Basic selective estrogen receptor degraders (B-SERDs) in combination with novel BET inhibitors in ER+ breast cancer: addressing resistance and brain metastases

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Background

Approximately 70% of breast cancer patients have estrogen receptor positive (ER+) tumors; and despite the success of endocrine therapy, 50% will progress to metastatic disease with resistance to tamoxifen (TAM) and aromatase inhibitors (AIs). Fulvestrant (FUL), a selective estrogen receptor degrader (SERD) able to ablate ER, was introduced to clinical practice in 2002 for advanced ER+ metastatic breast cancer. In August 2017, FUL was added to first-line therapy after demonstrating superiority to first-line AI therapy. Acquisition of FUL resistance in first-line setting (and in combination with CDK4/6I, i.e palbociclib) has arisen in many patients, which may be associated with the poor pharmacokinetics of FUL leading to sub-maximal dosing. Moreover, neither FUL, nor oral SERDs in current clinical trials address brain metastases. We have previously developed a novel family of oral SERDs, exemplified by G1T48 (ESMO Poster 340P).

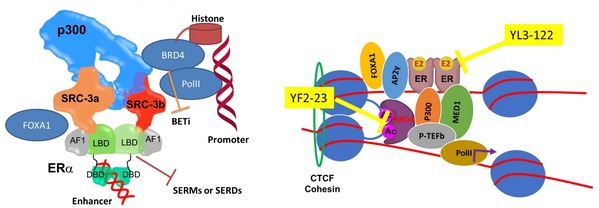
Objectives

1. To design and develop brain-bioavailable oral SERDs (B-SERDs) to treat metastatic ER+ breast cancer, addressing brain metastases that significantly degrade patient prognosis.

2. To explore epigenetic modulation in cancer by designing and developing novel bromodomain and extra-terminal inhibitors (BETi) that can effectively inhibit growth of TAM, FUL, and palbociclib (Palbo) resistant breast cancer.

3. To explore BETi in combination with SERDs and B-SERDs in endocrine resistant breast cancer to enhance efficacy, or prevent/delay resistance.

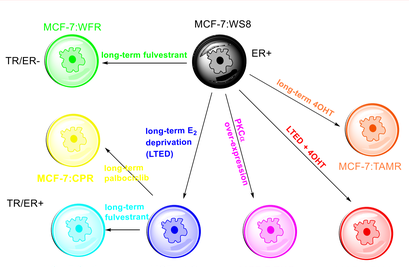
SERD/BETi Hypothesis



Co-targeting of ERα and transcriptional regulator (BET) has potential to: 1) increase efficacy in TAM/AI resistance setting; and 2) overcome acquired resistance to SERDs in ER+ breast cancer.

(Left figure adapted from cryo-EM structure published in Molecular Cell 57, 1047–1058)

Cell Model Systems (2D and 3D)



	TAM	AI	FUL	PALB
WS8	✓	✓	✓	✓
PKCα	✓	-	✓	✓
TAM1	×	×	✓	✓
TAMR	×	-	✓	✓
5C	×	×	✓	✓
CFR	×	×	×	✓
CPR	×	×	✓	×
WFR	×	×	×	✓

T47D-TYS (*ESR1-Y537S*); T47D-TDG (*ESR1-D538G),* and T47D-WT were a gift from David Shapiro, *Sci Rep* **2016,** 6, 34753.

Oral SERDs

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Novel Selective Estrogen Receptor Downregulators (SERDs) Developed against Treatment-Resistant Breast Cancer

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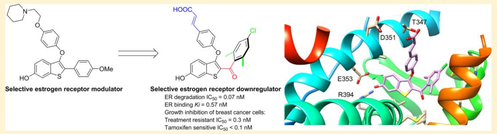
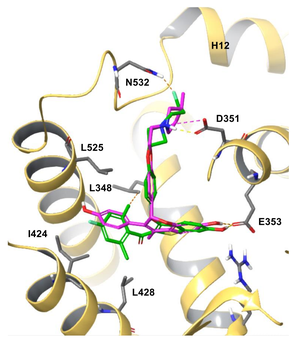
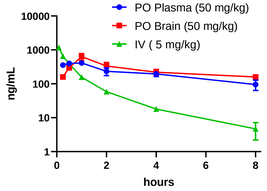
Supporting Information



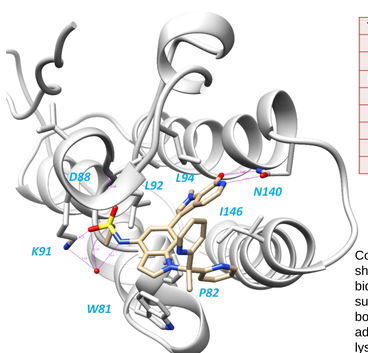
B-SERD Optimization





YF3-122 (50 mg/kg p.o.) pharmacokinetic parameters	
Oral bioavailability (F)	22%
$T_{1/2}$ (h)	4.48
C_{max} Ratio (Brain/Plasma)	1.54
C_{max} (ng/ml)	413
AUC_{Last} Ratio (Brain/Plasma)	1.26
AUC_{Inf} h*ng/mL	2292
T_{max} (h)	1.0

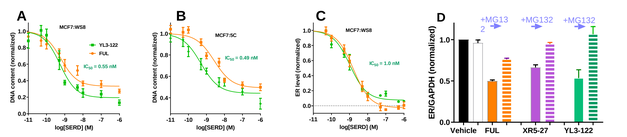
BETi Optimization



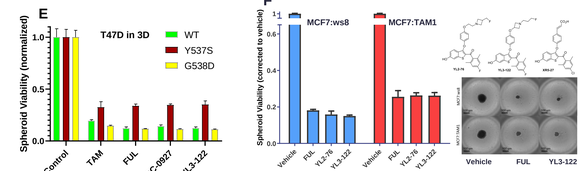
YF2-23 (30 mg/kg p.o.) pharmacokinetic parameters	
Oral bioavailability (F)	49%
$T_{1/2}$ (h)	2.9
hERG inhibition IC_{50} (μM)	29
C_{max} (ng/ml)	3090
Cl_obs (ml/min/kg)	25.5
Vss_obs (L/kg)	1.49
CYP inhibition	3A4 (10 μM); 2C8 (6 μM)
PPB	97.98%

Co-crystal structure of YF2-23 with BRD4-BD1 shows that the "warhead", pyridinone, forms bidentate H-bonds with ASN140. The sulfonamide in **YF-2-23** forms two hydrogen bonds with ASP88 and lysine 91 and two additional water-mediated hydrogen bond with lysine 91. The pyridine-indole ring system occupies the WPF shelf.

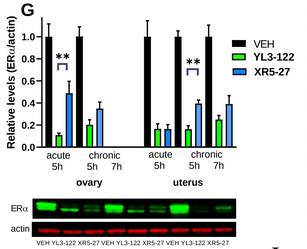
B-SERD Pharmacology



Summary of B-SERD in vitro activity. 2D growth inhibition (A, B). Data normalized to vehicle (1.0) and no cells (0.0) shown as mean ± SEM. (C) ER level following treatment for 24 hr by In-Cell-Western. Data corrected to vehicle (1) and 1 μM 2 shown as mean ± SEM.(D) ERα degradation after 24 h treatment of MCF7:WS8 cells with drugs (10 nM) measured by western blot and inhibited by proteasomal inhibitor MG-132 (1 μM) normalized to vehicle (1.0).

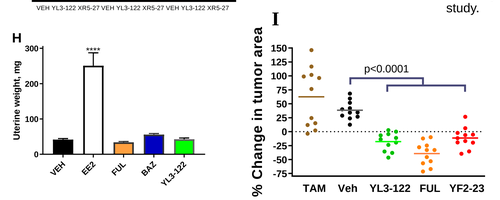


B-SERD activity in 3D spheroids modeling resistance to AI and TAM. (E) T47D:WT, T47D:TYS, and T47D:TGD cells were grown for 3 days to establish spheroids that were treated for a further 11 days with test compounds (10 nM). (F) Spheroid viability following treatment (10 nM) with representative images on day 10 of treatment. Luminescence normalized to vehicle/control (1.0) and background (0.0). Data shown as mean ± SEM Significance compared to vehicle/control by one-way ANOVA: p <0.0001.).



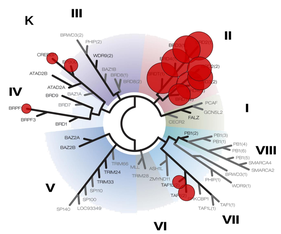
B-SERD activity in vivo. (G) ERα degradation after oral dosing of female mice with vehicle, YL3-122, or XR5-27 (50 mg/kg) measured by western blot of tissues, with representative immunoblots shown from individual mouse uteri. (H) Uterine weight from juvenile female rats dosed with YL3-122 or bazedoxifene (10 mg/kg), FUL (2 mg/kg) compared to EE2 (0.1 mg/kg) as a positive control. Cell culture data shown as mean ± SEM from three biological and analytical replicates. Statistical analysis by one-way ANOVA (** p < 0.01; **** p < 0.0001). (I) MCF7:TAM1 xenografts grown to 0.3 cm2 before treatment with FUL (5 mg s.c.), BETi (30 mg/kg), B-SERD (100 mg/kg) Individual tumor % area change after 4 weeks treatment: one-way ANOVA.

study.



(J) No gross toxicity from YF2-23 dosing in xenograft study .

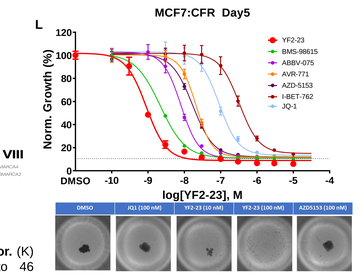
BETi Pharmacology



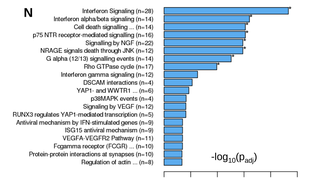
YF2-23 is a potent, selective BET inhibitor. (K) Selectivity from BROMOscan binding to 46 bromodomain proteins. (M) YF2-23 demonstrated 100x greater potency relative to the benchmark pan-BET inhibitor JQ-1 across BRD proteins.. .

M

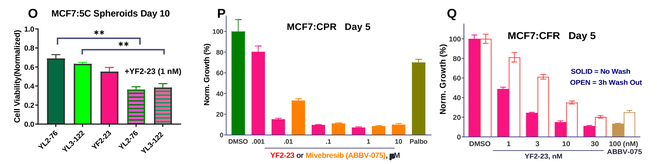
Bromodomain	YF-2-23, nM	JQ-1, nM
BRD2(BD1)	0.27	27
BRD2(BD2)	0.77	18
BRD2(BD1,2)	0.57	5.6
BRD3(BD1)	0.34	14
BRD3(BD2)	0.61	19
BRD3(BD1,2)	0.27	14
BRD4(BD1)	0.29	14
BRD4(BD2)	0.33	8.2
BRD4(BD1,2)	0.29	7.3
BRD4(full)	0.1	11
BRDT(BD1)	0.14	47
BRDT(BD2)	1.4	35
BRDT(BD1,2)	0.23	46



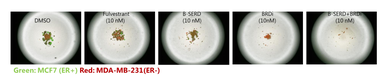
YF2-23 is effective in FUL-resistant breast cancer. (L) Superiority over other clinical stage BETi in MCF-7:CFR cells in 2D monolayer (conc-response) and in 3D spheroids.



YF2-23 response is not identical to JQ-1. (N) RNA seq analysis shows YF2-23 (100 nM) strongly downregulates interferon signaling compared to DMSO (not shown) and JQ1 (100 nM) in MCF-7:CFR cells.



YF2-23 inhibits cell growth: in combination with B-SERDs; and in both palbociclib and fulvestrant resistant breast cancer cells. (O) Spheroids treated at 24 h with B-SERDs (10 nM) ± BETi normalized to vehicle. (P) Subnanomolar potency of YF2-23 in growth inhibition of MCF7:CPR cells, superior to ABBV-075. (Q) To simulate drug clearance after daily dosing in vivo, drug was washed out at 3 h, showing persistent drug effect. Intermittent exposure driven by PK may be key to management of on-target toxicity seen for BETi in clinical trials. (R) Tumors containing ER+ and ER- cells respond to B-SERD/BETi combo.



Conclusions

1. B-SERDs were developed with good oral and brain bioavailability, evolved from the scaffold of G1T48. B-SERDs matched the efficacy and potency of FUL in cell cultures and xenografts resistant to TAM and in *ESR1* mutant cell lines. Side chain architecture led to SERD, SERM, and SERM/SERDs (the latter manifested lower efficacy in MCF7:5C cells). All SERMs, B-SERDs were ineffective in the MCF7:CFR cell line.
2. An orally bioavailable, selective BETi was identified with greater antiproliferative potency in MCF7:CFR cells than six BETi in clinical trials. On-target toxicity was not seen in xenograft studies, possibly due to PK properties. YF2-23 was differentiated from JQ1 in potency and transcriptional signature. Potent growth inhibition of MCF7:CPR cells and synergistic actions with B-SERDs in MCF7:5C cells were observed.
3. The antiproliferative actions of BETi are not limited to breast cancer cells, extending to pancreatic and other cell lines. Xenograft models are unlikely to recapitulate the immunomodulatory effects of BETi on tumor growth.

Future Directions

1. Testing of B-SERDs in ER+ breast cancer brain metastasis model using i.c.v. or intracarotid delivery of labeled MCF7:PKCα cells.
2. Comparison of B-SERD vs SERD in transcriptional complex dynamics and effect of added BETi.
3. Dose-ranging of B-SERD and BETi in multiple treatment-resistant ER+ breast cancer cell lines and in xenograft models.
4. Exploration of BETi beyond antiproliferative actions.

Acknowledgements

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