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APRIL 8-13, 2022 • #AACR22

NXP800: A first-in-class, orally active, small-molecule HSF1* pathway inhibitor

Paul Workman
CRUK Cancer Therapeutics Unit
The Institute of Cancer Research, London

For the Institute of Cancer Research and Nuvectis Project Team

*Heat Shock Factor 1

Abstract Number ND08

Disclosure information

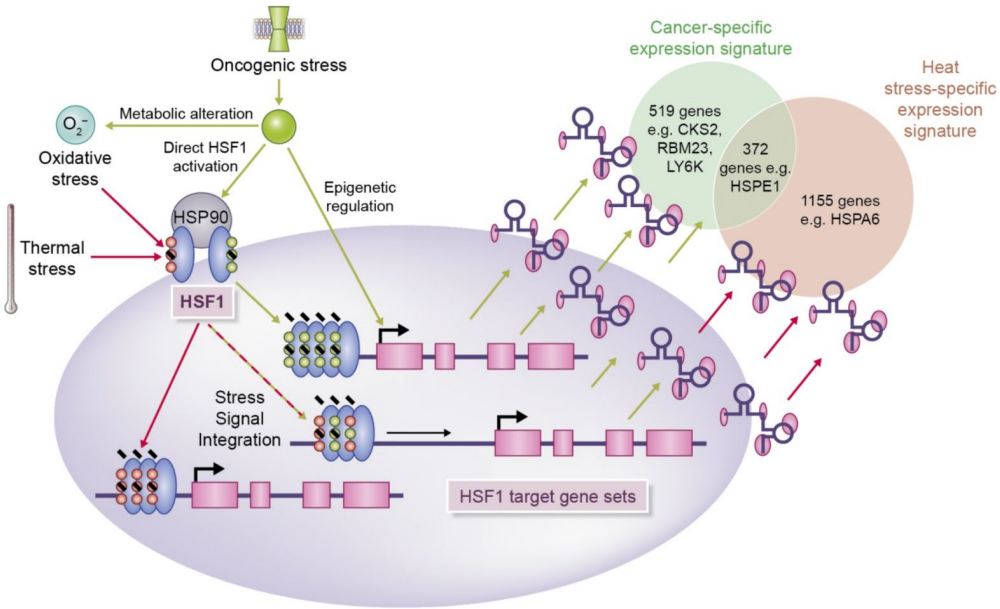
Paul Workman

I have the following relevant financial relationships to disclose:

- Consultant/SAB for Nextech (Science Partner), Astex Pharmaceuticals, CV6 Therapeutics, Black Diamond Therapeutics, Vividion Therapeutics, Storm Therapeutics, Alterome Therapeutics, Epicombi Therapeutics, Nuvectis Pharma
- Stockholder in: Chroma Therapeutics, Storm Therapeutics, Nextech
- Non-Executive Director of Storm Therapeutics
- Research funding and/or programme IP licensed to: Vernalis/Novartis, Merck KGaA, Cyclacel Pharmaceuticals, Piramed/Genentech/Roche, Astex/AstraZeneca, Sareum/Sierra Oncology, AstraZeneca, BACIT, CRT Pioneer Fund/Sixth Element Capital, Nuvectis Pharma
- Employee of ICR which has multiple commercial interactions and a rewards-to-inventors scheme
- Former employee of (Astra)Zeneca

HSF1 and the oncogene-associated stress response

- HSF1 is an ancient transcription factor regulating the classical eukaryotic Heat Shock Response (HSR) and is a master regulator of proteotoxic stress (Li et al *Trends Cell Biol* 2017)
- In response to oncogenic stress, HSF1 is hijacked in cancer cells to regulate a gene set overlapping with but distinct from the canonical HSR (Mendillo et al *Cell* 2012)
- Depleting HSF1 inhibits the oncogenic effects of p53 loss and RAS activation in mouse models (*Dai* et al *Cell 2007*) – indicating therapeutic potential
- HSF1 is amplified, over-expressed and activated in human cancers and these and the oncogenic stress signature predict clinical outcome in numerous human cancers, including ovarian (Mendillo et al *Cell* 2012; Powell et al *Trends Cell Biol 2017*) – indicating possible patient populations
- In flies and mice, HSF1 is dispensable for growth and survival (*Jedlicka et al EMBO J 1997; Xiao et al EMBO J 1999*) – indicating potential therapeutic index



Adapted from de Billy, Travers & Workman Oncotarget 3 741-743 2012
See also Workman and de Billy Nature Med 13 1415-1417 2007

HSF1 and the oncogene-associated stress response

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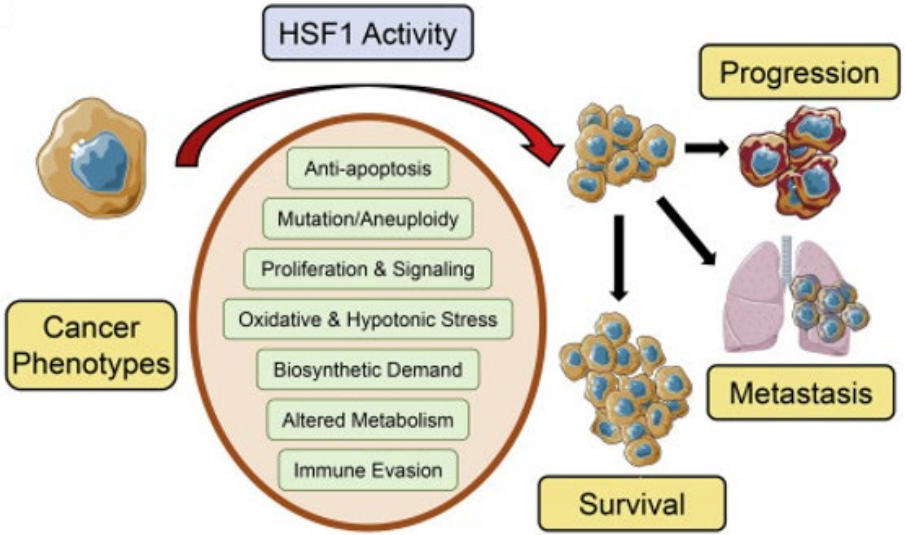
- In flies and mice, HSF1 is dispensable for growth and survival (*Jedlicka et al EMBO J 1997; Xiao et al EMBO J 1999*) – indicating potential therapeutic index
- An HSF1 pathway inhibitor could block multiple cancer hallmarks and exert powerful anticancer effects



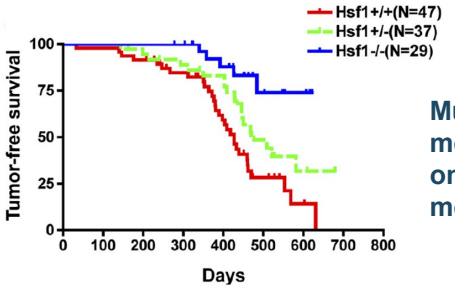
Dong et al Trends Pharm Sci 40 986-1005 2019

A wealth of data validates HSF1 as a cancer drug target

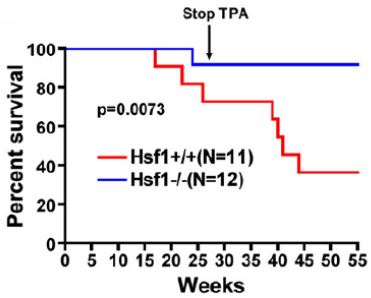
Heat Shock Factor 1 Is a Powerful Multifaceted Modifier of Carcinogenesis

Chengkai Dai,[1] Luke Whitesell,[1] Arlin B. Rogers,[3] and Susan Lindquist[1,2,*]
[1]Whitehead Institute for Biomedical Research, Cambridge, MA 02142, USA
[2]Howard Hughes Medical Institute, Chevy Chase, MD 20815, USA
[3]Divison of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
*Correspondence: lindquist_admin@wi.mit.edu
DOI 10.1016/j.cell.2007.07.020

Cell

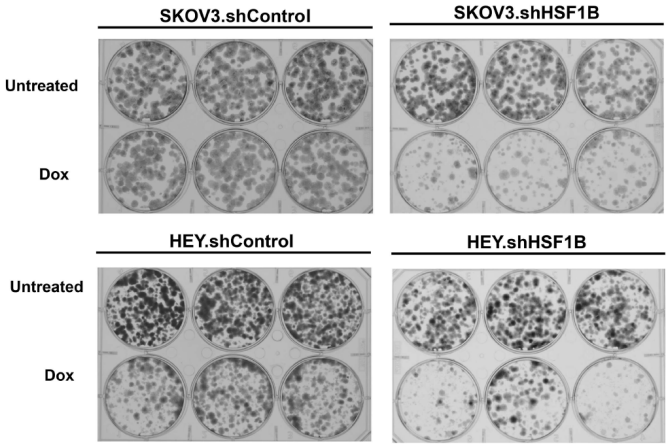


Mutant p53 mouse oncogenesis model



HRAS-driven mouse skin carcinogenesis model

Dai et al Cell 30 1005-18 2007

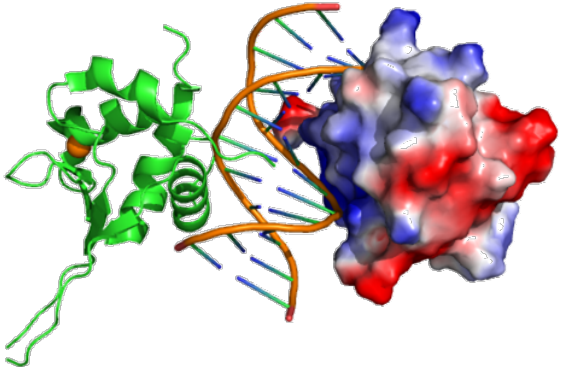


Human ovarian cancer cell lines

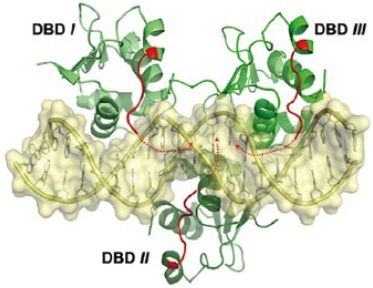
Powell et PLos One 11(12):e0168389 2016

- In cancer cells, HSF1 is validated as controlling proliferation, survival, migration, protein synthesis, metabolism, EMT and the tumor microenvironment, and immune evasion
- Example of exploiting 'non-oncogene addiction' and 'stress overload' (Luo/Elledge et al *Cell* 2009; Nager/Berns et al *EMBO Rep* 2016)

HSF1 is highly challenging to drug directly



Electrostatic surface
Red is negative charge
Blue is positive charge
White is hydrophobic



Feng et al iScience 24 102951 2021

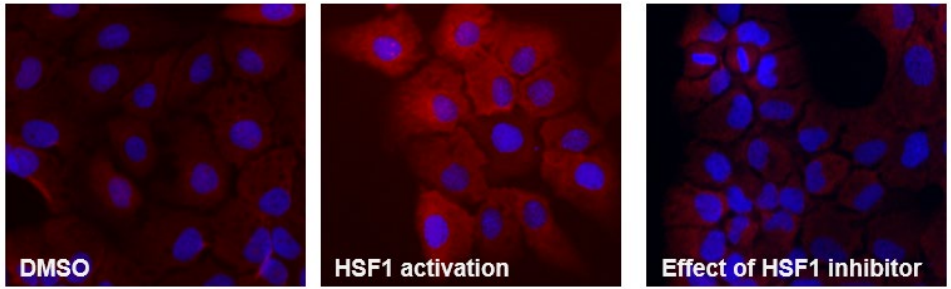
3D structure of HSF1 DBD dimer complex with DNA
PDB code: 5D5V

canSAR cansar.icr.ac.uk

- Only the DNA-binding domain is structurally characterized
- No druggable cavity detected in the limited structural space available
- Likely to need indirect ways to target HSF1
- We decided to take the approach of a cell-based phenotypic HSF1 pathway screen

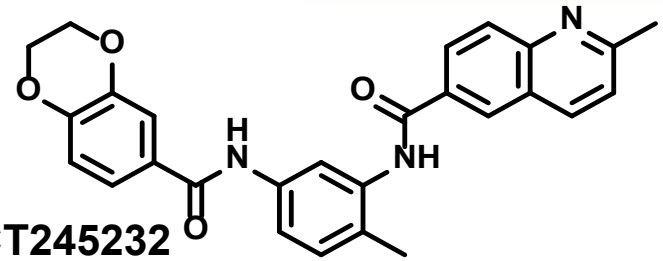
Discovery of bisamide HSF1 pathway inhibitors by phenotypic screening

Phenotypic screen with heat shock gene product reporter



Red=HSP72 Blue=DAPI (DNA)

- Above example: SK-OV-3 human ovarian cancer cells; IN Cell Analyser™
- For main screen: U2OS human osteosarcoma cells; ArrayScan™
- 200K compounds from AstraZeneca collection were screened



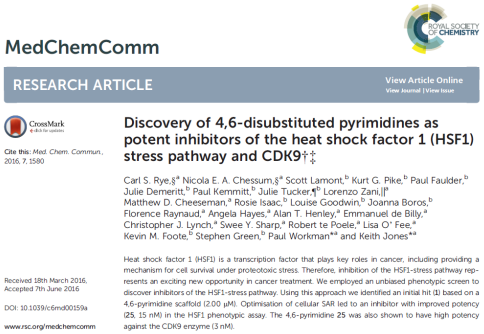
CCT245232

HSP72 IC_{50} = 2.8nM

U2OS cell GI_{50} =18nM

'Bisamide' N,N′-4-methyl-1,3-phenylenediamide core

- Very potent 'bisamide' inhibitor of the HSF1 pathway in cancer cells
- Potently inhibits cancer cell growth



MedChemComm

RESEARCH ARTICLE

View Article Online

Discovery of 4,6-disubstituted pyrimidines as potent inhibitors of the heat shock factor 1 (HSF1) stress pathway and CDK9†‡

Carl S. Rye,§* Nicola E. A. Chessum,§* Scott Lamont,[b] Kurt G. Pike,[b] Paul Faulder,[b] Julie Demeritt,[b] Paul Kemmitt,[b] Julie Tucker,¶[b] Lorenzo Zani,‖[a] Matthew D. Cheeseman,[a] Rosie Isaac,[b] Louise Goodwin,[b] Joanna Boros,[b] Florence Raynaud,[a] Angela Hayes,[a] Alan T. Henley,[a] Emmanuel de Billy,[a] Christopher J. Lynch,[a] Swee Y. Sharp,[a] Robert te Poele,[a] Lisa O' Fee,[a] Kevin M. Foote,[b] Stephen Green,[b] Paul Workman*[a] and Keith Jones*[a]

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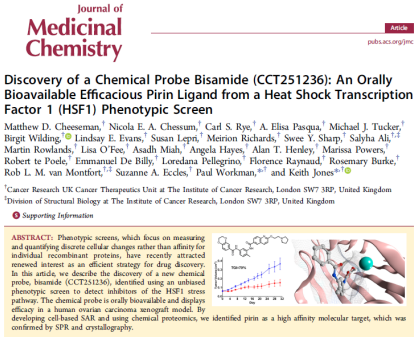
DOI: 10.1039/c6md00159a

www.rsc.org/medchemcomm

Heat shock factor 1 (HSF1) is a transcription factor that plays key roles in cancer, including providing a mechanism for cell survival under proteotoxic stress. Therefore, inhibition of the HSF1-stress pathway represents an exciting new opportunity in cancer treatment. We employed an unbiased phenotypic screen to discover inhibitors of the HSF1-stress pathway. Using this approach we identified an initial hit (1) based on a 4,6-pyrimidine scaffold (2.00 μM). Optimisation of cellular SAR led to an inhibitor with improved potency (25, 15 nM) in the HSF1 phenotypic assay. The 4,6-pyrimidine 25 was also shown to have high potency against the CDK9 enzyme (3 nM).



Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Discovery of a Chemical Probe Bisamide (CCT251236): An Orally Bioavailable Efficacious Pirin Ligand from a Heat Shock Transcription Factor 1 (HSF1) Phenotypic Screen

Matthew D. Cheeseman,[†] Nicola E. A. Chessum,[†] Carl S. Rye,[†] A. Elisa Pasqua,[†] Michael J. Tucker,[†] Birgit Wilding,[†] Lindsay E. Evans,[†] Susan Lepri,[†] Meirion Richards,[†] Swee Y. Sharp,[†] Salyha Ali,[†,‡] Martin Rowlands,[†] Lisa O'Fee,[†] Asadh Miah,[†] Angela Hayes,[†] Alan T. Henley,[†] Marissa Powers,[†] Robert te Poele,[†] Emmanuel De Billy,[†] Loredana Pellegrino,[†] Florence Raynaud,[†] Rosemary Burke,[†] Rob L. M. van Montfort,[†,‡] Suzanne A. Eccles,[†] Paul Workman,*[,†] and Keith Jones*[,†]

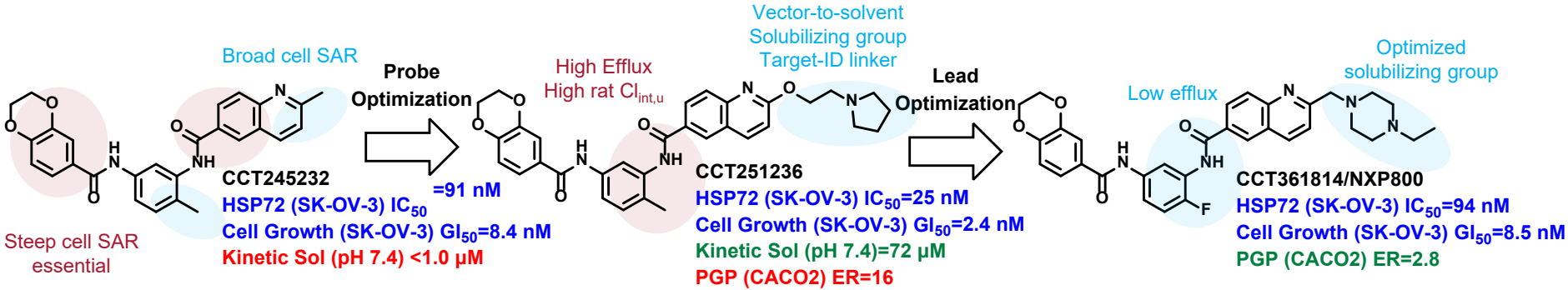
[†]Cancer Research UK Cancer Therapeutics Unit at The Institute of Cancer Research, London SW7 3RP, United Kingdom
[‡]Division of Structural Biology at The Institute of Cancer Research, London SW7 3RP, United Kingdom

Supporting Information

ABSTRACT: Phenotypic screens, which focus on measuring and quantifying discrete cellular changes rather than affinity for individual recombinant proteins, have recently attracted renewed interest as an efficient strategy for drug discovery. In this article, we describe the discovery of a new chemical probe, bisamide (CCT251236), identified using an unbiased phenotypic screen to detect inhibitors of the HSF1 stress pathway. The chemical probe is orally bioavailable and displays efficacy in a human ovarian carcinoma xenograft model. By developing cell-based SAR and using chemical proteomics, we identified pirin as a high affinity molecular target, which was confirmed by SPR and crystallography.

Rye et al Med Chem Commun.7 1580–1586 2016
Cheeseman et al J Med Chem 60 180-201 2017

From phenotypic screen to chemical tool to clinical candidate



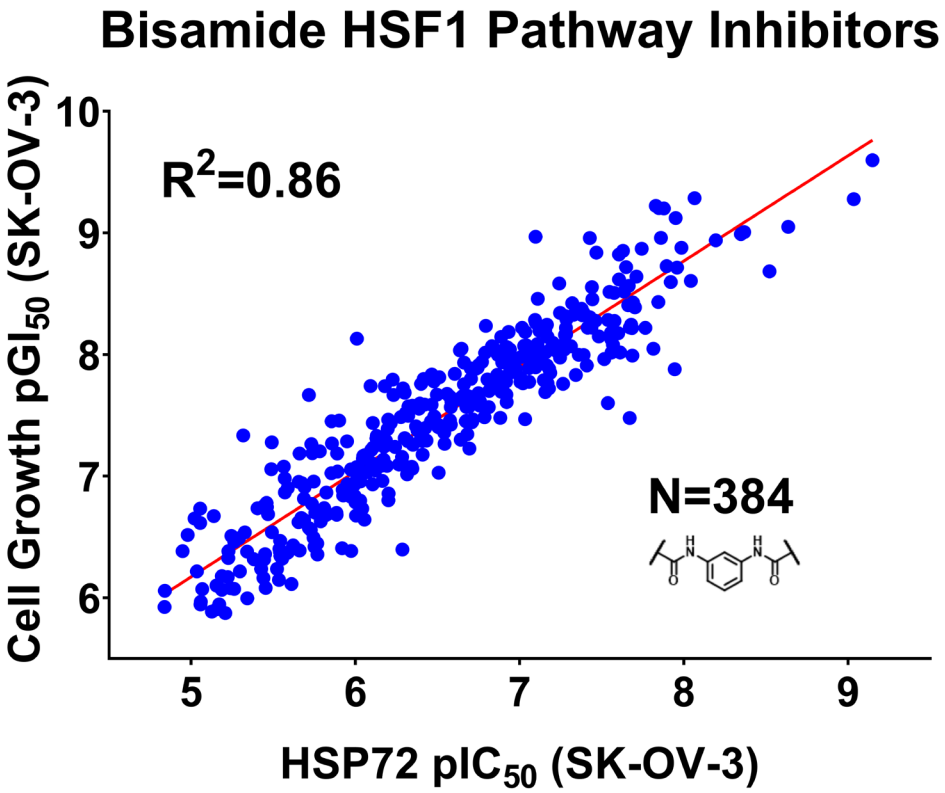
Hit
No efficacy from PO dose
Low Solubility

Lead & tool
Efficacy from PO dose
High PGP Efflux
High predicted human dose

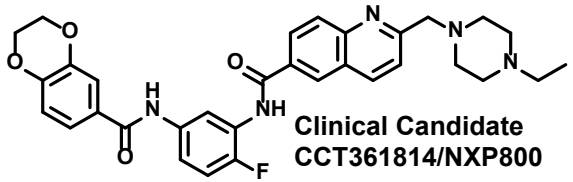
Clinical candidate
Tumor regressions from PO dose
Low PGP efflux
Low predicted human dose

Cheeseman et al J Med Chem 60 180-201 2017
Patent No. 9701664 2017
Pasqua et al in preparation

Good correlation between HSF1 pathway inhibition and cell growth inhibition



Pre-clinical profile of CCT361814/NXP800 clinical candidate

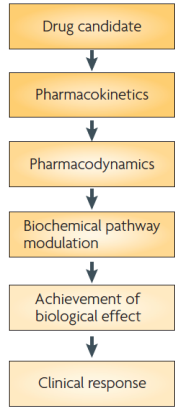


Clinical Candidate CCT361814/NXP800

Property	Value
Predicted Human Cl (mL/min/kg)	Cl_u = 100 (heps),[a] 170 (rat SSS)[b], $Cl_{tb}(f_{ub}=0.0054)$ = 0.54-0.92[c]
Predicted Human Half-life (h)	12 (rat)[d]
Thermodynamic Solubility FaSSIF, FeSSIF, FaSSGF (mg/mL)	0.27, 0.034, 0.92[e]
Predicted Human Dose (mg/person/day)	130-210[f]

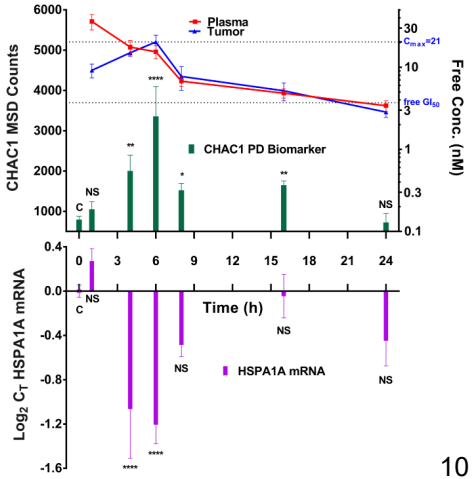
Pharmacologic Audit Trail

Yap et al Nature Rev Cancer 2010



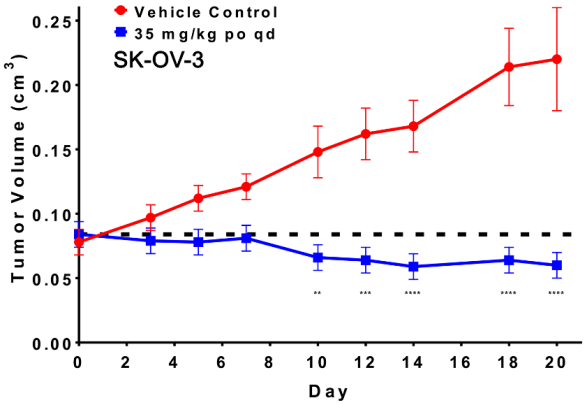
- Activity in multiple xenograft models of ovarian clear cell cancer – intrinsically resistant to platinum-based chemotherapy
- Acceptable oral absorption across the preclinical species, mouse, rat and dog
- Clean profile across in vitro hERG, Cyp450, kinase and Cerep-Safety-87 inhibition assays
- Acceptable toxicity profile and therapeutic index with dose-limiting toxicity studies complete in rat and dog
- Kilogram scale-up synthesis and solid state properties optimization complete to deliver GLP API suitable for human po dosing in capsules

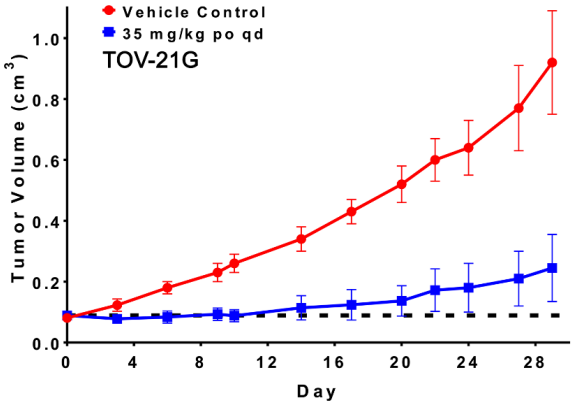
SK-OV-3 ovarian cancer xenograft PK/PD study

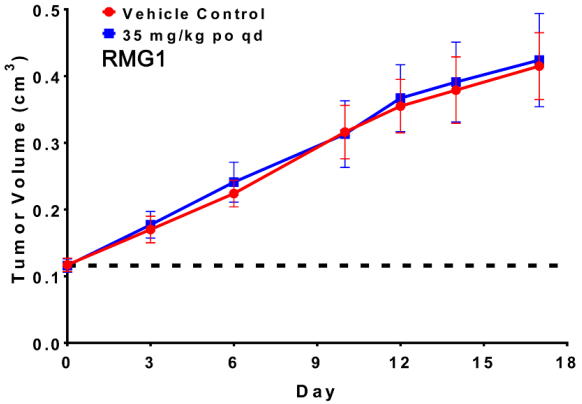


Identifying ARID1A as a predictive biomarker for NXP800

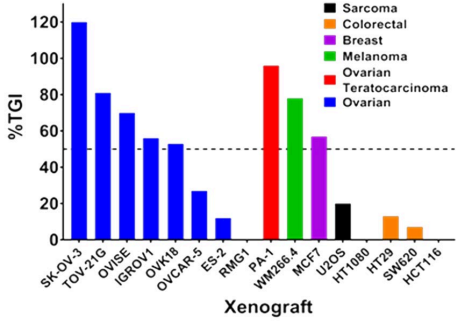
Response of three different human ovarian tumour xenografts







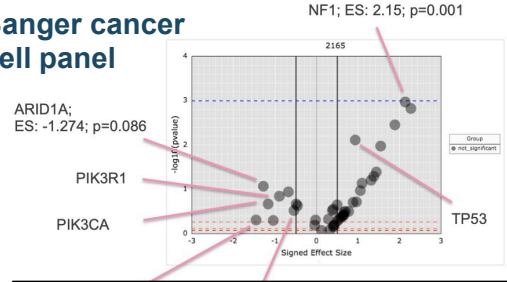
Response of larger tumour xenograft panel



Link to ARID1A status

Xenograft	Efficacy (% T/C)	ARID1A
SK-OV-3	25	Q586*
TOV-21G	27	Y551fs*72/Q758fs*75
IGROV-1	50	D1850fs*4
OVISE	53	Q542fs*80/H203fs*127
OVK18	52	p.Pro109fs*194
OVCAR-5	90	WT
ES-2	100	WT
RMG-I	100	WT

Sanger cancer cell panel

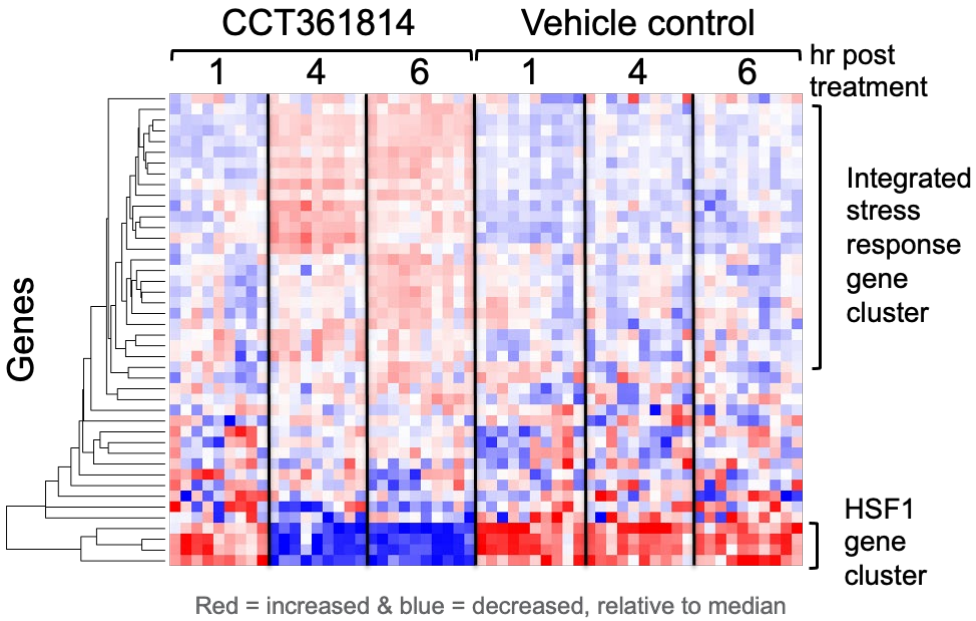


Further annotation & analysis showed a 4.7 fold difference in mean GI50 between the ARID1A mutant & WT ovarian cell lines p value =0.001

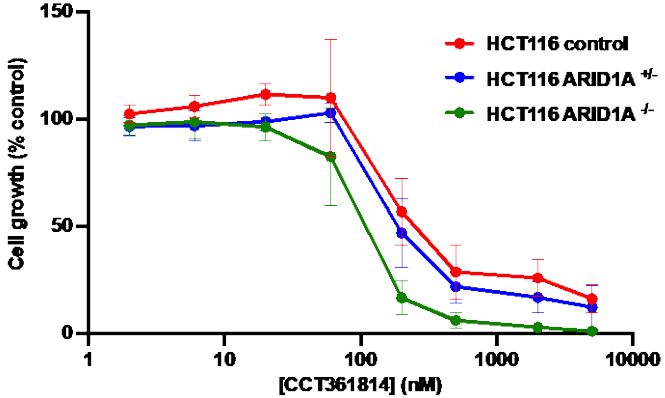
Identifying gene signature biomarkers for PD with NXP800

Mice with IGROV-1 human ovarian tumour xenografts treated with CCT361814/NXP800

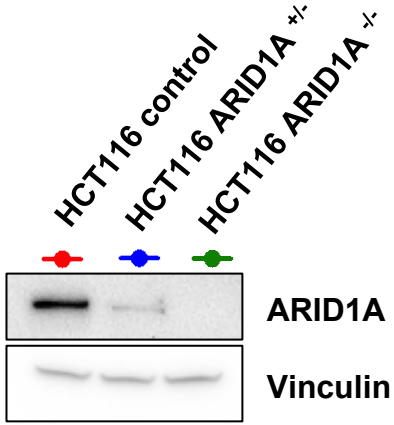
- NanoString gene signature established from microarray profiling experiments
- Heat map of all genes in the signature altered 4 and 6 hours post-treatment
- Genes encoding heat shock proteins are repressed and the integrated stress response activated eg CHAC1
- Provides biomarkers for PK/PD

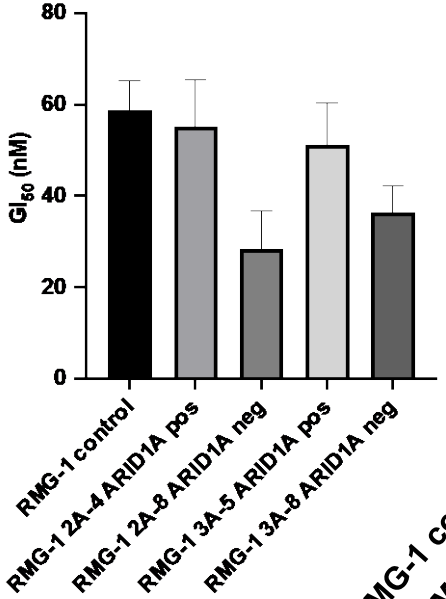


Further validation of ARID1A as a predictive biomarker in isogenic pairs

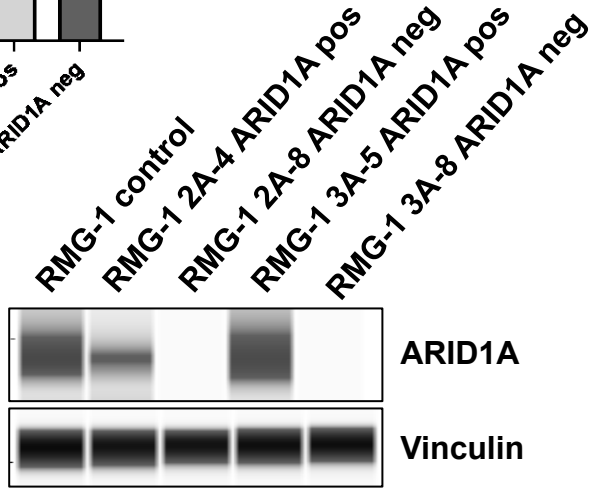


HCT116 human colon cancer line model





RMG-1 human ovarian cancer model



Phase 1 clinical trial of NXP800

- The Phase 1 trial commenced December 31, 2021 and first patient was treated in January 2022
- The study involves dose-escalation (Phase 1a) and expansion (Phase 1b) phases
- PK, safety and tolerability of NXP800 will be evaluated in patients with advanced solid tumors aiming to identify a dose and schedule for the Phase 1b
  - accelerated titration schedule with single-patient cohorts at the lowest dose levels
  - combination of rule-based and Bayesian approaches
- In the Phase 1b, the safety and preliminary anti-tumor activity of NXP800 will be evaluated in ARID1A biomarker-selected patients
  - initially those with ovarian clear cell carcinoma and endometrioid carcinoma (high unmet need)
- Builds on preclinical data and the Pharmacologic Audit Trail principles
- See www.clinicaltrials.gov/ct2/show/NCT05226507?term=hsf-1&draw=2&rank=3
- Future studies would look at drug combinations and additional cancer types

Summary and future plans

- NXP800 is a first-in-class, orally active small-molecule HSF1 pathway inhibitor
- Discovered at ICR and developed by Nuvectis Pharma
- Strong validation for modulating this pathway in a range of human cancers
- Bisamide series was discovered in phenotypic pathway screen
- Optimised through lead compound/chemical tool to clinical candidate
- Biomarkers discovered and validated to provide PK/PD relationship and Pharmacologic Audit Trail
- ARID1A loss cancers show increased sensitivity of NXP800
- Based on exciting preclinical activity, initial clinical studies will focus on ovarian clear cell carcinoma and endometrioid carcinoma – which have a very high unmet need
- Therapeutic potential in additional tumour types – being explored preclinically, also combinations
- Multiple orthogonal approaches are being applied to identify the precise key molecular target(s)

Thanks to the NXP800 drug discovery project team at ICR

AACR American Association for Cancer Research ANNUAL MEETING 2022 New Orleans

APRIL 8-13 • #AACR22

In vitro biology
Swee Sharp
Marissa Powers
Robert te Poele
Emmanuel de Billy
Alaide Morcanvallo
Kate Swabey
Paul Clarke

In vivo biology
Sharon Gowan
Loredana Pellegrino
Melanie Valenti
Suzanne Eccles
Paul Clarke

Medicinal chemistry
Nicola Chessum
Elisa Pasqua
Birgit Wilding
Lindsay Evans
Susan Lepri
Michael Tucker
Giampiero Columbo
John Caldwell
Carl Rye
Julian Blagg
Ian Collins
Matt Cheeseman
Keith Jones

DMPK
Asadh Miah
Angela Hayes
Florence Raynaud

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Martin Rowlands
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Rosemary Burke

Protein crystallography
Salya Ali
Rob van Montfort

Data management
Gary Nugent

Structural chemistry
Meirion Richards
Maggie Liu
Amin Mirza

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Michael Tucker
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Bugra Ozer
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Enrique Poradosu
Ron Bentsur
Consultant team

CRT Pioneer Fund/ Sixth Element Capital
Rob James
Ian Miscampbell

Battle Against Cancer Investment Trust (BACIT
Tom Henderson

Kidani Fund

AstraZeneca
Early stage collaboration

Clinical trial
Udai Banerji
ICR/Royal Marden
Drug Development Unit

CANCER RESEARCH UK

ICR The Institute of Cancer Research

Dedication

Susan Lindquist
1949–2016