Novel biomarkers for sulforaphane treated patients in ER+/HER2- metastatic breast cancer

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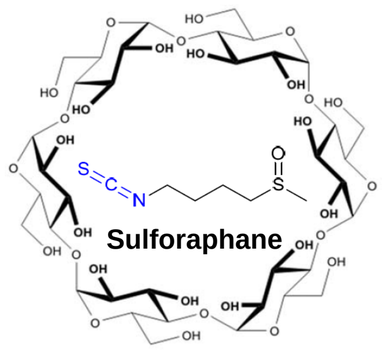
1. Project background

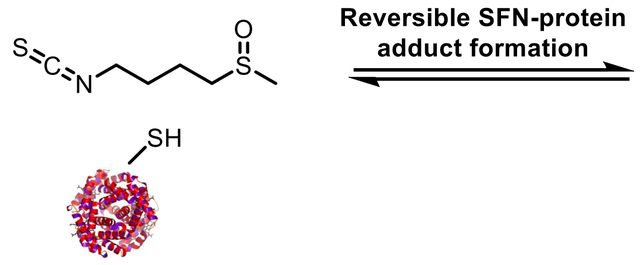
• **SFX-01** is a synthetic and stable formulation of sulforaphane (SFN) being developed by Evgen Pharma plc.

Evgen R&D pipeline – June 2018

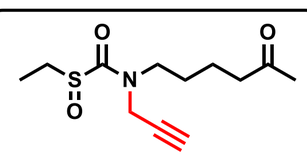
Drug (MoA)	Indication	Preclinical	Phase I	Phase IIa	Phase IIb	Phase III
SFX-01 (Nrf2)	Subarachnoid Haemorrhage					
SFX-01 (STAT3)	Metastatic Breast Cancer (ER+)					
SFX-01	Investigator-Initiated Clinical Studies† e.g. Triple Negative Breast Cancer, Ischaemic stroke					

Source: Evgen Pharma

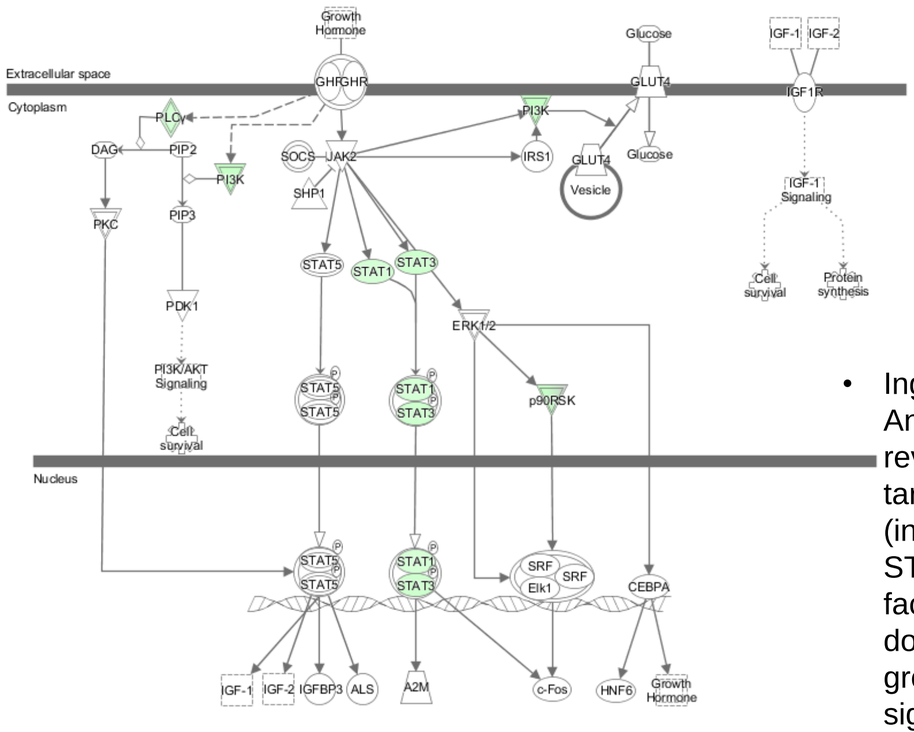




• Extending the work previously published by Ahn *et al.*[2], we have developed novel SFN activity-based probes (ABPs) that employ a more stable sulfoxycarbamate warhead and a click handle to identify known and novel targets of SFN.[3]

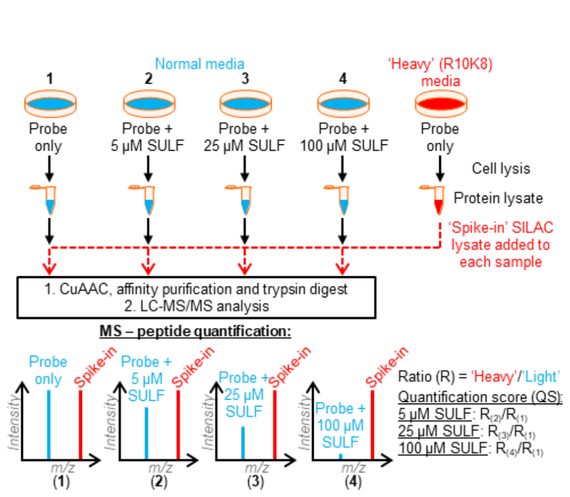


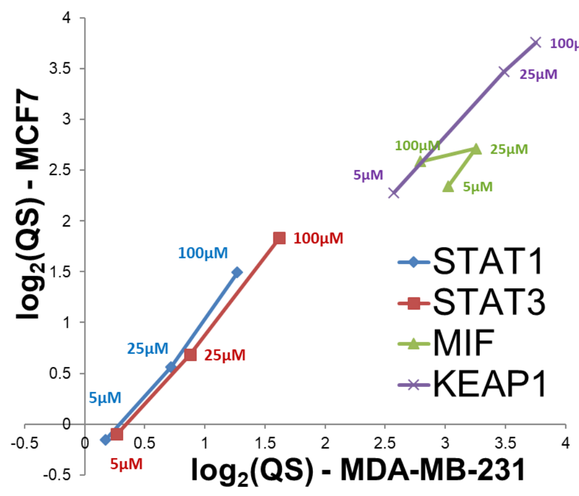
4. SFN targets in growth hormone signalling in MCF7



• Ingenuity Pathway Analysis (Qiagen) revealed that SFN target engagement (including inhibition of STAT transcription factors) would result in downregulation of the growth hormone signalling cascade.

2. Quantitative proteomics identifies SFN as a direct binder of STAT1 and STAT3 transcription factors

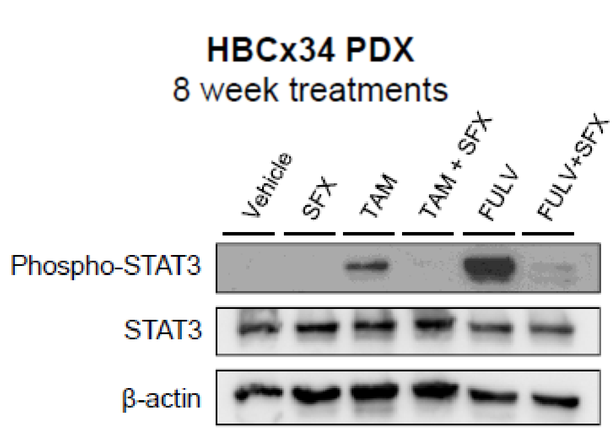


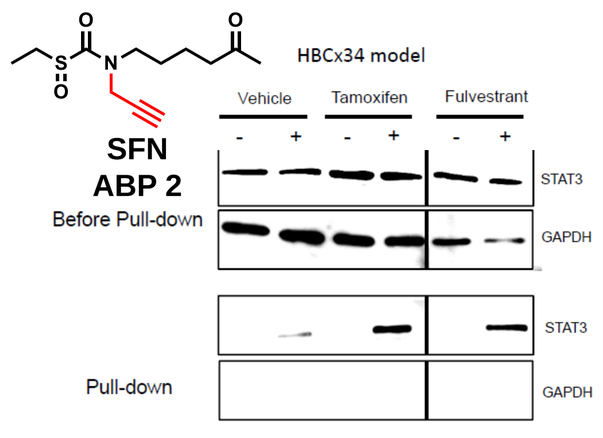


• While SFN inhibition of STAT3 signalling has been reported previously, the proposed mechanism of action is through the phosphorylation status of STAT3's activator kinase, JAK2.[4] Our data show sulforaphane also directly binds STAT1 and STAT3.

5. SFX-01 inhibits anti-estrogen activation of STAT3 signalling

• ER-positive breast cancers frequently develop resistance to anti-estrogen therapies (e.g. Tamoxifen, Fulvestrant), which is thought to be due to enhanced cancer stem cell (CSC) survival post treatment.





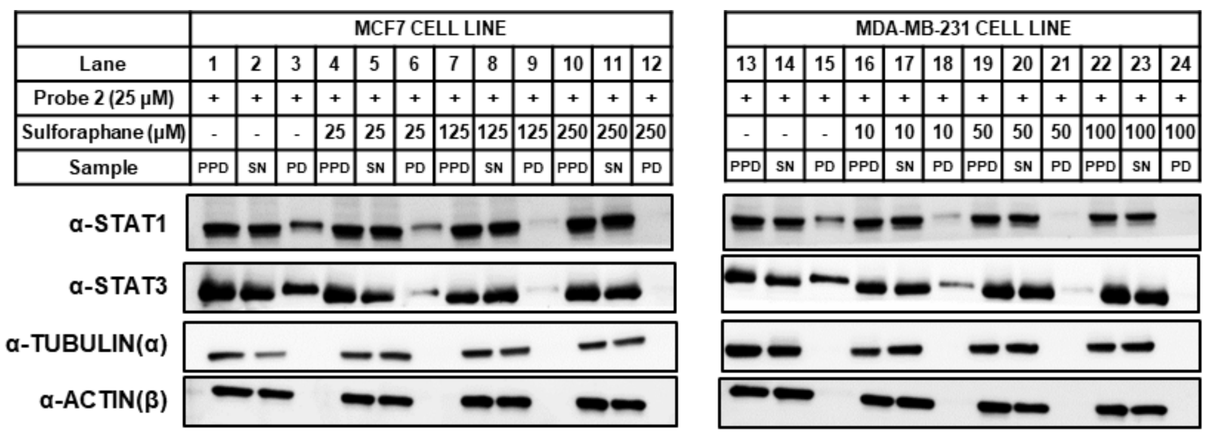
• **SFX-01** resensitises CSCs in human breast cancer patient-derived tumours (HBCx34 PDX), treated with either Tamoxifen (TAM) or Fulvestrant (FULV), via inhibition of STAT3 phosphorylation.

• SFN **ABP 2** was shown to directly engage STAT3 in HBCx34 PDX cells.

3. STAT1 and STAT3 validated as SFN targets

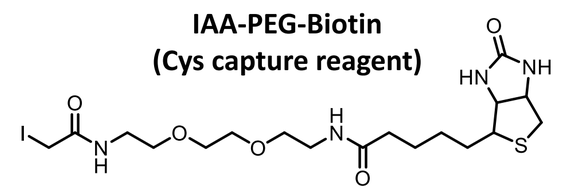
	MCF7 CELL LINE											
Lane	1	2	3	4	5	6	7	8	9	10	11	12
Probe 2 (25 µM)	+	+	+	+	+	+	+	+	+	+	+	+
Sulforaphane (µM)	-	-	-	25	25	25	125	125	125	250	250	250
Sample	PPD	SN	PD	PPD	SN	PD	PPD	SN	PD	PPD	SN	PD

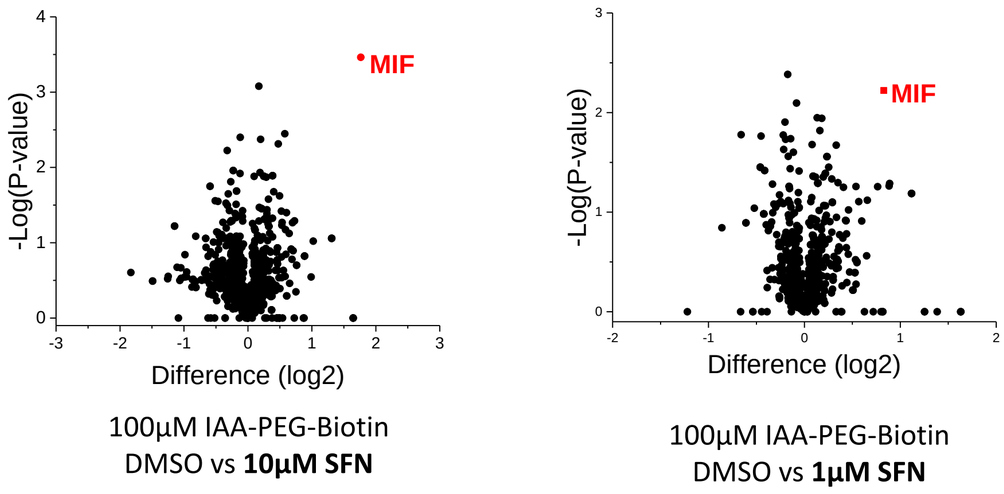
	MDA-MB-231 CELL LINE											
Lane	13	14	15	16	17	18	19	20	21	22	23	24
Probe 2 (25 µM)	+	+	+	+	+	+	+	+	+	+	+	+
Sulforaphane (µM)	-	-	-	10	10	10	50	50	50	100	100	100
Sample	PPD	SN	PD	PPD	SN	PD	PPD	SN	PD	PPD	SN	PD



Western Blot analyses for STAT1 and STAT3 proteins confirming them both as targets of **SFN ABP 2** and show that sulforaphane is capable of competing ABP labelling using an orthogonal technique to mass spectrometry-based proteomics across both the MCF7 and MDA-MB-231 cell lines. **PPD** = pre-pull down lysate, **SN** = supernatant lysate left after the affinity enrichment, and **PD** = proteins immobilised on the resin as a result of pulldown.

6. MIF as a potential biomarker for SFN engagement in breast cancer





• Employing a new method with lower (physiological) SFN concentrations, MIF was negatively enriched in a concentration dependent manner, when SFN was competed against the cysteine selective capture reagent (IAA-PEG-biotin), suggesting this protein could be used as a biomarker for clinical samples.

7. Summary

- Quantitative proteomics using SFN ABPs have allowed identification of novel SFN efficacy biomarkers (STAT1, STAT3, MIF)
- Pathway analysis suggests SFN influences growth hormone signalling
- Phospho-STAT3 may be a useful biomarker for response of anti-estrogen therapy resistant tumours to clinical SFN candidate **SFX-01**

8. References

(1) Evgen Product Pipeline http://evgen.com/pipeline/ (accessed Dec 27, 2018).
(2) Ahn, Y.-H., *et al. PNAS* **2010**, *107* (21), 9590–9595.
(3) Clulow, J. A. *et al. . Chem. Commun.* **2017**, *53* (37), 5182–5185.
(4) E.-R. Hahm *et al. Cancer Prev. Res.*, **2010**, *3*, 484–494.

Funding acknowledgements

evgen pharma
EPSRC