Immune-mediated anti-tumor activity with a clinical stage BET bromodomain inhibitor ODM-207 in pre-clinical models

Pratima Deshpande1, Ravi Krishna Babu2, Prashant Yallappa Vadnal3, Mahaboobi Jaleel2, Chandrasekhar Abbineni2, Anu Moolanen1, Pekka Kallio1, Murali Ramachandra2, Susanta Samajdar2

1Orion Corporation Orion Pharma, Espoo, Finland, 2Aurigena Discoveries Technologies Limited, Bangalore, India

Abstract L8:113

Building well-being

Immune-mediated anti-tumor activity with a clinical stage BET bromodomain inhibitor ODM-207 in pre-clinical models

Pratima Deshpande1, Ravi Krishna Babu2, Prashant Yallappa Vadnal3, Mahaboobi Jaleel2, Chandrasekhar Abbineni2, Anu Moolanen1, Pekka Kallio1, Murali Ramachandra2, Susanta Samajdar2

1Orion Corporation Orion Pharma, Espoo, Finland, 2Aurigena Discoveries Technologies Limited, Bangalore, India

Background

BET (brmodomains and extra-terminal) family proteins (BRD2, BRD3, BRD4, and BRD7) are acetyl-lysine readers that bind to acetylated lysine residues in histones and recruit proteins complexed to transcriptional elongation. In many cancers, BET proteins have been shown to regulate expression of MPC and other oncoprotective drivers that are important for cell proliferation and survival. Pharmacologic inhibition of the BET–brmodomain interaction has been shown to result in transcriptional downregulation of a number of oncogenes and inhibition of tumor growth providing a novel strategy for treatment of cancer.

ODM-207 is a potent and selective BET inhibitor that is structurally unrelated to the non-epigenetic-based inhibitors including CLI, IBBET2 and ITO-155. Phase I clinical trial is ongoing with this agent based on its potent anti-tumor activity in several in vitro and in vivo models of hematologic malignancies and solid tumors.

In view of the recent publications implicating a role for BET protein BRD4 in the suppression of PD-L1 expression, an immune checkpoint ligand for PD-L1, we sought to evaluate ODM-207 for its effect on immune-mediated anti-tumor efficacy in pre-clinical models.

Methods

Effectors cell phenotyping: CD8+ T-cells were isolated using commercially available isolation kit and stimulated for 24 h with coated anti-CD3 (1.5 µg/ml) and anti-CD8 (0.5 µg/ml). Following 3.5 h of incubation the cells were restimulated with PMA, ionomycin with brefeldin A and stained with anti-CD8 PerCP-Cy5.5, anti-granzyme B FITC and anti-IFN-γ PE antibodies.

Apoptosis assay: The assay was performed as described above. Following incubation the cells were stained with anti-CD3 APC, Annexin V and propidium iodide to measure the extent of apoptosis in the T-cell fraction.

Human whole blood assay: Blood was drawn from an healthy donor in a heparinized vacutainer tube and mixed well. 100 µl of whole blood was added to the flow-isotrimed 96-well anti-CD2 pre-coated plate (2.5 µg/ml) and 50 µl of ODM-207 diluted in X-VIVO media along with 50 µl of anti-CD28 antibody was added (0.15 µg/ml). Following 2.5 h incubation at 37°C in 5% CO2 atmosphere, supernatants were collected and cytokine levels were measured using EUSA.

Cell cytotoxicity assay: Indicated cancer cell lines or human PBMC were treated with either varying concentration of ODM-207 or 0.1% DMSO (control) for indicated time period, followed by terminal using the XTT cell viability assay.

Analysis of PD-L1 expression on tumor cell line: 0.2×10⁶ mouse colon carcinoma CT26 cells or human non-small lung carcinoma were seeded per well of 6-well plate and treated with indicated concentrations of ODM-207 for designated period. Following incubation, the cells were stained with anti-PDL1 antibody and the expression was assessed on a BD FACS caliber.

Subcutaneous CT26 syngeneic tumor model: Tumors were established by subcutaneous injection of CT26 cells into female BALB/c mice. After initial tumor growth, when the average tumor volume reached 100 mm³, and treatment with ODM-207 at 3, 10 and 30 µg/kg qo. was initiated and continued for 14 days. Tumor volume was measured with a caliper during the course of the study twice weekly. Statistical analysis: Paired t-test, ***p<0.001 vs vehicle control, Two way ANOVA followed by Dunnett’s test for ODM-207, p<0.05 vs baseline control. Two way ANOVA followed by Sidak test for p<0.05.

Measurement of immune PDL in PD-26 syngeneic tumor model: Tumors were established by subcutaneous injection of CT26 cells into male BALB/c mice. After initial tumor growth, when the average tumor volume reached 122 mm³, and treatment with ODM-207 at 30 µg/kg qo. or vehicle was initiated on day-1 and continued for 5 days. Samples (mouse, whole blood & plasma) were collected 24 h after the last dose of compound administration for bio-marker analysis by FACS. Statistical differences between vehicle control and treatment group were calculated by Maxin Whitney U test.

Results

1. ODM-207 activates mouse and human immune system in vitro cultures

ODM-207 enhances cytotoxic nature of cultured mouse CD8+ T-cells

ODM-207 decreases frequency of FOXP3+ cells and expression level in mouse splenocytes and LN cells

3. ODM-207 inhibits tumor growth in CT26 syngeneic model

ODM-207 modulates effector cytokine production in whole blood from healthy humans

Lack of direct cytotoxicity of ODM-207 in CT26 cell line

Conclusions

✓ ODM-207 activates mouse and human immune system in the in vitro cultures as demonstrated by the activation of effector function of mouse CD8+ T cells, reduction in the level and frequency of mouse FasD+ T cells and secretion of TNFα and IL-1β from human whole blood assay

✓ ODM-207 downregulates PD-L1 in mouse (CT26, colon carcinoma) and human (H1975, lung carcinoma) cell lines

✓ ODM-207 shows the anti-tumor activity in a syngeneic model of colon carcinoma in the absence of a direct anti-proliferative activity on tumor cells

✓ Observed tumor growth inhibition correlated with the in vitro activation of cytotoxic CD8+ T Cells supporting an immune-mediated effect leading to tumor growth inhibition

In view of the remarkable success with the immune-based therapeutic approaches, these findings are relevant in devising appropriate strategies for the continued clinical development of ODM-207