A Chimeric Monoclonal Antibody Specific for Kappa Multiple Myeloma Plasma Cells Mediates ADCC of Tumor Cells

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INTRODUCTION

While frontline therapies for multiple myeloma (MM) such as high dose chemotherapy and autologous stem cell transplant have improved progression free survival, the disease remains incurable. Although recent clinical trials with novel therapeutic agents exhibiting anti-angiogenic and proteosome inhibitory effects have provided encouraging results, there remains a need to explore alternative therapeutic strategies for this invariably fatal disease. Given the efficacy and safety of a number of monoclonal antibodies that have been used in the treatment of hematological malignancies such as NHL and CLL (Rituxan and Campath-1H respectively), antibody-mediated immunotherapy of MM represents an attractive therapeutic approach. 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 (MMk) plasma cells. Here, we show that the murine antibody, mKap, binds specifically to a range of kappa-type multiple myeloma (MMk) cell lines and mediate in vivo anti-tumor activity in a SCID mouse human myeloma xenograft model.

As part of the process of developing an antibody-based therapy for MM, we have generated a chimeric version of mKap, termed cKap. Data is presented indicating that cKap has identical antigen binding characteristics to mKap. Furthermore, cKap satisfies the therapeutic criteria of being able to trigger ADCC in MMk using human peripheral blood mononuclear effector cells. The data presented here highlights the potential of this chimeric antibody to provide an antibody-based therapy for MM.

METHODS AND RESULTS

mKap binds specifically to a range of MMk cell lines

The variable region genes of the heavy and light chains of mKap were cloned upstream of human constant region genes of y and k isotypes in two separate vectors. The heavy and light chain constructs were co-transfected into Chinese Hamster Ovary (CHO) cells. The expressed chimeric IgGs, cKap, was purified from CHO cell culture supernatants by protein A chromatography and the structural integrity of the antibody determined by SDS-PAGE.

The antigen binding specificity of cKap was confirmed by ELISA. The antibody was incubated with immobilized kappa Bence Jones protein (VOR and NAP), lambda Bence Jones protein (MOS) and human IgM kappa (huIgM). Bound chimeric antibody was detected with AP conjugated anti-human Fcy. The data show that cKap bound specifically to two free kappa light chain samples and did not react with kappa chains associated with heavy chain (huIgM) or with free lambda light chains.

cKap and mKap have similar antigen binding affinities

The binding affinities of mKap and cKap were measured using surface plasmon resonance on a Biacore 2000 biosensor. Kinetic rate analysis on mKap and cKap was performed as follows: mAbs (~1000RU) were immobilised to a Biacore chip using anti-Fc antibody. Antibodies (purified BSA in a mouse; 0 to 1000mM) were injected at 30mL/min across immobilised mAbs for 5 minutes then allowed to dissociate for 15 minutes. Binding curves for each antigen concentration were overlayed and the association (k_a) and dissociation (k_d) rate constants were calculated using BiaEvaluation software. The rate and equilibrium constants of cKap were very similar to those of the native murine antibody, k_a of cKap was 22.3nM, compared to 20.6nM for mKap.

In a separate Biacore experiment, competitive binding kinetics of mKap (red) and cKap (blue) were studied (Right hand figure). mAbs were injected at a concentration of 300mM over immobilized antigen (purified human BJkP) at 20pL/min for 14 minutes and then allowed to dissociate for 20 minutes. Figure A is an overlay of the antibody binding and dissociation phases, normalised to the start of the injection. Figure B is an overlay of the dissociation phases only, normalised to the to the start of dissociation phase.

cKap binds to JJN3 kappa myeloma cells

The binding of cKap to JJN3 induces ADCC

The ability of cKap to induce antibody-dependent cellular cytotoxicity (ADCC) was determined in vitro using freshly isolated human PBMCs as effector cells and JJN3 as the k Myeloma target. JJN3s were preincubated with 100µg/mL of cKap for 20 minutes at 37°C. Effector PBMCs were then added at effector to target ratios (E:T) ranging from 100:1 to 12.5:1, and incubated for 6 hours at 37°C. Cell death was measured by colorimetric LDH release assay (Promega). The graph above shows mean percentage cytotoxicity from 4 separate experiments using PBMCs from two different donors. Cytotoxicity due to cKap (hatched blue bars) was significantly higher than antibody-independent cytotoxicity (PBS; open red bars) for E:Ts of 100, 50 and 25:1. Error bars show SEM.

SUMMARY AND FUTURE WORK

The in vitro and in vivo specificity and functional activity of mKap make it a strong candidate for development as a therapeutic antibody

- cKap, a chimeric version of mKap, exhibits antigen binding specificity and affinity equivalent to that of mKap. In addition, cKap is capable of mediating ADCC by human effector cells.

PacMab Ltd is currently completing preclinical studies with a view to undertaking clinical trials of cKap in patients with kappa type multiple myeloma.

REFERENCES