Characterisation of a novel conformational epitope on human free *kappa* light chains defined by a monoclonal antibody with therapeutic potential for multiple myeloma

Ralitza Alexova[1], Andrew T Hutchinson[2], Darren R Jones[2], Cameron Jennings[2], Allen Edmundson[2], Paul Ramsland[3], Kevin W Broady[1,2] and Robert L Raison[1,2]

[1]*Department of Medical and Molecular Biosciences, University of Technology, Sydney;* [2]*Immune System Therapeutics Ltd,* [3]*Macfarlane Burnet Institute for Medical Research and Public Health, Heidelberg, Victoria*

I. INTRODUCTION

Multiple myeloma (MM), one of the most prevalent blood malignancies, is characterised by the uncontrolled proliferation and accumulation of plasma cells in the bone marrow. This leads to an increased secretion of monoclonal immunoglobulin and free immunoglobulin light chains in the serum and urine of patients. mKap is a murine monoclonal antibody, which binds a conformational epitope in the constant domain (CL) on free *kappa* light chains (κFLC), but not free *lambda* light chains or intact immunoglobulin[1]. In addition, mKap recognises a cell-surface antigen, KMA, associated with the plasma membrane of *kappa* myeloma cells[2]. Currently, a chimeric version of the antibody is undergoing clinical evaluation as a therapy for multiple myeloma.

In an attempt to better understand the unique specificity of mKap, a range of epitope mapping techniques was used to identify the amino acids on κFLC which comprise the conformational epitope recognised by mKap.

II. METHODS AND RESULTS

Epitope retention in κFLC fragments

mKap was found to retain reactivity towards fragments of κFLC with molecular weight of less than 15kDa. After passing through a mKap affinity column, these peptides were separated by 2D electrophoresis and identified by LC-MS/MS. All mKap-reactive peptides were found to have an N-terminus at Ile106, but were truncated at the C-terminus, excluding the possibility that this region contains the epitope (Figure 1).

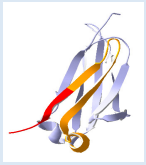

Figure 1: Ribbon diagram of the mKap-reactive κFLC fragments. The amino acids in red were not identified in any of the fragments, the amino acids in orange were truncated in three of the peptides.

Topographic epitope mapping

The binding of biotinylated κFLC to immobilised mKap was determined using the BIAcore biosensor (Figure 2). In this experiment biotinylation resulted in a significant decrease in the affinity of mKap to κFLC due to steric hindrance caused by the biotin attached to the light chain. This indicated that Lys residues were closely associated with the epitope recognised by mKap.

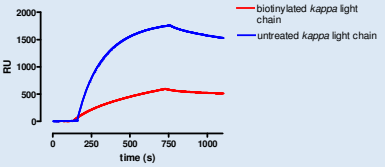

Figure 2: Binding of biotinylated κFLC (red) to mKap. mKap was immobilised on the surface of a CM5 chip in the BIAcore 2000 and κFLC diluted to 5μg/ml were injected for 10min at a flow rate of 20μl/min. Untreated κFLC (blue) was included as the maximum binding reference.

Epitope excision

Epitope excision is an epitope mapping technique which relies on the protection of the antigenic determinant from the action of proteolytic enzymes that occurs when an antigen-antibody complex is formed[3] (Figure 3). Two overlapping peptides identified as the switch region of κFLC and the N-terminus of the *kappa* CL were found to remain bound to mKap after proteolytic digestion (Figure 4).

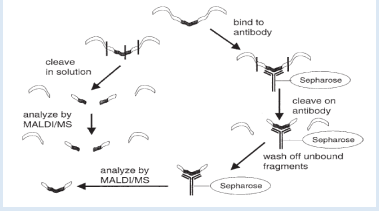

Figure 3: A typical epitope excision experiment. The antibody (mKap) is immobilised on Sepharose beads and incubated with the antigen (κFLC) before an overnight tryptic digestion[3]. The peptides still bound to the antigen are identified by MALDI/ MS. As a control a tryptic digestion of the unbound antigen is also performed.

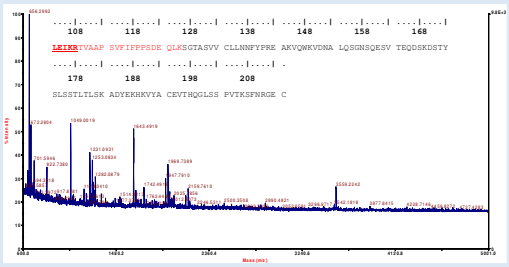

Figure 4: MALDI-MS spectrum of *kappa* light chain peptides protected from tryptic digestion by mKap. The insert shows the location of the identified peptides (red) of the constant domain (starting at Thr109) of the *kappa* light chain primary sequence. Amino acids found in several of the bound peptides are underlined.

Phylogenetic analysis and homology modeling

The availability of sequence data for *kappa* light chains from non-human primates provided an opportunity to use phylogenetic studies for further mapping of the mKap-defined epitope. The *kappa* light chain constant domains of primates are highly homologous, but show varying levels of amino acid substitution in several regions of the constant domain, which may result in disruption of the epitope (Figure 5A). This was confirmed by Western blotting and ELISA, where mKap bound only to human and chimpanzee *kappa* light chains.

Homology modeling, using the crystal structure of human *kappa* light chain as a template, revealed shifts in the topography of the CL domain around the changed amino acids at Ser171 when human and gorilla light chain were compared (Figure 5B).

A.

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                 112       122       132       142       152       162
             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                  5        15        25        35        45        55
human        RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG NSQESVTEQD
chimp        -TVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG NSQESVTEQD
gorilla      ---------- --PSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNVLQSG NSQESVTEQD
orangutan    GTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPRQVNVK WKVDNVEQSG NFQESATEQD
cynomol      RAVAPPSVFI FPPSEDQVTS GTVSVVCLLN NFYPREASVK WKVDGALKTG NSQESVTEQD
rhesus       -AVAAPSVFI FPPSEDQVKS GTVSVVCLLN NFYPREASVK WKVDGVLKTG NSQESVTEQD
marmoset     -AVAAPSVFI FQPSEEQVKS GTASVVCLLN DFYPRDVSVK WKVDDVVQSS NVQDSITEQD
ma's monk    -AVAAPSVFI FQPSEEQVKS GTASVVCLLN DFYPRDVSVK WKVDDVVQSS NVQDSITEQD
mouse        RADAAPTVSI FPPSSEQLTG GSASVVCFLN NFYPKDINVK WKIDGSERQN GVLNSYTDQD

                 172       182       192       202       212
             ....|....| ....|....| ....|....| ....|....| ....|..
                  65        75        85        95       105
human        SKDSTYSLSS TLTLSKADYE KHKVYACEVT HQGLSSPVTK SFNRGEC
chimp        SKDSTYSLSS TLTLSKADYE KHKVYACEVT HQGLSSPVTK -------
gorilla      SKDNTYSLSS TLTLSKAEYE KHKAYACEVT HQGLSS---- -------
orangutan    SKDSTYSLSS TLTLNNAEFQ KHKVYACEVT HQGLSS---- -------
cynomol      SKDNTYSLSS TLTLSSTEYQ SHKVYACEVT HQGLSSPVTK SFNRGE-
rhesus       SKDNTYSLSS TLTLSSTDYQ SHNVYACEVT HQGLSSPVTK SFNRGEC
marmoset     SKDNTYSLSS TLTLSSTEYQ RHKVYA---- ---------- -------
ma's monk    SKDNTYSLSS TLTLSSTEYQ RHKVYA---- ---------- -------
mouse        SKDSTYSMSS TLTLTKDEYE RHNSYTCEAT HKTSTSPIVK SFNRNEC
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B.

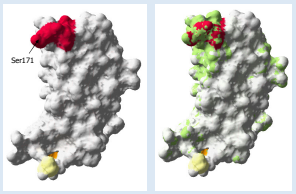

Figure 5: (A) Sequence alignment of the immunoglobulin *kappa* CL of primates. Amino acid differences from the human sequence are presented in red, allotype determining residues are shown in blue, '-' indicates residues for which no sequence is available. The number of amino acid differences compared to the human sequence are shown in brackets. **(B)** Homology models of the *kappa* light chain constant domain. After alignment of the human light chain (left) with the gorilla model (right) changes in the surface topology around Ser171 are visible (green). The C-terminal Cys214 is shown in yellow. Models were generated in SWISS-MODEL using *kappa* Fab (1clyL) as a template and aligned in DeepView v3.7.

Proposed region for the epitope of mKap

Based on the topographic mapping, epitope excision and homology modeling studies, an epitope located in a loop at the N-terminal end and the switch region of κFLC was proposed. These residues comprise a continuous surface that is normally involved in interaction with the adjacent light chain variable domain (Figure 6). It is possible that the closer association of the variable and constant domains in an Fab reduces the affinity of mKap to its epitope and completely prevents access of mKap to this region in an intact Ig molecule.

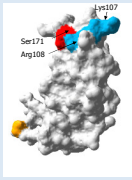

Figure 6: A surface model of the *kappa* light chain constant domain showing the proposed epitope recognised by mKap. The C-terminal Cys of the domain is shown in yellow.

Interaction of mKap with κFLC dimers

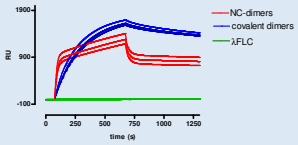

Figure 7: Binding of κFLC dimers to immobilised mKap. The interaction between mKap and κFLC dimers was assessed in the BIAcore 2000. Light chains diluted to 5μg/ml were injected over mKap immobilised on the sensor chip for 10min at a flow rate of 20μl/min and then dissociation was monitored for a further 10min. Lambda κFLC were included as a negative control.

In a BIAcore experiment (Figure 7) an apparent distinction between the binding of mKap to covalent and non-covalent κFLC dimers could be made, suggesting that the conformational difference in the light chain structure has affected the mKap-defined epitope. When the structure of the two light chain dimers was examined closely (Figure 8), a significant conformational difference around Ser171 was observed, supporting the proposed contribution of this region to binding to mKap.

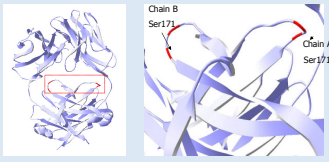

Figure 8: Ribbon diagram of (A) the non-covalent dimer Del (1B6D)[4] and (B) a magnified image of the loop containing Ser171 shows the conformational change of this region in both light chains forming the dimer.

III. CONCLUSIONS

- **mKap recognises a conformational epitope that includes residues Lys107, Arg108 in the switch region and Ser171 in the constant domain of *kappa* light chain**
- **Recognition of this unique epitope appears central to the therapeutic potential of mKap**
- **The reactivity of mKap confirms that subtle conformational differences exist between different forms of *kappa* light chain.**
- **The location of the epitope will be confirmed by site-directed mutagenesis and expression of recombinant *kappa* light chains**

References:

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2. Asvadi P, Jones D, Dunn R, Choo A, Raison M and Raison R (2004) 'A monoclonal antibody specific for free human kappa light chains induces apoptosis and exhibits anti-tumour activity *in vivo*' *American Society for Haematology*, San Diego 2004

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4. Roussel A, Spinelli S, Deret S, Navaza J, Aucouturier P and Cambillau C (1999) ' The structure of an entire non-covalent immunoglobulin *kappa* light chain dimer (Bence-Jones protein reveals a weak and unusual constant domains association' *European Journal of Biochemistry* **260:** 192-199