SFX-01 targets STAT3 signalling to inhibit stem-like cells in breast cancer patient-derived xenograft tumours

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Summary

• SFX-01 targets cancer stem-like cells in early and metastatic patient-derived breast cancer samples
• SFX-01 counteracts the effects of anti-estrogens on CSCs in patient-derived breast cancer xenografts
• Anti-estrogen activation of STAT3 signalling is inhibited by SFX-01

Background:
SFX-01 is a novel therapeutic comprising synthetic sulfophane (SFN) stabilised within α-cyclodextrin. Breast cancer stem-like cells (CSCs) have been identified in all molecular subtypes and are likely drivers of breast cancer metastasis and treatment resistance. We established previously that CSC activity in ER+ BC represents a source of therapeutic resistance (Simões et al, Cell Reports, 2015).

Material and methods:
We investigated SFX-01 effects on breast cancer activity using mammosphere formation efficiency (MFE) and aldehyde dehydrogenase (ALDH) activity using the ALDEFLUOR assay in patient samples and patient-derived xenograft (PDX) tumours. Cells from primary (n=12) and metastatic (n=15) samples were treated with SFX-01 (5 μM) or vehicle control. Using a 2 or 8 week in vivo treatment, early (HBCx34) and metastatic (BBRx31) ER+ PDX tumours were treated with SFX-01 (300mg/kg/day) alone or in combination with tamoxifen (Tam, 10 mg/kg/day) or fulvestrant (FULV, 200 mg/kg/week). Tumours were dissociated and MFE and ALDH activity assessed.

Results:
SFX-01 in vitro reduced MFE of both primary (0.19±0.02 vs control 0.52±0.06; p<0.001) and metastatic patient samples (0.43±0.04 vs control 0.93±0.07; p<0.001). TAM and FULV increased MFE and ALDH activity after 2 weeks of treatment in vivo, which was abrogated by combination with SFX-01; for example HBCx34 MFE with TAM alone: 0.81±0.07 vs TAM+SFX-01: 0.34±0.02 (p<0.01) and ALDH+ with TAM alone 10%±0.4 vs TAM+SFX-01 4.2%±0.4 (p<0.01). TAM+SFX-01 suppressed tumour growth at 8 weeks vs TAM alone in HBCx34 but not BBRx31. FULV treatment maintained tumour growth suppression at 8 weeks and no additive effect was seen with SFX-01, although MFE and ALDH activity were suppressed. Mechanistically, SFX-01 potently suppressed the increased expression in phospho-STAT3 after anti-estrogen treatments and we are currently investigating STAT3 signalling effectors through RNAseq data analysis.

Conclusions:
Our data demonstrate the potential of SFX-01 for clinically meaningful improvements to endocrine therapy in ER+ breast cancer by reversing CSC mediated resistance.

ABSTRACT

SFX-01 reduces breast CSC activity in primary and metastatic breast cancer patient derived samples

SFX-01 reduces breast CSC activity in primary and metastatic breast cancer stem cell patient derived samples.

SUMMARY

• SFX-01 targets cancer stem-like cells in early and metastatic patient-derived breast cancer samples
• SFX-01 counteracts the effects of anti-estrogens on CSCs in patient-derived breast cancer xenografts
• Anti-estrogen activation of STAT3 signalling is inhibited by SFX-01

CONCLUSION

SFX-01 may be of potential benefit in combination with anti-estrogens to overcome endocrine resistance of ER+ tumours

Figure 1: Mammosphere formation efficiency (MFE) of freshly isolated early (A) and metastatic (B) patient derived samples cultured in the presence of SFX-01 (5 μM, blue bars) or vehicle control (black bars). MFE data for each individual patient sample is represented. MFE was determined on day 7-9 and calculated by dividing the number of mammospheres formed (≥50 μm) by the original number of single cells seeded (500 cells/cm2) and is expressed as the mean percentage ± SEM. * p<0.05; ** p<0.01

Figure 2: Mammosphere formation efficiency (MFE) of estrogen receptor positive early (A) and metastatic (B) patient derived samples represented in Figure 1. Here, samples were also treated with tamoxifen alone (10 μM, red bars) or in combination with SFX-01 (5 μM, hatched bars). MFE data for each individual patient sample is represented. MFE was determined on day 7-9 and calculated by dividing the number of mammospheres formed (≥50 μm) by the original number of single cells seeded (500 cells/cm2) and is expressed as the mean percentage ± SEM. * p<0.05; ** p<0.01

Figure 3: A) HBBx31 and HBCx34 patient derived xenografts (PDXs) treated in vivo for 14 days with SFX-01 (300mg/kg/day, oral gavage) in the presence or absence of tamoxifen (10mg/kg/day, oral gavage). HBCx34 model was kindly provided by Dr Elisabetta Marangoni (Institute Curie, Paris). B) Quantification of K67 expression determined by immunohistochemistry showing that tamoxifen but not SFX-01 significantly decreases proliferation marker K67. C) Percentage of ALDH-positive cells was determined with ALDEFLUOR assay. ALDH-positive cells were discriminated from ALDH-negative cells using the ALDH inhibitor, DCAb. Mouse cells were excluded from the FACs analysis with anti-mouse MHC Class I (H-2Kd) antibody. D) Mammosphere formation efficiency was determined on day 7-9 and calculated by dividing the number of mammospheres formed (≥30 μm) by the original number of single cells seeded (500 cells/cm2) and is expressed as the mean percent of each treatment group. Representative micrographs are shown (scale bar 50 μm). E) Secondary transplantation of 500K, 100K, 20K and 4K cells after in vivo treatment. Experiment was carried out in NSG mice with 90-day slow release estrogen pellets. Tumor growth (>75 mm3) was assessed at day 90 and is represented as mice positive for each treatment group. ELDA of tumor-initiating cell frequency is shown. Data are represented as mean ± SEM. * p<0.05; ** p<0.01

Figure 4: A/B Tumour growth of HBCx34 PDX tumours treated in vivo for 56 days with tamoxifen (10mg/kg/day, oral gavage) or fulvestrant (200 mg/kg/week, subcutaneous injection) in the presence or absence of SFX-01 (300mg/kg/day, oral gavage). Individual tumours (n=10) treated with tamoxifen (A) or fulvestrant (B) are represented by squares and PDX tumours treated in combination with SFX-01 are represented by triangles. Red line represents average tumour growth for tamoxifen (A) or fulvestrant (B) and blue line represents average tumour growth for the combination treatment with SFX-01. C/D) Mice lungs were stained with anti-human mitochondrial antibody and micrometastasis with at least 10 cells were counted. Percentage of mice bearing micrometastasis for each treatment group is represented.

Figure 5: A) Phospho-STAT3 and total STAT3 protein expression levels determined by Western Blot in HBCx34 PDX treated in vivo in the presence or absence of tamoxifen or fulvestrant. β-actin was used as a reference for the loading control. B) Protein expression levels in tamoxifen-resistant (TamR) HBCx34 PDX treated in vivo for 56 days with SFX-01.