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Discovery and validation of biomarkers to support clinical development of NXP800: A first-in-class orally active, small-molecule HSF1 pathway inhibitor

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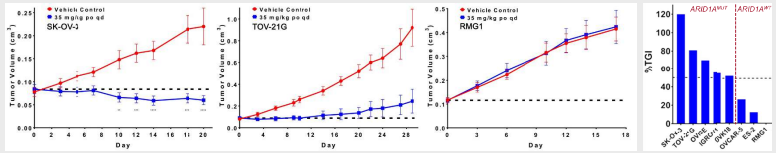
HSF1 and the oncogene-associated stress response

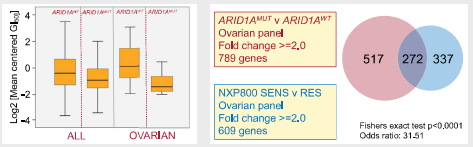
- HSF1 is an ancient transcription factor regulating the classical eukaryotic Heat Shock Response (HSR) and is a master regulator of proteotoxic stress[1]
- In response to oncogenic stress, HSF1 is hijacked in cancer cells to regulate a gene set overlapping with but distinct from the canonical HSR[2]
- Depleting HSF1 inhibits the oncogenic effects of p53 loss and RAS activation in mouse models[3] – 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[2,4] – indicating possible patient populations
- Preclinical toxicity indicates a potential therapeutic index - a phase 1 dose escalation, clinical study is ongoing

Identifying ARID1A as a predictive biomarker for NXP800



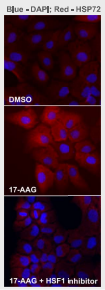
- NXP800 has activity in multiple xenograft models of ovarian clear cell cancer
  - all responsive ovarian cancer tumours xenografts (5 of 8; >50% Tumour growth inhibition) carried loss of function *ARID1A* mutations
  - ARID1A is a component of the large chromatin remodelling SWI/SNF complex that regulates transcription by altering their surrounding chromatin structure
  - ARID1A confers gene specificity to the SNF/SWI complex, particularly required for activation of genes normally repressed by surrounding chromatin structure
  - Impact of *ARID1A* mutation confirmed in isogenic models – ongoing; data not shown

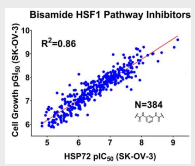
Sanger Institute cancer cell line panel

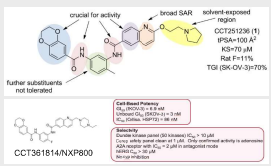


- An *ARID1A* mutation was a predictive biomarker for NXP800 sensitivity (4.7-fold; p_{adj}<0.001) in the ovarian cancer cell line subset
  - *ARID1A* mutation status not statistically predictive for the whole panel
  - possibly predictive for gastric and intestinal cancers
  - ovarian subset: 36% of basal genes associated with NXP800-sensitivity are also ARID1A-reg[4]

Discovery and profile of NXP800

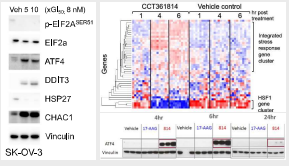




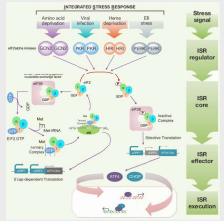


- HSF1 pathway inhibitors identified by the ability to block the induction of HSP72 by HSP90 inhibitor 17-AAG
  - screened 200K compounds from the AstraZeneca collection
  - discovered *N,N'*-4-methyl-1,3-phenylenediamide core structure
  - screen run in U2OS human osteosarcoma cells, followed up in SK-OV-3 human ovarian cancer cells
  - highly potent, low nanomolar hit against the HSF1 pathway and cancer cell growth inhibition
  - optimised through lead compound/chemical tool to clinical candidate[5,6]
  - NXP800 has a clean profile across *in vitro* hERG, Cyp450, kinase and Cerep-Safety-87 assays

Identifying protein and gene signature biomarkers for PD with NXP800



- Multiple cancer cell types treated with 5x GI_{50} NXP800 for 6 h and RNAseq profiled
  - significant enrichment of elevated integrated stress response (ISR) and ATF4-regulated genes
  - immunoblotting confirmed induction of ISR and ATF4
- NanoString probeset confirmed elevated ISR/ATF4 and repressed HSF1 activity *in vivo*
  - immunoblotting confirmed induction of ATF4 protein



- NXP800 treatment induces prolonged activation of the ISR[7]
  - ISR activation blocks HSF1 activation
  - prolonged ISR/ATF4 activation and CHOP induction can result in cell death

Phase 1 clinical trial of NXP800

- Phase 1 trial comprising a dose-escalation (Phase 1a) and expansion (Phase 1b) phases commenced and first patient treated in January 2022 (www.clinicaltrials.gov/ct2/show/NCT05226507)
  - 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

Summary and future plans

- NXP800 is the first-in-class, orally active inhibitor of HSF1 activation that is important for cancer cells
- Biomarkers discovered and validated to provide PK/PD relationship and Pharmacologic Audit Trail
  - strong validation for modulating HSF1 activation in a range of human cancers
  - biomarkers indicate a mechanism of action involving activation of the integrated stress response
- Initial clinical studies will focus on ovarian clear cell carcinoma and endometrioid carcinoma – with a very high unmet need
  - cancers with *ARID1A* loss show increased sensitivity to NXP800, providing a patient biomarker
  - therapeutic potential in additional tumour types
- Multiple orthogonal approaches are being applied to identify the precise key molecular target(s)

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References

1. Li J et al. 2017. Trends Cell Biol. 27:895.
2. Mendillo ML et al. 2012. Cell 150:549.
3. Dai C et al. 2007. Cell 130:1005.
4. Powell CD et al. 2015. PLoS 11:e168389.
5. Cheeseman MD et al. 2017. J. Med. Chem. 60:180.
6. Rye CS et al. 2016. Med. Chem. Comm. 2016 7:1580.
7. Pakos-Zebrucka K et al. 2016. EMBO Rep. 17:1374.

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