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 oncogenic 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 different 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, BRD1 BD1 and BRD4 full length recombinant proteins was tested by measuring the displacement of bromodomain/acylated peptide interaction using biotin-conjugated Acetyl-Histone H4(Lys8,12,16,20) peptide and the TR-FRET assay.

**Cell viability assay:** 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:** Maca266 tumors (Epidermolysis) 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 analysis:** MCF-7 and CAMA-1 cells were treated for 24h with vehicle control (DMSO), 1 μM ODM-207 or 1 μM JQ1 in triplicates. Gene set enrichment was analyzed by RNA-seq 30M reads/sample (Kumra et al.).

**Flow cytometry and western blotting:** For cell cycle analysis, cells were treated with indicated compounds for 48 hours, fixed in 70% ethanol, labelled with FISH/Cell cycle kit(Miltenyi) and analysed for DNA content on BD LSORTe flow cytometry. Data was analyzed using ModFit 5.0 software. For western blotting, samples were immunoblotted with the following antibodies: TOPBP1 (sc-272194), Santa Cruz and GAPDH (G8795, Sigma-Aldrich).

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

**Results**

1. **Biochemical activity of ODM-207**

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

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

3. **ODM-207 inhibits tumor growth in an ER+ breast cancer PDO 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

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

6. **ODM-207 synergizes with the PARP1/J2 inhibitor Olaparib at sub-IC50 concentrations in DNA repair proficient MCF7 cells**

**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 (NCT03205515).

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