Effects of G1T48, a novel orally bioavailable selective estrogen receptor degrader (SERD), and the CDK4/6 inhibitor, G1T38, on tumor growth in an animal model of tamoxifen resistant breast cancer

Abstract # 5641

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Abstract

Background: The combination of targeting the CDK4/6 and ER signaling pathways with palbociclib and fulvestrant is a proven therapeutic strategy for the treatment of ER positive breast cancer. However, the poor physicochemical properties of fulvestrant require monthly intramuscular injections to patients, which limit the pharmacokinetic and pharmacodynamic activity of the compound. Therefore, an orally available compound that more rapidly reaches steady state may lead to a better clinical response in patients. Here we report the preclinical characterization of G1T48, a novel, orally bioavailable, non-steroidal small molecule inhibitor of ERα, which is a potent, selective antagonist that down regulates ERα in vitro and in vivo in ER-positive models of breast cancer.

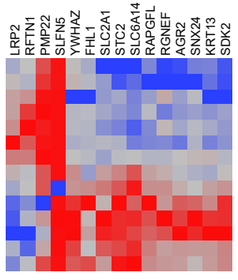
Methods: Breast cancer cells expressing clinically relevant ESR1 mutations (ER-Y537S, ER-D538G) were treated with G1T48, and mechanistically distinct SERMs/SERDs and cellular proliferation was assessed by measuring DNA content (Hoechst dye). Ovariectomized nu/nu mice bearing xenografts of tamoxifen (TamR) resistant ER+ breast cancer were treated with G1T38 and G1T48, alone or in combination, with clinically relevant comparators. Time to progression and tumor volume were assessed over a 4 week dosing period.

Results: G1T48 treatment led to dramatic reductions in ER protein levels. G1T48 significantly inhibited cellular proliferation of MCF7 breast cancer cells bearing endocrine resistant ER mutations, ER-Y537S and ER-D538G. Importantly, tumor growth inhibition was observed in mouse models of sensitive and resistant human breast cancer when G1T48 was dosed as a single agent or in combination with G1T38, a potent, selective CDK4/6 inhibitor.

Conclusions: G1T38, a novel CDK4/6 inhibitor, and G1T48, a novel SERD, either alone or in combination, demonstrated highly potent inhibition of tumor growth in an animal model of tamoxifen resistance. G1T48 also demonstrated activity in models of endocrine resistance mediated by ER mutation

Results

1 The ER target gene regulation profile of G1T48 resembles that of known SERDs.

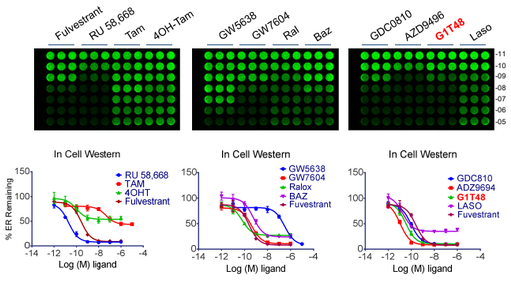


MCF7 breast cancer cells were plated in media supplemented with charcoal stripped serum 48 hours prior to 18 hours of treatment with ER ligands. mRNA expression of ER target genes previously found to be predictive of ER SERD/SERM pharmacology was analyzed by real time qPCR analysis. Relative mRNA levels were subjected to unsupervised clustering in JMP 13.

Results

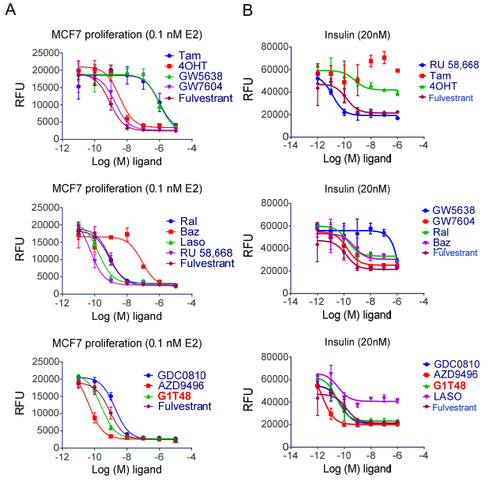
2 G1T48 exhibits SERD activity comparable to other clinically relevant SERDs.

MCF7 breast cancer cells were treated with ER ligands (10^{-11} – 10^{-5} M) for 18 hours prior to fixation of cells and detection of ER levels by In-Cell Western.



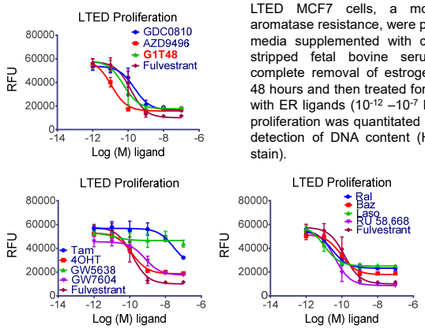
3 G1T48 inhibits ER-dependent MCF7 cell proliferation in response to estrogen or growth factors.

MCF7 breast cancer cells were treated for 7 days with 10^{-10} M 17β-estradiol (A) or 2 x 10^{-8} M insulin (B) in addition to ER ligands (10^{-11} – 10^{-5} M). Cell proliferation was quantitated through detection of DNA content (Hoechst stain).



Results

4 G1T48 inhibits the growth of long term estrogen deprived (LTED) MCF7 breast cancer cells.



LTED MCF7 cells, a model of aromatase resistance, were plated in media supplemented with charcoal stripped fetal bovine serum (for complete removal of estrogens) for 48 hours and then treated for 7 days with ER ligands (10^{-12} –10^{-7} M). Cell proliferation was quantitated through detection of DNA content (Hoechst stain).

5 G1T48 inhibits transcriptional activity of clinically relevant ESR1 mutations.

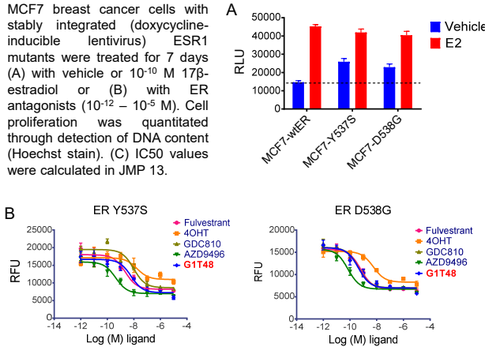
SKBR3 breast cancer cells were transfected with an estrogen-responsive reporter gene (3X-ERE-tata-Luc) together with wild type (wt) or mutant (Y537S or D538G) ER expression plasmids prior to 18 hours of treatment with 17β-estradiol (1.0 nM) and ER antagonists (10^{-11} – 10^{-5} M). Firefly and renilla luciferase activity were then assessed using dual luciferase reagent. IC50 values were calculated in JMP 13.

Compound	wtER	Y537S	D538G
G1T48	2.1E-09	1.6E-08	1.5E-08
GDC0810	1.1E-08	1.3E-06	2.5E-08
AZD9496	4.5E-10	2.6E-09	1.2E-09
Fulvestrant	4.5E-09	2.0E-08	1.0E-08
Raloxifene	2.7E-10	4.8E-09	5.8E-09
Bazedoxifene	7.1E-10	2.9E-08	8.0E-09
Lasofoxifene	3.8E-10	7.1E-09	1.4E-09
RU58,668	2.9E-10	2.9E-09	1.6E-09
Tamoxifen	2.7E-07	>10mM	2.9E-09
4OH-tamoxifen	3.3E-09	3.3E-09	3.1E-09
GW5638	>10mM	>10mM	>10mM
GW7604	2.5E-08	>10mM	2.3E-08

Results

6 G1T48 inhibits the growth of MCF7 breast cancer cells expressing resistance-associated ESR1 mutation.

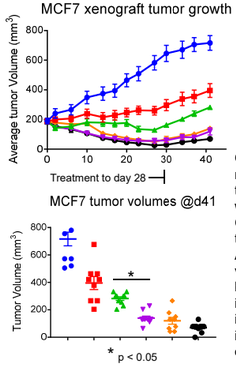
MCF7 breast cancer cells with stably integrated (doxycycline-inducible lentivirus) ESR1 mutants were treated for 7 days (A) with vehicle or 10^{-10} M 17β-estradiol or (B) with ER antagonists (10^{-12} – 10^{-5} M). Cell proliferation was quantitated through detection of DNA content (Hoechst stain). (C) IC50 values were calculated in JMP 13.



C

Compound	wtER	Y537S	D538G
G1T48	1.7E-11	6.1E-09	4.2E-10
Fulvestrant	7.7E-12	2.2E-09	6.2E-10
AZD9496	2.3E-12	4.3E-10	7.1E-11
GDC810	3.2E-11	2.2E-09	6.2E-10

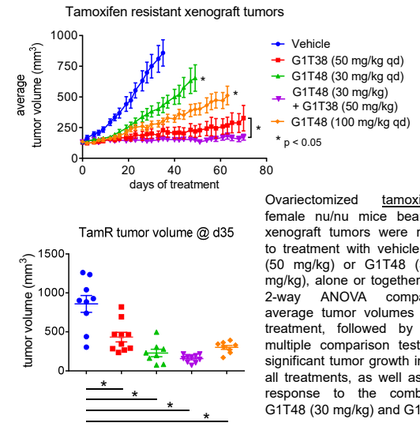
7 G1T48, alone or in combination with CDK4/6 inhibitor G1T38, inhibits the growth of estrogen-dependent MCF7 xenograft tumors.



Ovariectomized estrogen-treated female nu/nu mice bearing MCF7 xenograft tumors were randomized to treatment with vehicle, G1T38 (50 mg/kg) or G1T48 (30 or 100 mg/kg), alone or together, p.o. daily for 28 days. 2-way ANOVA comparison of average tumor volumes throughout treatment, followed by Bonferroni multiple comparison test, indicated significant tumor growth inhibition by all treatments, as well as increased response to the combination of G1T48 (30 mg/kg) and G1T38.

Results

8 G1T48 and G1T38 both inhibit the growth of tamoxifen resistant (TamR) xenograft tumors



Ovariectomized tamoxifen-treated female nu/nu mice bearing TamR xenograft tumors were randomized to treatment with vehicle or G1T38 (50 mg/kg) or G1T48 (30 or 100 mg/kg), alone or together, p.o. daily. 2-way ANOVA comparison of average tumor volumes throughout treatment, followed by Bonferroni multiple comparison test, indicated significant tumor growth inhibition by all treatments, as well as increased response to the combination of G1T48 (30 mg/kg) and G1T38.

Summary

- G1T48 is an orally bioavailable potent and efficacious antiestrogen with SERD activity.
- G1T48 regulates ER target gene transcription in a manner similar to fulvestrant, a pure antiestrogen with demonstrated efficacy in antiendocrine refractory breast cancer.
- G1T48 inhibits growth factor mediated breast cancer cell proliferation.
- G1T48 inhibits the growth of long term estrogen deprived cells, a model of aromatase resistance.
- G1T48 inhibits the transcriptional activity of ER mutants (Y537S, D538G) associated with endocrine-refractory breast cancer.
- G1T48 inhibits the ligand-independent growth of breast cancer cells expressing antiendocrine- resistant ER mutations (Y537S, D538G).
- G1T48, alone or in combination with the CDK4/6 inhibitor G1T38, inhibits the growth of estrogen-dependent MCF7 xenograft tumors.
- G1T48 and G1T38 both inhibit the growth of tamoxifen resistant (TamR) xenograft tumors
- G1T48 is currently completing IND enabling studies.
- G1T38 is currently being evaluated in combination with Faslodex in a Phase 1b/2a trial in ER+, HER2- breast cancer patients (NCT02983071).

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