Abstract 3970

Therapeutic targeting of ER+ breast cancer with the BET bromodomain inhibitor ODM-207

Julia Lindqvist[1, 2], Mari Björkman[1], Reetta Riikonen[1], Daniel Nicorici[1], Elina Mattila[1], Chandrasekhar Abbineni[3], Mahaboobi Jaleel[3], Juha Rantala[4], John Eriksson[2], Pekka Kallio[1], Anu Moilanen[1]

[1]Orion Corporation Orion Pharma, Espoo, Finland, [2]Turku Centre for Biotechnology, Åbo Akademi University and University of Turku, Finland, [3]Aurigene Discovery Technologies Limited, Bangalore, India, [4]Misvik Biology, Turku, Finland

ORION PHARMA
Building well-being

Background

The bromodomain and extra-terminal domain (BET) proteins are dual bromodomain-containing chromatin readers that recognize acetylated histones. BET proteins are abundant at promoter and enhancer regions of key oncogenes, where they drive oncogene transcription. Small molecule BET inhibitors displace BET proteins from the chromatin, causing growth inhibition in several pre-clinical cancer models through suppression of cell type-specific cancer drivers.

ODM-207 is a novel, highly selective BET bromodomain inhibitor structurally distinct from JQ1 and its benzodiazepine-related derivatives. Here we describe the pre-clinical activity of ODM-207 in ER+ breast cancer.

Methods

Biochemical activity: Binding of ODM-207 to BRD2 BD1, BRD3 BD1, BRD4 BD1, BRDT BD1 and BRD4 full length recombinant proteins was tested by measuring the displacement of bromodomain/acetylated peptide interaction using biotin conjugated Acetyl-Histone H4 [Lys5,8,12,16] peptide and the TR-FRET assay.

Cell viability assays: Cell lines and patient-derived cells harvested from pleural effusions or tumor biopsies were plated on multiwell plates and treated with ODM-207 in duplicate or triplicate for 3 to 4 days. Growth inhibitory effect of ODM-207 in tumor cell lines was measured using WST-1 assay (Roche). Growth inhibitory effect on patient-derived tumor cell cultures (Misvik Biology) was measured either by using CellTiter-Glo assay (for pleural effusions) or microscopic imaging of DAPI-stained cultures (for adherent cells from tumor biopsies). All data is presented as mean± S.E.

Patient-derived xenografts: Ma3366 tumors were implanted s.c. into nude female mice supplemented with E2 pellets. At day 18, mice were stratified into 3 treatment groups of 10 mice each. Tumor diameters were determined by caliper measurements 2 times weekly. Error bars represent SEMs.

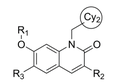
RNA sequencing and gene expression analyses: MCF-7 and CAMA-1 cells were treated for 24h with vehicle control (DMSO), 3 µM ODM-207 or 1 µM JQ-1 in triplicates. Gene set enrichment was analyzed by RNA-seq 30M reads/sample (Illumina HiSeq).

Flow cytometry and western blotting: For cell cycle analysis, cells were treated with indicated compounds for 48 hours, fixed in 70% ethanol, labelled with FxCycle PI/RNAse (Invitrogen) and analyzed for DNA content on BD LSRFortessa flow cytometer. Data was analyzed using ModFit 5.0 software. For western blotting, samples were immunoblotted with the following antibodies: Cyclin D1 (SC-8396; Santa Cruz), CDK4 (D9G3E; Cell Signaling) and beta-tubulin (Ab6046; AbCam).

Drug synergy calculation: Synergistic drug interactions were profiled based on five-concentration dose response matrices (WST-1 proliferation assay; Roche). Drug synergy score was calculated using the ZIP-method with SynergyFinder web application (https://synergyfinder.fimm.fi).

Results

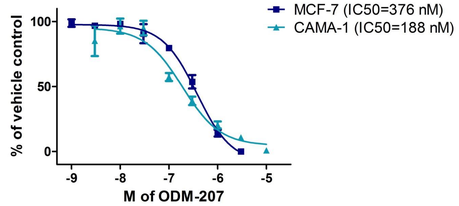
1. Biochemical activity of ODM-207



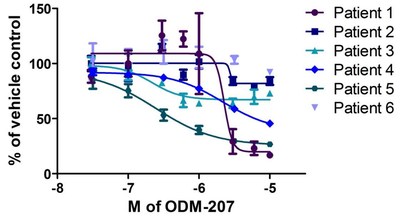
Bromodomain	IC50 (nM)
BRD4 BD1	116
BRD4 full length	89
BRD3 BD1	86
BRD2 BD1	61
BRDT BD1	89

2. ODM-207 shows antitumor activity in ER+ breast cancer cell lines and in patient-derived models

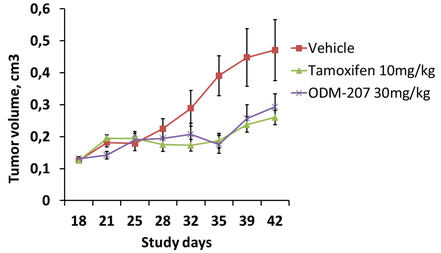
a) ODM-207 inhibits the proliferation of ER+ breast cancer cell lines



b) Effects of ODM-207 in ex vivo patient-derived ER+ breast cancer cells (3/6: cytotoxic response, 2/6: partial response, 1/6: no response)



c) ODM-207 inhibits tumor growth in an ER+ breast cancer PDX-model

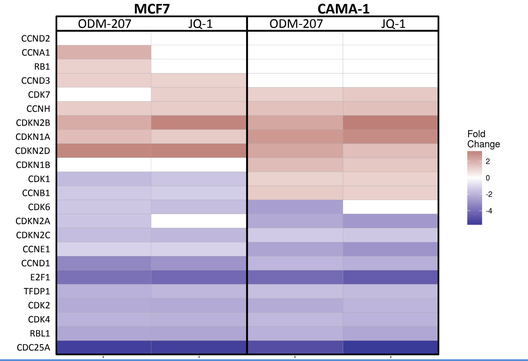


3. ODM-207 regulates signaling pathways involved in breast cancer cell cycle and survival

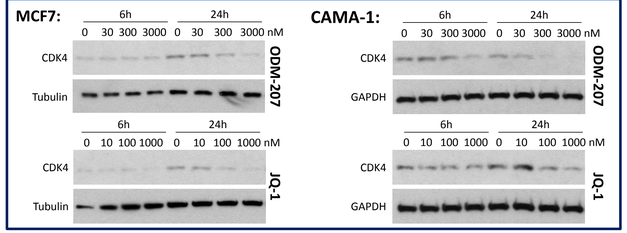
a) BET inhibition suppresses ER, MYC and cell cycle signatures

	MCF7 cells, 24 hours						CAMA-1 cells, 24 hours					
	ODM-207			JQ-1			ODM-207			JQ-1		
Name of enriched gene set (number of genes in set)	Affected genes	Direction	P-value	Affected genes	Direction	P-value	Affected genes	Direction	P-value	Affected genes	Direction	P-value
MYC_TARGETS_V2 (58)	99%	Down	0,001	93%	Down	0,001	89%	Down	0,001	90%	Down	0,001
ESTROGEN_ RESPONSE_EARLY (200)	90%	Down	0,001	88%	Down	0,001	79%	Down	0,001	80%	Down	0,001
E2F_TARGETS (200)	90%	Down	0,001	90%	Down	0,001	85%	Down	0,001	84%	Down	0,001
KEGG_CELL_CYCLE (123)	81%	Down	0,001	83%	Down	0,001	77%	Down	0,001	82%	Down	0,001
REACTOME_G0_AND_ EARLY_G1 (23)	100%	Down	0,001	95%	Down	0,001	87%	Down	0,001	87%	Down	0,001

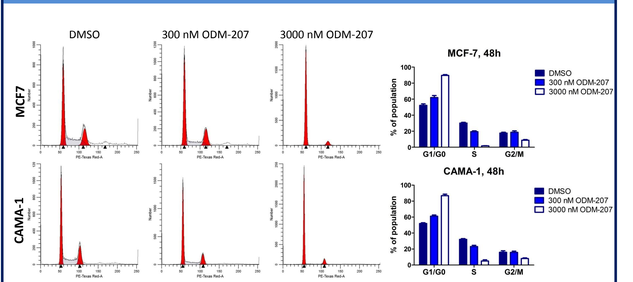
b) Fold change of genes in Biocarta CELLCYCLE_PATHWAY dataset



4. ODM-207 downregulates the protein expression of CDK4

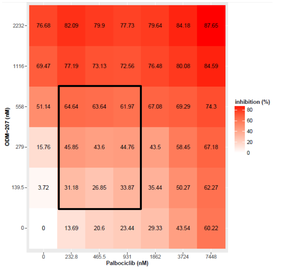


5. ODM-207 induces G1/G0 cell cycle arrest in breast cancer cells

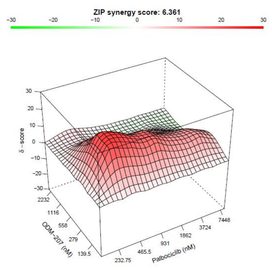


6. ODM-207 synergizes with Palbociclib at sub-IC50 concentrations in MCF7 cells

a) Dose response matrix (% inhibition)



b) ZIP interaction landscape



Conclusions

ODM-207 is a novel and structurally distinct BET inhibitor that

- ✓ inhibits the proliferation of ER+ breast cancer cell lines and patient-derived tumor models
- ✓ regulates signaling pathways involved in estrogen response, breast cancer cell cycle and survival, and causes G1/G0 cell cycle arrest
- ✓ synergizes with CDK4/CDK6 inhibitor Palbociclib in vitro

A clinical trial with ODM-207 is ongoing in patients with solid tumors (NCT03035591).

Poster presented at AACR 2018
Chicago, Illinois, USA 14-18 April

These studies were sponsored by Orion Corporation Orion Pharma