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TITLE PAGE

Full title: A reduction in B, T, and NK cells expressing CD38 by TAK-079 inhibits the induction and progression of collagen-induced arthritis in cynomolgus monkeys

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Running title: TAK-079 alleviates progression of collagen-induced arthritis

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A list of nonstandard abbreviations used in the paper:

distal interphalangeal (DIP)

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metacarpophalangeal (MCP)

proximal interphalangeal (PIP)

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ABSTRACT

Ectoenzyme CD38 is increased on lymphocytes in response to an antigenic challenge and it is hypothesized that targeting these activated lymphocytes could ameliorate pathologic activities in autoimmune diseases. The cynomolgus monkey is an appropriate model for assessing potential effects of targeting CD38 in humans because these species exhibit similar expression profiles. TAK-079 is a human monoclonal antibody (IgG_{1λ}) that binds to CD38 and lyses bound cells by CDC and ADCC. TAK-079 binds to monkey CD38 with an affinity at EC₅₀ 4.5 nM, and the potential activity of TAK-079 was investigated in a monkey collagen-induced arthritis model of autoimmune disease. Prophylactic administration of TAK-079 (3 mg/kg i.v. weekly) was well tolerated and prevented the arthritis development compared to vehicle-treated control animals, which exhibited progressive disease with radiographic damage and worsening clinical scores over the study course. Therapeutic treatment of arthritic monkeys with TAK-079 (3 mg/kg i.v. weekly) was also well tolerated and reduced disease progression and symptoms. Arthritis scores and joint swelling were significantly lower than the vehicle control, accompanied by decreases in blood levels of C-reactive protein, alkaline phosphatase, and natural killer, B, and T cells. Histopathology, morphometry, and radiology revealed significantly less joint damage in animals exposed prophylactically to TAK-079 treatment as compared to vehicle-treated animals and significantly less damage in animals treated therapeutically with TAK-079 or dexamethasone (0.1 mg/kg oral gavage daily), illustrating potential disease modifying activity. In conclusion, these data indicate that depletion of CD38-expressing cells could be a therapeutic mechanism for treating autoimmune diseases.

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SIGNIFICANCE STATEMENT

This study demonstrates that targeting CD38-expressing leukocytes with a cytolytic antibody can ameliorate autoimmune disease in cynomolgus monkeys. The study gives a unique perspective into this therapeutic strategy because the three other anti-CD38 cytolytic antibodies in clinical development (daratumumab, isatuximab, and MOR202) cannot be tested in similar models because they do not cross-react with CD38 expressed by new world primates.

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INTRODUCTION

Autoantibodies mediate the pathogenesis of many autoimmune diseases, including rheumatoid arthritis (RA) (Deane, 2014) and systemic lupus erythematosus (SLE) (Kurts et al., 2013), directly by binding to tissue antigens and indirectly via the deposition of immune complexes (Martin and Chan 2006). These autoantibodies are produced by transient, short-lived plasmablasts and plasma cells in secondary lymphoid tissues and by long-lived plasma cells and entrenched in supportive niches within the bone marrow (Nutt et al., 2015; Radbruch et al., 2006). Pharmacologic modulation of plasma cells is challenging because these cells are not actively cycling, cease expressing most surface antigens (e.g., CD20), and exhibit a long half-life (i.e., low turn-over). Consequently, most existing therapeutics affect plasma cells and antibody production indirectly by modulating progenitors of these cells, e.g., rituximab (anti-CD20) targets B lymphocytes and belimumab inhibits short-lived plasmablasts from proliferation (Hale et al., 2018). Both methods can reduce autoantibody production; however, this effect manifests slowly (e.g., months to years [Stohl et al., 2012]) because of the relatively long half-life of pre-existing plasma cells. In contrast, plasma cells are sensitive to proteasome inhibition. A clinical study has demonstrated that the proteasome inhibitor bortezomib can deplete plasma cells, decrease autoantibodies, and ameliorate disease activity in patients with refractory SLE within weeks. However, gastrointestinal and neurological adverse events restrict the use of bortezomib within these patients, presumably because of proteasome inhibition in neurons and epithelial cells (Alexander et al., 2015; Siowall et al., 2017). Consequently, a mechanism that specifically targets plasma cells could provide a safer and more effective method for reducing autoantibodies.

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The CD38 antigen is a type II glycoprotein, which is highly expressed by plasma cells and plasmablasts and at a lesser magnitude by B regulatory cells, natural killer (NK) cells, T regulatory cells, monocytes, and plasmacytoid dendritic cells in peripheral blood from healthy subjects (Krejci et al., 2016) and patients with RA or SLE (Cole et al., 2018; Banchereau et al., 2016; Kubo et al., 2017). Analysis of synovial biopsies revealed that CD38 is expressed at higher levels in patients with RA and SLE than those with undifferentiated arthritis, osteoarthritis, reactive arthritis, ankylosing spondylitis, psoriatic arthritis, and crystal-induced arthritis (Kraan et al., 1999; Vital et al., 2011). Recent transcriptional profiling studies of blood from patients have identified up to 13 distinct signatures (i.e., phenotypes) of SLE (Banchereau et al., 2016) and have concluded that disease activity correlated most positively with the level of CD38-expressing plasmablasts in peripheral blood.

In patients with SLE treated with rituximab and oral steroids, the level of CD38-expressing plasmablasts in peripheral blood was the best predictor of time to relapse. Moreover, circulating immunoglobulin (Ig)-secreting cells that express high levels of CD38 were identified in the peripheral blood of patients with active SLE, and the level of this plasma cell subset during treatment was associated with decreases in anti-double-stranded DNA (anti-dsDNA) antibody levels, proteinuria, and SLE disease activity (Grammer et al., 2003). Collectively, these data indicate that specifically reducing CD38-expressing cells could be an effective strategy for treating autoimmune diseases such as RA and SLE.

TAK-079 is a fully human IgG_{1λ} monoclonal antibody (mAb) that binds to both human and monkey CD38 and depletes cells by antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) (Smithson et al., 2017). The addition of TAK-079 to blood or bone marrow samples from patients with RA (manuscript in preparation) or SLE (Wang

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et al., 2016) depleted 80% of plasma cell populations, including short-and long-lived plasma cells. Additionally, the number of cells producing autoantigen-specific antibodies was dramatically reduced, with VH4-34 9G4+ antibodies, anti-Ro antibody, and anti-dsDNA antibody decreased by 70%, 70%, and 80%, respectively. The anti-human CD38 mAb daratumumab also depletes CD38-expressing plasmablasts and plasma cells in samples from patients with SLE and RA in a dose-dependent manner *in vitro* (Cole et al., 2018). In contrast to daratumumab (EMA, 2016), TAK-079 cross-reacts with CD38 expressed by cynomolgus monkeys, which provides a unique opportunity to determine if reducing the level of cells expressing CD38 would affect inflammation and tissue damage in a primate model of autoimmune disease.

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MATERIALS AND METHODS

Antibodies. TAK-079 was available at Takeda. Fluorochrome-conjugated anti-mouse IgG was purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). Pharm Lyse buffer was obtained from BD Biosciences (San Jose, CA). Alexa Fluor 647-conjugated TAK-079, unconjugated antibodies to the TAK-079, and a humanized control antibody with a different antigen specificity but the same IgG1 fragment crystallizable region (Fc) were generated at Takeda. The secondary antibody for the monkey tissue cross-reactivity investigation was rabbit anti-TAK-079 (generated at Takeda). The negative control human IgG1 was obtained from Millipore Bioscience Research Reagents (Temecula, CA).

Immunohistochemistry of Tissues. The expression profile of the CD38 protein was compared across 15 different types of tissues collected from healthy human and cynomolgus monkey donors using immunohistochemistry. The quality of each tissue for detecting CD38 was verified by using a positive-control antibody against the related transmembrane receptor CD31 (Dako North America, Inc., Carpinteria, CA). Sections (5 μm) were cut from fresh-frozen tissue samples embedded in OCT Compound (Sakura Finetek USA, Inc., Torrance, CA) and fixed in acetone for 10 min at room temperature. Just before staining, slides were fixed for 10 sec in 10% neutral buffered formalin. Acetone/formalin-fixed cryosections were rinsed twice in phosphate-buffered saline (PBS) and incubated for 20 min with a protein block (PBS; 0.5% casein; 5% human gamma globulins; 0.02% goat IgG; 1 mg/mL heat-aggregated human IgG) designed to reduce nonspecific binding. Unconjugated TAK-079 or a negative control human IgG1 were applied to sections at 5 or 25 $\mu\text{g}/\text{mL}$ and incubated at room temperature for 1 hour.

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The slides were then rinsed twice with PBS and an indirect immunoperoxidase procedure was performed to detect these primary reagents. The secondary antibody, rabbit anti-TAK-079, was then applied at 5 µg/mL for 30 min and rinsed twice with PBS. Endogenous peroxidase was blocked by incubating the slides for 5 min with the peroxidase solution provided in the Dako EnVision+ Kit and then rinsing twice with PBS. The slides were then treated for 30 min with the peroxidase-labeled goat anti-rabbit IgG polymer supplied in the Dako EnVision+ Kit, rinsed twice with PBS, and treated for 8 min with the substrate-chromogen (DAB+) solution supplied in the Dako EnVision+ Kit. All slides were rinsed with tap water, counterstained with hematoxylin, washed, “blued” in saturated lithium carbonate, washed, dehydrated through alcohols, cleared in xylene, and cover-slipped. Staining intensity was graded semi-quantitatively by a blinded American College of Veterinary Pathologists-certified anatomic pathologist.

TAK-079 Binding to Recombinant CD38. Chinese hamster ovary K1 (CHO-K1) cells stably expressing monkey CD38 were generated to study cell surface binding of anti-CD38 antibodies. CHO-K1 cells (Lonza, Morristown, NJ) were transfected with full-length cDNA clones of cynomolgus monkey CD38 (Origene Technologies, Rockville, MD). After selection, the pools were sorted by flow cytometry and the highest cynomolgus monkey CD38 expressing clones (top 15% mean fluorescence intensity) were utilized for binding studies. In a 96 well round bottom plate, 200,000 cells per well were plated and stained with 66.7 nM of directly conjugated Alexa Fluor® 488 to antibodies in 50 µL of FACS buffer (1% bovine serum albumin [BSA] in PBS) on ice for 30 min to 1 hour. Cells were washed 3 to 4 times in a final volume of 200 to 250 µL of FACS buffer. The final cell pellet was resuspended in 100 µL of FACS buffer containing 1% paraformaldehyde. Samples were evaluated on the FACS Canto II HTS (BD Biosciences) and analyzed using FlowJo software (Tree Star Inc., Ashland, OR).

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Flow Cytometry of Cell Lines and Whole Blood. For the staining of cell lines, cells were resuspended at 2×10^6 /mL in FACS buffer (5% fetal bovine serum and 0.05% sodium azide) in Dulbecco's phosphate buffers saline without calcium and magnesium (VWR, West Chester, PA) and 200- μ L samples were stained with the appropriate mAbs at 4°C for 30 min. Samples were washed with FACS buffer and analyzed by flow cytometry using BD FACSCalibur platform (BD Biosciences). For whole blood, 200- μ L samples from monkeys were stained with the appropriate mAb at 4°C for 30 min. Red blood cells (RBCs) were lysed with BD FACS lysing solution, and samples then washed with FACS buffer and analyzed by flow cytometry. In all cases, antibodies were used at saturating concentrations and, in many cases, up to 4 antibodies were used per sample.

TAK-079 Activity in Whole Blood from Cynomolgus Monkeys. Cynomolgus monkey whole blood was obtained from Charles River Laboratories (Wilmington, MA). For binding assays, anticoagulated cynomolgus monkey peripheral whole blood (100 μ L) was incubated with increasing concentrations of TAK-079 antibody (43 to 690 nM) for 30 min at room temperature. Binding of TAK-079 to the cells was assayed using a PE-labeled goat anti-human IgG Fc antibody (Thermo Fisher Scientific, Waltham, MA). Following binding of the antibodies, RBCs were lysed using fixative-free high yield lyse (Thermo Fisher Scientific). The cells were then washed twice with magnetic-activated cell sorting buffer containing 0.5% BSA (Miltenyi Biotec, Gaithersburg, MD). Cell staining data was collected by flow cytometry using Attune NxT Acoustic Focusing Cytometer (Thermo Fisher Scientific) and analyzed using FlowJo software (Tree Star Inc.).

For cytolysis assays, 90 μ L of the whole blood was plated into each well on the 96-well U-bottom plates. Immediately after plating, the whole blood was treated with 0.69, 2.06, 6.17,

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18.52, 55.56, 166.67, and 500 nM of TAK-079 or PBS as the control for 6, 24, and 48 hours. At each time point, control and testing molecule treated whole blood in the 96-well U-bottom plate was transferred to a deep-well plate for RBC lysis. After lysis, cells were resuspended in stain buffer with CD16-BV605, CD56-PE, and CD38-FITC antibodies for surface marker staining. Cells were protected against light and incubated at 4°C for 20 min. After incubation, cells were centrifuged at 350 g at room temperature for 5 min and washed with 1× Annexin V binding buffer. Then the cells were resuspended in 1× Annexin V binding buffer containing Annexin V Alexa Fluor 647 at room temperature for 20 min. The stained cells were analyzed by BD FACSCelesta flow cytometer (BD Biosciences), and the data were recorded with BD FACSDiva software, Version 8.0.1. The NK cell viability at each testing concentration and the half maximal inhibitory concentration values of TAK-079 NK cell depletion at each time point were graphed and fitted with GraphPad Prism 7.04 (GraphPad, La Jolla, CA).

Dose-range Finding Studies in Healthy Monkeys. In 2 investigations, groups (n = 10) of healthy, purpose-bred, experimentally naïve cynomolgus monkeys received vehicle control, 0, 0.1, 0.3, and 1 mg/kg of TAK-079 weekly or at 3, 30, and 80 mg/kg bi-weekly via a 20-min intravenous infusion (Charles River Laboratories). The animals were evaluated for changes in clinical signs including twice daily cage side observations, post-dose observations, weekly detailed examinations, food consumption, and weekly body weight. Blood samples were collected pre- and post-dose in for evaluation of pharmacokinetics (PK), pharmacodynamics (PD), and primate antihuman antibodies. Investigations were conducted in cynomolgus monkeys in accordance with the testing facility standard operation procedures (Charles River Laboratories), which adheres to the regulations outlined in the Animal Welfare Act, 9 CFR, Parts 1, 2, and 3 (United

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States Department of Agriculture, 1989) and the conditions specified in the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, 1996).

Bioanalytical Procedure for Determination of TAK-079 Serum Concentrations. The concentration of TAK-079 in cynomolgus monkey serum was determined by an indirect enzyme-linked immunosorbent assay (ELISA) method utilizing a 96-well microtiter format. The plate was coated with a mouse anti-idiotypic antibody against TAK-079. Blanks, standards, and quality control samples containing TAK-079 at various concentrations as well as monkey serum samples were added to the coated microtiter plate and incubated for 55 to 65 min at room temperature. After washing, the detection antibody (peroxidase conjugated AffiniPure mouse anti-human IgG) was added, and incubated on the plate for an additional 55 to 65 min. The plate was washed again, and tetramethylbenzidine was added to the wells to generate a chromophore, and the development of color was stopped by the addition of a stopping solution (2N sulfuric acid). The absorbance at 450 nm was measured, and the TAK-079 concentrations were calculated using a 4-parameter logistic weighted ($1/y^2$) standard calibration curve.

Bioanalytical Procedure for Determination of Anti-TAK-079 Antibodies. Anti-TAK-079 antibody screening of cynomolgus monkey serum was determined by a qualitative, enhanced chemiluminescence (ECL) method. Undiluted cynomolgus monkey serum samples were incubated with 300 mM acetic acid. After acid dissociation, samples were incubated in a mixture of biotinylated TAK-079, TAK-079 labeled with SULFO-TAG, and 1.5 mM Trizma base to neutralize the acid and form an immune complex. This complex was then added to a streptavidin-coated MSD plate (Meso Scale Discovery, Rockville, MD) and allowed to bind. After washing, the complex was detected by the addition of MSD read buffer T to the plate and subsequent excitation of the SULFO-TAG™ (Meso Scale Discovery) via an electrochemical

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reaction of Ru(bpy)₃ to generate luminescence, which was read using the MSD Sector 6000. The quantity of luminescence correlates with the level of cynomolgus monkey anti-TAK-079 antibodies present in the serum of individual samples. The minimum required dilution of cynomolgus monkey serum for this assay was set at 1/33. For graphical representation, results from each animal were plotted as a function of antibody titer and Enrollment day. Serum samples with values at or below the plate-specific cut-point were plotted using a nominal titer value to facilitate plotting.

Monkey Collagen-induced Arthritis Model. Ethically responsible use of nonhuman primates was ensured by modeling the PD responses of cynomolgus monkeys to TAK-079 and then extrapolating the minimum number of animals required per treatment group to yield statistically significant differences in PD responses. Thirty-five naïve female cynomolgus monkeys, ranging from 3 to 4 years old, 2.5 to 3.3 kg, were obtained from Biomedical Research (GZ) Ltd (SNBL, Guangdong, China). Upon receipt, a health inspection was performed on each animal by the contract research organization (PharmaLegacy Laboratories, Inc., Shanghai, China). The animals were housed one per cage and acclimated for a minimum of 14 days prior to the commencement of the experimental procedures. The animal room was maintained at a temperature of 20°C to 29°C with a relative humidity of 40 to 70%, and a 12-hour light/dark cycle. Animals were trained for receiving intravenous infusions or oral gavage before study initiation. Monkeys had *ad libitum* access to vegetables, fruit, chow (Shanghai Shilin Biologic Science & Technology Co. Ltd., Shanghai, China) and water according to a conventional protocol. Cages were stratified within the racks to reduce the effect of any environmental influences on the study. This experimental design, all study protocols, and experimental procedures were reviewed and

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approved by the host Ethics Committee (PharmaLegacy Laboratories, Inc.), in accordance with Chinese law on animal experimentation.

Monkeys were selected for the study based on pre-screening criteria. One naïve animal was not administered with collagen and maintained as negative control for disease induction. The remaining animals were administered with bovine type II collagen (Sichuan, China) dissolved in 0.01N acetic acid (SPGC Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) to a final concentration of 4 mg/mL (Mihara et al., 2001; Uchiyama et al., 2008a; Uchiyama et al., 2008b; Kato et al., 2008) on Days 0 and 21 subcutaneously. Collagen was emulsified with an equal volume of Complete Freund's adjuvant (Sigma-Aldrich, St. Louis, MO). The animals were pre-sedated with ketamine (4 mg/kg, intramuscular injection), and additional anesthesia was applied, if needed, with 1.5 to 5% isoflurane via an inhalation anesthesia machine, Midmark Matrx VIP3000 Isoflurane (Midmark, Torrance, CA), to effect with a 0.8- to 1.5-L flow rate of oxygen. Any ulcerative skin lesions developing at the immunization sites were treated with iodine each time that an animal was sedated to prevent infection.

Eight animals receiving collagen were assigned to the prophylactic TAK-079 group on Day 0. The prophylactic TAK-079 animals received weekly infusions of 3 mg/kg TAK-079 starting from Day 7 onward, with the last dose administered on Day 56, for a total of 8 doses. Animals in this group were sacrificed on Day 63. The remaining immunized animals were treated as one group and received a weekly 30-min intravenous (i.v.) infusion of vehicle (saline) starting from Day 7 until an animal reached or exceeded $\geq 15\%$ of the maximum Clinical Arthritis Score. At this point, the animal was enrolled into the vehicle control group (n = 5), the therapeutic TAK-079 group (n = 5), or the therapeutic dexamethasone group (n = 4), and enrollment continued on a rolling basis due to the time differences in disease onset. For animals enrolled in the vehicle

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control group, weekly infusions of vehicle were performed for 5 weeks. Animals in this group were sacrificed 7 days after the last dose. For animals enrolled in the therapeutic TAK-079 group, infusions were performed weekly for 5 weeks. Animals in this group were sacrificed 7 days after the last dose. Animals enrolled in the therapeutic dexamethasone group received daily oral gavage for 5 weeks and were sacrificed 1 day after the last dose to ensure that animals in the 3 therapeutic groups were exposed to agents for an identical duration (Figure 1). The animals were observed daily for signs of ill health and general reaction to treatments. All exceptions to normal healthy appearance and behavior were recorded and detailed in standard clinical observations forms.

Assessment of Arthritic Activity. Body weight of monkeys was measured once during the acclimation period (5 days prior to the start of the experiment), on the day prior to each cycle of disease induction, and then once weekly through the end of the study. The number of animals in a group with joint swelling was recorded on Days 0 and 21, and then daily through the end of study. The number of proximal interphalangeal (PIP) joints (each hand and foot, respectively) with joint swelling was recorded for each animal on Days 0 and 21, and then once weekly through the end of the study. The longitudinal and transverse axes of PIP joints of the fore and hind limbs (without thumb) were measured with calipers on Days 0 and 21, and then once weekly after disease onset through the end of study for all PIPs with arthritis. Mean oval area of 16 PIP joints was calculated and adopted as individual data. The oval area of each PIP was calculated using the following formula: $\text{Oval area} = \text{longitudinal axis} \times \text{transverse axis} \times 3.14 \times 1/4$. Percent oval area change and joint swelling was calculated using the following formula: $\% \text{ change of oval area} = (\text{mean oval area on Day X} / \text{mean oval area on the day of 1st sensitization})$

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$\times 100$; joint swelling = (mean oval area on Day X - mean oval area on the day of 1st sensitization).

Clinical Arthritis Score. The severity of arthritis of each limb in monkeys was scored on Days 0 and 21, and then once weekly through the end of the study according to these criteria: normal (0); mild arthritis, subtle but definite (1); moderate swelling (2); severe arthritis with significant swelling and/or noticeable joint deformity (3). The following joints of each paw were examined and scored: 15 joints in total including 5 metacarpophalangeal (MCP) joints, 4 PIP joints, 5 distal interphalangeal (DIP) joints, and 1 wrist/ankle. The knee/elbow of each limb was also evaluated for disease severity. The arthritic score of each animal was the total score of each individual joint with a maximum score of 192 ($16 \times 3 \times 4$; 16 = total number of joints plus the knee/elbow for each limb, 3 = maximum score for each individual joint, and 4 = number of limbs per monkey).

Blood Collection and Analysis. Blood samples were used for complete blood count, blood chemistry, and serum preparation for antibody, PK, and anti-drug antibody measurements, and were collected from animals at the time points indicated below. For animals from the naïve group and the prophylactic TAK-079 group, blood was collected twice, once prior to dosing and once on the following day, during Weeks 1 and 5, just before TAK-079 administration during Weeks 2, 3, 4, 6, 7, and 8, and when animals were terminated during Week 9. All other animals had weekly blood collections up until the time that the disease reached the threshold of 15% of maximum arthritis score. After assignment to the vehicle group, the therapeutic TAK-079 group, or the therapeutic dexamethasone group, blood samples were collected weekly just prior to dosing and at termination. In addition, samples were collected on the day after the first dose of drug and the day after the 5th dose. For the vehicle control, prophylactic TAK-079, therapeutic TAK-079, and dexamethasone groups, blood samples for flow cytometry were collected before

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the first dose (on the day of infusion), on the day following the first dose, before the second dose (on the day of dosing), before the 5th dose (on the day of dosing), and on the day of termination. For the therapeutic TAK-079 group, blood samples for flow cytometry were collected before the first dose of drug, on the day following first dose, before the 8th dose, before the 29th dose, and on the day of termination.

Radiographic Examination. Examinations were performed for each joint (DIP, PIP, 1st interphalangeal, and MCP - sites typically involved in human RA and lupus arthritis) on the hands and feet of live, anesthetized animals at study termination. Radiographic grading was carried out in a blinded fashion, based on a 0 to 4 grading system: normal (0); minor deformity in articular cartilage layers, and/or subchondral bone regions (1); severe deformity in articular cartilage layers and subchondral bone regions, a small amount of osteophytes present at periosteal surfaces and joint margins that are fuzzy but still visible (2); the same types of changes observed in grade 2 but advanced further and a large amount of osteophytes present at periosteal surfaces, the joint cavity is indistinguishable or invisible (3); the same type of changes as in grade 3 but advanced further, the joint cavity becomes undetectable, bones appeared sclerotic or ankylosing, major disfiguration occurs (4).

Histopathology and Histomorphometry of Tissue from Arthritic Monkeys. Animals were euthanized by exsanguination under anesthesia at the end of the study. The paws, spleen, colon, and lymph nodes (mesenteric and inguinal) were collected and fixed in neutral buffered formalin. PIP joints and DIP joints were decalcified with 15% EDTA, dehydrated, and embedded in paraffin (8 blocks/paw × 4 paws × 22 animals = 704 blocks). Frontal coronal sections of the joints were obtained using a rotary microtome (Leica Biosystems, Shanghai, China).

Histopathology scoring was performed using Toluidine Blue (Carolina Biological, Shanghai,

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China) stained sections (32×22 animals = 704 slides) by a bone histopathologist in a blinded manner. The histology sections were qualitatively assessed for cell infiltration, pannus, cartilage lesion, and bone resorption on a 5-point scale wherein a score of 0 = Normal/Negative for the feature, 1 = A few scattered features, 2 = Mild incidence, 3 = Moderate incidence, and 4 = Severe/Marked incidence. Quantitative histomorphometry was performed using Osteomeasure software (OsteoMetrics, Inc., Atlanta, GA) interfaced with a Nikon Eclipse E400 light/fluorescent microscope and video subsystem (Nikon Instruments, Inc., Shanghai, China). Histomorphometric measurements of the articular area and surface were performed blinded, by a bone histopathologist, using Toluidine Blue stained slides (32×22 animals = 704 slides).

Statistical Analysis. Data are presented as mean \pm standard error of the mean. Statistical analyses were conducted with GraphPad Prism on each parameter among naïve, model, and reference drug (dexamethasone) and test article groups. $p < 0.05$ was considered significantly different. Post-hoc multiple comparisons among the group data were conducted in this study for differences identified by ANOVA in order to identify which treatment groups differed.

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RESULTS

The Expression Profile of CD38 in Cynomolgus Monkey.

To determine if the cynomolgus monkey could be an adequate model for assessing the potential effects of targeting CD38 in humans, the expression profile of the CD38 antigen was compared across 15 different types of tissues collected from healthy human and cynomolgus monkey donors. Immunohistochemistry revealed that TAK-079 bound to mononuclear leukocytes in colon, stomach, small intestine, bone marrow, and lymph node, as well as some endothelial cells in the lamina propria of colon, stomach, and small intestine in both species (Table 1).

Conversely, in humans but not monkeys TAK-079 also bound to mononuclear leukocytes in lung, prostate, and uterus (cervix), and to prostate acinar epithelium. TAK-079-binding was not observed in heart, kidney, pancreas, skin, and endometrium of the uterus of either species.

TAK-079 staining was generally moderate to marked (3+ to 4+) in intensity in human tissues and minimal to mild (1+ to 2+) in intensity in cynomolgus monkey tissues (Table 1), which could reflect the difference in affinities of TAK-079 for human versus monkey CD38 and/or to differences in the magnitude of expression of CD38. The staining pattern was primarily cytoplasmic and cell membranes were stained in some cells (Table 1). Overall, it was concluded that the monkey was an adequate model species to assess the potential pharmacologic effect(s) of targeting CD38 in a model of autoimmune disease.

TAK-079 Binds to CD38 Expressed by Cynomolgus Monkeys.

The amino acid sequence of the human CD38 protein exhibits 91% amino acid identity with its cynomolgus monkey orthologue. A comparison of the binding epitope of TAK-079 in human

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CD38 with the corresponding sequence in monkey CD38 revealed a single amino acid substitution of a lysine with a glutamate at position 274. To determine if TAK-079 could be utilized for assessing the potential effects of targeting CD38 in monkeys, monkey CD38 was expressed in Chinese hamster ovary (CHO) cells. TAK-079 bound to monkey CD38 with a half-maximal effective concentration (EC_{50}) of 4.5 nM (Figure 2A), which is sufficient for assessing the potential effects of specifically targeting CD38 in monkeys.

TAK-079 also bound to monkey B, NK, and T cells in whole blood. NK cells exhibited a higher mean fluorescent intensity than B cells and T cells (Figure 2B), indicating that monkey NK cells express a higher density of CD38 per cell. After incubation within monkey whole blood *in vitro*, TAK-079 cytolysed NK cells in a dose-dependent manner (Figure 2C), exhibiting a mean EC_{50} of 29.6 nM. This cytolytic activity, as well as similarity in the expression profiles of CD38, illustrate that the cynomolgus monkey is a suitable model species for investigating the potential pharmacologic effect(s) of TAK-079 *in vivo*.

The Pharmacologic Effects of TAK-079 in Healthy Monkeys.

To characterize the pharmacologic effects of TAK-079 *in vivo*, healthy cynomolgus monkeys were infused i.v. with TAK-079 at 0.1, 0.3, and 1 mg/kg weekly and at 3, 30, and 80 mg/kg bi-weekly for 3 months, and a subset of animals were monitored for 3 months after the last dose (Figure 3A). For most animals, peak TAK-079 concentrations occurred at the end of the infusion and were dose proportional (Table 2). Exposure (area under concentration-time curve from dose administration to 366 hours post-dose [AUC_{366}]) at steady state generally increased in a dose-proportional manner in animals that did not exhibit anti-TAK-079 antibodies and accumulated 1.7- to 2.4-fold by Week 13 (Table 2), which is consistent with decreased clearance due to

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target-mediated drug disposition of TAK-079. The half-life after the last dose of 3 and 80 mg/kg TAK-079 in animals that did not exhibit anti-TAK-079 antibodies was 237 and 415 hours, respectively (Table 2). No gender differences in PK characteristics were observed.

The NK cell population expressed CD38 with a uniformly high density (Figure 2B) and was reduced in peripheral blood after the first infusion of TAK-079 with a median effective dose (ED₅₀) of 0.3 mg/kg (Figure 3B) at a corresponding maximum serum concentration (C_{max}) of 7.78 µg/mL and an overall mean exposure of 665 hr*µg/mL at Week 13 (Table 2). In contrast, the B cell population expressed CD38 heterogeneously with a lower median density than NK cells (Figure 2B) and was reduced in peripheral blood after the initial infusion of TAK-079 with an ED₅₀ of 1.0 mg/kg (Figure 3C) at a corresponding C_{max} of 28.6 µg/mL and an overall mean exposure of 2700 hr*µg/mL at Week 13 (Table 2). The T cell population also expressed CD38 heterogeneously with an even lower median density than that of B cells (Figure 2B) and was reduced in peripheral blood after the first infusion of TAK-079 with an ED₅₀ of 30 mg/kg (Figure 3D) at a corresponding C_{max} of 962 µg/mL and an overall mean exposure of 228,000 hr*µg/mL at Week 13 (Table 2). Collectively, these dose-range finding data indicated that a weekly dose of 3 mg/kg would sustain a reduction of the total NK, B, and T cell populations by greater than 80%, 60%, and 20% from baseline levels in peripheral blood, respectively.

The Profile of TAK-079 in Collagen-induced Arthritis.

Potential efficacy of TAK-079 was investigated with a collagen-induced arthritis (CIA) model in cynomolgus monkeys using clinical indices based upon the similarity of the monkey immune system, joints, and skeletal anatomy to human counterparts. To induce arthritis, monkeys were

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treated intradermally on Days 0 and 21 with type II collagen (Figure 1), and the successful immunization of each animal was confirmed by the emergence of anti-collagen antibodies (Supplemental Figure 1). One group of animals was treated on Day 7 with a 3 mg/kg prophylactic TAK-079 treatment regimen that continued for 8 weeks. In the 3 therapeutic groups, monkeys that had overt disease were randomized and administered a vehicle control, 3 mg/kg TAK-079, or 0.1 mg/kg dexamethasone. Therapeutic treatment continued for 5 weeks after initiation of treatment (Figure 1).

TAK-079 was well tolerated in both the prophylactic and therapeutic regimens. The healthy control monkey gained body weight over time, whereas the collagen-immunized monkeys in the other four groups started to lose body weight from Day 7 (Figure 4A), indicating a systemic impact associated with the development of arthritis. Based on the percentage change in body weight against baseline body weight upon enrollment, the animals in the prophylactic TAK-079 group, the therapeutic TAK-079 group, and the therapeutic dexamethasone group were found to regain their body weight beginning 14 days after the start of treatment, suggesting a therapeutic benefit from both TAK-079 and the dexamethasone positive control treatment. Weight gain approached near-normal levels in the TAK-079- and dexamethasone-treated monkeys compared with a healthy, untreated control, while arthritic animals treated with vehicle lost weight (Figure 4A).

The severity of arthritis was assessed using the Clinical Arthritis Score, which is a global assessment of disease activity that considers every measurable joint in the animal over the course of the study using a 192-point scoring system. Vehicle-treated animals exhibited progressive disease, with increasing clinical scores over the course of the study (Figure 4B). Prophylactic exposure to TAK-079 prevented the development of arthritis significantly ($p < 0.01$) as compared

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to the vehicle control. Similarly, therapeutic treatment with TAK-079 or dexamethasone significantly inhibited the development of arthritis as compared to vehicle control ($p < 0.05$) and reduced arthritis scores from the pre-treatment baselines (Figure 4B). Similar effects were observed in the subset of PIP joints with respect to both the number of inflamed PIP joints (Figure 4C) and the overall mean swelling of all PIP joints (Figure 4D). To obtain a comprehensive assessment of the arthritic joints and the potential for TAK-079 to have “disease modification” activity in human arthritis, radiographic examination was performed for every IP and MCP joint. Prophylactic exposure to TAK-079 prevented damage in PIP (Supplemental Figure 2), DIP (Figure 4E) and MCP (Figure 4F) joints significantly ($p < 0.01$) as compared to vehicle control. Similarly, therapeutic exposure to TAK-079 or dexamethasone resulted in significantly ($p < 0.05$) less damage in PIP (Supplemental Figure 2), DIP (Figure 4E), and MCP (Figure 4F) joints than in vehicle control animals.

To characterize components of the progressive arthritis, DIP and PIP joints were analyzed histologically with respect to cell infiltration, pannus severity, cartilage damage, bone resorption, and osteophyte formation (Figures 5 and 6). Prophylactic exposure to TAK-079 resulted in composite scores, which were significantly less ($p < 0.01$) than the vehicle control value and comparable in magnitude to animals not immunized with collagen (Figure 6A). TAK-079 had a similar effect on each component; scores were significantly less ($p < 0.01$) than the vehicle control value for pannus (Figure 6B), infiltrating leukocytes (Figure 6C), cartilage lesions (Figure 6D), bone resorption (Figure 6E), and osteophyte formation (Figure 6F). Similar differences of lesser magnitude were also observed for therapeutic treatment with TAK-079 or dexamethasone (Figure 6A to E), although not all differences reached a statistical significance.

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Quantitative histomorphometry was performed on all DIP and PIP joints in blind by a board-certified, veterinary pathologist, although only DIP results are presented in Figure 7, including total articular cartilage area (Figure 7A), thickness of damaged articular cartilage (Figure 7B), percentage of damaged articular surface (Figure 7C), and osteophyte area versus total periosteal surface (Figure 7D). All the parameters illustrated that prophylactic exposure to TAK-079 prevented the development of articular damage significantly. Therapeutic treatment with TAK-079 reduced the severity of disease, although some histomorphometric measures did not achieve statistical significance. The therapeutic effects of TAK-079 were similar to dexamethasone administered therapeutically.

Levels of C-reactive protein (CRP) (Figure 8A), alkaline phosphatase (ALP) (Figure 8B), and albumin (Supplemental Figure 3) correlated with disease severity. Prophylactic treatment with TAK-079 prevented the inflammation-associated increases in CRP (Figure 8A) and ALP (Figure 8B) observed in the vehicle control animal. Therapeutic treatment with TAK-079 caused a rapid decrease in CRP and ALP, whereas prolonged treatment with dexamethasone was required to reduce CRP and ALP levels (Figure 8A and B, respectively). No evidence for liver damage was found in serum chemistry parameters (e.g., increased alanine aminotransferase or aspartate transaminase), which suggests that the increased ALP was due to development of bone disease in animals with CIA, and the reduced ALP was due to reduced bone damage in TAK-079-exposed animals. Serum chemistry values for creatinine, blood urea nitrogen, glucose, and total protein varied over time but showed no consistent correlation with arthritis severity or treatment regimen (data not shown).

Hematology, complete blood count, and differential analysis were conducted during the study, and key parameters were reported. No change was observed in levels of RBCs after exposure to

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TAK-079 or dexamethasone (Figure 9A). Furthermore, the hematocrit count was decreased after development of arthritis and increased back toward the naïve control levels after treatment with either TAK-079 or dexamethasone (Figure 9B). Reticulocytes were elevated after development of arthritis, and both TAK-079 and dexamethasone treatment reduced the reticulocyte count toward the normal level found in the naïve control (Figure 9C). Both platelet and neutrophil levels were elevated with arthritis, and TAK-079 treatment reduced both platelet and neutrophil levels toward the naïve control, whereas dexamethasone had no effect (Figure 9D and E, respectively). In contrast, total lymphocyte levels were decreased with disease relative to the naïve control and treatment with TAK-079, as expected, further reduced lymphocyte levels, whereas treatment with dexamethasone had no effect (Figure 9F).

Among subsets of lymphocytes in peripheral blood, NK cells were reduced >95% below baseline levels (Figure 10A) within 24 hours of exposure to TAK-079, whereas baseline levels of B cells (Figure 10B), T cells (Figure 10C), and monocytes (Figure 10D) were reduced by a maximum of 60%, 55%, and 50%, respectively. NK and B cell reductions were sustained throughout dosing, whereas T-cell and monocyte reductions were transient and only observed after the first infusion. A similar reduction pattern was seen in each cell population after TAK-079 prophylactic treatment, except this group of animals had lower monocyte levels before treatment, and the monocyte levels did not change in response to treatment. No differences were seen in NK, B, T cells, or monocyte counts between vehicle and dexamethasone-treated animals (Figure 10A, B, C, and D, respectively).

Serum bioanalysis was conducted to measure concentrations of TAK-079 and anti-TAK-079 antibodies. Substantial exposure was achieved in each animal dosed prophylactically (Figure 11A) or therapeutically (Figure 11B) with the C_{max} ranging from 24 to 97 $\mu\text{g/mL}$. All

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animals exposed to TAK-079 exhibited trough concentrations that exceeded the EC₅₀ for CD38 saturation (Figure 2A) and NK cell lysis (Figure 2C) *in vitro*. Anti-TAK-079 antibodies were detected in 4 of 7 animals in the prophylactic group (Figure 11C) from 14 to 30 days after initial dosing and until the end of the study. Two animals exhibited high titers, which corresponded with low concentrations of TAK-079 in these animals by Day 31 (Figure 11A), indicating that these anti-TAK-079 antibodies may be affecting clearance. Anti-TAK-079 antibodies were also detected in and 4 of 5 animals in the therapeutic group (Figure 11D) from 21 days after initial dosing until the end of the study and two animals also exhibited high titers, which corresponded with low concentrations of TAK-079 in these animals by the end of the study (Figure 11B). Nonetheless, all animals were included in data analyses because most target cells remained reduced from baseline levels throughout the duration of the study (Figure 10A, B, and D) with the exception of T cells after the second therapeutic dose (Figure 10C).

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DISCUSSION

A deficiency of CD38 in mice was reported to result in attenuated forms of CIA (Rosal-Vela A et al. 2016), illustrating a nonredundant function(s) of this molecule in a model of autoimmune disease; however, the role of CD38 cells in primate models has not been investigated. In addition, numerous studies have indicated that the overall level of CD38 expression on peripheral blood cells correlates positively with disease activity in RA and SLE patients (Cole et al., 2018; Kraan et al., 1999; Vital et al., 2011). The anti-CD38 mAb TAK-079 depleted plasma cells (Smithson et al., 2017; Cole et al., 2018) in blood or bone marrow samples from patients with RA (manuscript in preparation) or SLE (Wang et al., 2016) *in vitro*. In contrast to other anti-CD38 mAbs in development (e.g., daratumumab, isatuximab, and MOR202), TAK-079 binds to CD38 expressed by cynomolgus monkeys and provides the unique opportunity to determine if reducing the level of cells expressing CD38 would prevent and/or ameliorate inflammation and tissue damage in a non-human primate model of autoimmunity.

The cynomolgus monkey was determined to be a suitable model for assessing potential effects of TAK-079 in an autoimmune disease because the CIA in these monkeys features a symmetrical small joint polyarthritis that resembles human RA and lupus arthritis (Mihara et al., 2001; Uchiyama et al., 2008a; Uchiyama et al., 2008b; Kato et al., 2008), the TAK-079-bound cells are similar between these species (Table 1), and TAK-079 binds to monkey CD38 with high affinity (Figure 2). We concluded that a weekly TAK-079 dose of 3 mg/kg would be adequate for assessing the potential role of CD38 in CIA because dose-range studies in healthy monkeys indicated that a weekly dose of 3 mg/kg of TAK-079 would reduce the total NK, B, and T cell populations by more than 80%, 60%, and 20% from baseline levels in peripheral blood, respectively (Figure 3B, C, and D). Similar reductions in NK, B, and T cells were achieved in the

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CIA model for prophylactic dosing (Figure 10A, B, and C) despite the emergence of anti-TAK-079 antibodies in some animals (Figure 11C and D). The decrease in concentration of TAK-079 over time in 3 animals exposed prophylactically (Figure 11A) indicate that these antibodies may have increased clearance. Nonetheless, NK, B, and T cells had not recovered to baseline levels by study termination (Figure 10A, B, and C), indicating that exposures to TAK-079 were sufficient to maintain PD effects throughout the investigation. These animals were therefore included in all analyses. This was also generally true for therapeutic treatment with TAK-079; NK and B cells had not recovered to baseline levels by study termination (Figure 10A and B), indicating that exposures to TAK-079 were sufficient to maintain PD effects throughout the investigation. In contrast, total T cell counts had recovered to baseline levels by study termination (Figure 10C), which may have resulted from reconstitution with naïve T cells that do not express CD38 and are not depleted by TAK-079.

Prophylactic administration of TAK-079 was included in this study as a comparator to assess the relative effectiveness of therapeutic administration. Prophylactic administration of TAK-079 prevented arthritis development, as illustrated consistently across all assessments, whereas therapeutic treatment with TAK-079 inhibited the development of arthritis and articular damage (Figures 4, 5, 6, and 7). Histologic assessment of joints demonstrated that TAK-079 exerted relatively broad effects, preventing pannus formation (Figure 6B), infiltration (Figure 6C), cartilage lesions (Figure 6D), bone resorption (Figure 6E), and osteophyte formation (Figure 6F). It is noteworthy that therapeutic treatment with TAK-079 and dexamethasone also inhibited these progressive histologic changes, albeit to a lesser magnitude than prophylactic administration. The arthritis scores decreased over time with therapeutic treatment (Figure 4B, C,

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and D), indicating potential reversal of damage. Collectively, these data demonstrated consistent disease-modifying effects with exposure to TAK-079 prophylactically and therapeutically.

The effect of these therapeutic TAK-079 and dexamethasone treatments appears comparable to one another for the regimes investigated. Therapeutic use of steroids, such as dexamethasone, is highly effective in treating human autoimmune diseases, including RA and SLE; however, deleterious side-effects (e.g., osteoporosis, hypertension, diabetes, weight gain, cataracts, glaucoma, thinning of the skin, and bruising) restrict chronic use of these therapeutics. The potential for targeted CD38 cell depletion to provide substantial clinical efficacy in human autoimmune diseases without the deleterious side-effects of steroids or other contemporary therapies warrants future clinical investigation.

The prophylactic and therapeutic effects of TAK-079 were associated with sustained reductions in the blood level of total lymphocytes (Figure 9F), NK, B, and T cells (Figures 10A, B, and C); a transient reduction in monocytes (Figure 10D); and no changes in RBCs (Figure 9A), platelets (Figure 9D), and neutrophils (Figure 9E). These PD data illustrate that differences exist in the sensitivity of cells expressing CD38 to reduction by TAK-079. Reduction generally correlated with the density of CD38 expression by the cell; the NK cell population uniformly expressed the highest median density of CD38 of all the cell types examined (Figure 2B) and was most sensitive to TAK-079 (Figures 3B and 10A). The highest density of CD38 expressed on B cells was approximately 3-fold lower than on NK cells (Figure 2B), and the B cell population was less sensitive to TAK-079 than NK cells (Figures 3C and 10A). CD38 was generally expressed at lower median densities on T cells than on B cells (Figure 2B), and T cells were less sensitive to reduction by TAK-079 than were B and NK cells (Figures 3D and 10C). Cells that express low densities of CD38 (e.g., RBCs) or do not express CD38 (e.g., neutrophils) were not affected by

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TAK-079 (Figure 9A and E). An exception were monocytes, which expressed CD38 uniformly at intermediate densities and were only transiently reduced by TAK-079 (Figure 10D). The kinetics of the transient reduction in monocytes is distinct from the sustained reduction observed for B, T, and NK cells and indicates distinct mechanisms. A direct cytolysis of NK cells by TAK-079 was observed *in vitro* (Figure 2C), and a sustained reduction in NK occurred after a single dose of TAK-079 *in vivo* (Figure 3 and Table 2), which indicates that CDC and/or ADCC mediated NK cell reductions *in vivo*. In contrast, monocytes were not cytolysed *in vitro* (data not shown) and did not exhibit sustained reduction *in vivo* (Figure 10D), which indicates an alternate mechanism (e.g., margination) mediated transient reductions *in vivo*. Resistance of human monocytes to cytolysis by TAK-079 has also been observed in healthy subjects (Fedyk et al., 2018) and described for daratumumab in multiple myeloma patients (Nijhof et al., 2016). The expression of inhibitors of CDC and ADCC by human monocytes did not correlate significantly with resistance to cytolysis by daratumumab, and it remains unknown why monocytes are relatively insensitive to daratumumab and TAK-079.

While the reduction in NK cells by TAK-079 is qualitatively consistent with NK cell reduction by daratumumab in refractory myeloma patients, quantitative differences exist. The i.v. infusion dose (0.3 mg/kg) and the maximal concentration ($C_{\max} = 4.32 \mu\text{g/mL}$) of TAK-079 required to maintain peripheral blood NK cells at 50% below baseline levels in monkeys (Figure 3B) were approximately 80- and 116-fold lower than the corresponding i.v. infusion dose (24 mg/kg) and the maximal concentration ($C_{\max} \sim 573 \mu\text{g/mL}$) required for comparable reductions of NK cells by daratumumab in refractory myeloma patients (Casneuf et al., 2017; Clemens et al., 2017). This difference does not appear to be attributable to differences in binding affinities; TAK-079 binds to CHO cells expressing monkey CD38 with an EC_{50} of 4.5 nM (approximately 700 ng/mL),

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which is similar to daratumumab binding to CHO cells expressing human CD38 ($EC_{50} = 470$ ng/mL) (Center for Drug Evaluation and Research, 2015). Alternatively, the observed difference may be due to another factor(s). Nonetheless, a more definitive comparison requires PK and PD data for TAK-079 in refractory myeloma patients because daratumumab does not cross-react with CD38 from mouse, rat, rabbit, pig, and cynomolgus and rhesus monkeys (EMA, 2016).

In conclusion, a reduction in cells expressing CD38 with the cytolytic antibody TAK-079 prevented the development of CIA in monkeys when administered prophylactically and reversed disease progression when administered therapeutically. A previous study utilizing blood and bone marrow samples from SLE patients demonstrated that TAK-079 depleted 80% of short- and long-lived plasma cells and reduced autoantibodies (e.g., VH4-34 9G4+, anti-Ro, and anti-dsDNA) *in vitro* (Wang et al., 2016). These collective data indicate that this therapeutic strategy may be effective in treating human RA and SLE, as well as other autoimmune diseases.

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REFERENCES

Alexander T, Sarfert R, Klotsche J, Kuhl AA, Rubbert-Roth A, Lorenz HM, Rech J, Hoyer BF, Cheng Q, Waka A, Taddeo A, Wiesener M, Schett G, Burmester GR, Radbruch A, Hiepe F, and Voll RE (2015) The proteasome inhibitor bortezomib depletes plasma cells and ameliorates clinical manifestations of refractory systemic lupus erythematosus. *Ann Rheum Dis* **74**: 1474-1478.

Banchereau R, Hong S, Cantarel B, Baldwin N, Baisch J, Edens M, Cepika AM, Acs P, Turner J, Anguiano E, Vinod P, Kahn S, Obermoser G, Blankenship D, Wakeland E, Nassi L, Gotte A, Punaro M, Liu YJ, Banchereau J, Rossello-Urgell J, Wright T, and Pascual V (2016) Personalized Immunomonitoring Uncovers Molecular Networks that Stratify Lupus Patients. *Cell* **165**: 551-565.

Casneuf T, Xu XS, Adams HC 3rd, Axel AE, Chiu C, Khan I, Ahmadi T, Yan X, Lonial S, Plesner T, Lokhorst HM, van de Donk NWCJ, Clemens PL, and Sasser AK (2017) Effects of daratumumab on natural killer cells and impact on clinical outcomes in relapsed or refractory multiple myeloma. *Blood Adv* **1**: 2105-2114.

Center for Drug Evaluation and Research (2015) Application Number: 761036Orig1s000 Pharmacology Review(s).

Clemens PL, Yan X, Lokhorst HM, Lonial S, Losic N, Khan I, Jansson R, Ahmadi T, Lantz K, Zhou H, Puchalski T, and Xu XS (2017) Pharmacokinetics of Daratumumab Following Intravenous Infusion in Relapsed or Refractory Multiple Myeloma After Prior Proteasome Inhibitor and Immunomodulatory Drug Treatment. *Clin Pharmacokinet* **56**: 915-924.

JPET #256602

Cole S, Walsh A, Yin X, Wechalekar MD, Smith MD, Proudman SM, Veale DJ, Fearon U, Pitzalis C, Humby F, Bombardieri M, Axel A, Adams H 3rd, Chiu C, Sharp M, Alvarez J, Anderson I, Madakamutil L, Nagpal S, and Guo Y (2018) Integrative analysis reveals CD38 as a therapeutic target for plasma cell-rich pre-disease and established rheumatoid arthritis and systemic lupus erythematosus. *Arthritis Res Ther* **20**: 85.

Deane KD (2014) Preclinical rheumatoid arthritis (autoantibodies): an updated review. *Curr Rheumatol Rep* **16**: 419.

EMA Committee for Medicinal Products for Human Use (CHMP) (2016) Assessment report: Darzalex; International non-proprietary name: daratumumab; Procedure No. EMEA/H/C/004077/0000. April 2016 EMA/278085/2016.

Fedyk ER, Berg D, Smithson G, Estevam J, Mclean L, Allikmets K, and Palumbo A (2018) A single administration of the cytolytic CD38 antibody TAK-079 to healthy subjects: tolerability, pharmacokinetics and pharmacodynamics. *Blood* **132**: 3249.

Grammer AC, Slota R, Fischer R, Gur H, Girschick H, Yarboro C, Illei GG, and Lipsky PE (2003) Abnormal germinal center reactions in systemic lupus erythematosus demonstrated by blockade of CD154-CD40 interactions. *J Clin Invest* **112**: 1506-1520.

Hale M, Rawlings DJ, Jackson SW (2018) The long and the short of it: insights into the cellular source of autoantibodies as revealed by B cell depletion therapy. *Curr Opin Immunol* **55**: 81-88.

Institute for Laboratory Animal Research, Committee for the Update of the Guide for the Care and Use of Laboratory Animals (1996) *Guide for the Care and Use of Laboratory Animals*, 8th

JPET #256602

ed. National Academy Press, Washington DC. <https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf>.

Kato A, Matsuo S, Takai H, Uchiyama Y, Mihara M, and Suzuki M (2008) Early effects of tocilizumab on bone and bone marrow lesions in a collagen-induced arthritis monkey model. *Exp Mol Pathol* **84**: 262-270.

Kraan MC, Haringman JJ, Post WJ, Versendaal J, Breedveld FC, and Tak PP (1999) Immunohistological analysis of synovial tissue for differential diagnosis in early arthritis. *Rheumatology (Oxford)* **38**: 1074-1080.

Krejci J, Casneuf T, Nijhof IS, Verbist B, Bald J, Plesner T, Syed K, Liu K, van de Donk NW, Weiss BM, Ahmadi T, Lokhorst HM, Mutis T, and Sasser AK (2016) Aratumumab depletes CD38+ immune regulatory cells, promotes T-cell expansion, and skews T-cell repertoire in multiple myeloma. *Blood* **128**: 384-294.

Kubo S, Nakayamada S, Yoshikawa M, Miyazaki Y, Sakata K, Nakano K, Hanami K, Iwata S, Miyagawa I, Saito K, and Tanaka Y (2017) Peripheral immunophenotyping identifies three subgroups based on T cell heterogeneity in lupus patients. *Arthritis Rheumatol* **69**: 2029-2037.

Kurts C, Panzer U, Anders HJ, and Rees AJ (2013) The immune system and kidney disease: basic concepts and clinical implications. *Nat Rev Immunol* **13**: 738-753.

Martin F and Chan AC (2006) B cell immunobiology in disease: evolving concepts from the clinic. *Annu Rev Immunol* **24**: 467-496.

Mihara M, Kotoh M, Nishimoto N, Oda Y, Kumagai E, Takagi N, Tsunemi K, Ohsugi Y, Kishimoto T, Yoshizaki K, and Takeda Y (2001) Humanized antibody to human interleukin-6

JPET #256602

receptor inhibits the development of collagen arthritis in cynomolgus monkeys. *Clin Immunol* **98**: 319-326.

Nijhof IS, Casneuf T, van Velzen J, van Kessel B, Axel AE, Syed K, Groen RW, van Duin M, Sonneveld P, Minnema MC, Zweegman S, Chiu C, Bloem AC, Mutis T, Lokhorst HM, Sasser AK, and van de Donk NW (2016) CD38 expression and complement inhibitors affect response and resistance to daratumumab therapy in myeloma. *Blood* **128**: 959-970.

Nutt SL, Hodgkin PD, Tarlinton DM, and Corcoran LM (2015) The generation of antibody-secreting plasma cells. *Nat Rev Immunol* **15**: 160-171.

Radbruch A, Muehlinghaus G, Luger EO, Inamine A, Smith KG, Dorner T, and Hiepe F (2006) Competence and competition: the challenge of becoming a long-lived plasma cell. *Nat Rev Immunol* **6**: 741-750.

Rosal-Vela A, Barroso A, Giménez E, García-Rodríguez S, Longobardo V, Postigo J, Iglesias M, Lario A, Merino J, Merino R, Zubiaur M, Sanz-Nebot V, and Sancho J (2016) Identification of multiple transferrin species in the spleen and serum from mice with collagen-induced arthritis which may reflect changes in transferrin glycosylation associated with disease activity: The role of CD38. *J Proteomics* **16**: 127-137.

Sjowall C, Hjorth M, and Eriksson P (2017) Successful treatment of refractory systemic lupus erythematosus using proteasome inhibitor bortezomib followed by belimumab: description of two cases. *Lupus* **26**: 1333-1338.

JPET #256602

Smithson G, Zalevsky J, Korver W, Roepcke S, Dahl M, Zhao L, Yuan J, McLean L, and Elias K (2017) TAK-079 is a high affinity monoclonal antibody that effectively mediates CD38+ cell depletion. *J Immunol* **198** (1 Supplement): 224.20.

Stohl W, Hiepe F, Latinis KM, Thomas M, Scheinberg MA, Clarke A, Aranow C, Wellborne FR, Abud-Mendoza C, Hough DR, Pineda L, Migone TS, Zhong ZJ, Freimuth WW, Chatham WW, BLISS-52 Study Group, and BLISS-76 Study Group (2012) Belimumab reduces autoantibodies, normalizes low complement levels, and reduces select B cell populations in patients with systemic lupus erythematosus. *Arthritis Rheum* **64**: 2328-2337.

Uchiyama Y, Yorozu K, Hashizume M, Moriya Y, and Mihara M (2008a) Tocilizumab, a humanized anti-interleukin-6 receptor antibody, ameliorates joint swelling in established monkey collagen-induced arthritis. *Biol Pharm Bull* **31**: 1159-1163.

Uchiyama Y, Koike N, and Mihara M (2008b) Anemia in monkey collagen-induced arthritis is correlated with serum IL-6, but not TNFa. *Rheumatol Int* **28**: 879-883.

United States Department of Agriculture (1989) *Animal Welfare Act (9 CFR, Parts 1, 2, and 3)*.
<https://www.nal.usda.gov/awic/final-rules-animal-welfare-9-cfr-parts-1-2-and-3>.

Vital EM, Dass S, Buch MH, Henshaw K, Pease CT, Martin MF, Ponchel F, Rawstron AC, and Emery P (2011) B cell biomarkers of rituximab responses in systemic lupus erythematosus. *Arthritis Rheum* **63**: 3038-3047.

Wang X, Dahl M, Nguyen D, Jenks S, Cashman K, Lee FEH, McLean L, and Sanz I (2016) The Anti-CD38 Monoclonal Antibody TAK-079 Depletes Antibody Secreting Cells from Normal

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and SLE Patients [abstract] *Arthritis Rheumatol* **68** (suppl 10): ACR/ARHP Annual Meeting,
Abstract 1085.

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FOOTNOTES

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FIGURE LEGENDS

Figure 1: Schedule of events.

Figure 2: A representative concentration-dependent binding curve of TAK-079 to CHO cells expressing recombinant cynomolgus monkey CD38 (**A**) and to endogenous cynomolgus monkey CD38 expressed on CD3⁺ T lymphocytes, CD3⁻CD20⁺ B lymphocytes, and CD3⁻/CD20⁻/CD16⁺ NK cells (**B**) (n = 3, error bars denote standard deviation from the mean). Mean levels of NK cells in whole blood from cynomolgus monkeys after incubation with TAK-079 for 48 hours in culture (**C**) (n = 3, error bars denote standard deviation from the mean).

CHO = Chinese hamster ovary; conc. = concentration; MFI = mean fluorescence intensity; n = number of observations; NK = natural killer.

Figure 3: The mean concentration of TAK-079 in serum from monkeys infused i.v. with TAK-079 (**A**) and percent change from pre-dose baseline in mean absolute cell counts of CD20⁻/CD3⁻/CD16⁺NK cells (**B**), CD3⁻/CD20⁺ B cells (**C**), and CD3⁺ T cells (**D**) in peripheral blood of monkeys after i.v. infusion to TAK-079. Cohorts denoted with white symbols (n = 7 animals) were dosed weekly and closed symbols (n = 5 animals) once every two weeks for 3 months. Error bars denote standard deviation.

i.v. = intravenously; NK = natural killer.

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Figure 4: Body weight measurements normalized for percent change from the time of enrollment (**A**). Mean Clinical Arthritis score from 16 joints (**B**). The number of PIP joints (**C**) with joint swelling. The mean oval area of 16 PIP joints (**D**) were calculated and reported as the Mean Joint Swelling for each animal. Radiographic examination was performed following X-ray imaging for every joint of DIP (**E**) and MCP (**F**). Data are the mean \pm standard error for each group (n = 1, 5, 7, 5, and 4, for non-arthritic control animal, untreated arthritic animals, animals treated prophylactically with TAK-079, animals treated therapeutically with TAK-079, and animals treated therapeutically with dexamethasone, respectively). * p<0.05 and ** p<0.01, as compared to the untreated group (One-ANOVA\Dunn's Multiple Comparison Test).

ANOVA = analysis of variance; DIP = distal interphalangeal; MCP = metacarpophalangeal; PIP = proximal interphalangeal.

Figure 5: A representative histopathology section from a PIP joint stained with Toluidine Blue from a naive animal at 1.25 \times (**A**) and 10 \times (**B**) magnification, which was scored as follows: cell infiltrate 1; pannus 1; cartilage lesion 0; bone resorption 0; and osteophyte 1. A PIP joint from an arthritic vehicle control monkey at 1.25 \times (**C**) and 10 \times (**D**) magnification, which was scored as follows: cell infiltrate 4; pannus 4; cartilage lesion 4; bone resorption 4; and osteophyte 4. A PIP joint from a monkey exposed to TAK-079 prophylactically at 1.25 \times (**E**) and 10 \times (**F**) magnification, which was scored as follows: cell infiltrate 1; pannus 1; cartilage lesion 0; bone resorption 0; and osteophyte 1. A PIP joint from a monkey treated therapeutically with TAK-079 at 1.25 \times (**G**) and 10 \times (**H**) magnification, which was scored as follows: cell infiltrate 2; pannus 2;

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cartilage lesion 2; bone resorption 1; and osteophyte 2. Black boxes in 1.25× magnification images denote the location of the corresponding 10× magnification image.

Figure 6: Quantitative histomorphometry of DIP performed blinded using Toluidine Blue stained sections (32 × 22 animals = 704 slides) to assess total grade (**A**), pannus (**B**), cell infiltration (**C**), cartilage lesion (**D**), bone resorption (**E**), and osteophyte formation (**F**). Data are the mean ± standard error for each group (n = 1, 5, 7, 5, and 4, for non-arthritic control animal, untreated arthritic animals, animals treated prophylactically with TAK-079, animals treated therapeutically with TAK-079, and animals treated therapeutically with dexamethasone, respectively). * p<0.05 and ** p<0.01, as compared to the untreated group (One-ANOVA\Dunn's Multiple Comparison Test).

ANOVA = analysis of variance; DIP = distal interphalangeal.

Figure 7: Quantitative histomorphometry of the articular area and surface of DIP joints was performed blinded by a bone histopathologist using Toluidine Blue stained slides (32 × 22 animals = 704 slides). The individual results from each animal were plotted as the mean ± standard error for each group. Statistical analysis was conducted as specified on the chart above: total articular cartilage area (**A**), thickness of damaged articular cartilage (**B**), percentage of damaged articular surface (**C**), and osteophyte area versus total periosteal surface (**D**). Data are the mean ± standard error for each group (n = 1, 5, 7, 5, and 4, for non-arthritic control animal, untreated arthritic animals, animals treated prophylactically with TAK-079, animals treated therapeutically with TAK-079, and animals treated therapeutically with dexamethasone,

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respectively). * $p < 0.05$ and ** $p < 0.01$, as compared to the untreated group (One-ANOVA\Dunn's Multiple Comparison Test).

ANOVA = analysis of variance; Dex. = dexamethasone; DIP = distal interphalangeal.

Figure 8: Serum chemistry levels of CRP (A) and ALP (B) over time. Data are the mean \pm standard error for each group (n = 1, 5, 7, 5, and 4, for non-arthritic control animal, untreated arthritic animals, animals treated prophylactically with TAK-079, animals treated therapeutically with TAK-079, and animals treated therapeutically with dexamethasone, respectively). * $p < 0.05$ and ** $p < 0.01$, as compared to the untreated group (One-ANOVA\Dunn's Multiple Comparison Test).

ALP = alkaline phosphatase; ANOVA = analysis of variance; CRP = C-reactive protein.

Figure 9: Levels of red blood cells (A), hematocrit (B), reticulocytes (C), platelets (D), neutrophils (E), and lymphocytes (F) in animals over time. Data are the mean \pm standard error for each group (n = 1, 5, 7, 5, and 4, for non-arthritic control animal, untreated arthritic animals, animals treated prophylactically with TAK-079, animals treated therapeutically with TAK-079, and animals treated therapeutically with dexamethasone, respectively). * $p < 0.05$ and ** $p < 0.01$, as compared to the untreated group (One-ANOVA\Dunn's Multiple Comparison Test).

ANOVA = analysis of variance; HCT = hematocrit; LYMPH = lymphocyte; NEUT = neutrophil; PLT = platelet; RBC = red blood cell; RET = reticulocyte.

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Figure 10: Levels of NK cells (**A**), B cells (**B**), T cells (**C**), and monocytes (**D**) in peripheral blood over time. Data are the mean \pm standard error for each group (n = 1, 5, 7, 5, and 4, for non-arthritic control animal, untreated arthritic animals, animals treated prophylactically with TAK-079, animals treated therapeutically with TAK-079, and animals treated therapeutically with dexamethasone, respectively). * $p < 0.05$ and ** $p < 0.01$, as compared to the untreated group (One-ANOVA/Dunn's Multiple Comparison Test).

ANOVA = analysis of variance; MONO = monocyte; NK = natural killer.

Figure 11: Serum concentrations of TAK-079 in individual monkeys when administered prophylactically (**A**) and therapeutically (**B**). Serum concentrations of anti-TAK-079 antibodies in individual monkeys when administered prophylactically (**C**) and therapeutically (**D**). Each symbol denotes data from an individual animal.

TABLES

Table 1: A comparison of TAK-079-bound cells in human and monkey tissues

Tissue	Human				TAK-079-Bound Cells	Monkey				TAK-079-Bound Cells
	TAK-079		Human IgG1 Control			TAK-079		Human IgG1 Control		
	5 µg/mL	25 µg/mL	5 µg/mL	25 µg/mL		5 µg/mL	25 µg/mL	5 µg/mL	25 µg/mL	
Bone marrow	3 C/M	2+ M	0	0	Bone marrow cells, possibly leukocytes	1+ C/M	1+ M	0	0	Few bone marrow cells, possibly leukocytes
Heart	0	0	0	0		0	0	0	0	
Colon	3+ C/M	4+ C/M	0	0	Leukocytes, including lymphocytes, in lamina propria; some endothelium	2+ C/M	0	0	0	Cells in lamina propria, possibly lymphocytes, some endothelium

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Tissue	Human				TAK-079-Bound Cells	Monkey				TAK-079-Bound Cells
	TAK-079		Human IgG1 Control			TAK-079		Human IgG1 Control		
	5 µg/mL	25 µg/mL	5 µg/mL	25 µg/mL		5 µg/mL	25 µg/mL	5 µg/mL	25 µg/mL	
Stomach	0	0	0	0	Leukocytes, including lymphocytes, in lamina propria; some endothelium	1+ C/M	1+ C	0	0	Spindle cells in lamina propria, apparently endothelium
Small intestine	4+ C/M	4+ C/M	0	0	Leukocytes, including lymphocytes, in lamina propria; some endothelium	2+ C/M	1+ C/M	0	0	Cells in lamina propria, possibly lymphocytes, some endothelium
Renal glomerulus	0	0	0	0	in lamina propria; some endothelium	0	0	0	0	
Renal tubule	0	0	0	0		0	0	0	0	

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Tissue	Human				TAK-079-Bound Cells	Monkey				TAK-079-Bound Cells
	TAK-079		Human IgG1 Control			TAK-079		Human IgG1 Control		
	5 µg/mL	25 µg/mL	5 µg/mL	25 µg/mL		5 µg/mL	25 µg/mL	5 µg/mL	25 µg/mL	
Liver	0	0	0	0		0	0	0	0	
Lymph node	3+ C/M	4+ C/M	0	0	Cells in medullary cords and sinuses; possibly plasma cells; few in cortex	4+ M	3+ C	0	0	Cells in medullary cords and sinuses, fewer in cortex
Lung	3+ C/M	3+ C/M	0	0	Interstitial and peribronchiolar cells	0	0	0	0	
Pancreas	0	0	0	0		0	0	0	0	
Prostate	3+ C	3+ C	0	0	Acinar epithelium; some interstitial leukocytes	0	0	0	0	

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Tissue	Human					Monkey				
	TAK-079		Human IgG1 Control		TAK-079-Bound Cells	TAK-079		Human IgG1 Control		TAK-079-Bound Cells
	5 µg/mL	25 µg/mL	5 µg/mL	25 µg/mL		5 µg/mL	25 µg/mL	5 µg/mL	25 µg/mL	
Skin	0	0	0	0		0	0	0		
Uterus - cervix	2+ C/M	1+ C/M	0	0	Submucosal leukocytes, probably lymphocytes	0	0	0		
Uterus - endometrium	0	0	0	0		0	0	0		

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Staining Intensity:

0 = negative	1+ = minimal	2+ = mild	3+ = moderate	4+ = marked
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C = Cytoplasmic staining pattern, M = Membranous staining pattern; IgG1 = immunoglobulin G1.

Staining pattern include cell type or tissue element (specifics of that cell type [e.g., epithelium] or tissue element [e.g., basement membrane] and subcellular or extracellular [membrane, cytoplasm, cytoplasmic filaments, basement membrane]).

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Table 2: Mean serum pharmacokinetic parameters of TAK-079 after an intravenous infusion into female cynomolgus monkeys

Dose (mg/kg)	Dose schedule	Sample Timing	N	C _{max} (µg/mL)	AUC ₃₆₆ (hr*µg/mL)	T _{1/2} (hr)
0.1	Weekly	Week 1	7	1.77	104	NC
		Week 13	1	2.49	218	NC
0.3	Weekly	Week 1	7	4.32	327	NC
		Week 13	2	7.78	665	NC
1.0	Weekly	Week 1	7	21.2	1630	NC
		Week 13	4	28.6	2700	NC
3	Bi-weekly	Week 1	3	62.1	7600	NC
		Week 13	3	90.1	18,400	237
30	Bi-weekly	Week 1	3	680	92,800	NC
		Week 13	3	962	228,000	NC
80	Bi-weekly	Week 1	3	1630	220,000	NC
		Week 13	3	3140	456,000	415

AUC₃₆₆ = area under concentration-time curve from dose administration to 366 hours post-dose, C_{max} = maximum serum concentration, hr = hour, N = number of animals, NC = no change, T_{1/2} = elimination half-life.

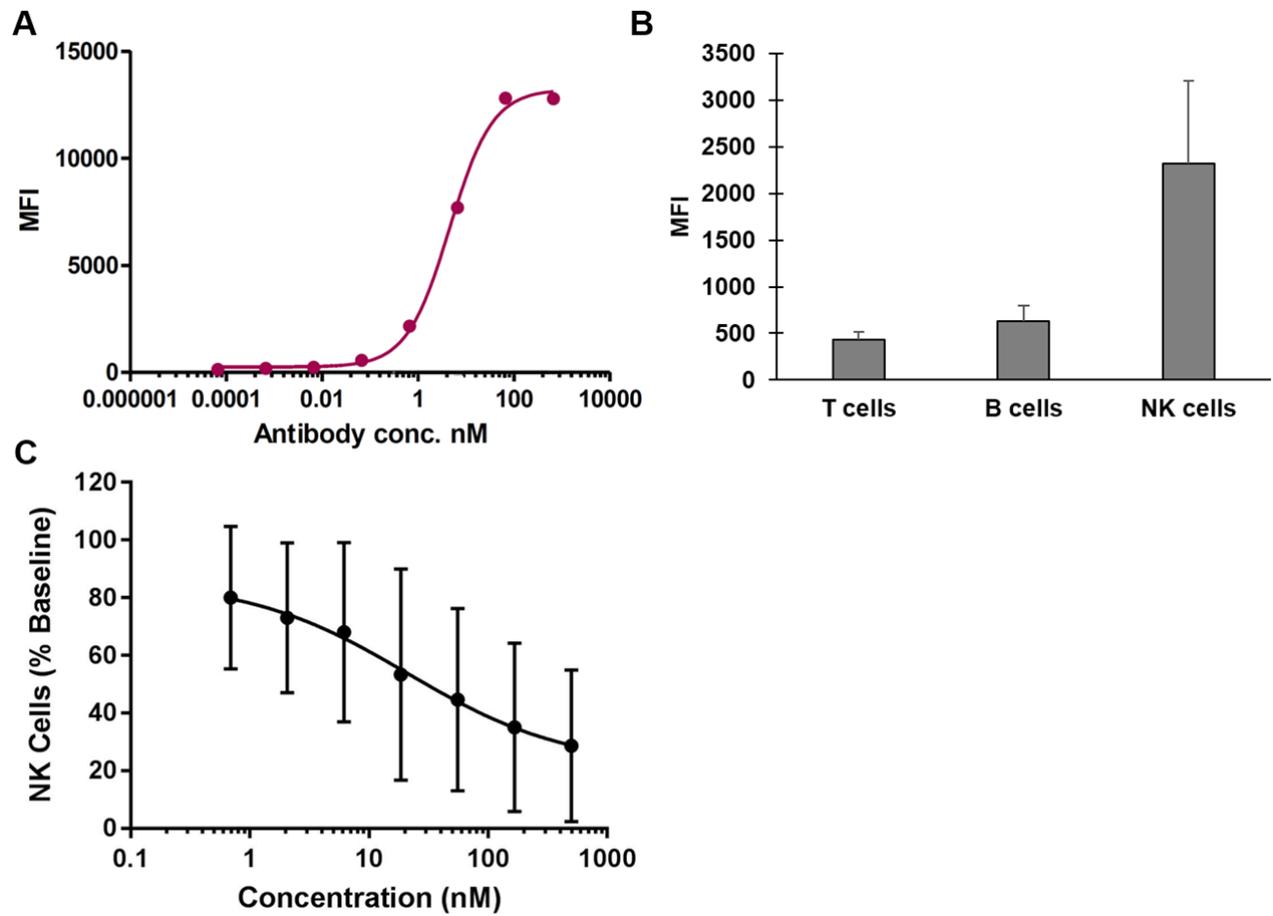


Figure 2

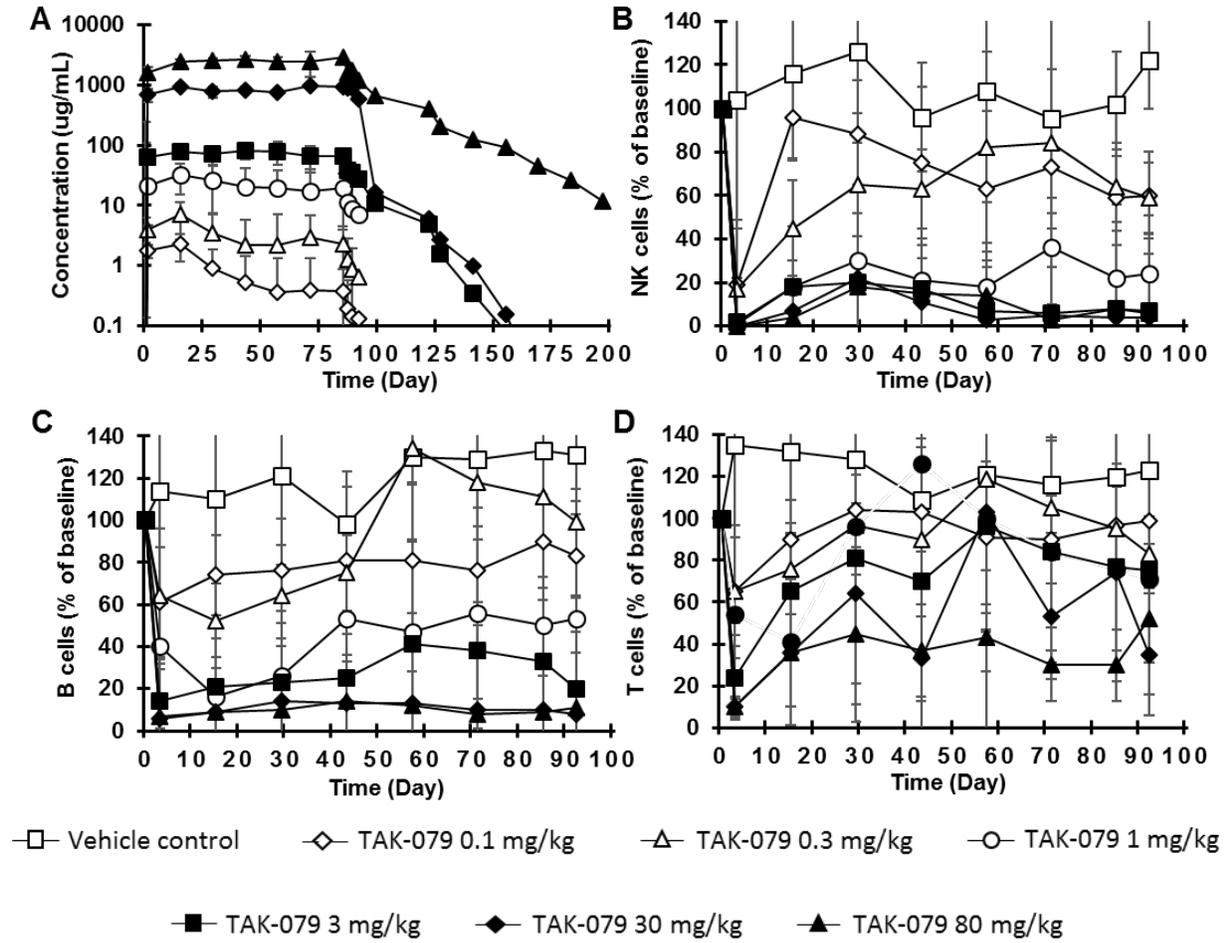


Figure 3

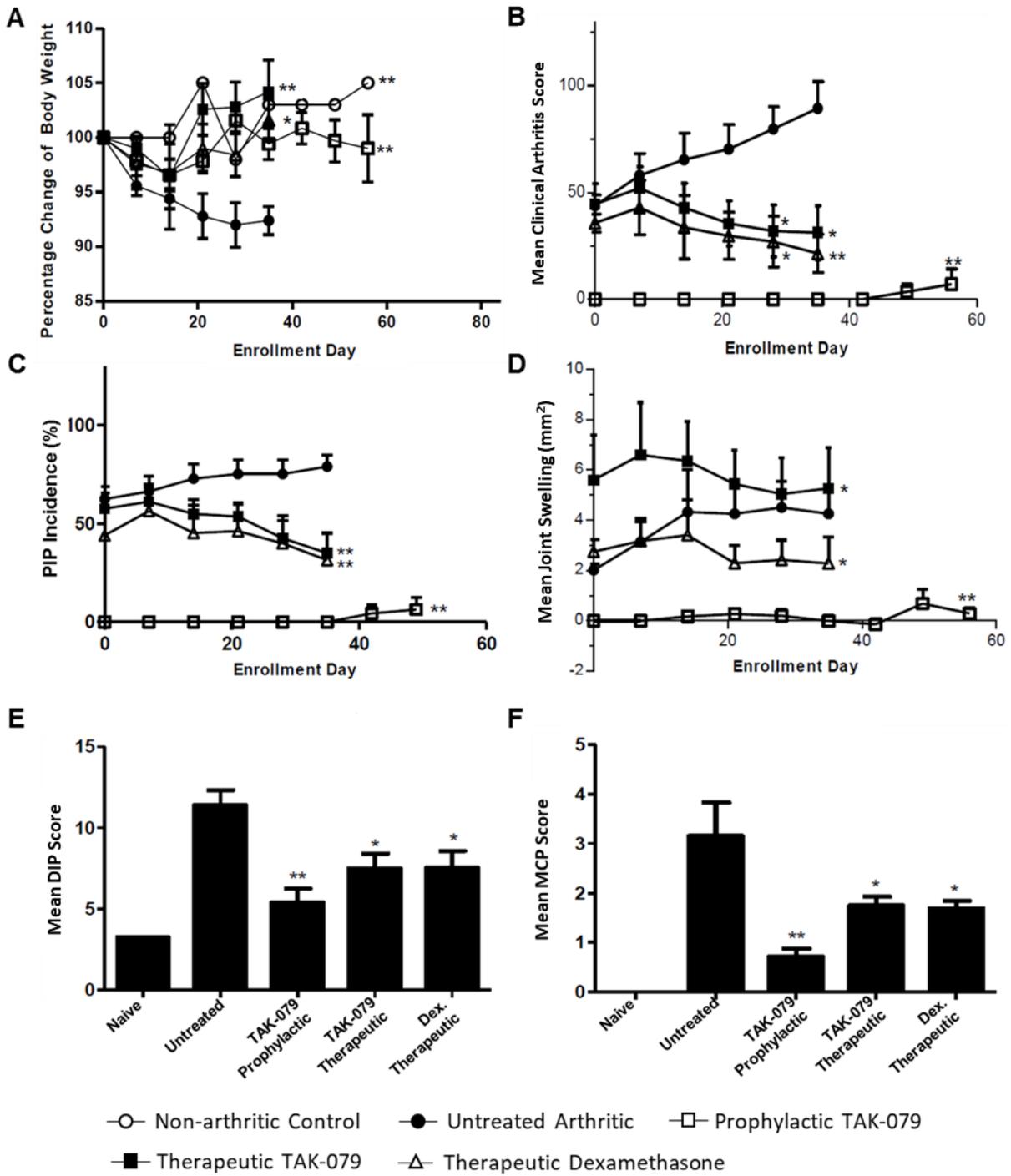


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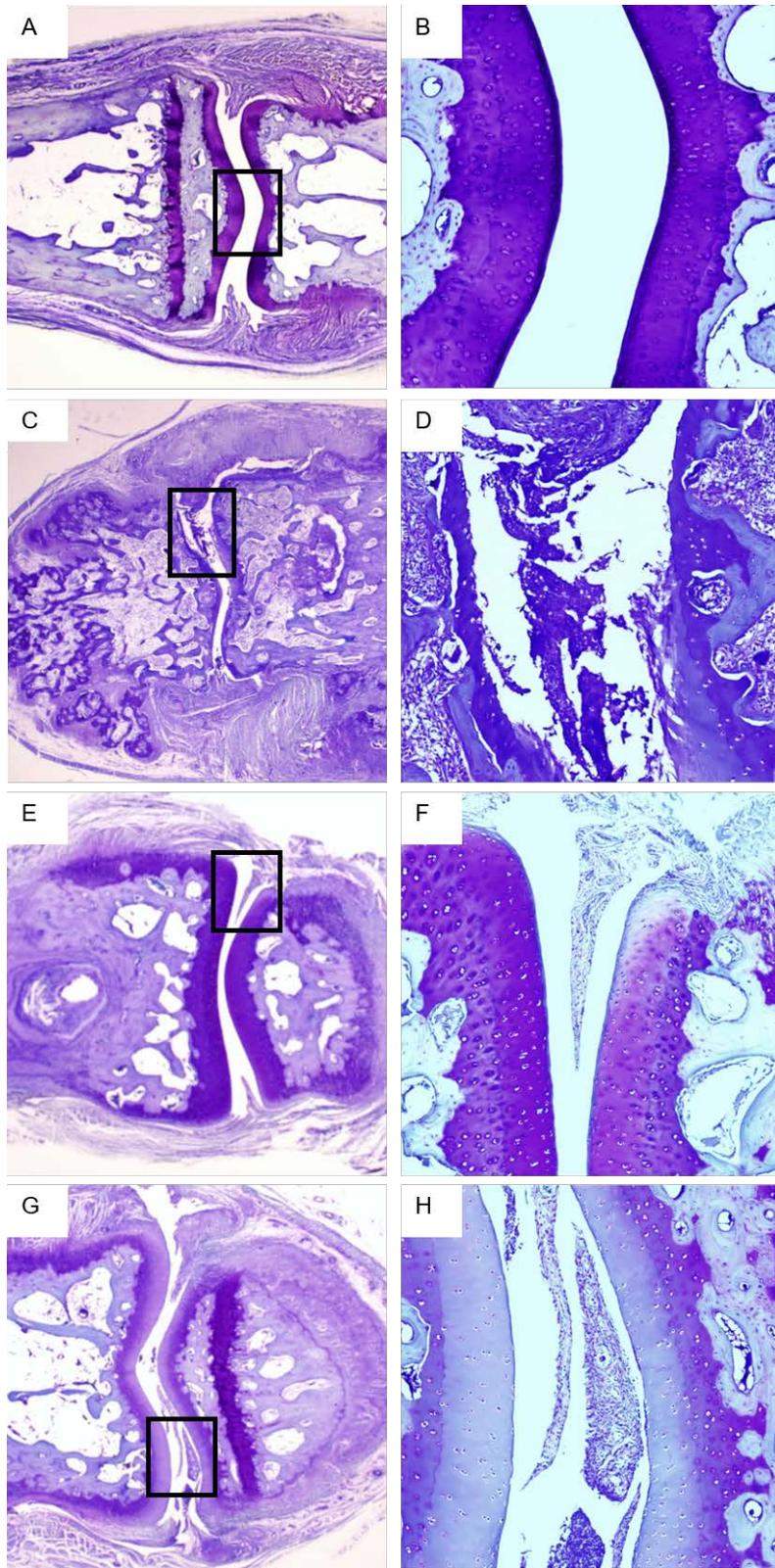


Figure 5

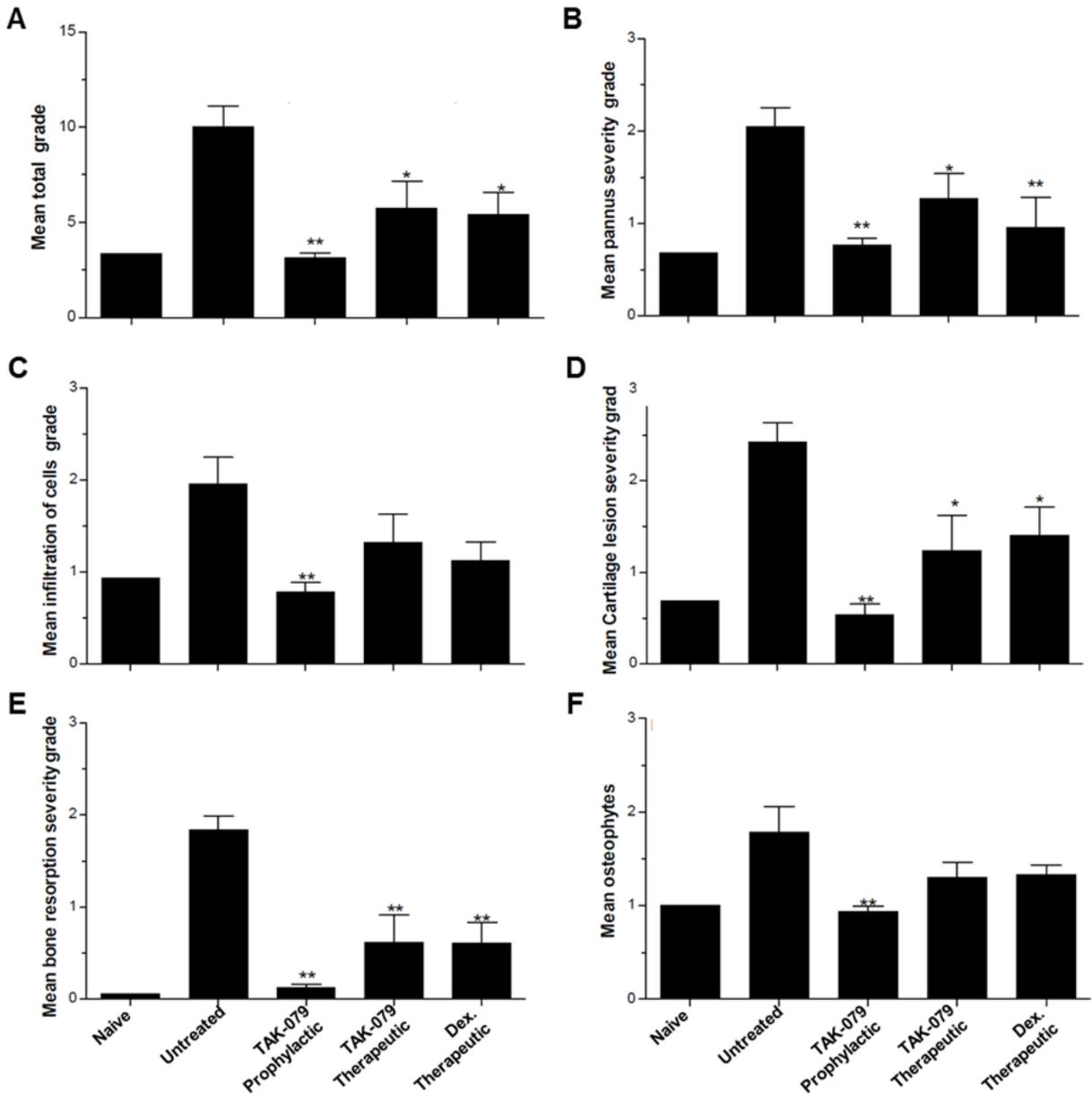


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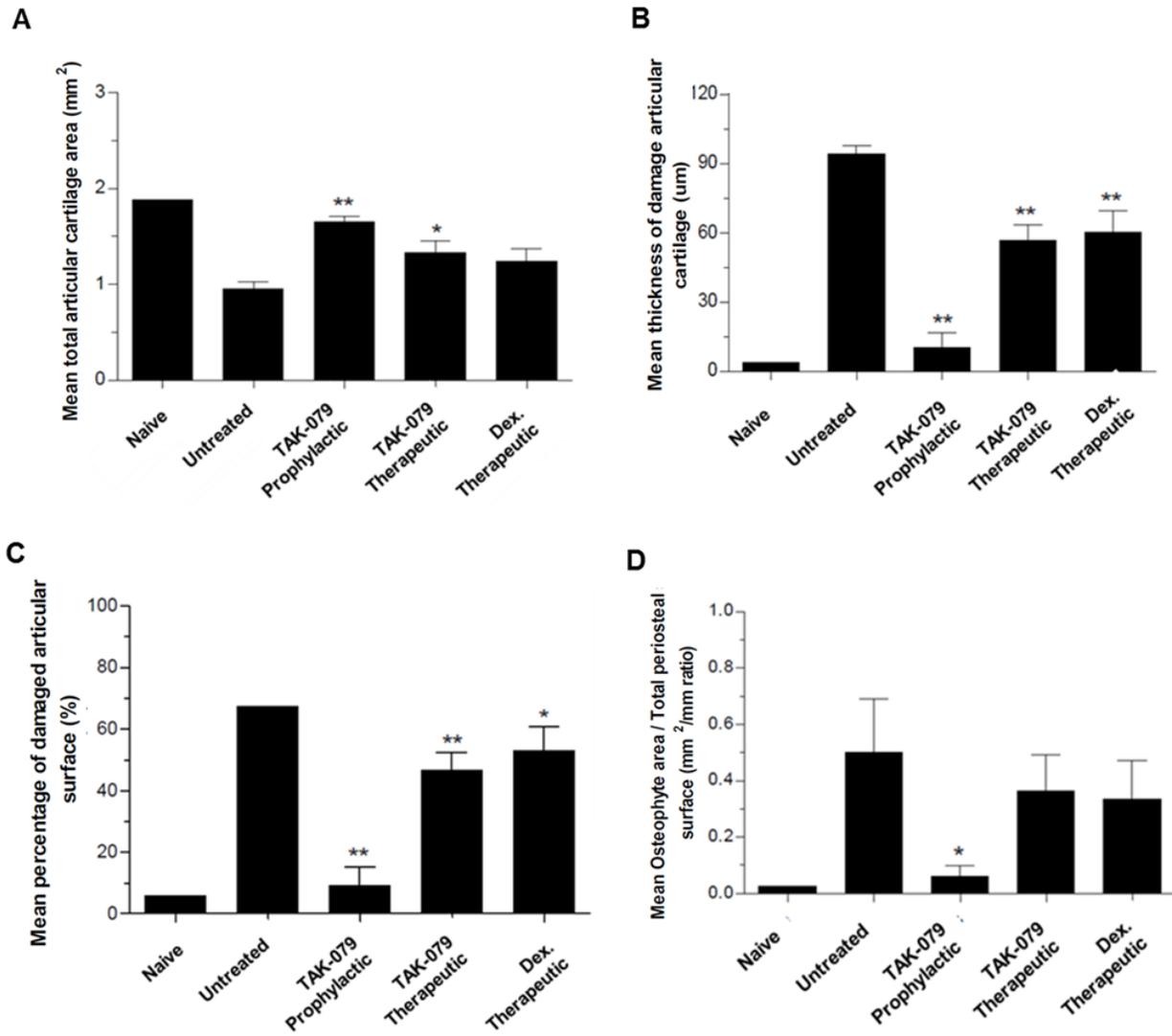


Figure 7

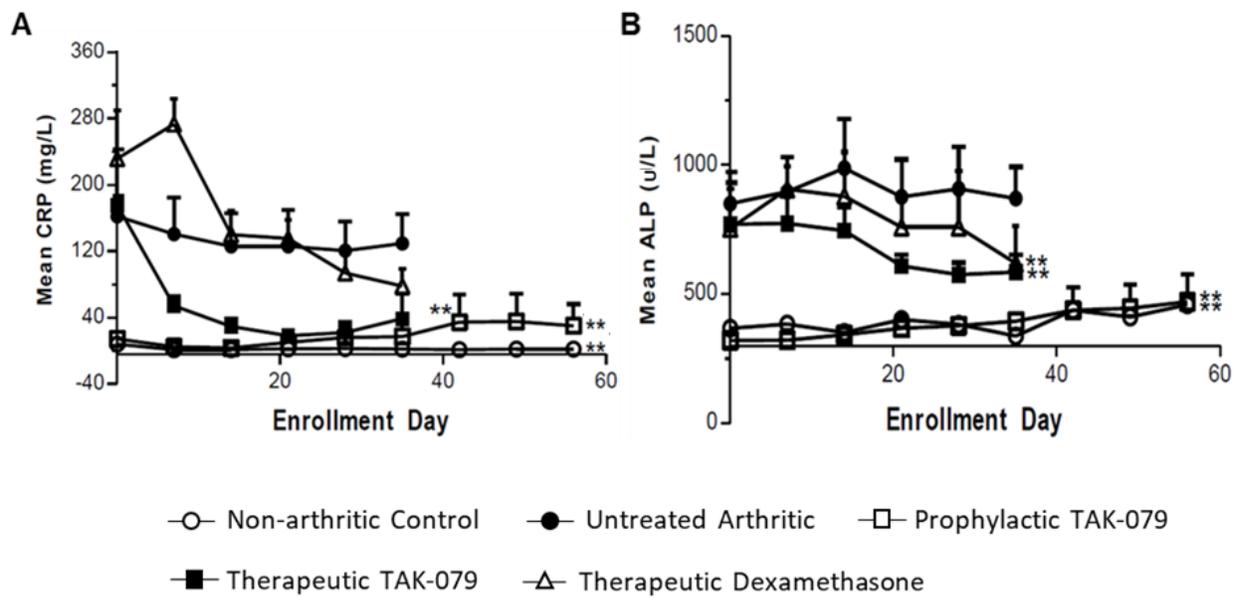


Figure 8

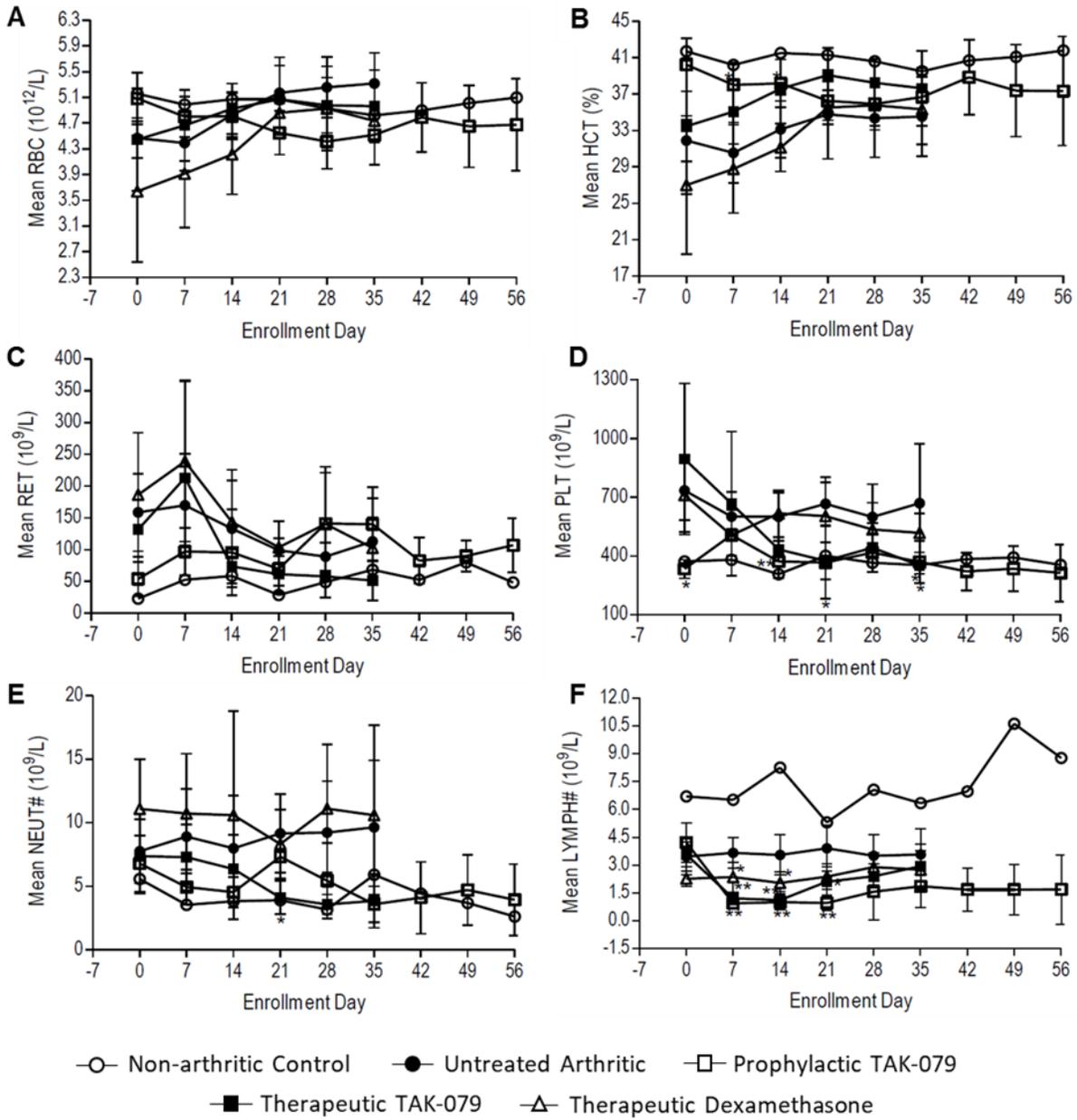


Figure 9

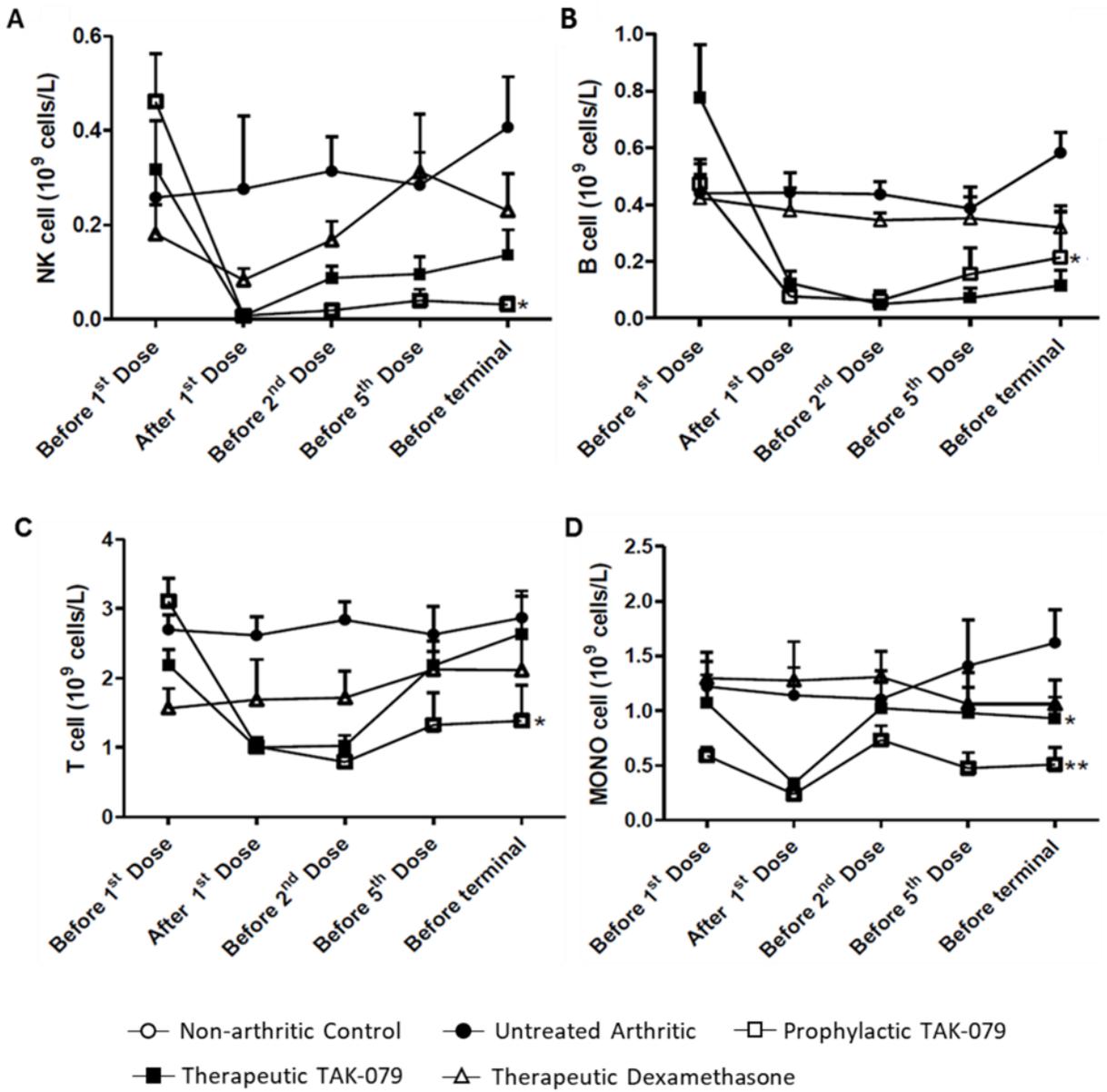


Figure 10

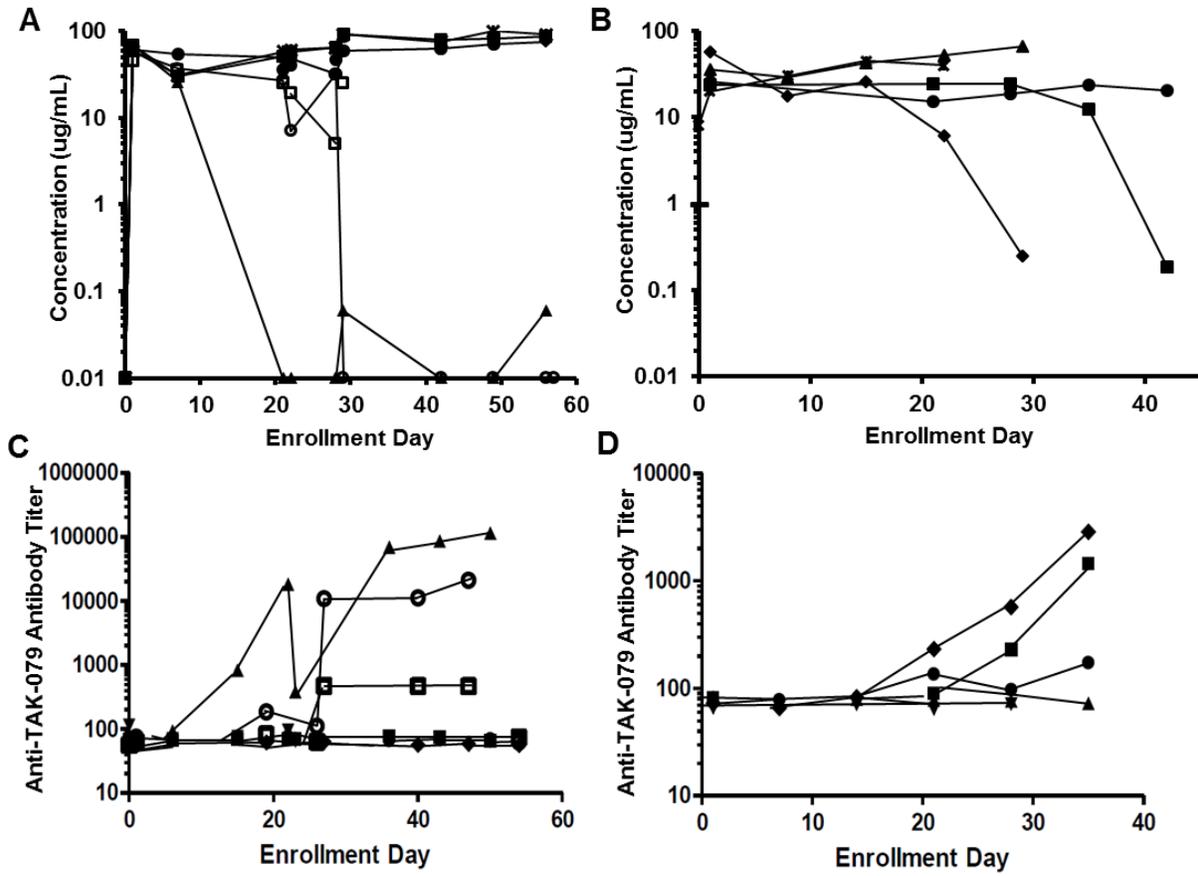


Figure 11