PRECLINICAL STUDIES USING MONOCLONAL ANTI-MUC1 AND DOCETAXEL: A NOVEL STRATEGY FOR TREATING ADVANCED EPITHELIAL OVARIAN CANCER

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Background: Epithelial ovarian cancer (EOC) is the most lethal gynecological malignancy. Despite advances in surgery and chemotherapy over the past 20 years, overall survival has not changed significantly for these patients and novel therapeutic strategies are needed to improve patient survival. MUC1 is a membrane bound glycosylated phosphoprotein and is over-expressed in EOC. Over-expressed of MUC1 is associated with aggressive biological behavior and poor clinical outcomes. Monoclonal antibody (MAb) C595, raised against the protein core of human MUC1, has been used for tumor imaging and targeted therapy in several cancers. We hypothesize that MAb C595 may be useful alone or in combination with docetaxel to improve the treatment of advanced EOC

Purpose: This study aimed to: 1) to evaluate the patterns of MUC1 (MAb C595) expression in primary tumors and metastatic lesions in advanced stages of EOCs and correlate the expression with clinicopathological parameters; 2) to test anti-tumor activity of anti-MUC1 MAb (C595) alone and in combination with docetaxel in vitro in EOC cell lines; and 3) to evaluate the efficacy of combined MAb C595 and docetaxel in an EOC mouse model produced using OVCAR-3 cells.

Methods: MAb C595 was used to determine MUC1 expression by immunohistochemistry in primary EOC (frozen sections n=42), and the matched metastatic lesions (n=30) as well as paraffin-embedded tissue sections from primary EOC (n=60), and normal ovarian tissues (n=20) using a semi-quatitative grading system. MUC1 expression in EOC cell lines (A2780, OVCAR-3, CAOV-3, IGROV-1, TOV21G, TOV112D, SKOV-3 and OV-90) was assessed using immunofluorescence and flow cytometry. The effect of MAb C595 alone or in combination with docetaxel on EOC cell lines was studied using MTT assays, clonogenic assays, and cell cycle analysis. Apoptosis was evaluated by terminal deoxynucleotidyl transferase-mediated deoxyuridinetriphosphate nick end-labelling (TUNEL) assay, and Enzyme-Linked Immunosorbent Assay (ELISA) for cytochrome c and caspase-3 (markers of apoptosis). Female nude mice (6~8 weeks old) developed ascites and peritoneal tumors comprised of human EOC cells (OVCAR-3) following intraperitoneal (i.p) inoculation, with 100% take rate after 2 weeks. Mice were treated with MAb C595 (10 mg/kg per week) and/or docetaxel (3 mg/kg per week) or vehicle [hydroperoxymethyl cellulose (HPMC) prepared as 0.5% in PBS] i.p for 3 weeks. Treated mice were killed 4 weeks post-treatment. Ascites volume, tumor number, and tumor weight were assessed. Microvessel density (MVD) and cell proliferation in tumor xenografts were assessed using antibodies to CD31 and Ki-67 respectively.

Results: MUC1 was expressed in 92% (39/42) primary EOC and 90% (27/30) matched metastatic lesions

respectively in frozen sections and in 95% (57/60) primary EOC and 5% (1/20) normal ovarian tissues respectively in paraffin sections. Most tumors showed moderate to strong immunostaining while normal ovarian tissues showed weak staining. The over-expression of MUC1 was significantly associated with various clinicopathological parameters such as tumor stage, grade, residual disease status and presence of ascites ($P < 0.05$). All EOC cell lines tested are MUC1 positive except for OV-90 cell line. Treatment with MAb C595 alone inhibited EOC cell proliferation in a dose- and MUC1-dependent manner. Low-dose MAb C595 (20 mg/mL) combined with different concentrations of docetaxel (10-5 nM to 10-12 nM) greatly enhanced levels of MUC1-positive EOC cell killing and induced apoptosis. This addictive effect was confirmed in a clonogenic survival assay.

Combined MAb C595/docetaxel induced G2/M cell arrest, and increased apoptosis (86% TUNEL+ cells) when compared to cells treated with MAbC595 (40%), docetaxel 63%) alone, or non-treated controls (2%) ($P < 0.05$). Cells treated with combined MAb C595/docetaxel also showed increased levels of cytochrome c and caspase-3. Vehicle-treated OVCAR-3 xenografted mice developed overt ascites requiring repeated aspiration, and exhibited increased numbers of metastases and tumor weight over 4 weeks after i.p injection. Ascites formation, number of metastases and tumor weight decreased in MAb C595/docetaxel-treated mice and expression of CD31 and Ki-67 in the combined MAb C595/docetaxel-treated mice were markedly reduced ($P<0.05$).

Conclusions: MUC1 is over-expressed in more than 90% of late-stage primary EOC and metastatic lesions, but not in normal ovarian tissues. Over-expression is correlated with EOC progression. Our in vitro and in vivo studies provide a rationale for using combined MAb C595 (targeting MUC1) and docetaxel in further clinical trials, as a potentially beneficial and novel treatment for patients with advanced EOC.