P0.205

INTENSITY OF EXPRESSION OF CD56 (NCAM) ON MYELOMATOUS PLASMA CELLS

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Background: It was shown that the absence of CD56 (neural cell adhesion molecule) on malignant plasma cells (PC) is a hallmark of plasma cell leukemia and of a special subset of multiple myeloma (MM) (Pellat-Deceunynck *et al.,* Leukemia 1998; 12:1977-82). It was also found that expression of CD56 correlates with the presence of lytic bone lesions in MM and distinguishes MM from monoclonal gammopathy of undetermined significance and lymphomas with plasmacytoid differentiation (Ely *et al.,* Am J Pathol 2002; 160: 1293-9). The aim of this study was to evaluate the intensity of CD56 expression on bone marrow (BM) myelomatous PC and to assess clinical correlations.

Materials and Methods: The study group consisted of 80 MM patients (42M 38F, median age 64, range 39-80yr ;12 at stage I, 18-II, 50 -III according to D.S.; 52 had osteolysis; monoclonal protein was IgG in 56 patients, IgA in 16, IgM in 1, Bence Jones in 7) and 12 had plasma cell leukemia (PCL) patients. Controls were 10 healthy subjects. Immunophenotyping was done on freshly collected BM samples using triple staining combination of CD138/CD56/CD38 monoclonal antibodies analyzed by flow cytometry (Cytoron Absolute and FACSCalibur-Becton Dickinson). Plasma cells were identified as cells showing high-density expression of CD38 and CD138 (syndecan-1). Antigen expression intensity was calculated as relative fluorescence intensity (RFI) and for direct quantitative analysis the QuantiBRITE test (Becton Dickinson) was applied. Mean channels of phycoerythrin fluorescence were defined and antibody bounding capacity (ABC) was then calculated using QuantiCALC software.

Results: In 54 patients (67.5%) PCs showed CD56 expression. Out of all CD38++/CD138+ BM cells, the mean proportion of PC with CD56 expression, was 79±23%, median 91%. RFI values ranged from 7.6 to 23.3 in particular patients (15.9±3.6, median 15.6) and the number of CD56 binding sites (ABC) on MM plasma cells ranged from 2255 to 58469 (14199±15038, median 8866). A correlation was found between RFI and ABC values (r=0.76; $p<0.05$). In 26 MM patients considered as CD56 negative, myeloma mean proportion of all BM $CD38^{++}$ cells with CD56 expression was 5.0±4.3%, median 4.0%. A correlation was found between proportion of all BM $CD38^{++}$ cells with CD56 expression and ABC (r=0.60) and RFI (r=0.61) indices ($p<0.05$). Normal PC did not express CD56. In patients with CD56+myeloma a correlation was found between proportion of CD56+cells and percentage of PC in BM smears in morphological analysis (r=0.65). No differences among analyzed cases were seen between occurrence of CD56 expression and presence of osteolysis, stage of disease and monoclonal protein isotype. Response rate to chemotherapy was similar in CD56 positive and CD56 negative plasma cell proliferation; 58 and 55% respectively. Of 12 PCL cases 6 showed CD56 expression on PC in BM and 3 on those in peripheral blood.

Conclusions: In two thirds of MM patients CD56 molecule could be considered as a *tumor associated antigen*. Intensity of CD56 expression on PCs varies among particular CD56 positive MM patients and differences in expression level may be as big as many times. There is a relationship between proportion of BM CD56 positive PCs and density (ABC) and intensity (RFI) of expression of this molecule. PCL cases show heterogeneity in expression of CD56.

P0.206

A CHIMERIC MONOCLONAL ANTIBODY SPECIFIC FOR KAPPA MULTIPLE MYELOMA PLASMA CELLS MEDIATES ANTIBODY-DEPENDENT CELL CYTOTOXICITY OF TUMOR CELLS

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Current front-line therapies for multiple myeloma such as high dose chemotherapy and autologous stem cell transplant have improved progression-free survival. However, patients invariably relapse with refractory disease and salvage therapies are generally ineffective. Antibody-mediated immunotherapy offers an attractive alternative; however, with the exception of idiotype, few antigen targets have been identified that would facilitate specific immunotherapy of MM. We have previously described a murine monoclonal antibody that recognizes a conformation-dependent epitope on free human kappa light chains and a cell surface antigen, KMA, expressed on kappa MM plasma (MM_κ) cells (Goodnow and Raison, J Immunol 1985; 135: 1276-80). Here we show that the murine antibody, mKap, binds specifically to a range of kappa-type multiple myeloma (MM_κ) cell lines and mediates *in vivo* anti-tumor activity in a SCID mouse human myeloma xenograft model.

With the rationale of producing a safe and effective therapeutic for MM_κ, a chimeric human IgG1 version of the murine antibody, cKap, was expressed in CHO cells. mKap and cKap exhibit similar antigen binding specificity to soluble and cell-associated antigen as determined by ELISA and flow cytometric analysis respectively. Kinetic analysis of antigen binding by cKap and mKap using surface plasmon resonance showed that cKap retains the affinity of the murine parent antibody (K_D cKap = 18.2nM; KD mKap = 20.9nM). *In vitro*, cKap mediates significant ADCC of MM_κ cells using human PBMC effectors at E:T ratios as low as 25:1. These results highlight the specificity and functional activity of cKap and indicate the potential of this chimeric antibody as an immunotherapeutic for MM_κ

P0.207

GENETIC ABNORMALITIES AND PATTERNS OF ANTIGENIC EXPRESSION IN MULTIPLE MYELOMA. A STUDY OF THE MYELOMA SPANISH GROUP (GEM-2000)

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Introduction: Myelomatous plasma cells (PC) show a high heterogeneity both in their immunophenotypic characteristics as well as in their cytogenetic features. So far, no extensive studies have been carried out to explore whether such antigenic diversity is associated with specific genetic characteristics. We have investigated the relationship between the immunophenotypic profile at PC and both their DNA ploidy status (evaluated by flow cytometry), and specific genetic features (ascertained by FISH) in a large series of 915