2.4 NONCLINICAL OVERVIEW

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List of Abbreviations and Definitions of Terms

ADA	Anti-drug antibodies
ADCC	Antibody-dependent cellular cytotoxicity
AUC	Area under the curve
CDC	Complement-dependent cytotoxicity
ELISA	Enzyme-linked immunosorbent assay
FACS	Fluorescence-activated cell sorting
IC_{50}	Half-maximal inhibitory concentration
MTD	Maximum tolerated dose
NOAEL	No observed adverse effect level
PBMC	Peripheral blood mononuclear cell
PD-1	Programmed cell death protein 1
PD-L1	Programmed death-ligand 1
PET	Positron emission tomography
SUV	Standard uptake value
TSH	Thyroid-stimulating hormone

1 Overview of Nonclinical Testing Strategy

The binding affinity of KN035 to PD-L1 was analyzed using two different methods: one was Fortebio (BLI, bio-membrane layer interference) and ELISA system; the other was through FACS detection of the binding between human PD-L1 expression cells and KN035 protein. The blocking effect of KN035 on PD-1/PD-L1 binding was conducted on both protein base and cell base. The activating effect of KN035 on the immune system was analyzed through the co-incubation of Jurkat T/Raji-PD-L1 and a different T cell activation system (CD4+ cells/allogeneic DC cells).

In the tumor microenvironment, tumor cells and tumor infiltrating immune cells can express PD-L1 and inhibit T-cell function. The mechanism of action of KN035 on tumor growth inhibition and elimination is to block the PD-1/PD-L1 binding. Consequently, KN035 activates the inactivated cytotoxic T cells. The immune checkpoint blockade drugs already out in the market, such as anti-PD-L1 and anti-CTLA-4, do not recognize the homologous target in mice, the preclinical pharmacology studies for them were mostly conducted in regular healthy mice with surrogate anti-mouse target antibody. However, because there are many differences between the surrogate anti-mouse target antibodies and the antibodies of real investigated drug candidates with regards to their structures, affinities, and epi-environment etc., the evaluation results were possibly not relevant to that of the drug property itself. Misjudgment could be made from the surrogate studies. In order to avoid possible errors from that type of study design, for KN035, we developed a new allogeneic transformation tumor model which was made through inoculation of premixed human PD-L1 expression cells A375-PD-L1 and human PBMCs into NOD/SCID mice subcutaneously. In the tumor model making procedure, the PBMC and tumor cell A375 were exposed to each other extensively. The specific tumor toxic T cells could be induced and function as the active T cell to kill cancer cells, after KN035 was applied to block the binding of PD-1/PD-L1 in the system.

The pharmacokinetics studies of KN035 include absorption and distribution studies in mice, rats and monkeys. The absorption study of KN035 was conducted in both Sprague Dawley rats and Cynomolgus monkeys (See Module 2.6.5 section 1). The routes of administration were subcutaneous and intravenous injections. Single and repeat doses were given to the animals in the studies. Bioavailability of KN035 was also investigated in Cynomolgus monkeys. The distribution study of KN035 was conducted in tumor bearing-mice and Cynomolgus monkeys (See Module 2.6.5 section 5) through intravenous injection of ^{89}Zr labeled KN035. The real time distribution of drugs was monitored.

According to relevant guidelines for the preclinical evaluation of biological products, the metabolism and excretion study of KN035 were not implemented. KN035 is an Fc fusion protein, which is expected to degrade into peptides and amino acids *in vivo*, and then be excreted or reused for the synthesis of proteins or peptides *in vivo*.

The toxicology studies of KN035 include single-dose studies 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.

In addition, the safety pharmacology, local irritation and immunogenicity of KN035 were incorporated into the repeat-dose toxicity studies in Cynomolgus monkeys.

According to relevant anti-cancer therapeutic biologics safety evaluation guidelines and regulations, genotoxicity and carcinogenicity study might not be required for IND filing, and were not conducted in approved similar therapeutic drugs (Opdivo/BMS, Keytruda/Merck, Yervoy/BMS and Tecentriq/Roche). As for reproductive toxicity study, PD-1/PD-L1 pathways play important roles in maintaining maternal fetal tolerance; blocking their interactions may cause abortion risk. The reproductive toxicity test for Opdivo in Cynomolgus monkeys suggested that Opdivo could lead to the increase of the mortality rate of embryo and newborn in the final stage of pregnancy. Therefore, using KN035 in pregnant women should be forbidden.

2 Pharmacology

2.1 Primary Pharmacodynamics

The pharmacological studies of KN035 included *in vitro* and *in vivo* studies. *In vitro*, the binding of KN035 to human, Cynomolgus monkey, and murine PD-L1 has been studied. Furthermore, other checkpoint proteins, such as B7/CD28 family (PD-L2, hB7H4, hCD28, hB7H3, hICOS) were also assessed. The inhibitory activity of KN035 by blocking the interaction of PD-L1 with PD-1 and CD80 (B7-1) has been also measured. The effect of KN035 on the activation of T cells was measured in MLR (CD4+ T cell/allogenic DC cell) and Jurkat/Raji-PD-L1 co-culture systems. The binding of Fc region of KN035 to FcγRs and FcRn was tested, respectively. ADCC and CDC activities have also been studied. The *in vivo* pharmacology of KN035 was conducted to evaluate the anti-tumor activity of KN035 in xenograft animal models generated by transforming the mixture of A375/PD-L1 and human PBMCs. In order to show the positive effect of KN035 on tumor suppression, Durvalumab (an anti-PD-L1 antibody 2.41H9OP, under development by AZ/Medimmune) was used as a reference for comparing *in vitro* and *in vivo* in several studies.

The summary of primary pharmacology is in Table 1.

Please refer to Module 2.6.2 Section 2 for more details.

Table 1 Summary of primary pharmacology of KN035

Study number	Study title	Test system	Noteworthy findings
RDR-KN035-QC-2016-010	Test Report of Binding Affinity of KN035 to PD-L1-Chis	Bio-Layer Interferometry	The average KD of KN035: (2.86±0.23)E-09M.
RDR-KN035-QC-2015-017	Test Report of binding affinities of KN035/2.41H9OP to hPD-L1	ELISA	The binding affinities of KN035 to hPD-L1-muFc was 1.5 times higher than that of Durvalumab.

Study number	Study title	Test system	Noteworthy findings
RDR-KN035-PD-2015-049	Binding Test of KN035 to PD-L1 on cell surface	Flow cytometer	The EC_{50} of KN035 binding to A375/hPD-L1 was 0.68ug/ml; KN035 does not bind to hPD-L2, hB7H4, hCD28, hB7H3, hICOS; KN035 binds to PHA activated monkey PBMCs; KN035 does not bind to mouse PD-L1.
RDR-KN035-QC-2015-018	ELISA Test Report of PD-1/PD-L1 Blockade by KN035	ELISA	mean IC_{50} of 6 product lots of KN035: 419-488ng/mL.
RDR-KN035-PD-2015-056	FACS Test of PD-L1/PD-1 blockade by KN035	Flow cytometer	KN035 blocks PD-1-muFc and 293T- hPD-L1 binding, IC_{50}: 0.25 ug/ml; KN035 blocks PD-L1-muFc and Jurkat-hPD-1 binding, IC_{50}: 13.42ug/ml.
RDR-KN035-QC-2016-006	Test Report of PD-L1/CD80 Blockade by KN035	ELISA	KN035 blocks PD-L1/CD80 binding, IC_{50}: 102.7ng/ml.
RDR-KN035-QC-2015-016	Binding affinity of KN035 to Fcγ Receptors	ELISA	Binding affinity of KN035/ to FcγR Ⅱa and FcγRⅢa is much lower than that of wtFc (wild type Fc).
RDR-KN035-PD-2015-057	Binding activity of KN035 to U937(Fc γ RI)	Flow cytometer	KN035 does not bind to U937 (CD64).
RDR-KN035-QC-2016-011	Binding affinity of KN035 to rhFcRn	Bio-Layer Interferometry	The average KD of KN035 to rhFcRn was (4.93±0.81)E-07M. The KD of KN015(wtFc) is 5.50E-07M.
RDR-KN035-PD-2015-050	ADCC activity assay of KN035	Raji-PD-L1	No ADCC activity
RDR-KN035-PD-2015-051	CDC activity assay of KN035	Raji-PD-L1	No CDC activity
DF-YX-KN01	KN035 MLR Results	CD4+ T cell/allogenic DC cell	KN035 stimulates CD4+ T cell to secrete IFN-γ with dose response. The activating effect of KN035 is stronger than that of Durvalumab.

Study number	Study title	Test system	Noteworthy findings
RDR-KN035-PD-2016-006	Activating effect of KN035 on T cells in Jurkat T/Raji-PD-L1 coculture system	Coculture Raji-PD-L1 cells and Jurkat T cells	KN035 stimulates Jurkat T cells to secrete IL-2 with dose level response. The activating effect of KN035 is stronger than that of Durvalumab.
RDR-KN035-PD-2015-015	Anti-tumor efficacy of KN035 on NOD-SCID xenograft of mixed A375-hPD-L1/human PBMC	NOD-SCID mouse; A375-PD-L1/human PBMC mixture xenograft animal model; IP delivery	KN035 reduced tumor growth in this animal model with no dose level relevance.
RDR-KN035-PD-2015-023	Anti-tumor efficacy of KN035 on NOD-SCID xenograft tumor model of A375-PDL1/human PBMC at different frequencies		KN035 reduced tumor growth with every dosing frequency in this animal model.
RDR-KN035-PD-2016-005	Anti-tumor efficacy of KN035 and 2.41H9OP in NOD-SCID xenograft model of A375-hPD-L1/human PBMC		At dose of 1mg/kg, KN035 showed similar anti-tumor efficacy when compared with Durvalumab. while the anti-tumor efficacy of KN035 was much stronger than that of Durvalumab at 0.3mg/kg and 0.1mg/kg.

Based on the results above, conclusion can be drawn that KN035 can specifically bind to hPD-L1, block PD-1/PD-L1 interactions, and to activate the functions of cancer antigen-specific T cells of cancer clearance. *In vivo* pharmacology study showed that in the dose range of 0.1-10mg/kg (QW, 4 weeks), KN035's tumor growth inhibition effects were observed very obviously. At the dose of 0.3mg/kg, even just a single dose administration, the tumor growth inhibition also can be observed. The results of parallel study between Durvalumab and KN035 suggested the initial tumor inhibition effective dose of KN035 was much lower than that of Durvalumab. At some dose level, the inhibition effect of KN035 was much stronger than Durvalumab. At the same effective level, the dose of KN035 was lower than that of Durvalumab.

2.2 Secondary Pharmacodynamics

No studies conducted.

2.3 Safety Pharmacology

Safety pharmacology studies of KN035 were incorporated into the 4-week repeated-dose toxicology studies in Cynomolgus monkeys. The studies included the evaluation of body temperature, blood pressure, and ECG. No notable abnormalities were observed. These results suggested KN035 has no adverse effect on the central nervous system and cardiovascular system. In 4-week general toxicology studies, the clinical observation results showed that no respiratory

adverse events were noticed in Cynomolgus monkeys. The NOAEL of KN035 in Cynomolgus monkeys was greater than 150mg/kg.

Please refer to Module 2.6.2 Section 4.

2.4 Pharmacodynamic Drug Interactions

No studies conducted.

3 Pharmacokinetics

The pharmacokinetics studies of KN035 include absorption and distribution studies in mice, rats and monkeys. The absorption study of KN035 was conducted in both Sprague Dawley rats and Cynomolgus monkeys (See Module 2.6.5 section 1). The routes of administration were subcutaneous and intravenous injections. Single and repeat doses were given to the animals in the studies. Bioavailability of KN035 was also investigated in Cynomolgus monkeys. The distributions of KN035 were conducted in tumor-bearing mice and Cynomolgus monkeys (See Module 2.6.5 section 5) through intravenous injection of ^{89}Zr labeled KN035. The real time distribution of drugs was monitored. The pharmacokinetics of KN035 is summarized in Table 2.

According to relevant guidelines for the preclinical evaluation of biological products, the metabolism and excretion study of KN035 were not implemented. KN035 is an Fc fusion protein, which is expected to degrade into peptides and amino acids *in vivo*, and then be excreted or reused for the synthesis of proteins or peptides *in vivo*.

Table 2 Summary of KN035 Pharmacokinetics Study

Study Number	Project	System	Routine	Test facilities
		Absorption		
RDR-KN035-PD-2014-015	A Single Dose Pharmacokinetic Study in Sprague Dawley (SD) rat	SD rat	intraperitoneal injection (i.p.)	Alphamab
RDR-KN035-PD-2015-011	A Single Dose Pharmacokinetic Study in Monkeys (Exploratory Study)	Cynomolgus monkey	SC, IV	JOINN& Alphamab
N2015042	A Single Dose Pharmacokinetic Study in Monkeys (Non-GLP Study)	Cynomolgus monkey	SC, IV	National Center for Safety Evaluation of Drugs
2015033-2*	Absorption after Repeated Doses	Cynomolgus monkey	SC	

Study Number	Project	System	Routine	Test facilities
		Distribution		
MIRT0005-1	The distribution and targeting analysis of ^{89}Zr-KN035 in tumor-bearing mice	NOD/SCID	IV	MITRO(Nanjing) Biotech Co., Ltd
MIRT0005-2	The distribution analysis of ^{89}Zr-KN035 in Cynomolgus monkeys	Cynomolgus monkey	IV	MITRO(Nanjing) Biotech Co., Ltd
		Metabolism		
		N/A		
		Exercitation		
		N/A		

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

3.1 Absorption

In the absorption study in Sprague Dawley rats (Module 2.6.4 Section 3), blood samples were collected after a single intraperitoneal injection of 10 mg/kg KN035. The half-life of KN035 was 72.02±26.82h in SD rats after a single i.p. dose at 10mg/kg. The C_{max} was 43.20±15.25 ug/ml, and AUC_{inf} was 3.04±0.73 h*mg/ml. No differences were noted in C_{max} and AUC_{inf} between male and female animals.

In the absorption studies of KN035 in Cynomolgus monkeys (Module 2.6.4 Section 3), blood samples were collected after single and repeated administrations. The pharmacokinetic parameters of KN035 were calculated based on the drug concentrations in serums. In the 5-50 mg/kg dose range, the C_{max} and $AUC_{(0-t)}$ of KN035 increased proportionally with dose. There were no significant differences between male and female animals. The absolute bioavailability of a single subcutaneous administration of 15mg/kg was 104.47%. In the 5-150mg/kg weekly dose range (5 doses in total), there was no significant accumulation of KN035 in both male and female animals.

Following a single subcutaneous administration of KN035, the half-lives of KN035 in the low dose group ($t_{1/2}$=31.30±24.85h) and middle dose group ($t_{1/2}$=26.48 + 13.59h) were significantly lower than that in the high dose group ($t_{1/2}$=155.81 + 29.33h). This may be attributed to the level of production of anti-drug antibodies (ADA) in test animals.

3.2 Distribution

^{89}Zr labeled DFO conjugated KN035 (^{89}Zr-KN035) was used for distribution studies. The procedures were: Inject ^{89}Zr-KN035 into tumor-bearing mice through IV; use MicroPET scan to identify and analyze the target orientation and specificity of KN035 in tumor tissue and its

distribution in main organs of the test animals (See Module 2.6.4 Section 4.1). Meanwhile, inject the labeled KN035 into Cynomolgus monkey through IV; use live imaging protocol to analyze the distributions of KN035 in live animals; offer real time distribution data for clinical references (See Module 2.6.4 Section 4.2).

The distributions of ^{89}Zr-KN035 in hPD-L1 positive tumor-bearing mice indicated that KN035 was specifically distributed in tumor tissues. It demonstrated that the %ID/g of KN035 was higher than that of Durvalumab at 1 and 2.5 hours post dosing, suggesting the tissue penetration ability of KN035 was better than Durvalumab.

The distribution volume of KN035 in Cynomolgus monkeys after single dose administration was 44.8ml/kg which was similar to that of the plasma volume, suggesting that the KN035 was mainly distributed in the circulating blood. Tissue cross-reactivity assay illustrated that except for thyroid follicular epithelial cell cytoplasm, there were no other organs found with specific cross reactions with KN035. In thyroid follicular epithelial cell cytoplasm, positive staining of KN035 was only found at high concentration of KN035. However, in the 4-week repeat dose toxicology study, the thyroid hormones levels were unaffected. The *in vivo* biological distribution of ^{89}Zr-KN035 in Cynomolgus monkeys also demonstrated that no significant radioactive substances distribution in major organs, except for liver and kidney. Other peer-reviewed research results showed ^{89}Zr labeled antibody drugs have also been observed in the liver and kidney tissues, the specific mechanism is unknown.

3.3 Metabolism

Please refer to Module 2.6.4 Section 5

3.4 Excretion

Please refer to Module 2.6.4 Section 6

4 Toxicology

4.1 Summary

The toxicology studies of KN035 include single-dose toxicity studies 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 cell. In addition, safety pharmacology, local irritation, toxicokinetics, and immunogenicity of KN035 were incorporated into the repeated dose toxicity studies in Cynomolgus monkeys. The studies were listed in Table **3**.

Table 3 **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.

4.2 Systemic Exposure

A toxicokinetic study was included in the 4-week toxicity studies in monkeys (Module 2.6.6 Section 3) to confirm systemic exposure.

4.3 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 15 or 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 was also incorporated in 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 150mg/kg. The MTD of KN035 should be greater than 150mg/kg in the Cynomolgus monkeys.

Please refer to Module 2.6.6 Section 2

4.4 Repeat-Dose Toxicity

A 4-week repeat-dose toxicity study was conducted to evaluate the toxicity of KN035 in Cynomolgus monkeys

Forty Cynomolgus monkeys, weighing 2.3-3.8 kg, 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.

The 4-week 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 these results of this study, the NOAEL of KN035 was determined at150mg/kg/week in Cynomolgus monkeys.

Please refer to Module 2.6.6 Section 3

4.5 Genotoxicity

Please refer to Module 2.6.6 Section 4

4.6 Carcinogenicity

No studies were conducted.

4.7 Reproductive and Developmental Toxicity

No studies were conducted.

4.8 Local Tolerance

No stand-alone local tolerance study was conducted with KN035. However, the injection sites were assessed in the 4-week repeat dose toxicity study conducted in monkeys. Visual observation and histopathological examination reveal no abnormal changes at or around the injection sites.

Please refer to Module 2.6.6 Section 7

4.9 Other Toxicity Studies

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. Please refer to Module 2.6.6 Section 8.1.

Hemolysis and local irritation: KN035 (49.2mg/ml, prepared in saline) were tested for hemolysis and coagulation in rabbit blood cell. No hemolysis and coagulation were observed. The results of histopathological examination showed that there was no abnormal change at or around the injection sites after the 4-week toxicity study in Cynomolgus monkeys. Please refer to Module 2.6.6 Section 8.2.

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 doses 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. Please refer to Module 2.6.6 Section 8.3.

Immuno toxicity: The 4-week toxicity study results suggested no treatment-related abnormal changes in white blood cells, CD4+/ CD8+ T cells, immune organs, and tissues. KN035 has no immuno toxicity in Cynomolgus monkeys. Please refer to Module 2.6.6 Section 8.4.

5 Integrated Overview and Conclusions

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 either single-dose or multiple 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 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 doseand 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.