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


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**Abstract 3970**

The bromodomains 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 disrupt 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**

**Biological activity:** Binding of ODM-207 to BET/BRD-8D3, BRD3-8D3, BRD4-8D3, BRD7-8D3 and BRD4 full length recombinant proteins was tested by measuring the displacement of bromodomain/BD-3/BD-3 complex peptide interaction using biotin conjugated anti-Brdu-dioleic acid (15s,15J,20j) peptide and the TR-FRET assay. Cell viability assays: Cell lines and patient-derived cells treated with vehicle or tumor bioropeurum were placed 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 CellTiter-Glo assay (Roche). Growth inhibitory effect on patient-derived tumor cell cultures (Mikkoiva Biology) was measured using CellTiter-Glo assay (for phosphofructose) or microscopic imaging of DNA-stained cultures (for adherent cells from tumor biopsies). All data is presented as means ± SE. Patient-derived xenografts: MCF7 tumors were implanted s.c. into nude female mice supplemented with E2 pellets. At day 18, mice were crossbred into 3 treatment groups of 10 mice each. Tumor diameters were determined using calipers measurements 2 times weekly. Error bars represent 50%.

**RNA sequencing and gene expression analyses:** MCF-7 and CAMA-1 cells were treated with 24h with vehicle control (DMSO), 1 µM ODM-207 or 1 µM JQ1-10 in triplicates. Gene set enrichment was achieved by Pick-seq. RNA expression data is from Kallionen.

**Flow cytometry and western blotting:** For cell cycle analysis, cells were treated with indicated compounds for 24 h, fixed in 70% ethanol, stained with propidium iodide (PI) (Invitrogen) and analyzed by FACS on BD LSRFortessa flow cytometer. Data was analyzed using FlowJo 5.0 software. For western blotting, samples were immunolabeled with the following antibodies: Cyclin D1 (SC-6949, Santa Cruz), CXR (D01, Cell Signaling and beta tubulin (Ab6646, Abcam).

**Drug synergy calculation:** Synergistic drug interactions were profiled based on free-concentration dose response matrices (ODM-207 proliferation assay, Roche). Drug synergy score was calculated using the ZOB method with SynergyFinder web application (https://synergypfinder.com).

**Results**

- **1. Biological activity of ODM-207**
  - **ODM-207 shows antitumor activity in ER+ breast cancer cell lines and in patient-derived models**
  - **ODM-207 inhibits the proliferation of ER+ breast cancer cell lines**
  - **Effects of ODM-207 on ex vivo patient-derived ER+ breast cancer cell lines**
  - **ODM-207 inhibits tumor growth in an ER+ breast cancer PDX model**

- **2. ODM-207 shows antitumor activity in ER+ breast cancer cell lines and in patient-derived models**
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- **3. ODM-207 regulates signaling pathways involved in breast cancer cell cycle and survival**
  - **BET inhibition suppresses ER, MYC and cell cycle signatures**
  - **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**
  - **Effects on G1/S distribution**

- **6. ODM-207 synergizes with Palbociclib at sub-IC50 concentrations in MCF7 cells**
  - **Dose response matrix (% inhibition)**
  - **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 human models.
- regulates signaling pathways involved in estrogen response, breast cancer cell cycle and survival, and causes G1/S cell-cycle arrest.
- synergizes with CDK4/CDK6 inhibitor Palbociclib in vitro.

A clinical trial with ODM-207 is ongoing in patients with solid tumors (NCT03225959).