Preclinical Characterization of SGN-70, a Humanized Antibody Directed against CD70

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Abstract

Purpose: CD70 (CD27L) is a member of the tumor necrosis factor family aberrantly expressed on a number of hematologic malignancies and some carcinomas. CD70 expression on malignant cells coupled with its highly restricted expression on normal cells makes CD70 an attractive target for monoclonal antibody (mAb)–based therapies. We developed a humanized anti-CD70 antibody, SGN-70, and herein describe the antitumor activities of this mAb.

Experimental Design: CD70 expression on primary tumors was evaluated by immunohistochemical staining of Hodgkin lymphoma, non-Hodgkin lymphoma, multiple myeloma, and renal cell carcinoma tissue microarrays. The CD70-binding and cytotoxic activities of SGN-70 were tested *in vitro* using a number of cell-based assays. The *in vivo* antitumor properties of SGN-70 were tested in severe combined immunodeficient mice bearing disseminated lymphoma and multiple myeloma xenografts. Mechanism-of-action studies were conducted using SGN-70v, a variant mAb with equivalent target-binding activity but impaired Fcγ receptor binding compared with SGN-70.

Results: Immunohistochemical analysis identified CD70 expression on ~40% of multiple myeloma isolates and confirmed CD70 expression on a high percentage of Hodgkin lymphoma Reed-Sternberg cells, non-Hodgkin lymphoma, and renal cell carcinoma tumors. SGN-70 lysed $CD70^+$ tumor cells via Fc-dependent functions, including antibody-dependent cellular cytotoxicity and phagocytosis and complement fixation. *In vivo*, SGN-70 treatment significantly decreased tumor burden and prolonged survival of tumor-bearing mice.

Conclusions: SGN-70 is a novel humanized IgG1 mAb undergoing clinical development for the treatment of $CD70^+$ cancers. SGN-70 possesses Fc-dependent antibody effector functions and mediates antitumor activity *in vivo*.

The advent of antibody engineering technologies has largely overcome the critical barrier of antibody immunogenicity and enabled the development and subsequent Food and Drug Administration approval of therapeutic antibodies for cancer and other diseases (1). To reduce tumor burden in patients, therapeutic antibodies use one or more mechanisms that include growth factor neutralization (bevacizumab) and receptor antagonism (trastuzumab, cetuximab); sensitization of tumor cells toward chemotherapy and radiation therapy (rituximab, trastuzumab, cetuximab); and recruitment of the innate immune system to mediate antibody-dependent cellular cytotoxicity (ADCC), complement fixation, and phagocytosis of tumor cells (rituximab, alemtuzumab, trastuzumab, cetuximab; ref. 1).

To achieve optimal targeting specificity and limit potential side effects, cell surface receptors that show restricted or no expression in normal tissues, including all vital organs, limited ability to be shed from cell surface, and increased or aberrant expression upon cellular transformation are attractive targets for antibody-based therapeutics (2). The tumor necrosis factor family member CD70 (3) possesses all of these characteristics.

Interaction between CD70 and its receptor CD27 contributes to robust immune responses, partly through costimulation of T and B lymphocyte maturation into effector as well as memory cells (4, 5). In normal tissues, CD70 expression is restricted to cells of hematopoietic origin, and it is only transiently induced upon activation of T and B lymphocytes (6, 7), natural killer cells (8, 9), and maturation of dendritic cells (10, 11). As a result, histologic analysis of normal tissues reveals rare CD70-expressing cells only in lymphoid tissues like the thymus, spleen, peripheral lymph nodes, and gut-associated lymphoid tissues (12, 13).

CD70 has been detected on transformed cells of both hematopoietic and epithelial origin, including the Reed-Sternberg cells

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Translational Relevance

Cell surface receptors that are preferentially expressed by cancer cells are sought after as targets for therapeutic antibodies. CD70 is a cell surface receptor whose expression is highly restricted in that it is only transiently induced on activated lymphocytes and mature dendritic cells and it has not been detected in any other normal, nonhematopoietic tissues. Interestingly, aberrant CD70 expression has been reported in both hematologic and solid tumors, including Hodgkin lymphoma, non-Hodgkin lymphoma, multiple myeloma, and renal cell carcinoma. McEarchern and colleagues report the development of SGN-70, a novel humanized IgG that targets CD70. SGN-70 exhibits potent antitumor activities *in vitro* and *in vivo* via Fc-mediated effector functions, including antibody-dependent cellular cytotoxicity, complement fixation, and opsonization. This is the first report to fully characterize and validate the potential of a humanized anti-CD70 as a therapeutic antibody and it also provides the rationale for clinical testing of antibody-based therapeutics targeting CD70.

of Hodgkin lymphoma (HL), a subset of high-grade non-Hodgkin lymphoma (NHL), Waldenström's macroglobulinemia (WM), T-anaplastic large-cell lymphoma, nasopharyngeal carcinoma, thymic carcinoma, glioblastoma, and renal cell carcinoma (RCC; refs. 14–21). Although differentiation arrest and subsequent cellular transformation may underlie sustained CD70 expression on some lymphoid malignancies, little is known about the mechanism(s) responsible for CD70 expression on transformed epithelial cells. Certain types of latent EBV infection involving expression of the viral latent membrane protein 1 may contribute to CD70 induction on nasopharyngeal carcinoma cells (15, 22), but this has not been reported for RCC or thymic carcinoma (16).

Several reports have provided convincing preclinical data in support of the therapeutic potential of anti-CD70 antibodies and anti-CD70 antibody-drug conjugates (20, 21, 23–25). We have previously described a chimeric anti-human CD70 monoclonal antibody (mAb) of IgG1 isotype that exhibits potent *in vitro* and *in vivo* antitumor activities through antibody effector functions, including ADCC, complement-dependent cytotoxicity (CDC), and antibody-dependent cellular phagocytosis (ADCP; ref. 25). In this study, we extended our assessment of CD70 expression to primary tumor isolates, including isolates from multiple myeloma (MM) patients in which CD70 expression has not been previously reported. With the ultimate goal of testing the therapeutic benefit of targeting CD70 in clinical trials, we have generated a humanized anti-CD70 mAb, SGN-70. We report that SGN-70 possesses all the antibody effector functions reported for chimeric anti-CD70 and shows *in vivo* antitumor activity in xenograft models of lymphoma and MM.

Materials and Methods

Cells and reagents. The cell lines used in this study were obtained from the American Type Culture Collection or the DSMZ. The MM.1S and MM.1R cell lines were a kind gift of Dr. Steven Rosen (Northwestern University, Chicago, IL). Cells were maintained in RPMI 1640 (Life Technologies, Inc.) supplemented with 10% fetal bovine serum. CD70 expression was quantified using the murine anti-CD70 antibody clone 1F6 and a Dako QiFi KiT flow cytometric indirect immunofluorescence assay (Dako). Monocyte-derived macrophages were prepared by culturing adherent leukocytes in 500 units/mL recombinant human granulocyte macrophage colony-stimulating factor (PeproTech) for 10 to 15 days as previously described (25). Cells recovered from these cultures were CD3/CD19 negative and expressed CD14, CD11b, CD16, CD32, and CD64 as determined by flow cytometry. Macrophage and T- and B-cell–specific fluorochrome-conjugated antibodies were purchased from BD Biosciences. CD138- and CD70-specific phycoerythrin-conjugated antibodies used to evaluate bone marrow from tumor-bearing mice were purchased from Fitzgerald Industries International and BD Biosciences, respectively. To induce CD70 on human and cynomolgus monkey T cells, peripheral blood mononuclear cells and splenocytes were added to tissues culture plates precoated with anti-human CD3ε (5 μg/mL; BD Biosciences). Murine and rat splenocytes were stimulated with 5 μg/mL species-specific anti-IgM $F(ab')_2$ (Jackson ImmunoResearch Laboratories, Inc.) and anti-CD40 (clone HM40-3, BD Biosciences). Anti-human CD2- and species-specific anti-B220 fluorochrome–conjugated antibodies were obtained from BD Biosciences. Anti-mouse CD70 (clone FR70) and recombinant human CD27-Fc were purchased from eBioscience and R&D Systems, respectively. Flow cytometric data were acquired with FACScan or LSRII flow cytometers (BD Biosciences) and analyzed with CellQuest or FACSDiVa software (BD Biosciences).

Immunohistochemical staining for CD70 and CD27. Formalin-fixed paraffin-embedded tissue microarrays were obtained from commercial sources (USBiomax, Tristar, and Petagen/Abxis). Slides were deparaffinized and processed for antigen retrieval using BondmaX system (Leica Biosystems). Nine MM samples were frozen, fixed in acetone for 10 min, and stained with no antigen retrieval. A murine anti-human CD70 mAb (SG-21.1C1) suitable for detection of CD70 in fixed tissues was generated and used as primary antibody. SG-21.1C1 binding was detected with the Bond Polymer Alkaline Phosphatase (AP) Red Detection kit (Leica Biosystems). For CD27 staining, EDTA-based antigen retrieval was done. A biotinylated goat anti-human CD27 antibody (R&D Systems) was used at 5 μg/mL and detected using Bond Intense R Detection kit (Leica Biosystems) with 3,3′-diaminobenzidine as substrate. Tissues were counterstained with hematoxylin. Slides were scored using a qualitative scoring scale as described in the figure legend. Images were acquired using a Zeiss Axiovert 200M microscope (Carl Zeiss Microimaging).

Generation of the humanized anti-CD70 antibody SGN-70. The complementarity-determining regions of the murine anti-CD70 clone 1F6 (25) were used to generate the humanized anti-CD70 antibody SGN-70. First, the complementarity-determining regions, as defined by Kabat et al. (26), of 1F6 V_L were grafted on to the framework regions of human germline exons B3 and Jκ1 obtained from V BASE[1] fused to the human κ constant domain. Likewise, the complementarity-determining regions of 1F6 V_H were grafted onto the framework regions of human germline exons V_H1-2 and J_H6 (V BASE) fused to the human IgG1 constant domains. In addition, a framework mutation, E46K (numbering scheme of Kabat et al.; ref. 26), was introduced into the humanized V_H domain to enhance antigen-binding activity. The resultant humanized anti-CD70 antibody, SGN-70, showed comparable antigen-binding activity to c1F6 in competition-binding assays with CD70-positive 786-O cells. A variant mAb, SGN-70v, impaired for Fcγ receptor (FcγR) binding was created by installing three previously described mutations into the heavy chain—E233P:L234V:L235A (27, 28). Recombinant SGN-70 and SGN-70v antibodies were expressed in CHO-DG44 (29) cell lines and purified by protein A chromatography.

Binding assays. To compare apparent antigen-binding affinities of chimeric anti-CD70 mAb (c1F6) and SGN-70, the mAbs were directly

[1] http://vbase.mrc-cpe.cam.ac.uk

labeled with europium according to the manufacturer's instructions (Perkin-Elmer), then applied in increasing concentration to $CD70^+$ 786-O cells. Following 1 to 2 h incubation on ice, the labeled cells were washed, resuspended in DELFIA enhancement solution (Perkin-Elmer), and fluorescence detected using a Fusion HT microplate reader (Perkin-Elmer) with an excitation wavelength of 335 nm and emission wavelength of 620 nm. To measure CD70 binding of SGN-70 and SGN-70v, $CD70^+$ WIL2-S cells were treated with increasing concentrations of mAb for 30 min on ice, washed, and detected by labeling with a FITC-conjugated goat $F(ab')_2$ anti-human IgG (Jackson ImmunoResearch Laboratories, Inc.). Fluorescence was measured using a FACScan flow cytometer. The relative ability of SGN-70 and SGN-70v to bind FcγR was assessed in a competition-binding assay using a CHO cell line transfected to express FcγRIIIA (CHO-CD16A). CHO-CD16A cells were treated with a mixture of 50 nmol/L AF488-conjugated SGN-70 and increasing concentrations of unlabeled SGN-70 or SGN-70v for 30 min on ice. Excess antibody was removed with washing and fluorescence was determined by flow cytometry.

In vitro *activity assays*. ADCC, CDC, and ADCP assays were done as previously described (25). For ADCC, $Na_2[^{51}Cr]O_4$-labeled 786-O target tumor cells were mixed with mAb (1 μg/mL) and $CD16^+$ effector cells from a normal donor at an effector-to-target cell ratio of 10:1. After 4-h incubation, the radioactivity released into the culture supernatant was measured and the percent specific cell lysis was calculated as (test sample cpm - spontaneous cpm) / (total cpm - spontaneous cpm) × 100. Spontaneous and total cpm values were determined from the supernatants of target cells incubated in medium alone and from target cells lysed with 1% Triton X-100, respectively. For CDC, $CD70^+$ MHH-PREB-1 target cells were treated with mAb (1.85 μg/mL) in an assay medium containing 10% normal human serum as a source of complement. After 2-h incubation, propidium iodide was added and the percent nonviable cells were identified by flow cytometry. To assess ADCP, $CD70^+$ MHH-PREB-1 tumor cells were labeled with the red fluorescent membrane dye PKH26 (Sigma), treated with mAb (2 μg/mL) for 30 min on ice, then mixed with monocyte-derived macrophages for 1 h at 37°C. The cell mixture was stained with Alexa Fluor 488–conjugated murine anti-CD11b, fixed with 1% paraformaldehyde, and analyzed by flow cytometry to detect $PKH26^+CD11b^+$ double-labeled fluorescent cells. Phagocytic activity was calculated as (percent $PKH26^+CD11b^+$) / (percent $CD11b^+$ cells) × 100. Identification of double-fluorescent events as phagocytosed tumor cells rather than macrophage-tumor cell pairs was previously confirmed by fluorescence microscopy (25). To determine the ability of SGN-70 to affect cell viability, 10^4 $CD70^+$ target cells in 0.2 mL of complete growth medium were treated with varying concentrations of SGN-70 or nonbinding control IgG in the presence or absence of a 4-fold excess of cross-linking $F(ab')_2$ goat anti-human IgG secondary antibody (Jackson ImmunoResearch Laboratories, Inc). The cytotoxic agent camptothecin was included as a positive control for cell killing. After 96-h incubation at 37°C, 5% CO_2, cell viability was assessed using the CellTiter-Glo Luminescent Cell Viability Assay (Promega) according to the manufacturers' instructions.

Xenograft models of lymphoma and MM. $CD70^+$ L-363 and MM.1S and Raji cell lines were used to establish disseminated plasma cell malignancy and lymphoma xenografts, respectively. To establish disseminated disease, 1×10^6 Raji or 1×10^7 L-363 or MM.1S cells in 0.2 mL PBS were injected into the lateral tail vein of C.B.-17 severe combined immunodeficient (SCID) mice (Harlan). Left untreated, >90% of engrafted mice required euthanization as a result of disease within 20 to 30 d (Raji) or 45 to 65 d (L363, MM.1S). Mice were monitored at least twice per week and were terminated when they exhibited signs of disease, including weight loss of 15% to 20%, hunched posture and lack of grooming, cranial swelling and hind limb paralysis, or, in the case of L-363–bearing mice, when the estimated size of palpable, disseminated lymphoid-tissue associated tumors approached 1,000 mm^3. Treatment schedules are indicated in figure legends. Statistical analyses of animal studies were conducted using the log-rank test provided in the Graphpad Prism Software version 4.01 (Graphpad). All animal experiments were conducted under Seattle Genetics' Institutional Animal Care and Use Committee guidelines and approval.

To evaluate tumor cell infiltration in the MM.1S model, representative mice exhibiting symptoms of disease were terminated. The brain, vertebrae, and long bones were collected and fixed in 10% neutral buffered formalin for at least 24 h. The vertebrae and long bones were decalcified over a period of 3 wk with weekly exchange of decalcification solution consisting of 14% EDTA in Tris-HCl (pH 7.0). Samples were paraffin embedded, sectioned, and stained with H&E at PhenoPath Laboratories. For fluorescence-activated cell sorting analysis of myeloma cell infiltrates, the tibiae and fibulae of mice were recovered and the marrow was flushed from the bones with PBS using a 30-gauge needle. The marrow was stained with fluorochrome-conjugated anti-mouse CD45 and anti-human CD70 and CD138 antibodies to identify MM.1S cells.

Results

CD70 expression on malignant cells. We have previously reported CD70 expression on tumor cell lines representative of HL, NHL, MM, and RCC, as determined by flow cytometry (25). Here, we have used immunohistochemistry to evaluate CD70 expression on patient tumor isolates. Consistent with our cell line data and with published findings on primary tumors (14, 18–20, 30, 31), examination of tumor tissue microarrays revealed a high percentage and intensity of CD70 expression in 97% of HL, 60% of NHL, and 70% of RCC samples (Fig. 1). In addition, we found 13 of 31 MM cases to be $CD70^+$ (Fig. 1), also consistent with our earlier observation that CD70 is expressed by MM cell lines. Figure 1B and Table 1 represent the mean staining intensity and the percent area of tumor that was positive for CD70, respectively. In HL, the Reed-Sternberg cells were uniformly $CD70^+$ with occasional CD70 expression identified on nonmalignant infiltrating cells. Although more heterogeneous in CD70 expression, most $CD70^+$ NHL and RCC cancers contained ≥50% antigen-positive cells. CD70 staining was predominantly membranous and cytoplasmic in nature, with some punctate perinuclear aggregates noted (Fig. 1C).

In addition to CD70 expression on primary tumor isolates, assessment of an expanded panel of tumor cell lines revealed that 64% of plasma cell malignancies, 78% HL, 75% of NHL, and 44% T-lineage lymphoma and leukemia cell lines were $CD70^+$. Receptor copy numbers were determined by quantitative flow cytometry and ranged from ~2,000 to >200,000 copies per cell (Supplementary Table S1). RCC cell lines were also highly positive for CD70 as we have previously described (88%; ref. 20). Cell lines that expressed CD70 were uniformly positive for this antigen.

Because CD70 and CD27 are reported to be coexpressed in some cases of B-cell lymphoma and leukemia (31–34), we also evaluated hematologic primary tumors and tumor cell lines for CD27 expression. CD27 was detected on infiltrating leukocytes, but not Reed-Sternberg cells, in the majority of HL primary tumor samples (Supplementary Table S2). A representative example of $CD27^+$ infiltrating leukocytes in a HL patient isolate is shown in Supplementary Fig. S1. CD27 was rarely expressed on HL, MM, and T lineage lymphoma and leukemia cell lines but was present on 11 of 15 $CD70^+$ NHL cell lines examined (Supplementary Table S1). Comparative staining of side-by-side MM and NHL tumor microarray sections revealed the presence of $CD70^+$ and $CD27^+$ cells in a subset of cases

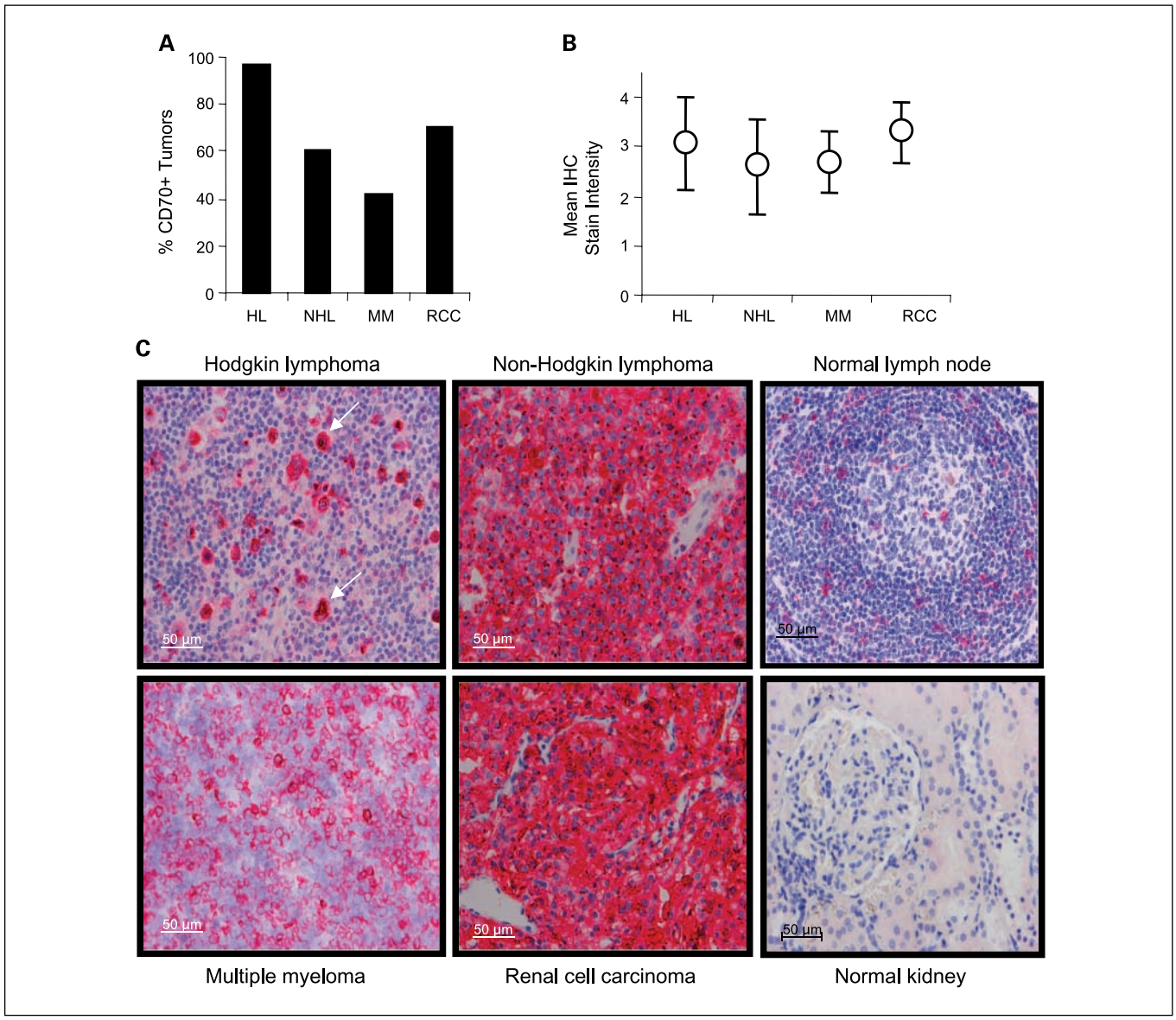

Fig. 1. Immunohistochemical analysis of CD70 expression on primary human tumors. Formalin-fixed paraffin-embedded tissue microarrays containing HL (n = 24), NHL (n = 119), MM (n = 22), and RCC (n = 20) tissues and an additional nine individual frozen MM cases were subjected to immunohistochemical analysis of CD70 expression. CD70 was detected with a murine anti-human CD70 mAb (SG-21.1C1) and visualized with a polymer alkaline-phosphatase detection system using Fast Red as a chromogen. *A,* frequency of CD70 expression in tumors examined. *B,* mean staining intensity of $CD70^+$ tumors. Intensity was scored as 1 = minimal, 2 = mild, 3 = moderate, 4 = strong. Bars, SD. *C,* representative examples of CD70 expression on different tumor types. Red stain, CD70 expression in both membrane and cytoplasm of tumor cells. In HL, Reed-Sternberg cells (*arrows*) are $CD70^+$. Tissues were counterstained using hematoxylin (*blue*). Scale bars, 50 μm.

(Supplementary Table S2). The relative scarcity and morphologic characteristics of the $CD27^+$ cells were consistent with infiltrating mature lymphocytes.

In contrast to constitutive expression on malignant cells, CD70 was not present on the majority of normal tissues (Supplementary Table S3). In accordance with known expression of CD70 on activated lymphocytes, CD70 was detected on scattered cells within lymphoid tissues (Fig. 1C; Supplementary Table S3).

In vitro *characterization of SGN-70 and SGN-70v.* To develop a CD70-targeting mAb suitable for clinical testing, and to minimize the risk for immunogenicity in humans, the murine anti-CD70 antibody 1F6 was humanized. The affinity of the humanized mAb, SGN-70, for CD70 was within 2-fold that of the chimeric version of the antibody (c1F6). Apparent K_d values were determined based on half-maximal binding in a cell-based saturation assay and were 0.97 and 0.55 nmol/L for SGN-70 and c1F6, respectively (Fig. 2A). As with c1F6 (25), SGN-70 lysed $CD70^+$ tumors via Fc-dependent mechanisms, including ADCC, CDC, and ADCP assays (Fig. 2C). To evaluate the importance of Fc-mediated functions to the activity of SGN-70 *in vivo*, we engineered a variant mAb, SGN-70v, with equal capacity to bind CD70 but impaired in its abilities to bind FcγR and mediate effector functions compared with SGN-70 (Fig. 2B-C). When added to tumor cell targets in the absence of effector cells or complement, SGN-70 did not induce tumor cell death either alone or when cross-linked with secondary antibody, whereas the cells were sensitive to killing mediated by

Table 1. Homogeneity of CD70 expression in primary tumor tissues

	Area of tumor CD70 positive*			
	Percent			
Cancer type	**1-25**	**26-50**	**51-75**	**76-100**
Hodgkin lymphoma †				33/33 (100)
Non-Hodgkin lymphoma	7/72 (10)	16/72 (22)	14/72 (17)	37/72 (51)
Multiple myeloma	4/13 (31)	2/13 (15)	4/13 (31)	3/13 (23)
Renal cell carcinoma	0/14 (0)	1/14 (7)	4/14 (29)	9/14 (64)

*Number of samples in percentile/total number of samples (percentage).
†Reed-Sternberg cells.

the positive control cytotoxic agent camptothecin (Fig. 2D; Supplementary Fig. S2). Because reverse signaling through CD70 has been reported (31, 35, 36), we also evaluated the effect of SGN-70 on phosphatidylinositol 3-kinase, mitogen-activated protein/extracellular signal-regulated kinase kinase, and nuclear factor-κB signaling pathways using lymphoma and MM cell lines. SGN-70 did not induce AKT and extracellular signal-regulated kinase phosphorylation or IκB degradation (Supplementary Fig. S3).

In vivo *activity of SGN-70.* The ability of SGN-70 to mediate tumor regression *in vivo* was examined in a disseminated lymphoma model initiated with CD70$^+$ Raji cells. The median survival of mice inoculated with Raji cells was ~20 days (Fig. 3, *left*). Whereas treatment with irrelevant control IgG did not prolong survival, 50% of mice were still alive at study end (103 days) when treated with SGN-70 on a multidose schedule of 10 mg/kg initiated 1 day after tumor cell implant. Survival of Raji-bearing mice was also prolonged (median = 59 days) when

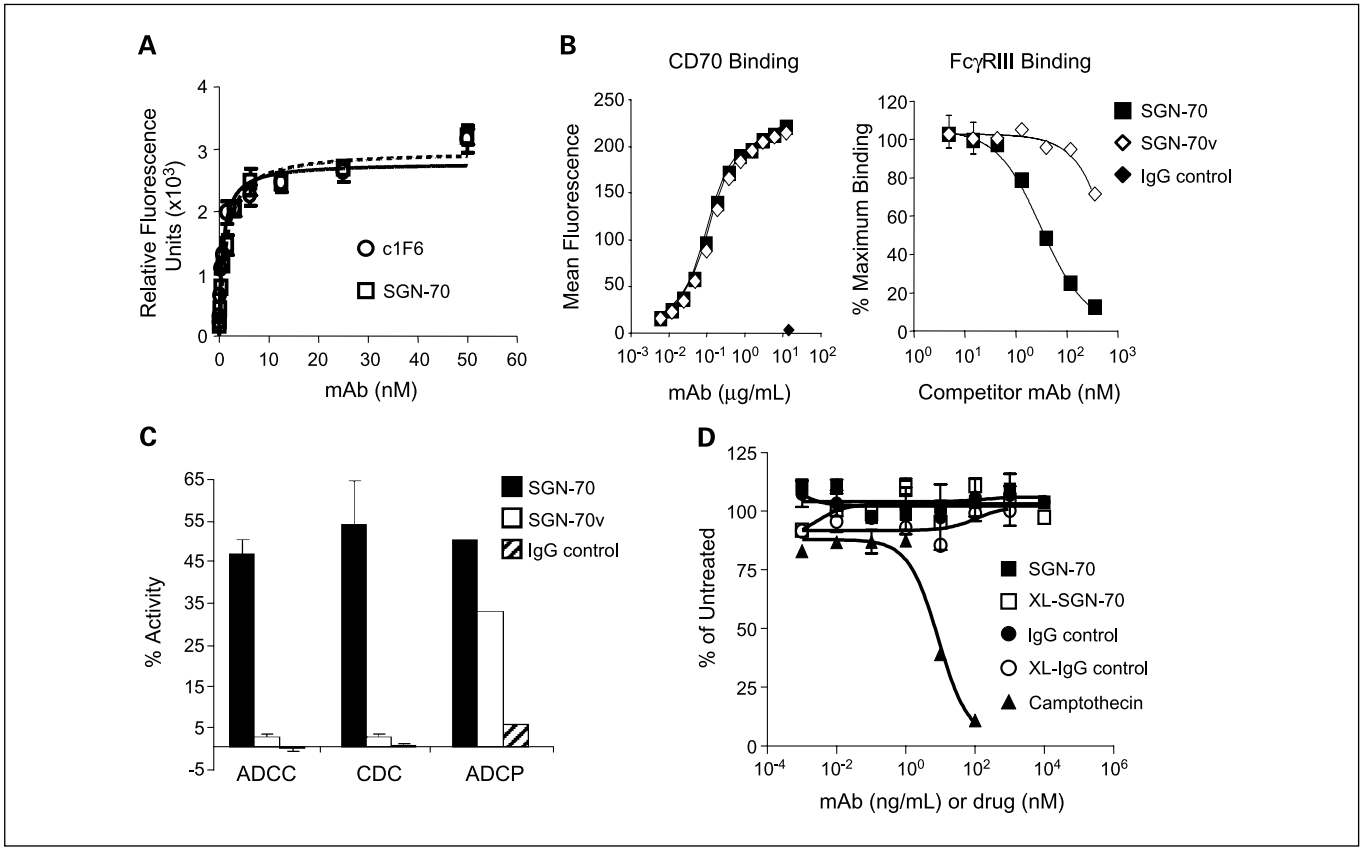

Fig. 2. Binding and functional activity of SGN-70 and SGN-70v. *A,* saturation binding comparison of chimeric 1F6 and SGN-70. CD70$^+$ 786-O tumor cells were mixed with varying concentrations of europium-labeled SGN-70 or c1F6 and the relative fluorescence was measured. *B,* binding of SGN-70 and SGN-70v to CD70$^+$ WIL2-S cells was detected with a fluorochrome-conjugated anti-human IgG antibody by flow cytometry. The relative ability of SGN-70 and SGN-70v to bind FcγRIIIA was determined in a competition-binding assay where CHO-CD16A cells were labeled with a mixture of fluorochrome-conjugated SGN-70 and increasing amounts of unlabeled SGN-70 or SGN-70v. *C,* SGN-70 and SGN-70v were tested for their ability to induce tumor cell lysis via ADCC, CDC, and phagocytosis as described in Materials and Methods. The CD70$^+$ target cells for ADCC and CDC, and ADCP were 786-O and MHH-PREB-1, respectively. *D,* CD70$^+$ L-363 cells were treated with SGN-70 or control IgG in the presence or absence of a 4-fold excess of a cross-linking secondary antibody (XL-SGN-70). Camptothecin was included as a positive control for cell killing. Cell viability was assessed after 96-h incubation. Bars, SD of triplicate samples.

a lower dose of 3 mg/kg SGN-70 was administered using the same schedule. We assessed the contribution of Fc-FcγR interactions to SGN-70 activity *in vivo* by treating Raji tumor-bearing mice with SGN-70v, the variant mAb with limited ability to bind FcγR and mediate effector functions (Fig. 3, *right*). Unlike mice whose survival was extended by treatment with SGN-70, treatment with SGN-70v provided no benefit (median survival of 25 days), confirming the functional importance of the Fc region of SGN-70.

The *in vivo* activity of SGN-70 was further tested in two plasma cell malignancy models established with $CD70^+$ L-363 and MM.1S lines. The MM.1S (37) and L-363 (38) cell lines were established from the peripheral blood of patients with MM and plasma cell leukemia, respectively. They are EBV negative, express phenotypic markers characteristic of plasma cell malignancies ($CD19^-$, $CD20^-$, $CD38^+$, $CD138^+$), and secrete monoclonal protein (λ light chain) detectable *in vitro* and in the serum and urine of tumor-bearing mice. Lambda light chain levels were a useful measure of tumor burden and could be readily distinguished from circulating SGN-70 (IgG1κ). When injected i.v. into SCID mice, L-363 establishes lymph node–associated masses, whereas MM.1S homes to bone marrow and invades the central nervous system (39). Histopathologic examination of skeletal tissues recovered from terminal MM.1S-bearing mice reflected generalized disease, with neoplastic cells filling intramedullary bone spaces, effacing normal hematopoietic cell populations, and invading adjacent extramedullary tissues (Fig. 4A, *left*). $CD70^+CD138^+$ tumor cell infiltration was also shown by flow cytometric analysis of marrow flushed from the long bones of MM.1S-bearing mice (Fig. 4A, *right*).

The median survival of untreated L-363- and MM.1S-bearing mice was between 45 days (MM.1S) and 55 days (L-363; Fig. 4B). Treatment with 10 mg/kg SGN-70 significantly increased the median survival of L-363- and MM.1S-bearing mice to 91 and 60 days, respectively. The effect of SGN-70 on tumor burden in the MM.1S model was also determined by measuring the percent myeloma cells in the bone marrow (Fig. 4C, *left*) and the λ light chain levels in the sera of individual mice (Fig. 4C, *right*). Fifty days after tumor cell implant, the percentage of myeloma cells in the bone marrow and the amount of circulating tumor-derived monoclonal protein were significantly lower in mice treated with SGN-70 compared with untreated mice or mice treated with nonbinding IgG control antibody. A reduction in tumor burden was not observed when mice were treated with the impaired Fc variant mAb SGN-70v (Fig. 4D).

Species cross-reactivity. Before the initiation of clinical trials, it is important to assess the safety of the intended drug in a relevant nonhuman host. To identify an appropriate species for toxicology studies, the ability of SGN-70 to bind rodent and nonhuman primate CD70 was assessed. Because CD70 is not found on normal tissues or on resting lymphocytes, SGN-70 binding was measured on lymphocytes from mouse, rat, and cynomolgus monkeys that were activated to induce CD70 expression. SGN-70 bound to CD70 on cynomolgus monkey T cells in a pattern similar to that observed with human activated T lymphocytes (Fig. 5B). SGN-70 did not bind rodent CD70 (Fig. 5A). SGN-70 cross-reactivity with nonhuman primate CD70 was confirmed in a cell-binding assay with 293 cells transfected with CD70 cDNA isolated from activated cynomolgus monkey splenocytes (Fig. 5B). Half-maximal binding of SGN-70 to these cells was within 4-fold of that achieved with a human $CD70^+$ cell line with similar antigen density (293-cynoCD70 9.6 nmol/L; RCC line A498, 2.8 nmol/L).

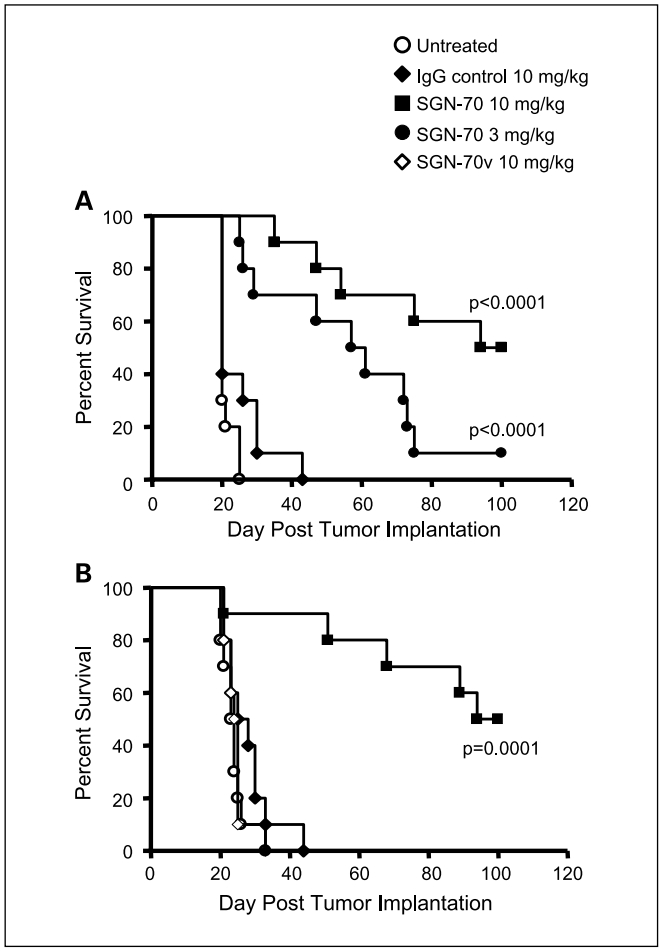

Fig. 3. Kaplan-Meier plot showing the antitumor effect of SGN-70 and SGN-70v in a disseminated lymphoma xenograft model. *A,* SCID mice were injected i.v. with 10^6 Raji B lymphoma cells then treated 1 d later with SGN-70 or control immunoglobulin at the doses indicated. Mice were treated weekly thereafter and received a total of four doses. *B,* Raji-tumor bearing mice treated with SGN-70, nonbinding control IgG, or SGN-70v. Mice received six doses of mAb once every 4 d starting 1 d after tumor injection. *P* values given are between SGN-70 – treated groups and control IgG – treated groups. (n = 10 mice/group).

Discussion

The promise of treating hematologic malignancies with mAbs that target cell surface antigens has been partly realized with the success of rituximab in treating NHL (40). Despite these advances, >18,000 people die from this disease yearly in the United States alone. For patients that are resistant to rituximab treatment and those with malignancies that do not express the rituximab target CD20, including MM, HL, and various T lymphomas, alternative treatments are needed. CD70 is an attractive target for mAb-based therapy because it is expressed on a number of malignancies but is absent on most normal tissues. With normal expression restricted to scattered cells in the lymphoid compartment, one might anticipate therapies targeting this antigen to have minimal side effects.

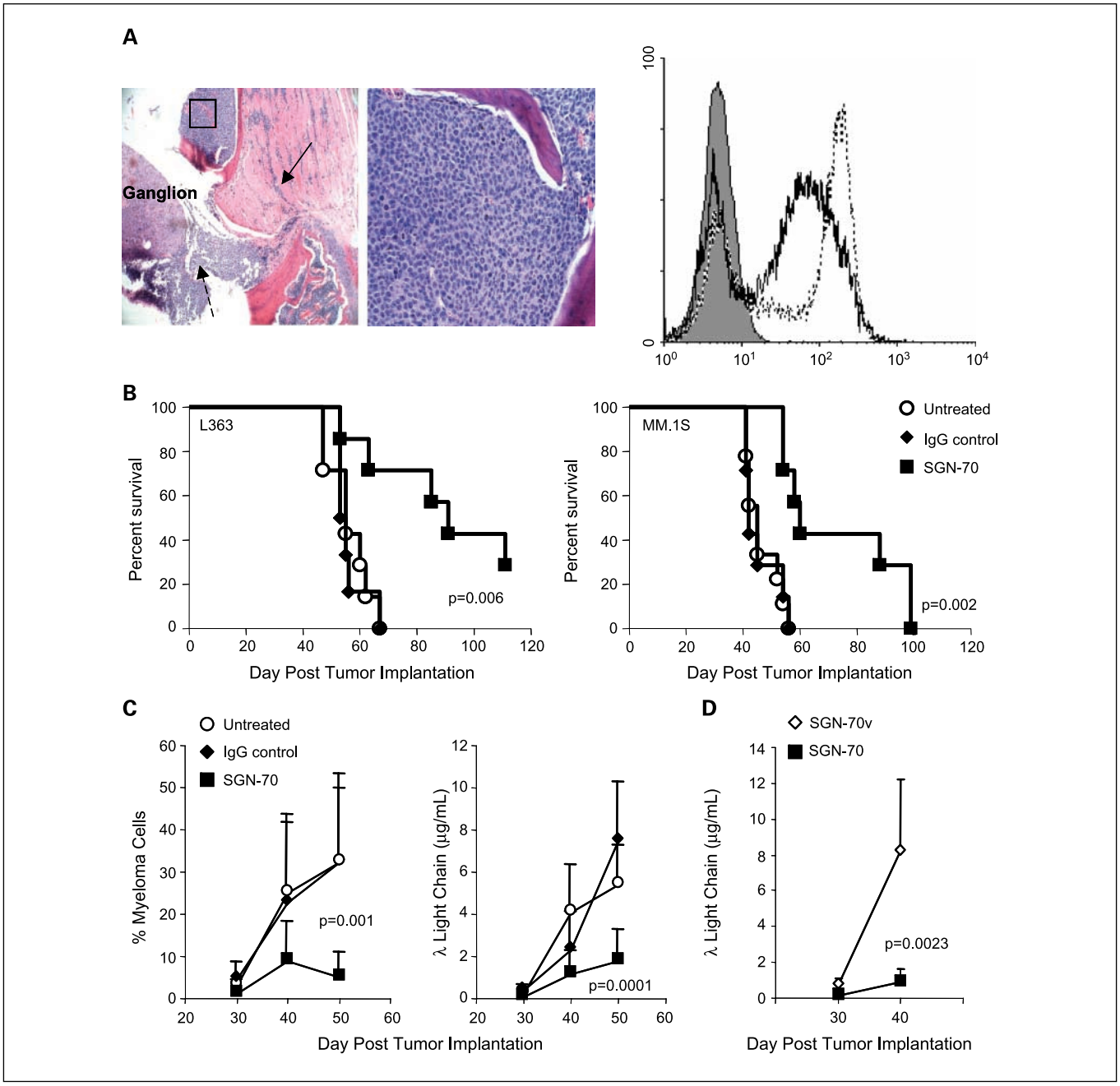

Fig. 4. Activity of SGN-70 in SCID mice bearing plasma cell malignancies. *A,* infiltration of bone marrow with MM.1S multiple myeloma cells. Left, H&E–stained section of the cervical vertebrae of a mouse with disseminated disease. Neoplastic round cell population fills and effaces bone marrow (*box, ×400 figure*) and invades the surrounding skeletal muscle (*arrow*) and adjacent ganglion (*dashed arrow*). Right, flow cytometric staining for CD70 (*solid line*) and CD138 (*dashed line*) on cells recovered from the marrow of MM.1S-bearing mice. Shaded histogram, isotype control. Small cells were excluded by forward scatter versus side scatter gating. *B,* survival curves of SCID mice bearing disseminated L-363 (*left*) or MM.1S (*right*) tumors. Mice injected i.v. with 10^7 tumor cells were untreated or given 5 weekly doses of SGN-70 or nonbinding control immunoglobulin starting 1 d (L-363) or 3 d (MM.1S) after tumor injection (n = 6-9 mice/group). *C,* MM.1S-bearing mice were untreated or treated with 5 weekly doses of nonbinding control immunoglobulin or SGN-70 starting 3 d after tumor injection. Tumor burden was assessed by the presence of $CD138^+$ human myeloma cells in the bone marrow (determined by flow cytometry; *left*) and λ light chain protein in serum (determined by ELISA; *right*); n = 6-14 mice per group. *D,* serum λ light chain protein levels in MM.1S-bearing mice treated with SGN-70 or SGN-70v as described in *C* (n = 6-7 mice per group). *P* values given are between SGN-70–treated groups and untreated (*B*), control IgG-treated (*C*), or SGN-70v (*D*) groups.

The favorable expression profile of this target prompted us to develop a humanized anti-CD70 antibody, SGN-70, suitable for clinical application. In this report, we describe the *in vitro* and *in vivo* antitumor properties of SGN-70 and expand our assessment of CD70 expression to primary tumor samples, including isolates from MM patients. In addition to confirming expression of CD70 on HL Reed-Sternberg cells, NHL, and RCC, we identified CD70 on ~42% of MM isolates tested, verifying by immunohistochemistry in a larger number of patient samples an observation we first made by flow cytometric analysis of MM cell lines and a small sampling of primary MM isolates (25, 41). These data, combined with our

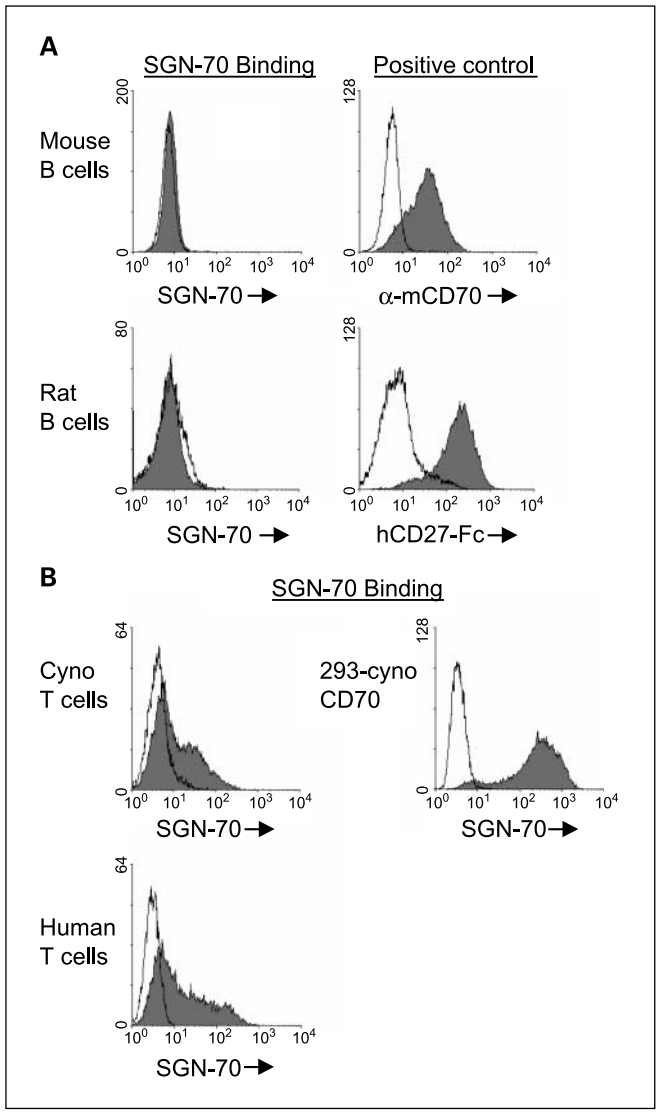

Fig. 5. SGN-70 does not bind mouse or rat CD70 but binds nonhuman primate CD70. *A,* murine and rat splenocytes isolated from immunocompetent animals were treated with anti-IgM + anti-CD40 to activate B cells and induce CD70 expression. To evaluate B cells, activated splenocytes were stained with species specific anti-B220 antibodies and histograms gated on $B220^+$ cells. Left, cells were stained with AF488-conjugated SGN-70. Right, murine cells were stained with biotinylated anti-mouse CD70 (clone FR70) and detected with AF488-conjugated avidin; rat CD70 was detected using a recombinant human CD27-Fc fusion protein followed by a fluorochrome-conjugated anti-Fc reagent. *B,* left, cynomolgus splenocytes and human peripheral blood mononuclear cells were stimulated with anti-CD3 for 5 d to induce CD70 expression. Cells were stained with anti-CD2 and histograms gated on $CD2^+$ cells. Right, 293 cells were transfected with CD70 cDNA isolated from cynomolgus monkey peripheral blood mononuclear cells activated to express CD70. Cells in *B* were stained with biotinylated SGN-70 and detected with AF488-conjugated avidin. *A* and *B,* CD70 (*filled histograms*) and isotype control (*open histograms*).

observation that SGN-70 prolongs survival and reduces tumor burden in NHL (Fig. 3), MM (Fig. 4), and HL (not shown) xenograft models, provide the rationale for clinical testing of SGN-70 in these indications.

Little is known about the induction of CD70 expression on cancer cells. CD70 has not been identified as a developmental or cell lineage marker but is best characterized as a cell surface protein expressed upon lymphocyte activation. Latent EBV infection is associated with the development of Burkitt's lymphoma, HL, and nasopharyngeal carcinoma (42), and could potentially contribute to CD70 expression in these cancers; however, no significant differences in CD70 expression have been found between latent membrane protein positive and negative HL cases (43). Furthermore, viral infection is unlikely to account for CD70 expression of MM, RCC, and thymic carcinoma, which are largely EBV negative (16). Chronic overexpression of CD70 by T cells in drug-induced lupus and lupus of unknown origin has been linked to hypomethylation of DNA sequences that flank the CD70 promoter (44). To our knowledge, the methylation status of the CD70 promoter in CD70-positive malignant cells has not been examined. It would be interesting to measure this and to determine if microenvironmental conditions, like hypoxia, that exist within solid tumors modulate promoter methylation and CD70 expression. If so, DNA-hypomethylating agents such as decitabine or 5-aza-deoxycytibine could up-regulate CD70 expression on transformed cells or induce more homogeneous expression throughout the tumor mass.

Our *in vitro* data show that SGN-70 facilitates elimination of $CD70^+$ tumor cells via Fc-dependent antibody effector functions that include ADCC and phagocytosis and complement fixation. *In vivo*, the inability of the Fc-modified antibody variant SGN-70v to promote survival in the tumor xenograft models tested provides convincing evidence that Fc–effector cell interactions are important to the activity of SGN-70. Because SCID mice retain natural killer cells, macrophages, and neutrophils that are capable of binding human IgG1, ADCC and phagocytosis are both likely to play a role in the activity of SGN-70 *in vivo*. We consider it unlikely that complement-dependent cell lysis contributed to tumor elimination in the xenograft models reported here, because the Raji and MM.1S tumor cells used in our studies are resistant to SGN-70–mediated CDC, possibly due to expression of complement regulatory proteins like CD59 (ref. 25; data not shown). Further, in cell lines that are efficiently lysed by SGN-70 in human serum, we have found murine complement to have little effect, perhaps due to decreased affinity of murine C1q for human IgG1. Although complement-mediated cytolysis of targeted cells did not occur, complement fixation and deposition of C3b could still contribute to antitumor activity by enhancing phagocytosis. The expression of complement regulatory proteins by human tumors may restrict SGN-70–mediated CDC in clinical application, as is true with other complement-fixing antitumor antibodies. A number of strategies designed to overcome the inhibitory effects of complement regulatory proteins are being tested preclinically (45, 46).

In addition to facilitating Fc-mediated effector functions, SGN-70 blocks CD70 binding to CD27 (Supplemental Fig. S4). Blockade of CD70-CD27 interactions between malignant cells and immune cells within the tumor microenvironment may be of clinical importance. In this study, we found coexpression of CD27 and CD70 on a subset of NHL cell lines derived from Burkitt's lymphoma, follicular lymphoma, and mantle cell lymphoma (Supplementary Table S1), confirming and extending previously published reports (31–34, 47). Although we have not observed reduced proliferation of $CD27^+CD70^+$ Raji lymphoma cells cultured in the presence of SGN-70 (Supplementary Fig. S2), our finding of CD70-CD27 coexpression is consistent with the potential of autocrine CD70-induced proliferation or survival of transformed lymphoma

cells via CD27 signaling. Apart from malignant cells, $CD27^+$ mononuclear infiltrates were present in HL, MM, and NHL primary patient isolates (Supplementary Table S2; Supplementary Fig. S1). Given the role of CD27 in lymphocyte costimulation, it is possible that interactions between $CD70^+$ tumor cells and $CD27^+$ infiltrating lymphocytes could provide a survival and/or proliferative advantage tumor cells through cytokine elaboration by $CD27^+$ lymphocytes. Treon and colleagues recently provided evidence for a CD70-mediated paracrine stimulatory loop in WM (48, 49). Mast cells isolated from WM tumors express CD70 and respond to WM-derived sCD27 by up-regulating the tumor necrosis factor ligands CD40L and APRIL (21, 49). These ligands in turn support the growth and survival of lymphoplasmacytic cells (48). SGN-70 prevented up-regulation of CD40L and APRIL on mast cells and blocked disease progression in SCID-hu mice bearing established WM (49). On the other hand, CD70 expression on malignant cells may contribute to immune suppression within the tumor microenvironment by inducing T cells with regulatory potential. In biopsy specimens from NHL patients, Yang et al. observed that a subset of tumor-infiltrating $CD4^+CD25^-$ cells expressed Foxp3 and had suppressive activity (50). They found that $CD70^+$ lymphoma B cells augmented activation-induced Foxp3 expression in these lymphocytes and that anti–CD70-mediated blockade abrogated this response (50). Thus, SGN-70 may act not only to deplete $CD70^+$ malignant cells but could also potentially block growth signals and/or impede acquisition of T-cell immune regulatory function within the tumor microenvironment.

In summary, we have generated a humanized anti-CD70 mAb, SGN-70, with antitumor activity *in vitro* and *in vivo*. To enable preclinical pharmacokinetic and toxicology studies of SGN-70, we have shown the binding of SGN-70 to cynomolgus CD70 (Fig. 5) and determined that the expression profile of CD70 in cynomolgus monkeys is remarkably similar to that of humans (data not shown). Pharmacokinetic and toxicology assessments are under way and should pave the way for clinical evaluation of SGN-70 in CD70-expressing cancers, either as monotherapy or in combination with standard therapeutic regimens.

Disclosure of Potential Conflicts of Interest

Most of the authors are employed by and have an ownership interest in Seattle Genetics. C.F. McDonagh and P.J. Carter were employed by Seattle Genetics at the time the work was conducted.

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