The Christie NHS
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The promises and pitfalls of liquid biopsy for the molecular portrait and monitoring of treatment response in NSCLC

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Consultant in Medical Oncology

Clinical Senior Lecturer in Experimental Cancer Medicine

15-18 April 2015, Geneva, Switzerland

Organisers IASLC International Association for the Study of Lung Cancer; ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE European Society for Medical Oncology

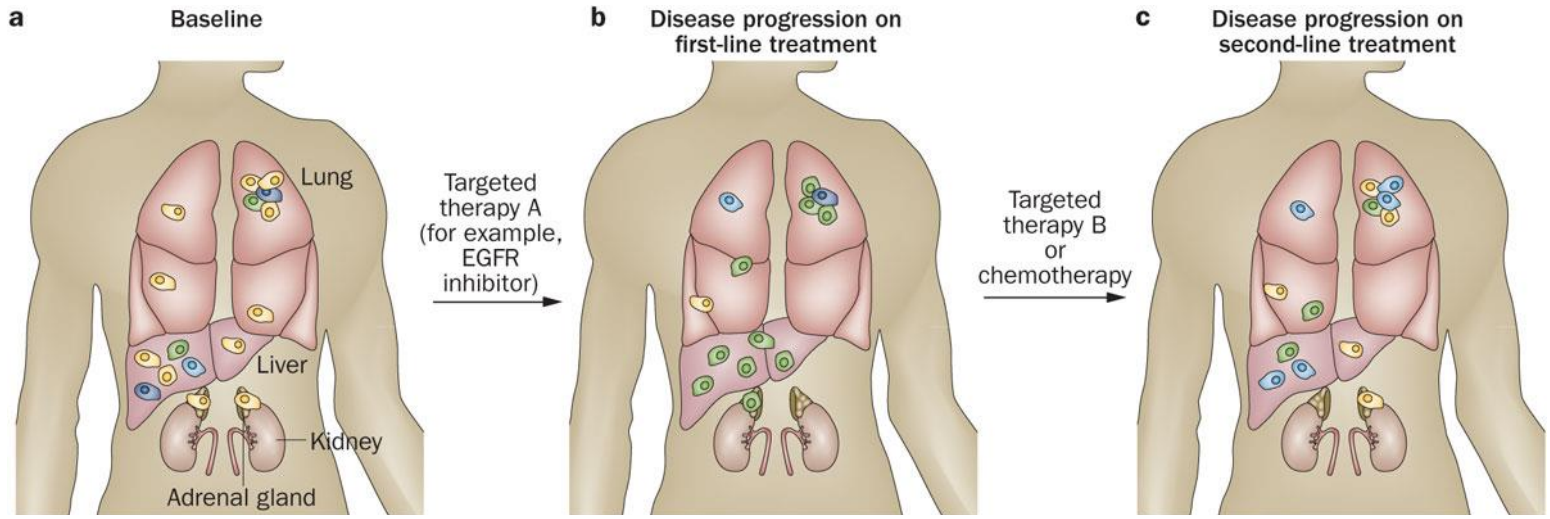
Partners ESTRO European Society for RADIOTHERAPY & ONCOLOGY; European Society of Thoracic Surgeons 1993; etop Information | Research

DISCLOSURE SLIDE

- Nothing to declare

THE AMBITION FOR PRECISION MEDICINE

monitoring of tumour clonal evolution



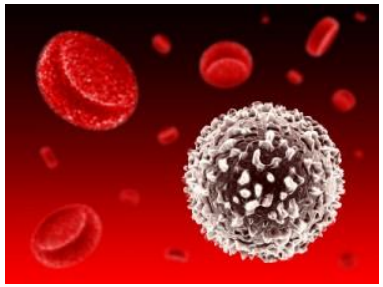
Krebs et al, Nature Reviews Clinical Oncology, 2014

THE AMBITION FOR PRECISION MEDICINE

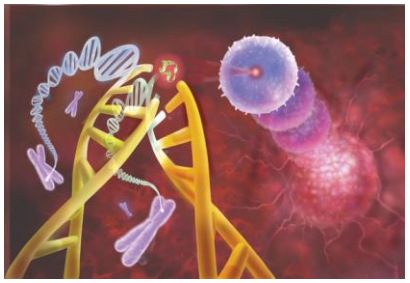
monitoring of tumour clonal evolution

BIOPSY IS GOLD-STANDARD FOR MOLECULAR CHARACTERISATION BUT…

- Invasive and sometimes difficult to obtain. **NOT without risk**
- Insufficient quantity of tissue from small biopsies / cytology
- Archival tumour may not reflect current status of tumour
- Tumour heterogeneity of primary and metastases
- Serial biopsies to assess PD activity/ molecular evolution of tumour/ resistance mechanisms are challenging



Circulating tumour cells

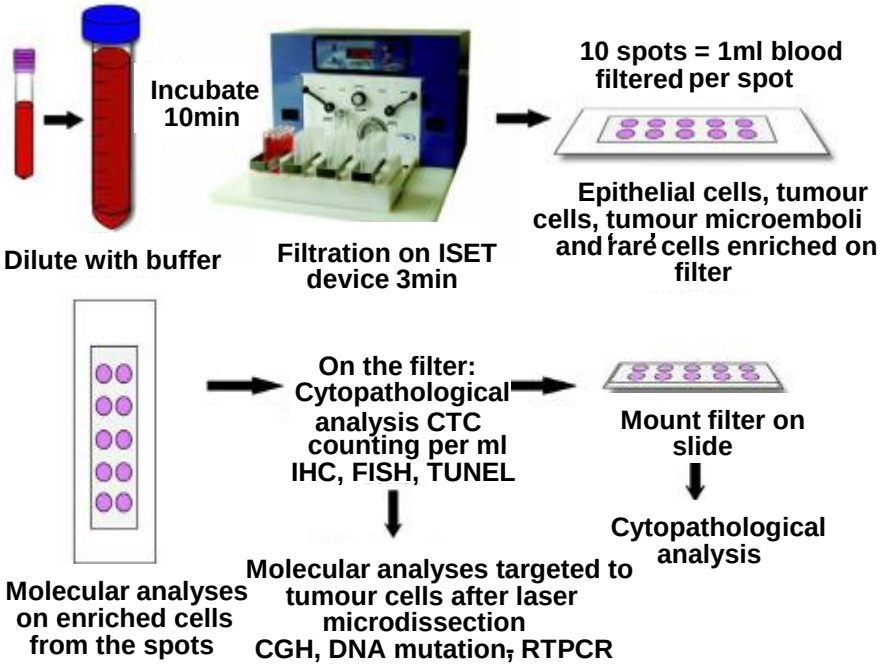


Circulating tumour DNA

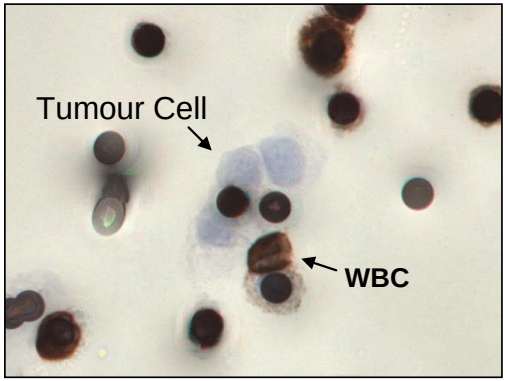
CTC ISOLATION TECHNOLOGIES

IMMUNOMAGNETIC CAPTURE - CELLSEARCH

FDA Approved



ISOLATION BY SIZE - ISET

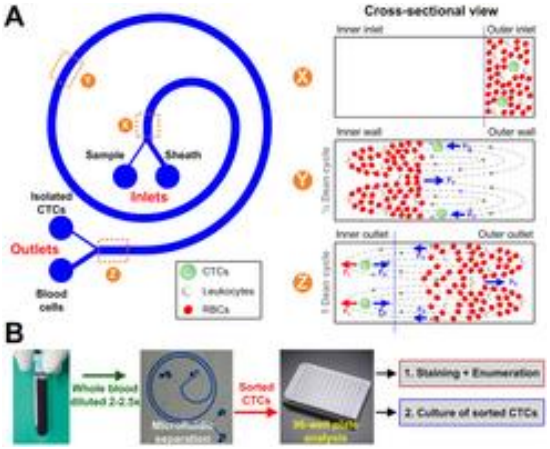


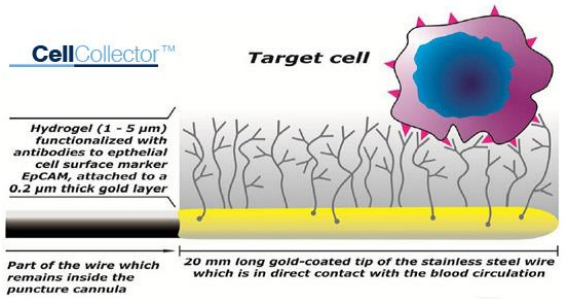
OTHER CTC TECHNOLOGIES

ISOFLUX

Parsortix

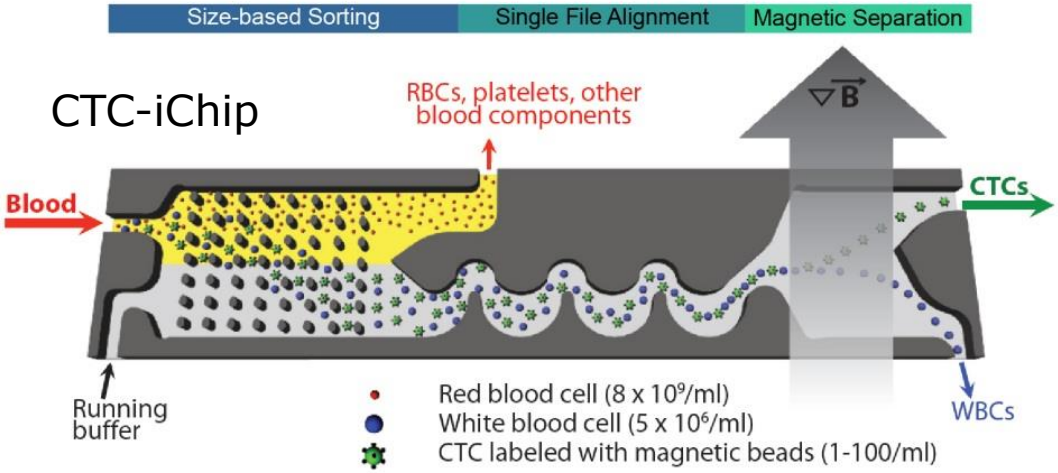
Clearbridge





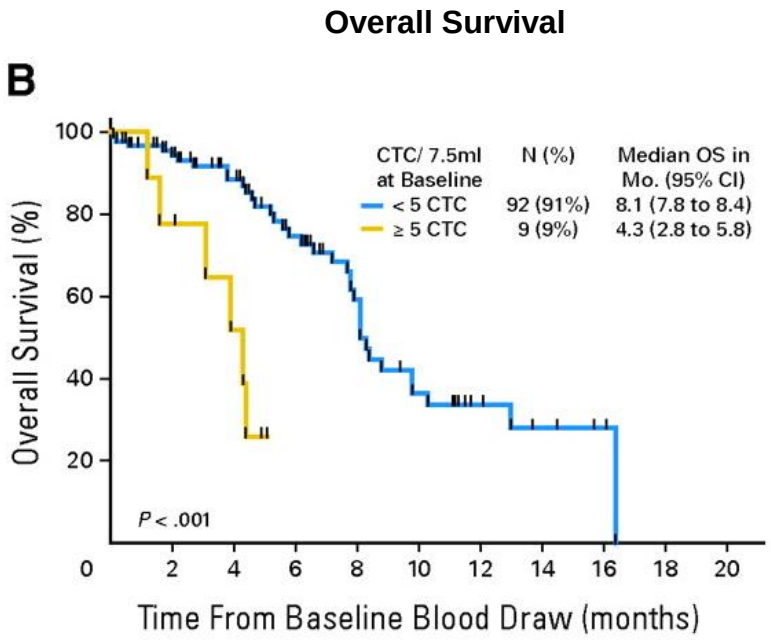
Gilupi

CTC-iChip



CTCS AS A PROGNOSTIC BIOMARKER IN NSCLC PATIENTS

≥5 CTCs is prognostic

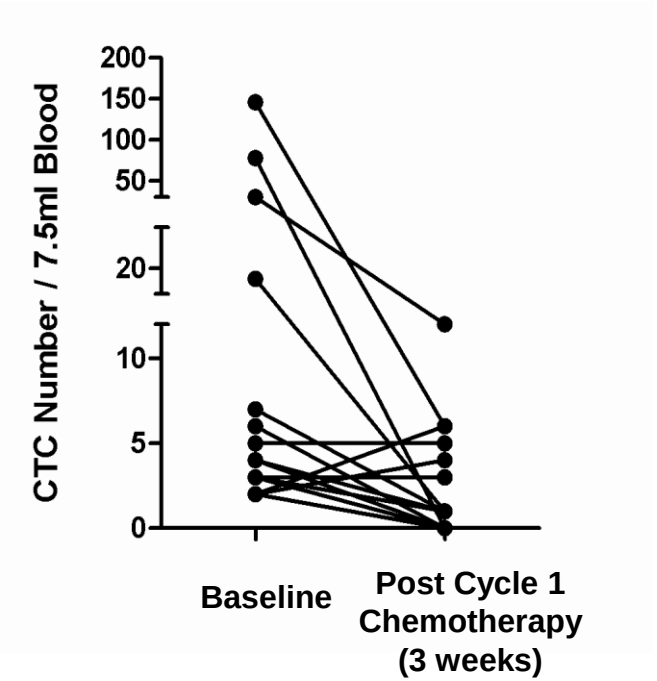


Median OS 8.1 vs 4.3 months

HR 5.98 (95%CI 2.25-15.87), P<0.001

Independent prognostic factor (HR = 7.92) for OS in multivariate analysis

CTC change on treatment



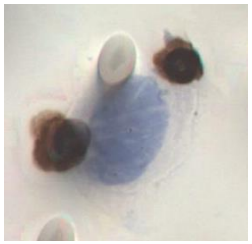
	Change	Median OS
72% (13/18)	Decrease	8.3 months
17% (3/18)	Increase	3.3 months
11% (2/18)	No change	3.9 months

Krebs et al, Journal of Clinical Oncology, 2011

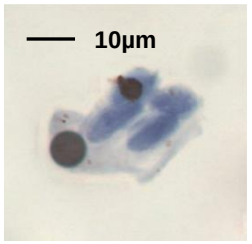
EXAMPLES OF CTCS AND CTM BY ISET IN NSCLC PATIENTS

CTM were observed in 15 (38%) of the 40 patients by ISET – not seen in matched CellSearch samples

PATIENT 101



PATIENT 83



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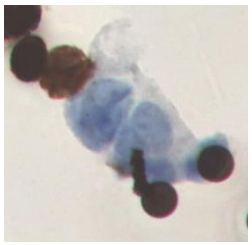

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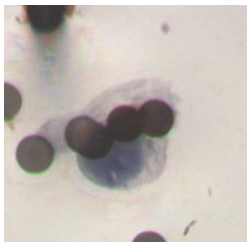

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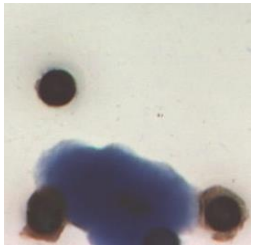

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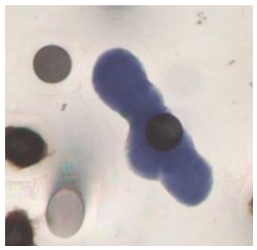

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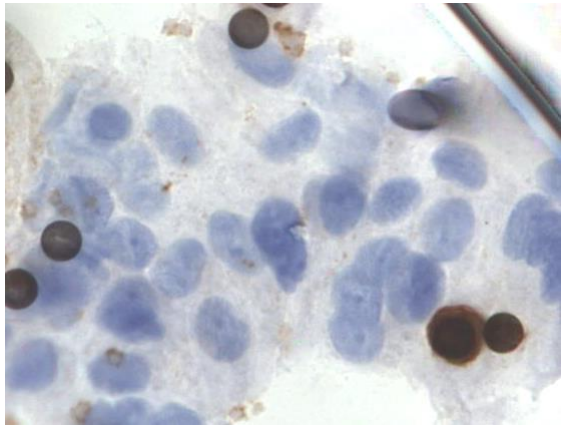

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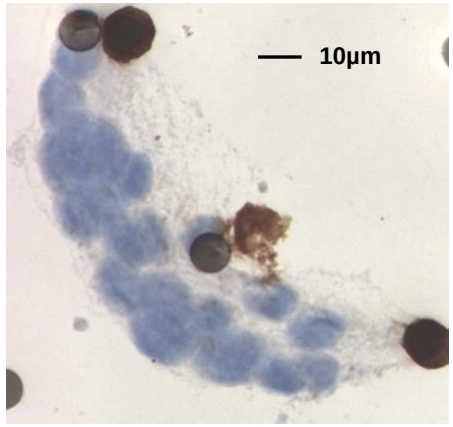

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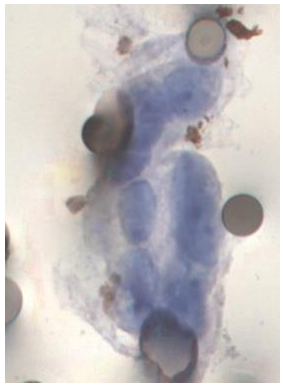

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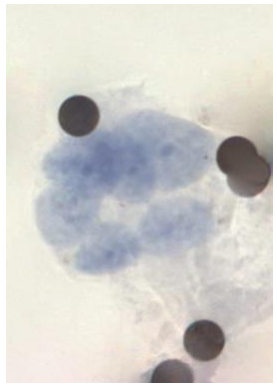

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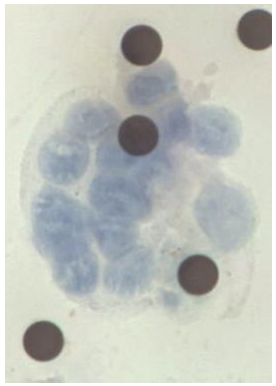

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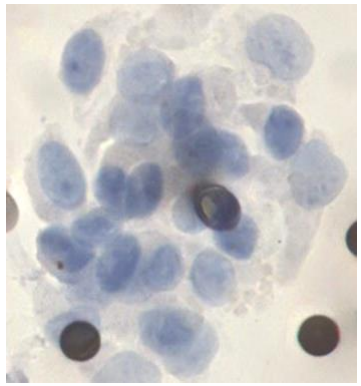


All images 40x magnification

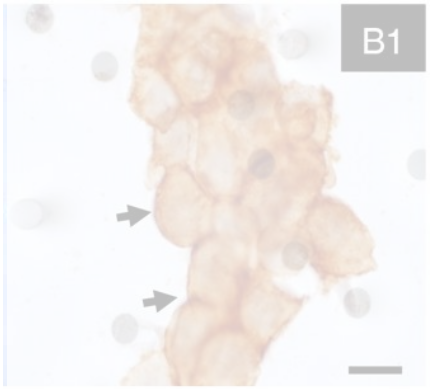
CD45 immunostaining with haematoxylin counterstain

ALK GENE REARRANGMENT IN ISET ISOLATED CTCS

***ALK*-gene rearrangement: a comparative analysis on circulating tumour cells and tumour tissue from patients with lung adenocarcinoma**

M. Ilie[1,2,3,4,5†], E. Long[1,2,4†], C. Butori[4], V. Hofman[1,2,3,4,5], C. Coelle[3], V. Mauro[3], K. Zahaf[4], C.H. Marquette[1,2,6], J. Mouroux[1,2,7], P. Paterlini-Bréchot[8] &

Annals of Oncology 23: 2907-2913, 20

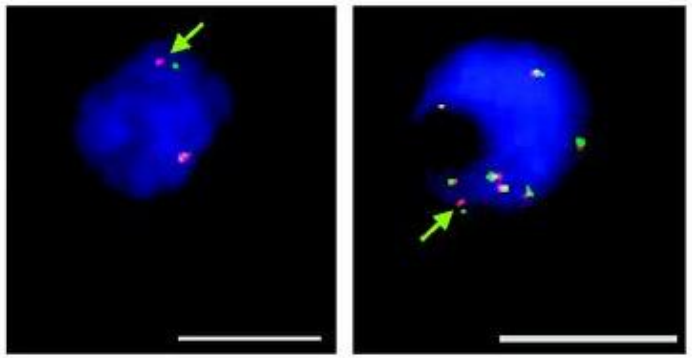


5/87 patients with *ALK* fusion disease. All 5 cases had strong ALK expression in CTCs by IHC and *ALK* gene re-arrangement by FISH.

Detection of Circulating Tumor Cells Harboring a Unique *ALK* Rearrangement in *ALK*-Positive Non–Small-Cell Lung Cancer

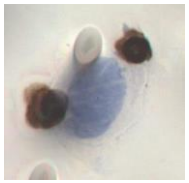
Emma Pailler, Julien Adam, Amélie Barthélémy, Marianne Oulhen, Nathalie Auger, Alexander Valent, Isabelle Borget, David Planchard, Melissa Taylor, Fabrice André, Jean Charles Soria, Philippe Vielh, Benjamin Besse, and Françoise Farace

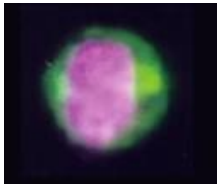
JOURNAL OF CLINICAL ONCOLOGY **31(18): 2273-2282, 2013**

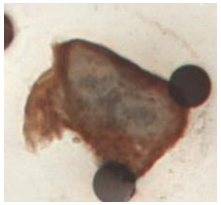


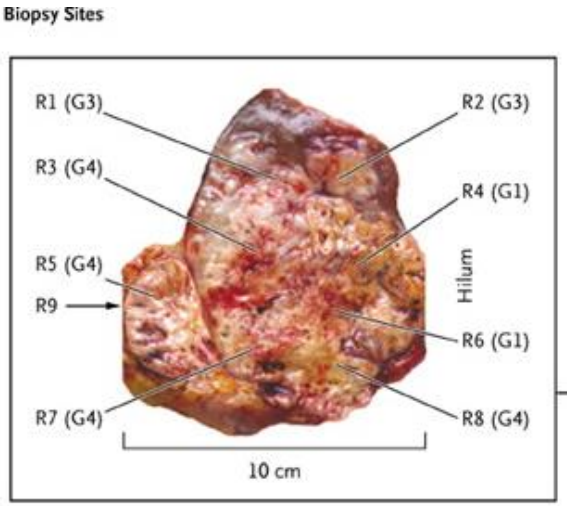
All *ALK* positive patients (n=18) had 4 or more *ALK* rearranged CTCs per ml of blood with unique 3’5’ split pattern seen in CTCs

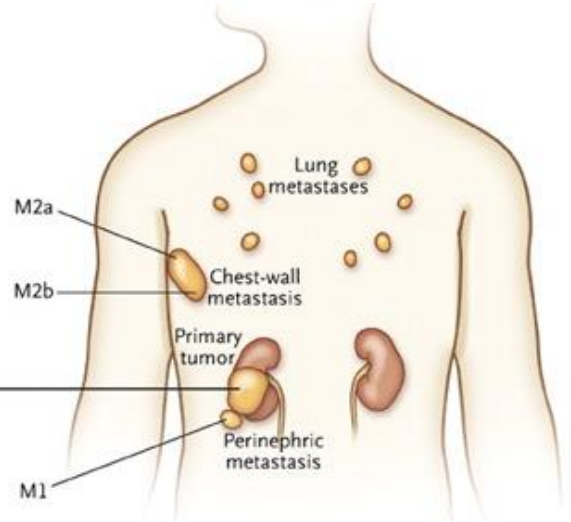
CIRCULATING TUMOUR CELL HETEROGENEITY

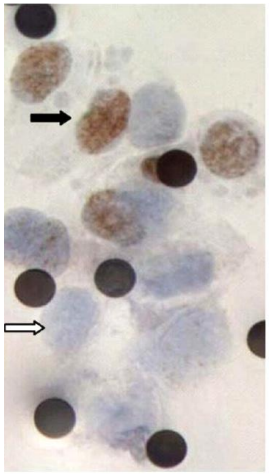


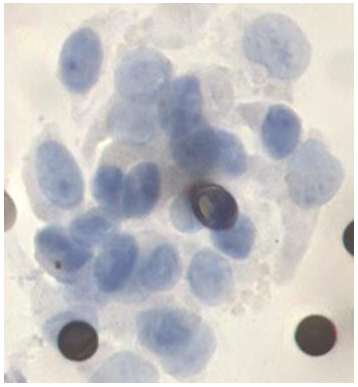












Questions

- Does the heterogeneity of CTCs reflect tumour heterogeneity
- Do the different populations of cells have unique driver mutations?
- Which of these survive and result in metastases?
- Single cell molecular analysis may help address these issues

Gerlinger et al, New England Journal of Medicine, 2012

FROM CTC ENRICHMENT TO SINGLE CELL ANALYSIS

1/10,000,000

Peripheral blood

1/1,000-10,000

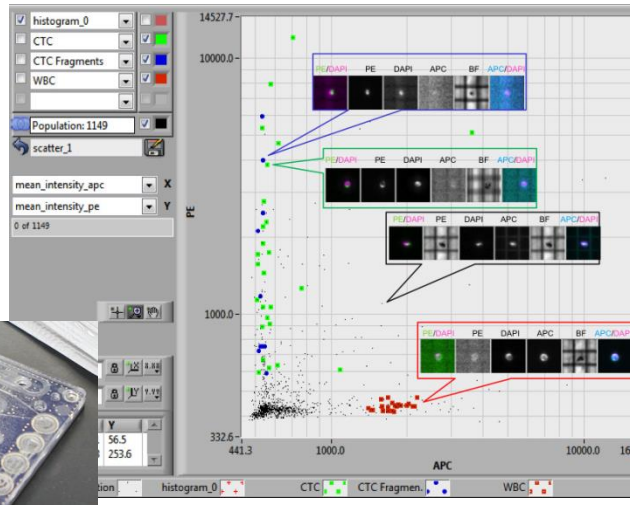
Enriched CTCs

silicon biosystems
THE LIVING-CELL COMPANY®

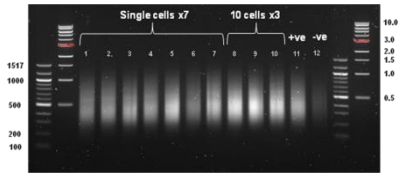
The DEPArray™

Enrichment / Sample-Prep

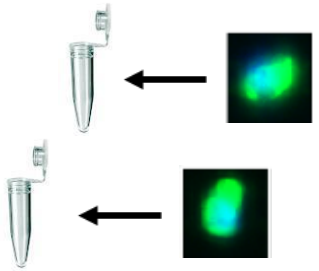
Veridex CellSearch®,
CTC Chips, Microfilters, etc.



Sequencing



Whole genome amplification

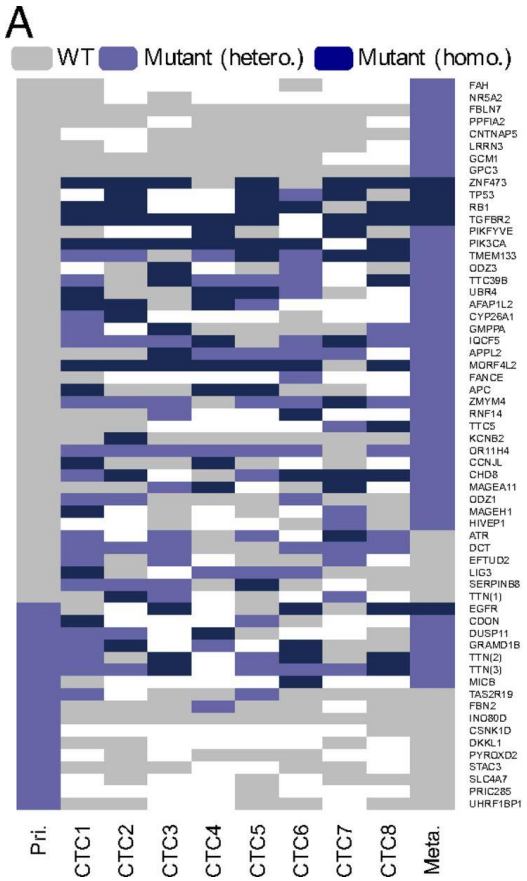


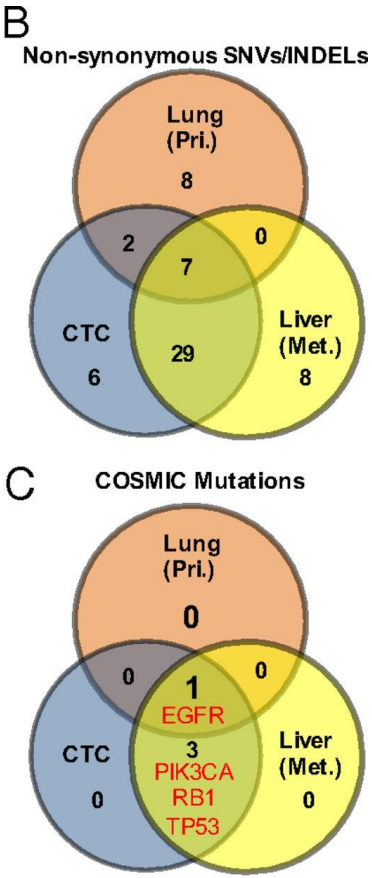
Reproducible copy number variation patterns among single circulating tumor cells of lung cancer patients

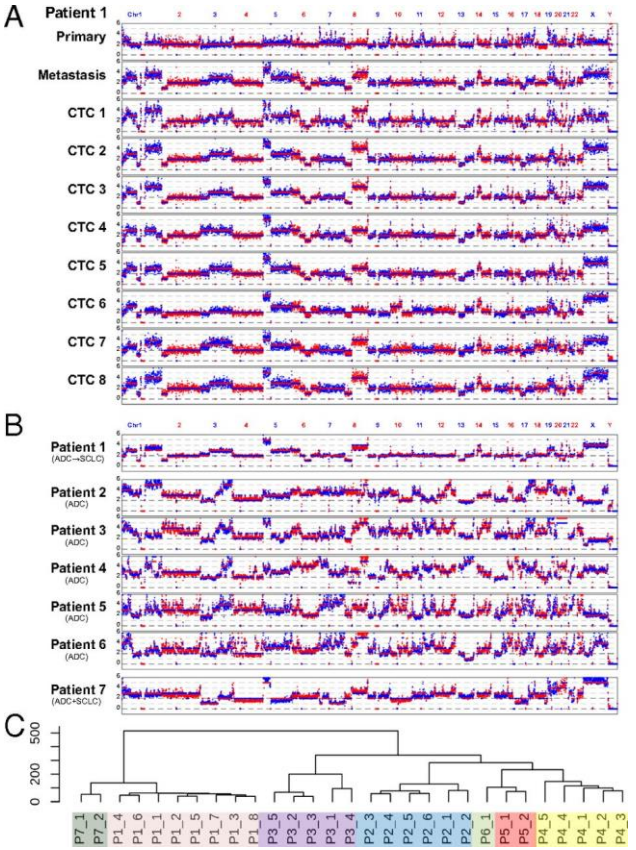
Xiaohui Ni[a,b,1], Minglei Zhuo[c,1], Zhe Su[a,1], Jianchun Duan[c,1], Yan Gao[a,1], Zhijie Wang[c,1], Chenghang Zong[b,1,2], Hua Bai[c], Alec R. Chapman[b,d], Jun Zhao[c], Liya Xu[a], Tongtong An[c], Qi Ma[a], Yuyan Wang[c], Meina Wu[c], Yu Sun[e], Shuhang Wang[c], Zhenxiang Li[c], Xiaodan Yang[c], Jun Yong[b], Xiao-Dong Su[a], Youyong Lu[f], Fan Bai[a,3], X. Sunney Xie[a,b,3], and Jie Wang[c,3]

PNAS

110:21083-21088, 2013







PROS AND CONS OF CTC ANALYSIS

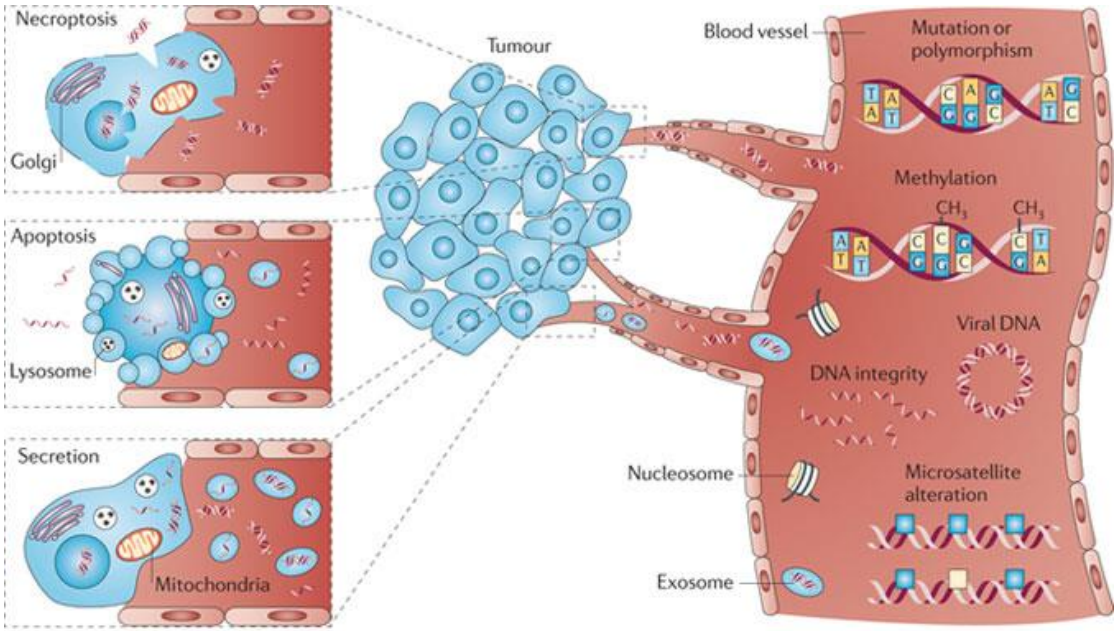
PROS

- **An excellent research tool**
- Powerful for investigating tumour heterogeneity and tumorigenicity (CTC derived xenografts)
- **Ability to assess morphology, protein expression and genetic aberrations all in the same cell**
- May help better understand mechanisms of metastasis and identify novel treatment targets

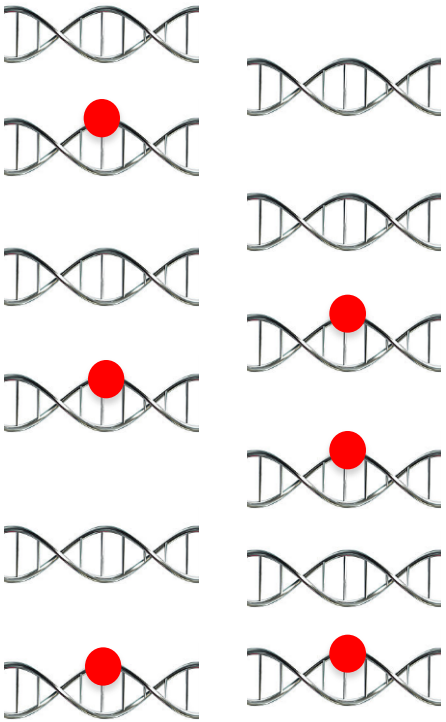
CONS

- **Lack of validation/qualification for most technologies**
- Expensive
- **Wide variations in CTC definition and technologies**
- Difficulty in reproducing assays across multiple sites limits ‘routine’ clinical use
- Need joined up approach across CTC community to validate assays

CIRCULATING TUMOUR DNA



Schwarzenbach et al, Nat Rev Cancer, 2011



Scorpion amplification refractory mutation assay (SARMS)

Sensitivity down to 1-2%

DETERMINATION OF EGFR STATUS BY ctDNA IN LUNG CANCER

- Phase IV study of gefitinib in EGFR mutation positive advanced NSCLC
- Compared EGFR status in tumour block and plasma using Scorpion ARMS assay

- 1033/1060 had evaluable tumour
- Mutation status determined for 859 patients
- 118/859 (13.7%) had EGFR mutation

- 803/1060 had 2 baseline plasma samples
- Mutation status determined for 784 patients
- 82/784 (10.5%) had EGFR mutation

Concordance = 94.3%

Sensitivity 66%

Specificity 100%

Douillard et al, Journal of Thoracic Oncology, Sep 2014

DETERMINATION OF EGFR STATUS BY ctDNA IN LUNG CANCER

- Phase IV study of gefitinib in EGFR mutation positive advanced NSCLC
- Compared EGFR status in tumour block and plasma using Scorpion ARMS assay

IRESSA receives CHMP positive opinion to include blood based diagnostic testing in European label

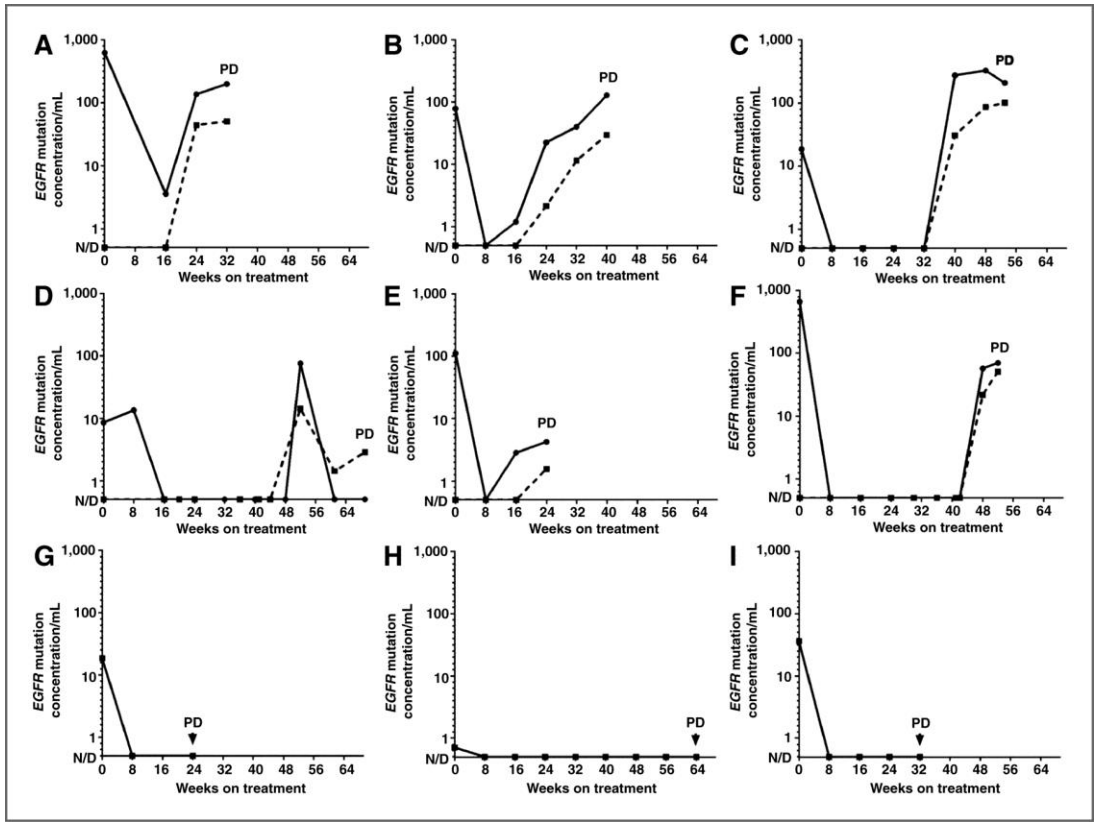
Fri, 26th Sep 2014

In cases where tumour material is not available, gefitinib may be started if EGFR mutation positive from ctDNA

Douillard et al, Journal of Thoracic Oncology, Sep 2014

DIGITAL PCR AND DISEASE MONITORING IN EGFR +VE DISEASE

- **DIGITAL PCR increases sensitivity further and provides quantification of mutated alleles**



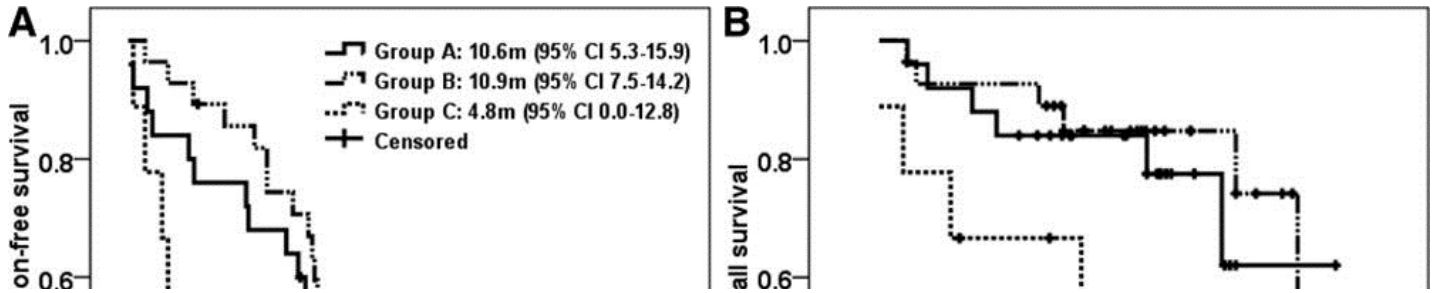
↑ in mutated ctDNA occured upto 16 weeks ahead of confirmed PD

T790M detectable ahead of clinical PD

Oxnard et al, Clinical Cancer Research, Mar 2014

FAILURE TO ERADICATE ctDNA = WORSE OS

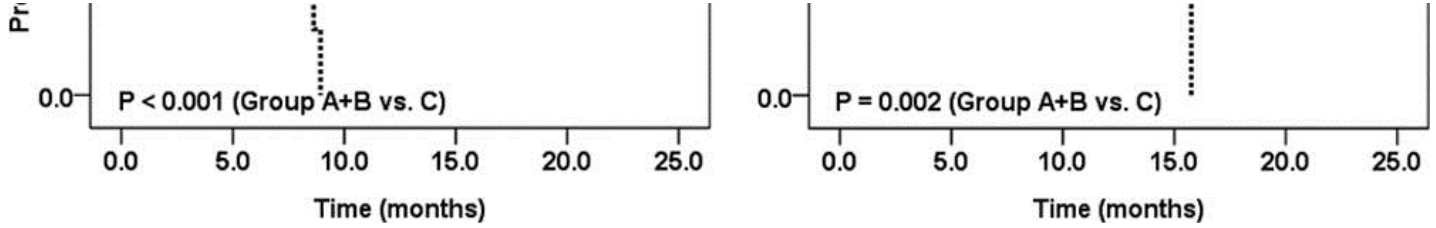
N=62



Clin Cancer Res. 2015 Mar 31. pii: clincanres.2594.2014. [Epub ahead of print]

Detection and Dynamic Changes of EGFR Mutations from Circulating Tumor DNA as a Predictor of Survival Outcomes in NSCLC Patients Treated with First-line Intercalated Erlotinib and Chemotherapy.

Mok TS[1], Wu YL[2], Soo Lee J[3], Yu CJ[4], Sriuranpong V[5], Sandoval-Tan J[6], Ladrera G[7], Thongprasert S[8], Srimuninnimit V[9], Liao M[10], Zhu Y[11], Zhou C[12], Fuerte F[13], Margono B[14], Wen W[15], Tsai J[16], Truman M[17], Klughammer B[18], Shames DS[19], Wu L[20].

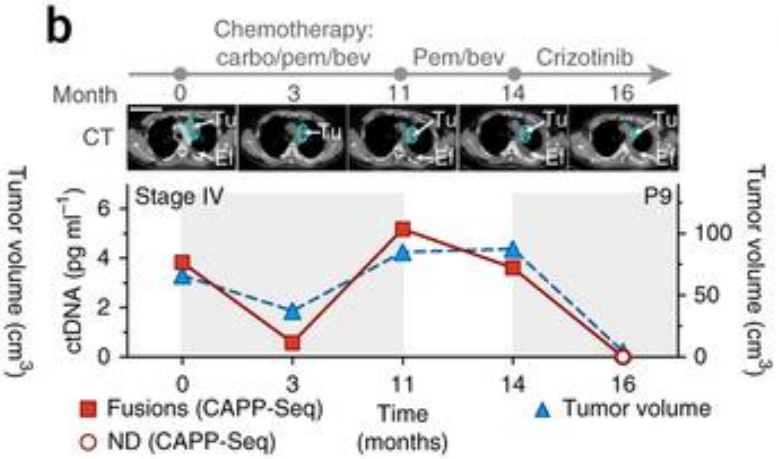


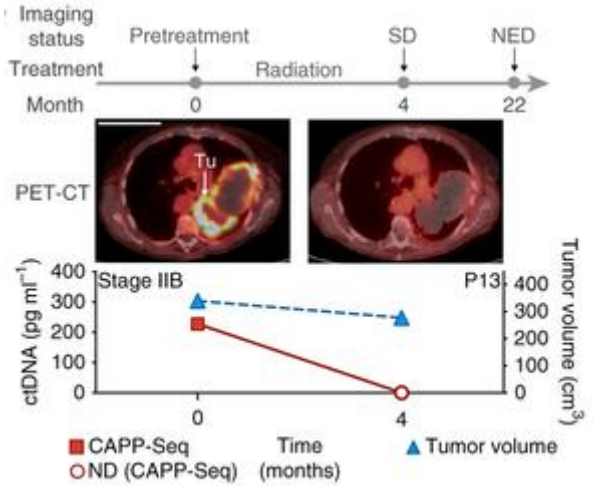
Group A = no mutation identifiable in ctDNA at baseline
Group B = EGFR mutation eradicated by 10 weeks on EGFR inhibitor
Group C = EGFR mutation not eradicated by 10 weeks on EGFR inhibitor

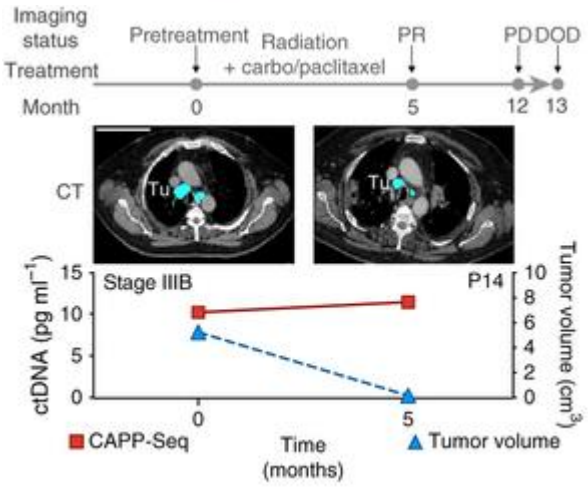
Tseng et al, Journal of Thoracic Oncology, Apr 2015 & Mok et al, Clinical Cancer Research, Mar 2015

QUANTIFICATION OF ctDNA IS COMPLEMENTARY TO IMAGING

Novel sensitive method for ctDNA quantification (CAPP-seq)







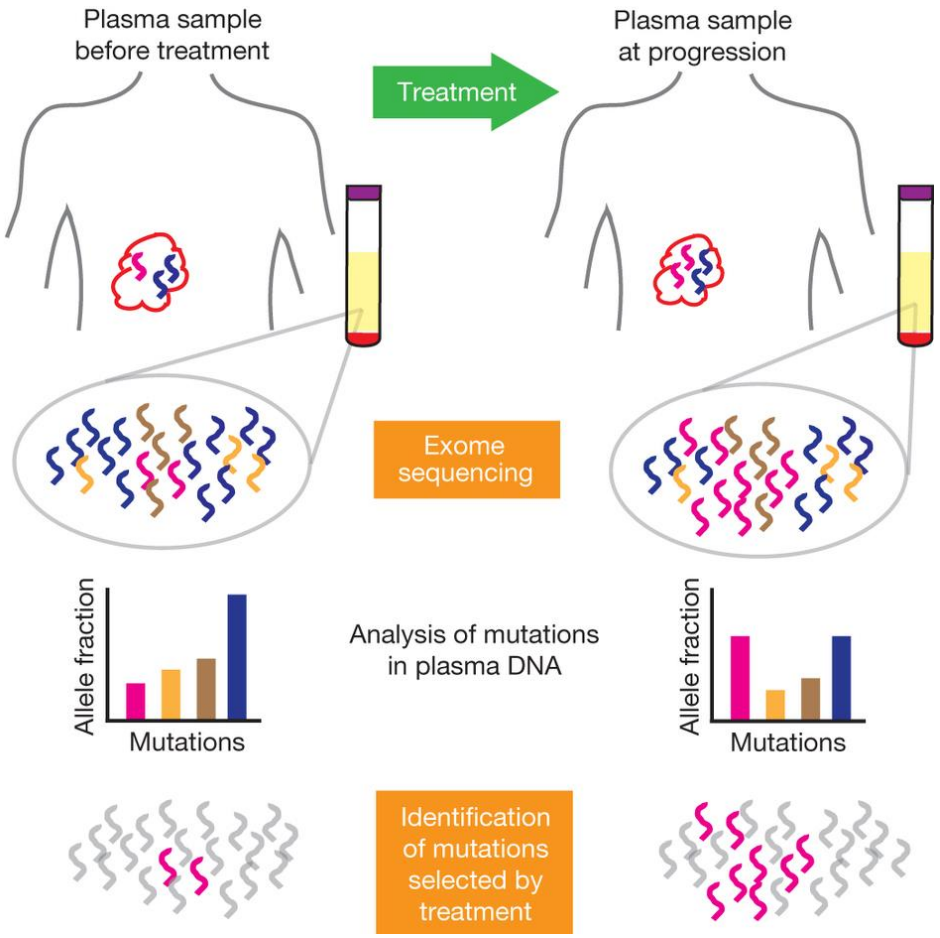
ctDNA

Imaging

nature medicine

Newman et al, May 2014

REISTANCE MECHANISMS FROM ctDNA – WHOLE EXOME SEQUENCING



‘Genome-wide representation of tumour in plasma’

‘Increased representation of mutant alleles in association with therapy resistance’

‘A new paradigm for the study of clonal evolution’

nature

Murtaza et al, May 2013

PROS AND CONS OF ctDNA ANALYSIS

PROS

- Various clinical applications of ctDNA –quantification and profiling
- **DNA easy to extract and profiling assays are much more amenable to validation and qualification than CTCs**
- Straight-forward blood test
- Already in clinic with EGFR mutation testing and gefitinib

CONS

- **Different methods across different centres**
- **Quantification and whole exome sequencing are still in their infancy** with reports including relatively small numbers of patients
- Technically challenging to look at whole exome from ctDNA
- **May not be suitable for every patient** – ? Stage IV/tumour burden; otherwise need biopsy
- Released from apoptosing cells - ? relevance

TUMOUR CHARACTERISATION TO GUIDE EXPERIMENTAL TARGETED THERAPY – THE TARGET TRIAL

Manchester Cancer Research Centre

TARGET

To molecularly profile ctDNA of all patients referred for consideration of phase 1 clinical trials
To select a relevant trial based on ctDNA ‘actionable aberrations’

Clinical trial selection

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MANCHESTER 1824
The University of Manchester

RECRUITNG CLINICAL FELLOWS AND SENIOR LECTURERS TO ECMT IN MANCHESTER

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elcc

15-18 April 2015, Geneva, Switzerland

Organisers IASLC International Association for the Study of Lung Cancer; ESMO European Society for Medical Oncology

Partners ESTRO European Society for Radiotherapy & Oncology; European Society of Thoracic Surgeons 1993; etop