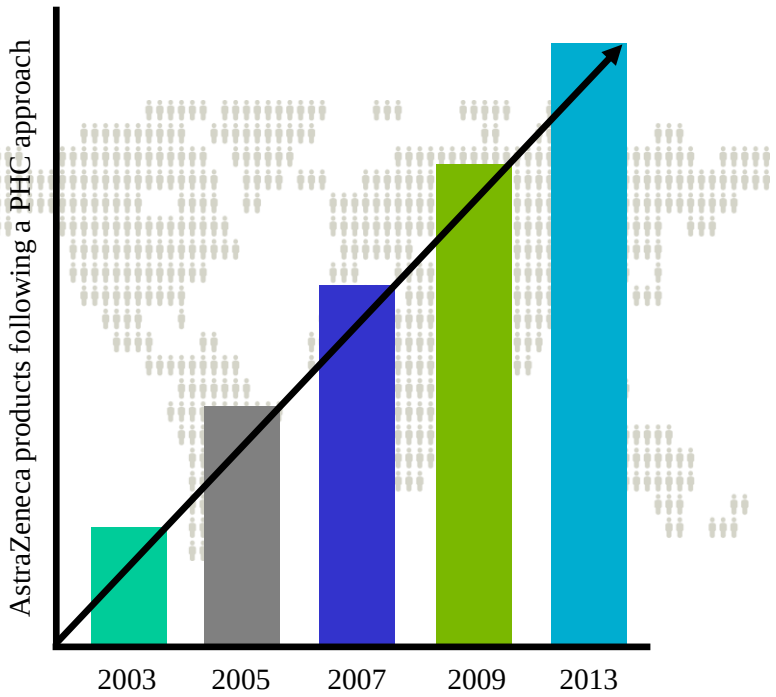
- Targeted/Singleplex assays

Drug	Aberration
Iressa	EGFR mutation
Olaparib	BRCA 1, 2 mutation
Selumetinib	Kras mutation
AZD4547	FGFR mutation, fusion, amplificactions
AZD9291	EGFR and T790M mutation

- Multiplex/NGS assays

Drug	Aberration
AZD1775	P53 mutations

AZ projects



- Currently ranked among the top 5 companies in the PHC field[1,2]

1. Diaceutics Pharma Readiness for Personalized Medicine 2011
2. PharmaTimes, April 2011

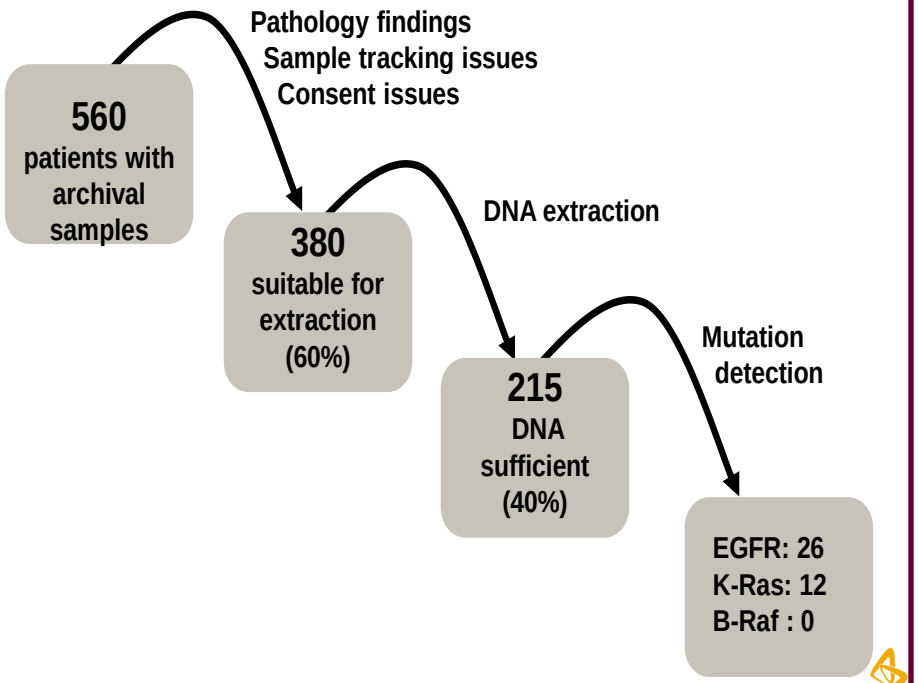
3. Single molecular aberration trials can be *very* inefficient

AZ recent experiences in conducting single molecular aberration precision medicine trials in **lung cancer:** ~23 patients "screened" for 1 "enrolled"

Iressa- Phase 3 trial

Attrition factors in EGFR mutation analysis:

Archival tissue

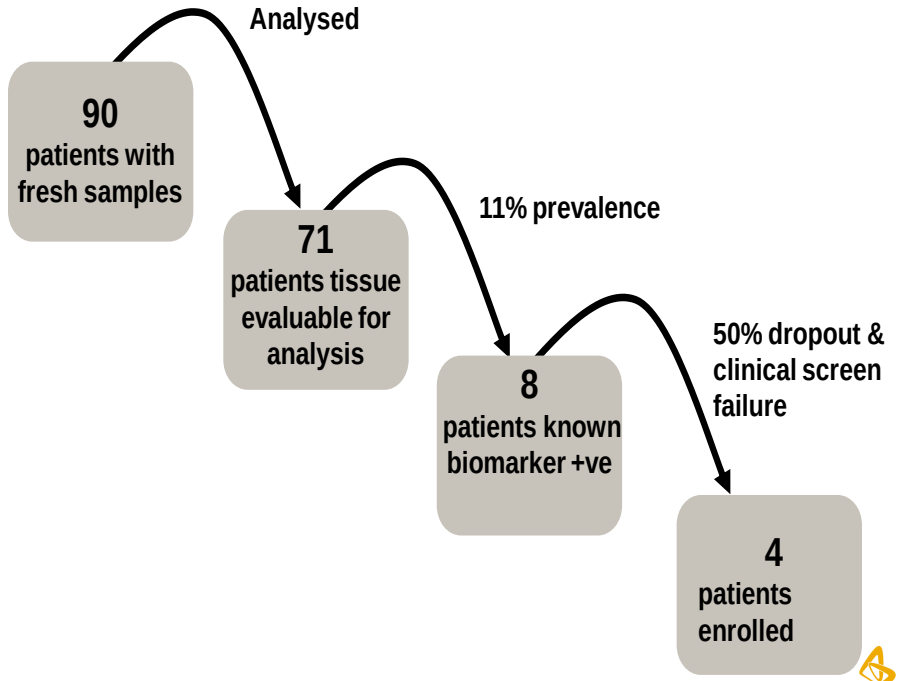


- Experience with **archival** samples from the ISEL study a double-blind, placebo-controlled Phase III survival study of gefitinib in 2L/3L Stage IIIB/IV NSCLC with1692 patients in 210 centres, 28 countries

AZD4547- Phase 1 trial

Attrition factors in FGFR amplification analysis:

Fresh tissue



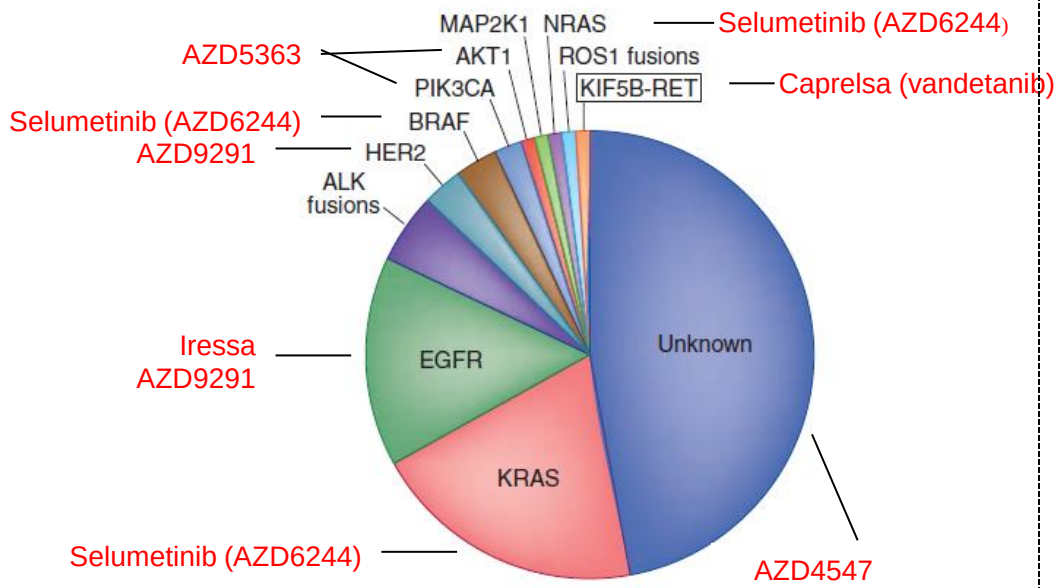
Experience with **fresh** samples from the AZD4547 open label study in 2L/3L Stage IIIB/IV squamous NSCLC in 19 centres

Thus $23K analytical screening costs (@ $1K/test) per patient enrolled

Stratified Medicine Requires Portfolio Approach

AZ / MedImmune portfolio well placed in Lung Cancer...

Adenocarcinoma



FGFR 2/3 mutation
FGFR1 amplification
FGFR translocations

Volitinib

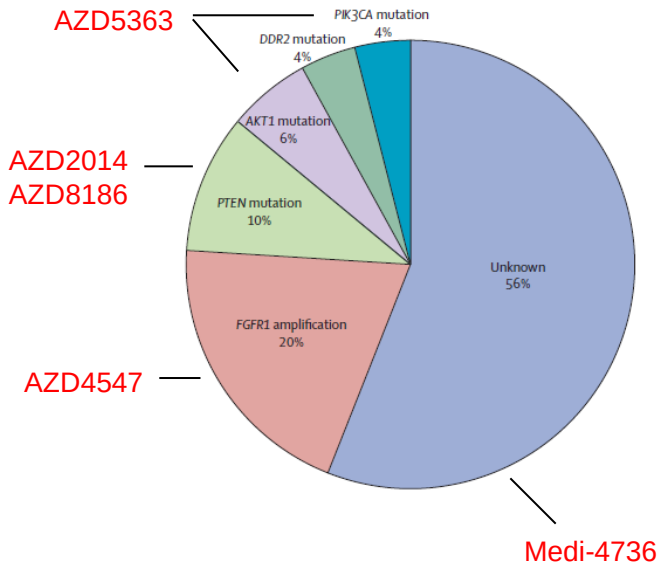
Met expression
Met amplification

Medi-4736

PDL-1 expression
PDL-1 amplification

William Pao & Katherine E Hutchinson
NATURE MEDICINE VOLUME 18 | NUMBER 3 | MARCH 2012

Squamous



Alexander Drilon, Natasha Rekhtman, Marc Ladanyi, Paul Paik
Lancet Oncol 2012; 13: e418–26

Lung Master Protocol – Friends Of Cancer Research.

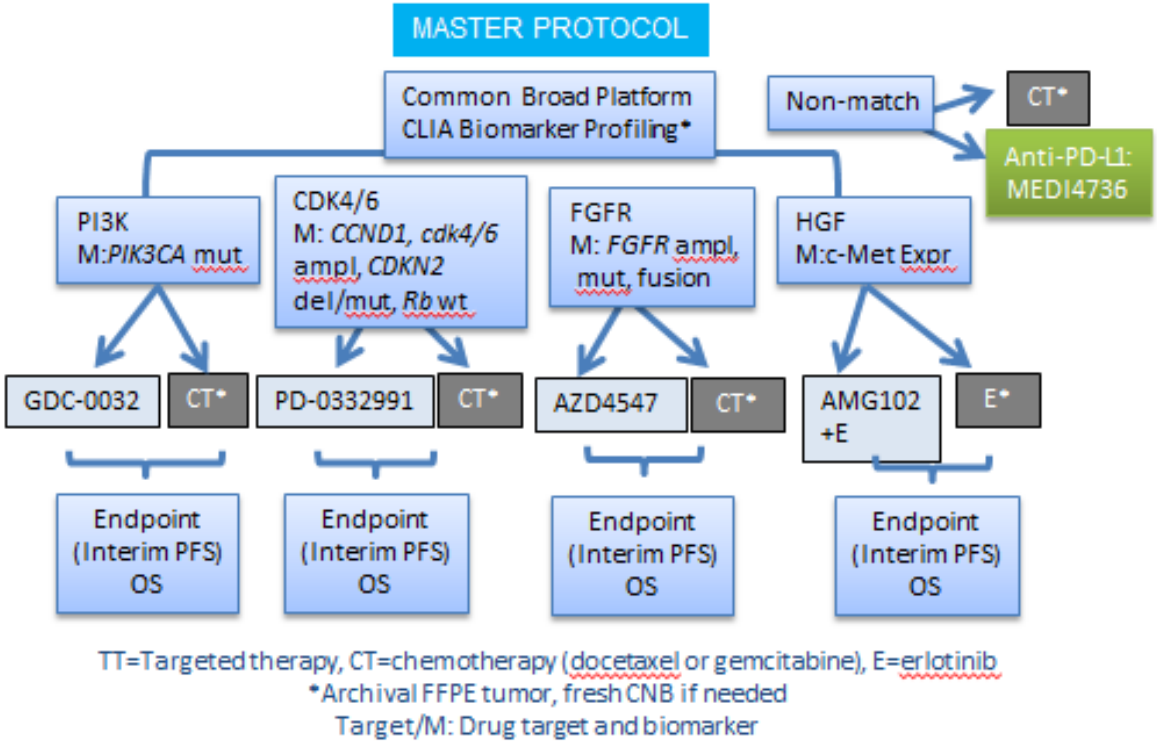
Squamous NSCLC

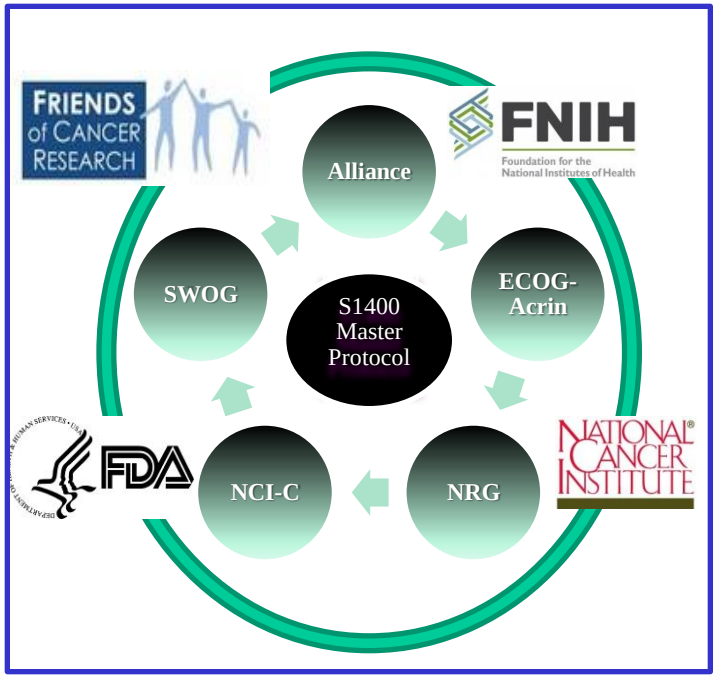
nature

'Master protocol' aims to revamp cancer trials (2013). Pilot project will bring drug companies together to test targeted lung-cancer therapies.

nature biotechnology

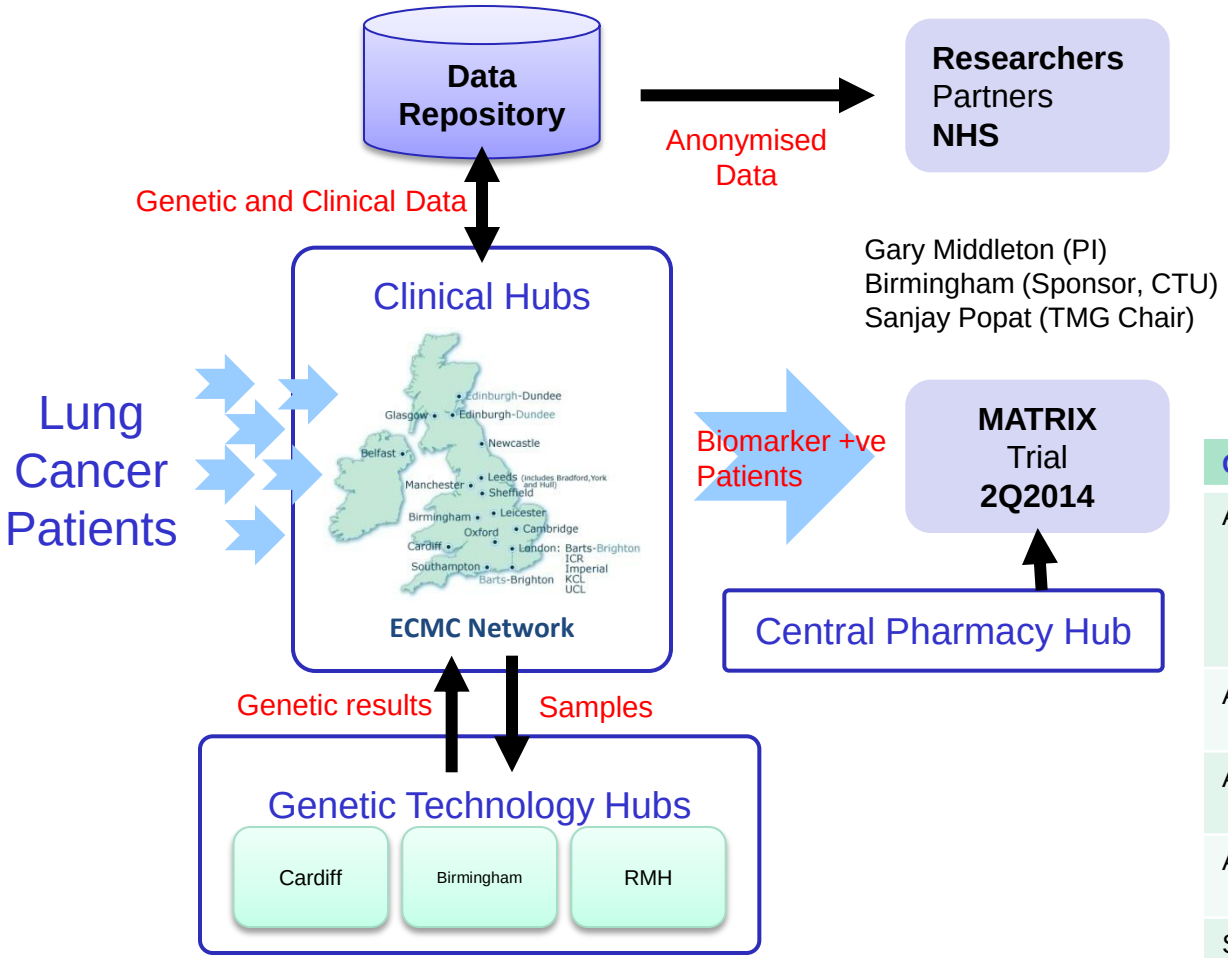
Master Protocol for squamous cell lung cancer readies for launch (2014). The master protocol is a "truly exciting development, one that will benefit industry and patients," says US Food and Drug Administration (FDA) Commissioner Margaret Hamburg.





MATRIX National Lung Trial – CRUK

Squamous and adenocarcinoma NSCLC

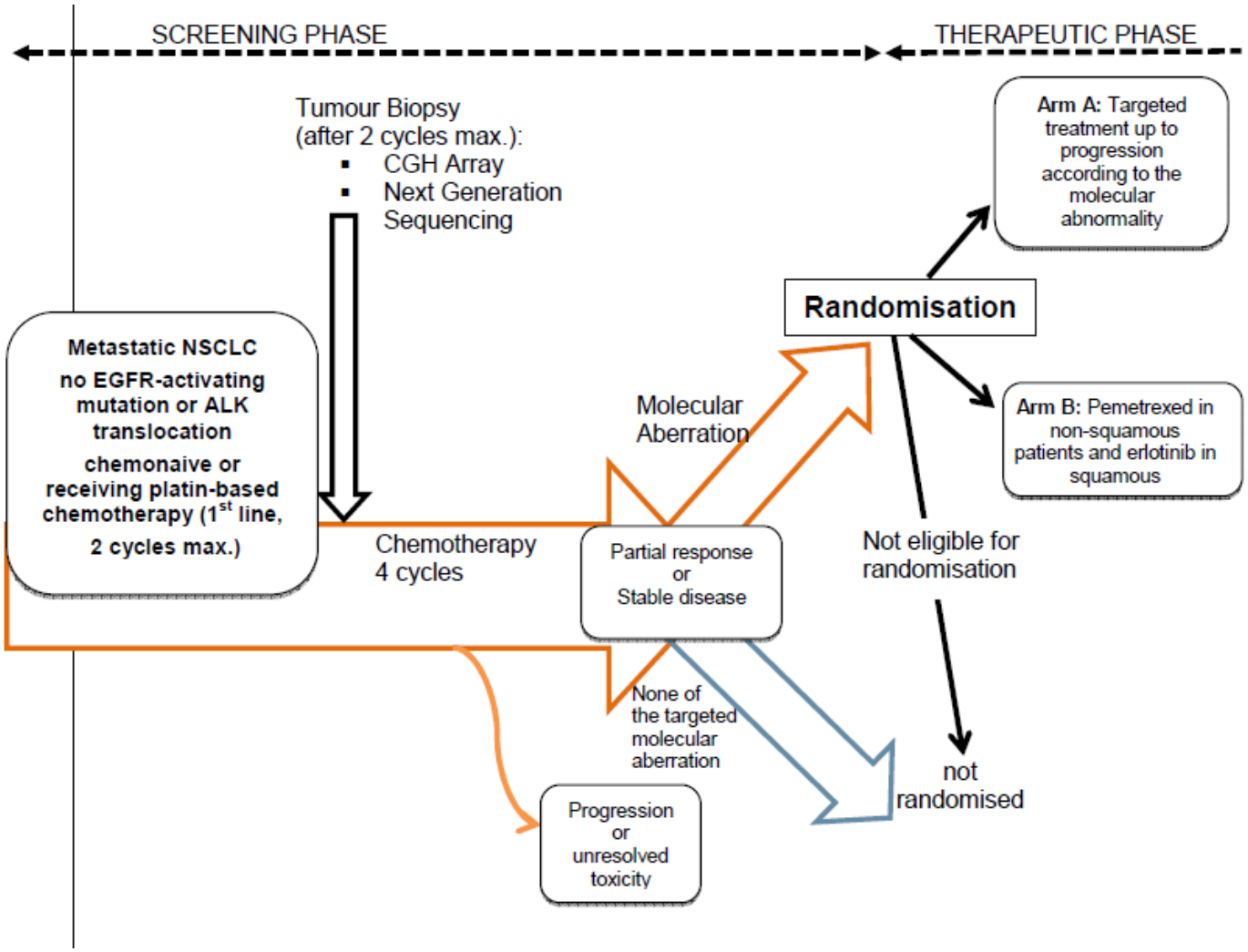


Gary Middleton (PI)
Birmingham (Sponsor, CTU)
Sanjay Popat (TMG Chair)

Compound	Molecular segment	Prevalence
AZD5363	PI3KCA mutation PIK3CA mutation AKT1 mutation PIK3CA amp PTEN null	4.6% 15.2% 0.9% 7.0% 7.9%
AZD4547	FGFR2/3 mutation FGFR2/3 mutation	3.3% 4.4%
AZD2014	LKB1 mutation TSC1/2 mutation	12.2% 8.9%
AZD9291	T790M (Her2 amp)	7.5% (5.0%)
Selumetinib/ docetaxel	KRAS wild type, NF1, NRAS, HRAS mutation	24.9%
MEDI4736	All markers negative (PD-L1 positive)	est. 40%

SAFIR02 Lung Trial – UNICANCER

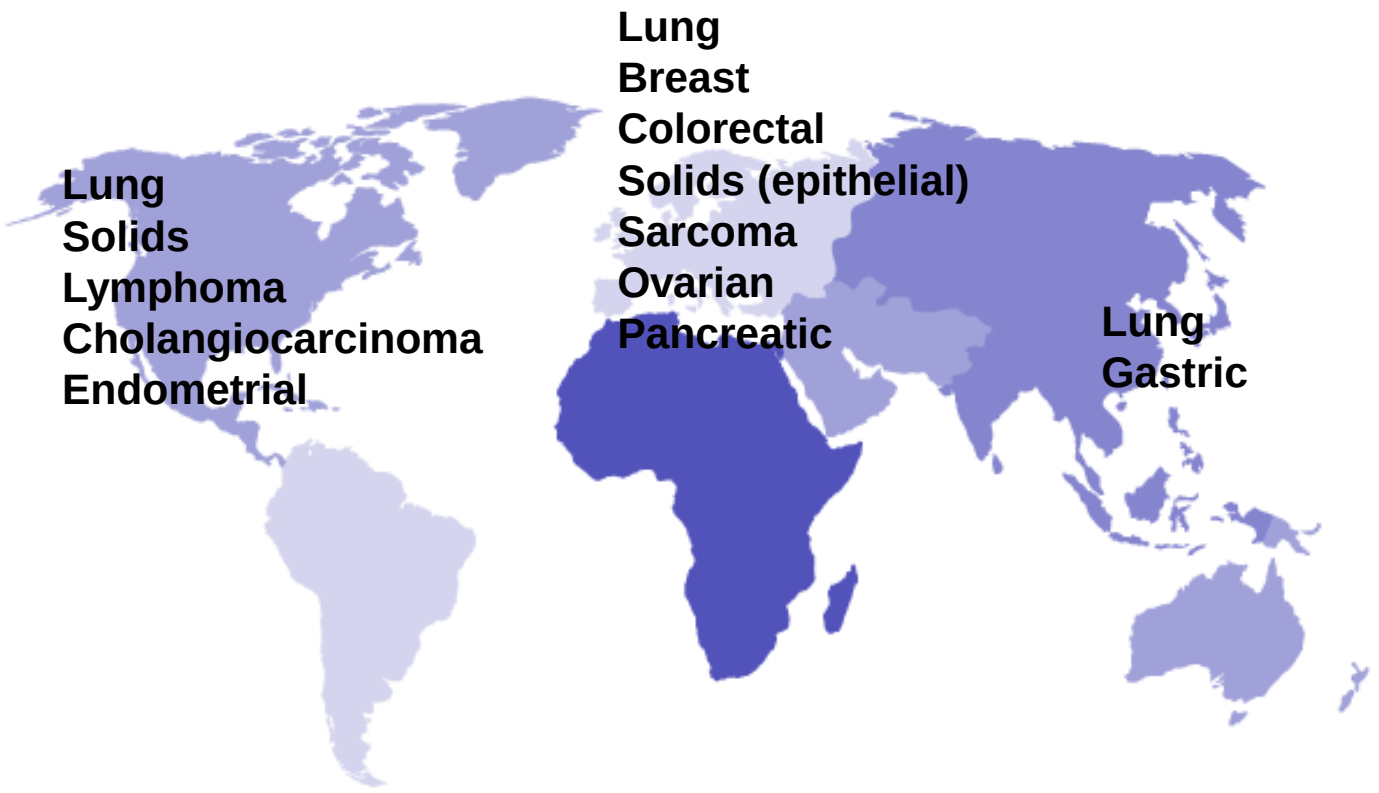
Squamous and adenocarcinoma NSCLC



Compound	Molecular segment
AZD5363	PI3KCA mutation AKT1 mutation PIK3CA amp PTEN loss PTEN mutation
AZD4547	FGFR1 amplification
AZD2014	LKB1 mutation
AZD8931	HER2 mutation HER2 amplification
Selumetinib	KRAS mutation BRAF mutation
Vandetanib	RET mutation

“Basket” Studies by Tumour Type and Region

AstraZeneca



www.clinicaltrial.gov

These studies are more patient efficient...although challenges remain...but the emerging science is promising

- Definition of “biomarker +ve” by NGS: understanding the clinical relevance of variants
- Necessitates a consortia approach
- Flexibility desirable for clinical patient selection decisions imposes statistical challenges
- Intent needs to be clear in the design- to signal search or adaptive with registration intent
- Lack of familiarity to IRB’s
- With multiple drugs, with multiple toxicities and disparate monitoring requirements- can attract regulatory concerns

Acknowledgements

MCRC

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Ruth March
Susan Galbraith
Antoine Yver
Max Kirkby
Anne Galer
Kevin Carroll*
Carl Barrett
Simon Hollingsworth
Elaine Kilgour
Iressa Product Team
AZD4547 Product Team

*formerly

Backups

COMBINED CTC cfDNA WORKFLOW

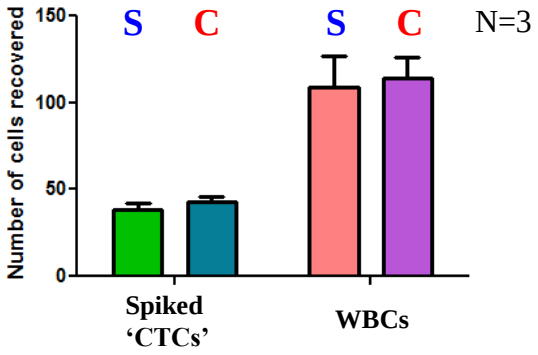
Example Workflow

Approach

- 100 H2009 cells spiked into HNV blood collected in either Streck Cell-Free DNA BCT® (**S**) or CellSave Preservative tubes (**C**) and left 96 hours at room temperature
- Remove plasma and process cells on Parsortix device
- Count retrieved spiked cells and white blood cells (WBCs)
- Whole genome amplify (WGA) pools of 40 cells and evaluate using PCR QC

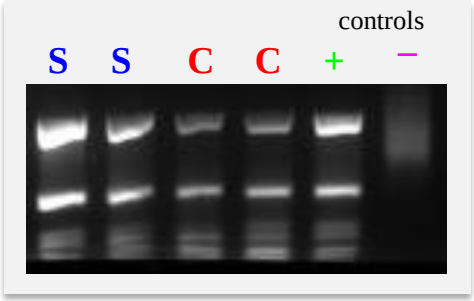
Cell Enrichment

- 'CTC' recovery >30%
- Total WBCs <200
- Streck and CellSave comparable



cfDNA

- Streck and CellSave comparable



Molecular Analysis

- Efficient WGA for Streck and CellSave
- Quantitative NGS underway

Considerations for Clinical Use

Single Tube for Plasma and Cells

- Reduces number of blood samples required
- Direct comparison of cfDNA and CTCs

Low WBC contamination (<200)

- Suitable for single cell isolation eg DEPArray
- Direct analysis of entire enriched population possible if assay sensitivity can detect at least 0.5 % tumour component (1 CTC amongst 200 WBCs)

Molecular CTC signature

- Based on:
  - common driver mutations eg KRAS in pancreatic cancer
  - sequence analysis of tumour
  - sequence analysis of CTCs or cfDNA
- Allows epitope independent CTC assessment

Courtesy Ged Brady

Sequenom Targeted Panels:

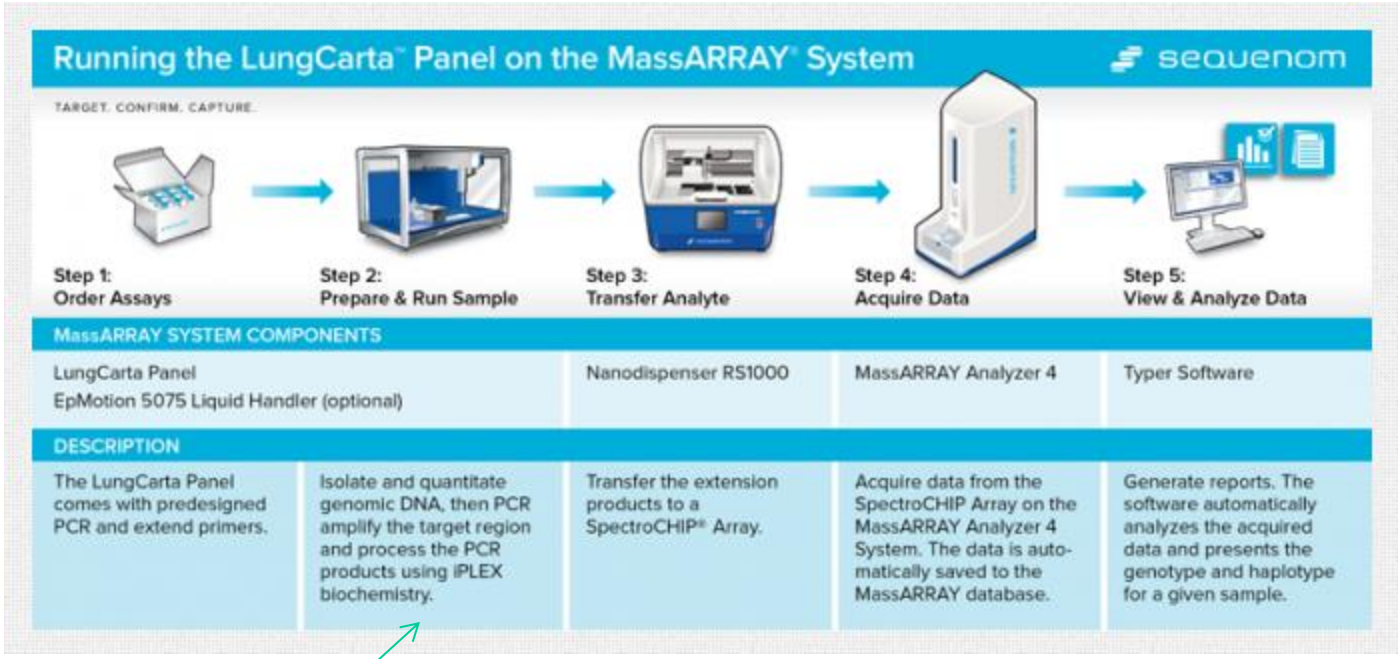
- High-throughput somatic mutation profiling for disease-specific genes of interest (e.g. Lung CancerResearch has the LungCarta Panel)
- The LungCarta Panel evaluates mutations in 2
  tumo of 214 soma

Genes Included in the LungCarta Panel:

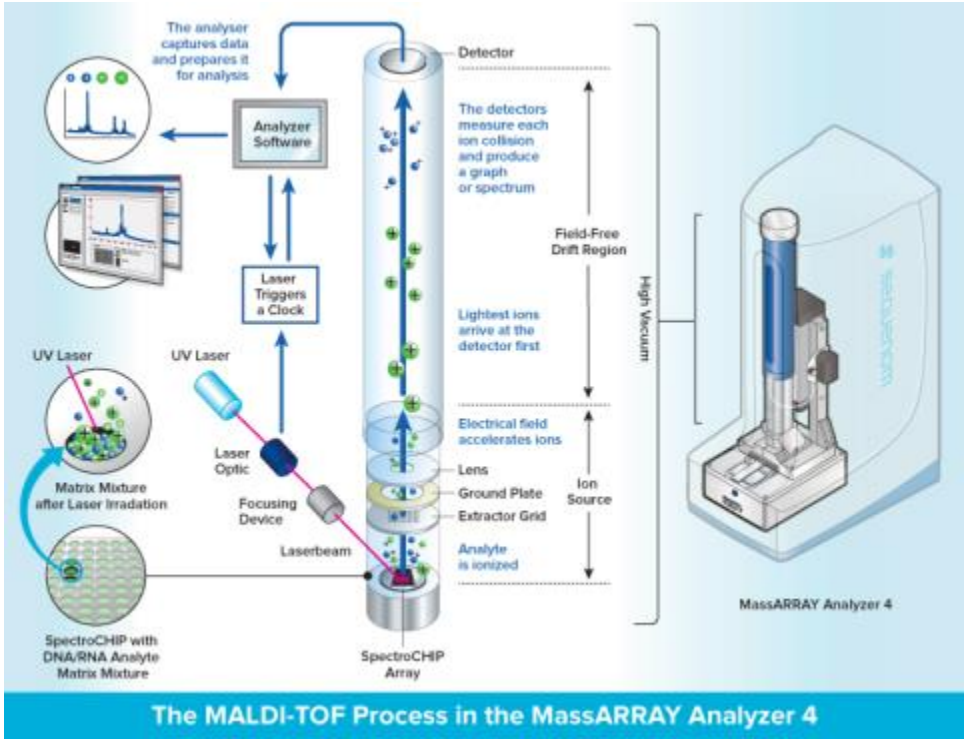
AKT1	*JAK2*	*NTRK3*
ALK	*KRAS*	*PIK3CA*
BRAF	*MAP2K1*	*PTCH1*
DDR2	*MET*	*PTEN*
EGFR	*NOTCH1*	*PTPN11*
EPHA3	*NRAS*	*PTPRD*
EPHA5	*NRF2*	*STK11*
ERBB2	*NTRK1*	*TP53*
FGFR4	*NTRK2*	

Gene	Mutations Detected with the LungCarta Panel
AKT1	E17K
ALK	C1156Y, L1196M
BRAF	D594G/V, G469S/E/A/V, L597Q/V, V600E/K/M
DDR2	C580Y, D125Y, G253C, G505S, G774E/V, I120M, I638F, L239R, L63V, T765P
EGFR	R108K, T263P, A289V, G598V, E709K/H, E709A/G/V, G719S/C/A/D, G719S/C/A/D, M766_A767insAI, D761Y/N, S768I, R776C/H, V769_D770insASV, V769_D770insCV, D770_N771>AGG/V769_D770insASV/V769_D770insASV, D770_N771insG, N771_P772>SVDNR, P772_H773insV, H773>NPY, H773_V774insNPH/PH/H, V774L, V774_C775insHV, T790M, L858R/M, L861Q, E746_T751del, E746_A750del, E746_T751del, E746_T751del, S752D, L747_E749del, L747_T750del, L747_S752del, L747_T751del, L747_S752del, P753S, A750P, T751A, T751P, T751I, S752I/F, S752_I759del, L747_Q ins, E746_T751del, I ins (combined), E746_A750del, T751A (combined), L747_E749del, A750P (combined), L747_T750del, P ins (combined), L747_S752del, Q ins (combined), T854A
EPHA3	A435S, D446Y, S449F, D806N, G187R, G518L, K761N, G766E, M269I, N379K, N85S, S229Y, T166N, T37K, T393K, W250R
EPHA5	D493Y, G582E, M1034I, N1032S, R1007Q, S566Y, S810I, T856I
ERBB2	M774_A775insAYVM, A775_G776insAYVM
FGFR4	P672T, H192fs*19
JAK2	L609S, P503L, R1122P, Y931C
KRAS	G12S/V/F/R/A/C/D, G13C/S/A/V/D, Q61L/R/P/H/E/K
MAP2K1	D67N, K57N, Q56P
STK11	A347fs*13, A43_L50del6, D327fs*10, E120*, E165*, E223*, E70*, E70fs*26, F354L, G163C, G188fs*99, G196V, G56fs*4, G56W, G91L, H174R, I26fs*25, K191*, K78E, L285Q, L50_D53del4, M51fs*14, P179L, Q123R, Q137*, Q159*, Q170*,Q220*, Q37L, R426W, R86G, V197fs*69, V236fs*30, Y272Y
MET	N375S, 982_1028del47
NOTCH1	H2276fs*79, D1643H, R2328W, T1997M, V1672I, V2444fs*35
NRAS	Q61E/K/H/L/R/P
NRF2	D29H, D77N/A, E79Q/K/G, G31A, G81D, R34Q
NTRK1	Q80*, R119H, S326R
NTRK2	Q666R, C45F, G261R, L138F, L670M, L755L
NTRK3	I769N, L152I, L248M, L270M, L336Q, S184C, T283K, V307L, R271F
PIK3CA	E542Q/K, E545Q/K, H1047Y/R/L
PTCH1	R1308G, R682L, S1326fs*46
PTEN	R233*
PTPN11	E76V
PTPRD	S1703R, T337A, V483E
TP53	G245C/S, G245D/V, R158C/G/L/P, R175L/H, R248G/L/Q/W, R249S/W/M, R273C/H/L/P, R282G/W, V157F, Y163C, R175L/H, Y220C

Panels consist of key mutations identified by sequencing discovery studies that affect key pathways in the disease of interest (e.g.



Each sample is PCR amplified (using gene-specific primers mentioned earlier), and then dispensed on the MassARRAY and analysed using Mass



Qiagen GeneRead:

- Another PCR-based target enrichment method
- Panels commercially available, include Lung Cancer, Colon Caner and a Comprehensive Cancer Panel.