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ARTICLE

First-in-human study of the safety, tolerability, pharmacokinetics, and pharmacodynamics of ALPN-101, a dual CD28/ICOS antagonist, in healthy adult subjects

Jing Yang[1] | **Jason D. Lickliter**[2] | **Jan L. Hillson**[1] | **Gary D. Means**[1] | **Russell J. Sanderson**[1] | **Kay Carley**[1] | **Almudena Tercero**[1] | **Kristi L. Manjarrez**[1] | **Jennifer R. Wiley**[1] | **Stanford L. Peng**[1]

[1]Alpine Immune Sciences, Inc., Seattle, Washington, USA

[2]Nucleus Network, Melbourne, Victoria, Australia

Correspondence
Jing Yang, Alpine Immune Sciences, Inc., 188 East Blaine Street, Suite 200, Seattle, WA 98012, USA.
Email: jing.yang@alpineimmunesciences.com

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Abstract

ALPN-101 (ICOSL vIgD-Fc) is an Fc fusion protein of a human inducible T cell costimulatory ligand (ICOSL) variant immunoglobulin domain (vIgD) designed to inhibit the cluster of differentiation 28 (CD28) and inducible T cell costimulator (ICOS) pathways simultaneously. A first-in-human study evaluated the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of ALPN-101 in healthy adult subjects. ALPN-101 was generally well-tolerated with no evidence of cytokine release, clinically significant immunogenicity, or severe adverse events following single subcutaneous (SC) doses up to 3 mg/kg or single intravenous (IV) doses up to 10 mg/kg or up to 4 weekly IV doses of up to 1 mg/kg. ALPN-101 exhibited a dose-dependent increase in exposure with an estimated terminal half-life of 4.3–8.6 days and SC bioavailability of 60.6% at 3 mg/kg. Minimal to modest accumulation in exposure was observed with repeated IV dosing. ALPN-101 resulted in a dose-dependent increase in maximum target saturation and duration of high-level target saturation. Consistent with its mechanism of action, ALPN-101 inhibited cytokine production in whole blood stimulated by *Staphylococcus aureus* enterotoxin B ex vivo, as well as antibody responses to keyhole limpet hemocyanin immunization, reflecting immunomodulatory effects upon T cell and T-dependent B cell responses, respectively. In conclusion, ALPN-101 was well-tolerated in healthy subjects with dose-dependent PK and PD consistent with the known biology of the CD28 and ICOS costimulatory pathways. Further clinical development of ALPN-101 in inflammatory and/or autoimmune diseases is therefore warranted.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

ALPN-101 is an Fc fusion protein of a human inducible T cell costimulatory ligand variant immunoglobulin domain designed to block the cluster of differentiation 28 CD28) and inducible T cell costimulator (ICOS) simultaneously, thereby inhibiting two key

costimulatory pathways in T lymphocytes. Although inhibitors of each pathway alone have been studied in humans, this is the first assessment of a dual antagonist in humans.

WHAT QUESTION DID THIS STUDY ADDRESS?

This first-in-human study assessed the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of ALPN-101 in healthy subjects. The PK-PD relationship was evaluated.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

ALPN-101 demonstrated favorable safety and tolerability profiles and dose-dependent PK and PD in healthy subjects. The dose-PK-PD analysis showed that the target saturation of ALPN-101 can be well-predicted based on PK data and the observed PD effects are consistent with the known biology of the CD28 and ICOS pathways.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

The results support further clinical development of ALPN-101 and the PK-PD relationship will guide dosing regimens in autoimmune and inflammatory diseases.

INTRODUCTION

Cluster of differentiation 28 (CD28) and inducible T cell costimulation (ICOS) are closely related costimulatory molecules that bind, respectively, the ligands CD80 (B7-1) and CD86 (B7-2), and ICOS ligand (ICOSL), and play partially overlapping roles in normal and pathogenic immune responses.[1] CD28 is recognized as a critical costimulatory signal for naïve T cell activation.[2] Abatacept and belatacept, both CTLA4-Ig fusion proteins that antagonize the CD28-CD80/86 interaction, are approved for the treatment of some inflammatory arthritis conditions (rheumatoid arthritis, juvenile idiopathic arthritis, and psoriatic arthritis) and for the prevention of renal allograft rejection, respectively.[3,4] However, abatacept and/or other inhibitors of the CD28 pathway alone have been unsuccessful in clinical trials for several other inflammatory diseases, including inflammatory bowel disease, systemic lupus erythematosus and lupus nephritis, and multiple sclerosis, suggesting that an additional costimulatory pathway(s) compensates in the presence of CD28/CD80 or CD86 blockade.[5–9] Indeed, CD28-negative T cells accumulate in various inflammatory diseases, correlating with disease activity and lack of responsiveness to abatacept.[10–16]

ICOS is the most closely related immunoglobulin superfamily member to CD28. Unlike CD28, it is not expressed in naïve T cells but rapidly upregulates after activation and may represent a key pathogenic pathway unaddressed by CD28 pathway antagonism. ICOS appears particularly important for the function of several activated and/or effector T cell subsets, including differentiated types 1, 2, 17, and follicular helper, as well as for T-dependent B cell and germinal center responses.[17] ICOS upregulation correlates with disease activity in several inflammatory diseases, and in preliminary clinical studies, the anti-ICOSL monoclonal antibody (mAb) prezalumab (AMG-557) demonstrated some beneficial activity for joint inflammation in patients with systemic lupus erythematosus (SLE).[18–22]

Combined blockade and/or knockout of both CD28 and ICOS has been demonstrated to be advantageous over intervention on either pathway alone in multiple animal models, such as for islet allografts,[23] inflammatory bowel disease,[24] delayed-type hypersensitivity,[25] and graft-versus-host disease (GVHD).[26] These observations support the hypothesis that combined inhibition of CD28 and ICOS may afford improved clinical outcomes in autoimmune and/or inflammatory diseases when compared with the therapeutics targeting the CD28 or ICOS pathway alone.

ALPN-101 (ICOSL vIgD-Fc) is an Fc fusion protein of a human ICOSL variant immunoglobulin domain (vIgD) designed to inhibit the CD28 and ICOS inflammation pathways simultaneously.[27] It is an Fc dimer of an ICOSL vIgD domain, derived by directed evolution to bind both CD28 and ICOS, fused to an effector function negative IgG1 Fc, with a predicted molecular weight of 80.8 kilodaltons. Nonclinical studies of ALPN-101 demonstrate potent inhibition of disease activity, in association with suppressed pathogenic T and/or B cell responses where appropriate, in mouse models of GVHD,[28] inflammatory arthritis,[29] sialoadenitis (Sjögren's syndrome), SLE,[30] inflammatory bowel disease,[31] and uveitis.[32] Furthermore, in preclinical studies in mice or with cells from patients with various inflammatory diseases, ALPN-101 exhibited immunomodulatory activity superior to that achievable by combining biologic inhibitors of the CD28 and ICOS pathways, suggesting a unique, possibly synergistic, advantage of ALPN-101 over currently available therapeutic reagents.[29,30] ALPN-101 was well-tolerated in nonclinical safety studies without evidence of cytokine release or target organ toxicities.

A first-in-human study was conducted to evaluate the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of ALPN-101 in healthy adult subjects after single intravenous (IV) infusion or subcutaneuos (SC) injection or multiple weekly (Q1W) or every-other-week (Q2W) IV infusions for up to 4 weeks.

METHODS

Study design

Overview

This phase I, randomized, placebo-controlled, dose-escalation trial (NCT03748836) was sponsored by Alpine Immune Sciences, and approved by the Alfred Health Human Research Ethics Committee (Melbourne, Australia) in accordance with the National Statement on Ethical Conduct in Human Research. It was conducted at the Centre for Clinical Studies at Nucleus Network Pty. Ltd., in compliance with ICH E6(R2), annotated with comments by the Australian Therapeutic Goods Administration (2018).

Subjects

All subjects provided written informed consent before enrollment. Key inclusion criteria included: healthy male and female volunteers between 18 and 65 years at the time of informed consent; body mass index (BMI) between 18 and 30 kg/m^2, inclusive. Key exclusion criteria included: any significant concomitant or history of clinically significant disease, condition or treatment that could interfere with the conduct of the study; clinically significant abnormalities in laboratory test results; and abnormal cardiovascular findings.

Dose regimens

Planned doses were selected based on the predicted human PK profile and CD28 target saturation, and anticipated safety margins relative to nonclinical safety studies. In order to minimize the possibility of experiencing unintended, excessive toxicities due to unpredicted agonistic effects, especially on CD28, as previously observed with TGN1412 (theralizumab),[33] a minimum anticipated biologic effect level, corresponding to a predicted CD28 target saturation of 26% at peak plasma concentration (C_{max}), was selected as the starting dose (0.001 mg/kg). In part A, 72 subjects ($N = 6$ per cohort) were randomized at 4:2 to receive a single dose of ALPN-101 or placebo (0.9% sodium chloride injection). There were 10 sequential ascending IV dose cohorts (0.001–10 mg/kg) and 2 SC dose cohorts (1 or 3 mg/kg). Sentinel dosing was implemented for each IV cohort: the first 2 subjects were randomized to receive ALPN-101 or placebo; after ~ 48 hours of observation, 4 additional subjects were randomized 3:1 to receive ALPN-101 or placebo. In part B, 24 subjects ($N = 8$ per cohort) were randomized 6:2 to receive repeated doses of ALPN-101 or placebo: 0.3 or 1 mg/kg Q1W IV for 4 doses, or 1 mg/kg Q2W IV for 2 doses.

Keyhole limpet hemocyanin immunization

At and above dose levels of 1 mg/kg in part A and all dose cohorts in part B, each subject received an SC administration of 1 mg keyhole limpet hemocyanin (KLH; Stellar Biotechnologies) within 2 h (IV cohorts) or 24–28 h (SC cohorts) following ALPN-101 or placebo administration.

Safety assessments

Safety evaluations were performed for all subjects including adverse events (AEs), clinical laboratory tests, vital signs, 12-lead electrocardiograms, and physical examinations. AEs were graded according to National Cancer Institute Common Toxicity Criteria for Adverse Events version 5.0 and coded using the Medical Dictionary for Regulatory Activities version 21.1.

Cytokines

Serial plasma samples for cytokines were collected in part A and part B as exploratory safety laboratory assessments. Thirty different cytokines were quantified using high-sensitivity multiplex immunoassay Human CytokineMAP A and B version 1.0 (Myriad RBM).

PK assessments

Serial dense PK serum samples in part A were collected at predose and postdose up to day 28. Serial dense PK serum samples in part B were collected following the first and last doses, whereas sparse PK serum samples were collected after the other doses. Concentrations of ALPN-101 were determined using a validated enzyme-linked immunosorbent assay (ELISA) with lower limit of quantification (LLOQ) of 175 ng/ml, where ALPN-101 was captured by mouse anti-ICOSL/CD275 (eBiosciences, Cat. #16-5889-82) and detected by mouse anti-human IgG-HRP (Jackson ImmunoResearch, Cat. #209-035-098).

PD assessments

Immunophenotyping and target saturation by flow cytometry

Serial whole blood samples for immunophenotyping and target saturation were collected at predose and postdose in part A and part B. Circulating lymphocyte cells and subsets were assessed by various T and B cell markers. Target saturation

by ALPN-101 on T cells were quantified by direct detection of ALPN-101 using a goat anti-human IgG Fc antibody. The details of immunophenotyping and target saturation by flow cytometry are provided in supplementary documents (Methods S1).

Ex vivo Staphylococcus aureus enterotoxin B-induced cytokine production

Serial whole blood samples for ex vivo Staphylococcus aureus enterotoxin B (SEB) stimulation were collected at predose and postdose in part A and part B using TruCulture blood collection tubes with or without SEB (Myriad RBM, Austin, TX). The cytokines in plasma were quantified by high sensitivity multiplex immunoassay Human CytokineMAP A (Myriad RBM).

Anti-KLH antibodies

Serum samples for anti-KLH antibody were collected at predose and postdose in part A and part B. Serum anti-KLH IgG and IgM were measured by a quantitative ELISA (Radboud University Medical Centre, Nijmegen, The Netherland) as described.[34] The LLOQ of anti-KLH IgG and IgM were 3 and 5 μg/ml, respectively.

Anti-drug antibody assessments

Serum samples for anti-ALPN-101 antibody assessment were collected at predose and postdose in part A and part B. Anti-ALPN-101 antibody were measured by a validated electrochemiluminescence (ECL) immunoassay using biotinylated and sulfo-TAG conjugated ALPN-101. The drug tolerance of this assay was 50 and 200 μg/ml in the presence of 100 and 50 μg/ml positive control antibodies, respectively. Samples that were confirmed positive for ADA were subsequently explored for neutralizing antibody (NAb) activity using a cell-based bioassay, as detailed in the supplementary documents (Method S2)

PK analysis

The PK population comprised subjects who received ALPN-101 and had sufficient serum concentration-time data to determine at least one PK parameter. Individual PK parameters were estimated by noncompartmental analysis using Phoenix WinNonlin (version 8.1; Certara). Dose proportionality of C_{max} and area under the curve (AUC) was assessed for the IV cohorts in part A using a power model with log-transformed PK parameters, as described previously.[35] Bioavailability of SC administration was estimated by computing the ratios of the geometric least square means (IV/SC) of AUC to infinity (AUC_{inf}) at 3 mg/kg. Accumulation index were calculated as the ratios of exposure between the last dose and the first dose for C_{max} and AUC of dosing interval (AUC_{τ}) in part B.

PD analysis

For target saturation calculation, median fluorescence intensity (MFI) of CD4+ or CD8+ T cells that stained positively for the anti-human IgG Fcγ (vs. fluorescence minus one control) was reported. Target saturation, as the measurement of the target engagement of both CD28 and ICOS, was determined using the following formula:

$$\%\,\text{Target Saturation} = \left(\frac{\text{MFI}_{\text{test sample}}}{\text{MFI}_{\text{ALPN-101 saturated sample}}}\right) \times 100\,\%$$

PD assessment of target saturation was primarily performed on the data obtained from CD4+ T cells due to the linear relationship between target saturation on CD4+ T cells and on CD8+ T cells (Figure S1). The PD parameters of the target saturation on CD4+ T cells were estimated based on mean target saturation versus nominal time for each cohort. Individual cytokines induced by SEB in each whole blood sample were calculated as $C_{\text{SEB induced}} = C_{\text{SEB treated}} - C_{\text{unstim}}$. PD parameters were estimated based on median IL-2 change from baseline versus nominal time for each cohort. PD parameters for target saturation and the change of IL-2 in ex vivo SEB assay were estimated by noncompartmental analysis using Drug Effect Model in Phoenix WinNonlin (version 8.1; Cetara). All statistical analysis were performed by GraphPad Prism 8.2.1 (GraphPad Software, www.graphpad.com).

RESULTS

Demographics and disposition

A total of 96 healthy adult male and female subjects were enrolled in part A ($N = 72$) or part B ($N = 24$) and 92 subjects completed the study. Demographic and baseline characteristics were well-balanced between ALPN-101 groups and placebo groups (Table S1). There were approximately equal numbers of men (51.0%) and women (49.0%), and most subjects were White (82.3%). The median age (range) of all subjects was 25.0 (18–60) years, the mean body weight (SD) was 69.95 (10.08) kg and mean BMI (SD) was 23.52 (2.82) kg/m^2. Four subjects withdrew from the study early: 3 subjects in part A (2 subjects received ALPN-101 at 0.003 mg/

kg and 1 subject received 1 mg/kg IV) withdrew due to personal reasons; 1 subject in part B (ALPN-101 1 mg/kg Q1W) withdrew early due to a grade 1 vessel puncture site reaction that was deemed not related to study drug. Among the 66 subjects (48 in part A and 18 in part B) randomized to receive ALPN-101, 5 subjects (1 in part A and 4 in part B) received less than the protocol planned treatments. The list of the subjects received partial dose and the impact on the PK and PD analysis are detailed in Table S2a.

Safety and tolerability

All 96 enrolled subjects were included in the evaluation of safety. There were no deaths, life-threatening AEs, grade 3 or higher AEs, or treatment-related serious AEs that occurred during the study. There were no clinically significant ALPN-101-related changes in vital signs, electrocardiogram parameters, or laboratory values.

Overall, 71.9% of subjects (69 of 96) reported at least one AE. The incidence was similar across the pooled ALPN-101 groups (74.2%, 49 of 66 subjects) compared with the pooled placebo groups (66.7%, 20 of 30 subjects). Most AEs were grade 1, occurring in 68.8% subjects (66 of 96), with a higher frequency reported in part B (87.5%) compared to part A (62.5%). The remaining AEs were grade 2, occurring in 21.9% (21 of 96), with a higher frequency reported in part B (29.2%) compared to part A (19.4%). There was one serious AE reported in the second lowest dose cohort 0.003 mg/kg in part A, an isolated grade 1 event of elevated troponin I that was not associated with any signs or symptoms. After an extensive diagnostic evaluation, this event was not associated with any evidence of cardiac damage or dysfunction and was assessed as not related to ALPN-101 treatment as well as not clinically significant. There were no additional elevations in cardiac enzymes in the study, and no other significant laboratory abnormalities. There were five events in three subjects that led to study drug interruption and four events in three subjects that led to study drug withdrawal, all of which were reported in part B and none of which were deemed related to ALPN-101 treatment (Table S2b).

In part A, the most common AEs by preferred term were headache (20.8% overall; 25.0% ALPN-101; and 12.5% placebo) and upper respiratory tract infections (18.1% overall; 22.9% ALPN-101; and 8.3% placebo). There were no clear associations of AEs with dose level and no events prompted cessation of dose escalation. The most common AEs in part B were administration site recall reactions related to KLH administration (16.7% overall; 22.2% ALPN-101; and 0% placebo), headache (45.8% overall; 44.4% ALPN-101; and 50.0% placebo), and aphthous ulcer (16.7% overall; 22.2% ALPN-101; and 0% placebo).

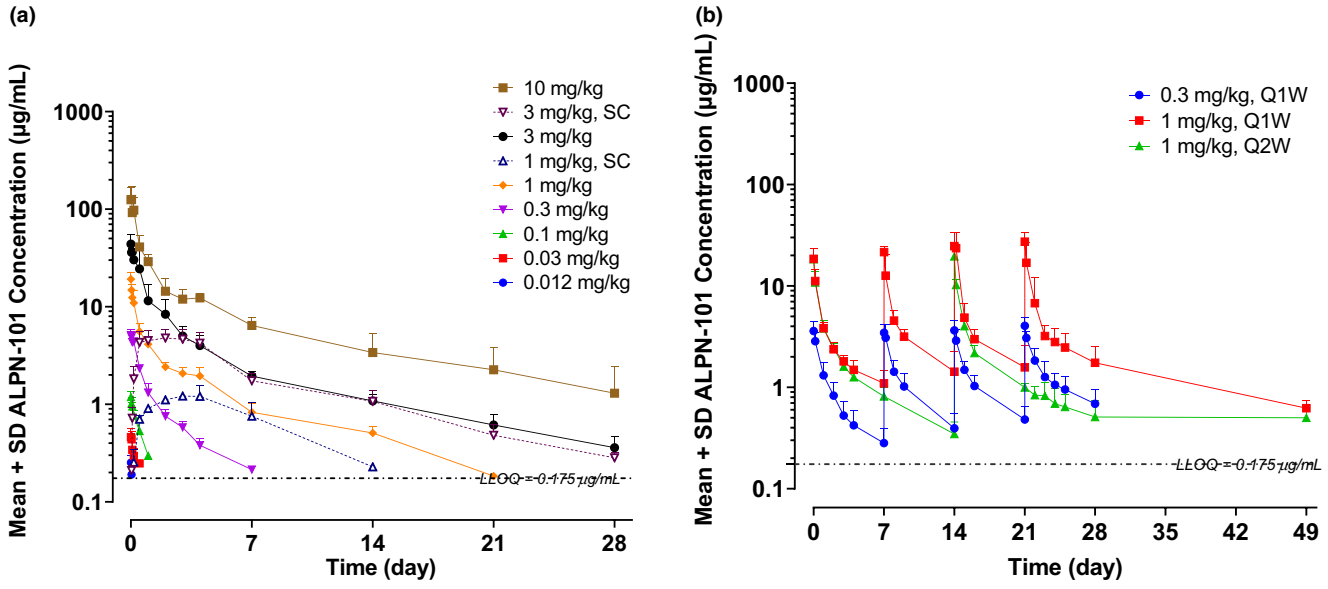

FIGURE 1 ALPN-101 serum concentration versus nominal time following single IV or SC dosing in part A (a) and repeated IV dosing in part B (b). All doses are IV unless indicated "SC" The concentrations below the lower limit of quantification (LLOQ) before dose administration were treated as "0" and after dose administration were treated as "missing." In (a), each cohort is presented as $n = 4$ except 0.012 mg/kg ($n = 2$), 0.03 mg/kg ($n = 3$), and 1 mg/kg SC ($n = 3$). Two subjects (1 in 0.012 mg/kg and 1 in 1 mg/kg SC) were excluded from the summary due to nonspecific enzyme-linked immunosorbent assay (ELISA) background interference. At 28 days following dose administration, the concentrations for most individuals fell below the LLOQ except at the following dose levels: 3 mg/kg SC ($n = 2$), 3 mg/kg ($n = 4$), and 10 mg/kg ($n = 4$). In (b), each cohort is presented as $n = 6$ except 1 mg/kg Q2W ($n = 5$), in which 1 subject was excluded from the summary due to nonspecific ELISA background interference. At 49 days following the first dose administration, the concentrations for most individuals fell below the LLOQ except 1 mg/kg Q1W ($n = 2$) and 1 mg/kg Q2W ($n = 2$)

TABLE 1 Mean (CV%) serum PK parameters for ALPN-101 following single IV or SC administration in part A

Dose (N)	C_{max}, µg/ml	T_{max}, day[a]	$AUC_{0\text{-last}}$, µg·day/ml	$AUC_{0-\infty}$, µg·day/ml	$T_{1/2}$, day	CL or CL/F, ml/day/kg	V_z or V_z/F, ml/kg
0.012 mg/kg ($N = 2$)[b,c]	0.253 (18.3%)	0.007 (0.007–0.007)	NC	NC	NC	NC	NC
0.03 mg/kg ($N = 3$)[b]	0.463 (22.1%)	0.0125 (0.007–0.042)	0.093 (75%)	NC	NC	NC	NC
0.1 mg/kg ($N = 4$)	1.19 (14.4%)	0.007 (0.007–0.007)	0.570 (11.9%)	NC	NC	NC	NC
0.3 mg/kg ($N = 4$)	5.45 (9.8%)	0.0236 (0.007-0.042)	5.11 (6.7%)	NC	NC	NC	NC
1 mg/kg ($N = 4$)	17.6 (23.1%)	0.0073 (0.007–0.042)	22.8 (12.2%)	25.4 (13.7%)	4.29 (30.8)	40.2 (13.7%)	242 (25.2%)
3 mg/kg ($N = 4$)	45.8 (21.6%)	0.0094 (0.007–0.083)	73.1 (27.1%)	77.8 (28%)	8.64 (20.4%)	40.8 (28.6%)	487 (10.9%)
10 mg/kg ($N = 4$)	133 (24.4%)	0.0417 (0.008, 0.042)	188 (17.1%)	210 (25.5%)	7.99 (39.3%)	49.6 (26.7%)	539 (25.0%)
1 mg/kg, SC ($N = 3$)[c]	1.33 (17.6%)	3.0 (3.0–4.0)	8.40 (40.9%)	NC	NC	NC	NC
3 mg/kg, SC ($N = 4$)	5.02 (20%)	2.52 (2.0–3.0)	42.9 (21.4%)	46.3 (18%)	6.88 (8.4%)	66.6 (19.1%)	666 (24.8%)

Note: Serum concentration of ALPN-101 were below LLOQ for all subjects in dose groups 0.001, 0.003, and 0.006 mg/kg.

Abbreviations: $AUC_{0-\infty}$, area under the concentration-time curve from time zero to infinity; $AUC_{0\text{-last}}$, area under the concentration-time curve from time zero to time of last measurable concentration; CL, apparent clearance for IV administration; CL/F, apparent clearance for SC administration; C_{max}, maximum observed concentration; CV%, percent coefficient of variation; LLOQ, lower limit of quantification; NC, not calculated due to insufficient data; PK, pharmacokinetic; $t_{1/2}$, elimination half-life at the terminal phase; T_{max}, time to maximum observed concentration; V_z, apparent volume of distribution at the terminal phase for IV administration; V_z/F, apparent volume of distribution at the terminal phase for SC administration.

[a]Median (minimum, maximum).

[b]Concentrations were below the LLOQ at all sample timepoints for two subjects (1 in each indicated dose group).

[c]PK analysis for total of two subjects (1 in each indicated dose groups) were not performed due to nonspecific enzyme-linked immunosorbent assay background interference at baseline (prior to the first dose).

No ALPN-101-related infusion-related reactions, hypersensitivity reactions, or significant change from baseline in any cytokine tested were observed.

PK of ALPN-101

After IV administration of ALPN-101, the serum concentration of ALPN-101 declined with time in a bi-exponential manner. The serum concentrations were measurable up to 1 h at 0.012 mg/kg to at least 28 days at 10 mg/kg after dosing in part A, and up to 28 days at 0.3 mg/kg Q1W dosing to at least 49 days at 1 mg/kg Q1W or Q2W in part B (Figure 1a,b).

After single IV infusion of ALPN-101, the mean clearance (CL) ranged from 40.2 ml/day/kg to 49.6 ml/day/kg in the dose range of 1 mg/kg to 10 mg/kg. The mean terminal half-life ($t_{1/2}$) was 4.29, 8.64, and 7.99 days in the 1, 3, and 10 mg/kg IV dose cohorts, respectively (Table 1). Dose proportional increases of ALPN-101 exposures were observed across the dose range of 0.03–10 mg/kg for C_{max} and the dose range of 1–10 mg/kg for $AUC_{0-\infty}$, with point estimates of 1.034 (90% confidence interval [CI]: 0.992–1.076) for C_{max} and 0.943 (90% CI: 0.802–1.084) for $AUC_{0-\infty}$ by the power model, respectively. PK parameters were not calculable for doses less than 0.012 mg/kg because concentrations were below the LLOQ at all sampling timepoints.

After single SC injection of ALPN-101, the time of maximum plasma concentration was observed between 2 and 4 days postdose (Table 1). The bioavailability of ALPN-101 following 3 mg/kg single SC administration was estimated to be 60.6%.

After repeated IV administration of ALPN-101, minimal to modest accumulation of C_{max} (mean accumulation index [AI]: 1.16–1.52) and AUC_{τ} (mean AI: 1.55–1.69) were observed at the 0.3 and 1 mg/kg Q1W, and minimal accumulation of C_{max} (mean AI: 1.10) and AUC_{τ} (mean AI: 1.13) were observed at 1 mg/kg Q2W (Table 2).

PD of ALPN-101

To characterize the biological activity of ALPN-101, target saturation, ex vivo SEB-induced IL-2 production, and

TABLE 2 Mean (CV%) serum PK parameters for ALPN-101 following repeated IV administration in part B

Dose	Dosing day 1		Dosing day 22				Dosing day 15			
	C_{max}, μg/ml	AUC_τ, μg·day/ml	C_{max}, μg/ml	AUC_τ, μg·day/ml	AI C_{max}	AI AUC_τ	C_{max}, μg/ml	AUC_τ, μg·day/ml	AI C_{max}	AI AUC_τ
0.3 mg/kg Q1W ($N = 6$)[a]	3.59 (23.0%)	5.21 (31.8%)	4.04 (20.3%)	8.21 (32.3%)	1.16 (23.4%)	1.69 (15.5%)				
1 mg/kg Q1W ($N = 6$)[a]	18.5 (25.4%)	18.4 (11.3%)	27.3 (22.2%)	29.0 (39.3%)	1.52 (24.7%)	1.55 (33.4%)				
1 mg/kg Q2W ($N = 5$)[a,b]	18.4 (8.8%)	20.8 (9.3%)					19.6 (15.9%)	23.4 (6.9%)	1.10 (16.6%)	1.13 (7.3%)

AI, accumulation index calculated as day 22 or day 15 over day 1 C_{max} or AUC_τ; AUC_τ, area under the concentration-time curve over doing interval of 7 days or 14 days; C_{max}, maximum observed concentration over the dosing interval; CV%, percent coefficient of variation; PK, pharmacokinetic.

[a]One subject (0.3 mg/kg Q1W) and two subjects (1 mg/kg Q1W) received three of the four planned doses. One subject (1 mg/kg Q2W) received one the two planned doses. The PK profiles and summary statistics by including these subjects were comparable to those by excluding these subjects. Therefore, the results are presented by including these four subjects.

[b]PK analysis for one subject was not performed due to nonspecific enzyme-linked immunosorbent assay background interference at baseline (prior to the first dose).

antibody responses to KLH immunization were assessed. These measurements were chosen based on the mechanism of action of ALPN-101: SEB-induced IL-2 measured the immediate downstream biological responses to ALPN-101 following target binding—inhibiting T cell activation by naïve T cells primarily through CD28 blockade as previously reported with anti-CD28 antibody[36]; anti-KLH antibodies measured the impact of CD28 and ICOS dual blockade by ALPN-101 on T cell-dependent B cell antibody response mediated by the interactions between T cells and germinal center B cells as previously reported with anti-CD28 or anti-ICOSL antibodies.[36–38]

Target saturation on CD4+ T cells

After single IV infusion of ALPN-101, target saturation peaked at the end of infusion. The mean maximum target saturation increased in a dose-dependent manner from 22.9% at 0.001 mg/kg to 99.2% at 0.1 mg/kg; and remained similarly high—ranging from 98.8% to 99.6% in subsequent dose cohorts 0.3–10 mg/kg. The duration of high target saturation (>95%) also increased in a dose-dependent manner from 1.39 days at 0.03 mg/kg to at least 28 days at 10 mg/kg. A simple binding model was used to correlate the IV dosing and maximum target saturation. The estimated binding effective dose for 50% of the population was 0.002 mg/kg. Single SC administration resulted in high target saturation (>95%) starting from 0.083 to 1-day postdose that lasted for 14.7 days at 1 mg/kg and at least 28 days at 3 mg/kg. The mean target saturation at 28 days postdose in part A decreased to less than 10% for doses below 1 mg/kg; and to 35.8% and 97.7% for 1 and 3 mg/kg SC, respectively; and to 58.1%, 82.8, and 99.4% for 1, 3, and 10 mg/kg, respectively (Figure 2a,c,d and Table S3).

After repeated IV administration, high target saturation (>95%) was achieved and maintained on average for 27 days at 0.3 mg/kg Q1W or 1 mg/kg Q2W and 41 days at 1 mg/kg Q1W. The mean target saturation at 49 days post first doses in part B decreased to 19.5%, 26.6%, and 91.7% for 0.3 mg/kg Q1W, 1 mg/kg Q2W, and 1 mg/kg Q1W, respectively (Figure 2b, Table S3).

Ex vivo SEB-induced cytokine production

Ex vivo stimulation of whole blood with SEB induced multiple cytokines, including IL-2, IL-6, IFN-γ, and TNF-α (Figure S2). PD assessment to valuate T cell specific activity was performed primarily based on IL-2, given its known mechanistic dependence on CD28.[39] ALPN-101 treatment resulted in a reduction in SEB-induced IL-2 (Figure 3a,b). Owing to the large variability of the assay and small sample size in each treatment group, statistically significant differences were only detected between some ALPN-101 treatment

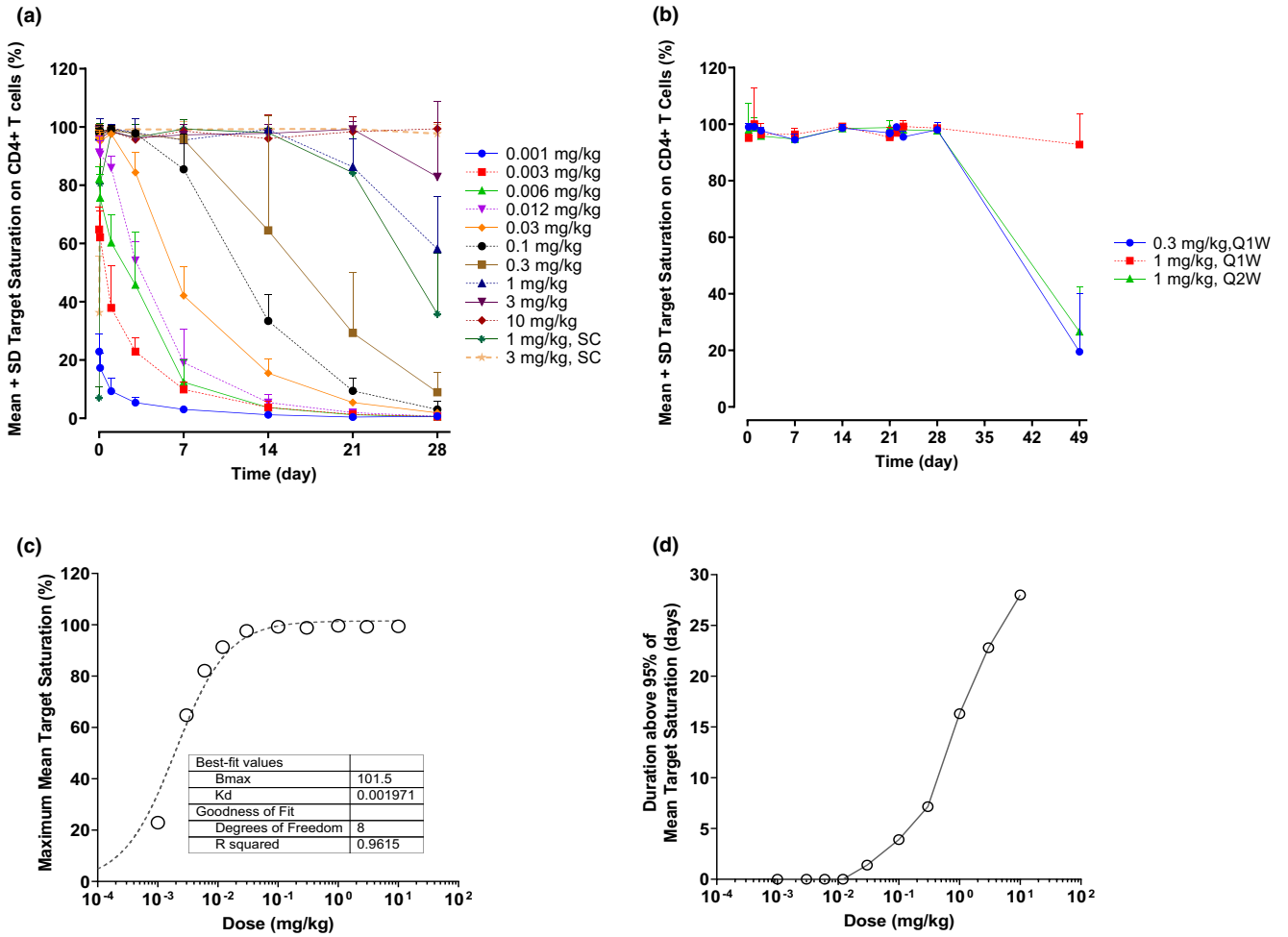

FIGURE 2 ALPN-101 target saturation on CD4+ cells following single IV or SC dosing in part A (a) and repeated IV dosing in part B (b). Maximum mean target saturation versus single IV doses fitted by a simple binding model (c) and the duration of high target saturation versus single IV doses (d) in part A. All doses are IV unless indicated "SC" The target saturations data at baseline and from subjects on placebo were low (<5%) and are not shown

groups and placebo (Figure S3). However, the magnitude and duration of the inhibition by ALPN-101 appeared to be dose-dependent and correlated with target saturation. Specifically, the maximum inhibition of IL-2 increased with increasing ALPN-101 dose from 0.001 mg/kg (−13% from baseline) to 0.03 mg/kg (−49.5% from baseline) and remained ~ −50% from baseline at doses above 0.03 mg/kg with the mean maximum target saturation greater than 95%. The duration of inhibition (defined as time ≤−25% reduction of IL-2 from baseline) also increased with increasing single ALPN-101 doses from 1.08 days at 0.003 mg/kg to 19.9 days at 10 mg/kg. The extended IL-2 reduction appeared to correlate with durable target saturation (Figure 3c,d, and Tables S3 and S4).

Anti-KLH antibodies

Elevated anti-KLH IgG and IgM were observed in the majority of placebo-treated subjects in response to KLH immunization, albeit with large intersubject variability. Single dose and repeated doses of ALPN-101 resulted in significant reductions in the anti-KLH IgG and IgM levels compared with placebo groups, indicating a potent suppression of T-dependent B cell responses by ALPN-101 (Figure 4a–d). The suppression sustained for at least 21 days for all dose groups tested (1–10 mg/kg in part A and all doses in part B). A trend toward dose-dependent inhibition of anti-KLH IgG and IgM, especially when evaluated at 28 days, was observed after single doses of ALPN-101. Specifically, some KLH responses were observed 28 days after a single ALPN-101 dose of 1 mg/kg SC, 1 and 3 mg/kg, when the target saturation decreased to 40%–90% (Figure 4e,f).

Immunophenotyping of T cell subsets

Following dose administration of ALPN-101, no significant changes from the baseline were observed in the absolute

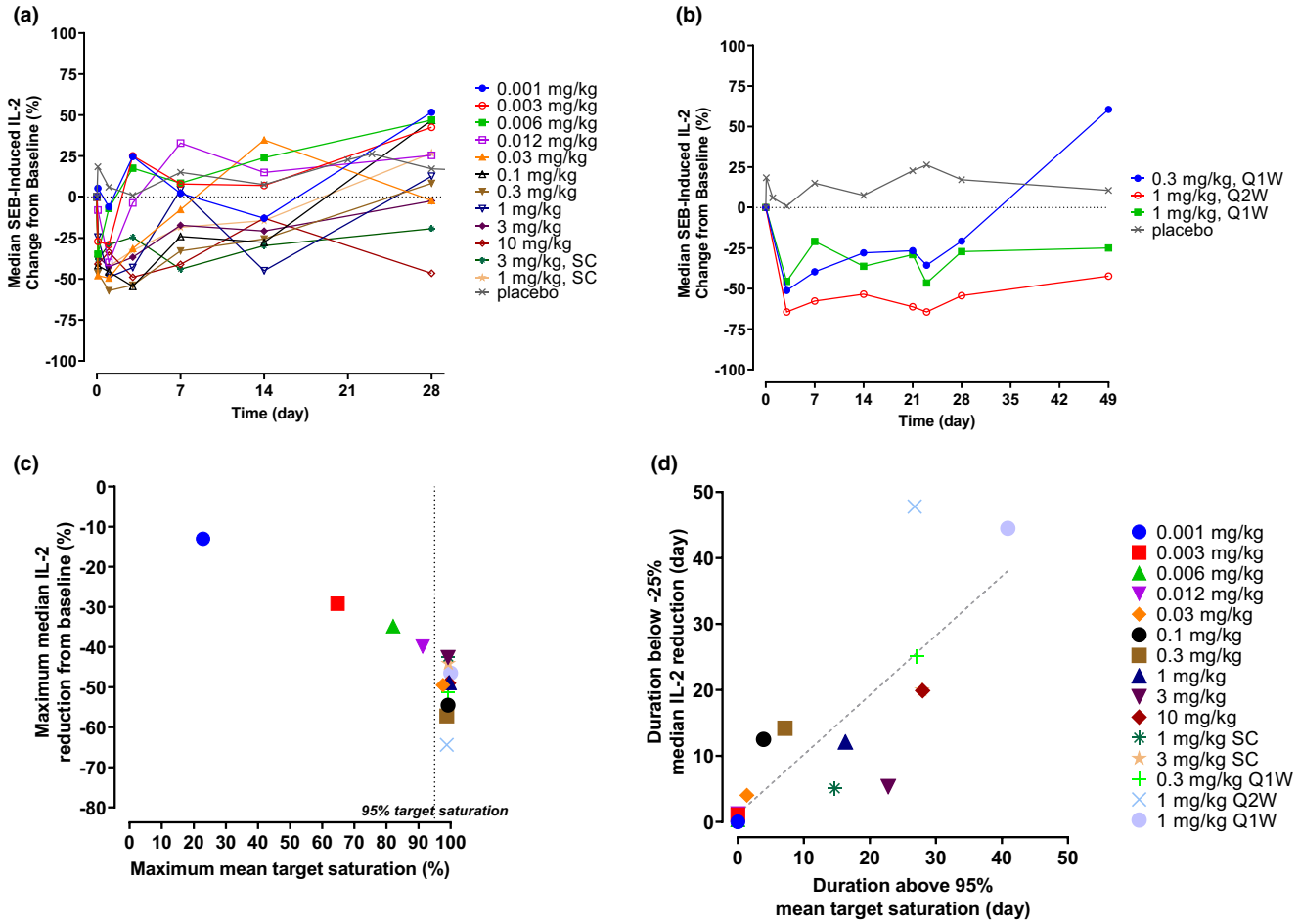

FIGURE 3 Median SEB-induced IL-2 change from baseline versus time profiles following single IV or SC dosing in Part A (a) and repeated dosing in Part B (b). Correlation of estimated PD parameters included maximum effect (c) and duration of effect above or below threshold (d) between target saturation and IL-2 reduction. All doses are IV unless indicated "SC". Dotted line represents 95% target saturation in (c) and linear regression line in (d). SEB, *Staphylococcus aureus* enterotoxin B

number or proportion of T cell subsets, including CD4+ helper subsets (Th1, Th2, Th17, Tfh, and Treg), naïve, central memory, effector memory, or regulatory T cells (data not shown).

Anti-drug antibodies

Twenty-five of the 48 subjects in the ALPN-101 groups (52.1%) tested ADA-positive after dosing in part A and 13 of 17 subjects in the ALPN-101 groups (76.5%) tested ADA-positive after dosing in part B. ADA titers were generally low with a range of 1 to 64 in part A and 1 to 128 except for 2 samples with titer greater than 128 in part B. Of these ADA-positive subjects, 2 of 25 (8%) in part A and 3 of 13 (23.1%) in part B tested positive for neutralizing activity by a cell-based functional assay.

There was no obvious dose relationship with the incidence of positive ADA or NAb activity following ALPN-101 dose administration. There was no apparent impact of ADA or NAb on the PK profiles, exposure of ALPN-101, and safety.

DISCUSSION

This is the first human study with a dual CD28/ICOS antagonist reported to date. ALPN-101 was generally safe and well-tolerated in healthy adult subjects after single doses up to 10 mg/kg or repeated doses up to 1 mg/kg Q1W for 4 weeks. In addition, ALPN-101 exhibited dose-proportional PK and dose-dependent PD, including target saturation, modulation of SEB-induced cytokine production ex vivo, and antibody responses to KLH immunization.

PK of ALPN-101 after IV dosing followed a typical bi-exponential decay, resembling that of common therapeutic antibodies. The estimated $t_{1/2}$ of 4–8 days and SC bioavailability of 60.6% are within ranges of such previously reported studies.[40] Dose proportional increases of ALPN-101

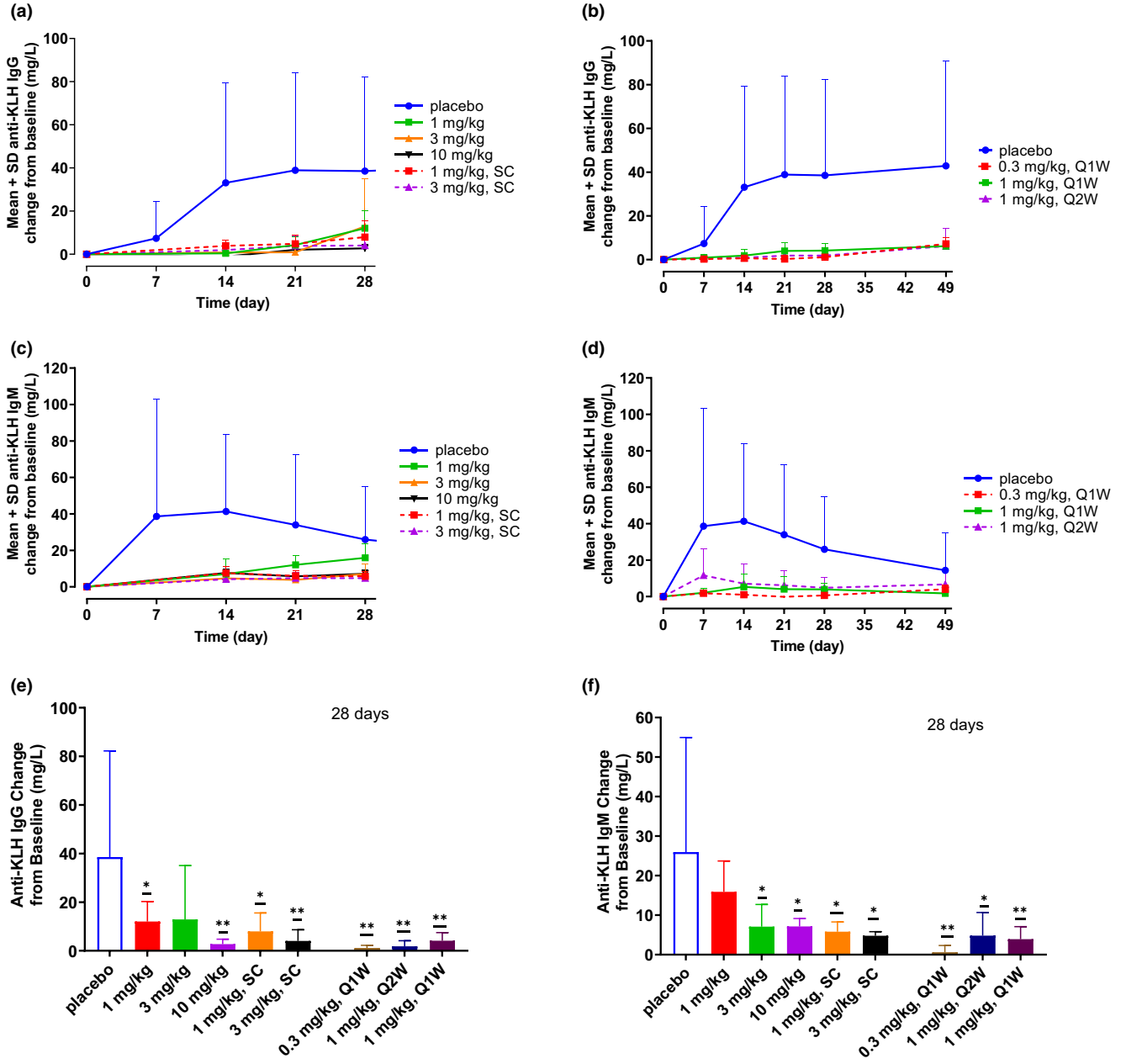

FIGURE 4 Anti-KLH change relative to the baseline versus time profiles after single IV or SC dosing in part A (a, c) and repeated dosing in part B (b, d), displayed as IgG (a, b) and IgM (c, d) anti-KLH responses. Mean (+SD) anti-KLH IgG (e) or IgM (f) change from baseline for each treatment groups at indicated time points after dosing. All doses are IV unless indicated "SC" Statistical analysis was performed by one-way analysis of variance (ANOVA) and unpaired *t*-test with Welch's correction. Statistical significances represent the comparison between placebo and ALPN-101-treated groups. * *p* value 0.033,** *p* value 0.002. KLH, keyhole limpet hemocyanin

exposures were observed across the dose range of 0.03–10 mg/kg for C_{max} and the dose range of 1 mg/kg to 10 mg/kg for $AUC_{0-\infty}$, suggesting a linear PK of ALPN-101 at doses greater than 1 mg/kg. In part B, 0.3–1 mg/kg Q1W or 1 mg/kg Q2W dosing resulted in minimal or modest accumulation in C_{max} and AUCτ, which are consistent with the estimated $t_{1/2}$ and the dosing intervals.

ALPN-101 resulted in a dose-dependent increase in the maximum target saturation and duration of high target saturation. Most importantly, the inhibition of SEB-induced IL-2 and inhibition of anti-KLH IgG and IgM by ALPN-101 were highly correlated with target saturation, suggesting that high levels of target saturation are required for maximal inhibition of T cell activation and T-dependent B cell responses.

Previous studies of biologics blocking either the CD28/CD80-CD86 (e.g., anti-CD28 antibody FR104, or monomeric anti-CD28 domain antibody lulizumab) or ICOS/ICOSL (anti-ICOSL antibody prezalumab or AMG 557) pathways showed that higher doses resulted in higher levels of receptor occupancy, correlating with the suppression of anti-KLH

antibody responses in humans, analogous to that observed with ALPN-101 in this study.[36–38] Because these different studies used different KLH methodologies (e.g., high molecular weight vs. subunit KLH, or primary vs. secondary antibody response assessments), a quantitative comparison of potency is not feasible, but all of these interventions exhibited potent inhibition of anti-KLH IgG responses. Interestingly, the anti-ICOSL mAb prezalumab resulted in no change of anti-KLH IgM compared to placebo. Anti-KLH IgM responses were not reported in the studies of the CD28 antagonists FR104 or lulizumab. The dose-dependent inhibition of both anti-KLH IgG and IgM by ALPN-101 is consistent with the mechanism of action, as a potent inhibitor of both CD28 and ICOS pathways, which could provide superior inhibition of pathogenic T cell-mediated immune responses, translating into improved clinical outcomes.

Although over half of ALPN-101-treated subjects developed low titer anti-ALPN-101 antibodies, it is recognized that a sensitive ECL-based assay was used in this study, which may not always correlate with clinically relevant immunogenicity.[41] Indeed, only a few subjects developed neutralizing antibodies during the study. In addition, low-titer ADA, as detected by sensitive methods, are common among several marketed biologics (e.g., certolizumab pegol, whose product label reports a of range 7%–23% incidence of ADA in studies using ELISA but a much higher incidence of 97% in a study using ECL[42]; adalimumab, whose product label reports a range of 3%–16% incidence of ADA using ELISA in various disease populations but biosimilar studies reported 70%–87% incidence of ADA using ECL).[43–45] Thus, the incidence of anti-ALPN-101 antibody is not atypical for humanized biologics, especially using a sensitive assay platform, such as ECL.[46] At the same time, the ADA formation may have been suppressed by ALPN-101, as predicted by its mechanism of action. Importantly, there was no obvious impact of ADA or NAb on the PK and PD of ALPN-101; there was no relationship between ADA or NAb and safety and tolerability. Continuing evaluation of ADA and NAb in the context of various diseases should be conducted in the future studies to fully characterize the immunogenicity of ALPN-101.

In conclusion, ALPN-101 showed favorable safety, tolerability profiles, and dose-dependent PK and PD in healthy subjects. These results support further clinical development of ALPN-101.

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CONFLICT OF INTEREST

Jing Yang, Jan L. Hillson, Gary D. Means, Russell J. Sanderson, Kay Carley, Almudena Tercero, Kristi L. Manjarrez, Jennifer R. Wiley, and Stanford L. Peng are employees of Alpine Immune Sciences. Jason D. Lickliter is a full-time employee of Nucleus Network.

AUTHOR CONTRIBUTIONS

J.Y., J.L.H., and S.L.P. wrote the manuscript. J.Y., J.L.H., and S.L.P. designed the research. J.Y., J.L.H., J.D.L., G.D.M., R.J.S., A.T., K.L.M., J.R.W., and S.L.P. performed the research. J.Y., J.L.H., G.D.M, R.J.S., and K.C. analyzed the data.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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