Apoptosis-Inducing ICAM-1 Antibody BI-505 Is a Potent Inhibitor of Multiple Myeloma

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Introduction

BioInvent has recently developed a fully human, high affinity IgG1 antibody (BI-505) specific for ICAM-1. The selected n-CoDeR® derived anti-ICAM-1 antibody designated BI-505 was found to induce apoptosis in a panel of malignant B cell lines of diverse origin including multiple myeloma and B-cell lymphoma, and was found to target an epitope up-regulated on tumor cells compared to normal B-cells. Several studies suggest that ICAM-1 is highly expressed and involved in the pathogenesis of human malignancies including multiple myeloma. Multiple myeloma cells from patients refractory to chemotherapy show increased expression of ICAM-1, and ICAM-1-mediated adhesion of myeloma cells to bone marrow stroma increases survival of myeloma cells and induces primary multidrug resistance. In addition, ICAM-1 and its counter receptor LFA-1 both participate in homing of multiple myeloma cells to the bone marrow. Therefore, we suggest that BI-505 could make a significant contribution to the treatment of the growing and clinically important group of patients with refractory and relapsed myeloma.

Aim

The aim of this study was to examine the efficacy and potency of n-CoDeR® derived human anti-ICAM-1 antibody BI-505 for treatment of multiple myeloma.

Methods and Results

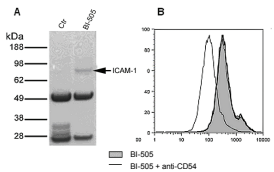

Figure 1. BI-505 shows specific binding to ICAM-1. (A) Raji lymphoma cells were lysed and immuno-precipitated with control antibody (Lane 1) or BI-505 (Lane 2). Antibody-specific bands were excised, trypsin digested, and analyzed by MALDI-TOF. The band in Lane 2 was identified as ICAM-1. (B) Binding of BI-505 to Ramos cells was inhibited by preincubation of cells with an excess of a commercially available anti–ICAM-1 antibody, demonstrating the specificity of BI-505 to ICAM-1.

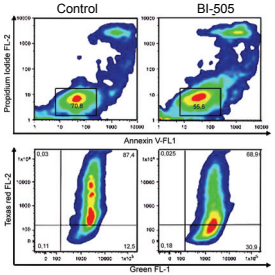

Figure 2. ICAM-1 is a B lymphoma associated cell surface receptor capable of mediating programmed cell death. BI-505 or control IgG_1 was added to CL-01 B lymphoma cells, incubated for 2 hr on ice, followed by addition of cross-linking secondary Fab'2 Goat anti-Human Fc antibody. Cells were incubated at 37°C for 6 hr and the effect of the antibody incubation was determined by two independent apoptosis 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 apoptosis is detected by a decrease in the red (y-axis)/green (x-axis) fluorescence intensity ratio.

Table I. Fully Human BI-505 Antibody Binds And Induces Apoptosis in Various B lymphoma cell lines

Tumor classification	Cell line	MFI[2]	Apoptosis induction[1]
Follicular lymphoma	DOHH-2	100	–
	WSU-NHL	0	–
	SC-1	0	–
	RL	100	–
Mantle cell lymphoma	Granta 519	260	+
	JVM-2	100	–
	Rec-1	380	–
	SP-53	90	–
	NCEB-1	340	+
	Jeko-1	30	+
Burkitt´s lymphoma	Ramos	100	++
	Raji	470	+
	Daudi	150	+
	BJAB	310	+
	CL-01	600	++
pre B cell leukemia	Reh/KM-3	20	–
Multiple myeloma	MC/CAR	120	+

[1] Apoptosis induction - determined as percentage of viable cells after 6-hr incubation with any of the human antibodies, crosslinked with Goat anti-Human (gamma) Fc specific antibody; –, viability not affected; +, 95-80%; ++, 79-60%; +++, 59-40% viable cells compared to control IgG_1. The results are based on duplicate samples from three independent experiments.

[2] MFI (Mean fluorescence intensity) of secondary RPE-conjugated Goat anti-Human IgG antibody. The cell line dependent MFI value of control human IgG antibody was subtracted from the MFI of each human antibody.

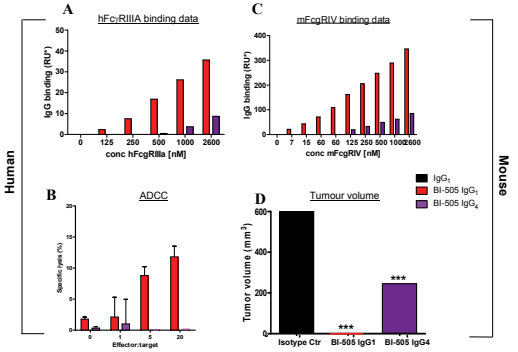

Figure 3. BI-505 has Fc-independent and Fc-dependent anti-tumor activity which correlates with FcγR-binding ability. Binding of BI-505 isotypes to different recombinant FcγRs was determined by Biacore analysis. ADCC was examined by using natural killer cells as effector cells at different ratios, and 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 IgG1 mediated FcγRIIIA-dependent ADCC of tumor cells. (C) BI-505 IgG1 showed strong binding to murine FcγRIV, a principal Fc-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 IgG1 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.

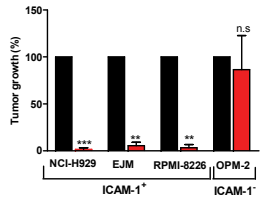

Figure 4. BI-505 has broad *in vivo* anti-myeloma efficacy and potency in the early scid/myeloma xenograft model. NCI-H929, EJM, RPMI-8226 or OPM-2 myeloma cells were injected subcutaneously into the left flank of scid mice at day 0. Antibody treatment with 2 mg/kg BI-505 (red bars) or control IgG1 (black 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.

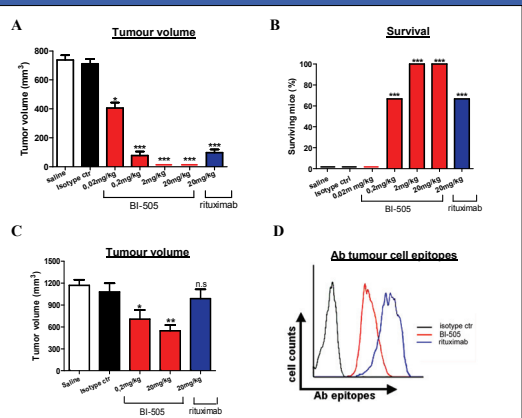

Figure 4. BI-505 demonstrates high efficacy and potency in the early *scid*/ARH-77 myeloma xenograft model. Mice received twice-weekly intraperitoneal (i.p.) injections with BI-505 at different doses. A) and B) Early tumor model: treatment started one day after myeloma cell inoculation and continued 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 100mm³. 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® derived IgG_1 was used as a negative control.

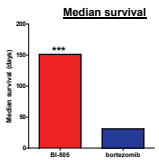

Figure 5. BI-505 confers protection against advanced experimental multiple myeloma. *Scid* mice were irradiated five days before i.v. tumor cell inoculation. Animals received i.p. injections with antibody at 2 mg/kg or bortezomib (Velcade) at 0.5 mg/kg on days 7, 10, 13, and 16). There were 8 mice per treatment group. Figure shows median survival in days, BI-505 treatment compared to bortezomib.

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 (Figure 6A) and plasma cells were counted optically in the bone marrow smear and by flow cytometry (Figure 6B). 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. (Abstract was written with the permission of the patient and with approval from the local ethical committee).

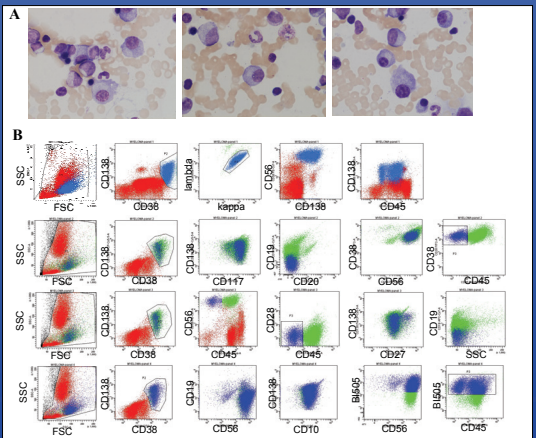

Figure 6A) The figure shows May-Giemsa stained bone marrow smear at 500 or 1000x magnification. Plasma cells were counted to 35-50% of all nucleated cells in different parts of the smear. The photos show typical plasma cells found in the smear. B) Multicolor phenotype-analysis of myeloma cells by flow cytometry. Bone marrow asprates were analyzed using four different panels of antibodies presented as individual rows in the figure. In the first row red dots indicate all cells, green color $CD38^+/CD138^+$ cells and blue color $CD38^+/CD138^+/kappa^+$ cells. In the second row red dots indicate all cells, green color $CD38^+/CD138^+$ cells and blue color a $CD38^+/CD138^+/CD45^-$ subpopulation. In the third row red dots indicate all cells, green color $CD38^+/CD138^+$ cells and blue color a $CD38^+/CD138^+/CD45^-/CD28^-$ subpopulation. In the last row red dots indicate all cells, green color $CD38^+/CD138^+$ and blue color $CD38^+/CD138^+/BI505^+$ cells.
The flow cytometry analysis showed a population of 24% with a myeloma cell phenotype, with high expression of CD138, CD38 and IgG kappa. All myeloma cells stained positive using the BI-505 antibody (80% with a high binding and 20% with intermediate binding).

Conclusion

The *in vivo* anti-myeloma efficacy and potency of the ICAM-1 specific antibody BI-505 was demonstrated in ICAM-1 positive *scid* models assessed as tumor growth inhibition and prolonged mouse survival. BI-505 dose-dependently inhibited tumor growth both in the early and advanced ARH-77/*scid* xenograft models and significantly increased survival in the disseminated experimental myeloma model. Mode-of-action studies demonstrated that *in vivo* anti-tumor activities involve both FcγR-independent and FcγR-dependent mechanisms eg. apoptosis by hyper-cross-linking and ADCC. Staining of myeloma patient bone marrow cells suggest that ICAM-1 is highly expressed in myeloma cells. Therefore, our results indicate that BI-505 could make an important contribution in the treatment of patients with multiple myeloma.

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