SUPPLEMENTAL DATA for article
JPET-AR-2023-001771R1 submitted to
Journal of Pharmacology and Experimental Therapeutics

First-in-Human Phase 1 Randomized Trial with the Anti-CD40 Monoclonal Antibody KPL-404: Safety, Tolerability, Receptor Occupancy, and Suppression of T-Cell–Dependent Antibody Response

Manoj Samant, John Ziemniak, and John F. Paolini

Kiniksa Pharmaceuticals, Lexington, Massachusetts, United States

APPENDIX S1
Inclusion and exclusion criteria for study participants

Inclusion Criteria
Participant must have met all of the following criteria to be enrolled in the study:

1. Healthy individual.
2. Aged 18 to 55 years, inclusive.
3. Body mass index in range of 18.0 to 32.0 kg/m^2, inclusive, at screening visit 1.
4. Female participants must have been:
   - Postmenopausal, defined as at least 12 consecutive months post-cessation of menses (without alternative medical cause) and confirmed by follicle-stimulating hormone test, or
   - Permanently sterile following documented hysterectomy, bilateral salpingectomy, bilateral oophorectomy, or tubal ligation, or having a male partner with vasectomy as affirmed by the participant, or
   - Nonpregnant, nonlactating, and have agreed to use a highly effective method of contraception (ie, condom for male partner combined with either hormonal contraception or intrauterine device) from screening until 5 months after study drug administration, if sexually active.

   Note: Females with same-sex partners were not required to use any methods of contraception. The principal investigator was responsible for determining risk of potential pregnancy for each female participant.
5. Male participants must have had documented vasectomy or used highly effective contraception with partners of childbearing potential (ie, condom plus hormonal contraceptive, condom plus intrauterine device, or condom alone if post-vasectomy) from screening visit 1 until 7 months after study drug administration. Male participants must agree to refrain from donating sperm from time of dose administration until 7 months post-dose.

   Note: Male participants with same-sex partners were required to use condom

only.

6. Able to comprehend and willing to sign an informed consent form and to abide by the study restrictions and requirements.
7. In good health as determined by no clinically significant findings from medical history, physical examination, 12-lead electrocardiogram, vital signs assessment, and clinical laboratory evaluations at screening visit and Day 1, as assessed by investigator in consultation with sponsor when appropriate.

Exclusion Criteria

Participants meeting any of the following criteria were excluded from the study:

1. Poor peripheral venous access that would interfere with study drug administration or blood sample collections.
2. Had a clinically significant illness within 4 weeks of dose administration, as determined by investigator in consultation with sponsor when appropriate.
3. Active or acute infection requiring systemic antibiotic treatment (oral or IV) within 2 weeks prior to screening visit.
4. Previous exposure to KLH.
5. Presence or history of severe adverse reaction to any drug or history of sensitivity to KLH.
6. Known allergy to shellfish.
7. Hospitalization in 3 months prior to screening visit.
8. Any history of anaphylaxis.
9. Previously received investigational product from this study.
10. Participated in a clinical study involving administration of an investigational drug (new chemical entity and/or biologic; ie, monoclonal antibody) and received a last dose of investigational drug within 30 days (or 5 half-lives, whichever is longer) prior to planned first dose of KPL-404.
11. Positive (or 2 intermediate) results for hepatitis B surface antigen, hepatitis B core antibody, or hepatitis C virus antibody at screening visit.
12. Positive results for Quantiferon Gold TB testing at screening.
13. Findings on chest x-ray (obtained at screening visit or within 3 months prior to screening) indicative of a preexisting acute or chronic process that, in the opinion of the investigator, poses a significant threat to the participant if dosed with investigational product at screening.
14. Human immunodeficiency virus infection at screening.
15. Was a heavy user of nicotine (>half a pack [>10 cigarettes] a day or nicotine equivalent of 6.25 g) or could not comply with restrictions of study site during in-unit evaluation.
16. Positive urine drug screen for opiates, methadone, cocaine, phencyclidine, or amphetamines at screening, or positive alcohol breath test result at Day –1. (Note: A positive test at screening may have been repeated at screening or Day –1 if a false-positive test was suspected.)
17. Had used cannabinoids within 7 days prior to dosing or did not agree to refrain from use of cannabinoids until Day 29 (cohorts IV 0.03 mg/kg and IV 0.3 mg/kg), Day 65 (cohorts IV 1 mg/kg, IV 3 mg/kg, and SC 1 mg/kg), Day 85 (cohort SC 5 mg/kg), or Day 113 (cohort IV 10 mg/kg) [ie, participants must have agreed

to not use cannabinoids from Day –7 to Day 29 (cohorts IV 0.03 mg/kg and IV 0.3 mg/kg), Day 65 (cohorts IV 1 mg/kg, IV 3 mg/kg, and SC 1 mg/kg), Day 85 (cohort SC 5 mg/kg), or Day 113 (cohort IV 10 mg/kg)].

18. Alcohol consumption of >14 units per week. One unit of alcohol equals 12 oz (360 mL) of beer, 1.5 oz (45 mL) of liquor, or 5 oz (150 mL) of wine.
19. History of alcoholism or drug/chemical abuse within 2 years prior to study drug administration.
20. A participant who, in the opinion of the investigator in consultation with the sponsor (when appropriate), should not have participated in this study.
21. Treatment with a live (attenuated) vaccine within 12 weeks before Day 1.
22. History of malignancy within 5 years prior to screening visit.
23. Significant history or clinical manifestation of any metabolic, dermatological, hepatic, renal, hematological, pulmonary, cardiovascular, gastrointestinal, neurological, endocrine, rheumatologic, or psychiatric disorder, as determined by the investigator in consultation with the sponsor, when appropriate.
24. Used or planned to use any prescription or nonprescription medications/products within 7 days prior to study drug administration until Day 29 (cohorts IV 0.03 mg/kg and IV 0.3 mg/kg), Day 65 (cohorts IV 1 mg/kg, IV 3 mg/kg, and SC 1 mg/kg), Day 85 (cohort SC 5 mg/kg), or Day 113 (cohort IV 10 mg/kg) unless deemed acceptable by investigator in consultation with sponsor, when appropriate.
    - Permitted medication included hormonal contraceptives and paracetamol (acetaminophen) in recommended doses (≤2 g per day).
25. History of COVID-19 or positive test at screening or baseline.

APPENDIX S2
Supplemental methods for KPL-404 serum and whole blood assays

KPL-404 Pharmacokinetic Assay (Serum)

The KPL-404 PK assay is a validated enzyme-linked immunosorbent assay (ELISA) that assessed calibration standard curve performance, precision, and accuracy; dilutional linearity; prozone effect; selectivity; specificity; stability; and robustness.

Briefly, KPL-404 was captured by an anti-idiotypic antibody (anti-ID) bound to microtiter plates and detected by a peroxidase-conjugated mouse anti-Hu IgG4 pFc. Prepared calibrators, quality control samples, and study human serum samples were diluted to a minimum required dilution (MRD) of 1:200 using assay diluent prior to loading onto the precoated plates. The plates were incubated to allow the KPL-404 present in the samples to bind to the anti-ID and subsequently were washed to remove unbound material. Mouse anti-Hu IgG4 pFc HRP was then added to the plate for detection of bound KPL-404, followed by incubation and a wash step. Tetramethylbenzidine (TMB), a chromogenic substrate for the horseradish-peroxidase (HRP) enzyme conjugate on the detection antibody, was then added to the plates, causing an enzymatic reaction and subsequent color change, measured as optical density (OD) where the intensity of the color is proportional to the amount of free KPL-404 present in the sample. The resulting OD values obtained from the calibration standards are fitted using a 4-PL logistic equation with $1/y^2$ to calculate the KPL-404 concentrations in the quality control and study samples. The calibration curve quantification range for this method is 0.08 µg/mL to 8 µg/mL in 100% human serum.

KPL-404 Antidrug Antibody Assay (Serum)

The KPL-404 ADA assay is a validated electrochemiluminescence (ECL) assay that assessed screening assay cut point factor (ACF), titration cut point factor (TCF), confirmatory assay cut point (CCP), inter- and intra-assay precision (screening and confirmatory), assay sensitivity (screening and confirmatory), titration precision, hook effect, drug tolerance, selectivity, stability, system and negative control suitability ranges, and robustness. Briefly, KPL-404 ADA were detected using the affinity capture and elution (ACE) procedure, where samples and controls were diluted 1:5 in acid to dissociate the KPL-404 Drug:ADA complex and afterward neutralized to capture the dissociated ADA with biotinylated drug following elution. The captured ADA were then pulled down on a pierce streptavidin plate followed by a second elution and neutralization to free the captured ADA. The eluted samples were then added onto a blocked MesoScale Discovery (MSD) streptavidin coated plate containing a Master Mix solution in neutralization buffer and allowed to incubate at ambient temperature with gentle shaking. The plates were washed, 2X Read Buffer added, and signal measured using a MesoScale Discovery (MSD) Sector Imager plate reader. The intensity of the light emitted is proportional to the amount of anti–KPL-404 antibodies present

in a sample or control. In the confirmatory assay, samples and controls were tested both with excess KPL-404 as determined in method development spiked into the label master mix and without excess KPL-404, as described in the screening assay method. Samples showing a specified reduction in signal in the presence of excess KPL-404 were designated as antidrug antibody (ADA) positive by the confirmatory test. In the titration assay, samples were serially diluted 2-fold into 100% normal human serum prior to being analyzed in the assay. The highest dilution that has a signal greater than or equal to the titration cut point was determined to be the titer and was reported as the reciprocal of the dilution.

CD40 Receptor Occupancy Assay (Whole Blood)

The RO was determined by flow cytometry-based assay performed on whole blood samples collected in Cyto-Chex BCT, measured as free CD40 on CD19-positive B cells. Briefly, whole blood collected in Cyto-Chex BCT was distributed in 96 deep-well plates. Before staining, nonspecific binding was blocked with human BD Fc Block solution.

Samples were stained with the antibody cocktail followed by lysis of red blood cells and fixation using BD FACS Lysing Solution. Samples were then washed twice before acquisition on the BD LSR Fortessa. The free CD40 on CD19-positive B cells was measured using Alexa Fluor 647 (AF647)-conjugated KPL-404 whose binding is inhibited by unconjugated KPL-404. The total CD40 on CD19-positive B cells was measured using a PE-conjugated anti-CD40 antibody whose binding is not inhibited by KPL-404. Binding of KPL-404 resulted in mean fluorescence intensity (MFI) reduction of CD40 on B cells. MFI for CD40 on B cells was converted into antibody binding capacity (ABC) using Quantum Simply Cellular (QSC) beads.

KPL-404 Anti–Keyhole Limpet Hemocyanin Assay (Serum)

KLH is an oxygen-transporting protein of the marine gastropod *Megathura crenulata*. It is recognized as a potent immunoactivator and therefore is widely used in research and clinical studies to demonstrate TDAR on B cells. This assay uses a commercial kit (Alpha Diagnostics Human Anti-Keyhole Limpet Hemocyanin [KLH] IgG ELISA kit), which employs a 96-well microtiter plate coated with immobilized KLH as the capture antigen and blocked by the manufacturer. The calibration curve range of this method is 10.0 U/mL to 100 U/mL. A blank (sample diluent) is included with a nominal value of 0.100 U/mL assigned to act as an anchor point. Since they are an arbitrary unit and the method is a quasi-quantitative method, this does not impact the method or data generated. Calibration standards are prepared per the kit instructions, with preparation of additional calibrators described in the test method. Control samples are diluted in sample diluent prior to loading onto the precoated plates. The plates are incubated to allow the anti-KLH present in the samples to bind to the target and subsequently washed to remove unbound material. Anti-human IgG HRP conjugate is added to the plate to detect the captured anti-KLH. The plates are further incubated, and then washed to remove any unbound materials. Substrate is added and the plate

further incubated, followed by addition of a stop solution to terminate the reaction. Plates are read using a SpectraMax plate reader (450 nm for detection and 630 nm for background). The optical density (OD) values obtained from the calibration standards are fit to a five-parameter logistic (5-PL) fit equation (Marquardt) with 1/y2 weighting to calculate the relative anti-KLH IgG concentrations in the quality control (QC) and unknown human serum samples.

APPENDIX S3
Noncompartmental Parameter PK Supportive Information

The primary purpose of the proposed Phase 1 FIH study was to evaluate safety and tolerability of single ascending intravenous (IV) and subcutaneous (SC) doses of KPL-404 in healthy volunteers, with the PK component intended to examine extent and duration of exposure. A model-independent NCA was conducted in order to characterize the exposure and elimination of KPL-404 in humans. The PK analysis examined exposure of KPL-404 at various doses following IV and SC administration. The NCA analysis allowed for a model-independent assessment of exposure and generation of classic parameters such as C_{max}, T_{max}, and AUC. Derived parameters such as $t_{1/2}$, Cl and VD were calculated relative to individual doses.

C_{max} was the observed maximal plasma concentration. Area under the concentration-time curve from time zero to the last measurable concentration, calculated by linear trapezoidal method when concentrations are increasing and by logarithmic trapezoidal method when concentrations are decreasing (Linear Up/Log Down Trapezoidal Method)

For generating $t_{1/2}$, points selected to represent the terminal phase were visually inspected and, if needed, manually adjusted by the pharmacokineticist, provided that the selection met the pre-specified SAP criteria: the number of data points used to calculate $\lambda z > 3$ (not including C_{max}) and the coefficient of determination (R2) value for $\lambda z > 0.800$.

Exposures of KPL-404 ($AUC_{0-\infty}$, AUC_{0-t}, and C_{max}) were tested for dose proportionality using a power model approach following IV administration (Part A). A power model was fitted to describe the relationship between Y (parameter) and X (dose) using the linear regression model [$\ln(Y) = \alpha + \beta^* \ln(X) + \varepsilon$], where the slope of the regression line β was presented along with the 90% CI of the slope. Dose proportionality was concluded if the 90% CI of the slope β lay entirely within [1+ln(0.5)/ln(r), 1+ln(2)/ln(r)], where r is a ratio that describes the dose range and is defined as the ratio of highest dose to lowest dose.

Dose Proportionality Assessment of Serum PK Parameters for KPL-404 Following IV Administration- Power Model (PK Population)

Dose Range	Parameter (units)	Intercept Estimate	Slope Estimate	Standard Error of Slope	90% CI of Slope

0.03 - 10 mg/kg, IV	C_{max} (ng/mL)	10.27	1.07	0.03	(1.02, 1.12)
	AUC_{0-t} (h*ng/mL)	14.35	1.90	0.06	(1.79, 2.00)
	$AUC_{0-\infty}$ (h*ng/mL)	14.46	1.79	0.06	(1.69, 1.90)

CI = confidence interval; IV = intravenous.
Note: Natural-log transformed pharmacokinetic parameters were analyzed using a power model where ln(parameter)= intercept + beta*ln(dose).
Dose proportionality would be concluded if the 90% confidence interval of the slope (beta) lies entirely within (0.8807, 1.1193) for dose range [i.e. (1+ln(0.5)/ln(r),1+ln(2)/ln(r))], where r is the dose range (highest dose/lowest dose).

The result of the statistical assessment of dose-proportionality showed C_{max} of KPL-404 increased almost dose-proportionally, and AUCs increased more than dose-proportionally over the 0.03 to 10 mg/kg dose range following IV administration. treatment group.

Dose Proportionality Assessment of Serum PK Parameters for KPL-404 Following SC Administration- ANOVA (PK Population)

		Dose-normalized Geometric LS Mean		Ratio of Dose-normalized Geometric LS Mean		
Parameter (units)	Comparison (Test/Reference)	Test	Reference	Estimate	Lower 90% CI	Upper 90% CI
C_{max} (ng/mL)	5 mg/kg / 1 mg/kg	8300	3410	2.43	1.44	4.09
AUC_{0-t} (h*ng/mL)	5 mg/kg / 1 mg/kg	3730000	618000	6.03	3.32	10.95
$AUC_{0-\infty}$ (h*ng/mL)	5 mg/kg / 1 mg/kg	3730000	553000	6.74	3.96	11.49

CI = confidence interval; LS = least squares; SC = subcutaneous.
Note: An analysis of variance (ANOVA) model was fitted to the natural-log transformed dose-normalized AUC and C_{max} data, with dose group fitted as a factor

The ratio of dose-normalized geometric mean showed a slightly greater than > 5 fold dose-proportional increase in serum KPL-404 PK exposure (C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$) when the SC dose of KPL-404 increased from 1 mg/kg to 5 mg/kg.

Given the observed dose-dependent changes seen in various PK parameters, the accuracy and reliability of NCA derived parameters reported herein should be viewed in the context of the doses administered and the plasma concentrations achieved.