

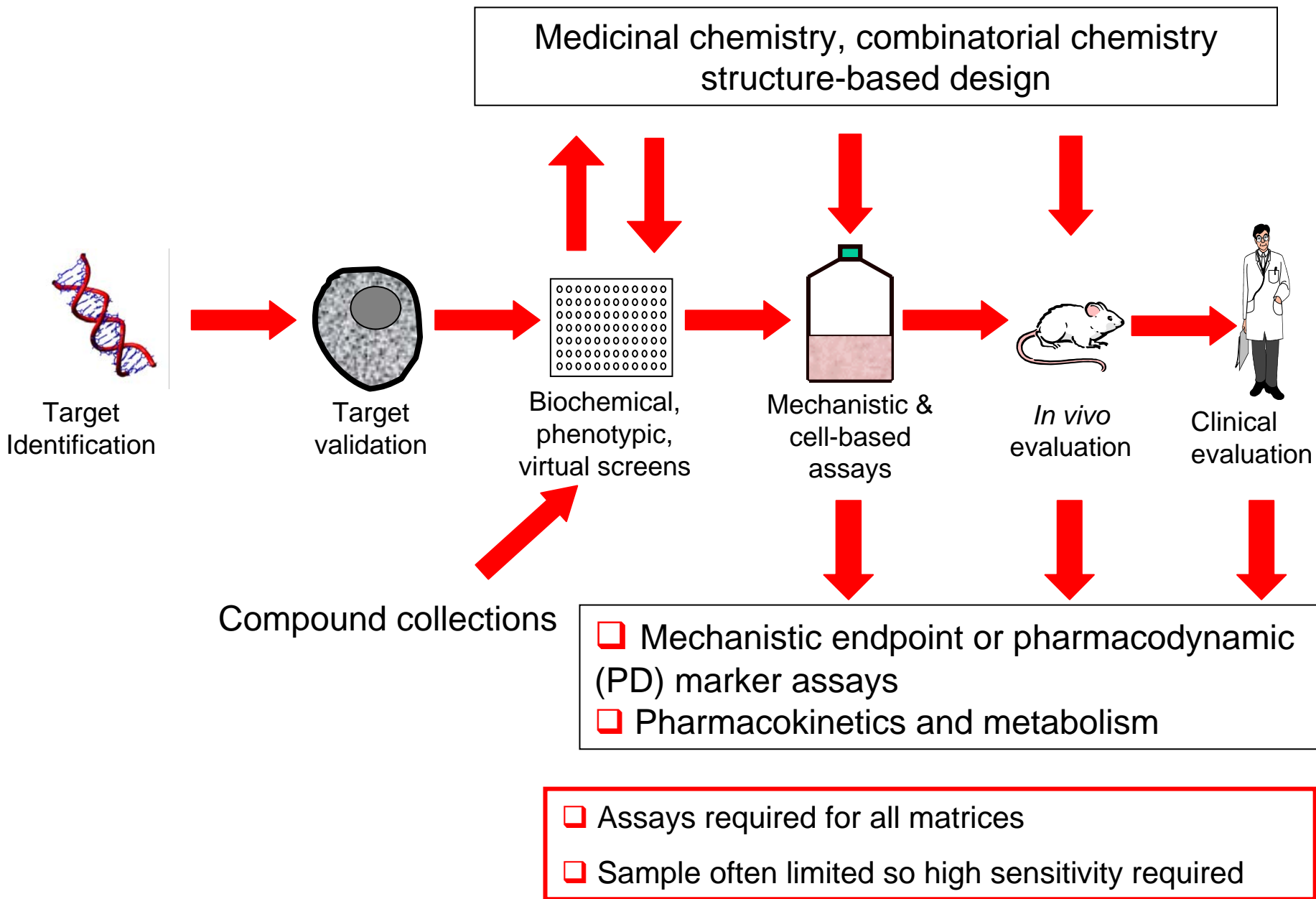
Pharmacodynamic markers of response to novel anticancer agents using a protein profiling sandwich immunoassay format

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Molecular mechanism-based drug discovery

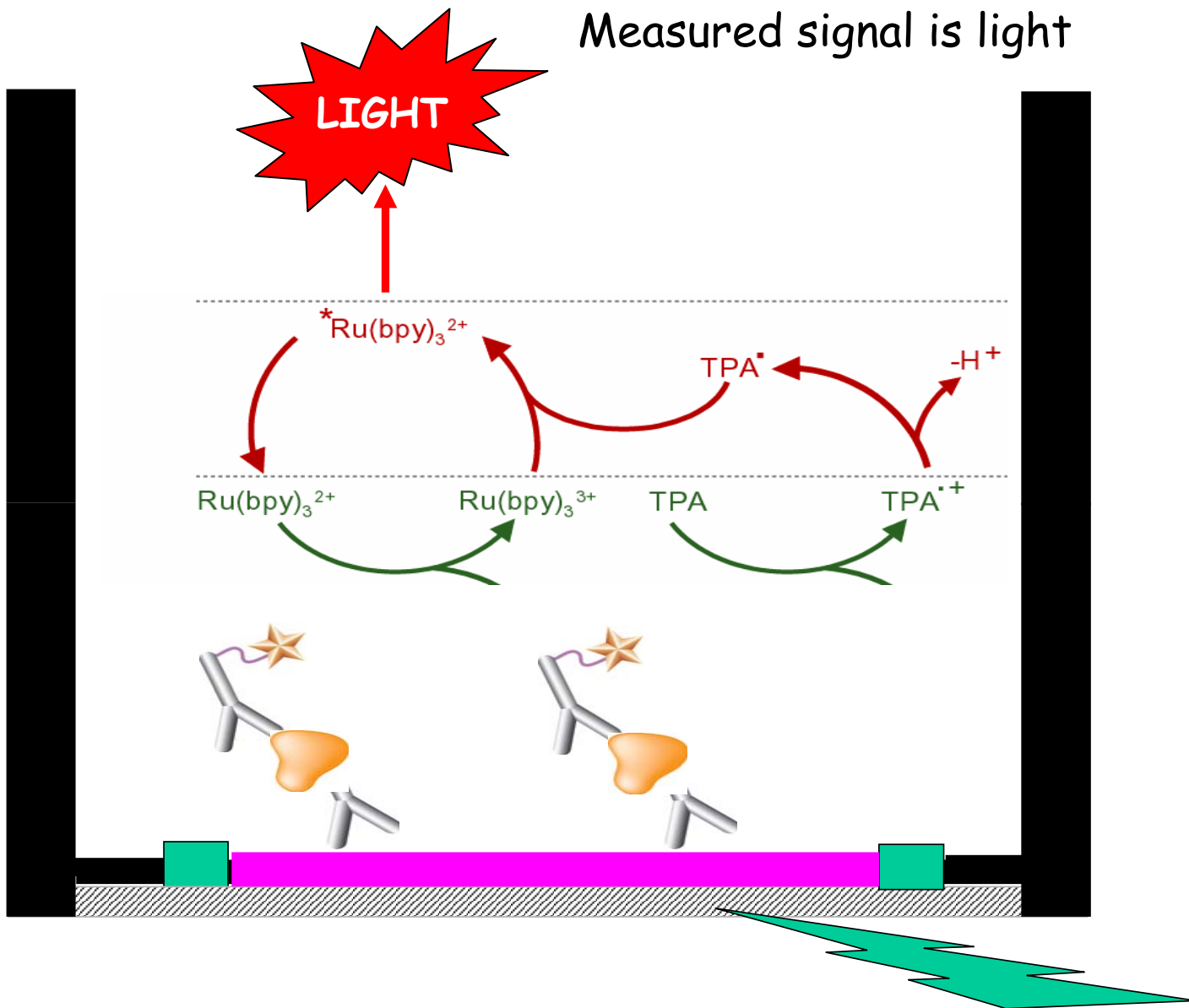


Techniques for PD marker measurement

- ❑ Western blotting (Gold Standard')
- ❑ Immunohistochemistry
- ❑ Flow cytometry
- ❑ Microplate immunoassays (ELISA)
 - ❑ MSD Protein Profiling sandwich immunoassays
 - Multiplex 'catalogue' assays
 - Single assay development 'in-house'
 - Option to multiplex 'in-house' and 'catalogue' assays

The ease of validation for GCLP is an important consideration for PD assays as compliance with regulatory requirements is essential

Meso Scale Discovery (MSD) technology



Can these assays be applied to different matrices?

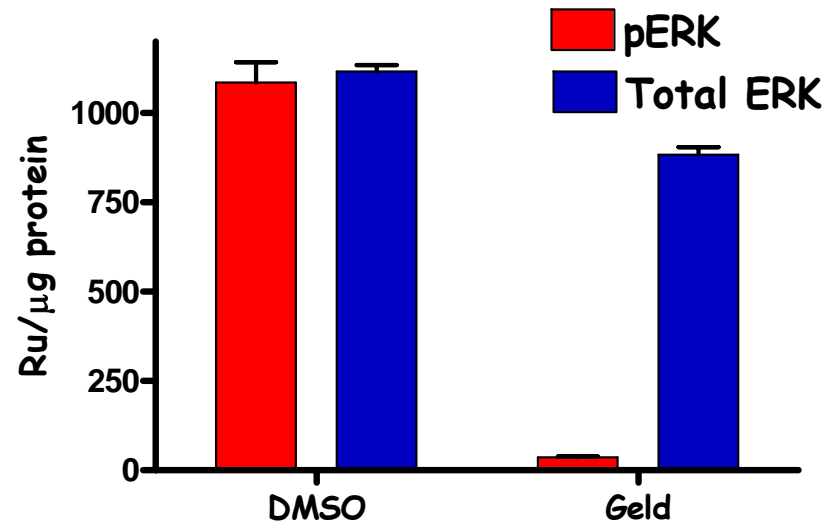
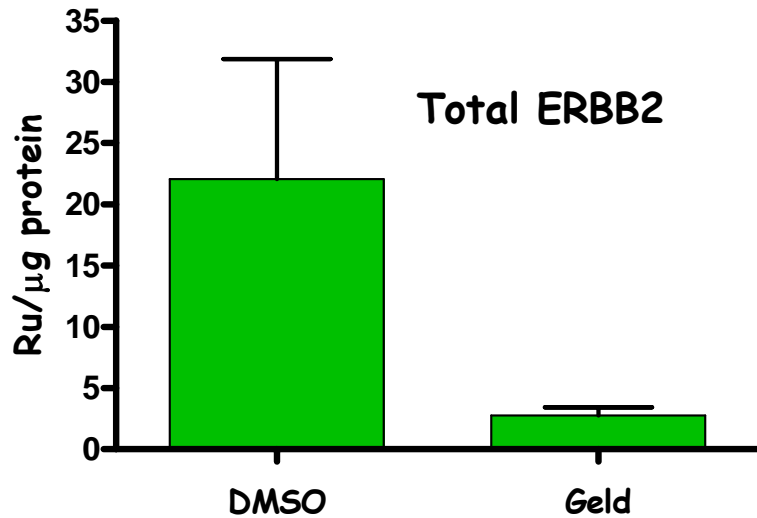
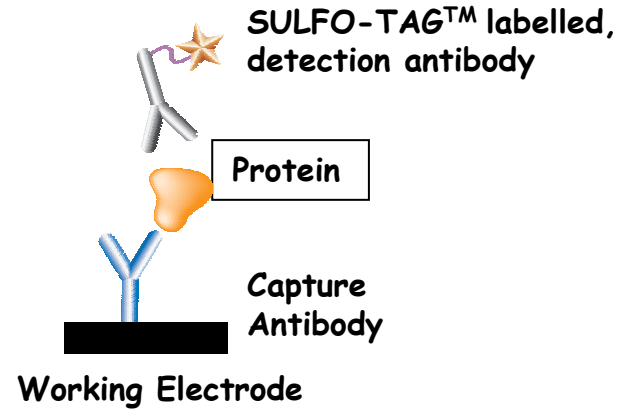
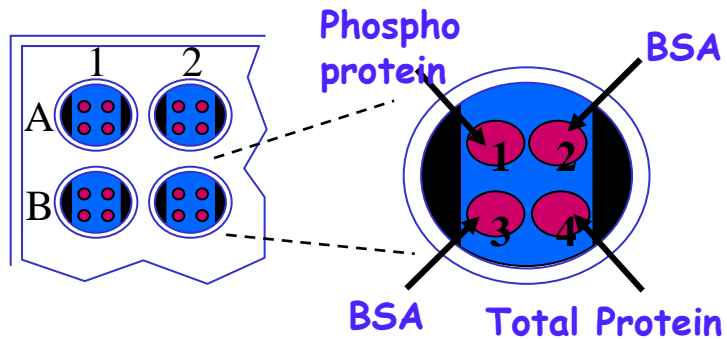
- In vitro* cell lysates
- Human tumour xenografts
- Clinical samples eg. PBMCs, tumours and plasma

Aim : to carry out a feasibility study with inhibitors
of HSP90 and HDAC

PD markers for inhibitors of HSP90

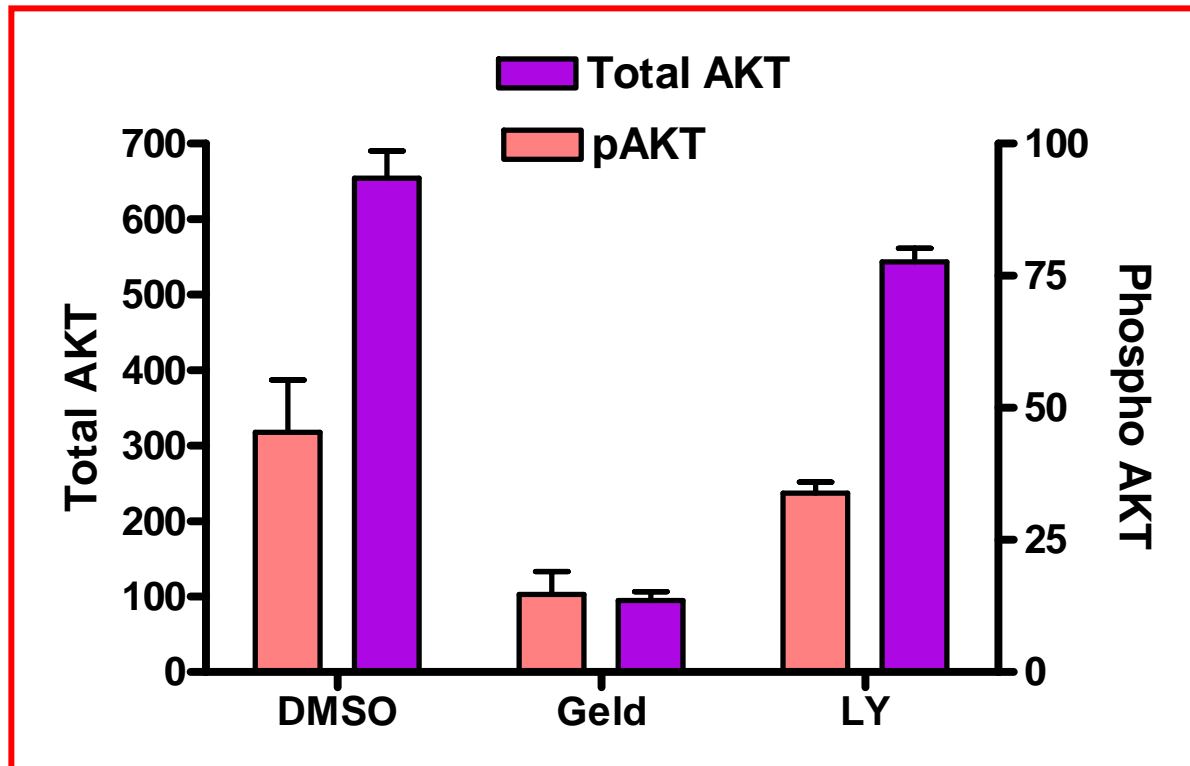
- ❑ The molecular chaperone HSP90 maintains the conformation, stability and function of oncogenic client proteins (eg. ERBB2, AKT and CDK4)
- ❑ HSP90 inhibitors cause degradation of client proteins, disruption of signalling pathways and antitumour activity
- ❑ Several agents currently in Phase I and II trials e.g. 17-AAG and 17-DMAG
- ❑ The molecular signature of HSP90 inhibition includes a fall in ERBB2, AKT and pERK and induction of HSP70

'Off the shelf' MSD duplexed assays for PD markers of HSP90 inhibition



HCT116 cells, 1 μM geldanamycin, 24h. 1.25-10 μg protein/well

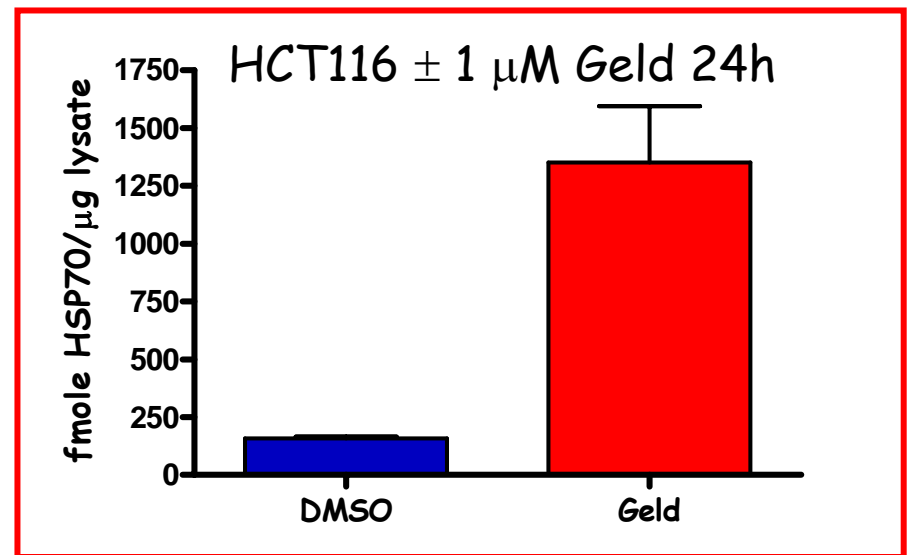
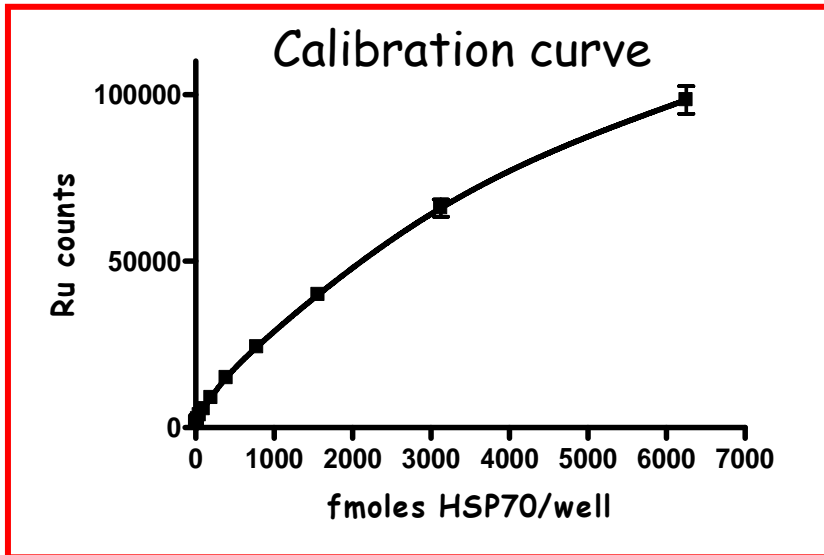
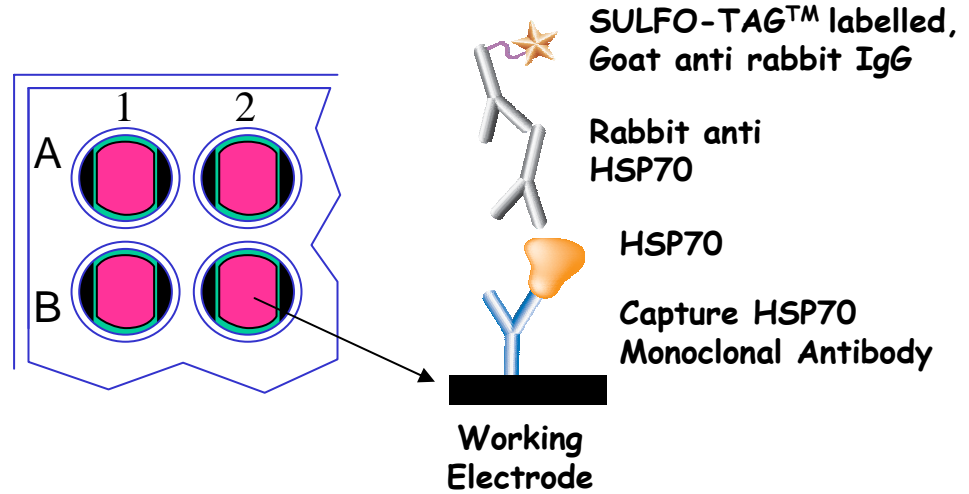
'Off the shelf' duplexed assays for PD markers of HSP90 inhibition



HCT116 cells, 1 μ M Geldanamycin, 20 μ M LY294002 (LY) 24h. 1.25-10 μ g protein/well

'In-house' MSD assay for HSP70

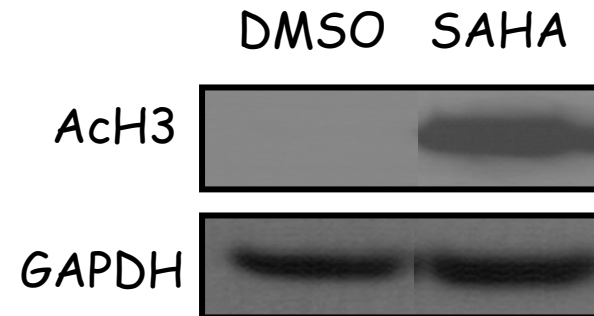
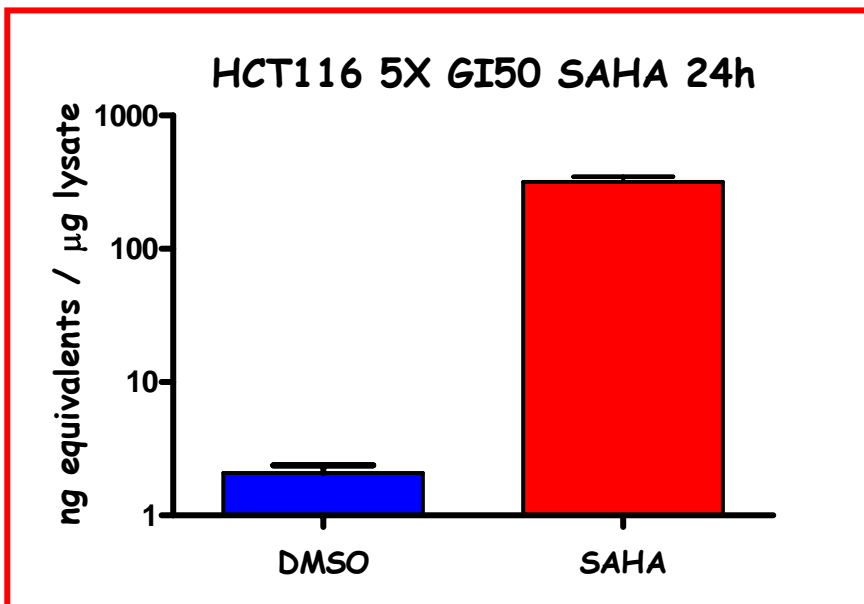
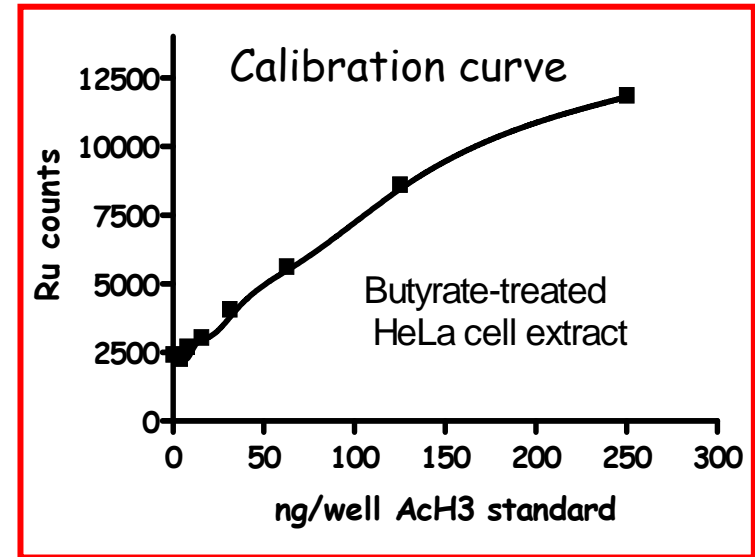
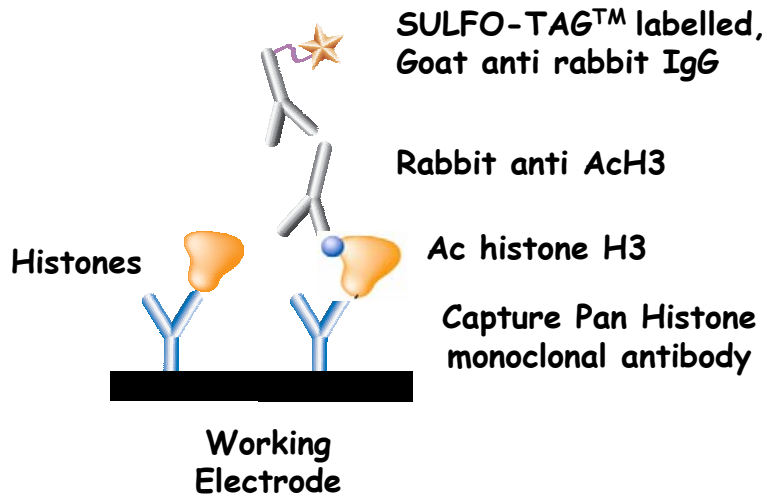
- ❑ DELFIA HSP70 assay validated to GCLP
- ❑ In use for clinical trials of HSP90 inhibitors
- ❑ Assay transferred to MSD
- ❑ Potential for multiplexing HSP70 with client protein expression



PD markers for HDAC inhibitors

- ❑ HDACs catalyse the deacetylation of histones and are important for the regulation of gene expression
- ❑ HDACs are involved in the development and progression of malignancy
- ❑ HDACIs display antitumour activity and several compounds are being evaluated clinically
- ❑ Their precise mechanism of action is not clear but the most obvious result of HDAC inhibition is hyperacetylation of histones
- ❑ Hyperacetylation of histone H3 is used as a PD marker for HDAC inhibition

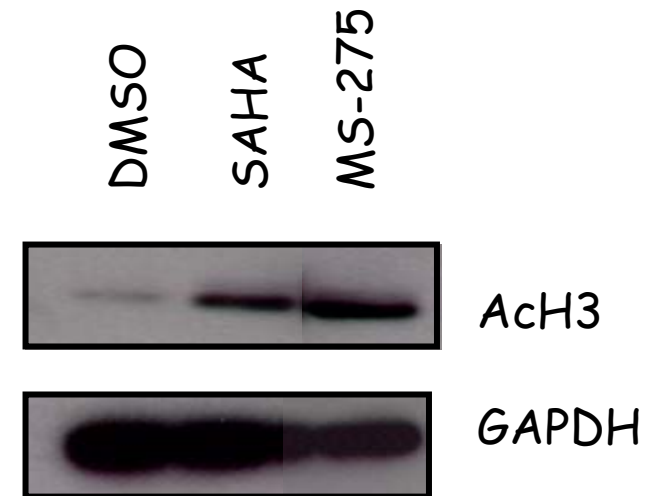
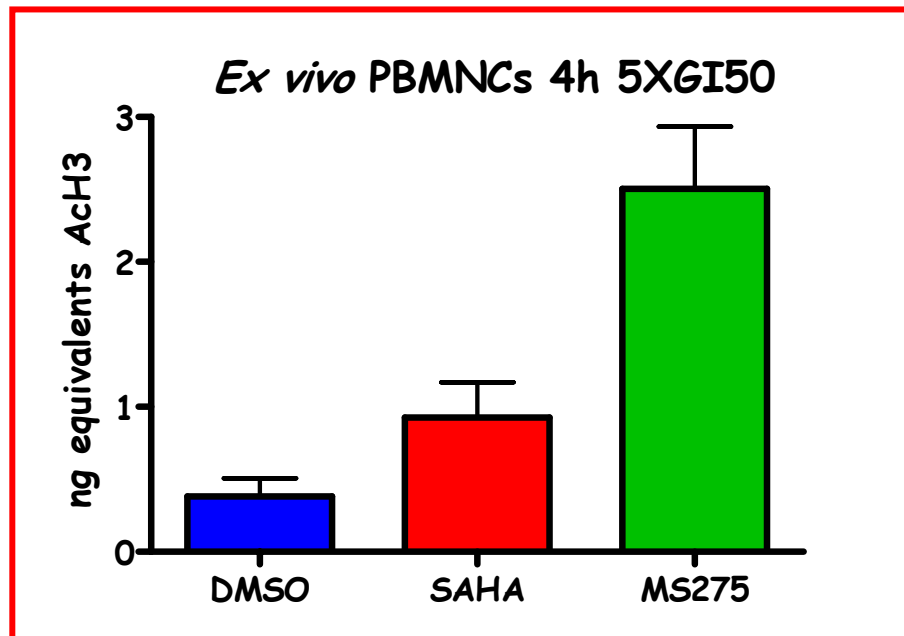
'In-house' assay for acetyl histone H3 (AcH3)



Assay being successfully used to measure the effect of novel HDACIs on AcH3 in human tumour xenografts

Ex vivo treatment of PBMCs with HDACIs

- ❑ PBMCs and plasma separated from whole blood
- ❑ HDACIs SAHA and MS-275 or DMSO (control) added
- ❑ Incubated at 37°C for 4h
- ❑ PBMCs separated, washed and lysed
- ❑ AcH3 in lysates measured by MSD assay and western blot



This assay is being validated to GCLP for use in clinical trials of HDACIs

Summary

- ❑ Experience with the MSD format is encouraging
 - ❑ robust
 - ❑ sensitive
 - ❑ minimal matrix interference
 - ❑ quantitative, higher-throughput alternative to western blots
 - ❑ both catalogue and in-house assays used
- ❑ Provided proof of principle for mechanism of action in 2 therapeutic areas, HSP90 and HDAC, in different sample matrices
- ❑ Now used in a number of drug discovery projects in the Centre
- ❑ Assays are amenable to GCLP validation to comply with regulatory requirements for clinical trials
- ❑ Multiplexing potential for 'in-house' assays to be investigated

Acknowledgements

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Paul Workman and Wynne Aherne**

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Tuc Ahmad from MSD

