Poster #4162

ODM-207 - a novel BET bromodomain inhibitor with antitumor activity in nonclinical models of ER+ breast cancer

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Background

The bromodomain and extra-terminal domain (BET) proteins are dual bromodomain-containing chromatin readers that recognize and bind to 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 the suppression of several different 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 activity of ODM-207 in pre-clinical models of estrogen receptor positive (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 were plated on multiwell plates and treated with ODM-207 in duplicate or triplicate for 3 to 5 days. Growth inhibitory effect of ODM-207 in tumor cell lines was measured using WST-1 assay (Roche) or microscopic imaging of Hoechst-stained cultures (for combination study). All data is presented as mean± S.E.

Patient-derived xenografts: Ma3366 tumors (EpoBerlin) 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 mean± S.E.

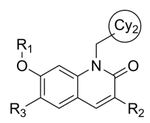
RNA sequencing and gene expression analyses: MCF-7 and CAMA-1 cells were treated for 24h with vehicle control (DMSO), 3 µM ODM-207 or 1 µM JQ-1 in triplicates. Gene set enrichment was analyzed by RNA-seq 30M reads/sample (Illumina HiSeq).

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: TOPBP1 (sc-271043; Santa Cruz), and GAPDH (G8795; Sigma-Aldrich).

Drug synergy calculation: Synergistic drug interactions were profiled based on five-concentration dose response matrices after 5 days of treatment. Drug synergy score was calculated using the ZIP-method with SynergyFinder web application (https://synergyfinder.fimm.fi).

Results

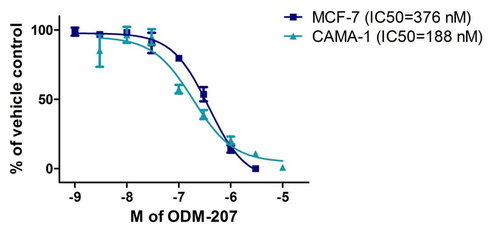
1. Biochemical activity of ODM-207



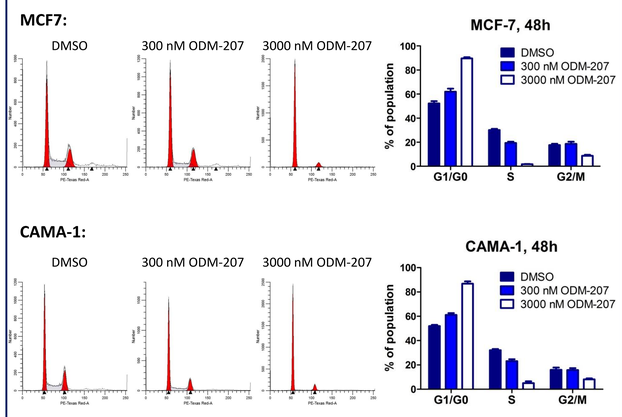
Bromodomain	IC50 (nM)
BRD4 BD1	116
BRD4 full length	89
BRD3 BD1	86
BRD2 BD1	61
BRDT BD1	89

2. ODM-207 shows antitumor activity in ER+ breast cancer cell lines

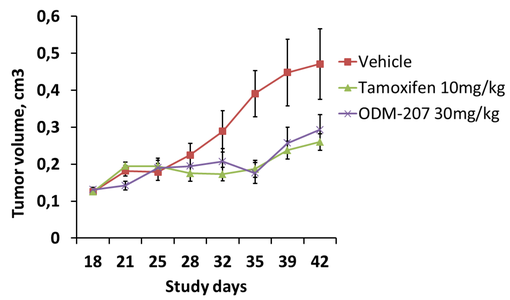
a) ODM-207 inhibits the proliferation of ER+ breast cancer cell lines



b) ODM-207 induces G1/G0 cell cycle arrest in breast cancer cells



3. ODM-207 inhibits tumor growth in an ER+ breast cancer PDX-model



4. ODM-207 regulates signaling pathways involved in breast cancer proliferation, survival and DNA repair

a) BET inhibition suppresses cell cycle and DNA repair signatures

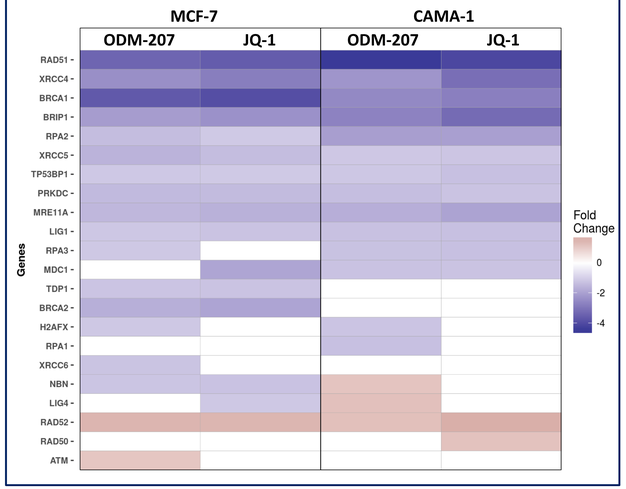
MCF7 cells, 24 hours

	ODM-207			JQ-1		
Name of enriched gene set (number of genes in set)	Affected genes	Direction of change	P-value	Affected genes	Direction of change	P-value
MSigDB_MYC_TARGETS_V2 (58)	99%	Down	0.001	93%	Down	0.001
MSigDB_ESTROGEN_RESPONSE_EARLY (200)	90%	Down	0.001	88%	Down	0.001
MSigDB_E2F_TARGETS (200)	90%	Down	0.001	90%	Down	0.001
KEGG_CELL_CYCLE (123)	81%	Down	0.001	83%	Down	0.001
MSigDB_DNA REPAIR (145)	83%	Down	0.001	90%	Down	0.008
Reactome_DOUBLE STRAND BREAK REPAIR (22)	96%	Down	0.001	78%	Down	0.001
KEGG_HOMOLOGOUS RECOMBINATION (27)	92%	Down	0.001	78%	Down	0.001
KEGG_NON HOMOLOGOUS END JOINING (12)	84%	Down	0.001	75%	Down	0.001

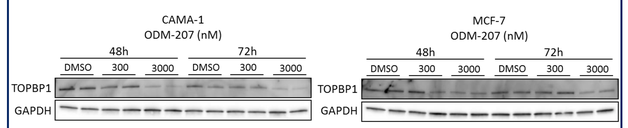
CAMA-1 cells, 24 hours

	ODM-207			JQ-1		
Name of enriched gene set (number of genes in set)	Affected genes	Direction of change	P-value	Affected genes	Direction of change	P-value
MSigDB_MYC_TARGETS_V2 (58)	89%	Down	0.001	90%	Down	0.001
MSigDB_ESTROGEN_RESPONSE_EARLY (200)	79%	Down	0.001	80%	Down	0.001
MSigDB_E2F_TARGETS (200)	85%	Down	0.001	84%	Down	0.001
KEGG_CELL_CYCLE (123)	77%	Down	0.001	82%	Down	0.001
MSigDB_DNA REPAIR (145)	81%	Down	0.001	80%	Down	0.002
Reactome_DOUBLE STRAND BREAK REPAIR (22)	82%	Down	0.001	69%	Down	0.001
KEGG_HOMOLOGOUS RECOMBINATION (27)	89%	Down	0.001	77%	Down	0.001
KEGG_NON HOMOLOGOUS END JOINING (12)	83%	Down	0.001	83%	Down	0.001

b) Fold change of genes in Reactome double strand break repair-gene set



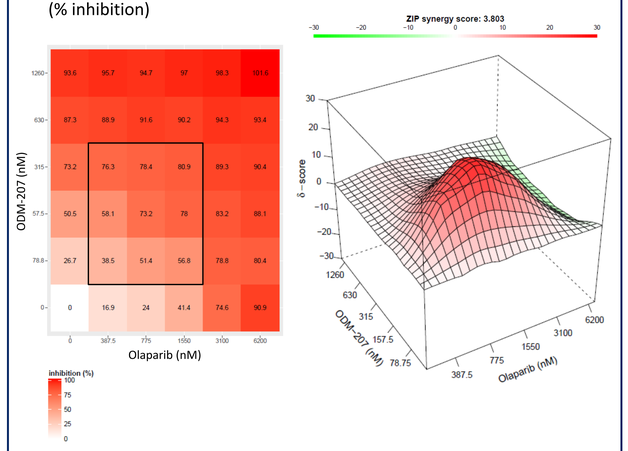
5. Effects of ODM-207 on DNA-damage response protein TOPBP1



6. ODM-207 synergizes with the PARP-1/2 inhibitor Olaparib at sub-IC50 concentrations in DNA-repair proficient MCF7 cells

a) Dose response matrix (% inhibition)

b) ZIP interaction landscape



c) Synergy scoring for ODM-207-Olaparib combination

Synergy calculation method	Overall synergy score	Most synergistic area score
Zero interaction potential (ZIP) model	3.80	13.01
Loewe additivity model	40.74	74.34
Bliss independence model	3.4	12.34

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 the DNA-damage response
- ✓ ODM-207 synergizes with the PARP-inhibitor Olaparib in MCF7 breast cancer cells

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

Poster presented at ESMO 2018
October 19-23, 2018 | Munich, Germany

These studies were sponsored by Orion Corporation Orion Pharma

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