The Effect of Bevacizumab (Avastin) in Combination with Doxorubicin on Tumor Growth of Malignant Fibrous Histiocytoma in the Animal Model.

INTRODUCTION
Bevacizumab (Avastin®), Roche Co, Basel, Switzerland), is one of the specific inhibitors for angiogenesis and a neutralizing antibody against vascular endothelial growth factor (VEGF), has recently been used as a drug against malignant tumors. We previously demonstrated that bevacizumab inhibited tumor growth of malignant fibrous histiocytoma (MFH) in vivo (1). Although bevacizumab is licensed for use in combination with chemotherapeutic agents for the first-line treatment of patients with metastatic colorectal cancer (2), advanced non-small-cell lung cancer (3) and metastatic breast cancer (4), there were few reports of combination therapy using bevacizumab against the soft tissue sarcoma. In this study, we evaluated the effect of bevacizumab in combination with doxorubicin (one of the most commonly used soft tissue sarcoma first line treatment) against MFH in the animal model.

MATERIALS AND METHODS
Animals. Male athymic BALB/c nude mice (6 week-old) were used. Animal maintenance was in accordance with institutional principals and procedures outlines in the Guide for the Care and Use of Laboratory Animals at our institution.

Implantation of tumor cells. The human MFH cell line, Nara H, was used in this study. We injected Nara H cells (1.2x10^7) subcutaneously to the dorsal area of nude-mice. Then, we measured body weight and tumor dimensions twice a week after implantation of tumor cells. Tumor volume was calculated according to the formula V=π/6×a×b×c, where a and b represent the shorter and the longer dimension of the tumor.

Treatment of established tumor in nude mice. Forty mice were randomly divided into four treatment groups (10 mice/group): (a) control (PBS in 0.05mL); (b) doxorubicin (1.2mg/kg in 0.05mL); (c) bevacizumab (2.0mg/kg in 0.05mL); and (d) combination (doxorubicin 1.2mg/kg + bevacizumab 2.0mg/kg in 0.05mL). We started treatment 2 days after implantation of tumor cells. Treatment repeated intraperitoneal injection twice a week for 6 weeks.

Statistical analysis. The statistical significance of the individual findings and their association indices were evaluated by the Student’s t-test. Overall survival duration was calculated from the start of treatment using the Kaplan-Meier method. Probability, p-values less than 0.05 were considered significant.

RESULTS
No significant difference in change of body weight was found among the four groups (Figure 1). And these treatments did not cause the loss of weight and the other significant side effects. During the experiment period, the mean of tumor volume in combination group was smaller than those in other groups (Figure 2). At the end point of our study, the tumor volumes were reduced to 28% in combination group, 41% in bevacizumab group and 62% in doxorubicin group compared with control group. After day 8 of treatment, the tumor growth was significantly inhibited in combination group compared with not only in control group but also in doxorubicin group. After day 11 of treatment, the tumor growth was also significantly reduced in bevacizumab group compared with control group. There were no significant differences to survival rate in four groups during our experimental period of 6 weeks.

DISCUSSION
In our study, single treatment of bevacizumab significantly inhibited the tumor growth in MFH animal model. Moreover, the tumor growth reduction in combination (doxorubicin + bevacizumab) group was observed earlier than that in bevacizumab group, but there was no significant difference in tumor growth between bevacizumab alone and combination groups. It showed that bevacizumab might be effective for MFH and combination treatment of bevacizumab and doxorubicin might delay the tumor growth than single treatment of bevacizumab for MFH. There were no significant difference to survival rate in four groups during 6 weeks in this study, but we must continue follow-up of survival rate and adverse event for a longer period.

References
1) Okada Y, Akisue T, Hara H et al. Anticancer Res. in press