MDX-1097 Binds Kappa Myeloma Cells Specifically and Its Anti-tumour Activity is Mediated by Multiple Effector Cells

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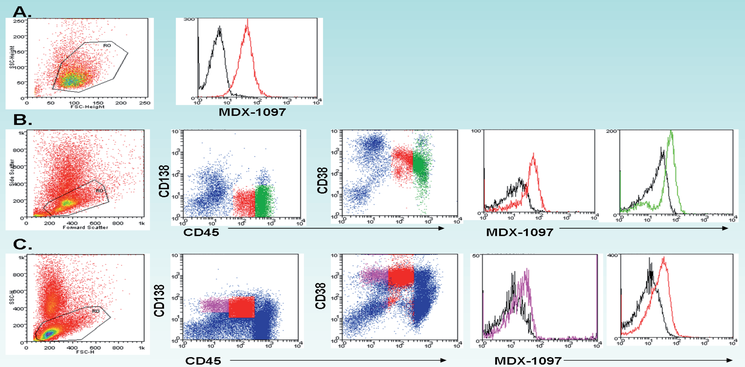
Introduction

MDX-1097 is a monoclonal antibody currently in clinical trial to evaluate its safety and efficacy in the treatment of kappa light chain restricted multiple myeloma (kMM) patients with stable measurable disease. MDX-1097 binds a conformational epitope on soluble free kappa light chain (kFLC) and the kappa myeloma antigen (KMA) which consists of membrane bound kappa light chain that is not associated with heavy chain. As a result, the antibody does not bind immunoglobulin. The presence of KMA has been demonstrated on plasma cells derived from the bone marrow (BM) of kMM patients, cells derived from bone marrow samples from patients with Waldenstrom's macroglobulinemia and on kMM cell lines.

Preclinical studies using the murine version of the monoclonal antibody, mKap, demonstrated that the antibody could induce growth inhibition and apoptosis of kMM cell lines. In addition, mKap was able to inhibit tumor growth in a SCID xenograft model of myeloma.

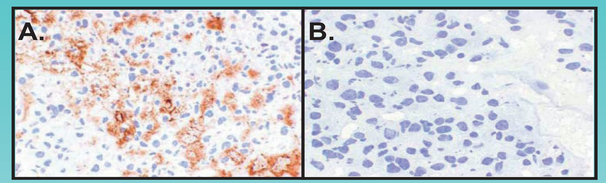
Data presented here demonstrates the presence of KMA and the co-expression of the B cell surface antigens CD45, CD38 and CD138 on cells derived from the bone marrow of kMM patients. Immunohistochemistry staining shows specific binding of MDX-1097 to plasma cells in bone marrow cross sections taken from patients with kappa plasma cell dyscrasia and no binding to normal human immune cells or tissue. *In vitro* data to ascertain the ability of MDX-1097 to bind kMM cells in the presence of patient sera containing a range of kFLC concentrations is also presented. Finally, the functional aspects of MDX-1097 binding to kMM cells are explored. The ability of MDX-1097 to mediate antibody dependent cellular cytotoxicity (ADCC) in the presence of human effector cells and antibody dependent cellular phagocytosis (ADCP) in the presence of macrophages isolated from normal human donors is demonstrated.

MDX-1097 binds to bone marrow cells derived from kappa multiple myeloma patients



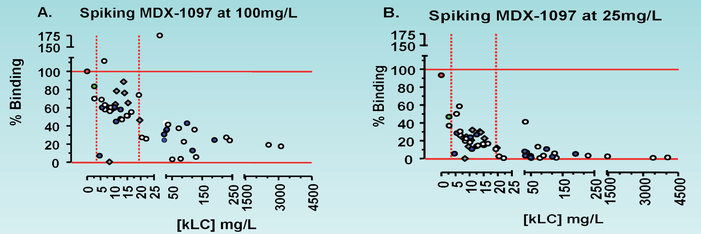
Panel A represents the JJN3 kMM human cell line while panels B and C represent patient bone marrow cells. All analyses were performed under the live lymphocyte gate (as shown). (A) JJN3 Cells were stained with MDX-1097-APC (red histogram) or isotype control ($IgG_{1\kappa}$-APC, black histogram). (B) BM cells were stained with CD45-FITC, CD138-PE, CD38-PerCP.Cy5 and MDX-1097-APC antibodies. KMA was detected on $CD45^{int}CD138^{-}CD38^{hi}$ (red shaded) and $CD45^{hi}CD138^{-}CD38^{hi}$ (green shaded) populations. The red and green histograms represent the KMA positive cells, in comparison to the isotype control, within the populations coloured similarly. (C) BM cells were stained as above. KMA was detected on $CD45^{-}CD138^{+}CD38^{hi}$ (pink shaded) and $CD45^{int}CD138^{+}CD38^{hi}$ (red shaded) populations. The pink and red histograms represent the KMA positive cells, in comparison to the isotype control, within the populations coloured similarly. In this study 8/9 kMM patient BM samples displayed surface expression of KMA. In 6 samples, KMA was present on the cells of the $CD45^{-}CD38^{+}CD138^{+}$ phenotype. The data shown here demonstrates the presence of KMA on $CD45^{+}$ and $CD138^{-}$ subpopulations.

MDX-1097 binds KMA in bone marrow tissue sections derived from kappa plasma cell dyscrasia



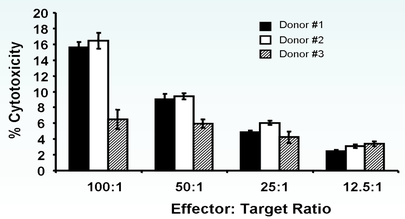
Tissue sections were fixed (MorphoSave) and stained with FITC labelled MDX-1097 followed by a mouse anti-FITC antibody and a peroxidase labelled goat anti-mouse IgG. All slides were counterstained with haemotoxylin and dehydrated. Representative photographs were taken of slides at 40X magnification. Panel A shows the staining (red) of a kappa plasma cell dyscrasia BM section while panel B demonstrates the lack of staining in a lambda plasma cell dyscrasia tissue section.

MDX-1097 binds kMM cells in the presence of kMM patient serum



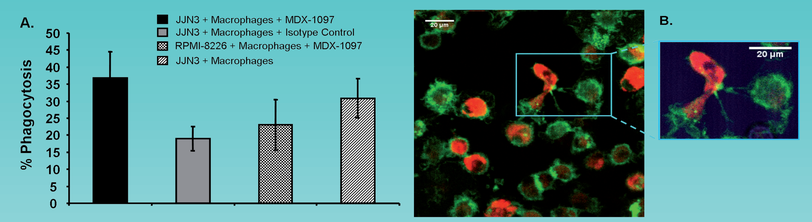
To simulate the *in vivo* environment for therapeutic intervention, individual sera from kMM patients (circles) or healthy blood donors (diamonds) spiked with MDX-1097-APC at final concentrations of 100 (A) and 25 mg/L (B) were added to JJN3 kMM cells and analysed by flow cytometry. The graphs show the results of the analysis of 35 kMM sera [23 kMM patients (white),12 MDX-1097 Phase 1 clinical trial patient sera (blue), one lambda patient serum (green)] and 10 healthy donors (grey). Maximum (100%) binding, as measured by a mean fluorescent intensity (MFI), was determined by spiking of MDX-1097 in FACS staining wash (FSW, red, mean of 7 replicates). Percent binding in patient sera was indexed against the maximal MFI obtained from FSW spiked with MDX-1097. Vertical dashed red lines indicate normal free kLC range (3.3-19.4 mg/L)[1].

MDX-1097 mediates antibody dependent cellular cytotoxicity (ADCC)



Healthy donor peripheral blood mononuclear (PBMCs; effector) cells were incubated with MDX-1097 labelled JJN3 kMM (target) cells at the ratios shown. Cytotoxicity was evaluated through a fluorescent measure of lactate dehydrogenase (LDH) release using the CytoTox-One™ reagent (Promega).

MDX-1097 induces macrophage mediated phagocytosis of kMM Cells



JJN3 (target) or RPMI-8266 (control λMM cell line) cells were labelled with pHRodo pH sensitive dye (Invitrogen) and primed with MDX-1097 or isotype control. The cells were then incubated with *in vitro* matured human macrophages (effector) at a 1:1 ratio. At the end point of the assay, the macrophages were stained with an Alexa-Fluor 488 anti-CD11b antibody and the mixture was analysed by confocal microscopy (40x magnification). (A) Total number of macrophages and macrophages containing ingested JJN3 cells were counted in 4 fields and % phagocytosis was calculated. Error bars represent the standard error of the mean. (B) A representative image of a phagocytosed MDX-1097 coated JJN3 cell is shown.

Conclusions

- MDX-1097 binds specifically to KMA, a membrane-associated antigen composed of free kappa light chains present on cells derived from kMM patient BM cells. In the majority of the BM samples analysed, KMA was present on cells of the $CD45^{-}CD138^{+}CD38^{hi}$ phenotype. KMA was also present on bone marrow subpopulations which were $CD45^{+}$ and $CD138^{-}$. It has been suggested that the $CD45^{+}$ phenotype in MM is associated with a higher proliferative index[2]. In addition, $CD138^{-}$ status has been shown to correlate with 'stem cell' like characteristics and also to confer drug resistance[3].
- MDX-1097 bound to kappa expressing cells in bone marrow samples obtained from patients with kappa plasma cell dyscrasia and did not bind to cells from patients with lambda plasma cell dyscrasia. No off target binding was observed in the normal human tissue panel.
- The ability of MDX-1097 to bind myeloma cells (JJN3) in the presence of kMM patient sera indicated that the antibody is able to engage membrane associated antigen (KMA) in the presence of soluble light chain. One explanation for this observation is that the antibody has a low affinity ($2x10^{-8}$ M) for a single epitope on the soluble antigen. This low affinity results in preferential binding of the membrane associated antigen due to avidity effects.
- *In vitro* experiments showed that MDX-1097 was able to mediate ADCC and this is probably one of the mechanisms that confer anti-tumour activity *in vivo*.
- Enhanced macrophage phagocytosis of MDX-1097 coated myeloma cells was demonstrated *in vitro*. This mechanism of action is likely to occur *in vivo* as macrophages are present in the haematopoetic islands within the bone marrow[4].

References :
[1] Katzmann *et al.*, 2002, Clinical Chemistry, 48: 1437-1440
[2] Kumar *et al.*, 2003, Blood, 102: 1075-1077
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