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.

Julia Lindqvist¹, Mari Björkman¹, Reetta Rikkinen¹, Daniel Nicorici², Elina Mattila¹, Mahaboobi Jaleesi³, Chandrasekhar Abbineni², Anu Moilanen¹

¹Orion Corporation Orion Pharma, Espoo, Finland, ²Aurigen Discovery Technologies Limited, Bangalore, India

Results

1. Structure and biochemical activity of ODM-207

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 PDO model

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

a) Fold change of genes in Biscarta CELLCYCLE_PATHWAY dataset
b) ZIP interaction landscape

4. ODM-207 synergizes with palbociclib at sub-IC50 concentrations in breast cancer colony assays

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

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/S cell cycle arrest
- synergizes with CDK4/6 inhibitor palbociclib in vitro to decrease cell survival

ODM-207 and palbociclib combination

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

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 BET, JQ-1 and JQ-2 domain and BRD4 full length recombinant protein was measured by displacement of bromodomain/intact peptide interactions using column conjugated recombinant His-tag (E. coli) peptide and Th-T.

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 cell proliferation assay, 500 cells were plated in 6-well plates and treated accordingly. Colorectal was stained, stained with crystal violet, imaged, and quantified using the Image J plug-in Colonnides. Annexin V cells were measured with Annexin V-Green stain (Eaton Biosciences) using flow cytometry (Eaton Biosciences) and cell number was determined at the 7 day endpoint (DNAS). All data is presented as mean ± S.E.

Patient-derived xenografts: MDA-MB-231 (grade II) and MDA-MB-237 (grade IV) were implanted s.c. into nude female mice supplemented with 12% caloric. At day 10, mice were stratified into treatment groups of 10 mice each. Tumor diameter were determined by caliper measurements two times weekly. Data is presented as mean ± S.E.

RNA sequencing and gene expression analysis: MCF-7 and CAMA-1 cells were treated for 24h with vehicle control (DMSO), 1 μM ODM-207 or 1 μM JQ-1 in triplicates. Gene set enrichment was studied by RNA-seq DMSO ready samples (illumina HiSeq). Flow cytometry: For cell cycle analysis, cells were treated with indicated compounds for 48 hours, fixed in 70% ethanol, labeled with Fixable Eva-Blue (Invitrogen) and analyzed for DNA content on BD LSRFortessa flow cytometer. Data was analyzed using FlowJo 10 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.com/sf).