MDX-1097 induces antibody-dependent cellular cytotoxicity against kappa multiple myeloma cells and its activity is augmented by lenalidomide

Parisa Asvadi,1 Andrew Cuddihy,2,3 Rosanne D. Dunn,1 Vivien Jiang,1 Mae X. Wong,1,3 Darren R. Jones,1 Tiffany Khong2,3 and Andrew Spencer2,3

1Immune System Therapeutics, Sydney, NSW, 2Malignant Haematology & Stem Cell Transplantation Service, Alfred Hospital, and 3Myeloma Research Group, Australian Centre for Blood Diseases, Monash University, Melbourne, VIC, Australia

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Correspondence: Parisa Asvadi, Immune System Therapeutics (IST) Ltd, Suite 145 National Innovation Centre, Australian Technology Park, 4 Cornwallis St., Eveleigh, NSW 2015, Australia.
E-mail: pasvadi@istl.com.au; pasvadi@gmail.com
and
Andrew Spencer, Myeloma Research Group, South Block, Alfred Hospital, 55 Commercial Rd., Melbourne, VIC 3004, Australia.
E-mail: aspencer@netspace.net.au

Summary

MDX-1097 is an antibody specific for a unique B cell antigen called kappa myeloma antigen (KMA) that consists of cell membrane-associated free kappa light chain (κFLC). KMA was detected on kappa human multiple myeloma cell lines (κHMCLs), on plasma cells (PCs) from kappa multiple myeloma (κMM) patients and on κPC dyscrasia tissue cryosections. In primary κMM samples, KMA was present on CD38+ cells that were CD138 and CD45 positive and/or negative. MDX-1097 exhibited a higher affinity for KMA compared to κFLC and the latter did not abrogate binding to KMA. MDX-1097-mediated antibody-dependent cellular cytotoxicity (ADCC) and in vitro exposure of target cells to the immunomodulatory drug lenalidomide resulted in increased KMA expression and ADCC. Also, in vitro exposure of peripheral blood mononuclear cells (PBMCs) to lenalidomide enhanced MDX-1097-mediated ADCC. PBMCs obtained from myeloma patients after lenalidomide therapy elicited significantly higher levels of MDX-1097-mediated ADCC than cells obtained prior to lenalidomide treatment. These data establish KMA as a relevant cell surface antigen on MM cells that can be targeted by MDX-1097. The ADCC-inducing capacity of MDX-1097 and its potentiation by lenalidomide provide a powerful rationale for clinical evaluation of MDX-1097 alone and in combination with lenalidomide.

Keywords: antibody therapy, immunotherapy, multiple myeloma.

Multiple myeloma (MM) is an incurable malignancy of terminally differentiated plasma cells (PCs) characterized by PC accumulation in the bone marrow (BM), lytic bone disease, renal insufficiency, anaemia, hypercalcaemia and immunodeficiency (Katzev et al, 2007; Palumbo & Anderson, 2011). The BM microenvironment, characterized by the presence of extracellular matrix proteins and accessory cells, such as BM stromal cells, osteoclasts and osteoblasts, is believed to play a significant role in the survival and proliferation of MM cells (Balakumaran et al, 2010; Palumbo & Anderson, 2011). In addition to the secretion of an array of cytokines that stimulate cells within the microenvironment, MM cells secrete monoclonal immunoglobulin (Ig) (M protein) and/or Ig light chains which constitute the laboratory hallmark of MM. The M protein is predominantly of the IgG isotype while the light chain isotype is either kappa (κ) or lambda (λ).

Until the 1990s, MM treatment was restricted to conventional chemotherapy, at which point high-dose therapy with autologous stem cell transplant (ASCT) and the use of bisphosphonates for treatment of MM-related osteolysis were integrated as standards of care (Kyle & Rajkumar, 2009). In the past decade, novel agents such as thalidomide, bortezomib and lenalidomide have been introduced into the clinic with significant benefit in terms of response rates and survival (Kyle & Rajkumar, 2009). Furthermore, the benefits of combining various anti-MM agents in comparison to sequential single agent therapy (Alexania et al, 1977; Lonial & Kaufman, 2012), and an increased understanding of the mode of action of novel anti-MM agents (Rajkumar et al, 2005; Quach et al, 2010; Mujtaba & Dou, 2011) has facilitated the use of rational approaches in the design of clinical trials. The use of oncogenomics for identification of high risk disease (Avet-Loiseau et al, 2007; Shaughnessy et al, 2007)
and flow cytometry for the detection of minimal residual disease (Bataille et al., 2006; Rawstron et al., 2008; Paiva et al., 2009) have enabled a more stringent interrogation of new treatments and their true impact on patient outcome. Coincidentally with the above, monoclonal antibodies have become an increasingly important class of anti-cancer therapy due to their demonstrable efficacy and favourable toxicity profiles. In this context, the increasing understanding of the biology of MM has resulted in the identification of multiple potential targets for antibody-based therapy of MM (Podar et al., 2009; Richardson et al., 2011; van de Donk et al., 2012).

MDX-1097 is an IgG1/κ chimeric antibody specific for free kappa light chain (κFLC), i.e., light chain not tethered to Ig heavy chain, and the unique cell surface antigen designated kappa myeloma antigen (KMA). The variable (V) region genes of MDX-1097 are derived from the mouse monoclonal antibody (mAb) mKap (K-121) (Dunn et al., 1996). In previous studies mKap has been shown to be effective in a xenograft model of MM in severe combined immunodeficient (SCID) mice (Raison et al., 2005) and KMA was shown to be present on cells derived from B cell lymphoproliferative disorders, such as lymphoma (Walker et al., 1985) and Waldenström macroglobulinaemia (Walker et al., 1985; Jones et al., 2007).

This study aimed to characterize MDX-1097 with respect to its cell surface antigen-binding characteristics and effector functions that could engender anti-MM activity in the clinical setting, in which it might be used alone or in combination with anti-myeloma agents constituting myeloma standard therapy. Immunomodulatory drugs (IMiDs), including lenalidomide, are one of the principle classes of therapeutic agents currently used to treat MM (Morgan, 2010; Chanan-Khan et al., 2013). Investigations into the mode of action of IMiDs have shown that lenalidomide inhibits MM cell proliferation and induces MM cell death both directly and indirectly via a number of mechanisms, including the augmentation of Natural Killer (NK) cell activation and cytotoxicity (Quach et al., 2010).

MDX-1097 has been used in single (Spencer et al., 2010) and multi-dose clinical trials (Dunn et al., 2013; unpublished observations) in κMM patients with stable measurable disease and the results presented here provide a compelling rationale for further clinical development of MDX-1097 in combination with established therapeutic agents to improve clinical outcome in κMM patients.

Materials and methods

Cell culture

KMS-11 and KMS-26 cells were a kind gift from Dr. Takemi Otsuki of the Kawasaki Medical School, Japan. All other kappa human myeloma cell lines (κHMCLs) were obtained from either the American Type Culture Collection (ATCC, Manassas, VA, USA) or Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) cell banks.

BM mononuclear cell and peripheral blood mononuclear cell isolation

Bone marrow aspirates and peripheral blood samples from lenalidomide-treated κMM patients were obtained, following patient consent with institutional ethics committee approval, and MNCs were isolated using density gradient centrifugation. Similarly, buffy coats or whole blood, obtained from the Australian Red Cross Blood Service, with institutional ethics committee approval, were used to isolate normal PBMCs.

MDX-1097 binding to KMA

Kappa human multiple myeloma cell lines were tested for KMA expression using APC-MDX1097 in both flow cytometry and confocal microscopy experiments. BM MNCs were stained with APC-MDX1097 (or isotype control). MDX-1097 was produced to current good manufacturing practice (cGMP) standards by Medarex Inc. (Princeton, NJ, USA) and labelled, along with its matched isotype, by Invitrogen (Carlsbad, CA, USA). The cells were simultaneously stained with anti-CD45-fluorescein isothiocyanate (FITC), anti-CD138-phycocerythrin (PE) and anti-CD38-peridinin chlorophyll-cyanin5.5 (PerCP-Cy5.5) and analysed by flow cytometry. For inhibition experiments, JN3 cells were incubated with biotinylated MDX-1097, alone or in the presence of hIgG/κ or κFLC, and analysed for MDX1097 binding.

Measurement of the affinity of MDX-1097 for κFLC and KMA

The affinity of MDX-1097 for κFLC was determined using kinetic surface plasmon resonance (SPR). MDX-1097 was captured on a BiaCore 2000 biosensor chip and κFLC binding curves were overlayed to calculate rate constants. The affinity of MDX-1097 for KMA, expressed on JJN3 and KMS-11 cells, was measured using an adaptation of the method developed by Bator and Reading (1989). Briefly, cells were incubated with a range of MDX-1097 concentrations (1–20 nmol/l) and the amount of un-bound antibody was measured. Un-bound and bound (total minus unbound) antibody concentration data was fitted with a non-linear regression curve (GRAPHPAD PRISM 5; GraphPad Software Inc., San Diego, CA, USA) and maximum specific binding (Bmax) and equilibrium binding constant (Kd) values were calculated.

Immunohistochemical analysis of MDX-1097 binding

As part of the pre-clinical development of MDX-1097 a Good Laboratory Practice (GLP) human tissue cross-reactivity
study was conducted by Charles River Laboratories Pathology Associates (Frederick, MD, USA). Briefly, cryosections from the BM of patients with κ or λ PC dyscrasias and a panel of normal human tissue were stained with FITC-labelled MDX-1097.

Myeloma drug treatment

JJN3 cells were treated with 1 μmol/l lenalidomide and dexamethasone alone or in combination for 5 d. PMBCs were treated with 1 μmol/l lenalidomide for 3 d. PBMCs were obtained from 3 MM patients (2 κMM and 1 λMM) prior to and following 1 month (two patients) or 3 months (one patient) of lenalidomide (initially 10 mg/d, escalating to 15 mg/d after 2 months) maintenance therapy. In addition to lenalidomide, the patients received prednisone, 50 mg/d.

Antibody-dependent cellular cytotoxicity assay

Antibody-dependent cellular cytotoxicity (ADCC) was measured using flow cytometry. Briefly, carboxyfluorescein succinimidyl ester (CFSE)-labelled, MDX-1097 (or isotype control) coated, target cells were incubated with peripheral blood mononuclear cell (PBMC) effector cells in a range of effector to target (E:T) ratios. Alternatively, a range of antibody concentrations and a fixed E:T ratio was used. 7-aminooactinomycin D (7-AAD) was used to measure cell death. To elucidate the effect of kFLC on MDX-1097-mediated ADCC, kFLC was added to the assay mixture and target cell toxicity was measured.

Results

MDX-1097 binds to KMA on κHMCLs and PCs from MM patient BM aspirates

Specific KMA binding by MDX-1097 was demonstrated using flow cytometry, confocal microscopy and immunohistochemistry. In flow cytometry experiments, κHMCLs showed differential surface expression of KMA (Fig 1A). JJN3, KMS-11 and KMS-26 cell lines expressed high levels of KMA but no KMA expression was detected on NCI-H929 cells.

Confocal microscopy experiments were utilized to illustrate and confirm the cell surface residence of KMA. In these experiments, co-staining for KMA and CD59 was carried out. CD59 was selected on the basis of information supporting its constitutive residence in membrane micro-domains known as rafts. Co-detection of KMA and CD59 was considered a preliminary step in investigating the molecular interactions at the cell surface that result in generation of KMA. KMA was detected on the JJN3 cell surface, both in domains that were positive for CD59 and in isolation (Fig 1B).

In the tissue cross reactivity study, MDX-1097 bound fixed BM sections from patients with κ but not λ isotype-restricted PC dyscrasias (Fig 1C) or normal BM samples. The tissue cross reactivity study used a panel of 35 human tissues obtained from three independent normal donors. No unexpected or off-target staining of the normal human tissue panel was observed (Figure S1). MDX-1097 staining of occasional mononuclear cells in the tonsil was interpreted to be the result of interaction with anatomically located B or PCs that produce kFLC. Low-level MDX-1097 staining of intravascular, interstitial and/or leaked proteinaceous material was deemed to be consistent with binding to residual kFLC in plasma, serum or tissue (Figure S1).

Analysis of κMM patient BM samples was undertaken to establish the presence of KMA on primary BM PCs and to investigate the CD38, CD138 and CD45 status of the KMA-positive population(s). Figure 2 shows representative flow cytometry profiles from two patients (Patients 18 and 19, Table I). In Fig 2A, the majority of CD38+ cells are CD138-negative, however, a proportion of cells are CD138+ and both these populations are CD45+ as well as KMA+. In addition KMA was detected on CD38+/CD138+-CD45+ cells. In Fig 2B, within the CD38+ population, both CD138– and CD138++ populations are positive for KMA (in this BM sample both the CD38+/CD138–/CD45+ and CD38+/CD138+/CD45– populations were KMA-positive). The results of the phenotypic analysis of KMA-positive cells from primary BM samples of 15 patients (Patients 5–19) are summarized in Table I. KMA was detected on samples from four patients (1–4) for which no CD38/CD138/CD45 information was available. In total, KMA was detected in 17 out of 19 (89%) samples. In one patient sample (Patient 11), lack of KMA detection was attributed to the very low proportion of PCs (1%). This data confirmed that KMA is present on malignant PCs with a variety of phenotypes.

MDX-1097 preferentially binds KMA over soluble kFLC

Given that MDX-1097 is specific for KMA as well as soluble kFLC, we evaluated whether the binding of MDX-1097 to KMA was influenced by the presence of kFLC, either spiked, at specific concentrations, into the assay mixture or as a component of serum derived from κMM patients. Only kFLC, and not IgG/κ, partially inhibited the binding of MDX-1097 to JJN3 and KMS-11 cells, reiterating the kFLC specificity of MDX-1097 (Fig 3A). Significantly higher molar ratios of kFLC to MDX-1097 (24:1) were needed to promote this effect and it was demonstrated that the affinity of MDX-1097 for kFLC was 20 nmol/l, whereas it was 4 nmol/l for KMA, i.e., five times higher (Fig 3B,C respectively). We therefore concluded that the relative lack of inhibition of KMA binding was due to the higher affinity of MDX-1097 for KMA compared to kFLC.

The kFLC used to generate this data was purified monoclonal Bence Jones protein (BJP). The effect of kFLC on MDX-1097 binding to KMA utilizing sera from κMM patients was also investigated. This confirmed the preferential MDX-1097 Induces Cytotoxicity Against κMM Cells
The presence of \( \text{kFLC} \) had a minimal effect on KMA binding by MDX-1097 with a modest reduction in the signal derived from MDX-1097-APC binding as the concentration of \( \text{kFLC} \) increased (Figure S2). Moreover, the signal registered in the assay was dependent on both the binding of MDX-1097 to KMA in the presence of \( \text{kFLC} \). More specifically, the presence of \( \text{kFLC} \) had a minimal effect on KMA binding by MDX-1097 with a modest reduction in

### Table 1. Phenotype of KMA positive BM PCs.

<table>
<thead>
<tr>
<th>Patient</th>
<th>M protein isotype</th>
<th>[M protein] (g/l)</th>
<th>[FLC] (mg/l)</th>
<th>%PC</th>
<th>KMA</th>
<th>KMA+ cell phenotype</th>
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<td>G</td>
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<td>2</td>
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<td>3</td>
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<td>6</td>
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<td>45</td>
<td>+</td>
<td>CD38+CD138+CD45+</td>
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KMA, \( \kappa \) myeloma antigen; BM, bone marrow; PC, plasma cell; FLC, free light chain; NA, not available; ND, not detected.

M protein isotype and concentration, FLC concentration and proportion of BM plasma cells (%PC) when BM aspirates were obtained are shown. The fluorescence-activated cell sorting profile of Patients 18 and 19 are shown in Fig 2.

*The phenotype of the PCs in sample 4 was CD38+CD138+CD45−.*
FLC concentration of the sera and the concentration of MDX-1097-APC indicating a dynamic interplay between the concentrations of MDX-1097 and FLC with respect to KMA binding.

MDX-1097 mediates ADCC of κMMCL cells

Antibody-dependent cellular cytotoxicity occurs when Fc receptor (FcR)-bearing immune effector cells and antibody-coated target cells are bridged, resulting in the release of cytotoxic enzymes from the effector cells. ADCC assays were done using both healthy donor PBMCs and NK cells as effector cells. When PBMCs were used as effector cells, MDX-1097-treated JJN3 cells were susceptible to PBMC-affected ADCC (Fig 4). Furthermore, this effect was recapitulated using alternative KMA expressing cells (KMS-26 and KMS-11) (Figure S3). Similar experimental set ups and a different target cell lysis measurement (lactate dehydrogenase release assay) produced comparable results (Figure S4). When soluble κFLC was included in ADCC assay mixtures, a modest dose-dependent reduction in target cell toxicity was observed (Figure S5).

In order to dissect the contribution of different immune effector cell populations to MDX-1097-mediated ADCC,
purified NK cells or monocytes were used as effector cells. This demonstrated that NK cells consistently elicited ADCC (Figure S6) whereas the ADCC levels elicited by monocytes were variable and, in some instances, negligible (data not shown).

Lenalidomide increases KMA expression and both in vitro and in vivo lenalidomide-exposed PBMCs elicit enhanced MDX-1097-mediated ADCC

*In vitro* experiments were conducted to simulate the clinical usage of lenalidomide, often in combination with dexamethasone, and its effects on KMA and other relevant PC markers. It was shown that lenalidomide exposure resulted in expression of statistically significant higher levels of KMA. Dexamethasone treatment decreased KMA expression levels, however, when compared to vehicle-treated cells, KMA levels remained higher in the lenalidomide + dexamethasone-treated cells (Fig 5A).

The effects on CD38 expression mimicked the effects on KMA expression, although CD38 expression post-lenalidomide + dexamethasone treatment was higher than KMA expression levels. CD138 expression levels were reduced by lenalidomide, dexamethasone or their combination.

When *in vitro* lenalidomide-exposed JJN3 cells were used in ADCC assays, a higher level of MDX-1097-mediated cell death was observed (Fig 5B). This increase in cytotoxicity could be attributed to the increase in KMA levels enabling increased MDX-1097 binding and therefore more potent immune effector cell engagement. Similarly, *in vitro* lenalidomide treatment of effector PBMCs resulted in higher levels of MDX-1097-mediated ADCC when compared to that seen with vehicle treated PBMCs (Fig 5C).

Importantly, given that lenalidomide is currently used for MM treatment, we investigated whether *in vivo* lenalidomide-exposed effector PBMCs from MM patients could elicit enhanced MDX-1097-mediated cytotoxicity of JJN3 cells. It was shown that PBMCs isolated from MM patients prior to lenalidomide therapy elicited MDX-1097-mediated ADCC of JJN3 cells at levels comparable to PBMCs obtained from normal donors, whereas PBMCs obtained from the same patients subsequent to initiating therapy with lenalidomide elicited significantly higher levels of MDX-1097-mediated ADCC (Fig 6). Notably, these patients were receiving, in addition to lenalidomide, a daily dose of prednisone. Therefore, it could be concluded that the *in vivo* presence of prednisone had not altered the immune potentiating effects of lenalidomide.

**Discussion**

The goal of antibody therapy for cancer is the specific targeting of malignant cells or the cellular processes that contribute to their survival and/or drug resistance. Here we have defined the functional characteristics of the chimeric mAb MDX-1097 and demonstrated the clear therapeutic potential of the antibody for the treatment of MM. Data is presented on the specific interaction of MDX-1097 with KMA on BM
MDX-1097 Induces Cytotoxicity Against kMM Cells

PCs from kMM patients and the absence of KMA on normal human tissue or other immune cells. The tissue cross reactivity study reported here identified rare tonsillar cells that express KMA. Similarly, Hutchinson et al (2014) reported locating KMA-positive cells in human tonsils. Comparison of the number of KMA-positive cells located in human tonsils (Figure S1) and the BM (Fig 1C) clearly indicates the rarity of tonsillar KMA-positive cells and the abundance of BM KMA-positive PCs. This suggests that in a clinical setting MDX-1097 would have a high degree of specificity against BM PCs, thus promoting a favourable efficacy and toxicity profile. The significance of the CD45 phenotype in MM stratification, disease stage, response to therapy and prognosis has been the subject of a number of studies (Joshua et al, 1996; Medina et al, 2002; Moreau et al, 2004; Kumar et al, 2008; Ishikawa et al, 2006). Furthermore, the CD138-negative phenotype has been suggested to be a marker of MM progenitor cells and as a factor affecting response to therapy (Matsui et al, 2008; Kawano et al, 2012). Therefore, the fact that MDX-1097 clearly binds to MM PCs expressing variations of both CD45 and CD138 may provide an important advantage in the clinical utility of the antibody.

Characterization of the MDX-1097 epitope on KMA has provided evidence regarding the conformational nature of the epitope and a structurally based rationale as to why this epitope is not accessible when kLC is coupled to an Ig heavy chain (Hutchinson et al, 2011). In the confocal microscopy experiments reported here, the detection of KMA in foci positive for CD39 suggests partial residence of KMA in raft cellular subdomains (Suzuki et al, 2012), which are rich in sphingomyelin and cholesterol (Brown & London, 2000) and believed to be involved in the organization of various signal transduction pathways (Simons & Toomre, 2000; Gao et al, 2011). This partial raft residence hypothesis correlates with the studies conducted by Hutchinson et al (2010) that provide substantial evidence for lipid (sphingomyelin)-protein (kFLC) interactions. Importantly, in our study, while the serum kFLC concentrations of patients studied ranged from 0-18 mg/l to >4375 mg/l (Table 1), KMA was detected in all but 2 instances (with serum kFLC concentrations of 43-1 and 65-4 mg/l). This indicates that the absolute level of soluble kFLC is not a major determinant of the quantity of cell surface KMA and suggests the involvement of alternative cellular mechanism(s) in retaining kFLC in the cellular membrane, thereby creating KMA.

In addition to binding to KMA, MDX-1097 binds soluble kFLC and an elevated level of serum FLC is a feature of MM. Importantly, however, our studies indicate that binding of MDX-1097 to KMA is not abrogated to any significant degree by soluble kFLC and provide a rationale, i.e., the higher affinity of MDX-1097 for KMA, for this occurrence. Considering that in the great majority of MM patients the serum kFLC concentrations are below 200 mg/l (Mead et al, 2004), it could be proposed that, in the clinical setting, the presence of soluble kFLC binding will not diminish the clinical potential of MDX-1097 when targeting KMA bearing MM cells. This proposal is also supported by the results from ADCC experiments in which kFLC were present in the assay mixture.

Binding of MDX-1097 to serum kFLC may have the potential for immune complex formation in vivo. However, previous studies with MDX-1097’s parent mAb (K121, mKap) indicate that, due to the existence of a single epitope on kFLC, only complexes with an overall composition of two antibody molecules bound to two kFLC monomers (180 kD) or two kFLC dimers (400 kD) were formed (Raison & Boux, 1985). Typically, serum kFLC exists as monomers, therefore it is unlikely that antibody-antigen complexes >400 kD would be formed. In addition, size exclusion chromatography studies using MDX-1097 have demonstrated that even in the presence of

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**Fig 4.** MDX-1097 mediates antibody-dependent cellular cytotoxicity of KMA-expressing kHMLC cells. (A) Labelled JJN3 cells were treated with MDX-1097 (black bars) or isotype control (white bars) before the addition of peripheral blood mononuclear cells (PBMCs). Data represents the average of six independent experiments ± standard error of the mean. Statistical significance (P value <0.05) is represented with one asterisk. (B) Labelled JJN3 cells were incubated with different concentrations of MDX-1097 (black bars) or isotype control (white bars) before adding PBMCs at a fixed 100:1 Effector:Target ratio. Statistical significance is represented with two asterisks (P value <0.001) or one asterisk (P value <0.05). KMA, κ myeloma antigen.
excess κFCLC, MDX-1097 forms an antibody-antigen complex of c. 180–200 kDa (data not shown). Finally, data from the phase I and IIa clinical trials of MDX-1097 (Spencer et al, 2010; Dunn et al, 2013) in kMM patients support these in vitro observations as there was no evidence of immune complex formation or organ damage.

MDX-1097 was shown to mediate ADCC, which is considered to be the major mechanism of action elicited by a range of therapeutic antibodies in various haematological malignancies (Cooley et al, 1999; Hayashi et al, 2003; Golay et al, 2006; van Meerten et al, 2006; Tai et al, 2008; Taylor et al, 2009). MDX-1097-mediated ADCC activity against MM cells was comparable to other anti-MM antibodies undergoing development, for example the anti-CD40 (Hayashi et al, 2003) and the anti-CS1 (elotuzumb) (Tai et al, 2008) mAbs. NK cells, but not monocytes, were capable of inducing ADCC against cells which expressed KMA with the difference in the ADCC-inducing capacity of NK cells and monocytes attributed to the divergence of the range of Fc receptors (FcRs) expressed by the two cell populations (Nimmerjahn & Ravetch, 2008).

Fig 5. In vitro lenalidomide exposure enhances KMA expression levels and MDX-1097-mediated ADCC. (A) Fold increase in the mean fluorescence intensity (MFI) of vehicle (dimethyl sulfoxide, DMSO), lenalidomide (Len), dexamethasone (Dex) or Len+Dex-treated JJN3 cells stained for KMA, CD38 and CD138 is shown. Data was collated from four independent experiments (error bars represent standard error of the mean [SEM]). Two-way analysis of variance (ANOVA) and Bonferroni post tests were used to determine statistical significance (t-test with P value <0.05; denoted with asterisks) in expression level changes. Reduction in the level of CD138 expression post-Len and -Dex treatment was also significant (not represented on the graph). (B) Len- (black and white bars) or DMSO- (grey and hatched bars) treated JJN3 cells were used in antibody-dependent cellular cytotoxicity (ADCC) assays. Data represent the average of three independent experiments ± SEM. (C) Len- (black and white bars) or DMSO- (grey and hatched bars) treated PBMCs were used in ADCC assays. Data represents the average of three independent experiments ± SEM. Tx, treatment.
Lenalidomide treatment of JJN3 cells increased KMA expression and this translated into increased MDX-1097-induced ADCC of the JJN3 cells. Lenalidomide treatment of JJN3 cells also increased CD38 expression levels in agreement with data characterizing the function of the anti-CD38 antibody daratumumab (Endell et al., 2012). The effect of dexamethasone on KMA expression was also examined because the anti-inflammatory and anti-proliferative properties of corticosteroids, such as dexamethasone, have made it part of the therapeutic regimen for MM. Although dexamethasone decreased KMA expression levels, it did not abrogate the augmenting effects of lenalidomide on KMA expression. Therefore, it can be anticipated that in a clinical setting when lenalidomide and corticosteroids are used in combination, the PC targeting of MDX-1097 is not significantly diminished. Furthermore, the in vitro or in vivo exposure of PBMCs to lenalidomide significantly potentiated the MDX-1097-mediated ADCC of KMA-positive target cells. Importantly, the net result of these two lenalidomide-mediated effects was an incremental increase in MDX1097-induced ADCC of KMA-positive MM cells which, similar to the case of other mAbs (van der Veer et al., 2011), is attributed to the potentiation of NK cell function by lenalidomide. Importantly, in the case of our in vivo results, the significant potentiation of NK cell capacity to elicit MDX-1097-mediated ADCC activity is present even when patients were receiving corticosteroid therapy. Although certain immune-potentiating effects of ImiDs on NK and T cells have been reported to be reduced by dexamethasone (Gandhi et al., 2010; Hsu et al., 2011), our data indicates that MDX-1097-mediated ADCC activity remains significantly augmented after exposure to both ImiDs and corticosteroids.

In summary, MDX-1097 specifically binds KMA on the surface of κHMCLs and primary κMM cells and induces ADCC against KMA bearing cells. Moreover, lenalidomide treatment of both immune effector and target cells, the latter via up-regulation of KMA expression, promotes the synergistic killing of KMA expressing MM cells. These data provide a compelling rationale for the implementation of clinical trials in κ-isotype restricted MM, incorporating a combination of lenalidomide and MDX-1097.

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Authorship and disclosures

PA designed and conducted experiments, analysed data, reviewed results and wrote the manuscript. AC designed and conducted experiments, analysed data, reviewed results and critically evaluated the manuscript. RD designed experiments, analysed data, reviewed results and critically evaluated this manuscript. AS designed experiments, reviewed results and edited the manuscript. VJ, MW and DJ performed experiments and collated data. TK assisted in planning experiments. All authors reviewed the manuscript. PA, RD and VJ are current, and MW and DJ are former employees of Immune System Therapeutics (IST) Ltd. RD owns IST shares. PA, VJ, MW and DJ as well as AC, TK and AS have no conflict of interest to disclose.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Materials and methods.

Fig S1. MDX-1097 shows no off-target binding in a human tissue cross-reactivity study.
**Fig S2.** MDX-1097 binds JNJ3 kHMCL cells in the presence of soluble kFLC.

**Fig S3.** MDX-1097 mediates toxicity against KMS-26 and KMS-11 kHMCLs.

**Fig S4.** MDX-1097 mediates ADCC against JNJ3 target as assessed using the LDH release assay.

**Fig S5.** Effects of kFLC on MDX-1097 mediated ADCC.

**Fig S6.** MDX-1097 mediates NK cell toxicity against JNJ3 cells.

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**References**


MDX-1097 Induces Cytotoxicity Against κMM Cells


