Abstract 3827

Antitumor activity of ODM-207, a novel BET bromodomain inhibitor, in nonclinical models of ER+ breast cancer as single agent and as a combination treatment

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Building well-being

Background

The Bromodomain and extraterminal (BET) family of proteins are chromatin readers that promote the transcription of several important cell identity genes. BET proteins also control expression of many genes that play an essential role in the pathogenesis of human cancer, including cell cycle- and proliferation-regulating genes. The small-molecule BET inhibitors block BET protein binding to chromatin and have shown antitumor activity in a variety of pre-clinical cancer models.

ODM-207 is a novel, highly selective BET bromodomain inhibitor structurally distinct from JQ-1 and its derivatives. Here we describe the pre-clinical activity of ODM-207 in ER+ breast cancer as single agent and in combination therapy.

Methods

Biochemical activity: Binding of ODM-207 to BRD2, -3, -4, and -T BD1 domain and BRD4 full length recombinant proteins was measured by displacement of bromodomain/acetylated peptide interaction using biotin conjugated Acetyl-Histone H4 [Lys5,8,12,16] peptide and TR-FRET.

Cell viability and health assays: Cell lines were plated on multiwell plates and treated with ODM-207 in triplicate for 4 days. Growth inhibitory effect of ODM-207 in tumor cell lines was measured using WST-1 assay (Roche). For colony formation assay, 950 cells were plated on 6 well plates and treated accordingly. Colonies were fixed, stained with crystal violet, imaged, and finally quantified using the ImageJ plugin ColonyArea. Annexin V+ cells were measured with Annexin V Green-stain (Essen Bioscience) using IncuCyte Zoom (Essen Bioscience) and cell number was determined at the 7 day endpoint (DRAQ5). 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. Data is presented as 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 studied by RNA-seq 30M reads/sample (Illumina HiSeq).

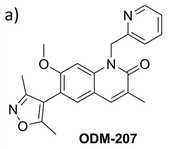
Flow cytometry: For cell cycle analysis, cells were treated with indicated compounds for 48 hours, fixed in 70% ethanol, labelled with FxCycle PI/RNAse (Invitrogen) and analyzed for DNA content on BD LSRFortessa flow cytometer. Data was analyzed using ModFit 5.0 software.

Drug synergy calculation: Synergistic drug interactions were profiled based on five-concentration dose response matrices and results were measured by cell count. Drug synergy scores were calculated using the SynergyFinder web application (https://synergyfinder.fimm.fi).

Results

1. Structure and biochemical activity of ODM-207

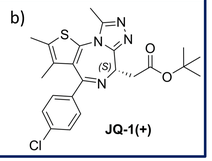
a)



ODM-207

Bromodomain	ODM-207 IC50 (nM)
BRD4 BD1	116
BRD4 full length	89
BRD3 BD1	86
BRD2 BD1	61
BRDT BD1	89

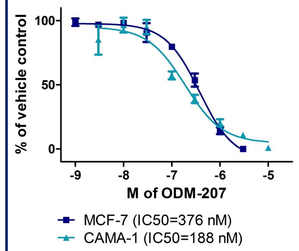
b)



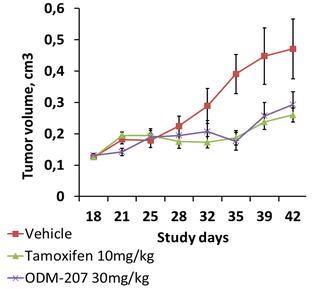
JQ-1(+)

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

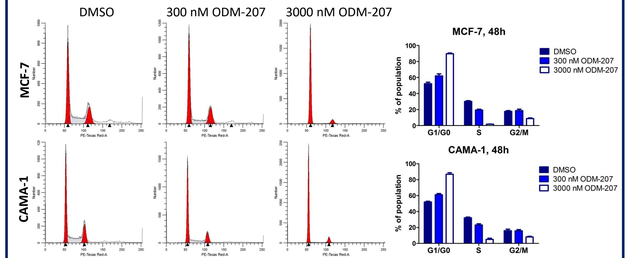
a) Inhibition of ER+ breast cancer cell proliferation



b) Tumor growth inhibition in an ER+ breast cancer PDX-model



c) ODM-207 induces G1/G0 cell cycle arrest

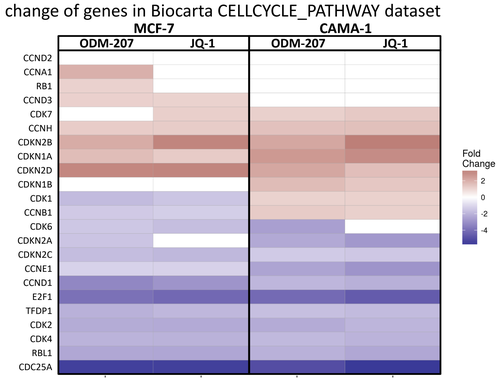


3. BET inhibition suppresses ER, MYC and cell cycle pathways

RNA sequencing	MCF-7 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
CELLCYCLE PATHWAY (23)	96%	Down	0,001	83%	Down	0,001	83%	Down	0,001	91%	Down	0,001

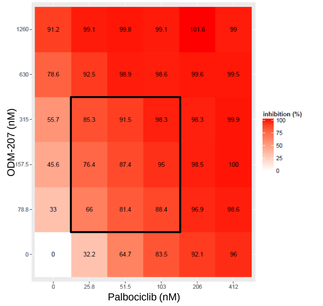
3. ODM-207 regulates genes involved in breast cancer cell cycle and survival

b) Fold change of genes in Biocarta CELLCYCLE_PATHWAY dataset

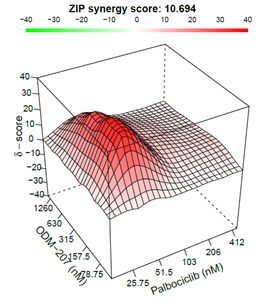


4. ODM-207 synergizes with palbociclib at sub-IC50 concentrations in MCF-7 cells

a) Dose response matrix (% inhibition)



b) ZIP interaction landscape



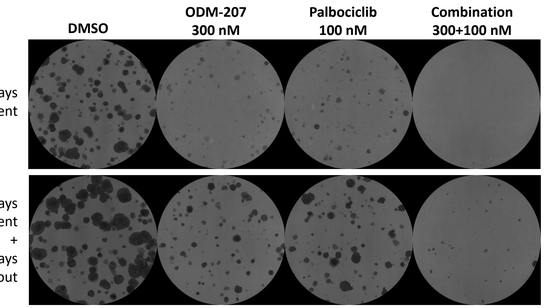
c) Synergy score calculations for ODM-207-palbociclib combination

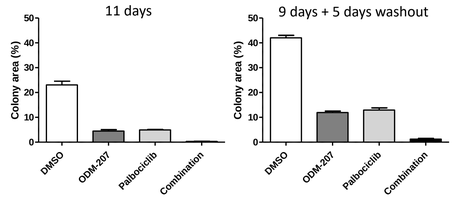
Synergy calculation method	Overall synergy score*	Most synergistic area score
Zero interaction potential (ZIP) model	10.69	19.35
Loewe additivity model	45.69	69.29
Bliss independence model	10.78	19.89

*synergy score indicates the inhibition % beyond the expected effect, which is calculated by each mathematical model (e.g. 0 = non-interaction)

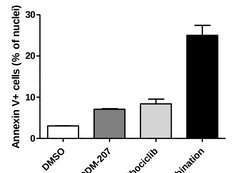
5. Effects of ODM-207 and palbociclib combination on cell survival

a) MCF-7 colony formation with or without treatment washout





b) Long term exposure of the ODM-207 and palbociclib combination treatment increases the proportion of Annexin V+ apoptotic cells



Conclusions

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

- ✓ inhibits the proliferation of ER+ breast cancer cell lines and tumor growth in a patient-derived tumor model
- ✓ 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 to decrease cell survival

Poster presented at AACR Annual Meeting 2019
March 29 - Apr 3 2019, Atlanta, Georgia, USA

These studies were sponsored by Orion Corporation Orion Pharma