A Monoclonal Antibody Specific for Free Human Kappa Light Chains Induces Apoptosis of Multiple Myeloma Cells and Exhibits Anti-Tumor Activity in vivo

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Introduction

Multiple myeloma (MM) is a clonal B-cell malignancy characterized by the accumulation of plasma cells in the bone marrow. The tumor disrupts normal bone marrow function and destroys the surrounding bone tissue. The other characteristic of MM tumors is the secretion of immunoglobulin (Ig) or an Ig component into the blood and/or urine.

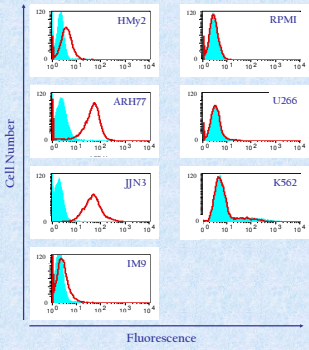
Therapeutic options available to MM patients include high-dose chemotherapy followed by autologous/allogeneic stem cell transplant and novel therapies such as thalidomide, the iMids, Velcade and Arsenic trioxide. The data from these clinical studies suggest that MM is almost invariably fatal despite the wide variety of chemotherapeutic and rescue treatment options.

Considering the significant efficacy of a number of antibodies in the treatment of hematological malignancies such as NHL and CLL (Rituxan and Campath-1H respectively), it is highly desirable to identify MM specific antigens against which antibody based therapeutics can be developed.

We have previously described a murine monoclonal antibody, now designated mKap, 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 [1,2]. Here we show that mKap is capable of killing MM_κ cells *in vitro* and inhibits tumor growth in a xenograft mouse model of MM.

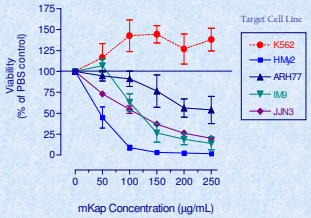
Methods and Results

mKap binds specifically to a range of MM_κ cell lines



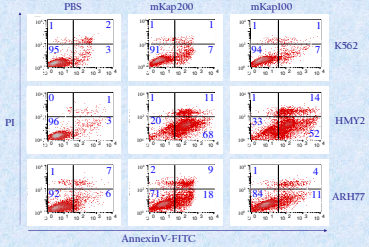
Solid histograms (blue) denote the isotype control and open histograms (red) denote mKap binding. mKap binds the MM_κ (HMy2, ARH77, IM9 and JJN3) cells and not the λ type MM (MM_λ: RPMI and U266) or the negative control (K562) cells. The antigen density on various MM_κ cell lines is variable as judged by the fluorescent shift arising from mKap binding.

mKap inhibits the growth of MM_κ cell lines



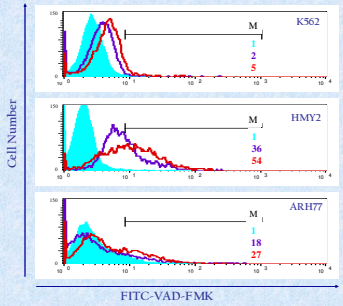
MM_κ (HMy2, ARH77, IM9 and JJN3) and negative control (K562) cells were incubated for 72 hours in the presence of various concentrations of mKap. Cell viability was measured using the MTS reagent in a colorimetric assay. Optical density of the PBS controls was taken to represent 100 percent viability, and viability of mKap treated samples were calculated accordingly.

mKap induces apoptosis in MM_κ cell lines



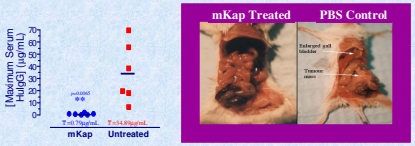
MM_κ (HMy2, ARH77) and negative control (K562) cells were incubated for 72 hours in the presence of mKap (200 and 100 μg/mL). The cells were subjected to AnnexinV-FITC/PI staining and analyzed by flow cytometry. Numbers denote the percentage cells falling within each quadrant. Apoptotic cells occur in the lower right quadrant (AnnexinV-FITC postive, PI negative). mKap induced apoptosis in the MM_κ cells in a dose dependent manner.

mKap induced apoptosis involves caspases

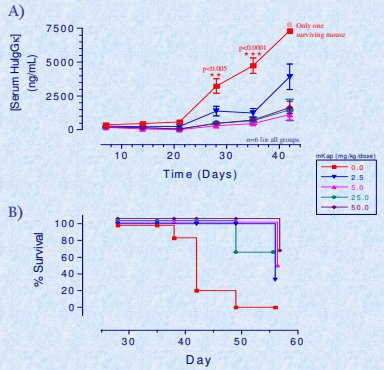


MM_κ (HMy2, ARH77) and negative control (K562) cells were incubated (48 hours) in the presence of mKap (200 and 100 μg/mL). The cells were stained with FITC -VAD-FMK (a fluorescenated analogue of Z-VAD-FMK caspase inhibitor which binds activated caspases) and analyzed by flow cytometry. Solid (blue) histograms represent PBS treated cells. The open histograms, purple and red, denote mkap treatment at 100 and 200 μg/mL respectively. Cells positive for active caspases fall within the marker (M). Percentage cells falling within M are shown in corresponding colors for each treatment.

mKap inhibits MM_κ cell line growth in a SCID xenograft model



To produce a tumor xenograft model HMy2 cells were intraperitoneally injected into SCID mice. Mice were then injected with 3 doses of either mKap (62.5mg/kg/dose) or PBS on days 1, 2 and 3. Tumor progression was monitored by measuring the serum concentration of HuIgG secreted by HMy2 cells. Mean Maximum HuIg attained in the untreated group was significantly higher than that of the mKap treated group. At day 36 surviving mice were sacrificed and inspected for tumor growth. Obvious granuloma, MM_κ cell line HMy2 masses and enlarged organs were observed in untreated mice, but mKap-treated animals appeared to be tumor-free.



In another *in vivo* experiment, the dose dependent effect of mKap on tumor growth was studied. HMy2 tumors were established in SCIDs on day 0. The mice then received mKap via i.p. injection at days 0, 1, 2 and 3 at doses ranging from 0 to 50mg/kg/dose. Tumor growth was monitored by measuring the serum concentration of HMy2-secreted HuIgG. By day 28, all mKap dose regimes had curtailed HMy2 tumor growth significantly compared to the no mKap control group (Fig. A). By day 49 all untreated mice had died, whereas most mKap treated mice had survived (Fig. B).

Summary and directions

mKap satisfies essential criteria as an immunotherapeutic for MM:

- It selectively targets MM cells
- It inhibits growth of MM cells independent of effector cells. The combination of this capability and other cell mediated effector functions should render mKap with significant therapeutic efficacy.

Currently, PacMab is developing therapeutic versions of mKap. PacMab expects to begin a Phase I clinical trial of its therapeutic antibody in late 2005.

References:

1) Raison, RL and Boux, HA. (1985) Mol Immunol, 22:1393

2) Goodnow, CC and Raison RL. (1985) J Immunol, 135(2):1276