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ARTICLE

A first-in-human study of KMRC011, a potential treatment for acute radiation syndrome, to explore tolerability, pharmacokinetics, and pharmacodynamics

Eunsol Yang[1] | Hyejung Choi[2] | Jin-Sol Park[2] | Young-Woock Noh[3] | Chi-Min Choi[4] | Woo-Jong Lee[5] | Jae-Wook Ko[2] | Jungryul Kim[2,6]

[1]Department of Clinical Pharmacology and Therapeutics, Seoul National University College of Medicine and Hospital, Seoul, Korea

[2]Department of Clinical Pharmacology and Therapeutics, Samsung Medical Center, Seoul, Korea

[3]New Drug Development Center, Osong Medical Innovation Foundation, Cheongju-si, Korea

[4]CONNEXT Co., Ltd., Yeongcheon-si, Korea

[5]Biomedical Manufacturing Technology Center, Korea Institute of Industrial Technology, Yeongcheon-si, Korea

[6]Department of Clinical Research Design & Evaluation, SAIHST, Sungkyunkwan University, Seoul, Korea

Correspondence
Jungryul Kim, Department of Clinical Pharmacology and Therapeutics, Samsung Medical Center, 81, Irwon-ro, Gangnam-gu, Seoul 06351, Korea.
Email: jungryul.kim@gmail.com

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Abstract

KMRC011 is a novel Toll-like receptor 5 agonist under development as a treatment for acute radiation syndrome (ARS). The aim of this first-in-human study was to investigate the tolerability, pharmacokinetics, and pharmacodynamics of a single intramuscular dose of KMRC011 in healthy subjects. A randomized, single-blind, placebo-controlled, single dose-escalation study was conducted with the starting dose of 5 μg. Eight (4 only for 5 μg cohort) subjects per cohort were randomly assigned to KMRC011 or placebo in a 3:1 ratio. Dose-limiting toxicity (DLT) was assessed throughout the study. Serum concentrations of KMRC011, granulocyte colony-stimulating factor (G-CSF), and interleukin-6 (IL-6) were measured up to 48 h post-dose. Based on safety review, the dose of KMRC011 escalated up to 20 μg, and consequently, a total of 4 dose levels (5, 10, 15, and 20 μg) were explored. The most common adverse event was injection site reaction, showing no dose-related trend. Three DLTs (2 cases of hepatic enzyme increased and 1 of pyrexia) were observed; 1 in the 15 μg cohort and 2 in the 20 μg cohort. A developed method could not detect any KMRC011 in serum. KMRC011 15 μg and 20 μg showed significant increases of G-CSF, IL-6, and absolute neutrophil counts, compared with the placebo. A single intramuscular administration of KMRC011 ranging from 5 to 15 μg was tolerated in healthy subjects. Doses of KMRC011 equal to or greater than 15 μg exerted TLR5 agonist-like activities by increasing serum G-CSF and IL-6. It suggests that KMRC011 has the potential for a treatment for ARS.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Toll-like receptor 5 (TLR5) can be a target for acute radiation syndrome (ARS), and KMRC011 is a novel TLR5 agonist being developed as a treatment for ARS. In animal models of irradiation, TLR5 agonists showed radioprotective and radiomitigative effect, and granulocyte colony-stimulating factor (G-CSF) and interleukin-6 (IL-6) have been proposed as efficacy biomarkers for TLR5 agonists. For further

development, properties of KMRC011 including tolerability in humans need to be evaluated.

WHAT QUESTION DID THIS STUDY ADDRESS?

Does KMRC011, a novel TLR5 agonist, show acceptable safety profiles and clinically meaningful changes in efficacy biomarkers in healthy humans?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

A single intramuscular administration of KMRC011 was tolerated in healthy humans. In addition, KMRC011 exerted TLR5 agonist-like activities by increasing the levels of serum G-CSF and IL-6. As a result, KMRC011 demonstrates acceptable tolerability and preliminary activity in humans as well as animal models, and thus, KMRC011 may have the potential for a treatment for ARS.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Our first-in-human study provided new clinical pharmacology information, such as safety and pharmacodynamics (PDs) of KMRC011 in humans. These findings are expected to be integrated with previous animal PD data, translate animal efficacy to humans, and ultimately contribute to the drug approval under the Animal Rule.

INTRODUCTION

Total-body or significant partial-body exposures to a high dose greater than 1 Gy of radiation can cause acute radiation syndrome (ARS).[1] ARS includes hematopoietic, gastrointestinal, and neurovascular sub-syndromes.[1,2] The hematopoietic effects of ARS are manifested as cytopenias, which increase the risk of sepsis and bleeding; the gastrointestinal manifestations of ARS are damage to the mucosal barrier and loss of intestinal epithelia, which increase the risk of peritonitis, various pathogens into the bloodstream, loss of fluid, and paralytic ileus.[1] Notably, the neurovascular sub-syndrome characterized by dizziness, headache, or decreased level of consciousness is regarded clinically unmanageable, while the other two sub-syndromes are more likely to respond to appropriate medical interventions.[2,3] Therefore, the hematopoietic and gastrointestinal sub-syndromes have been considered as potential targets for the development of treatments for ARS.

To date, three medical countermeasures for radiation have been fully approved by the US Food and Drug Administration (FDA).[4] All these agents, belonging to the colony-stimulating factor (CSF) family, have not been newly discovered but have been used for other indications over several decades.[4] In the development of new treatments for radiation, human efficacy studies cannot be conducted because it would be unethical to expose human subjects to a lethal or permanently disabling toxic radiological substance. So, efficacy data must be derived from adequate and well-controlled animal experimentation with the FDA Animal Efficacy Rule, which requires a good understanding of mechanistic knowledge in two or more animal models predictive of human response.[5]

Entolimod is a Toll-like receptor 5 (TLR5) agonist that binds to TLR5, thereby activating nuclear factor kappa-B (NF-κB) signaling. This signaling mobilizes an innate immune response that drives the expression of multiple genes encoding antioxidants, antiapoptotic proteins, antimicrobial peptides, and cytokines, particularly granulocyte CSF (G-CSF) and interleukin (IL)-6.[6,7] In animal models of irradiation and sepsis, activation of the NF-κB pathway promotes multi-organ tissue protection and regeneration.[8,9] Based on this mechanism of action, entolimod is being developed as a treatment for reducing the risk of death following exposure to lethal radiation. Entolimod showed radioprotective and radiomitigative efficacies in mice and nonhuman primates, and G-CSF and IL-6 are considered as potential biomarkers in mitigation of radiation-induced injury.[7,9,10] Two clinical studies were conducted in 150 healthy subjects to assess the safety of entolimod, indicating that the administration of entolimod appears to be tolerated within a specific dose range.[11,12] In these studies, the most frequent adverse events (AEs) were flu-like syndrome, decrease in blood pressure, and elevation of liver enzymes caused by cytokine storm, which is consistent with the mechanism of action of the compound.[12] As such, TLR5 agonist shows acceptable tolerability in humans and preliminary efficacy in animals,[9–12] however, there is no TLR5 agonist approved as a treatment for ARS yet.

KMRC011 is a novel TLR5 agonist, a slightly modified version of entolimod, and its efficacy was demonstrated in murine and nonhuman primate models of irradiation.[13–15] The purpose of this study was to investigate the tolerability, pharmacokinetics (PK), and pharmacodynamics (PDs) of KMRC011 following a single intramuscular administration in healthy subjects.

METHODS

The study protocol was approved by the institutional review board at Samsung Medical Center (Seoul, Republic of Korea) and the Ministry of Food and Drug Safety. This study was conducted in accordance with Korean Good Clinical Practice guidelines and tenets of Declaration of Helsinki. Prior to any study-related procedures, all subjects gave written informed consent.

Subjects

Healthy male adults aged 19 to 55 years with a body mass index of 18.5 to 27 kg/m^2 were eligible. Major exclusion criteria were: a history of infectious disease or severe trauma within 21 days before randomization; a history of allergic disease requiring treatment; blood aspartate aminotransferase, alanine aminotransferase or gamma-glutamyltransferase value 1.5-fold more than the upper reference limit; a significant electrocardiogram (ECG) result at screening, including a baseline Fridericia method-corrected QT interval (QTcF) of greater than 430 ms.

Study design

The study was designed as a randomized, single-blind, placebo-controlled, single dose-escalation study (ClinicalTrial.gov identifier NCT03585803). The no observed adverse effect level of KMRC011 in dog, which was the most sensitive species, was 0.05 mg/kg/day in nonclinical studies, and this dose corresponded to a maximum recommended starting dose of 0.09 μg/kg based on a safety factor of 30. Accordingly, the estimated starting dose of KMRC011 for intramuscular injection was about 5 μg, assuming a 60 kg adult man. The dose level cohorts were determined up to 50 μg by increasing the amount by 5 μg from the starting dose. Within each cohort, 8 (4 only for 5 μg cohort) subjects were randomly assigned to KMRC011 or placebo at a 3:1 ratio. The same volume of saline as KMRC011 dosed in each cohort was administered intramuscularly as the placebo.

Escalation to the next higher dose level occurred after all subjects at the current level completed tolerability assessment. If two or more subjects in the same cohort experienced dose-limiting toxicity (DLT)-related AEs, dose escalation was stopped. Based on the common terminology criteria for AEs (version 5.0) from the National Cancer Institute of the United States, a DLT was defined as an investigational product related grade 3 AE or any cytokine release syndrome (CRS) associated AE: oxygen supplementation at greater than or equal to 40% fraction of inspired oxygen (FiO_2), hypotension requiring high-dose or multiple vasopressors, or grade 3 or more increase in alanine or aspartate transaminase.

Assessments

Tolerability was assessed based on symptom reports, physical examinations, vital signs (systolic and diastolic blood pressures, and tympanic temperature), 12-lead ECGs, and clinical laboratory tests (hematology, clinical chemistry, coagulation, and urinalysis) throughout the study. All clinically significant findings were assessed by the investigators with respect to severity, outcome, seriousness, and relationship to the study drug. These were considered as AEs regardless of the relationship to the study drug.

Injection site reactions associated with intramuscular injection were examined for pain, tenderness, redness, and swelling by 4 grades at 24 and 48 h postdose, and at an additional 72 h postdose only in 5 μg cohort. Pain and tenderness were assessed by asking the subjects if they felt any pain without and with pushing the skin, respectively. Redness and swelling were assessed by measuring the diameter of the area showing a reaction.

Blood samples for PK assessment were collected at predose (0 h) and 0.5, 1, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, and 24 h postdose. In addition, serum concentrations of G-CSF and IL-6 were measured at predose (0 h) and 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24, and 48 h postdose for PD assessment.

Assay of KMRC011 and cytokines

In KMRC011 assay in serum samples, sandwich enzyme-linked immunosorbent assay was performed using a primary antibody (mouse anti-KMRC011 monoclonal antibody (3E3); custom made) and a secondary antibody (monkey anti-KMRC011 polyclonal antibody, biotin conjugated; custom made) with color development system streptavidin-HRP (Sigma-Aldrich, S2438) and TMB Microwell peroxidase substrate system (KPL, 50-76-00). Standard samples were prepared to have 0.020 to 10 μg KMRC011/ml by 2-fold serial dilution of 10 μg KMRC011/ml with human pooled serum (BioChemed, human serum, pooled/mixed gender). Absorbance was measured at 450 nm in the VersaMax microplate reader, and then data were analyzed by 4-parameter logistic model in the SoftMax Pro 5 GxP software (version 4.3.1; Molecular Devices, LLC). The assay was linear in the concentration range of 0.02 to 2.5 μg/ml. The lower and upper limits of quantification were 0.02 μg/ml and 2.5 μg/ml, respectively. Intrabatch and interbatch precision were 0.7 to 2.9% and 0 to 16.6%, and intrabatch and interbatch accuracy were −11.25 to 3.45% and −7.14 to 14.90%.

Serum concentrations of G-CSF and IL-6 were analyzed by using the Meso Scale Discovery (MSD) U-PLEX platform (Meso Scale Diagnostics, LLC) according to the manufacturer's instructions. The U-PLEX linker was coupled to the capture antibodies to prepare a multiplex coating solution. For coating the U-PLEX plate, 50 µl of the multiplex coating solution was added in each well of 96-well U-PLEX plate. The plate was sealed with an adhesive plate seal and incubated overnight at 2–8°C. After washing, 50 µl of standards or samples were loaded per well, and the plate was incubated for 1 h at room temperature. After another washing, 50 µl of Sulfo-tag labeled detection antibody solution was added to each well, followed by 1-h incubation and washing. Thereafter 150 µl of 2× Read buffer T was added to each well, and the plate was read on a MESO Sector S 600 instrument (Meso Scale Diagnostics). Data were calculated with MSD Workbench 4.0 software. The lower limit of detection was calculated by the concentration based on a signal of 2.5 standard deviations above the zero calibrators. The lower and upper limits of quantification were defined as the lowest and highest points of a calibration curve with the coefficient of variance of duplicates <20% and measured concentration between 80% and 120%.

Statistical analysis

The maximum concentration and area under the concentration-time curve for each analyte were used for PK/PD assessment. The parameters were obtained directly from the data or were calculated using noncompartmental analysis with linear trapezoidal rule.

Subjects were allocated into five intervention groups, including a placebo group. The PK and PD parameters were summarized by the intervention groups and were analyzed using the nonparametric methods, if necessary, followed by the Bonferroni adjustment for the multiple comparisons. In addition, subject characteristics and AEs were summarized by the intervention groups. Statistical analysis was carried out with the R package "PMCMRplus" in R (version 4.0.1),[16] and the significance level was set to 0.05.

RESULTS

Study population

A total of 26 subjects were enrolled, and two subjects withdrew their consent to participate in the study before drug administration: 17 subjects received KMRC011, and seven subjects received the placebo (Figure 1). Of those, two subjects discontinued the study due to AEs with pyrexia and hypotension. Consequently, 24 subjects were included in the tolerability assessment, and 22 subjects were included in the PK/PD analysis.

The characteristics of the subjects are summarized by the intervention groups, including the placebo group in Table 1. There was no significant difference between the intervention groups with respect to age, weight, height, and body mass index.

Tolerability

A total of 20 subjects (17 with KMRC011 and three with placebo) reported 97 AEs throughout the study. Of those, AEs reported in two or more subjects are shown in Table 2. The most common AE reported was injection site reaction, showing no trend in the incidence with increasing dose levels. Moreover, the main symptom was tenderness without accompanying erythema or sclerosis. Four AEs (hepatic enzyme increased [two cases], flushing [one], pyrexia [one]) were severe in its severity. There were no serious AEs reported. Two AEs, pyrexia in the 10 µg cohort and hypotension in the 20 µg cohort, resulted in withdrawal from the study. Collectively, three DLTs were observed: one of hepatic enzyme increased in 15 µg cohort, and one each of hepatic enzyme increased and pyrexia in the 20 µg cohort. Because two DLTs occurred in 20 µg cohort, the subject enrollment was discontinued.

The blood pressures and body temperatures from predose (0 h) to 24 h postdose are summarized by the intervention groups in Table 3. After a single intramuscular administration of KMRC011, the trends were observed for the blood pressures to decrease and for the body temperatures to increase, and the values returned to baselines about after 24 h postdose. Although the body temperatures at 4 to 6 h postdose were significantly higher than those at predose in 15 µg and 20 µg cohorts, at 24 h postdose, they dropped below 38°C, which is the criteria of CRS.[17]

Pharmacokinetics

We could not detect any significant amount of KMRC011 in serum using the developed method with the lower limit of quantification of 20 ng/ml.

Pharmacodynamics

G-CSF and IL-6

The mean serum concentrations of G-CSF and IL-6 over 48 h postdose are shown in Figure 2. The maximum effect (E_{max}) and area under the effect-time curve (AUEC) values for G-CSF and IL-6 are summarized in Table 4. Following

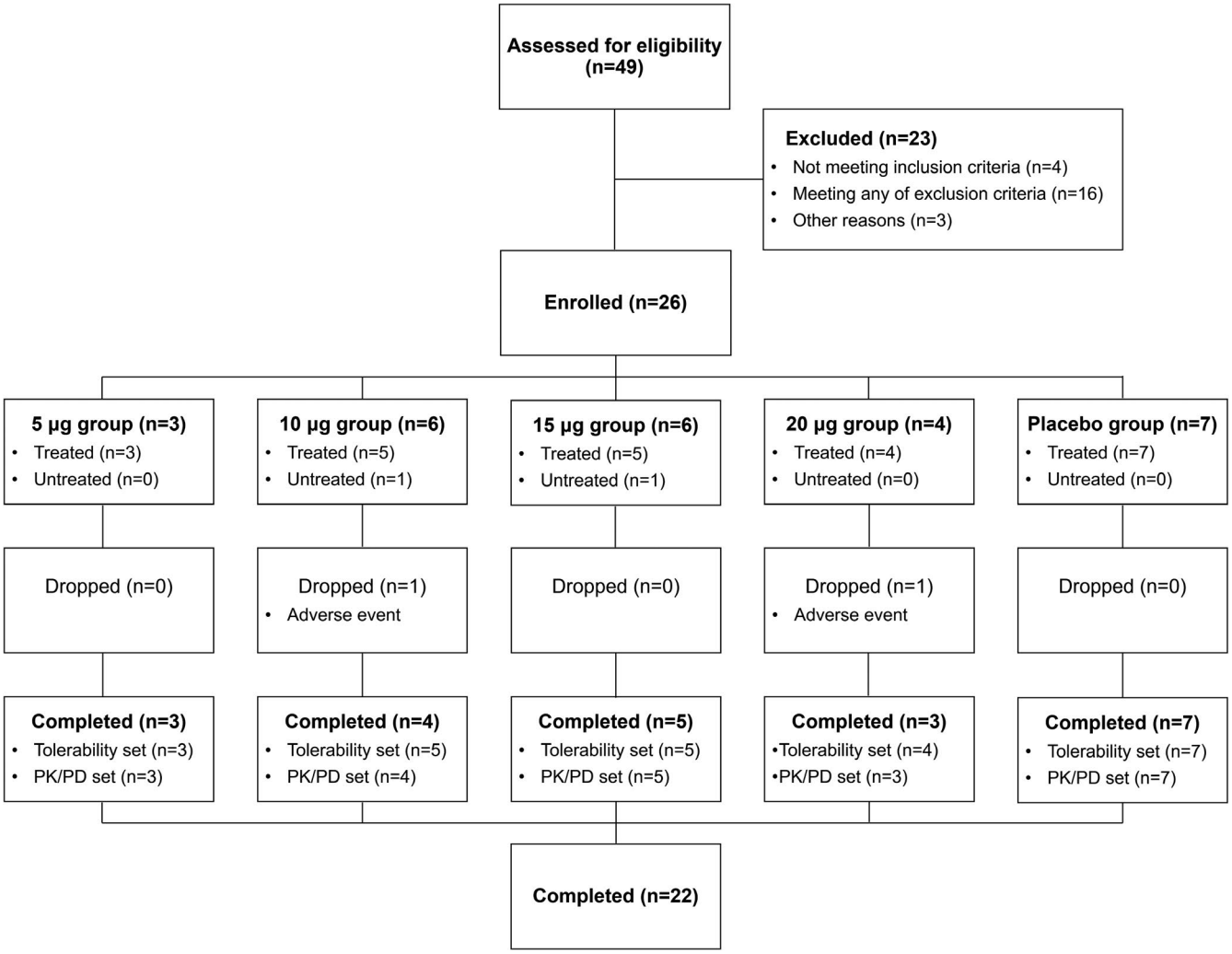

FIGURE 1 Subject disposition. PD, pharmacodynamics; PK, pharmacokinetics

TABLE 1 Subject characteristics by intervention groups

	5 µg ($N = 3$)	10 µg ($N = 6$)	15 µg ($N = 6$)	20 µg ($N = 4$)	Placebo ($N = 7$)	*p* value[a]
Age (years)	34.0 ± 7.8	39.2 ± 12.1	31.2 ± 8.2	32.3 ± 7.4	37.9 ± 11.5	0.8033
Weight (kg)	77.67 ± 3.20	78.73 ± 10.56	68.45 ± 11.11	68.10 ± 3.27	65.56 ± 4.84	0.0730
Height (cm)	176.57 ± 7.69	178.18 ± 8.42	171.35 ± 5.56	173.23 ± 6.34	170.24 ± 4.17	0.3221
Body mass index (kg/m^2)	24.93 ± 1.18	24.73 ± 2.06	23.22 ± 3.06	22.78 ± 2.00	22.67 ± 2.29	0.4199

Note: Data are expressed as mean ± standard deviation.

[a]Kruskal-Wallis test.

a single intramuscular injection of KMRC011, both G-CSF and IL-6 levels increased rapidly, peaking approximately at 4 to 6 h postdose, and were mostly recovered to their baseline levels at 48 h postdose. The E_{max} and AUEC values for G-CSF and IL-6 showed increasing trends with increasing dose levels (Figure S1). Two subjects in 15 µg cohort exhibited remarkably higher PD values, resulting in the means for the corresponding values greater than those in 20 µg cohort. In addition, KMRC011 15 µg and 20 µg cohorts resulted in the significantly higher E_{max} and AUEC values for G-CSF and IL-6 compared with the placebo group.

Absolute Neutrophil Counts

KMRC011 was associated with dose-related rises in absolute neutrophil counts (ANCs) (Figure 3). The mean ANC values at 24 h postdose were 5.52 (10^9/L), 8.02, 12.10,

TABLE 2 Summary of adverse events reported in two or more subjects by intervention groups

	5 μg (*N* = 3)	10 μg (*N* = 5)	15 μg (*N* = 5)	20 μg (*N* = 4)	Placebo (*N* = 7)
Anemia	0	0	1 (20)	0	1 (14.3)
Sinus tachycardia	0	0	0	2 (50)	0
Diarrhea	0	0	2 (40)	1 (25)	0
Nausea	0	1 (20)	1 (20)	1 (25)	0
Vomiting	0	0	2 (40)	1 (25)	0
Injection site reaction	3 (100)	5 (100)	3 (60)	4 (100)	0
Pyrexia	1 (33.3)	3 (60)	5 (100)	4 (100)	0
Pyuria	1 (33.3)	0	0	0	1 (14.3)
Oral herpes	0	0	1 (20)	1 (25)	0
C-reactive protein increased	2 (66.7)	3 (60)	5 (100)	4 (100)	0
Hepatic enzyme increased	0	0	1 (20)	1 (25)	0
International normalized ratio increased	0	0	1 (20)	1 (25)	0
Myalgia	1 (33.3)	1 (20)	4 (80)	2 (50)	0
Headache	0	1 (20)	3 (60)	3 (75)	1 (14.3)
Tension headache	2 (66.7)	0	0	0	0
Hypotension	0	0	2 (40)	1 (25)	0

Note: Data are expressed as n (%), where n is the number of subjects and % is based on the number of subjects in each intervention.

TABLE 3 Summary of vital signs following a single intramuscular administration of KMRC011 or placebo

	5 μg (*N* = 3)	10 μg (*N* = 5)	15 μg (*N* = 5)	20 μg (*N* = 4)	Placebo (*N* = 7)
Systolic blood pressure (mmHg)					
Predose (0 h)	116.7 ± 9.0	109.6 ± 12.6	125.0 ± 13.9	112.3 ± 8.5	116.4 ± 13.1
2 h	121.0 ± 14.7	118.6 ± 22.5	131.8 ± 18.5	118.5 ± 9.0	113.0 ± 12.2
4 h	113.7 ± 13.4	106.8 ± 14.5	111.8 ± 16.3	112.5 ± 23.5	114.7 ± 11.3
6 h	120.0 ± 16.5	101.8 ± 5.7	109.6 ± 18.1	99.0 ± 16.8	108.4 ± 12.8
12 h	113.3 ± 5.0	103.0 ± 7.6	111.8 ± 13.1	99.7 ± 9.1	106.7 ± 13.7
24 h	114.7 ± 8.1	104.3 ± 9.3	114.4 ± 16.2	113.0 ± 17.1	110.3 ± 15.0
Diastolic blood pressure (mmHg)					
Predose (0 h)	69.7 ± 11.7	79.8 ± 6.5	76.8 ± 6.3	77.3 ± 11.4	74.6 ± 7.4
2 h	70.0 ± 17.4	82.0 ± 11.5	71.6 ± 17.4	78.5 ± 11.6	73.1 ± 9.0
4 h	65.3 ± 12.9	66.0 ± 6.5	60.8 ± 12.9	71.8 ± 16.4	72.1 ± 7.2
6 h	68.0 ± 14.0	65.8 ± 3.9*	64.2 ± 9.4	65.8 ± 6.2	65.3 ± 4.2
12 h	65.3 ± 5.7	65.8 ± 5.9*	65.0 ± 7.7	68.7 ± 13.0	70.9 ± 9.8
24 h	70.0 ± 9.5	72.8 ± 2.5	70.0 ± 8.2	72.0 ± 11.1	70.3 ± 11.6
Body temperature (°C)					
Predose (0 h)	36.3 ± 0.3	36.5 ± 0.2	36.6 ± 0.1	36.3 ± 0.1	36.3 ± 0.2
2 h	36.3 ± 0.2	36.8 ± 0.8	37.4 ± 0.5	36.9 ± 0.2	36.2 ± 0.2
4 h	36.9 ± 1.0	37.8 ± 1.8	38.3 ± 0.3*	39.0 ± 0.9*	36.4 ± 0.2
6 h	36.8 ± 0.6	37.5 ± 0.8	38.0 ± 0.3*	38.3 ± 0.5*	36.5 ± 0.2
12 h	36.9 ± 0.2	37.2 ± 0.5	37.7 ± 0.5	37.7 ± 0.6	36.7 ± 0.1*
24 h	36.5 ± 0.4	36.7 ± 0.2	36.9 ± 0.2	36.5 ± 0.5	36.2 ± 0.2

Note: Data are expressed as mean ± standard deviation. The *p* values were adjusted for multiple comparisons using Bonferroni adjustment (*$p < 0.05$ vs. predose [0 h]).

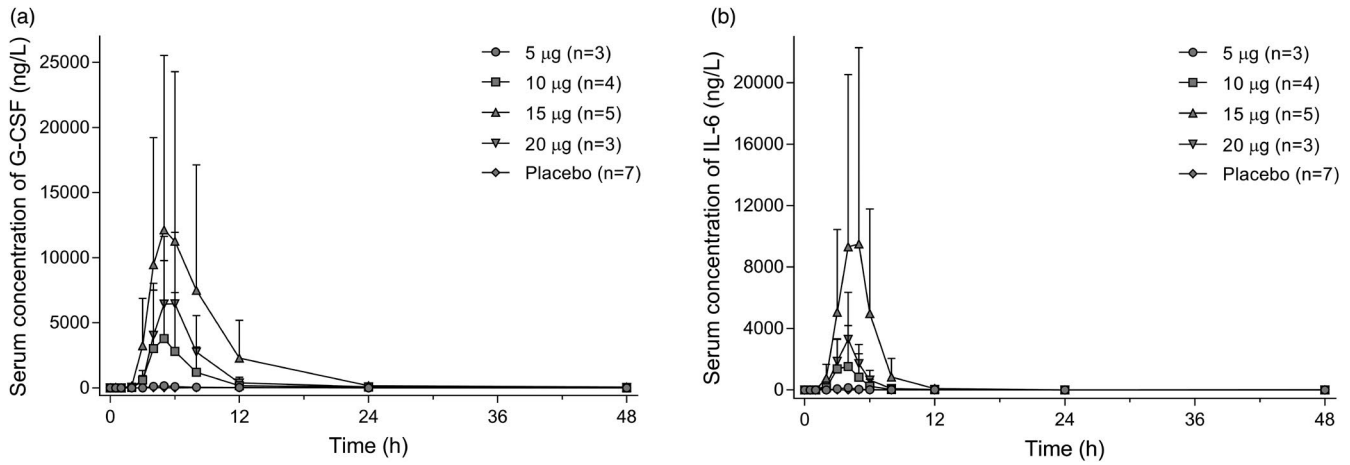

FIGURE 2 Mean serum concentration-time profiles of (a) G-CSF and (b) IL-6 following a single intramuscular administration of KMRC011 or placebo with linear scale. Error bars represent standard deviations

TABLE 4 Summary of pharmacodynamic parameters following a single intramuscular administration of KMRC011 or placebo

	5 μg (*N* = 3)	**10 μg (*N* = 4)**	**15 μg (*N* = 5)**	**20 μg (*N* = 3)**	**Placebo (*N* = 7)**
G-CSF					
E_{max} (μg/L)	0.15 ± 0.27 (173.2)	3.80 ± 5.99 (157.6)	12.22 ± 13.35* (109.2)	6.55 ± 5.37* (82.0)	0.02 ± 0.04 (173.4)
AUEC (h*μg/L)	0.76 ± 1.32 (173.2)	17.55 ± 27.19 (154.9)	86.11 ± 102.12* (118.6)	33.54 ± 32.37* (96.5)	0.44 ± 0.77 (172.8)
IL-6					
E_{max} (μg/L)	0.14 ± 0.23 (167.8)	1.70 ± 2.61 (153.9)	10.28 ± 12.12* (117.9)	3.29 ± 3.07* (93.2)	0.01 ± 0.02 (264.6)
AUEC (h*μg/L)	0.39 ± 0.66 (168.4)	4.38 ± 7.21 (164.7)	35.38 ± 43.91* (124.1)	8.53 ± 7.17* (84.1)	0.09 ± 0.25 (264.6)

Note: Data are expressed as mean ± standard deviation. The *p* values were adjusted for multiple comparisons using Bonferroni adjustment (*$p < 0.05$ vs. placebo).
Abbreviations: AUEC, area under the effect-time curve; E_{max}, maximum effect; G-CSF, granulocyte colony-stimulating factor; IL-6, interleukin 6.

and 12.23 in KMRC011 5, 10, 15, and 20 μg, respectively, whereas the corresponding value was 3.06 10^9/L in placebo. The mean ± standard deviation values of changes in ANC from baseline to 24 h postdose were 2.17 ± 0.66 (10^9/L), 4.09 ± 4.82, 8.68 ± 7.22, and 8.16 ± 2.92 in KMRC011 5, 10, 15, and 20 μg, respectively. Notably, changes in ANC from baseline to 24 h postdose in 15 μg and 20 μg cohorts were significantly higher compared with the placebo group (Bonferroni-adjusted *p* value = 0.0037 [15 μg vs. placebo], 0.0055 [20 μg vs. placebo]). At day 7 postdose, ANC levels were within its reference range in all dose levels.

DISCUSSION

This is a first-in-human study to investigate the tolerability of KMRC011 following a single intramuscular administration in healthy subjects. In addition, PK and PD assessments were performed by using the serum concentrations of KMRC011, G-CSF, and IL-6.

Cytokine release syndrome is a systemic inflammatory response triggered by the release of proinflammatory cytokines, including IL-6, and can include various symptoms ranging from mild and self-limiting to severe and life-threatening.[18] It is characterized by fever greater than or equal to 38°C, hypotension with systolic blood pressure less than 90 mmHg, and increased hepatic enzymes.[17] Especially, IL-6 is regarded as a critical factor for CRS pathophysiology, and IL-6 levels were markedly elevated in patients with CRS, whose median levels of IL-6 were about 10,000 ng/L.[19,20] In this study, two subjects in 15 μg cohort and one subject in 20 μg cohort showed IL-6 levels above 10,000 ng/L, and fever greater than or equal to 38°C and hypotension were reported in all of them. Hepatic enzymes were also increased in two subjects, one in 15 μg cohort and the other in 20 μg cohort. However, fever and hypotension responded to symptomatic therapies, and these observations were all lost at 48 h postdose. Considering

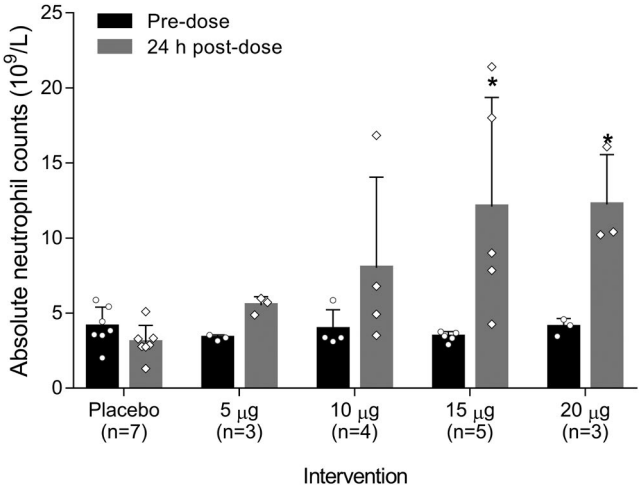

FIGURE 3 Mean absolute neutrophil counts at predose (0 h) and following a single intramuscular administration of KMRC011 or placebo. Error bars represent standard deviations. Open circles (○) and diamonds (◇) indicate the individual values. The *p* values were adjusted for multiple comparisons using Bonferroni adjustment (*$p < 0.05$ vs. placebo for changes in ANC from baseline to 24 h postdose)

that taking no action in an exigent ARS situation may ultimately result in death and KMRC011 is under development as a treatment for ARS, the AEs reported following a single intramuscular injection of KMRC011 in healthy subjects seem manageable and tolerable.

KMRC011 was designed by removing the N-terminal ancillary region of entolimod not related to the TLR5 activation.[14] In addition, the in vitro dissociation constant (K_d) of KMRC011 (108 pM) was smaller than that of entolimod (156 pM).[14] It suggests that KMRC011 has higher TLR5 binding affinities than entolimod due to its smaller and less complicated structure.

In this study, we could not detect any significant amount of KMRC011 in serum using the developed method with the lower limit of quantification of 20 ng/ml. Because the PD effects related to TLR5 stimulation were observed, it appears that KMRC011 reached the desired target site and played its agonistic function. Intramuscularly administered protein drugs pass by from the interstitial fluid to either the lymphatic system or the endothelial space to arrive at the systemic circulation, and predominantly undergo presystemic degradation by proteases and peptidases present ubiquitously throughout the body including the interstitial fluid.[21,22] KMRC011 would have acted on TLR5 expressed on the membrane of immune and epithelial cells in the interstitial fluid and lymphatic system, although being eliminated almost during the presystemic process.

The recommended doses of filgrastim, an available form of human G-CSF, are 5 μg/kg/day subcutaneous injection or intravenous infusion for chemotherapy-induced neutropenia and 10 μg/kg/day subcutaneous injection for hematopoietic ARS.[23] Although systemic exposures of G-CSF after a single intramuscular injection of KMRC011 were smaller than those after a single administration of G-CSF 5 or 10 μg/kg, KMRC011 15 μg resulted in the increase of ANC up to 12.10 10^9/L, which exceeds the criteria of G-CSF discontinuation for patients with neutropenia (ANC >10.00 10^9/L).[24] This indicates that the effects of KMRC011 on the increase of ANC in the present study can be clinically significant.

TLR5 is expressed on both hematopoietic and gastrointestinal tissues, whereas G-CSF receptors are mainly located in precursor cells of bone marrow.[25,26] Crypt damage, organ failure, and death under the gastrointestinal sub-syndrome can be prevented by inhibiting endothelial apoptosis,[27] and it has been reported that KMRC011 stimulated cell proliferation and showed anti-apoptotic effects in the small intestine of irradiated mouse model.[13] Accordingly, KMRC011 is also expected to be effective in not only hematopoietic but gastrointestinal sub-syndromes. In addition, due to its stability at room temperature and the intramuscular formulation with fixed-doses, KMRC011 is considered to be possible to self-dose through a portable device in an urgent situation exposed to radiation.

Responses to KMRC011 were highly variable within each dose cohort, with higher coefficients of variation of PD parameters. Because there were no meaningful differences among all subjects in either the demographic characteristics or the baseline G-CSF and IL-6 values, it seems that other individual or uncontrolled factors caused the observed fluctuations. Human TLR5 polymorphism, 1174C>T (rs5744168), is associated with the loss of responsiveness to flagellin, thus with an increased risk of infection.[28] Individuals with this polymorphism showed significantly lower G-CSF and IL-6 levels when exposed to bacteria.[29,30] On the contrary, other TLR5 polymorphism (rs5744174) is known to increase the response to flagellin.[31] Referring to these points, the interindividual genetic variations in TLR5 might have complexly affected the sensitivity of TLR5 to KMRC011.

It is of note that the results of this study were obtained only from male adults. Thus, extrapolation to children or old men as well as women should be performed with caution. Furthermore, the safety profile for KMRC011 may be different in those who have a compromised immune system or are currently suffering from ARS.

In conclusion, single intramuscular doses of KMRC011 ranging from 5 to 15 μg were tolerated in healthy subjects. KMRC011 in serum could not be measured with the currently developed assay, however, KMRC011 showed significant increases of serum G-CSF and IL-6 levels at doses equal to or greater than 15 μg. These findings suggest that KMRC011 has the potential for a treatment for ARS.

CONFLICT OF INTERTEST

C.C.M. has a patent KR 10-1744297 issued. L.W.J. reports grants from Civil and Military Dual-use Technology Program during the conduct of the study, and has a patent KR 10-1634380 issued and a patent KR 10-1744297 issued. All other authors declared no competing interests for this work.

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AUTHOR CONTRIBUTIONS

Y.E., C.H., P.J.S., N.Y.W., C.C.M., L.W.J., and K.J. wrote the manuscript. K.J.W. and K.J. designed the research. C.H., N.Y.W., C.C.M., L.W.J., K.J.W., and K.J. performed the research. Y.E., P.J.S., and K.J. analyzed the data.

REFERENCES

1. Singh VK, Newman VL, Romaine PL, Wise SY, Seed TM. Radiation countermeasure agents: an update (2011–2014). *Expert Opin Ther Pat*. 2014;24:1229-1255.
2. Donnelly EH, Nemhauser JB, Smith JM, et al. Acute radiation syndrome: assessment and management. *South Med J*. 2010;103:541-546.
3. Singh VK, Seed TM. A review of radiation countermeasures focusing on injury-specific medicinals and regulatory approval status: part I. Radiation sub-syndromes, animal models and FDA-approved countermeasures. *Int J Radiat Biol*. 2017;93:851-869.
4. Singh VK, Seed TM, Olabisi AO. Drug discovery strategies for acute radiation syndrome. *Expert Opin Drug Discov*. 2019;14:701-715.
5. *Guidance for industry: product development under the Animal Rule*. 2015. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/product-development-under-animal-rule. Accessed August 24 2020.
6. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. *Signal Transduct Target Ther*. 2017;2:17023.
7. Krivokrysenko VI, Shakhov AN, Singh VK, et al. Identification of granulocyte colony-stimulating factor and interleukin-6 as candidate biomarkers of CBLB502 efficacy as a medical radiation countermeasure. *J Pharmacol Exp Ther*. 2012;343:497-508.
8. Zhou J, Yang D, Liu K, Hou L, Zhang W. Systematic review and meta-analysis of the protective effect of resveratrol on multiple organ injury induced by sepsis in animal models. *Biomed Rep*. 2019;10:55-62.
9. Burdelya LG, Krivokrysenko VI, Tallant TC, et al. An agonist of toll-like receptor 5 has radioprotective activity in mouse and primate models. *Science*. 2008;320:226-230.
10. Krivokrysenko VI, Toshkov IA, Gleiberman AS, et al. The Toll-like receptor 5 agonist entolimod mitigates lethal acute radiation syndrome in non-human primates. *PLoS One*. 2015;10:e0135388.
11. Howell BA, Siler SQ, Shoda LK, Yang Y, Woodhead JL, Watkins PB. A mechanistic model of drug-induced liver injury AIDS the interpretation of elevated liver transaminase levels in a phase I clinical trial. *CPT Pharmacometrics Syst Pharmacol*. 2014;3:e98.
12. *Entolimod (CBLB502) Biodefense*. http://www.cbiolabs.com/entolimod-cblb502-biodefense/. Accessed August 24 2020.
13. Kim JY, Park J-H, Seo S-M, et al. Radioprotective effect of newly synthesized toll-like receptor 5 agonist, KMRC011, in mice exposed to total-body irradiation. *J Radiat Res*. 2019;60:432-441.
14. Song WS, Kim JH, Choi CM, Lee WJ, Yoon SI. TLR5 binding and activation by KMRC011, a flagellin-derived radiation countermeasure. *Biochem Biophys Res Commun*. 2019;508:570-575.
15. Lee HS, Cho D-W, Han J-S, et al. KMRC011, an agonist of toll-like receptor 5, mitigates irradiation-induced tissue damage and mortality in cynomolgus monkeys. *J Immunotoxicol*. 2020;17:31-42.
16. Pohlert T. *PMCMRplus: Calculate Pairwise Multiple Comparisons of Mean Rank Sums Extended*. 2020. https://CRAN.R-project.org/package=PMCMRplus. Accessed September 12 2020.
17. Riegler LL, Jones GP, Lee DW. Current approaches in the grading and management of cytokine release syndrome after chimeric antigen receptor T-cell therapy. *Ther Clin Risk Manag*. 2019;15:323-335.
18. Shimabukuro-Vornhagen A, Gödel P, Subklewe M, et al. Cytokine release syndrome. *J Immunother Cancer*. 2018;6:56.
19. Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med*. 2014;371:1507-1517.
20. Tanaka T, Narazaki M, Kishimoto T. Immunotherapeutic implications of IL-6 blockade for cytokine storm. *Immunotherapy*. 2016;8:959-970.
21. Zhao L, Ji P, Li Z, Roy P, Sahajwalla CG. The antibody drug absorption following subcutaneous or intramuscular administration and its mathematical description by coupling physiologically based absorption process with the conventional compartment pharmacokinetic model. *J Clin Pharmacol*. 2013;53:314-325.
22. Rathi C, Meibohm B. Pharmacokinetics of peptides and proteins. *Rev Cell Biol Mol Med*. 2015;1:300-326.
23. *NEUPOGEN® (filgrastim) injection, for subcutaneous or intravenous use, highlights of prescribing information*. 2018. https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/103353s5194lbl.pdf. Accessed August 24, 2020.
24. Lubenau H, Bias P, Maly AK, Siegler KE, Mehltretter K. Pharmacokinetic and pharmacodynamic profile of new biosimilar filgrastim XM02 equivalent to marketed filgrastim Neupogen: single-blind, randomized, crossover trial. *BioDrugs*. 2009;23:43-51.
25. Tsuji K, Ebihara Y. Expression of G-CSF receptor on myeloid progenitors. *Leuk Lymphoma*. 2001;42:1351-1357.
26. Yang J, Yan H. TLR5: beyond the recognition of flagellin. *Cell Mol Immunol*. 2017;14:1017-1019.
27. Paris F, Fuks Z, Kang A, et al. Endothelial apoptosis as the primary lesion initiating intestinal radiation damage in mice. *Science*. 2001;293:293-297.
28. Hawn TR, Verbon A, Lettinga KD, et al. A common dominant TLR5 stop codon polymorphism abolishes flagellin signaling and is associated with susceptibility to legionnaires' disease. *J Exp Med*. 2003;198:1563-1572.
29. West TE, Chantratita N, Chierakul W, et al. Impaired TLR5 functionality is associated with survival in melioidosis. *J Immunol*. 2013;190:3373-3379.
30. Dickey AK, Chantratita N, Tandhavanant S, et al. Flagellin-independent effects of a Toll-like receptor 5 polymorphism in the

inflammatory response to Burkholderia pseudomallei. *PLoS Negl Trop Dis*. 2019;13:e0007354.
31. Mukherjee S, Huda S, Sinha Babu SP. Toll-like receptor polymorphism in host immune response to infectious diseases: a review. *Scand J Immunol*. 2019;90:e12771.

SUPPORTING INFORMATION

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

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