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
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
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- An HSF1 pathway inhibitor could block multiple cancer hallmarks and exert powerful anticancer effects.
A wealth of data validates HSF1 as a cancer drug target

Heat Shock Factor 1 Is a Powerful Multifaceted Modifier of Carcinogenesis


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/Berms et al EMBO Rep 2016)
HSF1 is highly challenging to drug directly

- 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

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

Feng et al iScience 24 102951 2021
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

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

Steep cell SAR essential

CCT245232
HSP72 (SK-OV-3) IC_{50} = 91 nM
Cell Growth (SK-OV-3) GI_{50} = 8.4 nM
Kinetic Sol (pH 7.4) < 1.0 μM

CCT251236
HSP72 (SK-OV-3) IC_{50} = 25 nM
Cell Growth (SK-OV-3) GI_{50} = 2.4 nM
Kinetic Sol (pH 7.4) = 72 μM
PGP (CACO2) ER = 16

CCT261814/NXP800
HSP72 (SK-OV-3) IC_{50} = 94 nM
Cell Growth (SK-OV-3) GI_{50} = 8.5 nM
PGP (CACO2) ER = 2.8

Optimized solubilizing group

Probe Optimization
High Efflux
High rat Cl_{int,u}

Vector-to-solvent Solubilizing group
Target-ID linker

Lead Optimization

Low efflux

American Association for Cancer Research
AAGR
ANNUAL MEETING
2022 New Orleans
APRIL 8-13 • #AACR22

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

\[ R^2 = 0.86 \]

N = 384
Pre-clinical profile of CCT361814/NXP800 clinical candidate

- 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
Identifying ARID1A as a predictive biomarker for NXP800

Response of three different human ovarian tumour xenografts

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

Link to ARID1A status

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

Sanger cancer cell panel

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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

**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

**Biochemical assays**
Lisa O'Fee  
Martin Rowlands  
Mark Stubbs  
Rosemary Burke

**Protein crystallography**
Salya Ali  
Rob van Montfort

**Data management**
Gary Nugent

**Structural chemistry**
Meirion Richards  
Maggie Liu  
Amin Mirza

**Computational chemistry**
Michael Tucker  
Yi Mok  
Joshua Meyers  
Nathan Brown

**Bio- and Chemo-informatics**
Bugra Ozer  
Costas Mitsopoulos  
Bissan Al-Lazikani
Thanks to our development partner Nuvectis, funders and collaborators

Nuvectis Pharma
Shay Shemesh
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

Dedication
Susan Lindquist
1949–2016