Abstract LB:113

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

Pratima Deshpande[2], Ravi Krishna Babu[2], Prashant Yallappa Vadnal[2], Mahaboobi Jaleel[2], Chandrasekhar Abbineni [2], Anu Moilanen[1], Pekka Kallio[1], Murali Ramachandra[2], Susanta Samajdar[2]

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

Building well-being

Background

BET (bromodomain and extraterminal) family proteins (BRD2, BRD3, BRD4, and BRDT) are epigenetic readers that bind to acetylated-lysine residues in histones and recruit protein complexes to promote transcription elongation. In many cancers, BET proteins have been shown to regulate expression of MYC and other oncogenic drivers that are important for cell proliferation and survival. Pharmacologic inhibition of the BET–histone 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 benzodiazepine-based inhibitors including JQ1, I-BET762, and OTX015. 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-1, we sought to evaluate ODM-207 for its effect on immune-mediated anti-tumor efficacy in pre-clinical models.

Methods

Effector cell phenotype assay: CD8+ T-cells were isolated using commercially available isolation kit and stimulated for 2.5 d with coated anti-CD3 (2.5 µg/ml) and anti-CD28 (0.5 µg/ml). Following 3.5 d 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-IFNγ PE antibodies.

Regulatory T cell (Treg) assay: Spleens and lymph nodes (LNs) were harvested from an 8 week old male C57BL/6 mouse. After homogenization, the cells were counted and plated in a 96-well plate pre-coated with 2.5 µg/mL of anti-CD3 antibody. Soluble anti-CD28 was added at 1 µg/mL, and IL-2 and TGFβ at 2 ng/mL along with requisite concentrations of ODM-207. Following incubation period, cells were stained with anti-FOXP3 PE, anti-CD3 APC, anti-CD4 PerCP-Cy5.5 antibodies, and the data was acquired on a BD FACSCalibur™.

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 flat-bottomed 96-well anti-CD3 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 cocktail was added (0.5 µg/ml). Following 2.5 d incubation at 37^{0}C in 5% CO_2 atmosphere, supernatants were collected and cytokine levels were measured using ELISA.

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 termination using the XTT cell viability assay.

Analysis of PD-L1 expression on tumor cell line: 0.2 x 10^6 mouse colon carcinoma CT26 cells or human non-small lung carcinoma cells 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-PD-L1 antibody and the expression was assessed on a BD FACSCalibur™.

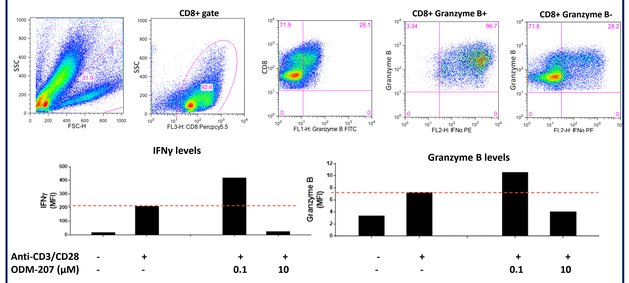
Subcutaneous CT26 syngeneic tumor model: Tumors were established by subcutaneous injection of CT26 cells into female BALB/c mice. After initial tumor growth, when the average tumor volume reached ~40 mm^3, oral treatment with ODM-207 at 3, 10 and 30 mg/kg qd, or vehicle was initiated and continued for 14 days. Tumor volume was measured with a caliper during the course of the study twice weekly. Statistical analysis: *p<0.05,***p<0.001, ****p<0.0001 vs Vehicle control, Two way ANOVA followed by Dunnett's test for ODM-207, #p<0.01 vs Isotype control, Two way ANOVA followed by Sidak test for J43

Measurement of immune PD in CT26 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, oral treatment with ODM-207 30 mg/kg qd or vehicle was initiated on day-1 and continued for 5 days. Samples (tumor, 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 tabulated by Mann-Whitney U test.

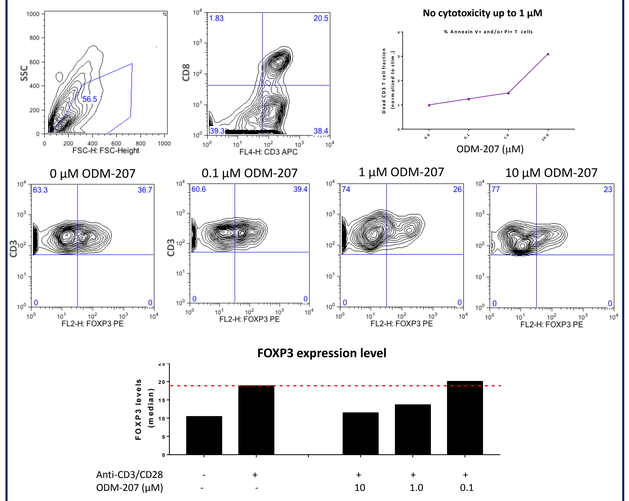
Results

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

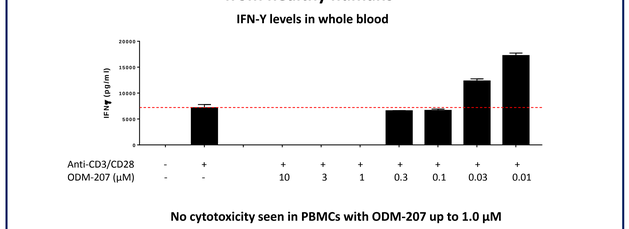
ODM-207 enhances cytotoxic nature of cultured mouse CD8+ T-cells



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



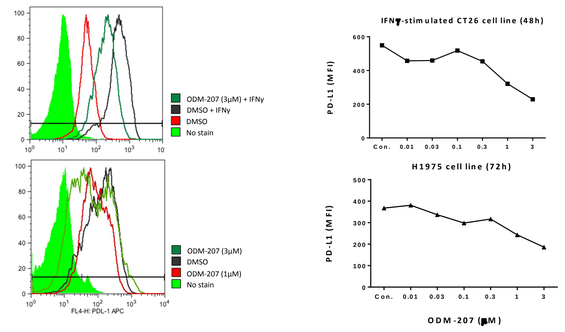
ODM-207 modulates effector cytokine production in whole blood from healthy humans



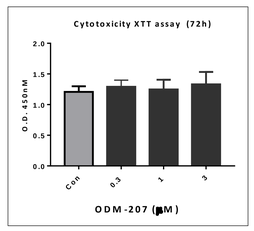
No cytotoxicity seen in PBMCs with ODM-207 up to 1.0 µM

2. ODM-207 downregulates PD-L1 in mouse CT26 colon carcinoma and human H1975 lung carcinoma cell lines

Inhibition of the cell surface expression of PD-L1

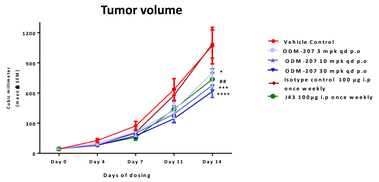


Lack of direct cytotoxicity of ODM-207 in CT26 cell line

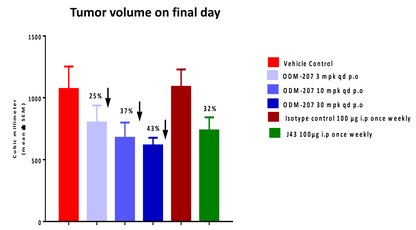


3. ODM-207 inhibits tumor growth in CT26 syngeneic model

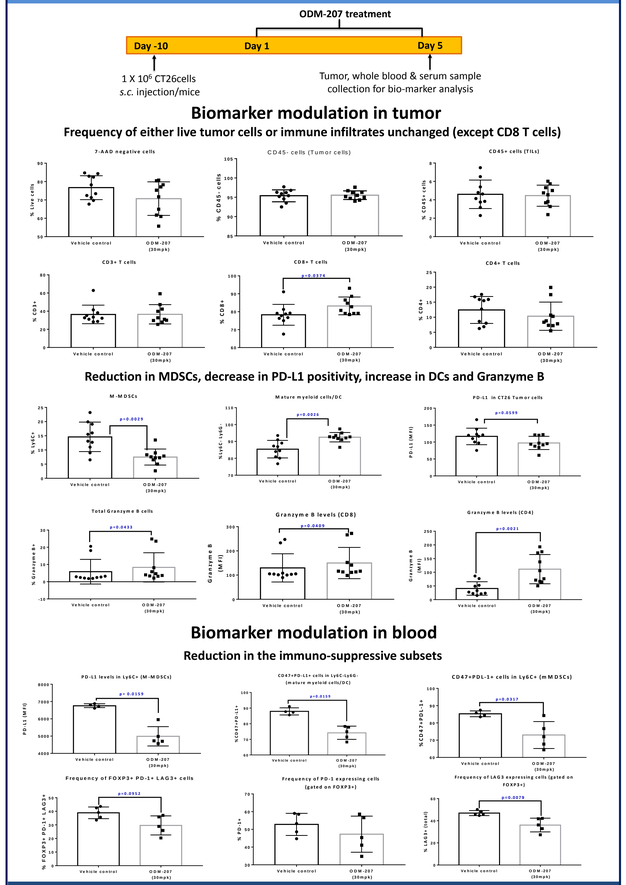
Tumor volume



Tumor volume on final day



4. Antitumor activity of ODM-207 correlates with immune pharmacodynamic effect in the tumor



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 FoxP3+ Treg cells and secretion of IFNγ cytokine in 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

Poster presented at AACR Symposium 2017
April 1 - 5, 2017. Washington, D.C., USA

These studies were sponsored by Orion Corporation Orion Pharma