

## INTRODUCTION:

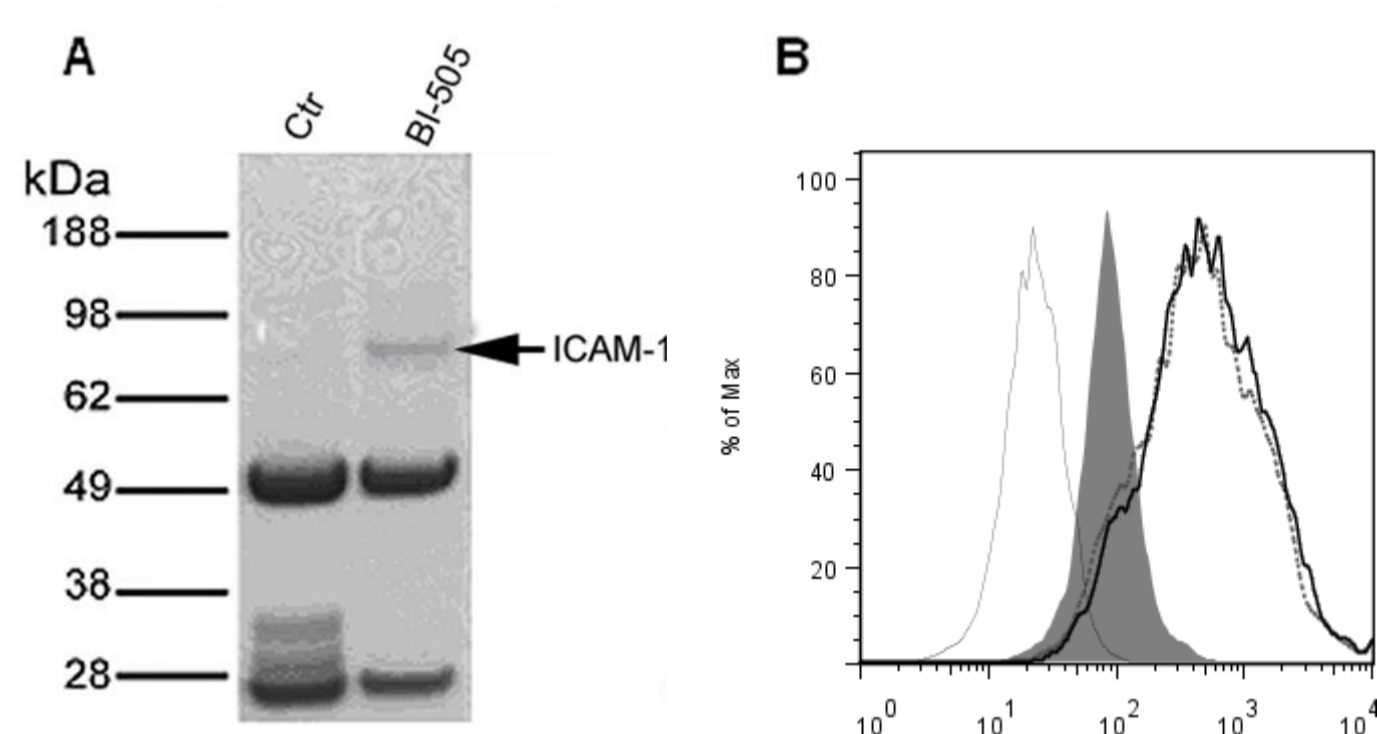
Bioinvent has isolated a novel tumor B cell-targeting antibody, BI-505, from a highly diversified human phage-antibody library (n-CoDeR<sup>®</sup>) using a pioneering 'function-first' approach involving screening for specificity for a tumor B cell surface receptor and induction of tumor programmed cell death (PCD). The fully human, high affinity IgG1 antibody BI-505 was found to be specific for Inter-Cellular Adhesion Molecule-1 (ICAM-1 or CD54) (Figure 1) and induce PCD in B-cell lines by binding (Figure 2) and by ADCC (Figure 3). The antibody was further tested *in vivo* in several different murine myeloma xenograft models and was found to have anti-myeloma properties (Figure 4 and 5).

## OBJECTIVE:

The aim of this study was to examine the expression ICAM-1 on bone marrow multiple myeloma cells from treated and untreated multiple myeloma patients.

## RESULTS:

The BI-505 epitope of ICAM-1 was found to be highly expressed in most multiple myeloma patients (Table 1) as analyzed by multi-color staining and flow cytometry (Figure 6). The expression was found to be preserved during disease progression and relapse (Figure 7).

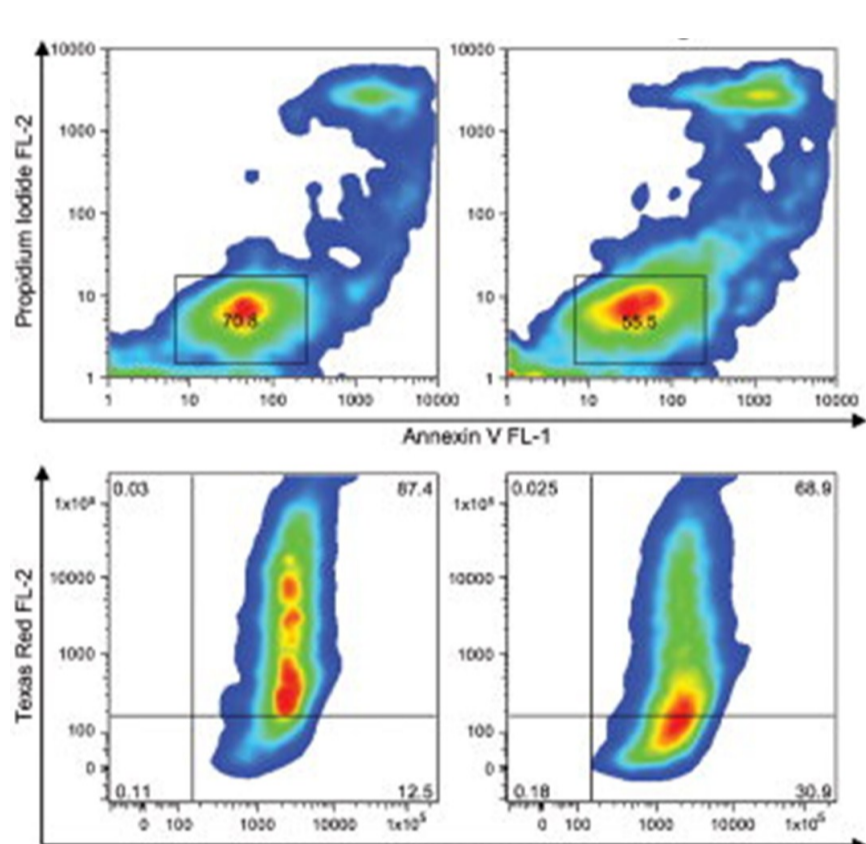


**Figure 1. BI-505 shows specific binding to ICAM-1.** (A) Raji lymphoma cells were lysed and immuno-precipitated with control antibody (Lane 1) or BI-505 (Lane 2). Antibody-specific bands were excised, trypsin digested, and analyzed by MALDI-TOF. The band in Lane 2 was identified as ICAM-1. (B) Binding of BI-505 to PC-3 cells was inhibited by preincubation of cells with an excess of sICAM-1 (filled grey line), but not by VCAM (dotted line). Black solid line indicates maximal binding and grey line isotype control.

**Table 1.** Multiple myeloma patient characteristics. Patients at the Hematology clinic, Skåne University Hospital, Lund, were investigated during 2009 and 2010 using bone marrow aspirates that were analysed with multi-color flow cytometry for ICAM-1 expression on multiple myeloma cells.

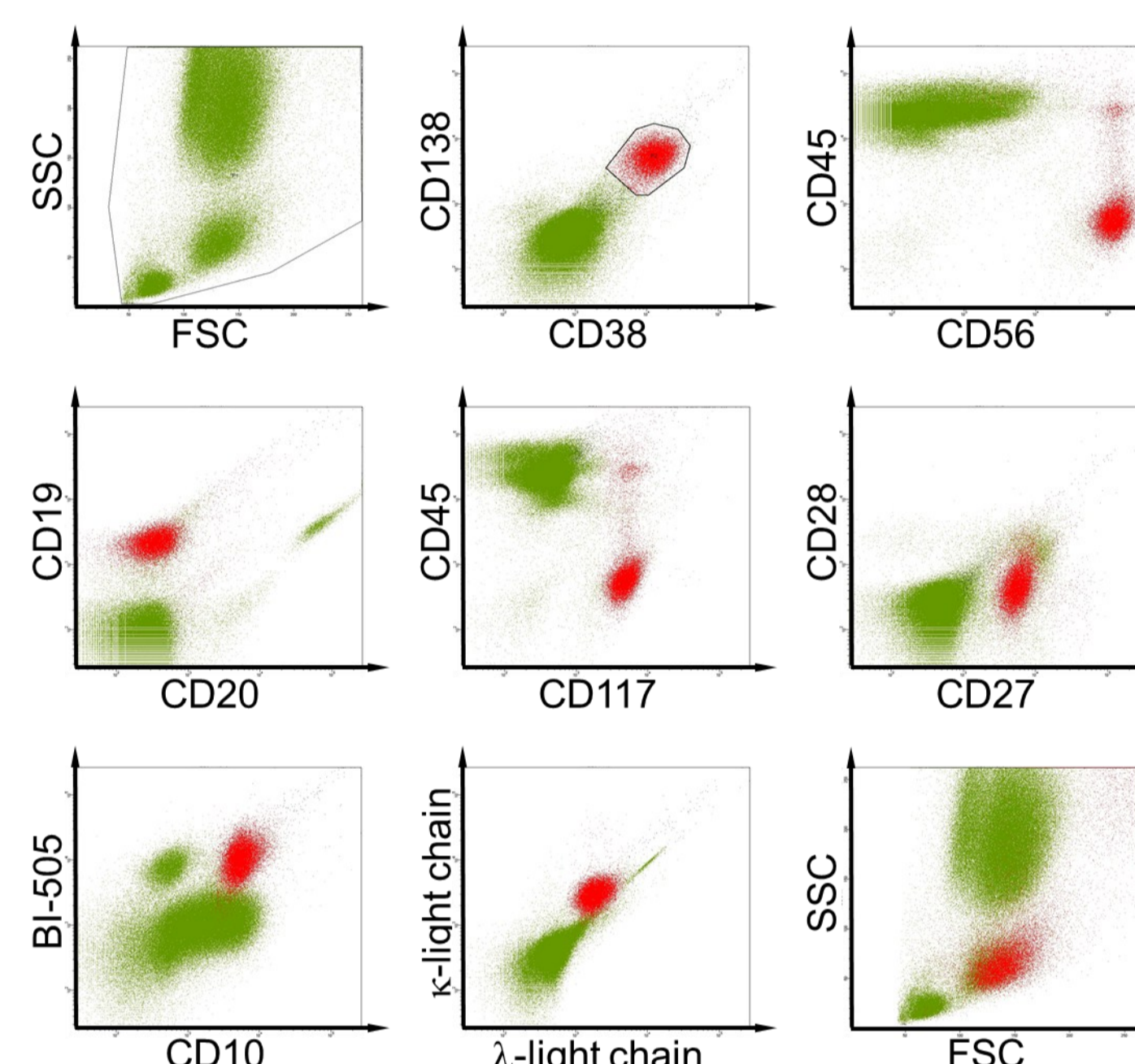
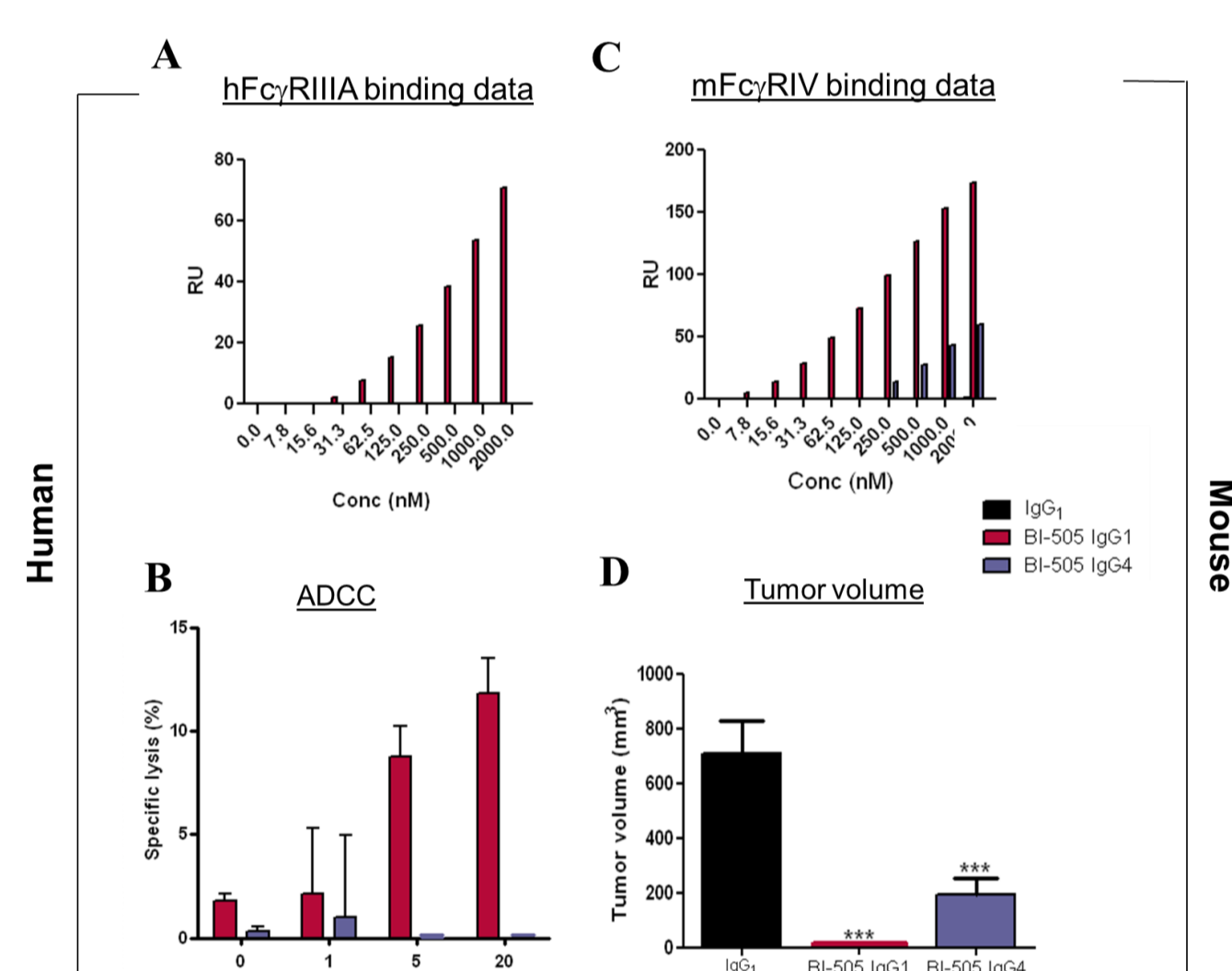
Multiple myeloma patient characteristics and BI-505 epitope expression										
age	sex	Ig	M (g/L)	skeletal destr.	Plasma cells morph	Plasma cells (%) FACS	BI-505	ISS	T	diagnosis
38	M	G	10	0	14	2	+++	I	0	MM
46	M	G	38	0	34	20	+++	II	0	MM
53	F	G	14	>10	6	22	+++	I	0	MM
54	M	-	0	3	22	5	+++	III	2	non secretory MM
59	M	G	32	>10	29	7	++	I	0	MM
60	F	G	4	3	2	0,1	+++	II	1	MM
60	F	A	26	1	10	2	+++	I	0	MM
61	M	G	28	0	23	9	+++	II	0	MM
62	M	G	69	>10	30	10	++	II	0	MM
62	F	G	70	>10	80	35	+	III	1	MM
68	M	A	36	>10	60	6	+++	I	0	MM
69	M	-	-	3	50	2	+++/*	I	1	non secretory MM
71	M	G	26	0	30	11	+++	I	0	MM
72	M	G	13	0	29	10	+++	I	0	MM
74	M	G	20	0	23	7	+++	I	0	MM
75	M	A	40	0	78	77	++	II	0	MM
77	M	G	45	0	34	13	+++	II	0	MM
79	F	G	23	0	16	6	+++	I	0	MM
79	M	G	24	7	50	24	+++	III	0	MM
82	M	A	29	0	44	20	+++	III	0	MM
83	F	G	39	0	89	64	+++	III	0	MM
84	M	A	17	0	38	22	+++	III	0	MM

M (M-component); skel. destr. (skeletal destructions measured by skeletal X-ray); ICAM-1 (ICAM B505 epitope expression, measured by FACS); ISS (international staging system); T (number of treatment regimes before ICAM-1 analysis); diagnosis MM (multiple myeloma), ns MM (non-secretory multiple myeloma).

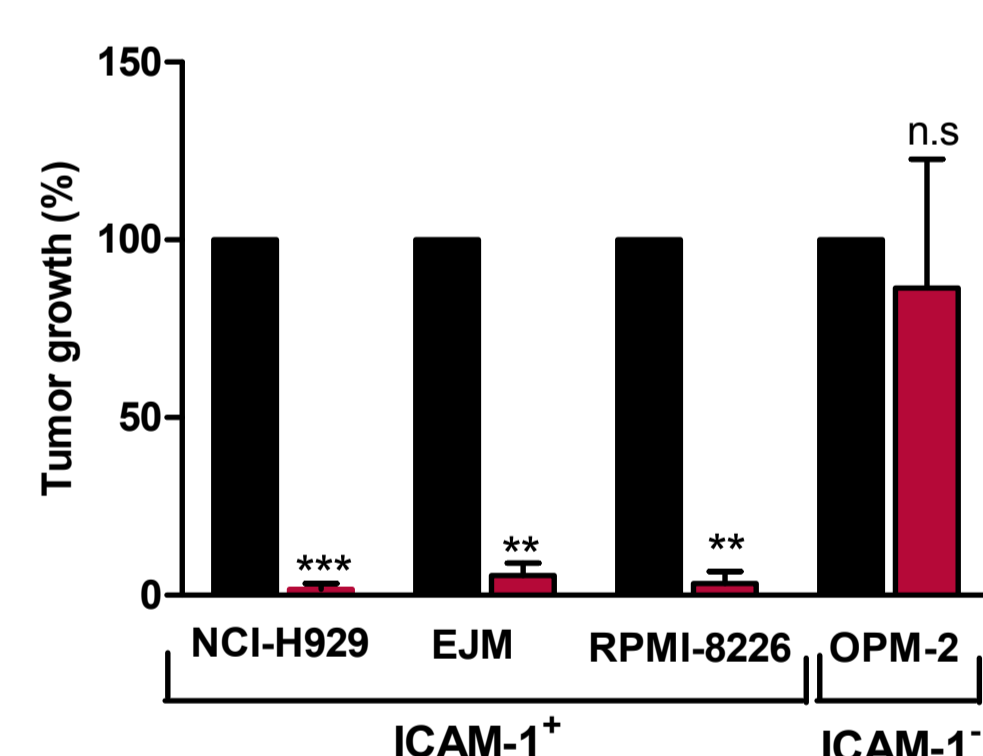


**Figure 2. ICAM-1 is a B lymphoma associated cell surface receptor capable of mediating programmed cell death.** BI-505 or control IgG<sub>1</sub> was added to CL-01 B lymphoma cells, incubated for 2 hr on ice, followed by addition of cross-linking secondary Fab'2 Goat anti-Human Fc antibody. Cells were incubated at 37°C for 6 hr and the effect of the antibody incubation was determined by two independent cell death assays. Cells were stained either by AnnexinV/PI (upper panel) or by incubation with the mitochondrial membrane depolarisation reagent JC-1 for 30 min at RT (lower panel). Induction of cell death is detected by a decrease in the red (y-axis)/green (x-axis) fluorescence intensity ratio.

**Figure 3. BI-505 anti-tumor activity correlates with FcγR-binding ability.** Binding of BI-505 isotypes to different recombinant FcγRs was determined by Biacore analysis. ADCC was examined by using natural killer cells as effector cells at different ratios, and the B-lymphoma cell line (CL-01) as target cells. (A) BI-505 isotypes bound human FcγRIIIA, a principal human ADCC-mediating receptor, with different affinities. (B) As expected, only BI-505 IgG<sub>1</sub> mediated FcγRIIIA-dependent ADCC of tumor cells. (C) BI-505 IgG<sub>1</sub> showed strong binding to murine FcγRIV, a principal Fcγ-receptor involved in ADCC in mouse. (D) ARH-77 myeloma cells were injected subcutaneously into the left flank of *scid* mice (n = 8 per group). Antibody treatment with 2 mg/kg BI-505 isotypes or control IgG<sub>1</sub> was started at day 1, and was continued on a twice-weekly i.p. dosing regimen. A correlation between ADCC activity and anti-tumor efficacy was observed.

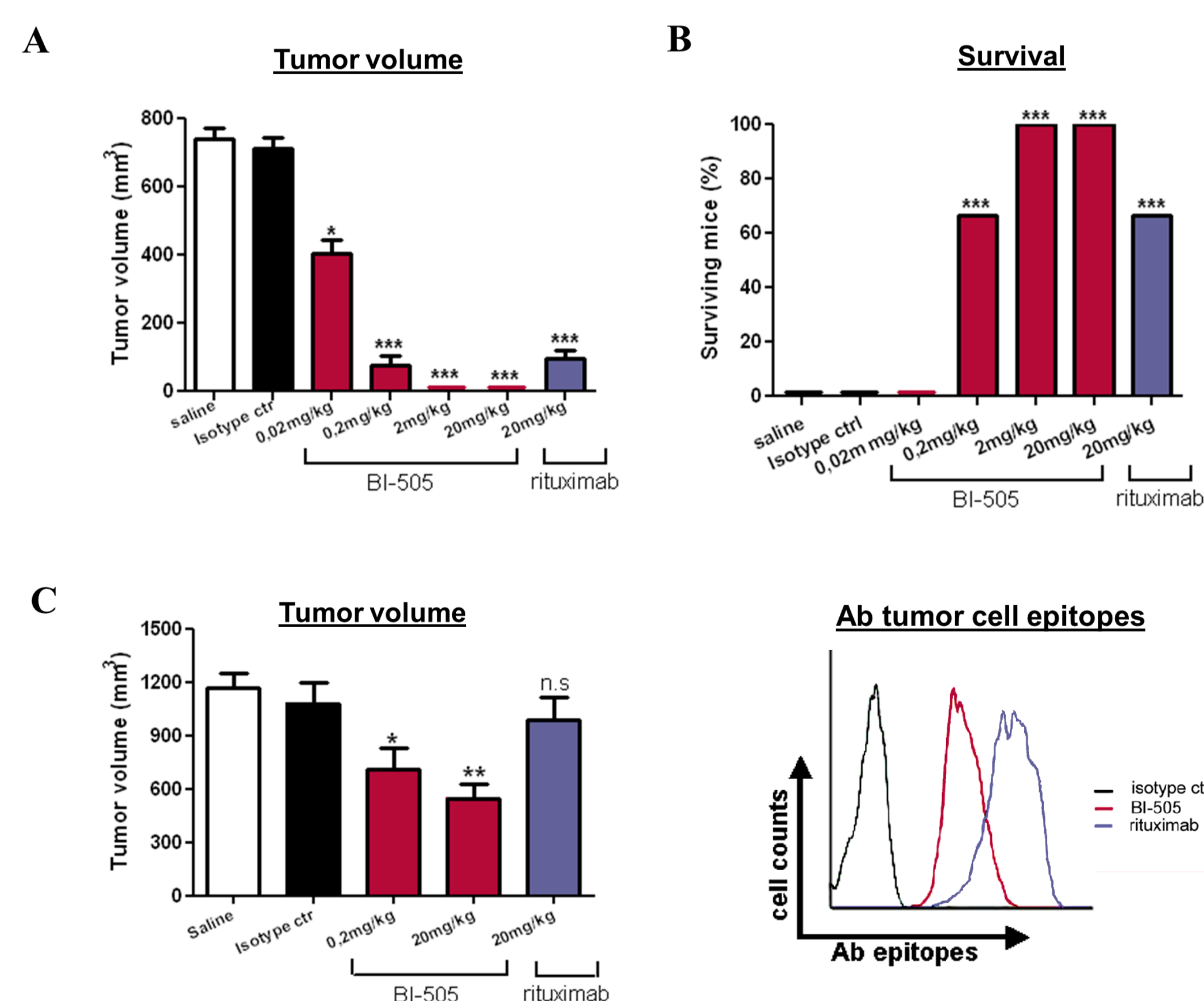


**Figure 6. Multi-color FACS analysis of bone marrow multiple myeloma cells from patients.** Cells were gated based on high expression of CD38, CD138 and CD56 and loss of CD45. Monoclonality was confirmed with intra cellular lambda and kappa staining.

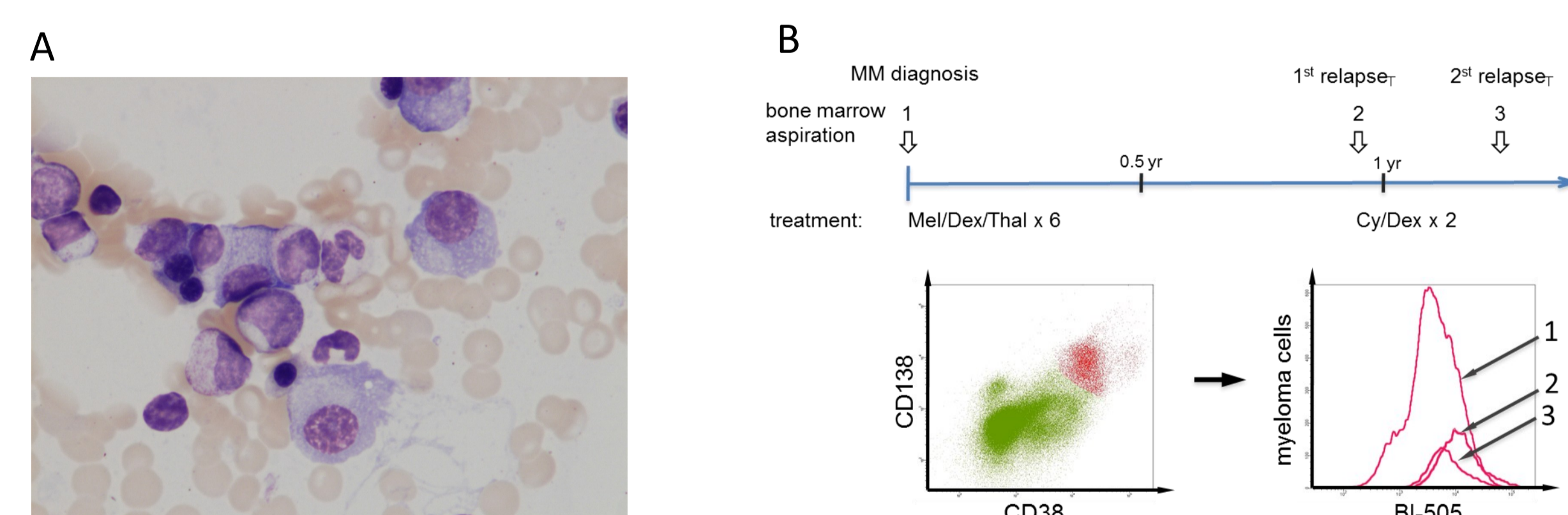


**Figure 4. BI-505 has broad *in vivo* anti-myeloma efficacy and potency in the early *scid*/myeloma xenograft model.** NCI-H929, EJM, RPMI-8226 or OPM-2 myeloma cells were injected subcutaneously into the left flank of *scid* mice at day 0. Antibody treatment with 2 mg/kg BI-505 (red bars) or control IgG<sub>1</sub> (black bars) was started at day 1, and was continued on a twice-weekly i.p. dosing regimen. Mice were sacrificed when tumor sizes reached the ethical limit. BI-505 had no effect on tumor growth in animals xenografted with the ICAM-1 negative cell line OPM-2, demonstrating that anti-myeloma activity was ICAM-1 dependent.

**Figure 7. Case Report.** A 78-year-old male, in previously good health, presented with a two-month history of progressive back pain. Serum protein electrophoresis showed a peak with a broad gamma band, and immunofixation revealed an immunoglobulin G-kappa monoclonal component of 24g/L. X-ray of the skeleton showed seven lytic destructions in several ribs, left and right femur and right humerus. A bone marrow aspiration was performed (A) and plasma cells were counted in the bone marrow smear and by flow cytometry (B). The bone marrow analysis showed an increased ratio of plasma cells (35-50% by morphology and 24% by flow cytometry) and the patient was diagnosed with multiple myeloma. The patient was treated with melphalan/ prednisolone/thalidomide and also received the osteoclast-inhibitor; pamidronate. ICAM-1 expression was measured at diagnosis and after the first and second relapse (B). (With the permission of the patient and with approval from the local ethical committee).



**Figure 5. BI-505 demonstrates high efficacy and potency in the early *scid*/ARH-77 myeloma xenograft model.** Mice received twice/weekly i.p. injections with BI-505 at different doses. (A) and (B) Early tumor model: treatment started one day after myeloma cell inoculation and continued until tumor volumes reached the ethical limit. Tumor volume (A) as well as mouse survival (B) were monitored. (C) Advanced tumor model: treatment started when the tumor volume reached approximately 100 mm<sup>3</sup>. (D) BI-505 potency over rituximab is not due to higher epitope expression. The ARH-77 cells were stained with BI-505 (ICAM-1 expression) and rituximab (CD20 expression) and analyzed by flow cytometry. Human n-CoDeR<sup>®</sup> derived IgG<sub>1</sub> was used as a negative control.



## CONCLUSIONS:

Collectively, our results demonstrate proof-of-principle for the function-first approach in the search for new efficient anti-tumor antibodies, and provide a rationale for further clinical evaluation of BI-505 in the treatment of MM. An open label multicenter phase I dose-escalation study with BI-505, approved by the Swedish Medical Product Agency and in accordance with the United States Food and Drug Administration's (FDA) guidance, is currently enrolling relapsed/refractory MM patients in Salt Lake City and Baltimore, United States, and in Lund, Sweden (NCT01025206, <http://clinicaltrials.gov/>). Results from the clinical trial is expected during 2011.