2.6.6 TOXICOLOGY WRITTEN SUMMARY

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1 Brief Summary

The toxicology studies of KN035 consist of single-dose study in Sprague Dawley rats and Cynomolgus monkeys, repeat-dose toxicity studies in Cynomolgus monkeys, cross-reactivity studies with frozen fresh tissue of normal human and Cynomolgus monkey, and hemolysis assay with rabbit red blood cells. In addition, the safety pharmacology, local irritation and immunogenicity of KN035 were incorporated into the repeat-dose toxicity studies in Cynomolgus monkeys. The studies are listed in Table 1.

Table 1 Summary of KN035 Toxicity Studies

Study number	Project	Species	Grouping	Routine	Frequency	Dose	CRO	GLP
RDR-KN035-PD-2014-015	Single dose toxicity study	Sprague Dawley rat	5/sex/group	intraperitoneal injection	Single dose	10mg/kg	Alphamab	No
RDR-KN035-PD-2015-011		Cynomolgus monkey	1/sex/group	Intravenous injection; subcutaneous injection	Single dose	15 mg/kg (IV) 15, 30 mg/kg (SC)	JOINN& Alphamab	No
2015033-2*	Repeated dose toxicity study	Cynomolgus monkey	5/sex/group (control, low dose, medium dose, and high dose groups)	Subcutaneous injection	QW ×5	5, 30, 150 mg/kg	NCSED	Yes
2015046	Hemolysis	Rabbit blood cells	negative control, positive control and treatment groups	*in vitro*	-	49.2mg/ml	NCSED	Yes
337-0002-IM	Tissue Cross-Reactivity	Human	3x32 tissues /individual	*in vitro*	-	5, 25μg/ml	Wuxi Apptec	Yes
337-0003-IM		Cynomolgus monkey	3x32 tissues /individual	*in vitro*	-	5, 25μg/ml	Wuxi Apptec	Yes
RDR-KN035-PD-2015-011	Immunogenicity	Cynomolgus monkey	1/sex/group	Intravenous injection; subcutaneous injection	Single dose	15 mg/kg (IV) 15, 30 mg/kg (SC)	Alphamab	No
RDR-KN035-PD-2014-015		Sprague Dawley rat	5/sex/group	intraperitoneal injection	Single dose	10mg/kg	Alphamab	No
N2015042 appendix 1		Cynomolgus monkey	3/sex/group	Intravenous injection; subcutaneous injection	Single dose	15mg/kg (IV) 5, 15, 50mg/kg (SC)	Alphamab	No
2015033-2* appendix V		Cynomolgus monkey	5/sex/group (control, low dose, medium dose, and high dose groups)	Subcutaneous injection	QW ×5	5, 30, 150mg/kg	Alphamab	No
2015033-2*	Immune toxicity	Cynomolgus monkey	5/sex/group (control, low dose, medium dose, and high dose groups)	Subcutaneous injection	QW ×5	5, 30, 150mg/kg	NCSED	Yes

*As there is no report number from NCSED, this number is assigned by 3D Medicines.

2 Single-Dose Toxicity

2.1 Single-Dose Toxicity in Sprague Dawley Rats

Single-Dose Toxicity in Sprague Dawley (SD) rats was evaluated in the course of single-dose PK study (study#: RDR-KN035-PD-2014-015). In this study, 10 SD rats (5/sex) were administered with single-dose of KN035 at 10mg/kg through intraperitoneal injection (i.p.), blood samples were collected from the experimental animals at the time points of 0 min, 15min, 1h, 2h, 8h, and 1, 2, 4, 7, 11, 15, 21, 28days post drug injection; ELISA methods were used to quantitate the concentrations of KN035 in the serum of the collected blood samples. Based on the observations, there were no abnormal clinical phenomena noted during the experiment, such as appearance, activity, coat, urine, feces, etc.

2.2 Single-Dose Toxicity in Cynomolgus Monkeys

Single-dose toxicity of KN035 in Cynomolgus Monkeys was observed in the course of dose range finding pharmacokinetics study (RDR-KN035-PD-2015-011).Six Cynomolgus monkeys, with weights from 3.16 to 3.83kg, were randomly assigned into three groups (1/gender/group). Animals in Group 1 and Group 2 received single dose of KN035 at 15and 30mg/kg by subcutaneous injection (SC), respectively. Animals in Group 3 received single dose of KN035 at 15mg/kg by intravenous injection (IV).There were no abnormal clinical signs observed in the animals during the study, such as unusual behavior and changes in food consumption.

Single-dose toxicity study of KN035 in Cynomolgus monkeys was also incorporated into the 4-week repeat-dose toxicity studies in Cynomolgus monkeys (Study #: 2015033-2).The test animals were observed closely twice a day for one week. No abnormalities, such as unusual behavior, changes in food consumption and body temperature, sleeplessness, etc. were observed even at the highest dose of 150mg/kg. The MTD ofKN035 should be greater than 150mg/kg in the Cynomolgus monkeys.

3 Repeat-Dose Toxicity Study in Cynomolgus Monkeys

A 4-week repeat-dose toxicity study was conducted to evaluate the toxicity of KN035 in Cynomolgus monkeys (Study #: 2015033-2).

Forty Cynomolgus monkeys, weighing 2.3-3.8kg, were randomly assigned into four groups (5/sex/group). The animals received repeated doses of KN035 by subcutaneous injection at 0 (control), 5, 30, and 150 mg/kg/week for four weeks, for a total of five injections. The animals were then put in recovery for 4 weeks.

During the experimental period, the clinical signs, local irritations of injection sites, food consumption, body weight, body temperature, ECG, blood pressure, urinalysis, ophthalmological, hematology, serum biochemistry, CD4+/CD8+ T cells, anti-drug antibody productions, and toxicokinetic parameters were observed and evaluated. Serum thyroid hormones (T3, T4, and TSH) were also studied due to the positive results between KN035 and thyroid tissue staining

identified in drug-tissue cross-reactivity studies. Organ:body weight ratios were calculated as well. Gross pathology and histopathology were performed on terminated animals at the end of the 5thdosing and recovery period.

The repeat-dose toxicity study results showed that no severe adverse event occurred during the experiment. Through observation and analysis of clinical symptoms, food consumption, body weight, ophthalmic examination, body temperature, electrocardiogram, blood pressure, urine, hematology, blood biochemistry, blood coagulation, and organ weights of the test animals, no KN035-induced abnormality was noted. In the detection of serum thyroid function hormone, no abnormal changes in T3 and T4 were found. However, due to the limitation of the TSH detection method, most of the detection values of TSH were too low (<0.005 mIU/L) to analyze. Anatomical and microscopic examinations demonstrated that the administration of KN035 did not induce any pathological alterations in the animals.

Based on the results of this study, the NOAEL of KN035 was determined at150mg/kg/week in Cynomolgus monkeys.

For more details, please refer to Module 2.6.7 section 7.

4 Genotoxicity

KN035, as a monoclonal antibody, is not expected to interact directly with DNA or other chromosomal materials. Genotoxicity studies were not conducted as they were not required based on ICH S6 guidelines.

5 Carcinogenicity

No studies were conducted.

6 Reproductive and Developmental Toxicity

No studies were conducted.

7 Local Tolerance

No stand-alone local tolerance study was conducted with KN035. However, the injection sites were assessed during the 4-week repeat-dose toxicity study conducted in monkeys. There was no test article-related irritation observed in the animals.

8 Other Toxicity Studies

8.1 Tissue Cross-Reactivity Studies

8.1.1 Cross-Reactivity Study with Cynomolgus Monkey Tissue

Immunohistochemistry staining method was used to assess the cross-reactivity of KN035 with 32 different normal tissues from freshly frozen Cynomolgus monkeys (Study #: 337-0003-IM).

The following tissues of Cynomolgus monkeys were investigated for cross-reactivity with KN035 in these studies: adrenal gland, heart, small intestine (duodenum), bladder, kidney (renal glomerulus and renal tubules), skin, bone marrow (femur), liver, spinal cord (thoracic), breast, lung, spleen, blood cells, lymph node (SMA), testis, brain cortex, ovary, thymus, cerebellum, pancreas, thyroid gland, colon, pituitary gland, tonsil, endothelial (aorta), prostate and ureter, eye, muscle (skeletal muscle), uterus (cervix, endometrium), fallopian tube and stomach. For more details, please refer to Module 2.6.7 section 17.Threerepeated tests were conducted on each kind of tissue from 3 different animals. KN035 was labeled with Biotin and detected with Streptavidin-Peroxidase. The color was developed in DAB. Two concentrations of KN035 were test, respectively, at 5ug/ml and 25ug/ml.

The results showed that at 25ug/ml concentration, KN035 specific staining was seen in the cytoplasm of thyroid follicular epithelial cells. The staining intensity was from 1+ to 3+ and the frequency of the stained cells was rare. At the concentration of 5ug/ml, no KN035 staining was found in any animal tissues. Except in the cytoplasm of thyroid follicular epithelial cells, no KN035 was found in any other tissues.

8.1.2 Cross-Reactivity with Human Tissue

Immunohistochemistry staining method was used to assess the cross-reactivity of KN035 with 32 different normal tissues from freshly frozen human species (Study #: 337-0002-IM).

In this experiment, the following human tissues have been studied for cross reactivity with KN035: adrenal gland, heart, small intestine, bladder, kidney (renal glomerulus and renal tubules), skin, bone marrow, liver, spinal cord, breast, lung, spleen, blood cells, lymph node, testis, brain cortex, ovary, thymus, cerebellum, pancreas, thyroid gland, colon, pituitary gland, tonsil, endothelial (aorta), prostate and ureter, eye, muscle, uterus (cervix, endometrium), fallopian tube and placenta. For more details, please refer to Module 2.6.7 section 17. Three repetitions were conducted for each kind of tissue from 3 different individuals. KN035 was labeled with Biotin and detected with Streptavidin-Peroxidase. The color was developed in DAB. Two concentrations of KN035 were tested, respectively, at 5ug/ml and 25ug/ml.

The results showed that at 5ug/ml and 25ug/ml concentrations, the specific staining in the cell membrane of syncytiotrophoblast were observed. The staining intensity was 1+and 2+ at the concentrations of 5ug/ml and25 ug/ml, respectively. The frequency of the stained cells was frequent. At 25ug/ml concentration, 2/3 of individuals were observed with specific staining in the cytoplasm of the thyroid follicular epithelial cells. The staining intensity was 1+ or 2+ and the frequency of stained cells was rare and infrequent. At 5ug/ml concentration, no specific staining was found in the cytoplasm of the thyroid follicular epithelial cells. Except for the observations stated above, no other specific staining was found.

8.2 Hemolysis Assay

The objective of this study was to determine the potential of KN035 for causing the hemolysis of New Zealand rabbit blood cells (Study #: 2015046).

De-fibrinogen treated rabbit blood was washed with 0.9% sodium chloride injection buffer several times and re-suspended to be 2% of red blood cell suspension. KN035 was diluted with 0.9% sodium chloride buffer at a ratio of 1:3. Diluted KN035, 0.9% sodium chloride buffer, and sterilized injection water were used as test article (TA), negative control, and positive control, respectively. The effectiveness of the samples on blood cell hemolysis and coagulation was evaluated within 3 hrs. The experiment procedures were: 1) add 2.5 ml of 2% of the red blood cell suspension to each of the seven tubes; 2) add 2.0, 2.1, 2.2, 2.3, 2.4, and 2.5 ml of 0.9% sodium chloride injection buffer and 2.5 ml of sterilized injection buffer to each of the above tube, respectively; 3) add 0.5, 0.4, 0.3, 0.2, 0.1, and 0 ml of RA and 0 ml of sterilized injection buffer; 4) gently mix the tubes very well; 5) put the test tubes into the incubator at 37^{o}C; 6) check the cell lysis progress every 15 minutes during the first 1 hour, and every hour during 1-3 hours after incubation.

The results show that at 49.2 mg/mL concentration, KN035 did not cause the hemolysis of rabbit red blood cell. For more details, please refer to Module 2.6.7 section 17.

8.3 Studies of Immunogenicity

8.3.1 Single-Dose Immunogenicity Study in Sprague Dawley Rat

The single-dose immunogenicity study of KN035 in Sprague Dawley rat was incorporated with the single-dose PK studies of KN035 in Sprague Dawley rat (study#: RDR-KN035-PD-2014-015). The bridging ELISA method was applied to test anti-KN035 antibodies (ADA) and assess the immunogenicity. Results obtained from the studies showed that, at 10mg/kg intraperitoneal injection of KN035, the positive rate of immunogenicity for 15d was 4/10; the positive rate of immunogenicity for 28d was 9/10.

8.3.2 Single-Dose Immunogenicity Study in Cynomolgus Monkeys

The bridging ELISA method was applied to test anti-KN035 antibodies (ADA) and assess immunogenicity of KN035in Cynomolgus monkey serum.

Single-dose immunogenicity data were obtained from two single-dose studies in Cynomolgus monkeys following either subcutaneous or intravenous administration.

In a dose range finding pharmacokinetics study (Study #: RDR-KN035-PD-2015-011), six Cynomolgus monkeys, weighing 3.16-3.83kg, were randomly assigned into three groups (1/sex/group).The animals received a single dose of KN035 at 15 and 30mg/kg by subcutaneous injection (SC), and a single dose at 15mg/kg by intravenous injection (IV), respectively. The time points of blood sample collection and the results of anti-drug antibody (ADA) positive are listed in the table below (Table 2). The results showed that anti-drug antibody (ADA) positive in animals No.1 and No. 3 on Days 17 and 24, animal No. 2 on Day 32, animal No. 5 on Day 24, and animal No. 6 on Days 10, 14, and 24. Animal No. 4 showed ADA negative at all detection time points.

Table 2 The Days of Sample Collection & ADA Positive in Dose Range finding PK Study

Dose (mg/kg) & Route	Animal #	Sample collection time	ADA Positive
15, SC	1	Days 0, 17, 24	Positive on Days 17 and 24
15, SC	2	Days 0, 24, 32	Positive on Day 32
30, SC	3	Days 0, 17, 24	Positive on Days 17 and 24
30, SC	4	Days 0, 17，24, 32	Negative
15, IV	5	Days 0, 17, 24	Positive on Day 24
15, IV	6	Days 0,,10,14,24	Positive on Days , 10, 14 and 24

In another pharmacokinetics study of KN035 (N2015042), twenty-four Cynomolgus monkeys, weighing 2-5kg, were randomly assigned into four groups (3/sex/group). One group received a single dose of KN035 at 15mg/kg by intravenous injection. The other three groups received a single dose at 5, 15, and 50mg/kg by subcutaneous injection, respectively. The sample collection time points for immunogenicity analysis were at 10 minutes to 1 hour Pre-dosing, and then at 9, 17, 28, and 37days post-administration. The appearances of ADA positive in each group are listed in the following table (Table 3). For more details, please refer to Module 2.6.7 section 17.

Table 3 Sample Collection Date & ADA Positive Rate in Single-Dose PK Study

Dose (mg/kg) & Route	Sample collection days	ADA Positive Rate
15, IV	0, 9,17, 28, 37	1/6 on Day 9; 1/6 on Day 17; 4/6 on Day 28; 6/6 on Day 37
5, SC	0, 9,17, 28, 37	1/6 on Day 9; 5/6 on Day 17; 5/6 on Day 28; 5/6 on Day 37
15, SC	0, 9,17, 28, 37	1/6 on Day 9; 2/6 on Day 17; 4/6 on Day 28; 4/6 on Day 37
50, SC	0, 9,17, 28, 37	Negative

8.3.3 Repeat-Dose Immunogenicity Study in Cynomolgus Monkeys

In this study, forty Cynomolgus monkeys, weighing 2.3-3.8kg, were randomly assigned into four groups (5/sex/group). The animals received repeated doses of KN035 by subcutaneous injection at 0 (control), 5, 30, 150 mg/kg/week for four weeks, for a total of five injections. The animals were then put in recovery for 4 weeks(Study #: 2015033-2).The results suggested that following 2 dose administrations of KN035 at 5 and 30mg/kg, the ADA positive rates were 3/10 and 1/10, respectively. After 4 dose administrations, the ADA positive rates were 4/10 at 5 mg/kg and 0/10 at 30 mg/kg. After a 4-week recovery period, the ADA positive rates were at 5 and 30mg/kg were 3/4 and 0/4, respectively. At the 150mg/kg dose level, ADA was all negative after 2 and 4 doses and after the recovery period. The results demonstrated that ADA positive was not

detected following the treatment of KN035 at 150 mg/kg, which was the highest dose level used on the test animals. For more details, please refer to Module 2.6.7 section 17.

8.4 Immune-toxicity Studies

8.4.1 Repeat-Dose Immune Toxicity

Forty Cynomolgus monkeys, weighing 2.3-3.8kg, were randomly assigned into four groups (5/sex/group). The animals received repeated doses of KN035 by subcutaneous injection at 0 (control), 5, 30, 150 mg/kg/week for four weeks, and then followed by a 4-week recovery period (Study #: 2015033-2).

White blood cells (neutrophils, eosinophilic granulocyte, basophilic leukocyte, lymphocytes, and mononuclear cells), CD4+ and CD8+ T cells were checked twice during the quarantine acclimation period, ~24 hours after the last drug administration and at the end of the recovery period. Major immune system organs, such as the spleen and thymus, were dissected and weighed. The heart:organ weight ratios, such as heart:body weight ratio and heart:brain weight ratio were calculated. The histopathology of immune organs, such as thymus, mesenteric and inguinal lymph nodes, etc. was evaluated at the end of drug administration and the end of recovery period.

The results showed that the lymphocyte count was significantly lower than normal in the low dose group after 4 doses ($P<0.01$or $P<0.05$). However, after further analysis, it was considered as individual bias and not relevant to KN035 treatment. Compared with the control group, the CD8+ T cells, CD4+ T cells as well as CD4+/CD8+ T cells in each dose group showed no significant differences. At the end of the dosing and recovery period, in each dose group, the weight of the animal immune organs, such as thymus and spleen, showed no significant differences. The histopathology of immune system organs and tissues, such as the thymus, mesenteric lymph nodes, and inguinal lymph node tissue, showed that there were no KN035-related pathological alternations. This study indicated that KN035 did not produce significant immune toxicity in monkeys.

9 Discussion and Conclusions

Single-dose toxicity: Single-dose Toxicity of KN035 was evaluated in SD rats at 10mg/kg via intraperitoneal injection (i.p.), and in Cynomolgus Monkeys at 15or 30mg/kg by subcutaneous injection (SC), and 15mg/kg by intravenous injection (IV), no abnormal clinical signs were observed in these studies. In addition, single-dose toxicity study of KN035 was also incorporated into the 4-week repeat-dose toxicity studies in Cynomolgus monkeys (Study #: 2015033-2).The test animals were closely monitored twice a day for one week after the first dosing. No abnormalities, such as unusual behavior, changes in food consumption and body temperature, sleeplessness, etc. were observed even at the highest dose of 150mk/kg. The MTD of KN035 should be greater than 150mg/kg in the Cynomolgus monkeys.

Repeat-dose toxicity: In a 4-week repeat dose toxicity study, Cynomolgus monkeys were dosed at 0 (control), 5, 30, 150 mg/kg/week for four weeks (5 subcutaneous injections in total), and

then followed by a 4-week recovery period. No severe adverse effects were noted in clinical signs, food consumption, body weight, body temperature, ECG, blood pressure, urinalysis, ophthalmological examination, hematology, serum biochemistry, CD4 + / CD8 + T cells, , serum thyroid function hormone T3 and T4, organ weights, and gross and histopathological examinations. Based on these results, the NOAEL of KN035 was determined at150mg/kg/week in Cynomolgus monkeys.

Special toxicity studies: No mutagenicity was performed since KN035 will not directly interact with DNA or other chromosomal materials. No reproductive toxicity or carcinogenicity potentials were evaluated at this stage.

Tissue cross-reactivity: The tissue cross-reactivity studies show that at the concentration of 5 and 25ug/ml, KN035 specific staining was observed in human trophoblast cell membrane, and 3/3 of individuals were observed specific staining. KN035 specific staining was also found in the cytoplasm of human thyroid follicular epithelial cells with 2/3 rate at the concentration of 25ug/ml. For Cynomolgus monkey tissues, at the concentration of 25ug/ml, KN035 specific staining was observed in the cytoplasm of thyroid follicular epithelial cell. Staining with placental tissue of Cynomolgus monkey was not investigated because of the missing tissue. No other specific staining was found except the observations mentioned above.

Hemolysis and local irritation: KN035 (49.2mg/ml, prepared in saline) were tested for hemolysis and coagulation in rabbit blood. No test articles related hemolysis and coagulation were observed. The results of histopathological examination showed that there was no test articles related abnormal change at or around the injection sites after the 4-week toxicity study in Cynomolgus monkeys.

Immunogenicity: Immunogenicity parameters were evaluated in three single-dose and a multiple-dose toxicity studies. The single-dose study of KN035 in Sprague Dawley rat showed at single doses of 10mg/kg intraperitoneal injection of KN035, the positive rate of immunogenicity for 15d was 4/10; the positive rate of immunogenicity for 28d was 9/10.A dose range finding study showed at single dose of 15 and 30mg/kg through subcutaneous injection, the ADA positive rates were 2/2 and 1/2, respectively. The pharmacokinetics study showed that at single dose of 5, 15, and 50mg/kg through subcutaneous injection, the ADA positive rates were 5/6, 4/6 and 0/6, respectively. At the end of the 4-week repeated dose toxicity studies, the ADA rate for each dose level was 4/10, 0/10, and 0/10, and at the end of the recovery period, the ADA rates were 3/4,0/4, and 0/4, respectively.

Immune toxicity: The 4-week toxicity study results show no treatment-related abnormal changes in the white blood cells, CD4+/CD8+ T cells, immune organs, and tissues. KN035 has no immune toxicity in Cynomolgus monkeys.

In summary, a series of toxicology studies were conducted to support the proposed Phase I clinical study. It appears that the monkey is the most relevant animal species. Although ADA was detected in the single-dose and repeat-dose toxicity studies in monkeys, the appearance of ADA did not cause any toxicological abnormality, suggesting that there were very low concentrations of ADA in the monkeys tested.

KN035 did not induce apparent toxicity at the highest dose of 150 mg/kg in the 4-week GLP toxicity study in monkeys. Therefore, 150 mg/kg was considered as the NOAEL in monkeys, it is already 30 times of the clinical dose for most antibody drugs (5mg/kg). Based on FDA guidance for “Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers”, one tenth of the NOAEL can be used as starting dose for healthy volunteers, it would be 15 mg/kg for KN035. If ICH guideline S9 “Nonclinical Evaluation for Anticancer Pharmaceuticals” is referred, then 1/6 the highest non-severely toxic dose (HNSTD) is used as starting dose, it would be higher than 25 mg/kg for KN035. However, the effective dose in pharmacology study of KN035 was much lower. When both the effective dose and the NOAEL are considered, 1 mg/kg is selected as the safe starting dose for the phase 1 clinical trial. This will be a very safe starting dose. It is more than 150 times lower than the NOAEL in monkeys.