Background

The bromodomain and extra-terminal domain (BET) proteins are dual bromodomain-containing chromatin readers that recognize acetylated histones. BET proteins are abundantly present 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 benzamidopyridine-related derivatives. Here we describe the pre-clinical activity of ODM-207 in ER+ breast cancer.

Methods

Biochemical activity: Binding of ODM-207 to BRD2-BSI, BRD3-BSI, BRD4-BSI, BRD8-BSI and BRD4 full length recombinant proteins was tested by measuring the displacement of bromocriptine/competed peptide interaction using bacterially expressed human-brain derived M1 and SH-SY-5Y peptide and the TR-RET assay.

Cell viability assays: Cell lines and patient-derived cells harvested from pleural effusions or tumor biopsies were plated 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 WST-1 assay (Roche). Growth inhibitory effect on patient-derived tumor cell cultures (Mikael Bögl) was measured using either CellTiter-Glo assay (for plated cells) or microscopic imaging of DAPI-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 12% gel. At day 18, mice were stratified into 3 treatment groups of 10 mice each. Animal diameters were determined by using measurements 2 times weekly. Error bars represent 50%.

RNA sequencing and gene expression analyses: 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 PathwayEMD, Enrichr (Mikael Bögl).

Flow cytometry and western blotting: For cell cycle analysis, cells were treated with indicated compounds for 24h, fixed in 70% ethanol and permeabilized with 0.05% RNaseA (Invitrogen) and analyzed for DNA content by 66000/70000 flow cytometer. Data were analyzed using ModFit 5.0 software. For western blotting, samples were immunoblotted with the following antibodies: Cyclin D1 (SC-3949, Santa Cruz), Dicate, Cyclin E1 (Bioss, Cell Signaling) and β-tubulin (Abcam, Ab6466, Abcam).

Drug synergy calculation: Synergistic drug interactions were profiled based on fixed-concentration dose response matrix (ODM-207: proliferation assay, JQ1: drug synergy score was calculated using the Z-scan method with SynergyFinder web application (https://synergyfinder.biocarta.com).

Results

1. Biochemical activity of ODM-207

2. ODM-207 shows antitumor activity in ER+ breast cancer cell lines and in patient-derived models

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

b) Effects of ODM-207 in ex vivo patient-derived ER+ breast cancer cells

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

a) BET inhibition suppresses ER, MYC and cell cycle signatures

4. ODM-207 downregulates the protein expression of CDK4

5. ODM-207 induces G1/G0 cell cycle arrest in breast cancer cells

6. ODM-207 synergizes with Palbociclib at sub-ICSI concentrations in 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 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 (NCT03035591).