Targeting PI3K/AKT: Biomarkers for PhAT Studies

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Professor in Experimental Cancer Medicine
The Institute of Cancer Research, and
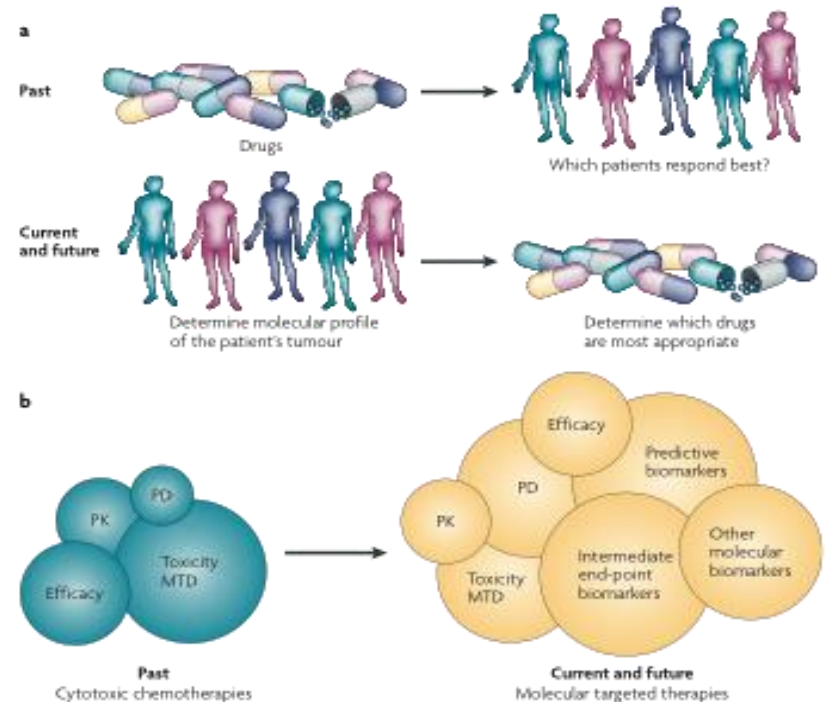The Royal Marsden Hospital

Sitges 2014

Overview

- The PhAT
- Predictive biomarkers
- Pharmacodynamic biomarkers
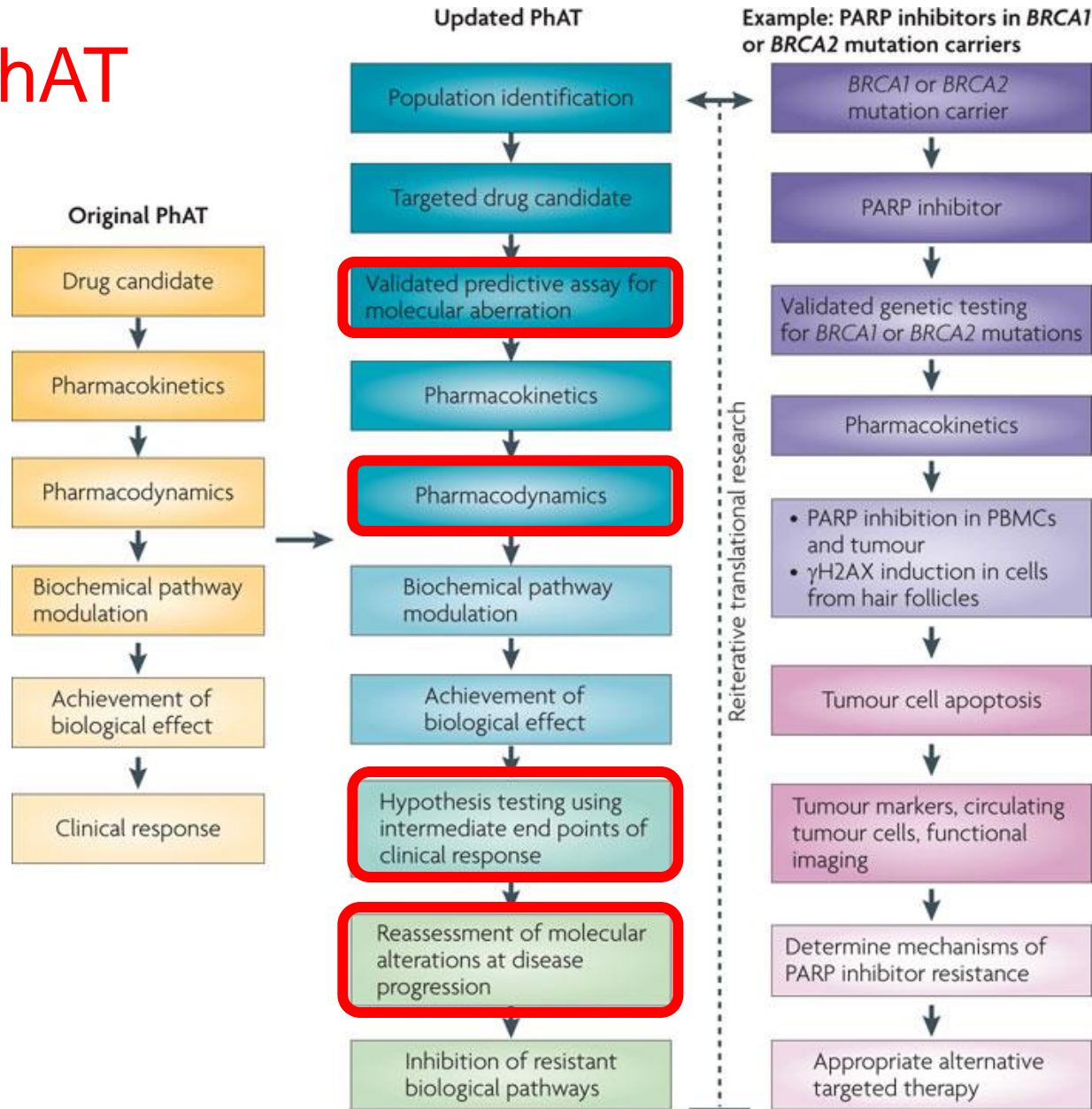- Circulating biomarkers
- Clonal evolution

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The Pharmacological Audit Trail (PhAT)

- PhAT
  - Biological basis
  - Patient population
  - Identify target & drug
  - Hypothesis testing trials using biomarkers

Yap, Sandhu, Workman & de Bono, Nature Reviews Cancer 2010

The PhAT

Original PhAT

Drug candidate → Pharmacokinetics → Pharmacodynamics → Biochemical pathway modulation → Achievement of biological effect → Clinical response

Updated PhAT

Population identification → Targeted drug candidate → Validated predictive assay for molecular aberration → Pharmacokinetics → Pharmacodynamics → Biochemical pathway modulation → Achievement of biological effect → Hypothesis testing using intermediate end points of clinical response → Reassessment of molecular alterations at disease progression → Inhibition of resistant biological pathways

Reiterative translational research

Example: PARP inhibitors in *BRCA1* or *BRCA2* mutation carriers

BRCA1 or *BRCA2* mutation carrier → PARP inhibitor → Validated genetic testing for *BRCA1* or *BRCA2* mutations → Pharmacokinetics → • PARP inhibition in PBMCs and tumour • γH2AX induction in cells from hair follicles → Tumour cell apoptosis → Tumour markers, circulating tumour cells, functional imaging → Determine mechanisms of PARP inhibitor resistance → Appropriate alternative targeted therapy

Nature Reviews | Cancer

It ain't over until the PhAT lady sings!

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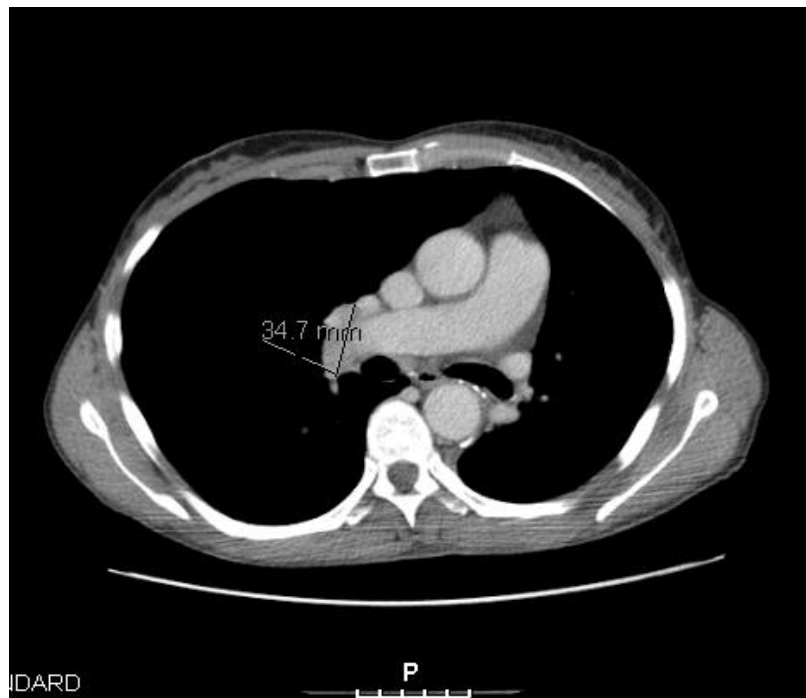- Circulating biomarkers
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Patient Enrichment/Putative Predictive Biomarkers for PI3K/AKT inhibitors

- Mutation: eg PIK3CA, AKT

- Amplification: eg PIK3CA, AKT

- Loss of function: eg PTEN, INPP4B, PHLLP1, FBXW7

For Drug combinations eg MEK/AKT
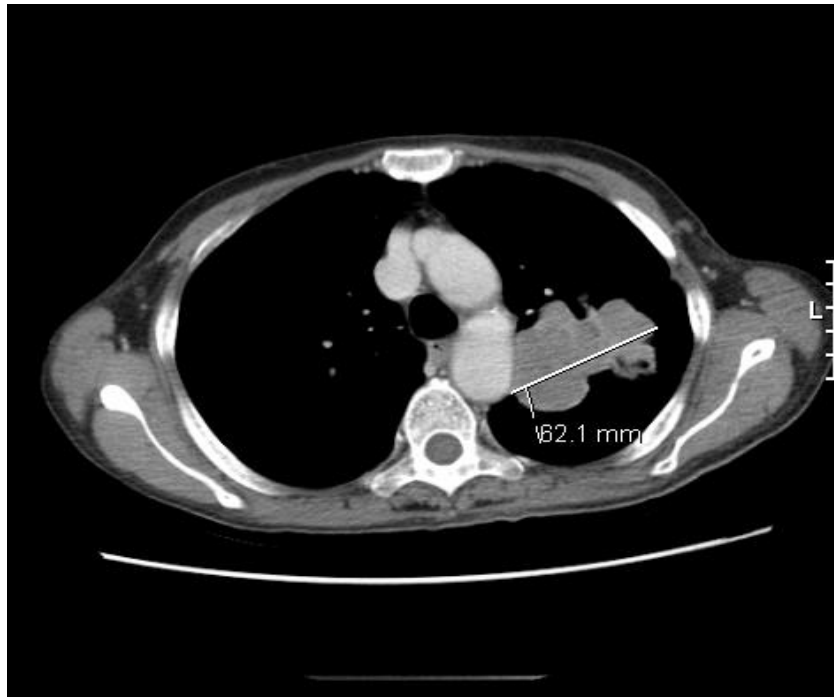
- KRAS mutation

KRAS Mutant NSCLC: MEK and AKTi

January 2011

December 2011

KRAS Mutant NSCLC: MEK + AKTi

October 2012

December 2012

Mutation detection

- Archival tissue
- Fresh tissue at treatment preferable
- Liquid biopsies

Case study: RMH Patient

- 72 year-old male with stage 4 pancreatic cancer with hepatic and peritoneal metastases

- Multiple prior therapies

- *PIK3CA* and *KRAS* not mutated (plasma DNA analyses)

- Loss of PTEN expression (tumor)

Molecular Biomarkers Group, **The Institute of Cancer Research, UK**
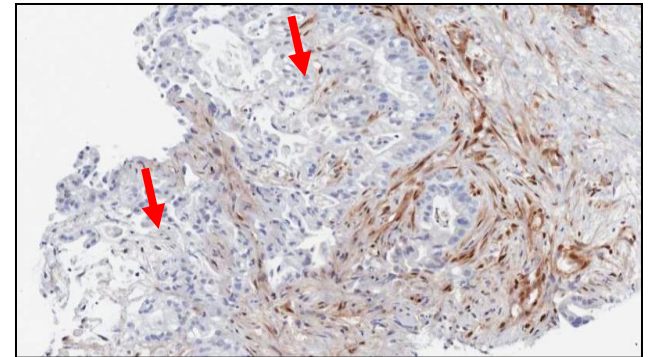
Case study - 60 mg QOD MK2206 PD

Tumor and normal tissue PD
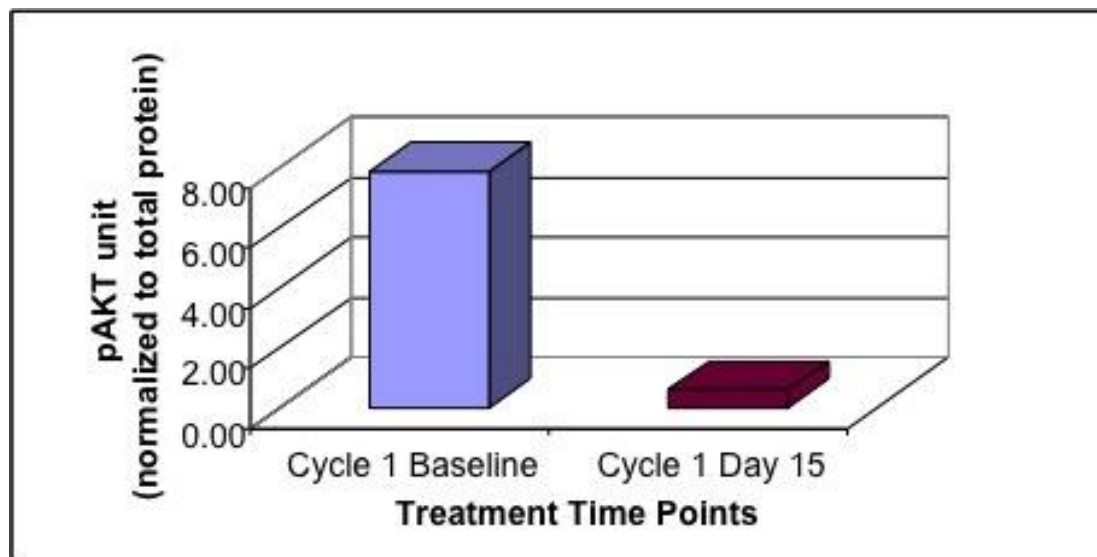
Tumor

↓ pSer473 AKT

Clinical Development Lab, Merck   MSD® platform
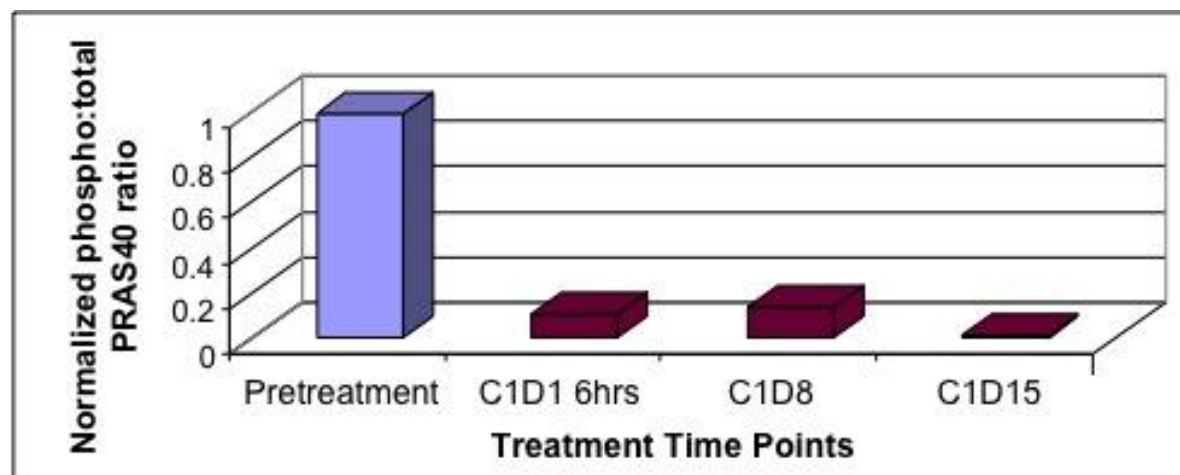
Hair follicles

↓ pThr246 PRAS40

Immunofluorescence analysis

Clinical PD Biomarker Group, The Institute of Cancer Research

Case study

60 mg QOD MK2206 treatment:
- 50% shrinkage of the largest hepatic lesion after 4 months on treatment, RECIST response of 23%
- ~60% decrease in CA19-9 tumor marker levels

4.5 cm

2.0 cm
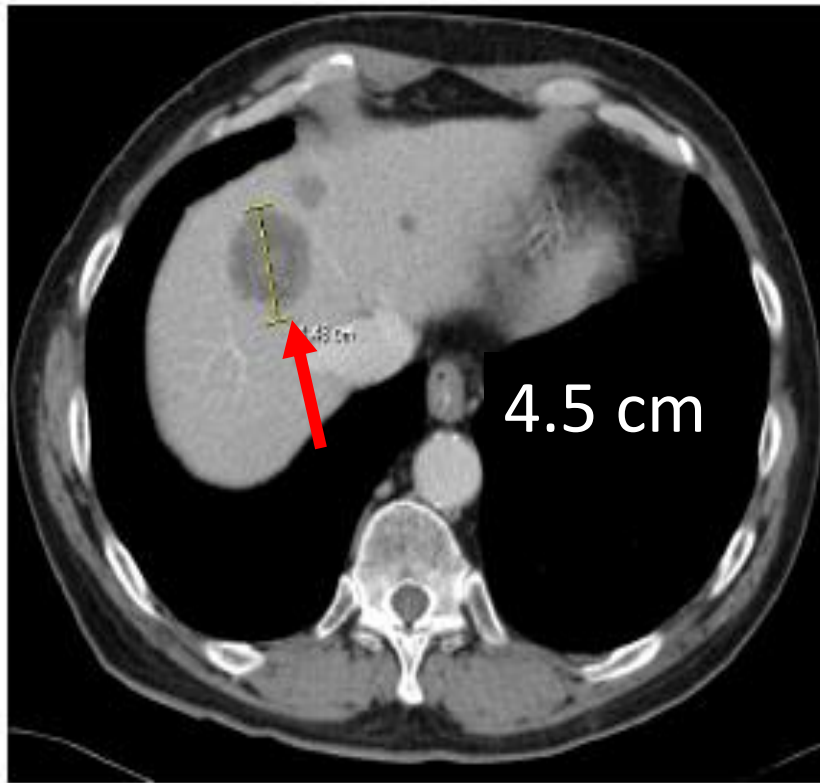
Baseline
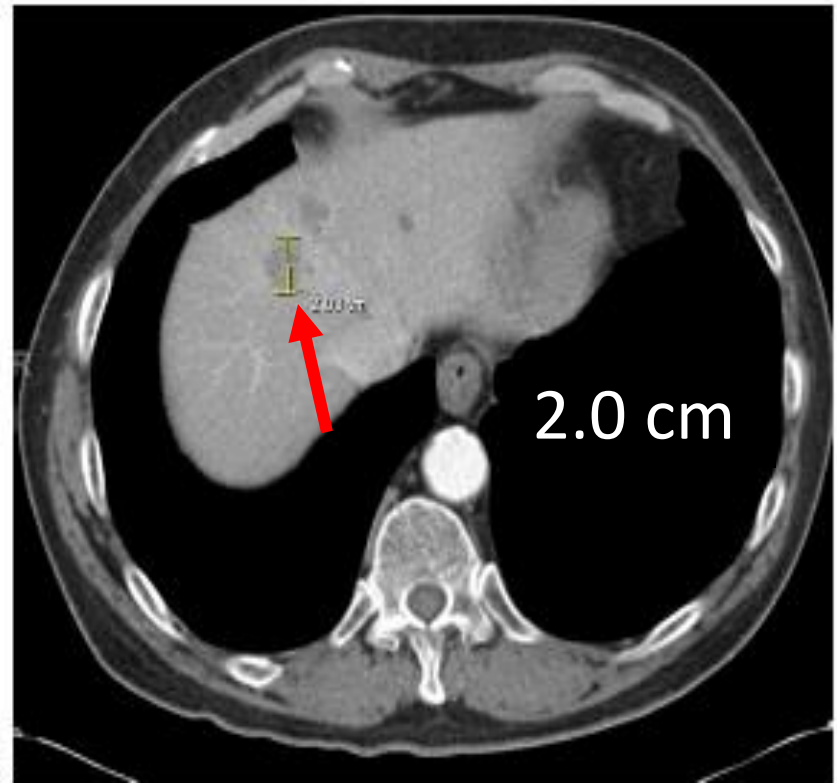
4 months post-treatment

Some Challenges

- Defining 'cut off' for patient selection
  - Eg How much PTEN loss is enough? IHC/IF not quantitative
- Multiple genetic changes can activate pathway
  - Eg PTEN wt may still be pathway activated
- Redundancy
  - Eg If one inhibits p110beta in PTEN loss cancers will p110a take over signaling? p110a mutation may not signal just through AKT

Abiraterone Sensitivity & PTEN Loss (ESMO 2013)

ABSTRACT ID: 2.930

The ROYAL MARSDEN
NHS Foundation Trust

ICR | The Institute of Cancer Research

Clinical benefit on abiraterone acetate (AA) in patients (pts) with PTEN loss castration-resistant prostate cancer (CRPC)

Omlin A, Pezaro C, Reid A, Nava Rodrigues D, Riisnaes R, Miranda S, Tunariu N, Lorente D, Attard G, de Bono J

Prostate Cancer Targeted Therapies Group, The Royal Marsden NHS Foundation Trust and The Institute of Cancer Research, Sutton, Surrey

Introduction

The CYP17A1 inhibitor AA is an effective but costly treatment for CRPC, which is a molecularly heterogeneous disease. It has been postulated that upregulated AKT pathway signalling through PTEN loss results in resistance to AA (Carver Cancer Cell 2011). We therefore aimed to evaluate the impact of PTEN loss on the anti-tumour activity of AA in CRPC in an attempt to deliver more precise treatment for this disease.

Methods

- Patients were identified from a population of men with CRPC treated at the Royal Marsden NHS Foundation Trust. Eligible patients had at least one tissue sample available for analysis.
- All patients signed ethics approved consent.
- Tissue samples of hormone sensitive (HS) and castration resistant (CR) disease were collected.

PTEN Analysis
- Four uM tissue sections of formalin-fixed and paraffin-embedded tissue were cut and immunostained for PTEN (Cell Signaling Technology #9559)
- Standard heat induced antigen retrieval methods was used as described earlier (Reid Mod. Pathology 2012).
- PTEN was scored on a minimum of 100 cancer cells per slide and an H-score was calculated for each patient sample.
- H-score >30 was considered positive. Cases were analysed only if positive internal controls were present.

Abiraterone response criteria
- Baseline clinical and laboratory variables were collected from the hospital electronic record system.
- Biochemical response to abiraterone was defined as per PCWG2 and soft tissue responses were defined as per RECIST 1.1.

Statistical analysis
- Treatment duration and survival were estimated using the Kaplan Meier method.
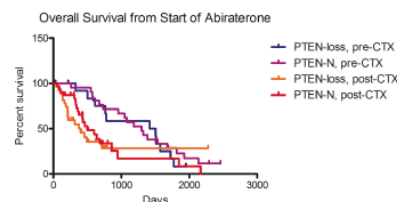- Descriptive statistics and survival analyses were performed using IBM SPSS Statistics v20 (IBM).

NHS

Patient Characteristics

	AA & PTEN normal (N=52)	AA & PTEN loss (N=41)
Median age at diagnosis (years)	63.1	60.6
- Range	43.9– 79.9	43.6– 75.2
Median Gleason Score	8	7
- Range	4–10	5–9
AA pre-Docetaxel (N=35)		
	PTEN normal N=21	**PTEN loss N=14**
ECOG Performance status at AA, N (%)		
- 0	11 (52)	7 (50)
- 1	10 (48)	6 (43)
- 2		1 (7)
Metastases at AA, N (%)		
- Bone	16 (76)	11 (79)
- Nodal	10 (48)	5 (36)
- Visceral	3 (14)	2 (14)
Median PSA at AA (µg/l)	54	119.5
- Range	11.2 – 964	8.8 – 499
AA post-Docetaxel (N=58)		
	PTEN normal N=31	**PTEN loss N=27**
ECOG Performance status at AA, N (%)		
- 0	7 (23)	5 (19)
- 1	21 (68)	17 (63)
- 2	2 (6)	4 (15)
- NA	1 (3)	1 (4)
Metastases at AA, N (%)		
- Bone	28 (90)	25 (93)
- Nodal	19 (61)	14 (52)
- Visceral	2 (6)	10 (37)
Median PSA at AA (µg/l)	413	318
- Range	47 – 6385	22 – 10335

Clinical Activity by PTEN loss

	AA pre-docetaxel				p
	PTEN normal N=21		PTEN loss N=14		
PSA decline	N	%	N	%	
≥50%	16	76	9	64	
≥90%	7	33	4	29	
Time on AA, m	11.4		12.0		P=0.56
Median Survival, m	42.7		48.0		P=0.64
	AA post-docetaxel				
	PTEN normal N=31		PTEN loss N=27		
PSA decline	N	%	N	%	
≥50%	14	45	13	48	
≥90%	3	10	6	22	
Time on AA, m	5.4		5.0		P=0.56
Median Survival, m	16.4		13.2		P=0.53

Overall Survival from Start of Abiraterone

PTEN-loss, pre-CTX
PTEN-N, pre-CTX
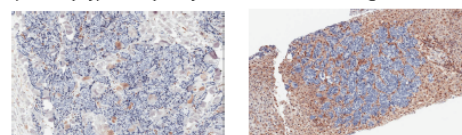PTEN-loss, post-CTX
PTEN-N, post-CTX

Examples of PTEN staining

Patient with normal PTEN by IHC in HS (primary prostate biopsy) and CR tissue (lymph node biopsy)
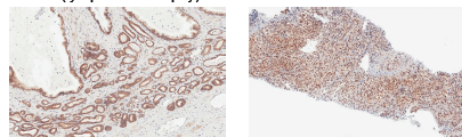
Patient with PTEN loss by IHC in HS (primary prostate biopsy) and CR tissue (liver biopsy), the hepatocytes shows normal staining for PTEN.

Results

- Patient-matched HS and CR tissue samples were available for 49 pts.
- HS tissue showed staining consistent with PTEN loss in 25/49 pts (51%).
- In CR samples PTEN loss was identified in 28/49 (57%).
- Heterogeneity was evident between HS and CR samples, with changed classification from PTEN normal to PTEN loss in 3 pts (6%) and conversely from PTEN loss to PTEN normal in another 3 pts (6%).
- AA activity and survival data in 93 pts stratified by PTEN status are presented in the table 2 and show no difference in time on AA or median overall survival from start of AA in both chemotherapy-naïve and post-docetaxel patients. The rates of PSA declines of ≥50% are also similar in all four cohorts.

Conclusion

- PTEN status does not significantly change with development of castration resistance.
- AA retains significant activity in patients with PTEN loss, both in chemotherapy naïve and docetaxel pre-treated patients in our population.
- Further biomarker studies, including markers that associate specifically with activation of the Pi3K/AKT pathway are urgently required.

Acknowledgements

Bottom Line: PTEN loss does not impact abiraterone sensitivity. Abi and enza combos ongoing

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Pharmacodynamic Biomarkers: Challenges

- Key to Go/No Go decisions
    - No target modulation – drug development termination

Pharmacodynamic Biomarkers: Challenges

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- How?
  - Normal tissue: Platelet rich plasma, hair follicles preferable
  - Tumour tissue key…..but intrapatient heterogeneity
  - Molecular imaging

Pharmacodynamic Biomarkers: Challenges

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- How much knockdown necessary for tumour cell kill?
  - Need for xenograft data

Pharmacodynamic Biomarkers: Challenges

- Key to Go/No Go decisions
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  - Tumour tissue key…..but intrapatient heterogeneity
  - Molecular imaging
- How much knockdown necessary for tumour cell kill?
  - Need for xenograft data
- Feedback loops:
  - Desensitization? Homeostatic feedback.

Other MAJOR Challenges

- Schedule selection

- Can we target tumour tissue enough?
  - Does toxicity allow it? More selective inhibitors have advantages as may be less toxic but may allow redundant leakage of signal.
    - p110 beta inhibition may be preferable for PTEN loss cancers
    - Selective p110 alpha inhibition may be preferable for PIK3CA mutant cancers

Two examples of trials I have conducted of a p110α inhibitor and of an AKT inhibitor

Example 1: Skin rash with AKTi MK2206

Toxicity may limit drug dosing that inhibits intratumoral target hard enough

It may be easier to block normal cell target: Better drug distribution

MK2206: PK-PD
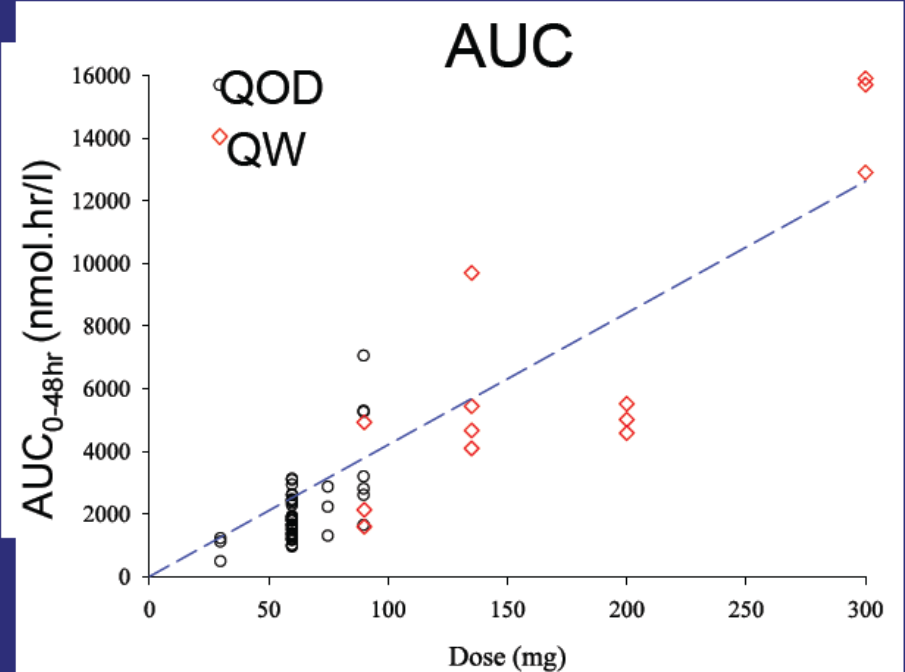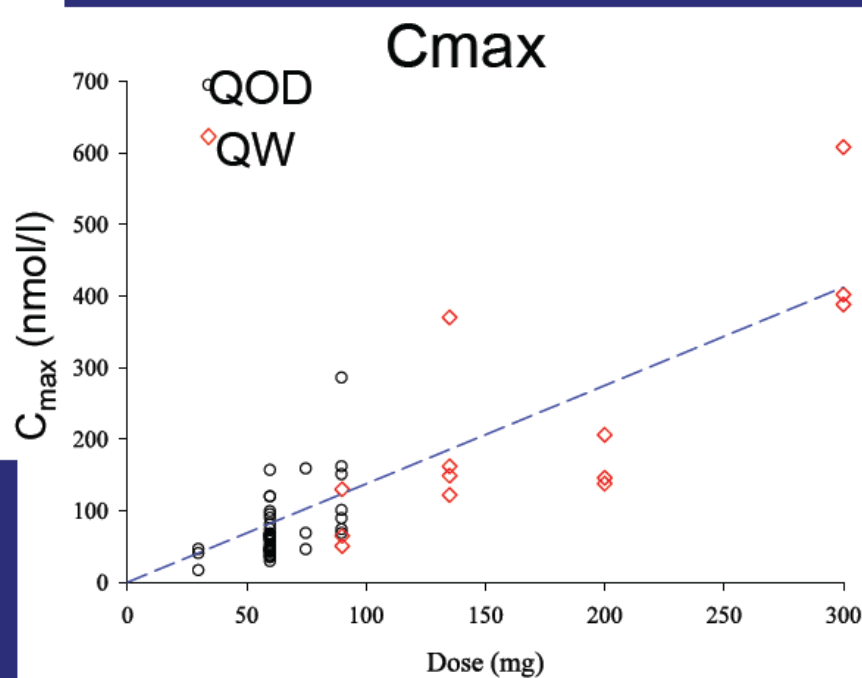
- Long terminal half-life ($t_{1/2}$) of 60–80 hours
- No substantial departure from dose-proportionality
- PK data support QOD and QW dosing

Normal tissue Pharmacodynamics - Platelet-rich plasma (60mg QOD MK2206): How much is enough?!!!!!
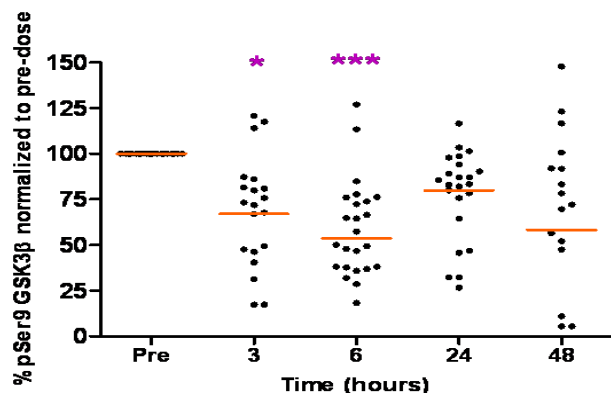
pSer473 AKT (n=25)

pSer9 GSK3β (n=25)

pThr246 PRAS40 (n=9)

☞ *Enough?*

Paired t-test (predose vs treated) *p<0.05, **p<0.01, ***p<0.001

Clinical PD Biomarker Group,

The Institute of Cancer Research, UK
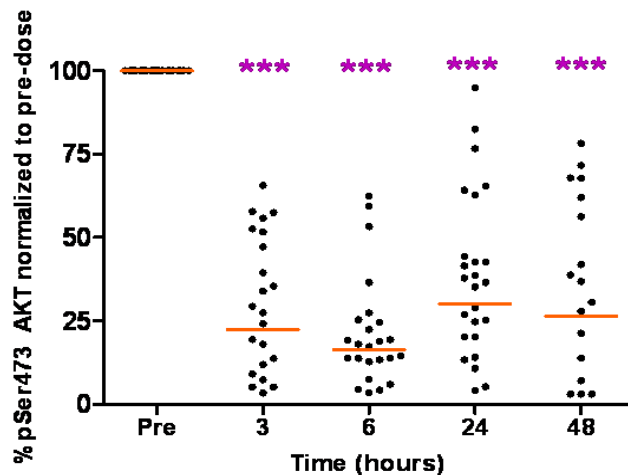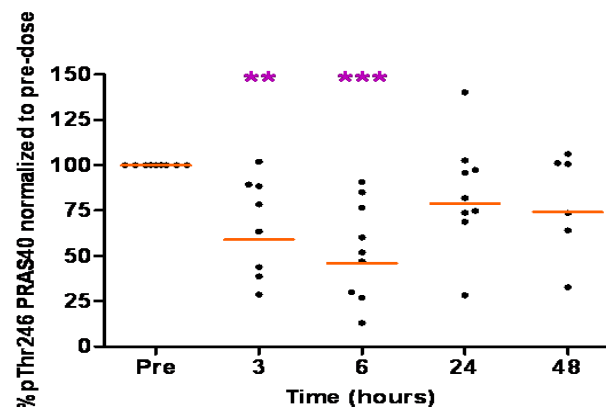
Normal tissue Pharmacodynamics - Platelet-rich plasma (QW MK2206): How much is enough?!!!!!!

pSer473 AKT
QW MK2206
(n=11)
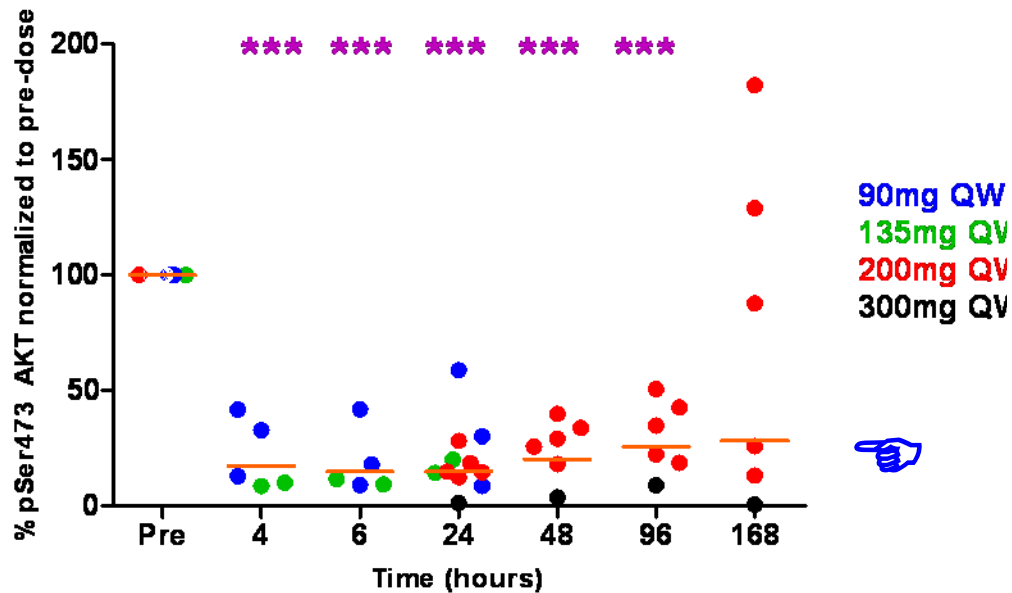
pSer473 AKT
200mg QW MK2206
(n=5)

90mg QW
135mg QW
200mg QW
300mg QW
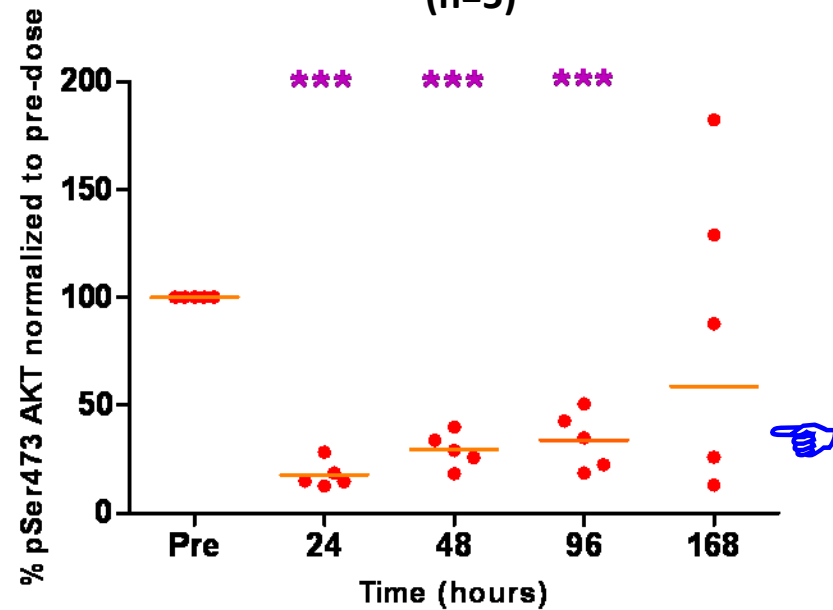
AKT target modulated ☞ *Enough?*

Paired t-test (predose vs treated) ***p<0.001

Tumor PD (MSD assay)
60 mg QOD (MTD)
N = 12
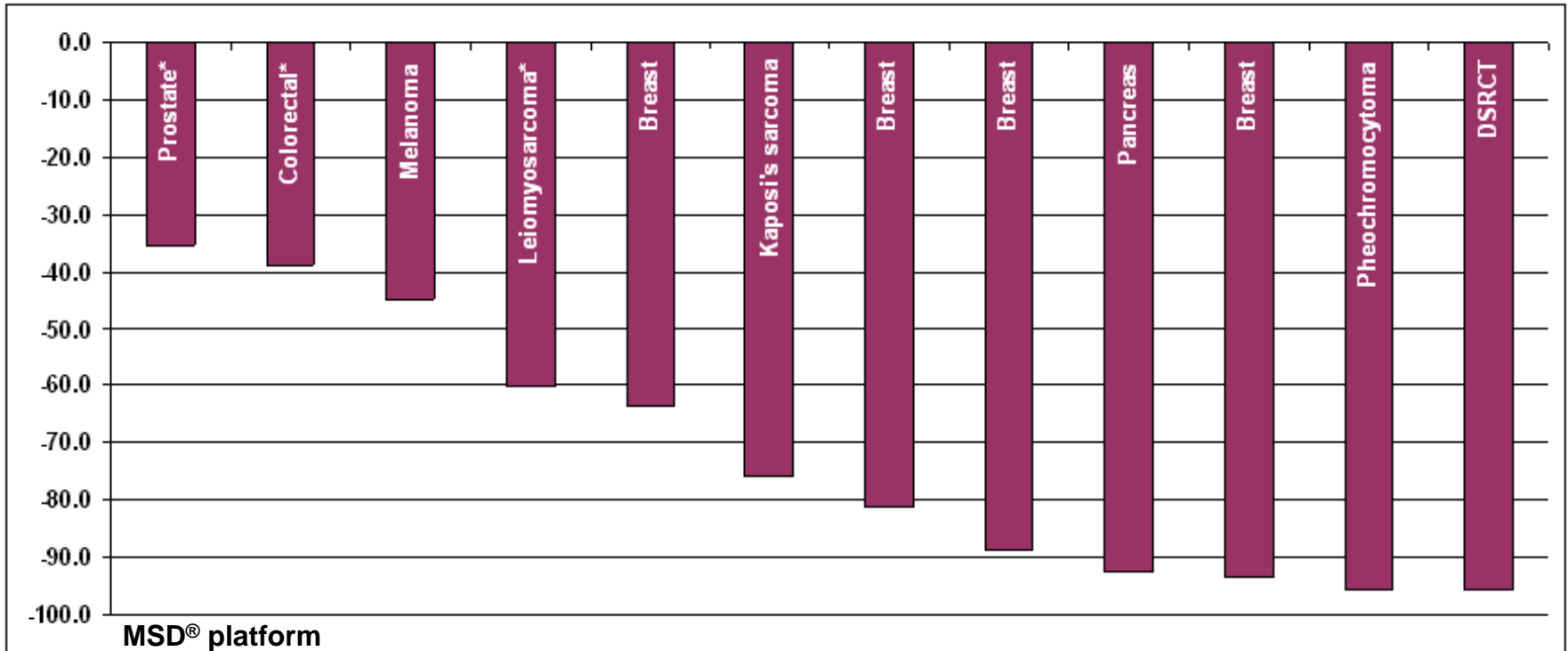
- Tumor pSer473 AKT decreased post-MK2206 in all 12 patients
- $\geq$ 50% decrease of pSer473 AKT in 9 of 12 patients
- AKT target modulated in tumor

Impact of AKT blockade by MK2206: Feedback in cell lines and patient biopsies
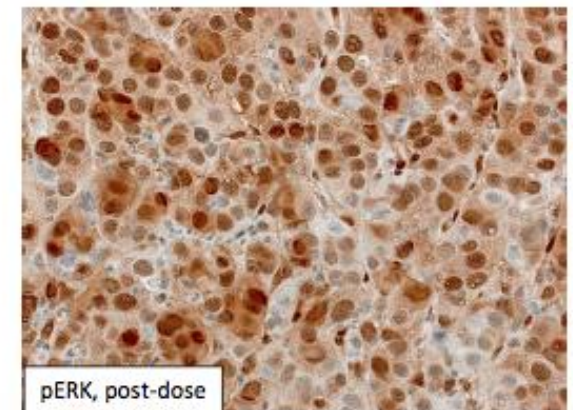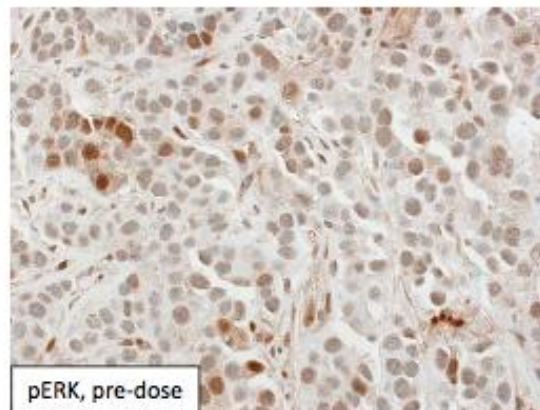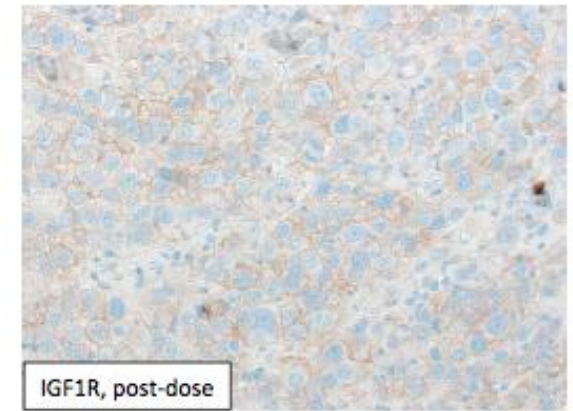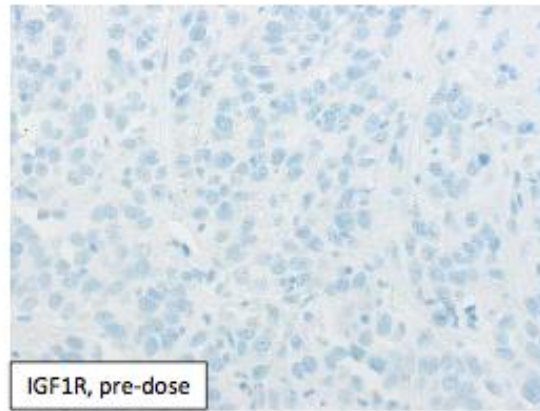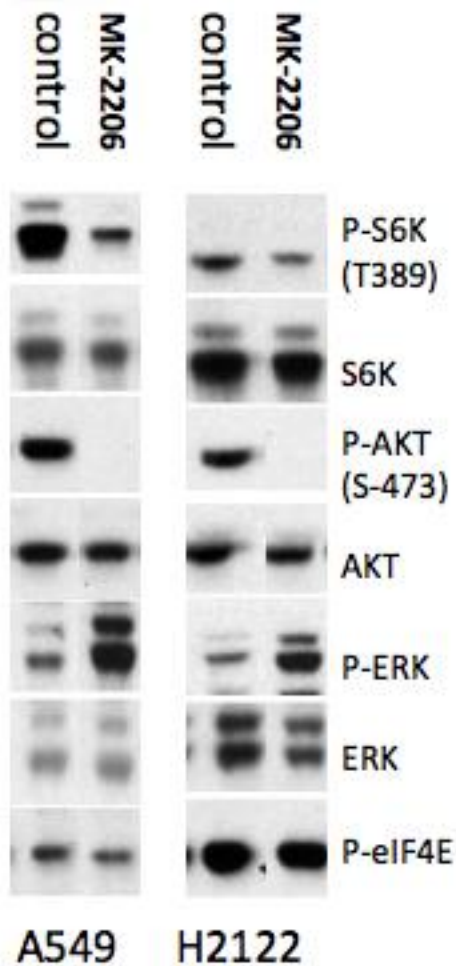
Schedule Selection

- Randomised Phase II studies preferable comparable schedule but not usually pursued
- Evaluation in Phase I studies in parallel cohorts
  - Second best but better than not studied at all

Example 2: Pictilisib (GDC-0941) Phase I

• Drug designed by ICR scientists

PK Data
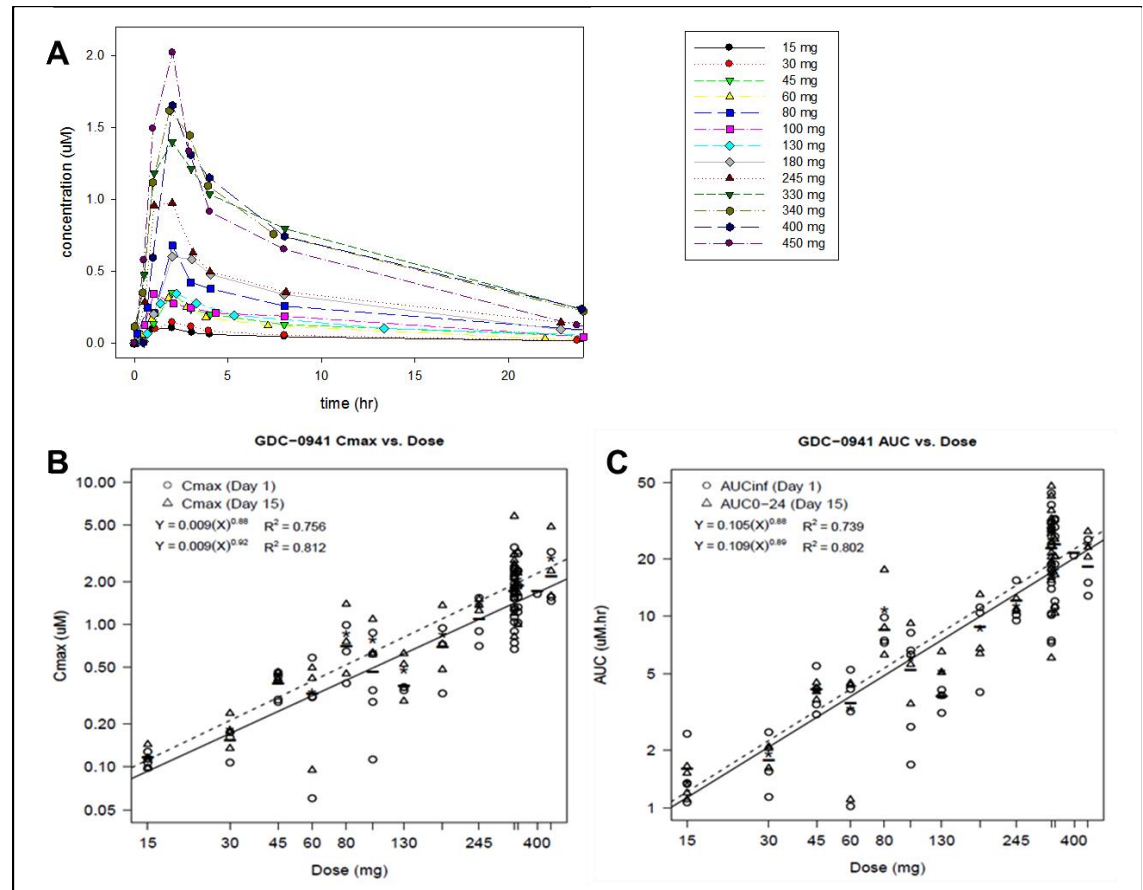
Example 2: Pictilisib (GDC-0941) Phase I

- Drug designed by ICR scientists; outlicensed to Genentech
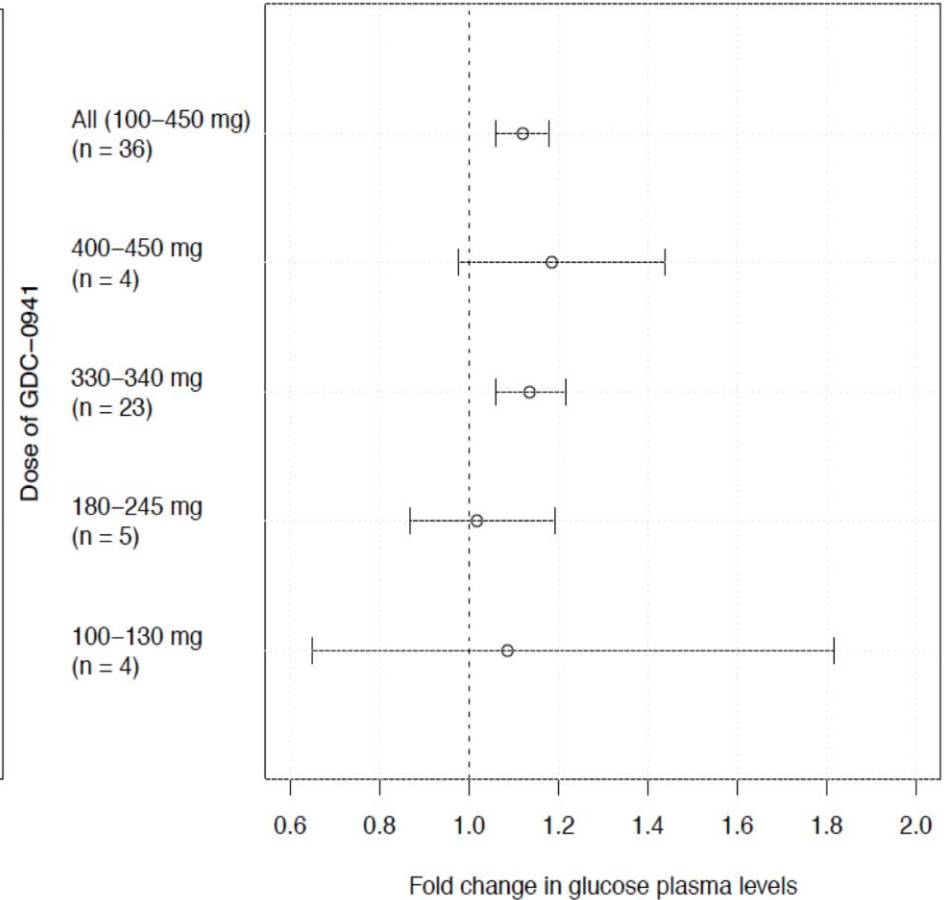
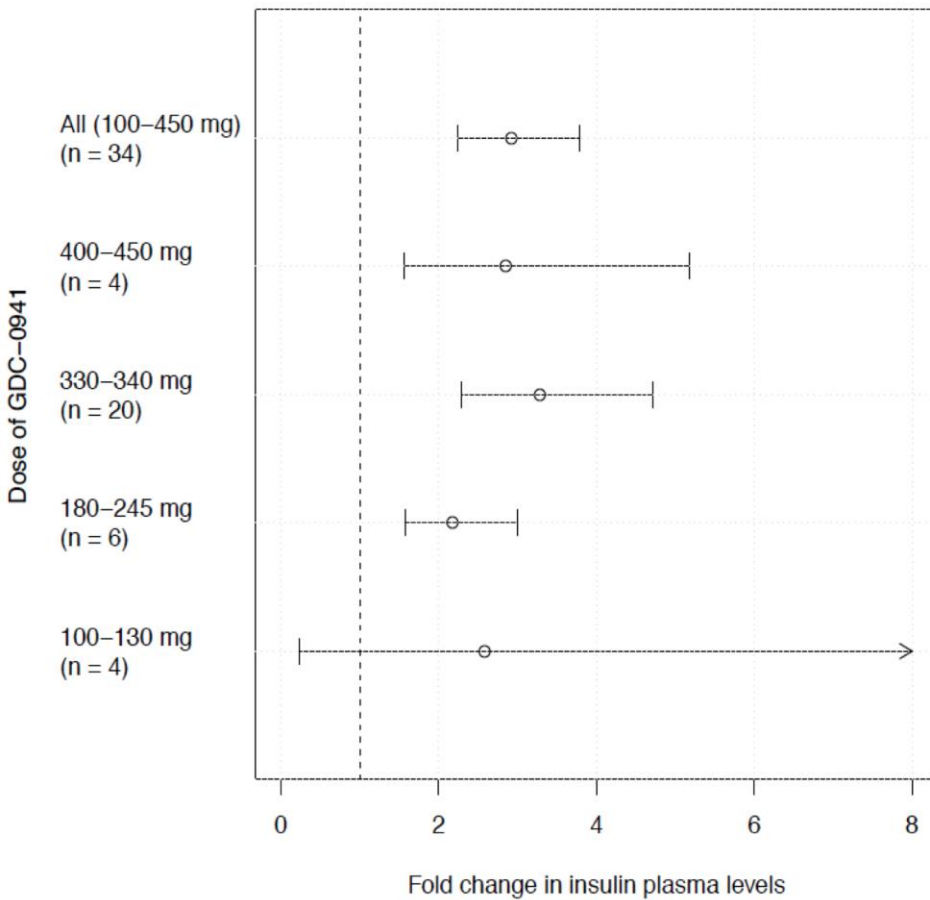*PK-PD Data*
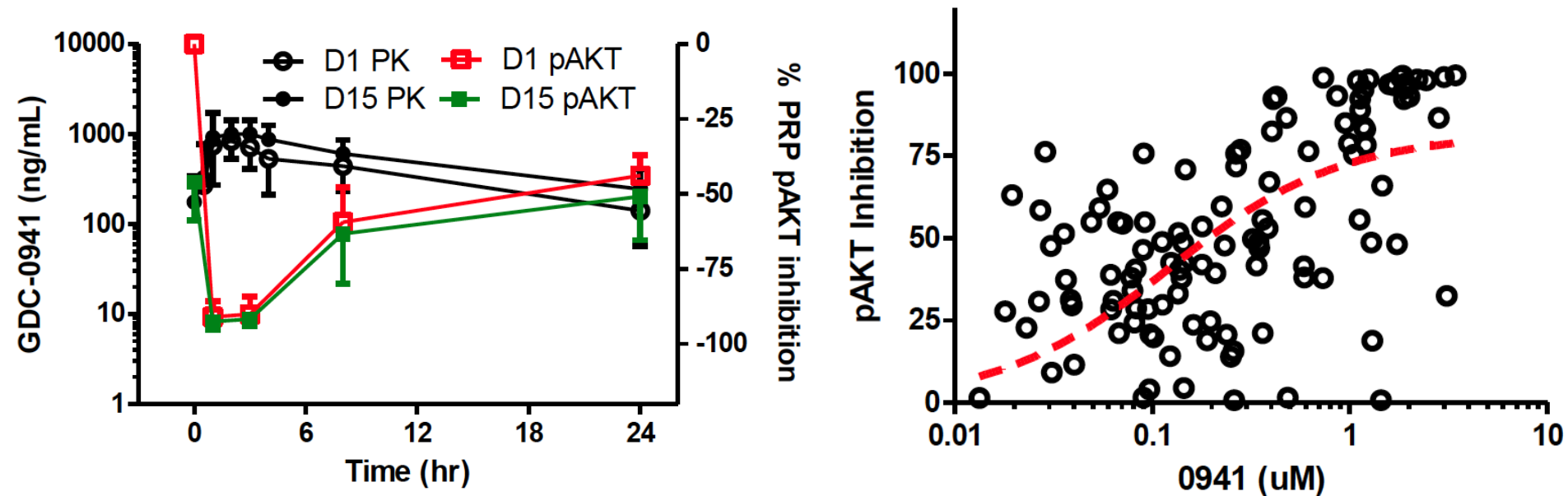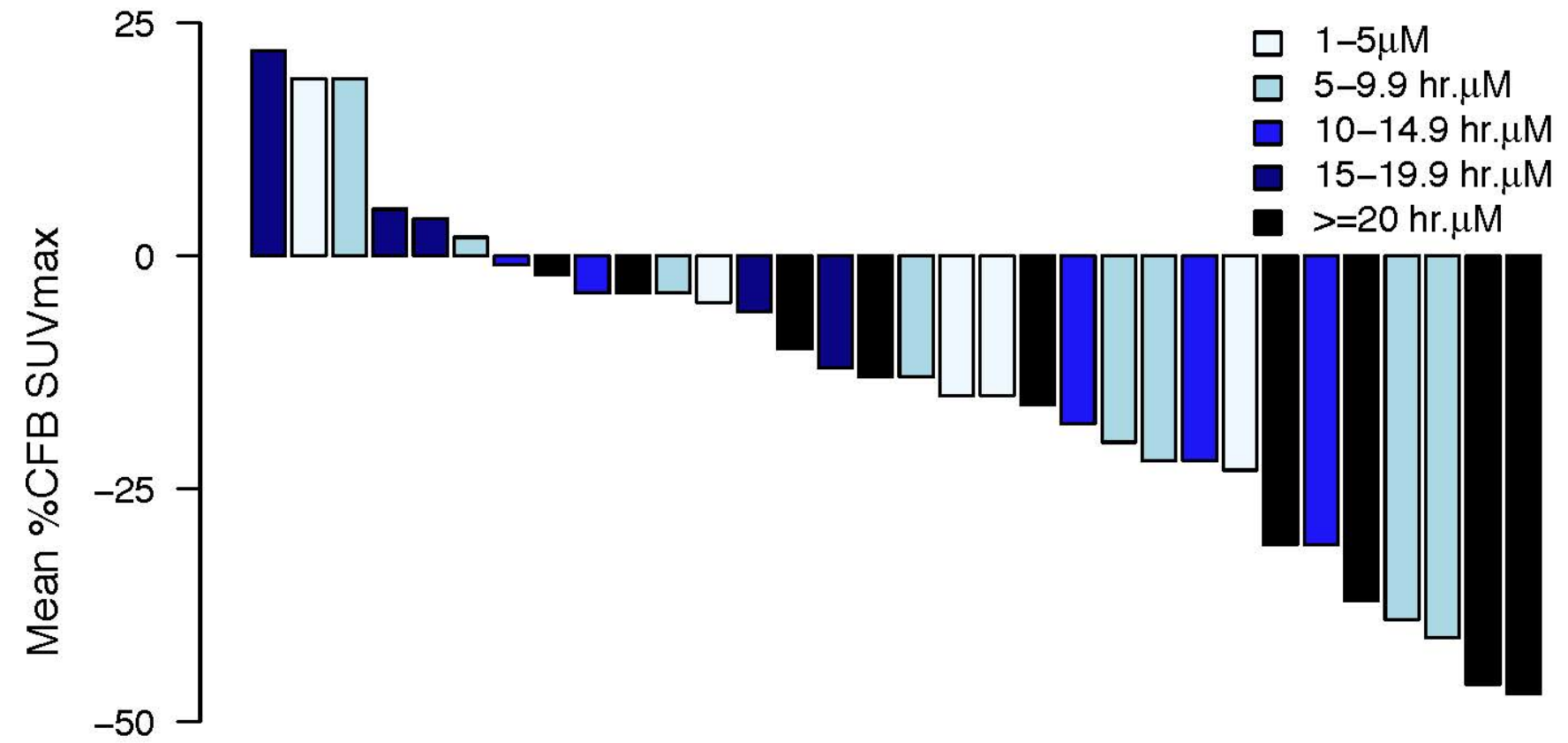
Tumour Biopsy PD
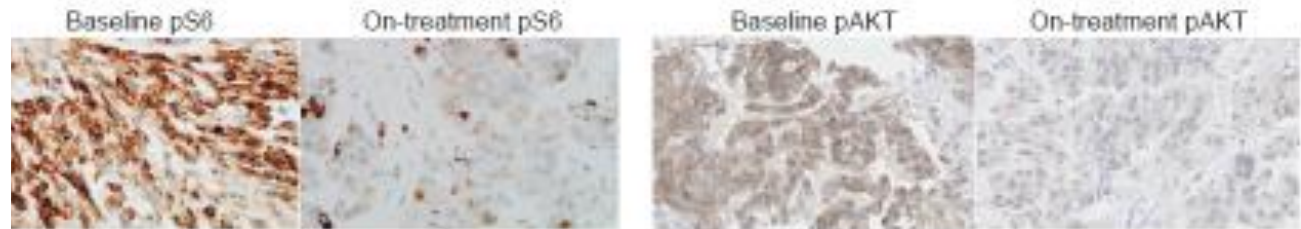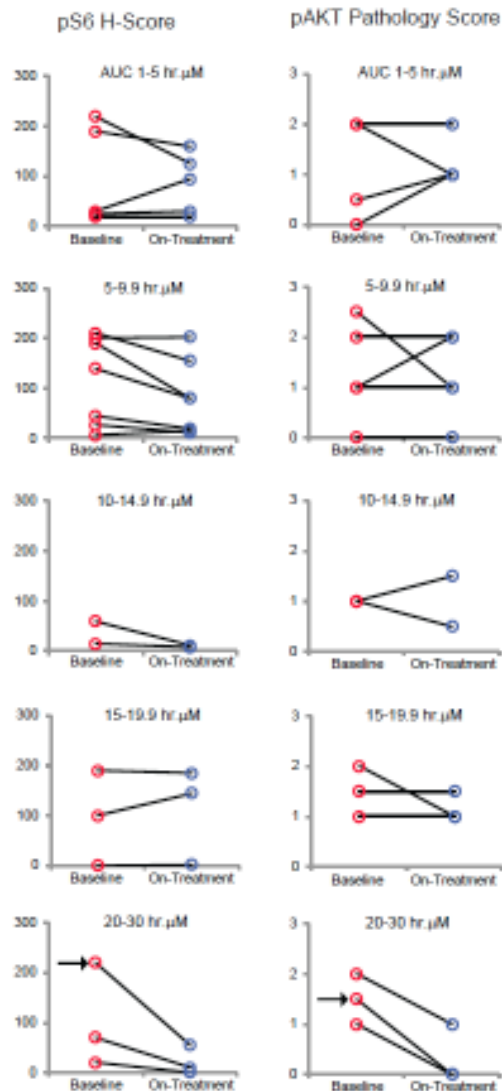
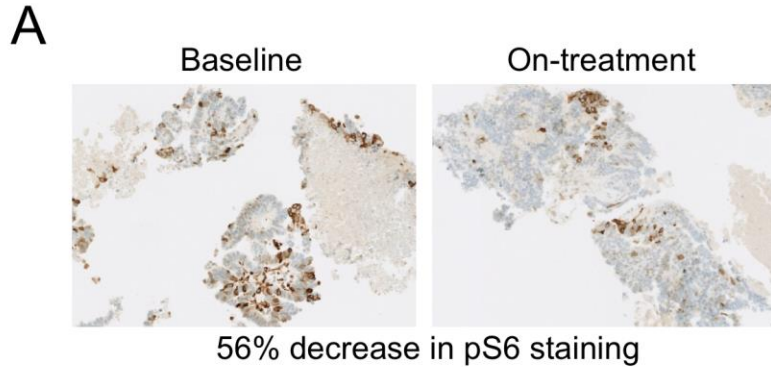*Patients treated at GDC0941 RP2D*
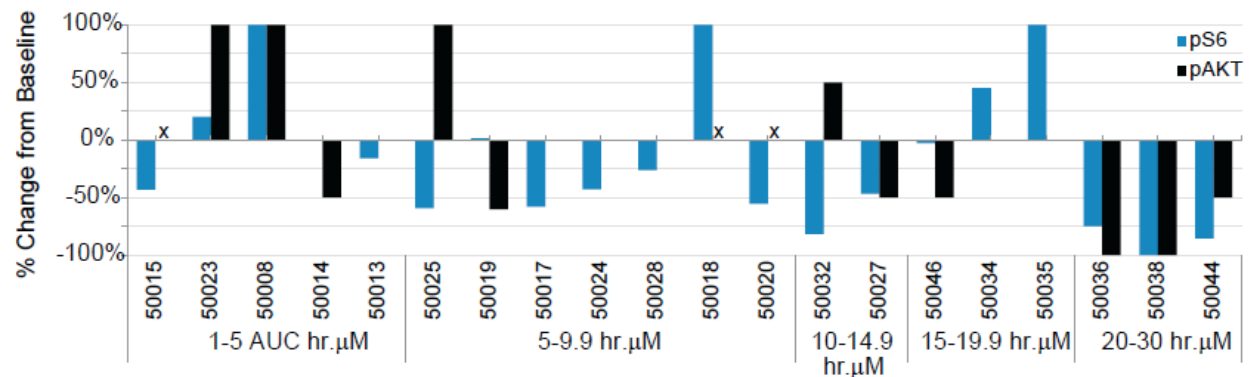
Impact on Plasma Insulin and Glucose

Waterfall plot of changes in^{18}F-FDG-PET SUV$_{max}$ grouped according to pictilisib AUC

Responding Ovarian Patient

A

Baseline · On-treatment
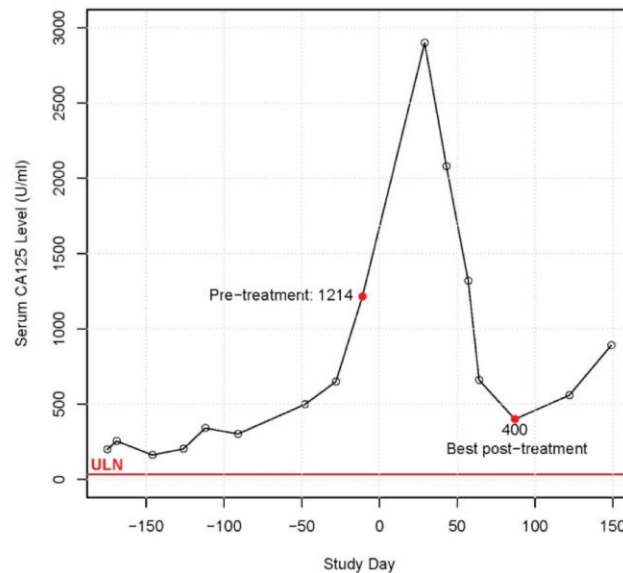
56% decrease in pS6 staining

49y old, platinum-refractory ovarian; 5 prior lines of chemo; following pictilisib (100mg once-daily) there was a 56% reduction in pS6 by IHC(tumour), a 30% reduction in mean SUV_{max} (FDG-PET) and serum CA125 fall (1214 to 400). Tumour analyses revealed *PIK3CA* amplification by FISH with PTEN loss (IHC).
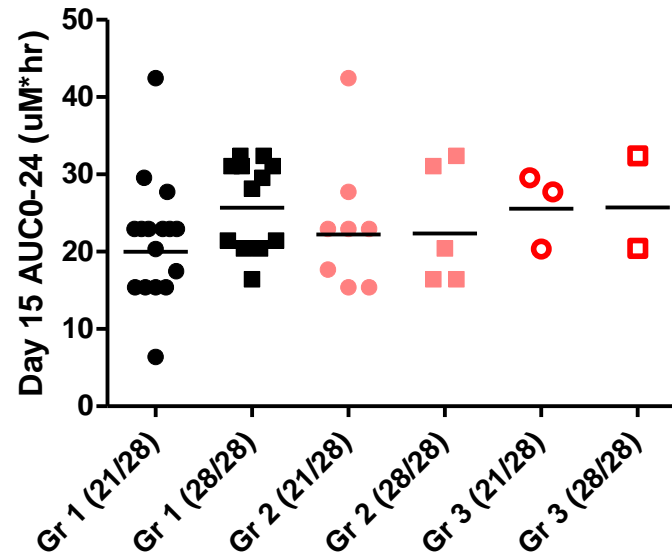
B

30% decrease SUVmax in perihepatic disease
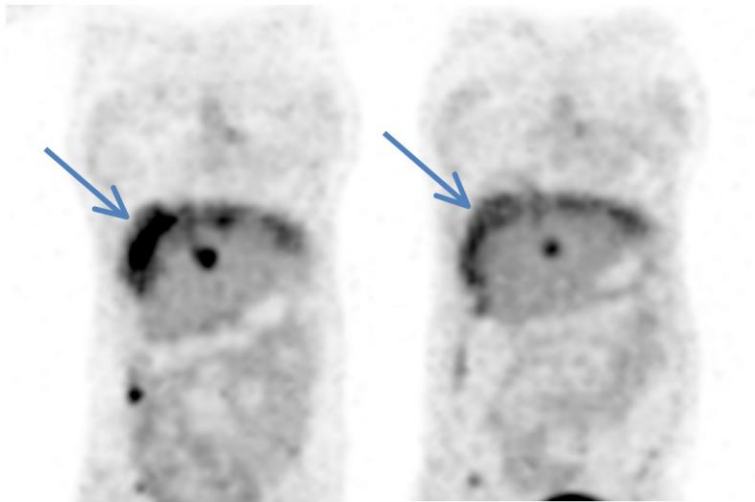
C

CA125 Response Observed

Serum CA125 Level (U/ml)

Pre-treatment: 1214

ULN

400
Best post-treatment

Study Day

Toxicity can also serve as PD biomarker

Skin rash post-GDC0941
Two schedules: 21/28 and 28/28 (continuous)

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Circulating Biomarkers

- Plasma: DNA, RNA, Proteins, Metabolome
- Whole blood: Expression Array Profiling
- Circulating Tumour Cells

Plasma: Next Generation Single Plex

JS Frenel

DNA extraction from 2ml of Plasma

Quantification with Picogreen high sensitivity
Concentration of the sample

Targeted sequencing with the PGM Ion Torrent platform:
   - Input DNA of 10ng
   - Sequencing of plasma DNA, germline DNA and tumor DNA

PIK3CA FFPE and Plasma Sequencing Data: 80 Breast Pts Referred for Phase I Trials.

PIK3CA mutation data: NGS >500x coverage

Characteristic	N	%
Plasma Analyzed	76	95.0%
FFPE Analyzed	54	67.5%
PIK3CA Mutations	**12**	**15.0%**
Plasma PIK3CA	8/76	10.5%
FFPE PIK3CA	9/54	16.6%
PIK3CA in FFPE + Plasma	5	41.7%
PIK3CA in FFPE alone	4	33.3%
PIK3CA in Plasma alone	1	8.3%
PIK3CA in Plasma, no FFPE sample	2	16.4%

Plasma DNA levels may be low missing mutations
Alternatively clonal evolution may mean new mutations evolve

Plasma: Next Generation Sequencing Multiplex; average coverage >1000x

Patient DF, colon Cancer, C° 25.6 ng/ml

Gene	Type	TUMOR		PLASMA	
		Var Freq	Coverage	Var Freq	Coverage
APC	A1492fs*15	44	989	Not found	
KRAS	G12V	47	820	13	218
TP53	R248E	31	991	13	549
TP53	R158fs*11	50	825	23	446
ALK	DEL TC>T	Not found		19	165
RB1	INS TA>T	19	1252	16	393

Work conducted in de Bono lab by Jean Sebastien Frenel
(ESMO George Mathe Translational Research Fellowship)

Circulating Biomarkers

- Plasma: DNA, RNA, Proteins, Metabolome
- Whole blood: Expression Array Profiling
- Circulating Tumour Cells

Back to back papers: Lancet Oncology October 2012

SEOM
Sociedad Española de Oncología Médica

Prognostic value of blood mRNA expression signatures in castration-resistant prostate cancer: a prospective, two-stage study

David Olmos*, Daniel Brewer*, Jeremy Clark*, Daniel C Danila, Chris Parker, Gerhardt Attard, Martin Fleisher, Alison H M Reid, Elena Castro, Shaheen K Sandhu, Lorraine Barwell, Nikhil Babu Oommen, Suzanne Carreira, Charles G Drake, Robert Jones, Colin S Cooper, Howard I Scher, Johann S de Bono

Summary

Background Biomarkers are urgently needed to dissect the heterogeneity of prostate cancer between patients to improve treatment and accelerate drug development. We analysed blood mRNA expression arrays to identify patients with metastatic castration-resistant prostate cancer with poorer outcome.

Methods Whole blood was collected into PAXgene tubes from patients with castration-resistant prostate cancer and patients with prostate cancer selected for active surveillance. In stage I (derivation set), patients with castration-resistant prostate cancer were used as cases and patients under active surveillance were used as controls. These patients were recruited from The Royal Marsden Hospital NHS Foundation Trust (Sutton, UK) and The Beatson West of Scotland Cancer Centre (Glasgow, UK). In stage II (validation-set), patients with castration-resistant prostate cancer recruited from the Memorial Sloan-Kettering Cancer Center (New York, USA) were assessed. Whole-blood RNA was hybridised to Affymetrix U133plus2 microarrays. Expression profiles were analysed with Bayesian latent process decomposition (LPD) to identify RNA expression profiles associated with castration-resistant prostate cancer subgroups; these profiles were then confirmed by quantitive reverse transcriptase (qRT) PCR studies and correlated with overall survival in both the test-set and validation-set.

Findings LPD analyses of the mRNA expression data divided the evaluable patients in stage I (n=94) into four groups. All patients in LPD1 (14 of 14) and most in LPD2 (17 of 18) had castration-resistant prostate cancer. Patients with castration-resistant prostate cancer and those under active surveillance comprised LPD3 (15 of 31 castration-resistant prostate cancer) and LPD4 (12 of 21 castration-resistant prostate cancer). Patients with castration-resistant prostate cancer in the LPD1 subgroup had features associated with worse prognosis and poorer overall survival than patients with castration-resistant prostate cancer in other LPD subgroups (LPD1 overall survival 10·7 months [95% CI 4·1–17·2] vs non-LPD1 25·6 months [18·0–33·4]; p<0·0001). A nine-gene signature verified by qRT-PCR classified patients into this LPD1 subgroup with a very low percentage of misclassification (1·2%). The ten patients who were initially unclassifiable by the LPD analyses were subclassified by this signature. We confirmed the prognostic utility of this nine-gene signature in the validation castration-resistant prostate cancer cohort, where LPD1 membership was also associated with worse overall survival (LPD1 9·2 months [95% CI 2·1–16·4] vs non-LPD1 21·6 months [7·5–35·6]; p=0·001), and remained an independent prognostic factor in multivariable analyses for both cohorts.

Interpretation Our results suggest that whole-blood gene profiling could identify gene-expression signatures that stratify patients with castration-resistant prostate cancer into distinct prognostic groups.

Funding AstraZeneca, Experimental Cancer Medicine Centre, Prostate Cancer Charity, Prostate Cancer Foundation.

A whole-blood RNA transcript-based prognostic model in men with castration-resistant prostate cancer: a prospective study

Robert W Ross*, Matthew D Galsky*, Howard I Scher, Jay Magidson, Karl Wassmann, Gwo-Shu Mary Lee, Leah Katz, Sumit K Subudhi, Aseem Anand, Martin Fleisher, Philip W Kantoff, William K Oh

Summary

Background Survival for patients with castration-resistant prostate cancer is highly variable. We assessed the effectiveness of a whole-blood RNA transcript-based model as a prognostic biomarker in castration-resistant prostate cancer.

Methods Peripheral blood was prospectively collected from 62 men with castration-resistant prostate cancer on various treatment regimens who were enrolled in a training set at the Dana-Farber Cancer Institute (Boston, MA, USA) from August, 2006, to June, 2008, and from 140 patients with castration-resistant prostate cancer in a validation set from Memorial Sloan-Kettering Cancer Center (New York, NY, USA) from August, 2006, to February, 2009. A panel of 168 inflammation-related and prostate cancer-related genes was assessed with optimised quantitative PCR to assess biomarkers predictive of survival.

Findings A six-gene model (consisting of ABL2, SEMA4D, ITGAL, and C1QA, TIMP1, CDKN1A) separated patients with castration-resistant prostate cancer into two risk groups: a low-risk group with a median survival of more than 34·9 months (median survival was not reached) and a high-risk group with a median survival of 7·8 months (95% CI 1·8–13·9; p<0·0001). The prognostic utility of the six-gene model was validated in an independent cohort. This model was associated with a significantly higher area under the curve compared with a clinicopathological model (0·90 [95% CI 0·78–0·96] vs 0·65 [0·52–0·78]; p=0·0067).

Interpretation Transcriptional profiling of whole blood yields crucial prognostic information about men with castration-resistant prostate cancer. The six-gene model suggests possible dysregulation of the immune system, a finding that warrants further study.

Funding Source MDX.

Work conducted in de Bono lab by Dr David Olmos (funded by SEOM)

Stage I: differentially expressed probe-sets

Group	CRPC	Surveillance	Dif. Expr. probesets
LPD 1	14	0	2740
LPD 2	17	1	541
LPD 3	15	16	2179
LPD 4	12	9	10063
10 patients Unclassified			
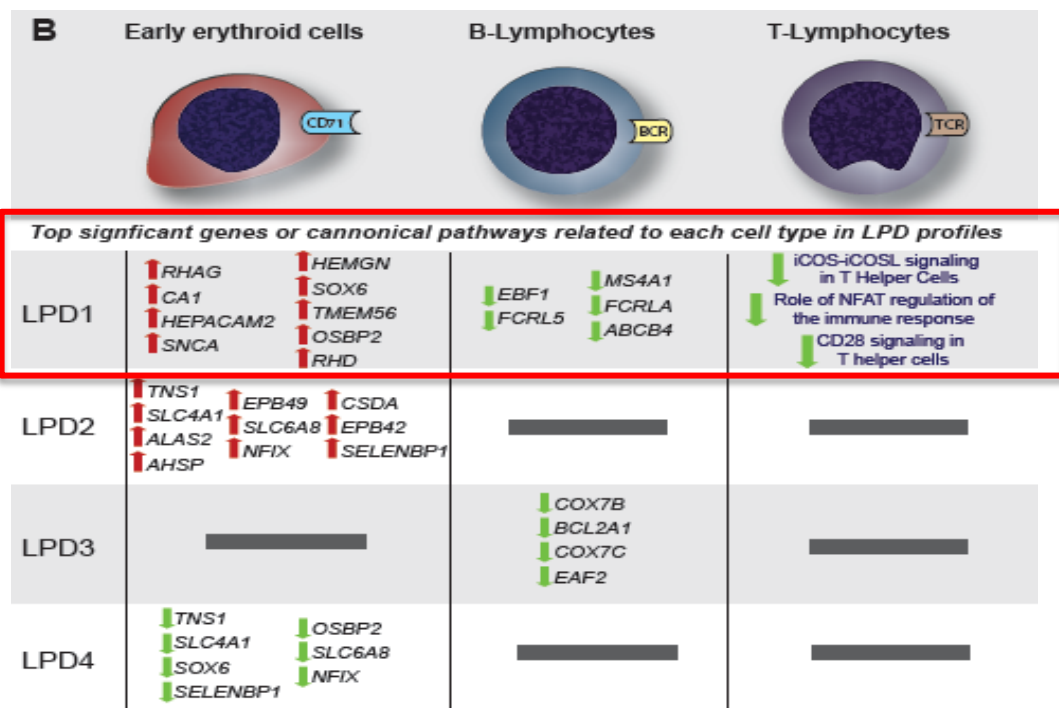
B

Early erythroid cells   B-Lymphocytes   T-Lymphocytes

Top signficant genes or cannonical pathways related to each cell type in LPD profiles

LPD1
↑ *RHAG*   ↑ *HEMGN*
↑ *CA1*   ↑ *SOX6*
↑ *HEPACAM2*   ↑ *TMEM56*
↑ *SNCA*   ↑ *OSBP2*
   ↑ *RHD*

↓ *EBF1*   ↓ *MS4A1*
↓ *FCRL5*   ↓ *FCRLA*
   ↓ *ABCB4*

↑ iCOS-iCOSL signaling in T Helper Cells
↓ Role of NFAT regulation of the immune response
↓ CD28 signaling in T helper cells

LPD2
↑ *TNS1*   ↑ *EPB49*   ↑ *CSDA*
↑ *SLC4A1*   ↑ *SLC6A8*   ↑ *EPB42*
↑ *ALAS2*   ↑ *NFIX*   ↑ *SELENBP1*
↑ *AHSP*

LPD3

↓ *COX7B*
↓ *BCL2A1*
↓ *COX7C*
↓ *EAF2*

LPD4
↓ *TNS1*   ↓ *OSBP2*
↓ *SLC4A1*   ↓ *SLC6A8*
↓ *SOX6*   ↓ *NFIX*
↓ *SELENBP1*

Circulating Biomarkers

- Plasma: DNA, RNA, Proteins, Metabolome
- Whole blood: Expression Array Profiling
- Circulating Tumour Cells

Evaluation of PTEN status in circulating tumor cells (CTCs) and matched tumor tissue from patients with castrate resistant prostate cancer

Elizabeth Punnoose[1], Eric Tucker[2], Dena Marrinucci[2], Edith Szafer-Glusman[1], Lukas Amler[1], Hartmut Koeppen[1], Premal Patel[1], Yibing Yan[1], Ruth Riisnaes[3], Gerhardt Attard[3], Johann deBono[3]

[1]Genentech, San Francisco, CA   [2]Epic Sciences, La Jolla, CA   [3]The Institute for Cancer Research and the Royal Marsden, Sutton, U

Introduction

PTEN loss occurs frequently in prostate cancer (PCa) and may trigger progression to CRPC through PI3K/AKT pathway activation. A blood-based assay that determines PTEN status could provide a non-invasive real-time evaluation in the metastatic setting that leads to informed treatment decisions such as the use of a PI3K-targeted therapy. Here we evaluate PTEN status on CTCs from CRPC patients with radiographic evidence of mets and compare these results to primary tissue and metastatic bone biopsies.

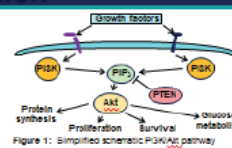Figure 1: Simplified schematic PI3K/Akt pathway

CTC Characterization

Blood from Stage IV PCa patients was plated onto glass slides and CTCs were identified as DAPI+/CK(cytokeratin)+/CD45- cells by immunofluorescence staining, followed by fluorescent in situ hybridization (FISH) using probes against chromosome 10 centromere (CEP10) and PTEN. Cells with equal number of signals (copy number) for PTEN and CEP10 were scored as PTEN-Normal, cells with low PTEN to CEP10 signal were scored as heterozygous PTEN-loss, and cells with no PTEN signal were scored as homozygous PTEN-loss. Scoring of PTEN status on white blood cells (WBCs) was used as internal control, and defined the rate of PTEN false positives (FP) across all samples. Based on a 10% FP rate, samples with > 10% loss and ≥ 3 CTCs exhibiting the abnormal genotype were categorically called heterozygous or homozygous loss.
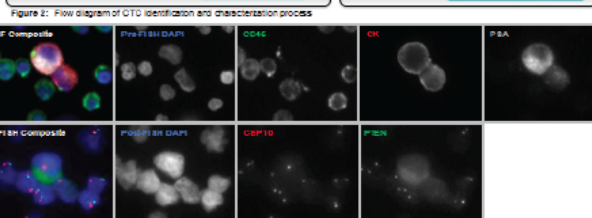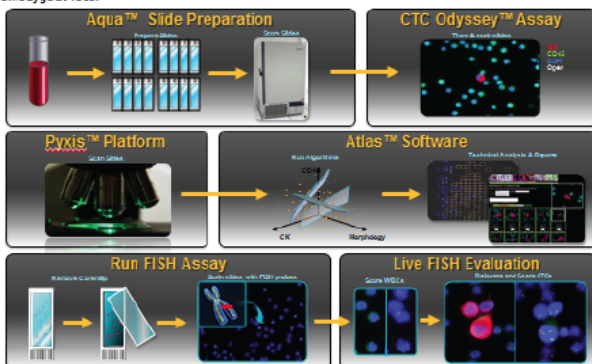
Aqua™ Slide Preparation
CTC Odyssey™ Assay
Pyxis™ Platform
Atlas™ Software
Run FISH Assay
Live FISH Evaluation

Figure 2: Flow diagram of CTC identification and characterization process

IF Composite | Pre-FISH DAPI | CD45 | CK | PSA
FISH Composite | Post-FISH DAPI | CEP10 | PTEN

Figure 3: Example of 4-color IF and 2-color FISH assays on PSA+/PTEN- CTC cluster.

PTEN Results from CTC Samples

Of 49 patient samples tested for CTC enumeration, 34 (70%) contained enough CTCs (≥ 4 CTCs) for PTEN evaluation. 19 patients (56%) exhibited significant PTEN loss: 8 (24%) homozygous loss and 11 (32%) heterozygous loss. Changes in ploidy were frequently observed and broad heterogeneity seen both within and between patients in both PTEN copies and ploidy. The most frequent abnormal genotypes detected in CTCs (diploid and homozygous PTEN-loss; triploid and homozygous PTEN-loss; triploid and PTEN-normal) were never observed in WBCs.

Figure 4: Patient CTC count distribution

Homozygous Loss | Heterozygous Loss | PTEN-Normal

Figure 5: Distributions of PTEN status within and between patients, sorted by level of PTEN loss.

1 CEP10, 1 PTEN | 1 CEP10, 0 PTEN | 2 CEP10, 0 PTEN | 3 CEP10, 0 PTEN | 3 CEP10, 3 PTEN | 4 CEP10, 2 PTEN | 1 CEP10, 0 PTEN

Figure 6: Representative IF and FISH images of various CTC genotypes
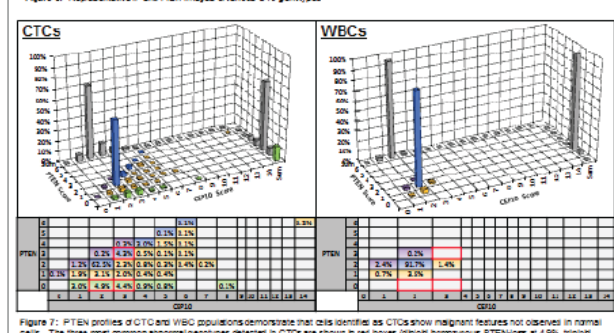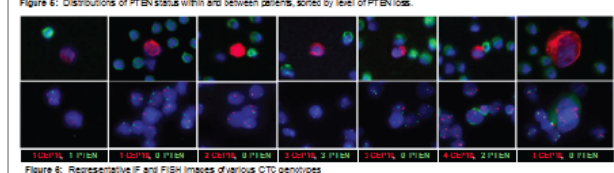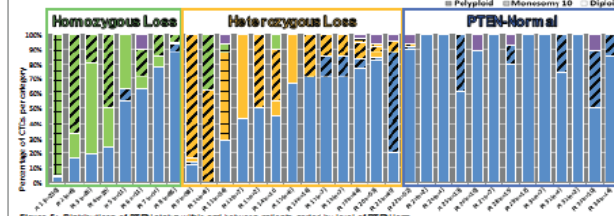
CTCs
WBCs

Figure 7: PTEN profiles of CTC and WBC populations demonstrate that cells identified as CTCs show malignant features not observed in normal cells. The three most common abnormal genotypes detected in CTCs are shown in red boxes (diploid homozygous PTEN-loss at 4.9%, triploid homozygous PTEN-loss at 4.4%, and triploid PTEN-normal at 4.3%) were not observed in over 1000 WBCs evaluated across patients.

Comparison to Tissue Biopsies

To date, matched CTC and tissue samples are available for PTEN analysis in 9 patients. PTEN status determined by Epic's CTC PTEN FISH assay is 75% (3/4) concordant with PTEN FISH results from primary tissue and 100% (6/6) concordant with results from metastatic bone biopsies.

Other | Homozygous Loss | Heterozygous loss | PTEN-Normal | Polyploid | Monosomy 10 | Diploid

Homozygous Loss | Heterozygous Loss | PTEN-Normal

	Pt 5 (n=11)	Pt 6 (n=11)	Pt 7 (n=14)	Pt 9 (n=58)	Pt 11 (n=34)	Pt 14 (n=11)	Pt 19 (n=44)	Pt 28 (n=15)	Pt 31 (n=8)
Patient ID									
Archival FISH		N/A		N/A	HET 67%	N/A	HET 9%	Normal	N/A
Bone Met FISH	HO 100%	HO 100%	N/A	HO 87%	HE 91%, HO 87%	HE 91%	Normal	Normal	N/A
Archival IHC H-score	N/A	180	300	100	100	180	300	300	270
Bone Met IHC H-score	N/A	N/A	N/A			N/A			

Figure 8: PTEN results from matched CTC, archived prostate, and bone metastasis samples. PTEN IHC H-scores in tissue can have a max range of 0-300 and is calculated from the % of tumor cells expressing an IHC stain of 0,1+, 2+, 3+.

Archived Primary Prostate
Green = CEP10, Red = PTEN
Bone Metastasis
Green = CEP10, Red = PTEN
CTCs
Red = CEP10, Green = PTEN

Patient 6 | Patient 14 | Patient 19

Figure 9: Images of PTEN FISH in matched archived prostate, fresh bone biopsies, and fresh CTC specimens

Conclusions

Our results show that PTEN status determined by CTC analysis correlates strongly with matched metastatic biopsies. This data illustrates the potential for using CTCs as a non-invasive real-time biopsy to determine a patient's PTEN status. IHC, FISH and CTC assays are used to determine PTEN status in an ongoing AKT inhibitor Phase 1b/II trial.

Archived Primary Prostate	Bone Metastasis	CTCs
Green = CEP10, Red = PTEN	Green = CEP10, Red = PTEN	Red = CEP10, Green = PTEN

No change

Two cases

Homozygous Loss — Homozygous Loss — Homozygous Loss

4 CEP10 / 0 PTEN   3 CEP10 / 0 PTEN   3 CEP10 / 0 PTEN   4 CEP10 / 0 PTEN

Archived Primary Prostate	Bone Metastasis	CTCs
Green = CEP10, Red = PTEN	Green = CEP10, Red = PTEN	Red = CEP10, Green = PTEN

PTEN status change: Uncommon

Normal   Heterozygous Loss   Normal   Heterozygous Loss   Normal

3 CEP10 / 3 PTEN   4 CEP10 / 2 PTEN   3 CEP10 / 3 PTEN   4 CEP10 / 2 PTEN   3 CEP10 / 3 PTEN

Overview

- The PhAT
- Predictive biomarkers
- Pharmacodynamic biomarkers
- Circulating biomarkers
- Clonal evolution

Clonal Evolution

- Genomic instability – intra-patient heterogeneity

- Drug resistance may be related to emerging clones
  - But drug may still have had antitumour activity imparting benefit against some clones
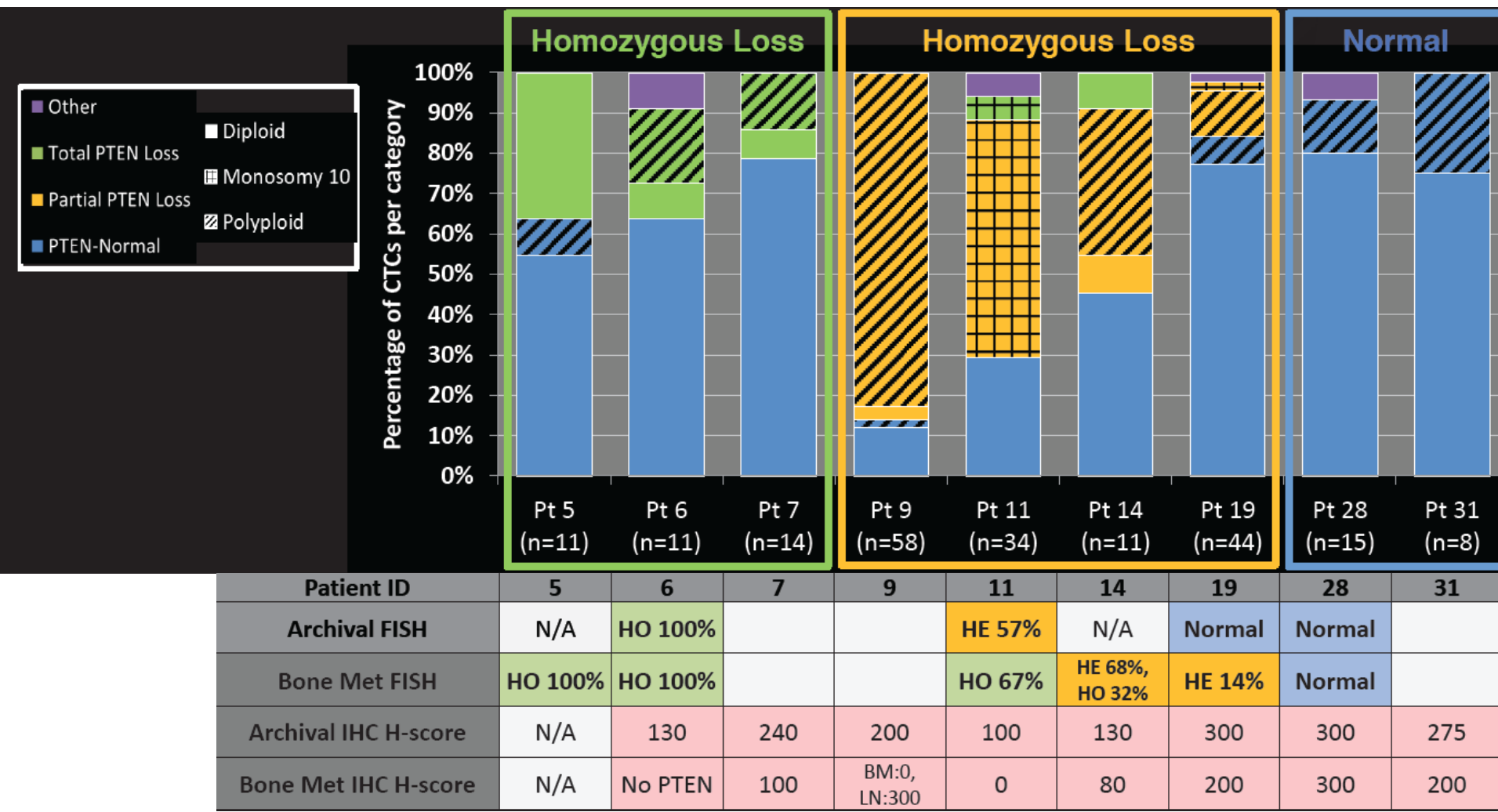
- We need to study this in more detail
  - Circulating biomarkers
    - CTC, plasma DNA, molecular imaging

CTC PTEN Loss: Intrapatient Heterogeneity

- PTEN loss in fresh CRPC biopsies always seen in CTCs
  - We are utilizing CTC PTEN analyses in RP2 AKTi trials

Patient ID	5	6	7	9	11	14	19	28	31
Archival FISH	N/A	HO 100%			HE 57%	N/A	Normal	Normal	
Bone Met FISH	HO 100%	HO 100%			HO 67%	HE 68%, HO 32%	HE 14%	Normal	
Archival IHC H-score	N/A	130	240	200	100	130	300	300	275
Bone Met IHC H-score	N/A	No PTEN	100	BM:0, LN:300	0	80	200	300	200

Cell Free DNA as a Biomarker

ORIGINAL ARTICLE

Analysis of Circulating Tumor DNA to Monitor Metastatic Breast Cancer

Sarah-Jane Dawson, F.R.A.C.P., Ph.D., Dana W.Y. Tsui, Ph.D., Muhammed Murtaza, M.B., B.S., Heather Biggs, M.A., Oscar M. Rueda, Ph.D., Suet-Feung Chin, Ph.D., Mark J. Dunning, Ph.D., Davina Gale, B.Sc., Tim Forshew, Ph.D., Betania Mahler-Araujo, M.D., Sabrina Rajan, M.D., Sean Humphray, B.Sc., Jennifer Becq, Ph.D., David Halsall, M.R.C.Path., Ph.D., Matthew Wallis, M.B., Ch.B., David Bentley, D.Phil., Carlos Caldas, M.D., F.Med.Sci., and Nitzan Rosenfeld, Ph.D.

ABSTRACT

BACKGROUND

The management of metastatic breast cancer requires monitoring of the tumor burden to determine the response to treatment, and improved biomarkers are needed. Biomarkers such as cancer antigen 15-3 (CA 15-3) and circulating tumor cells have been widely studied. However, circulating cell-free DNA carrying tumor-specific alterations (circulating tumor DNA) has not been extensively investigated or compared with other circulating biomarkers in breast cancer.

METHODS

We compared the radiographic imaging of tumors with the assay of circulating tumor DNA, CA 15-3, and circulating tumor cells in 30 women with metastatic breast cancer who were receiving systemic therapy. We used targeted or whole-genome sequencing to identify somatic genomic alterations and designed personalized assays to quantify circulating tumor DNA in serially collected plasma specimens. CA 15-3 levels and numbers of circulating tumor cells were measured at identical time points.

RESULTS

Circulating tumor DNA was successfully detected in 29 of the 30 women (97%) in whom somatic genomic alterations were identified; CA 15-3 and circulating tumor cells were detected in 21 of 27 women (78%) and 26 of 30 women (87%), respectively. Circulating tumor DNA levels showed a greater dynamic range, and greater correlation with changes in tumor burden, than did CA 15-3 or circulating tumor cells. Among the measures tested, circulating tumor DNA provided the earliest measure of treatment response in 10 of 19 women (53%).

CONCLUSIONS

This proof-of-concept analysis showed that circulating tumor DNA is an informative, inherently specific, and highly sensitive biomarker of metastatic breast cancer. (Funded by Cancer Research UK and others.)
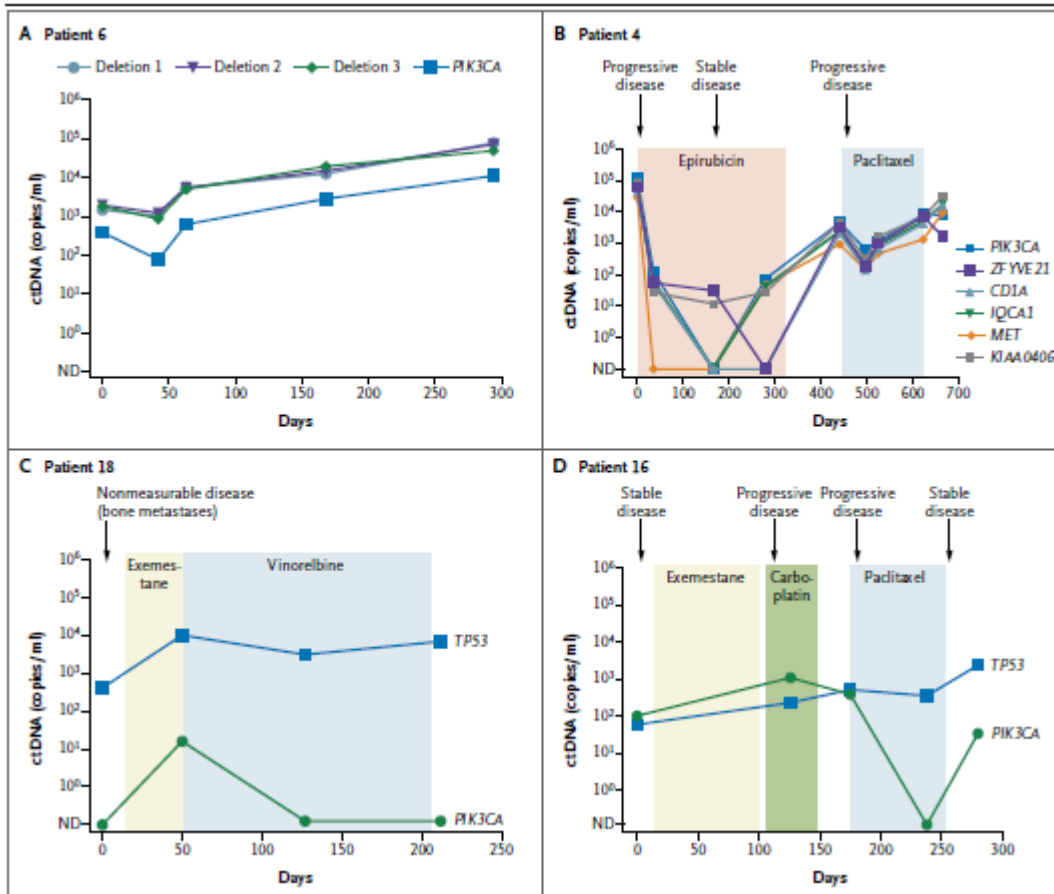
Emergence of *AR L702H* on treatment is not the only mechanism of resistance in CRPC
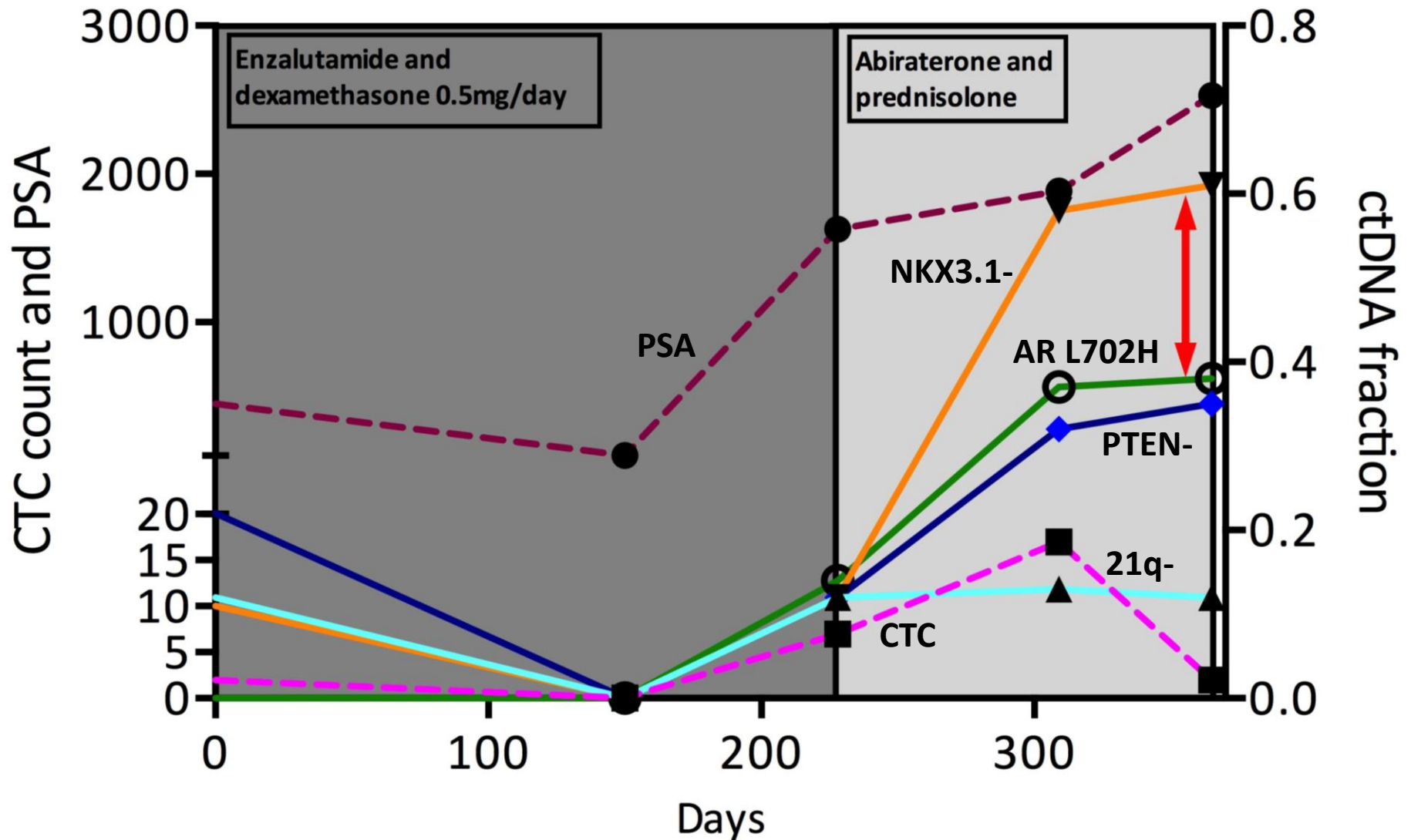
Conclusions

- Pharmacological Audit Trail requires the utilization of multiple types of biomarkers
  - Predictive: PIK3CA or AKT mutation; PTEN loss etc
  - Pharmacodynamic: phospho-protein analyses; FDGF-PET; toxicity
- Drugs targeting PI3K/AKT pathway have modest antitumour activity as single agents but have activity with MEKi against some KRAS mt cancers
- Concerns remain that toxicity may limit intratumoral target blockade and antitumor activity
- Circulating biomarkers have much promise in the study of the PhAT and clonal evolution