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Targeting cancer with a novel BET bromodomain inhibitor

Anu Moilanen[1], Mari Björkman[1], Reetta Riikonen[1], Chandrasekhar Abbineni [2], Mahaboobi Jaleel [2], Sivapriya Marappan[2], Tarja Ikonen[1], Girish Daginakatte[2], Aravind A B [2], Elina Mattila[1], Juha Rantala[3], Susanta Samajdar[2], Murali Ramachandra[2], Pekka Kallio[1]

[1]Orion Corporation Orion Pharma, Espoo, Finland, [2]Aurigene Discovery Technologies Limited, Bangalore, India, [3]Misvik Biology, Finland

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Background

Bromodomain and extra-terminal (BET) family proteins are dual bromodomain-containing epigenetic readers that bind to acetylated-lysine residues in histones at gene promoter and enhancer elements and recruit protein complexes to promote transcriptional elongation. Recent evidence demonstrates that BET bromodomain inhibition leads to anti-proliferative activity in pre-clinical models of many hematological malignancies and solid tumors. Selective inhibition of BET bromodomains by small molecule inhibitors has emerged as a promising therapeutic strategy for the treatment of cancer. In this study, we evaluated the antitumor activity of ODM-207, a novel, potent and highly selective BET bromodomain inhibitor.

Methods

Biochemical activity: Binding of ODM-207 to BRD2 BD1, BRD3 BD1, BRD4 BD1, BRDT BD1 and BRD4 full length recombinant proteins was tested by measuring the displacement of bromodomain/acetylated peptide interaction using biotin conjugated Acetyl-Histone H4 [Lys5,8,12,16] peptide and the TR-FRET assay.

Cell viability and apoptosis assays: Cell lines and patient derived cells harvested from pleural effusions or tumor biopsies were plated on multiwell plates and treated with 8-point semi-log dilution series of ODM-207 in duplicate or triplicate for 3 to 4 days. Growth inhibitory effect of ODM-207 in solid tumor cell lines was measured using WST-1 Cell Proliferation Assay (Roche) and in hematological cancer-derived cell lines using CellTiter-Glo (Promega) assay (ProQinase). Growth inhibitory effect on patient-derived tumor cell cultures (Misvik Biology) was measured either by using CellTiter-Glo® assay (for pleural effusions) or microscopic imaging of DAPI stained cultures (for adherent cells from tumor biopsies). Apoptosis in relation to live cells was measured using ApoTox-Glo assay (Promega).

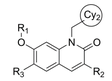
Immunofluorescence and cell cycle analysis: Expression of Myc was detected by immunolabelling the cells with Anti-Myc antibody [Y69] (Abcam, ab32072). DAPI was used to identify the cells for automated image analysis. Analysis of cell cycle was performed by flow cytometry using propidium iodide staining.

Chromatin binding assay: In situ cell extraction was performed essentially as described by Zhan et al. 2015 Epigenetics & Chromatin. Anti-BRD4 antibody (Abcam, ab128874) was used to detect chromatin bound BRD4.

Antitumor activity: Tumors were established by subcutaneous injection of 22Rv1 or MV4-11 cells into male athymic nude mice. After initial tumor growth, when the average tumor volume reached 127 mm3 (22Rv1) or 159 mm3 (MV4-11), oral treatment with ODM-207 30 mg/kg qd, enzalutamide 20 mg/kg qd or vehicle (22Rv1) or ODM-207 30 mg/kg qd, OTX015 40 mg/kg qd or vehicle (MV4-11) was initiated and continued for 27 (22Rv1) or 16 (MV4-11) days. Tumor volume was measured with a caliper during the course of both studies. Statistical analysis: 1-way ANOVA, Dunnett's test: **, p<0.01, ***, p<0.001, ****, p<0.0001.

Results

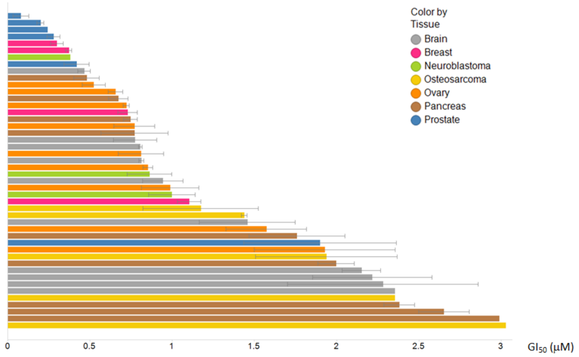
1. Biochemical activity of ODM-207



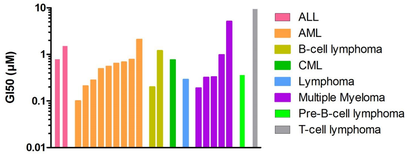
Bromodomain	IC50 (nM)
BRD4 BD1	116
BRD4 full length	89
BRD3 BD1	86
BRD2 BD1	61
BRDT BD1	89

2. In vitro antiproliferative activity of ODM-207 across multiple tumor types

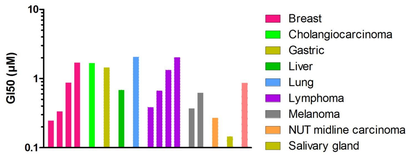
a) Growth inhibition (GI_{50}) values for ODM-207 in a broad panel of cancer cell lines



b) Sensitivity of hematological cancer cell lines to ODM-207

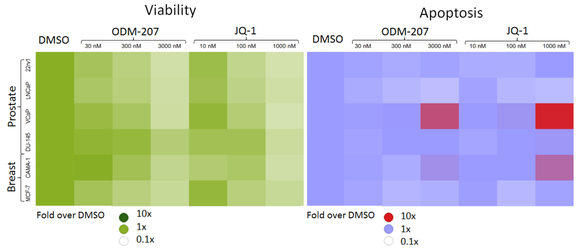


c) Growth inhibition (GI_{50}) values observed for ODM-207 in patient-derived tumor cell cultures

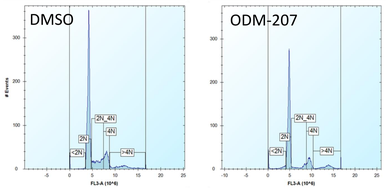


3. ODM-207 inhibits cell growth and induces apoptosis in a subset of prostate and breast cancer cell lines

a) Effect of ODM-207 on viability and apoptosis of androgen sensitive prostate and estradiol sensitive breast cancer cell lines

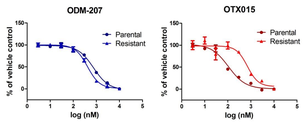


b) Effect of ODM-207 on cell cycle. Representative FACS histograms for VCaP prostate cancer cells

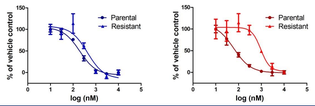


4. OTX015 resistant LNCaP prostate cancer cells maintain sensitivity to ODM-207

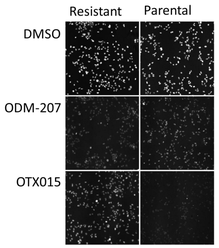
a) Inhibition of LNCaP cell proliferation



b) Inhibition of Myc expression

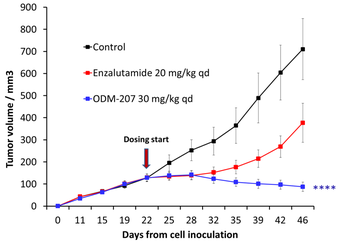


c) Displacement of BRD4 from chromatin

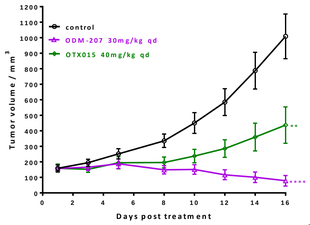


5. ODM-207 is efficacious as a single agent in xenograft models

a) Efficacy of ODM-207 in AR-V7 expressing 22Rv1 prostate cancer xenograft



b) Efficacy of ODM-207 in MV4-11 acute myeloid leukemia xenograft



**, p<0.01; ***, p<0.001; ****, p<0.0001

Conclusions

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

- ✓ Induces cell cycle arrest and shows broad and potent antiproliferative activity against a wide range of different hematological and solid tumors in vitro and in vivo.
- ✓ Inhibits proliferation of patient-derived cancer cells representing various tumor types.
- ✓ inhibits proliferation and downregulates Myc levels in prostate cancer cells that have acquired resistance to BET-inhibitor OTX015.

A clinical trial with ODM-207 is ongoing in patients with solid tumors (NCT03035591).

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