Giant cell tumor of bone (GCTB) is a locally aggressive bone tumor, which is composed of neoplastic mononuclear cells and non-neoplastic osteoclast-like giant cells.

**Denosumab treatment induces significant morphological change**
Denosumab, a monoclonal antibody that inhibits receptor activator of NF-κB ligand (RANKL), is applied for treatment of GCTB, which suppresses osteoclastic activity. As a result, denosumab-treated GCTB (dGCTB) characterized by giant cell depletion and new bone formation (Fig.1).

**H3F3A pG34W mutation highly characterizes GCTB**
Recently, H3 histone family member 3A pG34W (H3F3A G34W) mutation was identified in the majority of GCTBs (Fig.2). An anti-H3G34W mutation-specific antibody is a powerful tool for detecting the resultant abnormal protein (H3G34W), which has been shown to detect the mutation in approximately 90% of GCTBs.

In this study, we examined cytological change in dGCTB by immunocytochemistry using an anti-H3G34W antibody.

**Materials and Methods**
Crushed cytology specimens of 8 surgically resected or biopsied dGCTBs in 7 patients were examined (Table.1). They were compared with cytological specimens of pre-denosumab treatment in each case.

**Immunocytochemistry**
Three liquid biopsy cytology (Surepath, Becton Dickinson) samples (case 2-3,7) and 1 crushed cytology samples (case 6) of dGCTBs were subjected to immunocytochemistry using the anti-H3G34W antibody for detecting the mutation in approximately 90% of GCTBs.

**Results**

**dGCTs exhibits spindle cell morphology with a marked decrease of osteoclast-like giant cells**
All cases showed the cytological appearance of typical GCT before denosumab treatment (Fig.3). Cellularity of dGCTBs was low or moderate. All GCTBs showed an increase of fibroblast-like spindle cells and a marked decrease of round cells and osteoclast-like giant cells which were present in conventional GCT (Fig.4). Residual giant cells underwent degenerative change (Fig.5). They also contained the various extent of macrophages and histiocytes; however, it was different from secondary aneurysmal bone cyst (ABC). Histologically, osteoid formation was observed in all dGCTs (Fig.6). Cytological features were summarized in Table 2.

**Neoplastic mononuclear cells survive continuously with changing their shape after denosumab treatment**
By immunocytochemistry, H3G34W was positive in the nuclei of most of the spindle and ovoid cells in all the examined cases except for case 2. These results directly indicate that these are neoplastic cells changed their shape (Fig.7, Table 2).

**Conclusions**
In diagnosing GCT, one should keep it in mind that the cytological appearance of dGCTs is different from typical GCTs with many giant cells. It needs to be distinguished from a variety of spindle cell lesions of both benign and malignant with/without bone formation, such as reactive fibroblast lesions, fibrous dysplasia, ABC and osteosarcoma (low grade and fibroblastic). Clinical information about denosumab treatment is indispensable for the differential diagnosis.

Immunocytochemistry of H3G34W is useful adjunct for cytological evaluation of dGCTB, as it detects neoplastic mononuclear cells regardless of their cellular shape.