

# Development of an ICOSL and BAFF bispecific inhibitor AMG 570 for systemic lupus erythematosus treatment

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## Objective

Systemic lupus erythematosus (SLE) is a heterogeneous disease lacking highly effective treatment options. Here we tested if targeting both BAFF and ICOSL has superior efficacy than single target inhibition in the mouse arthritis and lupus models. We also generated AMG 570, an ICOSL and BAFF bispecific inhibitory molecule, for potential treatment of autoimmune diseases such as SLE.

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## Methods

Murine BAFF/ICOSL bispecific, combination of BAFF and ICOSL inhibitors or single inhibitor was evaluated in the sheep red blood cell (SRBC) challenge model, mouse collagen induced arthritis (CIA) model, or NZB/NZW lupus models. AMG 570 was tested for human and cyno BAFF and ICOSL binding affinities by Kinexa A. AMG 570 dual target blocking activities was evaluated in human and cyno BAFF and ICOSL mediated B cell and T cell assay, respectively. Pharmacodynamics effect of AMG 570 was evaluated in cynomolgus monkey.

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## Results

Treatment with murine ICOSL/BAFF bispecific or combination therapy was more efficacious than single ICOSL or BAFF inhibitor in mouse NZB/NZW lupus model. Dual ICOSL and BAFF inhibition was also more effective in the mouse collagen induced arthritis (CIA) model. AMG 570 was developed as the clinical bispecific lead. AMG 570 inhibits human and cynomolgus monkey ICOSL and BAFF. B cell reduction was observed after AMG 570 treatment in cynomolgus monkeys, consistent with the pharmacological effect of BAFF inhibition.

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## Conclusion

By targeting both ICOSL and BAFF, AMG 570 has the potential to achieve superior efficacy in treatment of autoimmune diseases such as SLE and rheumatoid arthritis.

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## Key words

systemic lupus erythematosus, ICOSL, BAFF, T cell, B cell

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## Introduction

Systemic lupus erythematosus (SLE) remains an indication with high unmet medical needs. Benlysta® (belimumab), an anti-BAFF antibody with modest efficacy, provides clinical validation for the BAFF pathway yet also indicates the need for additional therapeutics with better efficacy (1). SLE is a heterogeneous disease. Thus, desirable efficacy is likely achievable by targeting multiple pathways. Among many cell types and pathways involved in SLE pathogenesis, aberrant B cells and T cells are critical drivers in autoantibody production, inflammatory cytokine production, and tissue damage.

ICOSL is critical for T<sub>HH</sub> cell development and T memory cell homeostasis (2). ICOSL is mainly expressed on B cells and antigen presenting cells, whereas its receptor ICOS is expressed on activated T cells. ICOSL knockout (KO) mice or mice administered with anti-ICOSL antibody had an impaired T-dependent antibody response (3-5). Elevation of ICOS expression on T cells was reported in patients with SLE, RA and Sjögren's syndrome (6-8). Evidence of the role of ICOS/ICOSL in human immune function comes from both patients deficient in ICOS function as well as in lupus patients treated with anti-ICOSL antibody. ICOS null patients are immunodeficient showing impaired T effector memory cell homeostasis and humoral immune responses (9, 10). AMG 557 is a fully human IgG2 antagonist antibody against ICOSL and is currently being investigated in a Phase 1 clinical trial in lupus arthritis patients and primary Sjögren's syndrome (pSS). Single and multiple doses of AMG 557 demonstrated ICOSL occupancy on B cells in the peripheral blood of SLE subjects in a dose-related and reversible manner. A significant reduction in anti-KLH (Keyhole Limpet Hemocyanin) IgG response was observed after AMG 557 treatment (11). AMG 557 showed safety and potential efficacy in patients with active lupus arthritis (12).

BAFF (TNFSF13B) supports B cell survival through binding to BAFF receptor (BAFFR) expressed on B cells (13). BAFF is a type II transmembrane protein mainly expressed by monocyte

and dendritic cell. It is released by proteolytic cleavage and functions in the periphery (14). BAFF inhibition results in a significant reduction of B cell number in human, cynomolgus monkey, and rodents, a hallmark of pharmacodynamics (PD) for BAFF inhibition (15-17). BAFF blocking antibody Benlysta is approved for SLE treatment, albeit with modest efficacy.

Given both aberrant B cell and T cell are involved in SLE pathogenesis, we hypothesise that addition of a BAFF inhibitor to an anti-ICOSL antibody, is likely to achieve superior efficacy than BAFF and ICOSL inhibitor alone in SLE treatment. We demonstrated that dual ICOSL and BAFF inhibition, either by bispecific or combination treatment, was more efficacious than single inhibitor treatment in lupus and arthritis models in mice. AMG 570 was selected as the clinical lead. By targeting both BAFF and ICOSL, AMG 570 has the potential to achieve superior efficacy in treatment of autoimmune diseases such as SLE and rheumatoid arthritis.

## Material and methods

### Reagents

Recombinant human, cynomolgus monkey and murine BAFF or ICOSL-Fc protein were generated at Amgen. Anti-mouse ICOSL antibody 1B7 was described previously (Hu *et al.*, 2009). Anti-mouse BAFF peptide was described previously (Hsu *et al.*, 2012). Murine ICOSL/BAFF bispecific molecule was generated by fusion of two tandem copies of BAFF-binding peptides (Hsu *et al.*, 2012) to the C-terminus of anti-mouse ICOSL blocking antibody 1B7 as described (Hu *et al.*, 2009). AMG 570 was generated by fusion of two tandem copies of BAFF-binding peptides to the C-terminus of anti-ICOSL antibody AMG 557 (Cheng *et al.*, 2018). PE labelled anti-B220, FITC labelled anti-CD3 mAb, PE labelled anti-CD69 mAb, PE labelled anti-CD62L, FITC anti-CD44 were from BD Biosciences. Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA) were from Difco. Bovine type II collagen were from Dr. Marie Griffiths University of Utah. Cynomolgus monkey spleen was provided

Competing interests: All the authors were Amgen employees and stock holders at the time when this research work was conducted.

by Shin Nippon Biomedical Laboratories. Human PBMC for T cell and B cell purification was from Amgen donors.

#### *Binding affinities*

The binding affinities of AMG 570 to human and cynomolgus monkey BAFF were measured by KinExA™. In brief, AMG 570 was incubated with various concentrations of human or cynomolgus monkey BAFF before running through the human or cynomolgus monkey BAFF-coated Reacti-Gel 6x beads. The amount of the bead-bound AMG 570 was quantified by fluorescent (Cy5) labelled goat anti-human-Fc antibody. The binding signal is proportional to the concentration of free antibody at equilibrium with a given BAFF concentration. Dissociation equilibrium constant (Kd) was obtained from non-linear regression of the competition curves using a curve one-site homogeneous binding model provided in KinExA™ software.

The binding affinities of AMG 570 to human and cynomolgus monkey ICOSL were measured using BIAcore based methods. Briefly, AMG 570 was incubated with various concentrations of human or cynomolgus monkey ICOSL-Fc protein before injection over human or cynomolgus monkey B7RP-1-Fc immobilised surface, respectively. Binding affinity was obtained from nonlinear regression of the competition curves using a curve one-site homogeneous binding model provided in KinExA™ software.

#### *In vitro B cell and T cell assays*

Human B cells were purified from peripheral blood mononuclear cells (PBMCs) using a human negative B cell Isolation Kit II according manufacturer's suggestions (Miltenyi Biotech, Auburn, CA). Purified ( $10^5$ ) B cells were cultured in RPMI media plus 10% heat inactivated FBS in duplicate in 96-well flat-bottom plates in the presence of 50 ng/ml human BAFF protein, 2 µg/ml Goat F(ab')<sub>2</sub> anti-human IgM from Jackson ImmunoResearch (Cat # 109-006-129, West Grove, PA) and a serially diluted amount of AMG 570. The plates were incubated at 37°C and 5% CO<sub>2</sub> for 96 hours. Proliferation was measured by the uptake of radioactive <sup>3</sup>H thymi-

dine in the last 18 hours of pulse. For cynomolgus monkey BAFF assay, cynomolgus monkey splenocytes were cultured in RPMI media plus 10% heat inactivated FBS in duplicates in 96-well flat-bottom plates in the presence of 100 ng/ml cynomolgus monkey BAFF protein, 10 µg/ml Goat F(ab')<sub>2</sub> anti-human IgM from Jackson ImmunoResearch, (Cat.# 109-006-129, West Grove, PA) and a serially diluted AMG 570. For mouse BAFF assay, mouse spleen B cells were stimulated with 200 ng/mL of murine BAFF and 0.1 µg/mL Goat F(ab')<sub>2</sub> anti-IgM. B cell proliferation was measured by <sup>3</sup>H thymidine incorporation as described above.

For ICOSL mediated T cell assay, 96-well tissue culture plates were first coated with 2 µg/mL anti-CD3 and 10 µg/mL anti-human Fc overnight at 4°C. The plates were washed with 200 µl of PBS, and coated with 3 µg/mL human ICOSL-Fc for 4 hours at 37°C. Approximately  $10^5$  purified T cells from PBMC using Pan T cell isolation kits (Miltenyi Biotec, Auburn, CA) were added to anti-CD3/ICOSL-Fc coated plates in the presence or absence of ICOSL/BAFF bispecific or anti-ICOSL antibody. After 48 hours of incubation, 1 µCi/well of <sup>3</sup>H-thymidine was added to the T cells. Plates were incubated for another 16 hours. Proliferation was measured by the uptake of radioactive <sup>3</sup>H thymidine in the last 18 hours of pulse. Cynomolgus monkey T cells were isolated from cyno spleen cells, and added to anti-CD3/ICOSL-Fc coated plates. Proliferation was measured as described above. For mouse ICOS occupancy measurement, biotin labelled murine ICOSL-Fc was first incubated with indicated concentrations of murine ICOSL/BAFF bispecific or anti-ICOSL antibody (1B7) for 30 minutes on ice. The mixture was further incubated with anti-CD3 activated mouse spleen cells for 30 minutes. The ICOSL binding on T cells were measured by FACS.

#### *SRBC challenge in mice*

BALB/c mice were purchased from Taconic Bioscience. Experiments were conducted under protocol 2007-00071 approved by Institutional Animal Care and Use Committee at the Laboratory

Animal Research Facility at Amgen Thousand Oaks. Mice (female, 6-8wk old) were immunised with a single intraperitoneal injection of  $2 \times 10^8$  SRBC (Lampire Biological Laboratories, Pipeville, PA) in 0.2 ml of PBS at day 1 and were boosted with  $2 \times 10^8$  SRBC on day 28. Mice were treated with anti-ICOSL, anti-BAFF peptide, combination of anti-ICOSL and anti-BAFF inhibitors, murine ICOSL/BAFF bispecific, mouse IgG1 isotype control twice per week until day 35. On day 35, spleen cells were collected for FACS analysis. In brief, splenocytes were pre-incubated with unlabelled anti-CD16/32 to block nonspecific binding of the staining mAbs to FcγR. Cells were incubated on ice with 1:100 diluted FITC anti-B220 for 30 minutes for B cell staining; 1:100 diluted FITC anti-CD44 and 1:200 diluted PE anti-CD62L for 30 minutes for effector memory T cell staining. B cell were defined as B220<sup>+</sup> cells, effector memory T cells (T<sub>EM</sub>) were defined as CD3<sup>+</sup>CD4<sup>+</sup>CD44<sup>hi</sup>CD62L<sup>lo</sup> cells and activated T cells were defined as CD3<sup>+</sup>CD69<sup>+</sup> cells. The flow cytometry was performed with FACS Calibur (BD Biosciences, San Jose, CA) and the data were analysed using the FlowJo program (Tree Star, Inc.).

#### *NZB/NZW lupus model*

Female New Zealand Black/New Zealand White (NZB/NZW) F1 mice were ordered from Taconic Bioscience. Experiments were conducted under protocol 2006-00066 approved by Institutional Animal Care and Use Committee at the Laboratory Animal Research Facility at Amgen Thousand Oaks. In brief, 4.5 months old female NZB/W F1 mice were randomly divided into six groups (20 mice in each group) for administration intraperitoneally with anti-ICOSL (clone 1B7, 14 mg/kg), anti-BAFF (5.6 mg/kg), combination of anti-ICOSL (14 mg/kg) and anti-BAFF (5.6 mg/kg), ICOSL/BAFF bispecific (15 mg/kg) or mouse IgG1 isotype control (15 mg/kg) twice per week for 4 months. Doses were adjusted based on molecular weight to be molar equivalents (bispecific MW 160KDa, anti-ICOSL MW 150KDa, anti-BAFF MW 64KDa).

Peripheral blood was collected after one-month treatment for B cell number and ICOSL occupancy measurement by FACS. B cells were gated on B220+ cells with APC-conjugated anti-B220 antibody. ICOSL occupancy (free ICOSL on B cells) was measured by staining B cells with biotinylated anti-ICOSL antibody followed by streptavidin PE. For anti-dsDNA antibody measurement, serum samples were collected after 4 months treatment and added to the 96 well plate coated with 100 µl per well (100ug/ml) of salmon sperm DNA (Rockland Immunochemicals Inc, Boyertown, PA 19512). After incubation 2 hours at room temperature, bound dsDNA specific IgG was detected with HRP conjugated goat anti-mouse IgG (Southern Biotech, Birmingham, AL). The substrate reaction was performed with Sureblue TMB microwell peroxidase substrate (KPL, Gaithersburg, MD) and the OD was read using Spectrum Max (Molecular Devices).

Proteinuria was measured using Albustix (Bayer) every 2 weeks starting at 4.5 months of age until study termination at 14 months of age. The incidence of proteinuria was expressed as percentage of mice where urine protein was at least 300 mg/dl in two consecutive measurements. Kidneys from all mice in the study, including deceased mice before study termination, were collected for histology analysis for renal lesion severity.

#### *Collagen induced arthritis model*

DBA/1 mice were purchased from Charles River Labs. Experiments were conducted under protocol 2007-00025 approved by Institutional Animal Care and Use Committee at the Laboratory Animal Research Facility at Amgen Thousand Oaks. Bovine type II collagen was dissolved at 2 mg/ml concentration in 0.1M acetic acid overnight at 4°C using end-to-end low speed rotator. The collagen was emulsified 1:1 volume with either the 2X complete Freund's adjuvant (CFA) or incomplete Freund's adjuvant (IFA). On day 0, ten weeks old male DBA/1 mice were injected intradermally at the base of the tail with 100µg of bovine type II collagen emulsified in CFA. Mice were

given a booster injection on day 21 with 100µg of bovine type II collagen emulsified in IFA. Mice were randomised to 5 groups (n=15) for administration intraperitoneally with 5mg/kg of anti-ICOSL antibody, anti-BAFF peptibody, combination of anti-ICOSL and anti-BAFF peptibody, or isotype control two times per week for 6 weeks. Arthritis scores were evaluated 2 times per week. A score of 0= no swelling, 0.5=swollen toes only, 1= noticeable swelling of the ankle/paw, 1.5= noticeable swelling or pronounced swelling in the paw and swollen toes, 2=pronounced swelling of the ankle/paw, 2.5= gross swelling of ankle/paw with no toe swelling or 3=gross swelling of paw/toes and deformity. Scores from each limb was evaluated, therefore arthritis scores from a mouse can vary from 0 to 12. Percent incidence is quantified when an animal achieved a score of 1 on any particular paw.

#### *PKPD in cynomolgus monkeys*

Study (529-353) was performed at MPI Research, Inc., Mattawan, MI. Animal welfare for the study followed the U.S. Department of Agriculture's (USDA) Animal Welfare Act (9 CFR Parts 1, 2 and 3). Naive male cynomolgus monkeys (n=4) were given a single bolus intravenous or subcutaneous dose of 10mg/kg AMG 570. Blood and serum samples were collected pre-dose (day-5 and day-1) and at various timepoints post-dosing for standard haematology evaluation performed MPI Research, FACS and PK studies performed at Amgen. Total B cells (CD20<sup>+</sup>), naïve B cells (CD20<sup>+</sup>CD27<sup>-</sup>) and memory B cells (CD20<sup>+</sup>CD27<sup>+</sup>) in blood were measured by FACS analysis. An average of B cell numbers at day-5 and day-1 pre-dosing was used as baseline. Total serum AMG 570 was measured by anti-human IgG antibody capture and detection. Intact AMG 570 was measured by capturing with ICOSL-Fc capture and detection with biotinylated BAFF

## **Results**

### *Generation of murine ICOSL/BAFF bispecific for proof-of-concept studies in vitro and in vivo*

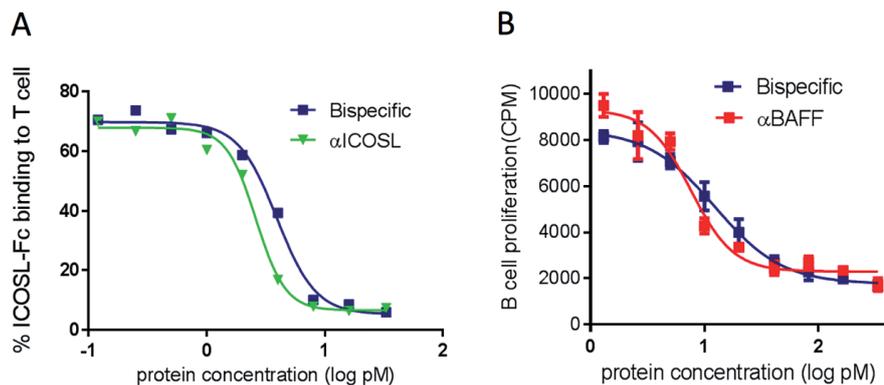
A murine ICOSL/BAFF bispecific was

generated by fusion of BAFF inhibitory peptides (Hsu *et al.*, 2012) to the C-terminus of each heavy chain of an anti-mouse ICOSL antibody 1B7 (Hu *et al.*, 2009). The murine ICOSL/BAFF bispecific inhibited ICOSL binding to ICOS on activated T cells, with potency comparable to the parental ICOSL antibody (Fig. 1A). The murine ICOSL/BAFF bispecific also inhibited murine BAFF mediated B cell proliferation with potency comparable to the parental BAFF peptibody (Fig. 1B).

We next studied whether the ICOSL/BAFF bispecific treatment was capable of impacting both ICOSL and BAFF activities using the sheep red blood cell (SRBC) immunisation model in mice. SRBC immunisation in mice induces significant expansion of effector memory T cell (T<sub>EM</sub>) population, which was reported to be ICOSL dependent (Hu *et al.*, 2009). Consistent with the ICOSL inhibition mechanism, the murine BAFF/ICOSL bispecific inhibited T<sub>EM</sub> cell expansion induced by the SRBC immunisation, with similar effectiveness to the parental anti-ICOSL antibody 1B7 treatment alone (Fig. 2A). Anti-BAFF peptibody treatment alone, on the other hand, did not inhibit T<sub>EM</sub> induction after SRBC challenge. B cell reduction is the pharmacodynamics hallmark of BAFF inhibition. Murine ICOSL/BAFF bispecific showed similar B cell reduction as anti-BAFF treatment alone, while treatment with anti-ICOSL antibody alone did not show spleen B cell reduction (Fig. 2B). Taken together, murine ICOSL/BAFF bispecific has dual ICOSL and BAFF inhibitory activities *in vitro* and *in vivo*.

### *Dual ICOSL and BAFF inhibition is more efficacious than single target inhibition in NZB/NZW lupus mice*

It was previously reported that treatment with anti-mouse ICOSL antibody 1B7 or anti-BAFF peptibody alone was efficacious in the NZB/NZW mice, a mouse model of spontaneous lupus (Hsu *et al.*, 2012; Hu *et al.*, 2009). Therefore, we compared the effects of the bispecific or combination treatment with single ICOSL or BAFF inhibitor treatment in the NZB/NZW mouse lupus model. NZB/NZW female mice at



**Fig. 1.** Murine ICOSL/BAFF bispecific inhibits mouse ICOSL and BAFF *in vitro*.

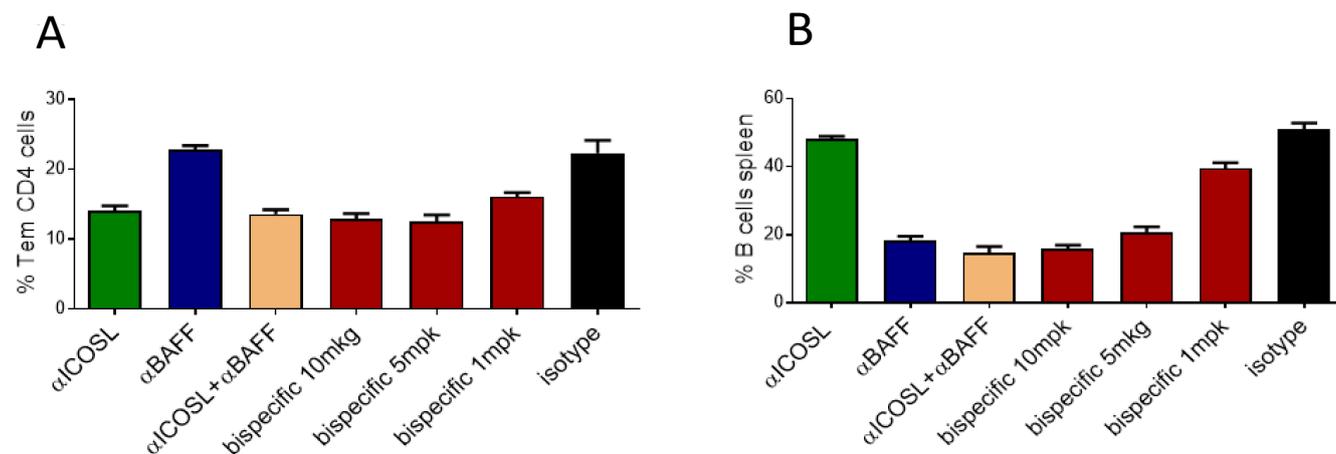
**A:** Biotinylated murine ICOSL-Fc was used to stain anti-CD3 activated mouse T cells in the presence of indicated concentrations of murine ICOSL/BAFF bispecific or anti-ICOSL antibody. The ICOSL-Fc binding on activated T cells were measured by FACS.

**B:** Purified B cells were stimulated with 200 ng/mL of murine BAFF and 0.1  $\mu$ g/mL Goat F(ab')<sub>2</sub> anti-IgM in the presence of indicated concentrations of ICOSL/BAFF bispecific or anti-BAFF peptide. B cell proliferation was measured by <sup>3</sup>H thymidine incorporation.

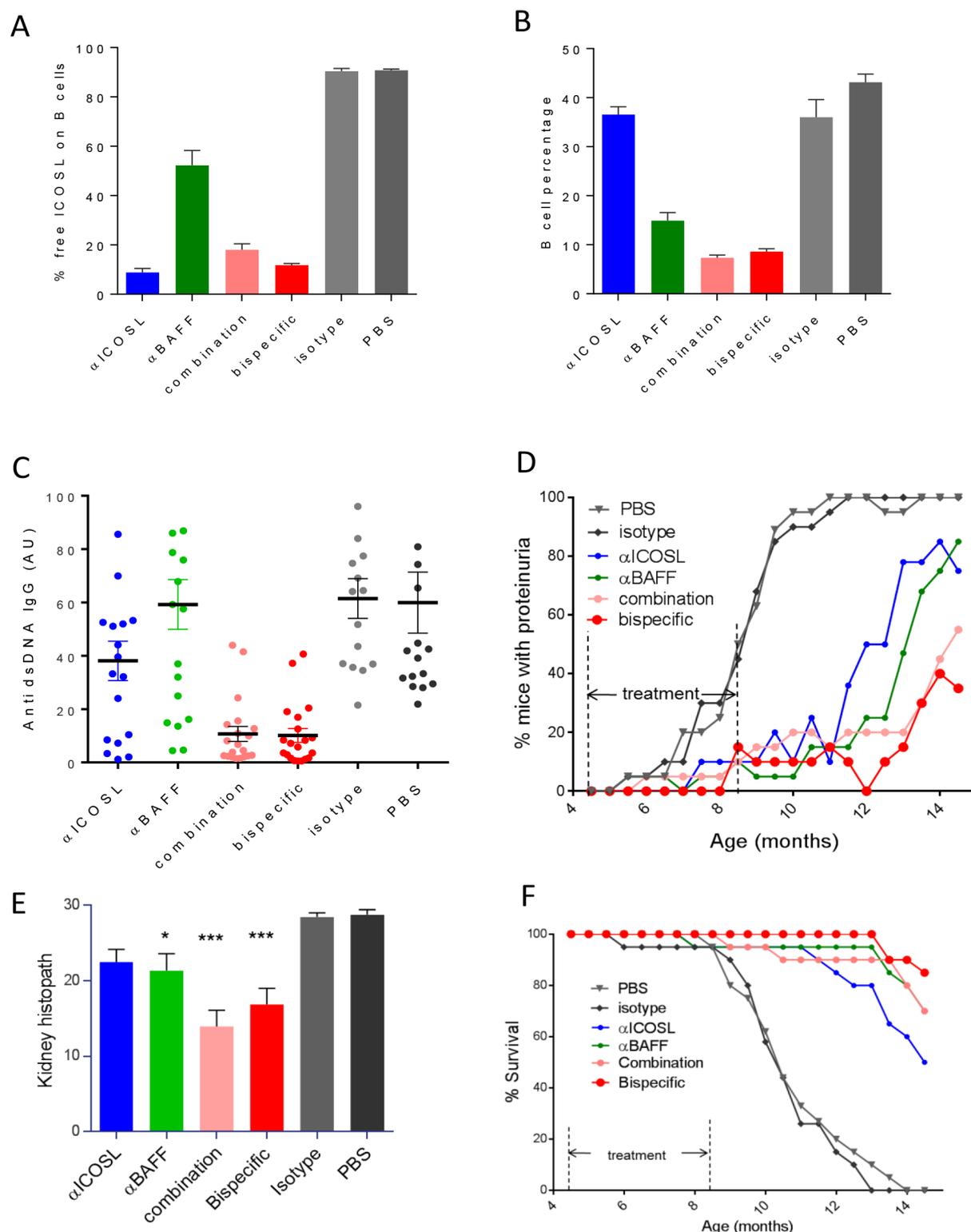
4.5 months of age were given ICOSL/BAFF bispecific, combination or single inhibitors twice per week for 4 months. ICOSL occupancy and B cell number in peripheral blood were monitored after one-month treatment. Treatment with bispecific or combination had similar ICOSL occupancy on peripheral blood B cells, comparable to anti-ICOSL antibody treatment alone (Fig. 3A). Moderate reduction of ICOSL occupancy was observed in anti-BAFF treatment group, likely due to the dynamic changes of B cell subpopulation with various ICOSL expression levels after BAFF inhibition. Treatment with

bispecific or combination also had similar level of B cell number reduction (Fig. 3B). Interestingly, slightly more B cell reduction was observed in both bispecific and combination treatment groups compared to anti-BAFF treatment alone, likely due to dual ICOSL and BAFF inhibitory effects. No significant B cell reduction was observed in anti-ICOSL treatment group (Fig. 3B). To investigate the effects of ICOSL/BAFF inhibition on autoantibody production, sera from all treated mice were tested for anti-dsDNA IgG after 4 months treatment. Anti-ICOSL treatment resulted in moderate inhibition of

anti-dsDNA IgG, whereas no significant inhibition of anti-dsDNA antibody was observed by anti-BAFF treatment alone. Both bispecific and combination treatment groups had significant inhibition of anti-dsDNA IgG (Fig. 3C). All mice were monitored for proteinuria development. During the four months treatment period, both single and dual ICOSL/BAFF inhibition treatment effectively inhibited proteinuria development. However, after the discontinuation of treatment, the single ICOSL or BAFF inhibition groups rapidly developed proteinuria, whereas the mice in the dual inhibition groups had significantly slower onset of proteinuria (Fig. 3D). Kidneys from all mice in the study were collected for histology analysis of renal lesion. Consistent with the decreased proteinuria, both murine bispecific and combination treatment groups had significantly less severe renal tissue damage (Fig. 3E). All therapeutic agent treatment groups had significantly prolonged survival compared with isotype control groups (Fig. 3F). No significant difference was observed between the dual targets *versus* single inhibition at the time of study termination. Overall, treatment with dual ICOSL and BAFF inhibition, either by bispecific or combination therapy, was more efficacious than single ICOSL or BAFF inhibition in the NZB/NZW lupus model.



**Fig. 2.** Dual target inhibition pharmacodynamics by murine ICOSL/BAFF bispecific in mice. Balb/c mice (n=5) were immunised with sheep red blood cells (SRBC) on day 0 and boosted on day 28. Mice were treated with 5 mg/kg of mouse IgG1 isotype control, anti-ICOSL antibody 1B7, anti-BAFF peptide, combination of anti-ICOSL antibody and anti-BAFF peptide, or indicated doses of murine ICOSL/BAFF bispecific twice per week starting on day 0 until day 35. On day 35, spleen cells were collected for FACS analysis. **A:** ICOSL inhibition PD effect was measured by CD4<sup>+</sup>CD44<sup>hi</sup>CD62<sup>lo</sup> T<sub>EM</sub> cell numbers. **B:** BAFF inhibition PD effect was measured by B220<sup>+</sup> B cell numbers.



**Fig. 3.** Murine ICOSL/BAFF bispecific treatment attenuates disease development in NZB/NZW mouse lupus model. Female NZB/NZW F1 mice (n=20/group) were treated with anti-ICOSL antibody, anti-BAFF peptibody, combination of anti-ICOSL antibody and anti-BAFF peptibody, ICOSL/BAFF bispecific, mIgG1 isotype or PBS control twice per week from age 4.5 month to age 8.5 month. Doses were adjusted based on MW to be molar equivalents.

**A:** ICOSL occupancy on peripheral blood B cells were measured by FACS after one-month treatment. **B:** B cells from peripheral blood were measured by FACS after one-month treatment. **C:** Anti-dsDNA IgG was measured at the age of 8.5 months by ELISA.

\*\*\*<0.0001, \*<0.05 for indicated treatment compared to isotype control (*t*-test). **D:** Proteinuria was measured every 2 weeks from age 4.5 month until 14.5 months age. *p*<0.0001 bispecific vs. isotype control; *p*<0.001 bispecific vs.  $\alpha$  ICOSL; *p*<0.01 bispecific vs.  $\alpha$  BAFF; *p*=0.59 bispecific vs. combo; *p*<0.0001  $\alpha$  ICOSL vs. isotype; *p*<0.0001  $\alpha$  BAFF vs. isotype (Kaplan-Meier plot). **E:** Kidney histology from all treated mice, including early diseased mice and remaining mice at the end of the experiment (14.5 months age), were collected and evaluated for histology analysis. \*\*\*<0.001, \*<0.05 1way ANOVA comparison to mIgG1 control group. **F:** Survival was monitored in the study.

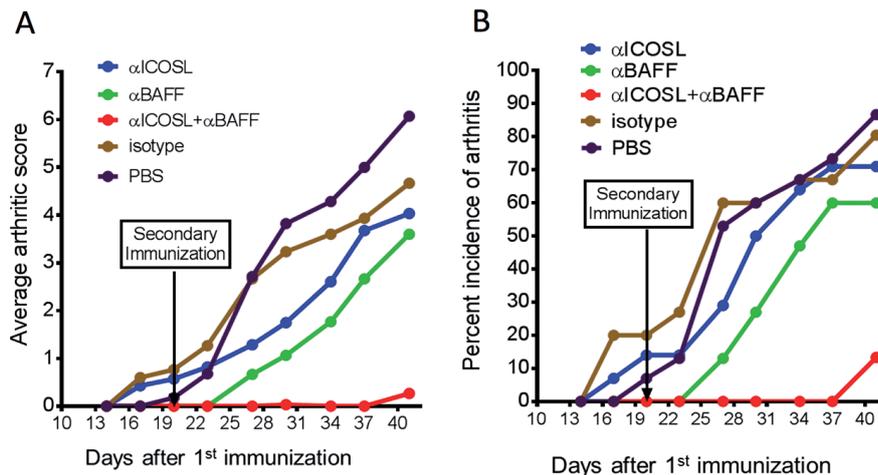
### Dual ICOSL and BAFF inhibition is more efficacious than single inhibition in collagen induced arthritis models in mice

To further investigate the effects of ICOSL and BAFF dual inhibition in autoantibody mediated disease models, we compared ICOSL and BAFF dual inhibition with single inhibition in the collagen induced arthritis (CIA) model. Development of high anti-drug antibody response against the bispecific molecule was observed in mice treated with complete Freund's adjuvant, precluded testing the bispecific in the CIA model. Therefore, ICOSL and BAFF dual inhibition in CIA model was evaluated by comparing combination of anti-ICOSL and anti-BAFF treatment with single inhibitor treatment. The combination of anti-ICOSL and anti-BAFF inhibitors had greatly reduced arthritis severity and incidence compared to the isotype, anti-ICOSL alone or anti-BAFF alone treatment (Fig. 4).

### Development of AMG 570, a bispecific molecule inhibiting human ICOSL and BAFF

We generated a bispecific molecule AMG 570 targeting human ICOSL and BAFF. AMG 570 is a human IgG2 containing two tandem copies of BAFF-inhibitory peptides to the C-terminus of each heavy chain of anti-ICOSL antibody AMG 557. AMG 570 has a 28 pM Kd human ICOSL binding affinity and 1.36 nM IC50 in ICOSL mediated T cell proliferation cellular. AMG 570 has a 29 pM Kd human BAFF binding affinity measured by KinExA™ and 0.86 nM IC50 in inhibition of BAFF mediated B cell proliferation.

Since AMG 570 also binds cynomolgus monkey ICOSL and BAFF with 49.4 pM and 22.3 pM Kd respectively, AMG 570 was tested in cynomolgus monkeys to understand the pharmacokinetic and the pharmacodynamics properties. Naïve male cynomolgus monkeys (n=4) were given a single bolus intravenous or subcutaneous dose of 10mg/kg AMG 570. Pharmacokinetics properties of AMG 570 were similar as the anti-ICOSL antibody AMG 557 in cynomolgus monkeys (Table I). We also examined if AMG 570 retains dual ICOSL and BAFF binding



**Fig. 4.** Dual ICOSL and BAFF inhibition was more efficacious than single inhibition in mouse collagen induced arthritis. 10 weeks old male DBA/1 mice were immunised at day 0 with bovine collagen in complete Freund's adjuvant at day 0. Mice were boosted with bovine collagen emulsified in incomplete Freund's adjuvant on day 21. Mice (n=15) were treated with 5mg/kg of anti-ICOSL antibody 1B7, anti-BAFF peptibody, combination of anti-ICOSL and anti-BAFF peptibody, or isotype control intraperitoneally two times per week for 6 weeks. **A:** Arthritis scores were evaluated two times per week. **B:** Percent incidence is quantified when an animal achieved a score of one on any paw.

**Table I.** Pharmacokinetics of AMG 570 in cynomolgus monkey.

Parameter	AMG 570		AMG 557	
	10mpk IV	10mpk SC	10mpk IV	10mpk SC
Cmax (ug/ml)	323	90	264	112
Tmax (hr)		45		72
AUC <sub>0-inf</sub> (ug*hr/mL)	33800	20300	26100	23800
MRT <sub>0-inf</sub> (hr)	136	132	138	144
CL (ml/hr/kg)	0.303	0.491	0.388	0.427
Vss (ml/kg)	42.5		52.1	

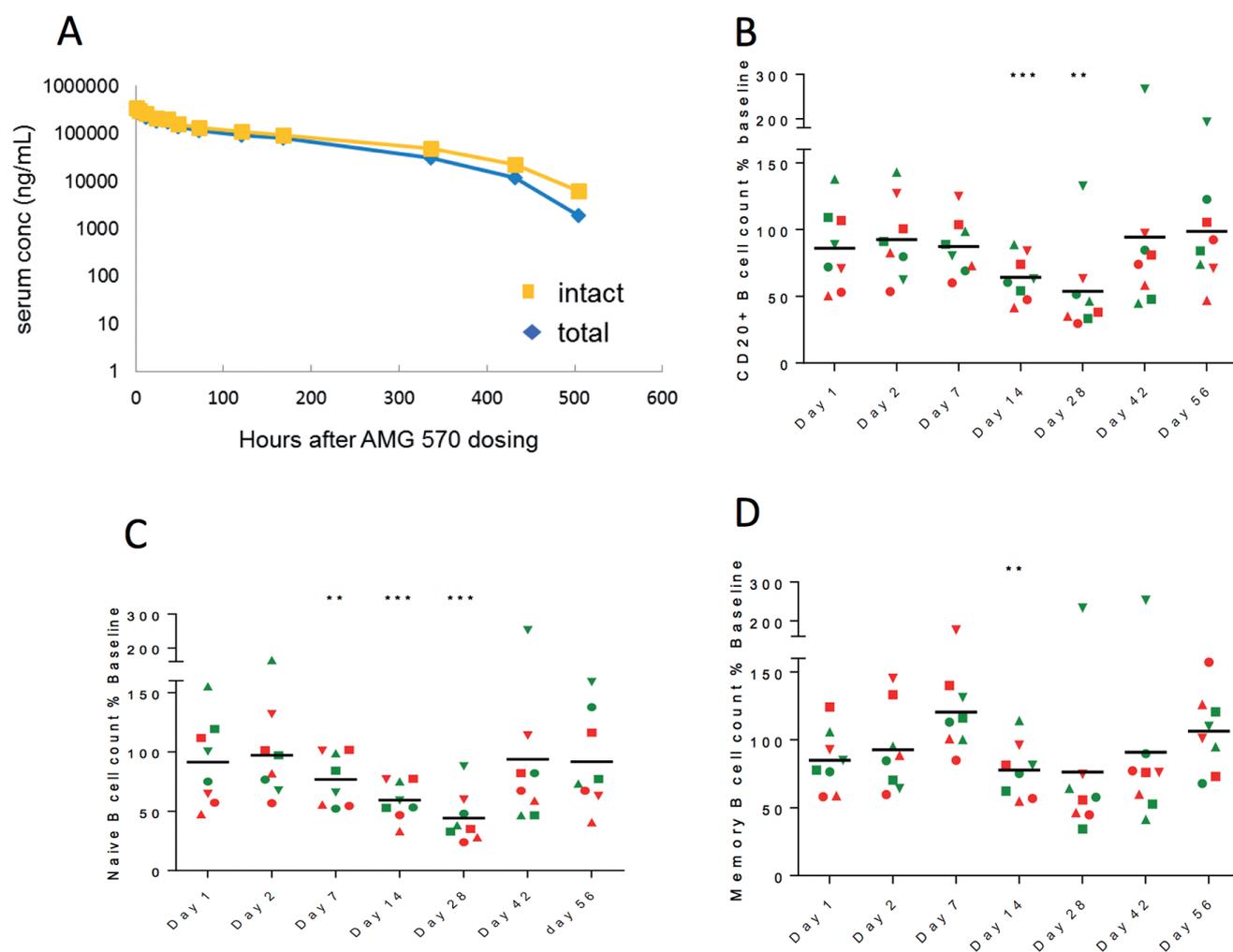
properties after administration in cynomolgus monkeys. The intact AMG 570 was measured by capturing with plate-bound BAFF and detecting with ICOSL-Fc, whereas the total AMG 570 level was measured by anti-human IgG capture and detection. The intact AMG 570 level was similar as the total AMG 570 level in serum, suggesting AMG 570 remained active in circulating blood in cynomolgus monkeys (Fig. 5A).

Pharmacodynamics effects on BAFF inhibition in cynomolgus monkeys were also investigated by monitoring peripheral immune cell populations by FACS analysis. Significant reduction of total CD20<sup>+</sup> B cells in peripheral blood was detected on day 14 after single dose treatment of AMG 570 by either subcutaneous or intravenous administration (Fig. 5B). Total B cells reached approximately the maximal reduction of 50% compared to baseline on day 28, and recovered by day 56. Naïve B cell reduc-

tion was detected on day 7 and reached maximal on day 28. Memory B cells had a trend of increase on day 7, but then reduced below baseline with maximal 50% reduction observed on day 28. No significant changes in other immune cell population, including T cells, in peripheral blood were observed. The B cell reduction kinetics need to be further examined in the repeat dose studies of AMG 570 in cynomolgus monkey and in clinical studies.

### Discussion

SLE remains a disease indication with high unmet medical needs. Given auto-reactive T cells are critical for autoimmune disease pathogenesis, we hypothesise that addition of T cell modulator to clinically validated BAFF inhibitor is likely to achieve efficacy superior to Benlysta. ICOSL is required for T<sub>FH</sub> cell development, which is critical for B cell activation and autoantibody generation.



**Fig. 5.** Single Dose AMG 570 PKPD study in cynomolgus monkey. Naive male cynomolgus monkeys ( $n=4$ ) were given a single bolus intravenous dose of 10mg/kg AMG 570. **A:** Total serum AMG 570 was measured by anti-human IgG antibody capture and detection. Intact AMG 570 was measured by capturing with ICOSL-Fc capture and detection with biotinylated BAFF. **B:** Total B cells ( $CD20^+$ ) (**C**) Naive B cells ( $CD20^+CD27^-$ ) and (**D**) memory B cells ( $CD20^+CD27^+$ ) in blood were measured by FACS analysis after treatment. B cell numbers at indicated timepoints after AMG 570 treatment relative to baseline for each animal was indicated. Red symbol represented intravenous administration. Green symbol represented subcutaneous administration. Shape represented individual animal. \*\* $p<0.01$ , \*\*\* $p<0.001$  student *t*-test comparison to baseline.

Compared to T cell inhibition by CT-LA4-Fc, ICOSL blockage has moderate effect in inhibition of T cell activation, therefore, is likely safe when combined with BAFF inhibitor for SLE or other autoimmune disease treatment.

Treatment with either mouse surrogate ICOSL/BAFF bispecific or combination were more effective in the inhibition of anti-dsDNA antibody, proteinuria development and renal tissue damage compared to single ICOSL or BAFF inhibition. Interestingly, both dual or single target inhibition had profound effects in the inhibition of proteinuria development during the initial four months treatment period. However, more sustained inhibition of proteinuria was observed in the dual

target inhibition groups compared to single target inhibition groups after the discontinuation of treatment. We speculate the dual ICOSL and BAFF inhibition had more profound effects in autoreactive B cell and T cell inhibition in NZB/NZW mice, therefore further delayed the spontaneous lupus development. Indeed, dual ICOSL and BAFF inhibition by either surrogate bispecific or combination treatment had more significant inhibition in anti-dsDNA antibody.

In addition to the lupus model, we also observed profound effect of dual ICOSL and BAFF inhibition in the collagen induced arthritis mouse model. Of note, high anti-drug antibody against the surrogate bispecific was

observed in wildtype mice without arthritis treated with complete Freund's adjuvant. Given the larger size and dual target binding of the surrogate bispecific molecule compared to the single inhibitors, we speculate that the surrogate bispecific might be more immunogenic in the presence of potent non-specific adjuvant such as complete Freund's adjuvant. The profound effect by the dual ICOSL and BAFF inhibition in the collagen induced arthritis model suggest ICOSL/BAFF bispecific may be used for other autoimmune and inflammatory disease treatments, such as rheumatoid arthritis (RA). A novel T cell subset known as T peripheral helper cell ( $T_{PH}$ ) was recently reported expanded in the synovium of RA patients and drove au-

toantibody generation (18). These T<sub>PH</sub> cells share some similarities with T<sub>FH</sub> phenotypically, including ICOS expression on cell surface. It remains to be studied if ICOSL is involved in these T<sub>PH</sub> cells development and activation in RA or other inflammatory diseases. If confirmed, ICOSL/BAFF bispecific will be suitable for RA treatment.

B cell reduction is the hallmark of BAFF inhibition. Reduction of total B cells and naïve B cells were reported approximately 12 weeks after belimumab treatment in cynomolgus monkeys and in clinical studies (15, 19). In our single dose AMG 570 study in cynomolgus monkey, reduction of total B cells and naïve B cells were observed 2 weeks after single dose AMG 570 administration. Since B cell development and activation are regulated by T cells, we speculate that inhibition of T cell co-stimulation could predispose B cells more susceptible to BAFF inhibition, thus resulting in earlier B cell reduction after AMG 570 treatment compared to BAFF inhibition alone.

Unlike total or naïve B cells, memory B cells increased initially after treatment with BAFF inhibitor belimumab (Benlysta) or tabalumab (15, 20). The memory B cell numbers gradually returned to baseline but did not further reduce below baseline in the reported studies. The mechanism of memory B cell increase after BAFF inhibition remains unclear. Interestingly, in our single dose AMG 570 study in cynomolgus monkey, memory B cell had a trend of increase on day 7, but then decreased below baseline similar as total and naïve B cells on day 14. Given the memory B cells are increased in SLE patients (21), treatment with AMG 570 may have additional benefit in controlling autoreactive B cells compared to single BAFF inhibitor treatment.

In our preclinical studies, we demonstrated dual ICOSL and BAFF inhibition has superior efficacy compared to single target inhibitor. Dual ICOSL and BAFF inhibition in clinical development could be achieved by combination of two single inhibitors or by a single bispecific molecule. A bispecific molecule also has more straightfor-

ward clinical development path compared to combination of two single inhibitors which need multiple cohorts to compare combination treatment *versus* each single inhibitor treatment at various doses. In addition, a bispecific molecule, as a single new molecular entity (NME), has lower cost of goods and administration convenience compared to combination treatment with two single inhibitors.

We successfully developed AMG 570, a bispecific molecule with high potencies of ICOSL and BAFF dual inhibitions. In addition, AMG 570 has antibody like pharmacokinetic properties. We believe AMG 570 has the potential to achieve superior efficacy in treatment of autoimmune diseases such as SLE and rheumatoid arthritis.

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