

Preclinical Development of a Bispecific Antibody that Safely and Effectively Targets CD19 and CD47 for the Treatment of B-Cell Lymphoma and Leukemia



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Abstract

CD47, an ubiquitously expressed innate immune checkpoint receptor that serves as a universal "don't eat me" signal of phagocytosis, is often upregulated by hematologic and solid cancers to evade immune surveillance. Development of CD47-targeted modalities is hindered by the ubiquitous expression of the target, often leading to rapid drug elimination and hemotoxicity including anemia. To overcome such liabilities, we have developed a fully human bispecific antibody, NI-1701, designed to coengage CD47 and CD19 selectively on B cells. NI-1701 demonstrates favorable elimination kinetics with no deleterious effects seen on hematologic parameters following single or multiple administrations to nonhuman primates. Potent *in vitro* and *in vivo* activity is induced by NI-1701 to kill cancer cells across a plethora of B-cell malignancies and control tumor growth in xenograft mouse models. The mechanism

affording maximal tumor growth inhibition by NI-1701 is dependent on the coengagement of CD47/CD19 on B cells inducing potent antibody-dependent cellular phagocytosis of the targeted cells. NI-1701-induced control of tumor growth in immunodeficient NOD/SCID mice was more effective than that achieved with the anti-CD20 targeted antibody, rituximab. Interestingly, a synergistic effect was seen when tumor-implanted mice were coadministered NI-1701 and rituximab leading to significantly improved tumor growth inhibition and regression in some animals. We describe herein, a novel bispecific antibody approach aimed at sensitizing B cells to become more readily phagocytosed and eliminated thus offering an alternative or adjunct therapeutic option to patients with B-cell malignancies refractory/resistant to anti-CD20-targeted therapy. *Mol Cancer Ther*; 17(8); 1739–51. ©2018 AACR.

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Introduction

The incidence of hematologic malignancies has been on the rise for the last 30 years, and accounts for approximately 9% of all cancers (1). Of the hematologic malignancies, lymphoma is the most common type. B-cell lymphomas are far more frequent than T-cell lymphomas accounting for around 85% of all non-Hodgkin lymphomas (NHL). The introduction of rituximab, the first anti-CD20 mAb, has revolutionized the management of B-cell lymphomas (2). Rituximab plus the "CHOP" (i.e., cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy regime is the frontline treatment for B-cell lymphomas (3). However, 30%–60% of patients with indolent NHL are resistant to rituximab at baseline and up to 50% of patients suffer relapses after anti-CD20 therapies and become refractory to their treatment (4).

Two major mechanisms underlying rituximab relapse/refractory responses are low CD20 expression levels in some patients with lymphoma and downregulation of CD20 expression post anti-CD20 treatment (5, 6). CD19, a B-cell-specific marker, has

been considered to be a promising target to overcome the anti-CD20-resistant/refractory situation. CD19 is a transmembrane glycoprotein of the immunoglobulin (Ig) superfamily. It is expressed during different stages of B-cell development, starting from pre-B-cell stage till being downregulated in early plasma cells (7). Furthermore, CD19 is broadly expressed in B-cell malignancies including those which are CD20 positive [e.g., NHL and B-chronic lymphocytic leukemia (B-CLL)] and those which may be CD20 low or negative [e.g., B-acute lymphoblastic leukemia (B-ALL); ref. 8]. Consistent with its broad expression spectrum in B-cell malignancies, targeting CD19 with different strategies (e.g., CD3/CD19 bispecific, CD19 CAR T cells) to harness B-cell killing has generated promising results in several clinical trials (9–11).

The emergence of "checkpoint inhibitors", for example, antibodies that block the interaction of PD-1 with its ligand PD-L1, thereby unleashing the natural brake on T cells and boosting the immune response represent a paradigm shift in our approach to treating cancer (12). In addition to harnessing the adaptive immune response to fight malignant cells, attention has turned to the innate immune system, in particular macrophages, a cell population that is abundant in the tumor microenvironment and that plays a specific role in phagocytosing cancer cells (13).

Macrophages express signal regulatory protein α (SIRP α) that interacts with CD47, an ubiquitously expressed protein that mediates a "don't eat me" signal. Cancer cells have evolved to hijack this interaction by upregulating the expression of CD47 on their cell surface, thus counterbalancing phagocytic signals and increasing the chance of evading innate immune surveillance (14). Therefore, blockade of the CD47/SIRP α interaction represents a promising strategy to increase the phagocytic clearance of tumor cells from the body. Several mAb and fusion proteins that target this interaction are in early clinical development (clinicaltrials.gov; e.g., NCT02953509, NCT03013218, NCT02367196, and NCT02890368). One limitation of this approach is that CD47, while upregulated on tumor cells (15), is also ubiquitously expressed on all cells of the body, including relatively high levels on erythrocytes and platelets (16, 17). Monospecific agents targeting CD47 would thus be expected to exhibit poor pharmacokinetic properties due to target-mediated drug disposition (TMDD) and possible side effects including anemia.

We have recently described a fully human bispecific antibody (biAb) format, the κ L-body (18). Using this format, we generated a panel of biAb comprising a high-affinity CD19-targeting arm combined with CD47-blocking arms with a range of affinities, on a human IgG1 Fc backbone to impart full effector mechanisms (19). The resultant biAbs are able to selectively block the interaction CD47/SIRP α on CD19⁺ cells and induce tumor cell killing *in vitro* and *in vivo*. From the panel of biAbs, we selected the CD47 arm with the appropriate affinity needed to balance efficacy on CD19⁺ cells against "off-target" effects, that is, an affinity that is weak enough to result in a fast off-rate for CD47 on non-CD19⁺ cells.

Here we describe the preclinical characterization of NI-1701, which induces potent macrophage-mediated phagocytosis of tumor cell lines and primary samples representing various B-cell malignancies. We also present *in vivo* proof of efficacy data using several mouse models. Together with nonhuman primate studies, these data suggest that NI-1701 may be an

effective and safe anticancer therapeutic both as a monotherapy and in combination.

Materials and Methods

Cell lines and primary samples

The Burkitt lymphoma Raji (CCL-86) and Ramos (CRL-1596) cell lines, the B-ALL NALM-6 (ACC-128), and the DLBCL SUDHL-4 (CRL-2957) cell lines were obtained from the ATCC. B-CLL MEC-2 cell line (ACC 500) was obtained from DMSZ. Cells were obtained between 2010 and 2014, authenticated and mycoplasma tested by the suppliers. Cell lines have been retested internally before the experiments. After thawing, cells were cultured between two weeks and two months. The Raji CD47-silenced cell line was derived from original wild-type (wt) Raji cells transfected with short hairpin (sh) RNA, subcloned, screened by flow cytometry, and finally selected as the most silenced stable clone by quantification of CD47 receptors using QIFIKIT (Dako). Cells were cultured at 37°C and 5% CO₂. Primary samples were collected from Conversant Biologics, the Leukemia Research Center (Glasgow, United Kingdom), the Geneva University Hospital (Geneva, Switzerland), the Josep Carreras Leukaemia Research Institute (Barcelona, Spain), and the CeVi collection of the Institute Carnot/CALYM (ANR, Rennes1 University, France). The ethics approvals were obtained from appropriate research ethics committees. Samples were obtained in agreement with the principles of the Declaration of Helsinki. Each patient provided and signed a written informed consent. The study was approved by the Institutional Review Board of the different collaborating hospitals.

Reagents

NI-1701, CD47 monovalent Ab, CD19 monovalent Ab, and CD19/CD47^{hi} biAb were generated using a fixed Ig heavy-chain variable domain (VH) library construction and produced as described in detail by Fischer and colleagues (18). Human (h) IgG1 isotype-matched control mAb was produced and purified at Novimmune from Chinese Hamster Ovary (CHO) culture supernatants. The CD19 monovalent Ab contains the same anti-CD19 arm as NI-1701 and an irrelevant nonbinding arm, while the CD47 monovalent Ab contains the same anti-CD47 arm as NI-1701 and an irrelevant nonbinding arm. The bivalent CD19 Ab (i.e., a mAb) contains the same anti-CD19 arm as NI-1701. The CD19/CD47^{hi} biAb contains the same anti-CD19 arm as NI-1701 and an anti-CD47 arm with a higher affinity than NI-1701 for CD47. Clinical-grade rituximab (anti-CD20 hIgG1 mAb) was obtained from FarmaMondo. The neutralizing anti-hCD47 mAb mouse B6H12 was cloned and expressed as hIgG1 in CHO cells (hB6H12). The anti-CD47 mAb, 5F9 (20), was cloned and expressed as hIgG4 in CHO cells.

Quantification of cell surface receptor density

Receptor density was quantified following manufacturer's instruction (QIFIKIT, Dako). Briefly, following incubation with Fc Receptor (FcR) Blocking Reagent (Miltenyi Biotec, catalog no. 130-059-901), primary antibodies (50 μ g/mL), anti-hCD19 (BD Biosciences, catalog no. 555410), anti-hCD47 (eBiosciences, catalog no. 11-0478-42), and anti-hCD20 (R&D Systems, catalog no. MAB4225) were added to the samples (whole blood or cells) for 30 minutes at 4°C. One-hundred microliters of Calibration beads were washed along with the cells and treated identically.

One-hundred microliters of secondary antibody (1/50 in PBS BSA 2%) were added for 30 minutes at 4°C. Cells were washed and resuspended in 130 µL of CellFix (BD Biosciences) and acquired on FACSCalibur (BD Biosciences). Analysis was performed and mean fluorescence intensity (MFI) was determined. A linear regression was performed using MFI values from the calibration beads. Receptor density per cell was extrapolated from this regression line.

Whole-blood binding

Human whole-blood samples were collected from healthy donors in citrate at the Blood Transfusion Center in Geneva. Samples were mixed with 3 µg/mL of AF488-coupled NI-1701, hB6H12, or isotype control [using the Alexa Fluor 488 Protein Labeling Kit (A10235, Thermo Fisher Scientific)] and surface staining antibodies for 30 minutes at 4°C. More precisely, samples were incubated with PE anti-hCD41a (#555467, BD Biosciences), APC-Cy7 anti-hCD3 (#557757, BD Biosciences), and with BV510 anti-hCD20 (#302311, BioLegend) Abs. Whole blood was then divided in two samples: 5 µL were diluted in PBS for erythrocytes analysis while 150 µL were incubated with erythrocyte lysing solution (#349202, BD Biosciences), and washed for leukocytes and platelets analysis. Samples were then acquired on a CytoFLEX instrument (Beckman Coulter) and analyzed with FlowJo software.

Affinity measurements

The affinity (K_d) for the binding to CD19 was determined on a 3200 KinExA (Sapidyne) while the binding to CD47 was determined using surface plasmon resonance (SPR) on a Biacore T200 instrument (GenScript). The affinity for CD19 was determined on CD19⁺ Raji B cells with a F(ab')₂ of the CD19 arm, while the affinity for CD47 was determined using recombinant hCD47 protein with NI-1701.

Antibody-dependent cellular phagocytosis

Human peripheral blood mononuclear cells (PBMC) were isolated from buffy coats by Ficoll gradient. Classical macrophages (M0) were prepared as described previously (19). M1 macrophages were generated from PBMCs with 20 ng/mL recombinant hM-CSF for 14 days with the addition of 50 ng/mL of hIFN γ during the last 18 hours of culture. For M2a or M2c polarization, macrophages were generated from PBMCs supplemented with 20 ng/mL recombinant hM-CSF for 14 days with the addition of either 20 ng/mL of hIL4 or 10 ng/mL of hIL10 + 20 ng/mL of hTGF β during the last 18 hours of culture, respectively (21–23). Target cells stained with 0.2 µmol/L CFSE (Invitrogen) were opsonized with the tested Abs for 15 minutes at 37°C. The flow cytometry-based phagocytosis assay used is described elsewhere (19). In here, the ratio between effector (macrophages) and target cells was 1:5. The percentage of phagocytosis is defined as the percentage of macrophages having engulfed at least one target cell and identified as CD14⁺ CFSE⁺ double-positive events among the total live CD14⁺ macrophages. In some experiments, the FlowSight imaging flow cytometer was used to investigate the phagocytosis index defined as the number of target cells engulfed per 100 macrophages. Fluorescence microscopy was also used. Here, monocytes were isolated from healthy donor buffy coats using the Human Pan Monocyte Isolation Kit (Miltenyi Biotec). A total of 1.5×10^4 monocytes were plated in 16-well chamber slide, in

100 µL of RPMI/10% heat-inactivated FCS with 50 ng/mL hM-CSF + (h-IL4 and h-IL10; 20 ng/mL each) to obtain M2 macrophages. At day 10, M2 macrophages were washed and stained with 0.2 µmol/L CFSE. On the day of the phagocytosis assay, B cells from lymph node biopsies were purified using the Human B cell Isolation Kit II (Miltenyi Biotec). Purified B cells were stained with 60 ng/mL of pHrodo solution (#P36600, Thermo Fisher Scientific, 12 minutes, room temperature) opsonized with 10 µg/mL of NI-1701, rituximab, or hIgG1 isotype control antibody. Cells were then added on macrophages to obtain the final 1:5 ratio between effector and target cells. Cells were cocultured in chamber wells (3 hours, 37°C) and analyzed by fluorescence microscopy using FIJI software. The percentage of phagocytosis is defined as the percentage of macrophages that have engulfed at least one target cell and identified as pHRodo⁺ CFSE⁺ double-positive events among 100 counted macrophages.

Antibody-dependent cellular cytotoxicity

A total of $1-2 \times 10^6$ cells/mL healthy PBMCs were activated overnight at 37°C with RPMI/10% heat-inactivated FCS supplemented with 10 ng/mL of recombinant hIL2. The next day, 5,000 Raji cells were incubated with 100 µCi ⁵¹Cr (Perkin Elmer, 37°C, 1 hour). After washing, cells were opsonized with NI-1701 or hIgG1 control (30 minutes, 37°C). A total of 5,000 Cr51-loaded Raji cells were then mixed with 4×10^5 PBMCs to obtain the final 80:1 ratio between effector (PBMC) and target cells (Raji cells). The cell mixture was incubated for 4 hours at 37°C before being centrifuged for 10 minutes at 1,500 rpm. Supernatant was transferred into a LumaPlate (coated with scintillant) and counted in a γ -counter. Negative controls (spontaneous ⁵¹Cr release) consisted of ⁵¹Cr-loaded target cells incubated with medium in the absence of effector cells. Total lysis control consisted of ⁵¹Cr-loaded target cells incubated with 5 µL of cell lysis solution (Triton X-100). Nonspecific lysis control (baseline) consisted of ⁵¹Cr-loaded target cells incubated with effector cells, without antibody. The ADCC percentage was calculated using the following formula: % specific ADCC = ((sample counts per minute (cpm) – nonspecific lysis control cpm)/(total lysis control cpm – negative control cpm)) \times 100%.

Cell-derived xenograft model in mice

In accordance with the Swiss animal protection law, 5×10^6 Raji cells were injected subcutaneously into the flank of 6- to 10-week-old NOD/SCID mice (Charles River Laboratories). Tumor volume was measured using a caliper and calculated using the formula (width \times length \times height) \times $\pi/6$. Mice received 4 or 5 doses of 10 mg/kg or 20 mg/kg of antibodies by intravenous (i.v.) administration (tail vein) once a week during 4 or 5 weeks. Mice were euthanized when the set endpoint of the experiment was reached (tumor volume \approx 1,400 mm³) or at the end of the study. Percentage of tumor growth inhibition, in comparison with hIgG1 control group was determined using the formula: %TGI = $\{1 - [(T_t - T_0)/(V_t - V_0)]\} \times 100$, where T_t = mean tumor volume of treated at time t , T_0 = mean tumor volume of treated at time 0, V_t = mean tumor volume of control at time t , and V_0 = mean tumor volume of control at time 0. Survival time is the time between the initiation of the treatment to the euthanasia of the mice when the set endpoint of the experiment was reached (tumor volume \approx 1,400 mm³).

Patient-derived xenograft model in mice

This experiment was performed in accordance with the United States animal protection law and Institutional animal guidelines. NSG mice irradiated 2 days earlier (275 cGy) were injected intravenously with 1.8×10^6 PBMCs from an ALL patient (invaded with 90% of cancer cells). Mice were intravenously treated with hlg1 control or NI-1701 antibodies (20 mg/kg) at days 7, 14, 21, 28, and 35. Upon sacrifice, peripheral blood (PB), spleen, liver, and bone marrow (BM) were harvested and subjected to flow cytometry to detect the human ALL cells identified as hCD45⁺, hCD3⁻, hCD14⁻ hCD56⁻ using fluorescence-labeled antibodies.

Pharmacokinetics and dose range-finding studies in cynomolgus monkeys

A single-dose (SD) pharmacokinetics study and a dose range-finding study (DRF) were performed in cynomolgus monkeys at Covance Laboratories test facilities. All procedures in the studies were in compliance with the German Animal Welfare Act or the UK Animals (Scientific Procedures) Act 1986 and were approved by the local Institutional Animal Care and Use Committee. For the single-dose study, 2- to 3-year-old females ($n = 3/\text{dose group}$) were injected by intravenous bolus with either 0.5 mg/kg or 10 mg/kg of NI-1701 or CD19/CD47^{hi} biAb. For the DRF study, one male and two females of 2- to 3-year-old received weekly intravenous bolus injections of NI-1701 or vehicle for 4 weeks. The NI-1701-treated animals received two injections at 30 mg/kg (days 1 and 8) followed by two injections at 100 mg/kg (days 15 and 22). Control group animals received intravenous bolus injections of vehicle only on days 1, 8, 15, and 22. For both studies, the animals were assessed twice daily for clinical signs and blood samples collected at different time points for hematologic and pharmacokinetic analyses. NI-1701 and CD19/CD47^{hi} biAb serum concentrations were measured by ELISA at Novimmune with in-house developed assay. IL6 serum concentrations were measured using MILLIPLEX MAP Multiplex Immunodetection Kits (Millipore) and analyzed on the Luminex instrument. The evaluation of the pharmacokinetic data was conducted at Novimmune using WinNonlin software (Professional Version 6.3, Pharsight).

Statistical analysis

GraphPad Prism 6 was used for all statistical analysis depicted in each figure legend. Data are expressed as mean \pm SEM or mean \pm SD, as indicated.

Results

The anti-CD19/CD47 biAb, NI-1701, specifically binds to B cells in whole blood

As CD47 is a ubiquitously expressed molecule (24, 25), we designed a biAb, NI-1701, that consists of a CD47-binding arm with an affinity that affords rapid engagement/disengagement kinetics when binding CD47 in a monovalent setting. This arm is paired with a high-affinity CD19-binding arm, in a fully human biAb, the $\kappa\lambda$ -body (18). More precisely, the affinity to CD19 was measured to be subnanomolar (i.e., 0.6 nmol/L) while the affinity to CD47 is 500 nmol/L. To assess the specificity of NI-1701, the binding profile to B cells was assessed in whole blood using flow cytometry. While CD47 is expressed on various cells in whole blood, including T cells, platelets, and erythrocytes (Supplementary Table S1), NI-1701 substantially shifted the signal for binding only to the B cells and rather weakly if at all to platelets, T cells, and erythrocytes as compared with the irrelevant isotype control (Fig. 1). Conversely, the CD47 mAb, hB6H12, used as a control for CD47 expression, significantly bound to all cell populations (Fig. 1).

The specific blockade of CD47/SIRP α interaction on CD19-expressing cells mediates potent macrophage-dependent phagocytosis of the target cells

Having demonstrated the specific binding of NI-1701 to B cells, we then assessed the *in vitro* efficacy of this compound to mediate phagocytosis of CD19⁺ B cells by macrophages. For this, CFSE-labeled Raji cells (Burkitt lymphoma) were used as target cells in an antibody-dependent cellular phagocytosis (ADCP) assay using hM-CSF-differentiated macrophages as effector cells. The results demonstrated a dose-dependent phagocytosis of Raji cells mediated by NI-1701, with an EC₅₀ of 7.44 ng/mL (Fig. 2A) and a maximum of 42% of phagocytic macrophages. Furthermore, using video microscopy to view phagocytosis in real-time, we

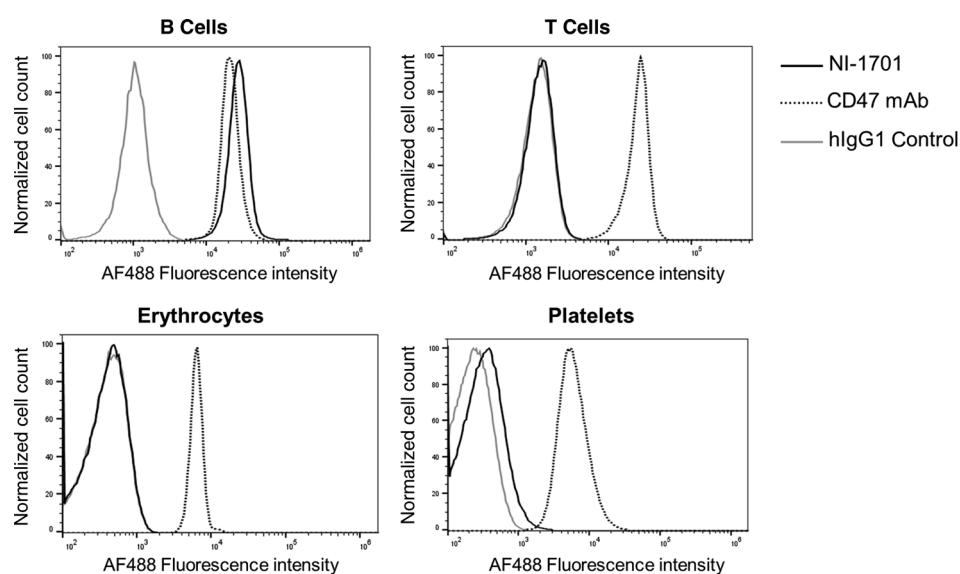


Figure 1.

NI-1701 binds selectively to B cells. Human whole-blood samples were incubated with antibodies to CD20 (for B cells), CD3 (for T cells), and CD41a (for platelets) in combination with either AF-488-labeled isotype control Ab (gray line), AF-488-labeled NI-1701 (dark line), or AF-488-labeled anti-hCD47 mAb (hB6H12, dashed line). Erythrocytes were gated on the basis of SSC/FSC parameters. Samples were analyzed by flow cytometry.

observed that these phagocytic macrophages sequentially engulfed not only one but several tumor target cells in response to NI-1701 treatment (Supplementary Video S1). In contrast, in the presence of a hIgG1 isotype control, no phagocytic activity was observed (Supplementary Video S2).

To demonstrate the requirement of the coengagement of both targets by NI-1701 to reach the maximal potency, NI-1701-mediated phagocytosis was compared with control monovalent variants. The CD47 monovalent variant contains only the anti-CD47-binding arm of NI-1701 (CD47 monovalent Ab) while the CD19 monovalent variant contains the anti-CD19-binding arm of NI-1701 (CD19 monovalent antibody) combined to an irrelevant arm. The control monovalent antibodies elicited a weaker potency of phagocytosis as compared with NI-1701, with a 4.2-fold decrease for the CD19 monovalent Ab (EC_{50} of 31.11 ng/mL) and a 52-fold decrease for the CD47 monovalent antibody (EC_{50} of 385.3 ng/mL; Fig. 2A). Interestingly, the bivalent CD19 antibody (containing the same anti-CD19 arm as NI-1701) induces similar potency to NI-1701 (i.e., EC_{50} at 7.7 ng/mL), although less effective at inducing maximum phagocytosis (Fig. 2A).

To demonstrate the contribution of CD47/SIRP α interaction to phagocytosis, we generated Raji cells with a minimal

expression level of CD47, while the levels of CD19 and CD20 remain unchanged (Fig. 2B). We then assessed the activity of rituximab on the wt versus the CD47-silenced Raji cells. While rituximab mediated efficient phagocytosis of the wt Raji cells, the knockdown of CD47 on the target cell surface results in an enhanced potency of rituximab by 5-fold (Fig. 2C), demonstrating that the reduction of the CD47/SIRP α interaction enhances CD20-mediated targeted cell killing. A similar result was seen with the CD19 monovalent antibody, in that silencing of CD47 enhanced antibody-mediated phagocytosis. In contrast, and as expected, NI-1701 demonstrated a similar potency on both cell populations that was equal to the efficacy of the anti-CD19 monovalent antibody killing of CD47-silenced Raji cells (Fig. 2D).

Next, the ability of NI-1701 and rituximab to mediate phagocytosis was compared using human CD19⁺/CD20⁺ cancer cell lines derived from patients with NHL (Raji and Ramos), ALL (NALM-6), CLL (MEC-2), or DLBCL (SUDHL-4). NI-1701 was as potent as rituximab at mediating phagocytosis of the Raji, Ramos, and MEC-2 cell lines (Fig. 3A). NALM-6 cells, which express a low level of CD20 (Supplementary Table S2), were weakly phagocytosed in the presence of rituximab,

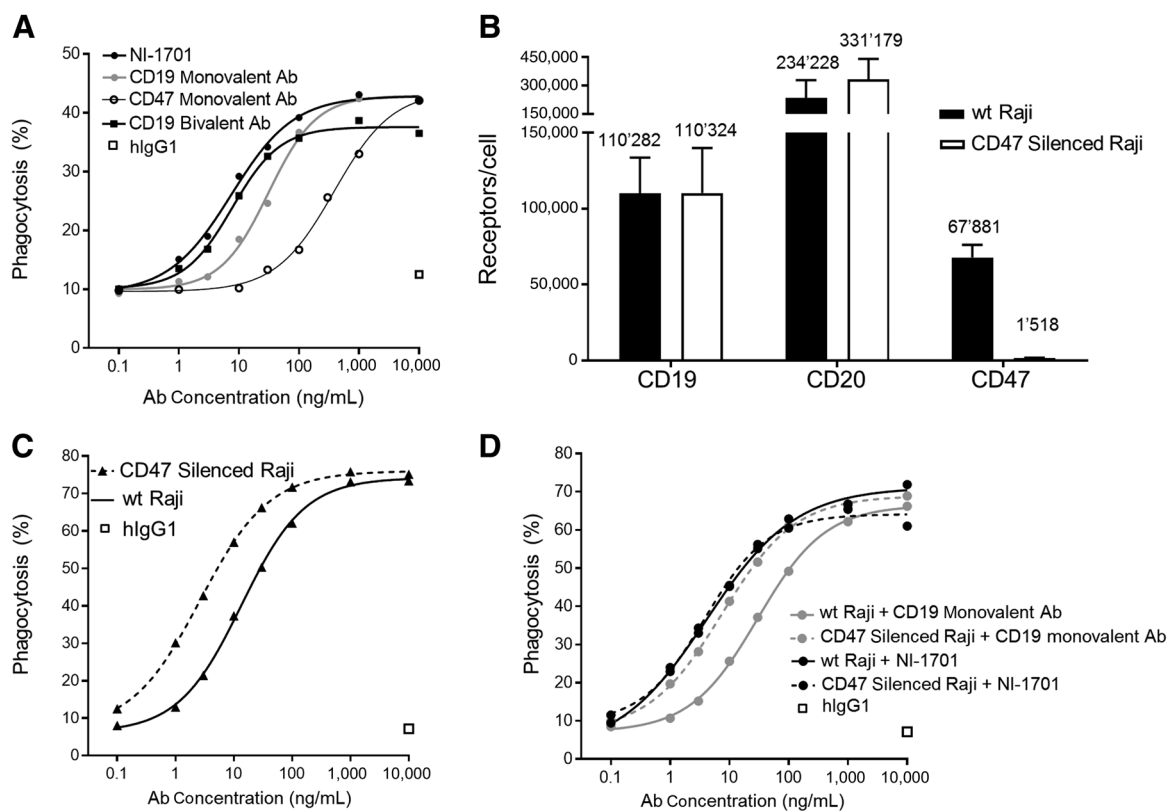
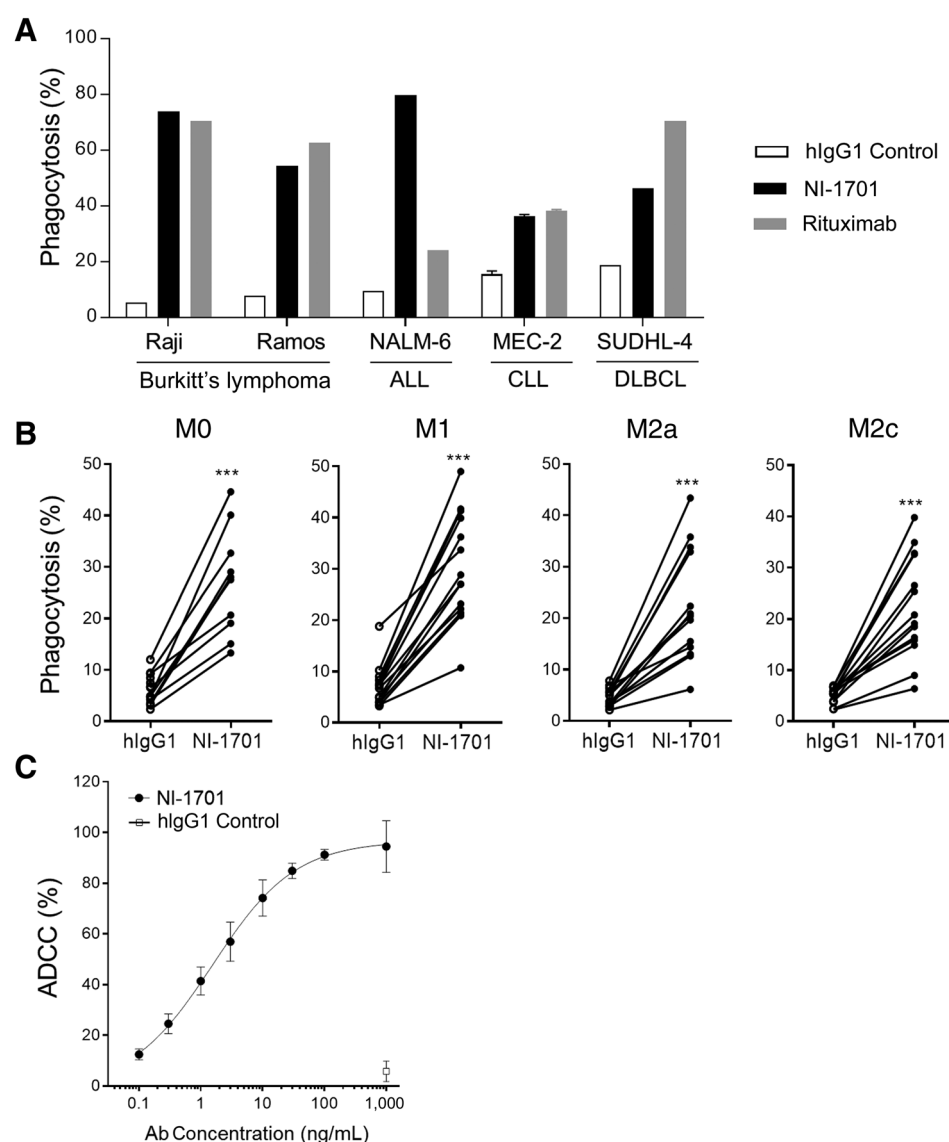


Figure 2.

Blockade of CD47/SIRP α enhances phagocytosis of tumor B cells. **A**, Percentage of phagocytosis by macrophages of CFSE-labeled Raji cells after treatment with 10 μ g/mL of hIgG1 control, or a dose range of NI-1701, the CD47 monovalent antibody, the CD19 monovalent antibody, and the CD19 bivalent antibody. **B**, The number of CD19, CD20, and CD47 per cell on Raji cells or the CD47-silenced clone Raji cells was quantified using the Qifikit. Histograms represent the mean \pm SD of three experiments. The mean is indicated above the histograms. **C**, Percentage of phagocytosis by macrophages of CFSE-labeled Raji cells or CFSE-labeled CD47 silenced Raji cells after treatment with a dose range of rituximab. **D**, Percentage of phagocytosis by macrophages of CFSE-labeled Raji cells or CFSE-labeled CD47-silenced Raji cells after treatment with a dose range of NI-1701 or the monovalent CD19 antibody. **A**, **C**, and **D**. Data were obtained by flow cytometry; percentage of phagocytosis is expressed as the percentage of CFSE/CD14 double-positive events among CD14⁺ macrophage cells. Graphs depict a representative dose-response curve of a minimum of three independent experiments.

**Figure 3.**

NI-1701 mediates killing of B lymphoma and leukemic cell lines by different subtypes of macrophages or killing by IL2 stimulated PBMCs by ADCC. **A**, Phagocytosis of CFSE-labeled Raji, Ramos, NALM-6, MEC-2, and SUDHL-4 cells by macrophages after treatment with 10 $\mu\text{g}/\text{mL}$ of hlgG1 control antibody, NI-1701, or rituximab. Phagocytosis was assessed by flow cytometry and expressed as a percentage of CFSE/CD14 double-positive events among CD14⁺ macrophages cells. Histograms are representative of 2–6 independent experiments, depending on the cell lines. **B**, Phagocytosis of CFSE-labeled Raji by macrophages differentiated into M0, M1, M2a, or M2c subset after treatment with 10 $\mu\text{g}/\text{mL}$ of hlgG1 control or NI-1701. A minimum of 13 donors were used to differentiate macrophage subtypes and each point represent one unique donor. Phagocytosis was assessed as above. Statistical analysis was performed using the paired Student *t* test. ***, $P < 0.001$. **C**, ⁵¹Cr-labeled Raji cells were incubated with a fixed concentration of hlgG1 control Ab (1 $\mu\text{g}/\text{mL}$), or a dose range of NI-1701 (15 minutes, room temperature). These cells were then incubated (4 hours, 37°C) in the presence of IL2-stimulated PBMC as a source of effector cells (E:T 80:1). The graph depicts the % of specific ADCC with the mean \pm SD. Graphs depict a representative dose–response curve of minimum five independent experiments.

whereas NI-1701 mediated a potent effect (Fig. 3A). SUDHL-4 cells were efficiently phagocytosed by both NI-1701 and rituximab, although the anti-CD20 mAb was slightly more effective (Fig. 3A), which may be explained by the high level of CD20 (Supplementary Table S2).

Using *in vitro* polarized macrophages to mimic the situation of the tumor microenvironment, the ability of NI-1701 to engage different subtypes of macrophages to mediate phagocytosis of cancer target cells was assessed. Classical M-CSF-cultured macrophages (M0), M1 macrophages polarized using IFN γ as well as M2 phenotypes, defined as M2a and M2c, generated by the addition of IL4 or IL10 + TGF β , respectively, were used. NI-1701 mediated a similar level of phagocytosis of Raji cells by all subsets of macrophages with up to 40% to 50% of the macrophages being phagocytic (Fig. 3B). A 5.4-, 4.3-, 4.7-, or 4.2-fold increase in phagocytosis by NI-1701 opsonized-Raji cells was observed as compared with the isotype control for the *in vitro* macrophages polarized into M0, M1, M2a, or M2c subtypes, respectively (Fig. 3B).

Finally, as a fully human IgG1 antibody, we wanted to investigate whether NI-1701 was able to mediate other Fc-dependent effector mechanisms such as antibody-dependent cellular cytotoxicity (ADCC). As expected, a dose-dependent killing by ADCC of Raji cells in the presence of NI-1701 was observed (Fig. 3C).

NI-1701 controls tumor growth *in vivo*

We next sought to assess the *in vivo* antitumor killing efficacy of NI-1701 in a mouse xenograft model using NOD/SCID mice implanted with Raji cells. NI-1701 was significantly efficacious in controlling tumor growth, with a final tumor growth inhibition (TGI) of 79% as compared with the control (Fig. 4A, top). The monovalent variants of NI-1701, that is, CD47 and CD19 monovalent antibodies, showed a partial effect on tumor growth with a final TGI of 54% and 46%, respectively. The bivalent CD19 antibody only demonstrated a partial effect with a final TGI of 36%. Tumor growth was monitored beyond the end of the treatment period demonstrating that NI-1701 affords a longer period of slowing tumor growth compared with both monovalent

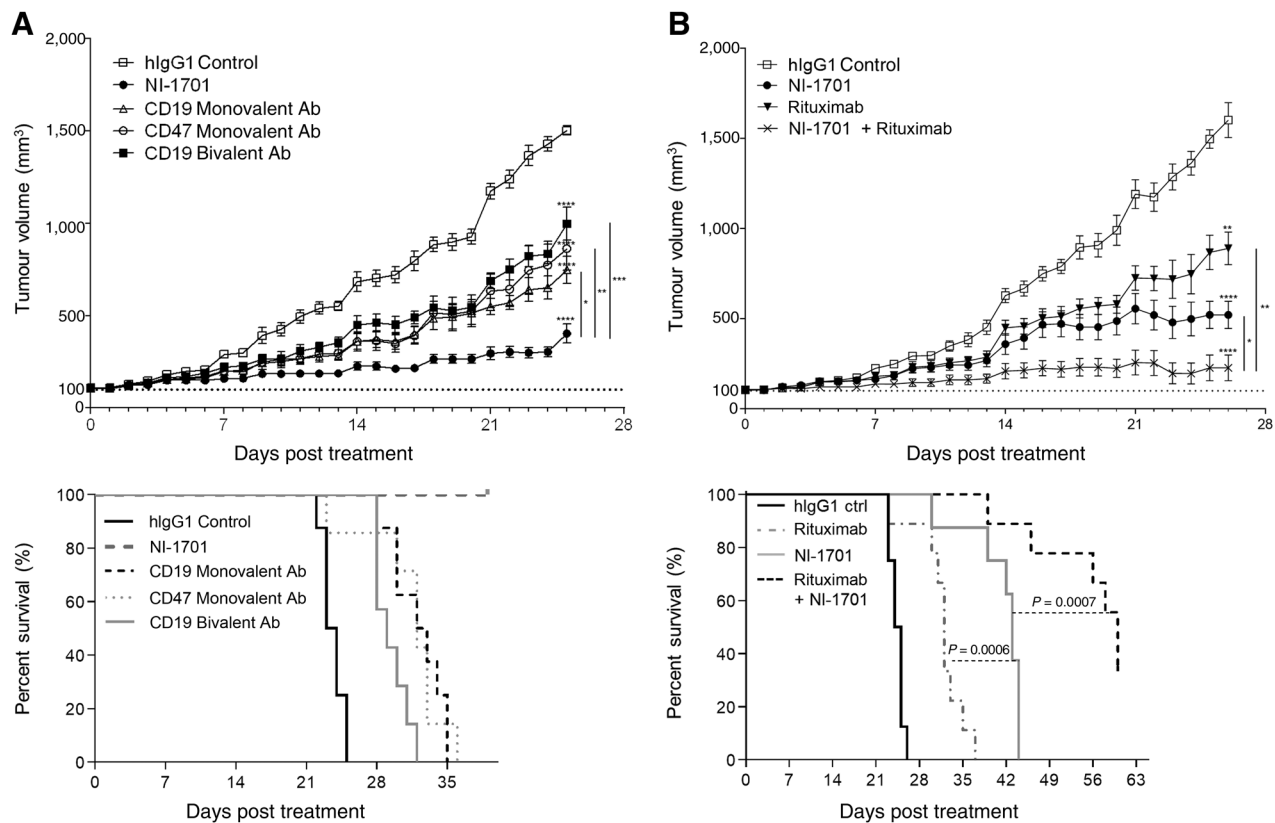


Figure 4.

NI-1701 controls tumor growth in a subcutaneous xenograft model and synergizes with rituximab. **A**, NOD/SCID mice were subcutaneously (s.c.) injected with 5×10^6 Raji cells. When tumors reached a volume of 100 mm^3 , mice were randomized into the following treatment groups: hlgG1 control ($n = 8$); NI-1701 ($n = 8$); CD19 monovalent antibody ($n = 8$), CD47 monovalent antibody ($n = 8$), or CD19 bivalent antibody ($n = 7$). Antibodies were injected intravenously at 20 mg/kg , once a week (days 0, 7, 14, and 21; **A**, top). The mean tumor volume ($\pm \text{SEM}$) per group is depicted over time. Statistical analyses were performed on the calculated area under the curve using a one-way ANOVA followed by a Tukey multiple comparison test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$ (**A**, bottom). Tumor growth was monitored beyond the end of the treatment period until day 39. Mice were euthanized when the endpoint was reached (tumor volume $\approx 1,400 \text{ mm}^3$) or at the end of the study (day 39). For comparison of survival curves (Kaplan-Meier curves), a log-rank (Mantel-Cox) test was performed. $P < 0.05$ is considered to be statistically significant. **B**, NOD/SCID mice were subcutaneously (s.c.) injected with 5×10^6 Raji cells. When tumors reached a volume of 100 mm^3 , mice were randomized into the following treatment groups: hlgG1 control ($n = 8$); NI-1701 ($n = 8$); rituximab ($n = 8$); or the combination of NI-1701 + rituximab ($n = 8$). Antibodies were injected intravenously at 20 mg/kg for single treatment or $10 \text{ mg/kg} + 10 \text{ mg/kg}$ for combination, once a week (days 0, 7, 14, and 21; **B**, top). The mean tumor volume ($\pm \text{SEM}$) per group is depicted over time. Statistical analyses were performed on the calculated area under the curve using a one-way ANOVA followed by a Tukey multiple comparison test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$ (**B**, bottom). Tumor growth was monitored beyond the end of the treatment period until day 60. Mice were euthanized when the endpoint was reached (tumor volume $\approx 1,400 \text{ mm}^3$) or at the end of the study (day 60). For comparison of survival curves (Kaplan-Meier curves), a log-rank (Mantel-Cox) test was performed. $P < 0.05$ is considered to be statistically significant.

antibodies and the bivalent CD19 antibody (Fig. 4A, bottom). These results confirmed that coengaging both CD47 and CD19 by NI-1701 promotes a more potent antitumor effect.

Next, the activity of NI-1701 was compared with the anti-CD20 mAb, rituximab. As combination therapies are widely used in oncology clinical practice, we tested the hypothesis that targeting two different B-cell epitopes in conjunction with blockade of the CD47/SIRP α interaction may lead to enhanced tumor control. As expected, rituximab significantly reduced tumor growth compared with hlgG1 control-treated group (Fig. 4B, top). Interestingly, NI-1701 was superior to rituximab at reducing tumor growth (72% vs. 48% of TGI for NI-1701 and rituximab, respectively). Finally, the combination therapy of NI-1701 and rituximab led to higher inhibition (92%) of tumor growth until the end of the treatment period at day 26. Tumor growth was monitored beyond the end of the treatment period and the data

demonstrate that NI-1701 affords a longer period of slowing tumor growth than rituximab, and that the combination of the two resulted in a 2.45-fold increase in the survival time from a median of 24.5 days for isotype control-treated animals to 60 days for the combination therapy treated group (Fig. 4B, bottom).

NI-1701 demonstrates a favorable pharmacokinetic and safety profile in nonhuman primates

To evaluate pharmacokinetic and safety parameters, NI-1701 was administered to cynomolgus monkeys. The anti-CD19 arm of NI-1701 is not cross-reactive to cynomolgus monkey, while the CD47 arm has a similar binding profile for cynomolgus monkey and human CD47. This allowed us to test the hypothesis that the affinity for CD47 was sufficiently low to circumvent potential liabilities of targeting the ubiquitously expressed CD47, that is, rapid drug elimination kinetics through TMDD

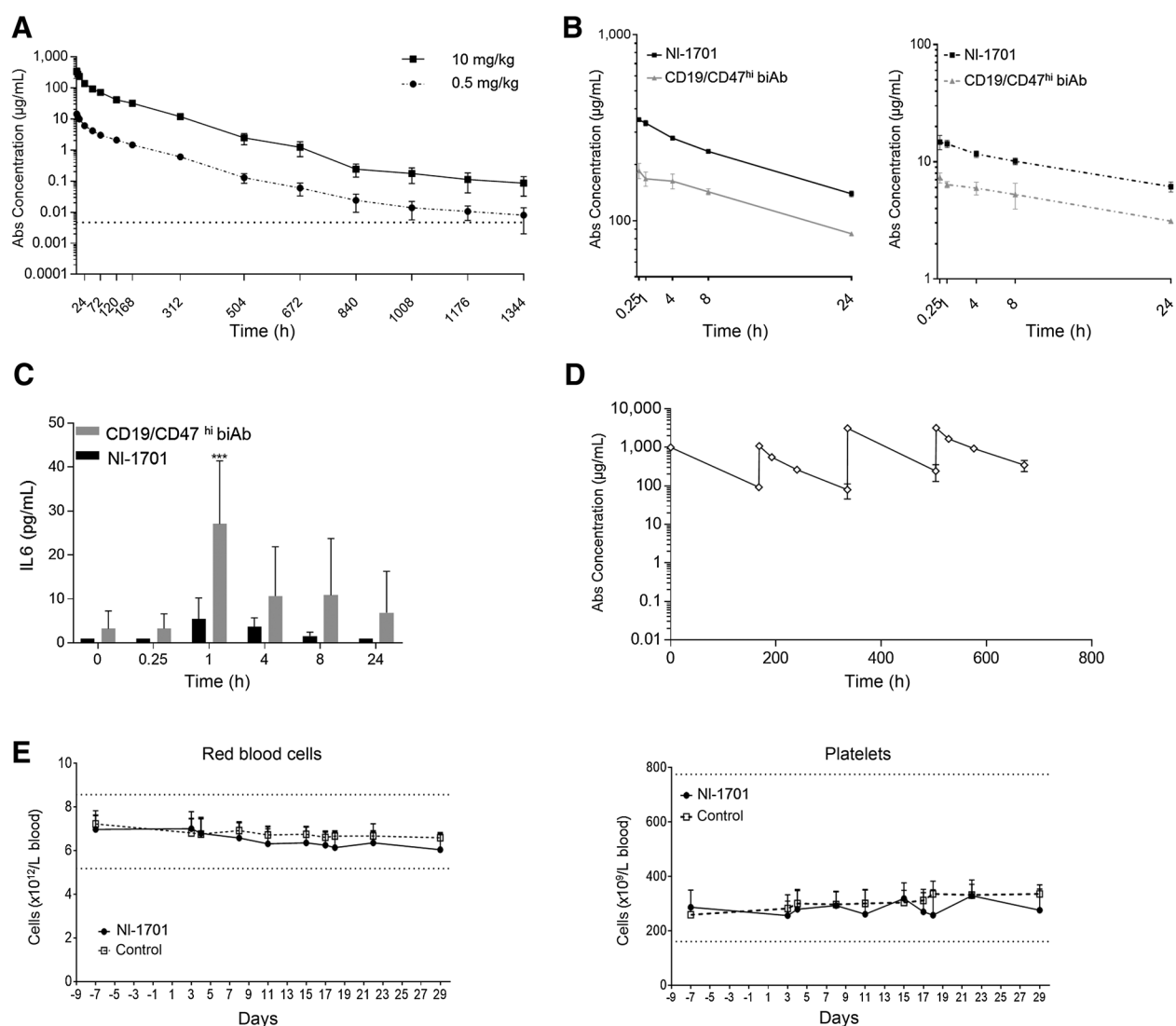


Figure 5. Nonhuman primate studies demonstrate favorable pharmacokinetics and normal hematologic parameters of NI-1701 following one single injection or multiple weekly injections. **A–C**, NI-1701 and/or CD19/CD47^{hi} were administered to cynomolgus monkeys as a single intravenous bolus at 0.5 or 10 mg/kg (*n* = 3 animals per treatment group per dose group). An ELISA assay was developed in-house to measure NI-1701 or CD19/CD47^{hi} biAb serum concentration. **A**, The elimination profile of NI-1701 at both doses are shown. The horizontal dotted line represents the lower limit of quantification (LLOQ) of the ELISA assay. **B**, The distribution phase (up to 24 hours) of the pharmacokinetic profiles of NI-1701 and CD19/CD47^{hi} at high doses (10 mg/kg, left) and at low doses (0.5 mg/kg, right) are compared. **C**, IL6 serum concentrations were quantified for NI-1701 and/or CD19/CD47^{hi}-treated animals for the dose at 10 mg/kg. Statistical analysis was performed using Mann–Whitney test. ***, *P* < 0.001. **D** and **E**, A DRF study was conducted. Cynomolgus monkeys (2 females and 1 male per group) were administered weekly as intravenous bolus doses of vehicle or NI-1701. Treated animals received two escalating doses of NI-1701: 2 injections at 30 mg/kg (days 1 and 8) followed by 2 injections at 100 mg/kg (days 15 and 22). **D**, The elimination profile of NI-1701 following two injections at 30 mg/kg and two injections of 100 mg/kg is shown. **E**, Hematologic parameters were monitored predose and over 4 weeks of dosing; RBC and platelet counts are shown. The horizontal dotted lines indicate the normal reference values for this species.

and target-related toxicity including anemia. NI-1701 was administered once as an intravenous bolus, at a high (10 mg/kg) or low (0.5 mg/kg) dose, and serum concentrations of the compound were measured. The terminal elimination profile of NI-1701 was parallel for both doses, suggesting that no target-mediated mechanisms contributed to the clearance of the biAb (Fig. 5A). Furthermore, the pharmacokinetic parameter estimates for NI-1701, that is, half-life and clearance, were 110 hours and 12.24 mL/day/kg, close to those of other hIgG1

molecules that have been reported during preclinical and clinical development (26).

To evaluate the impact that a higher affinity binding to CD47 may have on antibody clearance, we compared the pharmacokinetics of NI-1701 with another bispecific molecule having the same (non-cross reactive) CD19-targeting arm but a CD47-blocking arm with a higher affinity (i.e., 100 nmol/L), noted as CD19/CD47^{hi} biAb. NI-1701 and CD19/CD47^{hi} biAb have similar functional potency (Supplementary Fig. S1). The

pharmacokinetic profile of the latter has been described elsewhere (19). The two biAbs have similar half-lives (110 hours for NI-1701 and 106 hours for CD19/CD47^{hi} biAb), but the clearance parameters are different, with the CD19/CD47^{hi} biAb exhibiting a high clearance value (i.e., 18.24 mL/day/kg) as compared with 12.24 mL/day/kg for NI-1701. Examination of the distribution phase (i.e., alpha phase) of NI-1701 versus the CD19/CD47^{hi} biAb during the first 24 hours following intravenous bolus revealed that, at both high and low doses, the initial serum concentration measurements for NI-1701 were higher than for CD19/CD47^{hi} biAb (Fig. 5B). This result suggests an early binding event that impacts the distribution phase and results in a higher clearance for the higher affinity CD47-binding molecule. The ratio of AUC_{0-inf} shows that an even greater proportion of CD19/CD47^{hi} biAb is undetected at the low dose versus the high dose compared with NI-1701 (59% at 0.5 mg/kg compared with 67% at 10 mg/kg), suggesting that a target-mediated event occurs (Supplementary Table S3). Finally, while no hematologic toxicity was observed, an increase in circulating IL6 levels following CD19/CD47^{hi} biAb injection was detected (Fig. 5C).

A DRF study was conducted with NI-1701 in cynomolgus monkeys where treated animals received 2 intravenous bolus injections at 30 mg/kg at weeks 1 and 2, followed by two injections at 100 mg/kg at weeks 3 and 4. The mean C_{max} values on weeks 1 and 3 (post the first dose at 30 and the first dose at 100 mg/kg, respectively) were 1,003.47 and 3,149.36 µg/mL, respectively, showing dose proportionality with the single dose study (Supplementary Table S4). Furthermore, a minimal accumulation of NI-1701 was seen between the first and second injection at each dose level (Fig. 5D). No signs of anemia or thrombocytopenia were observed as red blood cell and platelet levels remained within normal ranges throughout the study (Fig. 5E). There were no drug-related observations. NI-1701 was well tolerated at doses up to 100 mg/kg. Complete hematologic results from the study are tabulated in Supplementary Table S5. Further *in vitro* safety assessments were performed on human and/or cynomolgus whole blood to assess hemagglutination and platelet aggregation, and no findings related to NI-1701 were observed, whereas anti-CD47 mAbs showed effects on both parameters (Supplementary Fig. S2A–S2D).

NI-1701 effectively kills primary leukemia and lymphoma cells

To demonstrate the clinical relevance of targeting CD47 on CD19⁺ cancer cells, the *in vitro* studies were extended to primary cells obtained from patients with B-cell leukemias or lymphomas. NI-1701 mediated an efficient and potent phagocytosis of primary cells from 24 individual CLL patients (Fig. 6A). Imaging confirmed the engulfment of the target cells by visualizing the double labeling of the CD14⁺ phagocytes and the CFSE⁺ target cells (Fig. 6B, left). Calculating the phagocytic index revealed that a mean of 175 (±80) B-CLL cells opsonized with NI-1701 were phagocytized by every 100 macrophages (Fig. 6B, right). Similar results were obtained with rituximab (Fig. 6A and B).

Cells from bone marrow aspirates of 12 and whole blood of 3 patients with B-ALL, a leukemia with limited treatment options, were also obtained and used in the ADCP assay. A significantly enhanced phagocytosis of target cells in the presence of NI-1701 was consistently observed with a maximum percentage of phagocytosis ranging from 46.4% to 86.3% and a mean value of 62.5% (±13.6). The activity of NI-1701 was three times higher than the isotype control and significantly higher than rituximab (43.26%

± 19.1%; Fig. 6A). NI-1701-induced phagocytosis was also tested using samples from 13 patients with cancer diagnosed with different subtypes of NHL including the following cases: 3 marginal zone lymphomas (MZL), 2 Waldenstrom macroglobulinemias (WM), 3 mantle cell lymphomas (MCL), 4 follicular lymphomas (FL), and 1 DLBCL. A significantly higher and similar phagocytosis was induced following NI-1701 or rituximab treatment as compared with the control IgG1, with maximum percentage ranging from 58.3% to 97.9% and from 55.3% to 98.5%, respectively (Fig. 6A). To further investigate the capacity of NI-1701 to mediate phagocytosis of NHL tumor cells, purified B cells isolated from lymph node biopsies of 10 FL patients were used as target cells. NI-1701 and rituximab induced equivalent phagocytosis of these patients' FL B cells (Fig. 6C).

Using a patient cell-derived model (PDX) generated with PBMCs from a B-ALL patient, the ability of NI-1701 to kill primary tumor cells *in vivo* was evaluated. Mice were intravenously injected with patient-derived B-ALL cells, and 7 days later treated either with NI-1701 or isotype control antibody. Analysis of the bone marrow, peripheral blood, spleen, and liver demonstrated that, while B-ALL cells were present in these compartments following hIgG1 control antibody treatment, NI-1701 eradicated the tumor burden (Fig. 6D).

Discussion

Approaches targeting CD47 face considerable development challenges due to the ubiquitous expression pattern of the target on healthy cells. As such, hematotoxicity and poor pharmacokinetics are common unwanted secondary effects that have been described in studies conducted in mice and nonhuman primates (20, 27–30). The primary differentiating characteristic of the biAb approach is its increased selectivity for the target, that is, CD19⁺ B cells. While CD47 is expressed on all cell types in whole blood, including T cells, platelets and erythrocytes, flow cytometry analysis demonstrates that NI-1701 binds strongly to human B cells with no detectable binding to T cells and erythrocytes and weak binding to platelets. The lack of detectable binding to erythrocytes is consistent with the lack of hemagglutination seen in the presence of NI-1701. The weak NI-1701-binding activity on platelets is most probably due to the coengagement of the Fc portion of the biAb interacting with the low-affinity FcγRIIA (31) and the low-affinity CD47 arm on the same cell. This binding event has no functional consequence, such as platelet aggregation or activation *in vitro*. Furthermore, single or multiple doses of NI-1701 in nonhuman primates are well tolerated, demonstrating favorable pharmacokinetic profiles with proportionality between the doses and, importantly, no hematologic toxicity. Disrupting the CD47/SIRPα axis with the "right" affinity CD47-binding arm is key to the biAb approach. Indeed, in the process of selecting a lead candidate for preclinical development, a higher affinity CD47-binding arm was tested in the biAb format (i.e., CD47/CD19) and administered to NHP. Interestingly, while no hematologic toxicity was observed, the higher CD47 binding resulted in rapid decrease in initial plasma concentration (alpha phase). We hypothesize that this drop in the alpha phase concentration is due to a binding event in blood leading to a faster clearance, which is supported by our *in vitro* observation in whole blood demonstrating increased binding to erythrocytes and platelets. Furthermore, these data corroborate previous published results of a biAb with reduced affinity for CD47, which allows for selective binding

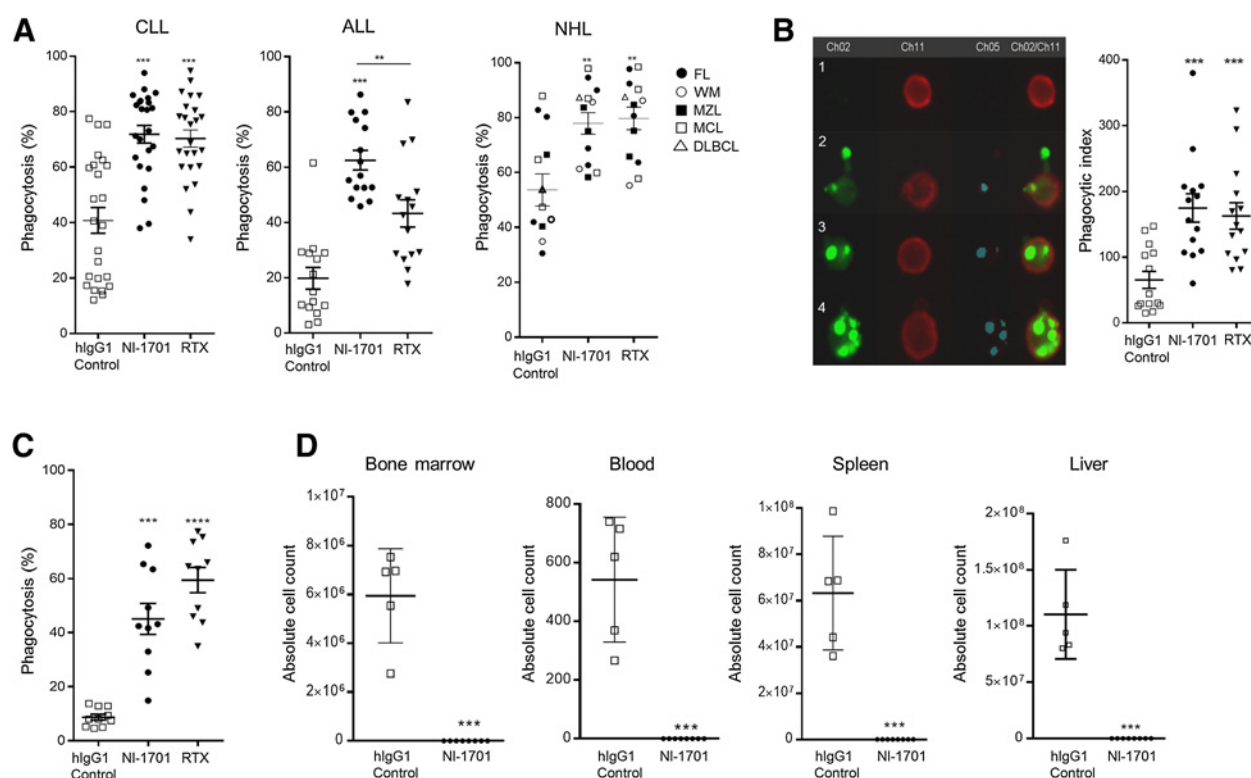


Figure 6.

NI-1701 mediates effective killing of B cells from a range of primary human samples *in vitro* and *in vivo*. **A**, Percentage of phagocytosis by macrophages of CFSE-labeled primary samples from 24 patients with CLL, 15 patients with ALL, and 12 patients with NHL including 3 MZL, 2 WM, 3 MCL, 4 FL, and 1 DLBCL. Primary samples were treated with 10 $\mu\text{g}/\text{mL}$ of hlgG1 control, NI-1701, or rituximab (RTX). Phagocytosis % was assessed by flow cytometry and expressed as the percentage of CFSE/CD14 double-positive events among CD14⁺ macrophages cells. Each symbol corresponds to one sample, the lines represent the mean of phagocytosis % \pm SEM. **B**, Examples of FlowSight acquisitions showing target cells (stained with CFSE, green fluorescence, Ch02) and macrophage (stained with CD14-APC, red fluorescence, Ch11). Ch05 corresponds to the IDEAS software quantification counting the number of B-CLL cells inside macrophages. Ch02/Ch11 corresponds to the merged acquisition between Ch02 and Ch11. Row 1 is representative picture of empty macrophages. Row 2 demonstrated the specific counting of engulfed target cells versus nonengulfed ones. Rows 3 and 4 show representative pictures of macrophages with several engulfed target cells. Graph represents the phagocytosis index for hlgG1 control antibody, NI-1701, and rituximab (RTX)-treated B-CLL primary samples ($n = 14$) tested at 10 $\mu\text{g}/\text{mL}$. Each symbol corresponds to one sample, the lines represent the mean of phagocytosis % \pm SEM. **C**, Percentage of phagocytosis by M2 macrophages of pH-rodo-labeled purified B cells from 10 FL primary samples and treated with 10 $\mu\text{g}/\text{mL}$ of hlgG1 control, NI-1701 or rituximab (RTX). Phagocytosis % was assessed by fluorescent microscopy with the acquisition of 100 macrophages. Each symbol corresponds to one sample, the lines represent the mean of phagocytosis % \pm SEM. **D**, NSG mice irradiated 2 days earlier (275 cGy) were injected intravenously with 1.8×10^6 PBMC from an ALL patient. Seven days later, the first dose of hlgG1 control ($n = 5$) or NI-1701 ($n = 8$) was administered intravenously (20 mg/kg). Dosing has continued once a week throughout the study that was terminated at day 38. The absolute number of B-ALL tumor cells following flow cytometry acquisition (based on hCD45⁺ staining) in one femur for the bone marrow (BM), peripheral blood (PB; per μL), spleen and liver from mice treated with NI-1701 or hlgG1 isotype control are depicted. Each dot represents an individual mouse and the horizontal bar the mean \pm SD. Statistical analyses were performed using paired one-way ANOVA followed by multiple comparison tests (**A–C**) or unpaired *t* test (**D**; **, $P < 0.01$; ***, $P < 0.001$; and ****, $P < 0.0001$).

to dual antigen-expressing cells in the presence of a large CD47 sink (32). Finally, the lower affinity CD47-binding arm of NI-1701 was far less prone to inducing cytokine release *in vitro* as compared with the CD19/CD47^{hi} biAb. Taken together, the selectivity afforded by the high affinity CD19-binding arm coupled to a low affinity CD47-binding arm has the potential to significantly widen the safety margin of therapeutic CD47 targeting in patients.

Therapeutic antibodies targeting B cells exert antitumor activities through various Fc-mediated mechanisms including ADCP and ADCC. These attributes have been extensively described preclinically as key mechanisms in the elimination of cancer B cells by anti-CD20 (33) and anti-CD19 Abs (34, 35). However, to avoid indiscriminate elimination of healthy host cells by CD47-targeting mAbs through Fc-mediated effector functions, the Fc domains of the anti-CD47 mAbs have been selected for reduced

effector functions, for example, IgG4 (20). This limits the tumor-killing capacity of the anti-CD47 mAbs as a monotherapy. In contrast, due to its selective targeting to CD19⁺ cells, NI-1701 is a fully hIgG1 with the whole spectrum of Fc-mediated effector function. We show here that NI-1701 mediates effective killing of primary and immortalized cancer cells via ADCP and ADCC. The ability for NI-1701 to harness the antitumor potency of the tumor-infiltrating myeloid cells through blockade of CD47/SIRP α interaction was demonstrated via effective *in vitro* phagocytosis of cancer cells taken from a plethora of B-cell malignancies. In addition, NK cells were shown to be efficient killers by ADCC of malignant B cells in the presence of NI-1701, in line with several reports demonstrating the importance of these cells for the cytotoxic activity of several mAbs (36–38).

We extended our *in vitro* findings to demonstrating efficacy induced by cotargeting CD47/CD19 *in vivo*. NI-1701

administration to Raji B-cell-transplanted NOD/SCID mice resulted in control of tumor growth and a significant increase in median survival time as compared with isotype control-treated mice. Coengagement of CD47/CD19 is obligatory for maximal efficacy as the CD19 monovalent biAb, the CD47 monovalent biAb, and the bivalent CD19 allow only for partial control of tumor growth. Interestingly, the monovalent CD47 antibody showed equivalent activity to the CD19 monovalent antibody *in vivo*. This result was not predicted by the *in vitro* killing data, where the latter was more potent. Nonetheless, the *in vivo* observation is explained by the high dose of CD47 monovalent antibody given to mice allowing exposure levels to reach antibody concentrations known to induce maximal ADCP activity *in vitro*. The potent *in vivo* antitumor effect of NI-1701 was also demonstrated in a patient-derived xenograft model of B-ALL in which NI-1701 reduced tumor across the various organs tested. Studies are underway to dissect underlying mechanism. Nonetheless, the data herein derived from xenograft experiments in NOD/SCID or NSG mice, characterized by impaired T/B development, reduced NK-cell function (39, 40), and the ability for mSIRP α to bind hCD47 with high affinity (41), suggest that macrophages will be the main driver for the observed tumor control *in vivo*. Tumor-associated macrophages (TAM), displaying diverse phenotypes (42), represent key regulators of the complex interplay between the immune system and cancer in humans and mice (43, 44) and consequently efforts are focusing on targeting TAMs in oncology (reviewed in ref. 45). In general, M2 macrophages, which exert anti-inflammatory and protumorigenic activities, are described to support tumor growth as opposed to the M1 macrophages understood to induce inflammatory responses (43, 46). Our *in vitro* analysis demonstrated that monocyte-derived M1 and M2 macrophages effectively kill Raji B cells in the presence of NI-1701. The results suggest that NI-1701 may reeducate M2 macrophages, thus disrupting the protumor-favoring microenvironment (X. Chauchet and colleagues, manuscript in preparation).

The most widely used treatment for B-cell lymphomas is R-CHOP (3). However, a large number of patients become refractory to rituximab with time thus representing a growing population with an unmet medical need (4). Several emerging therapies for the treatment of relapsed or refractory B-cell lymphomas are in development (47), including the biAb approach presented here. A plethora of ongoing trials are evaluating the efficacy of rituximab combined with checkpoint inhibitors and other immune therapies for lymphoma (48). As such, we show that combination studies of rituximab with NI-1701 in xenograft mouse model resulted in a significantly improved tumor growth inhibition as compared to either treatment. The complementary mechanisms of action afforded by the two approaches certainly explain the significant benefit over stand-alone therapies and further investigations are ongoing to dissect mechanisms. These results also reinforce the concept of targeting two distinct cell-surface antigens to increase the likelihood of cancer control in situations with preexisting epitope variants or loss, such as reported in patients with rituximab-refractory NHL (4, 49). Furthermore, NI-1701 was shown to be superior to rituximab in killing B cells from B-ALL patients and the B-ALL-derived cell line, NALM-6 cells. Data from our laboratory and others (50) have demonstrated the lower proportion of ALL patients expressing CD20 on B cells, which may explain the improved efficacy afforded by NI-1701.

The study here describes the development of a novel bispecific antibody approach harnessing macrophages through the block-

ade of CD47 in B-cell malignancies. By targeting CD19 with high affinity, NI-1701 aims to selectively inhibit the CD47 "don't eat me" signal on B cells. This approach offers an alternative treatment for patients resistant and/or refractory to anti-CD20 therapy. Clinical experience will validate the safe and selective strategy afforded by cotargeting CD47 and CD19 on B cells.

Disclosure of Potential Conflicts of Interest

V. Buatois is a project leader at NovImmune S.A. Z. Johnson has ownership interest (including patents) in Company stock. S. Salgado-Pires is a unit head non-clinical safety (former employee, until April 2017), has ownership interest (including patents), and is a consultant/advisory board member for NovImmune. A. Papaioannou is a senior research associate at NovImmune S.A. E. Hatterer is a head, *in vitro* pharmacology at NovImmune. X. Chauchet is a head, *in vivo* pharmacology at NovImmune. F. Richard is a senior research associate, *in vitro* pharmacology section at NovImmune S.A. Leticia Barba is a senior research assistant at NovImmune S.A. B. Daubeuf is a senior research associate at NovImmune S.A. and has ownership interest (including patents) in NovImmune S.A. L. Cons is a senior research assistant at NovImmune S.A. L. Broyer is a research associate at NovImmune S.A. M.H. Kosco-Vilbois is a chief scientific officer at NovImmune S.A. and has ownership interest (including patents) in NovImmune S.A. K. Masternak is a head of biology at NovImmune S.A. N. Fischer is an employee at NovImmune S.A. L. Shang is a head of pharmacology section at NovImmune S.A. W.G. Ferlin is a department head at NovImmune S.A. No potential conflicts of interest were disclosed.

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