2.6.4. PHARMACOKINETICS WRITTEN SUMMARY

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1 Brief Summary

The pharmacokinetics studies of KN035 were mainly included the studies of the absorption and distribution of KN035 in rodent animals and non-human primates (NHPs). 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 drug administration were subcutaneous and intravenous injections. Single and repeat doses were given to the animals in the studies. In the meantime, the bioavailability of KN035 in Cynomolgus monkeys was also investigated during this study. The distribution study of KN035 was conducted in tumor-bearing mice and Cynomolgus monkeys (See Module 2.6.5 section 5), respectively, through intravenous injection of ^{89}Zr (T1/2: 78.4h) labeled KN035.The real time distribution of drugs was monitored.

Following the guidelines for the safety evaluation of biological products, the metabolism and excretion 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*.

2 Methods of Analysis (See Module 2.6.5 section 2)

2.1 Serum Drug Detection Methods (2015006 and 2016009)

Ninety-six well ELISA plates were coated with human PD-L1. The test serum samples were added. HRP-labeled goat anti-human Fc antibody was used for detection. After development with TMB, the OD value was read, and KN035 concentrations were determined on a standard curve obtained by plotting OD versus concentration using a four-parameter logistic curve-fitting program.

The measurements of the drug concentrations in the serum were performed with the validated ELISA protocol created by the National Center for Safety Evaluation of Drugs, except for those in dose range finding studies. The precise measurement ranges of the method are 0.75-50ng/ml. The drugs in the serum of the test animals were verified to be stable for up to 6 hours under room temperature, 6 hours at2-8℃, and 251 days under -70℃ with 3 freeze-thaw cycles.

2.2 Detection Methods for Serum Anti-drug Antibody (ADA)

Bridging ELISA was used in detection of the anti-drug antibody (ADA) in Cynomolgus monkey serums. The procedures were the following: 1) coat a 96-well ELISA plate with KN035;2) add the test serum;3) add Biotin-labeled KN035; develop color with HRP-labeled streptavidin and media reagent; 4) after color has fully developed, read OD absorbance. When $OD_{\text{after KN035applied}}/OD_{\text{before KN035applied}}>1.5$, the ADA was determined as positive. Otherwise, it was considered to be negative.

3 Absorption

The absorption studies of KN035were conducted in Cynomolgus monkeys and Sprague Dawley Rats. After single and repeat administrations of KN035 to animals, the serums were detected for the concentration of the drug. The pharmacokinetic parameters of KN035 were calculated based on the drug concentrations in serums.

3.1 Single-Dose Pharmacokinetic Study in Sprague Dawley Rats (RDR-KN035-PD-2014-015)

The purpose of this study was to explore the pharmacokinetic profiles of KN035 in Sprague Dawley rats after a single intraperitoneal injection.

In this study, 10 Sprague Dawley 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 method was used to quantitate the concentrations of KN035 in the serum of the collected blood samples. The mean drug concentration-time curve was shown in Figure 1.For more detailed data refer to Module 2.6.5 section 3.

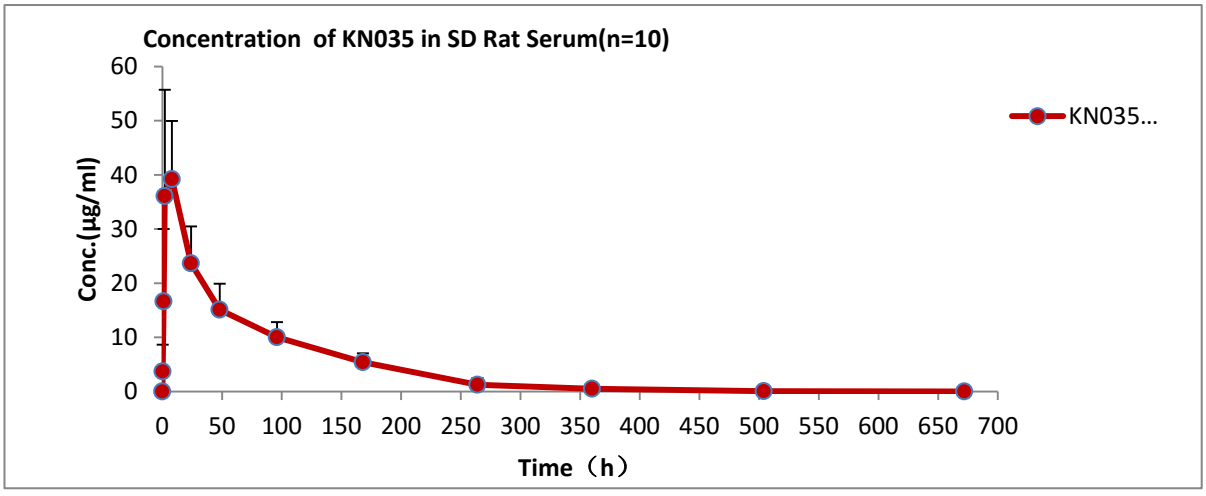

Figure 1 Average drug-time curve of KN035 in the serum of SD ratsafter 10mg/kg IP administration（n=10）

The half-life of KN035 was72.02±26.82h in SD rats after a single i.p. dose at 10mg/kg. The C_{max} levels were 43.20±15.25ug/ml， and the AUC_{inf} was 3.04±0.73 h*mg/ml. Both the C_{max} and AUC_{inf} were no different between male and female animals in the experiment.

3.2 Single-Dose Pharmacokinetic Study in Cynomolgus Monkeys (RDR-KN035-PD-2015-011)

This study was conducted as dose range finding pharmacokinetics study. The aim of this study was to explore the pharmacokinetic profiles of KN035 in Cynomolgus monkeys after a single subcutaneous or intravenous administration.

Six Cynomolgus monkeys, with the weight from3.16to 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 doseofKN035 at 15mg/kg by intravenous injection (IV). For SC groups, blood samples were collected at 0, 1, 4, 8, 12, and 24 hours post dosing, and then at 2, 3, 5, 7, 10, 14, 17, 24, and 32dayspostdosing. For IV group, blood samples were collected at 0, 0.25, 2, 8, and 24 hours post dosing, and then at 3, 7, 10, 14, 17, 24, and 32dayspostdosing. Serum drug concentrations at different time points were shown in Figure 2 and Figure 3.For more detailed data refer to Module 2.6.5 section 3.

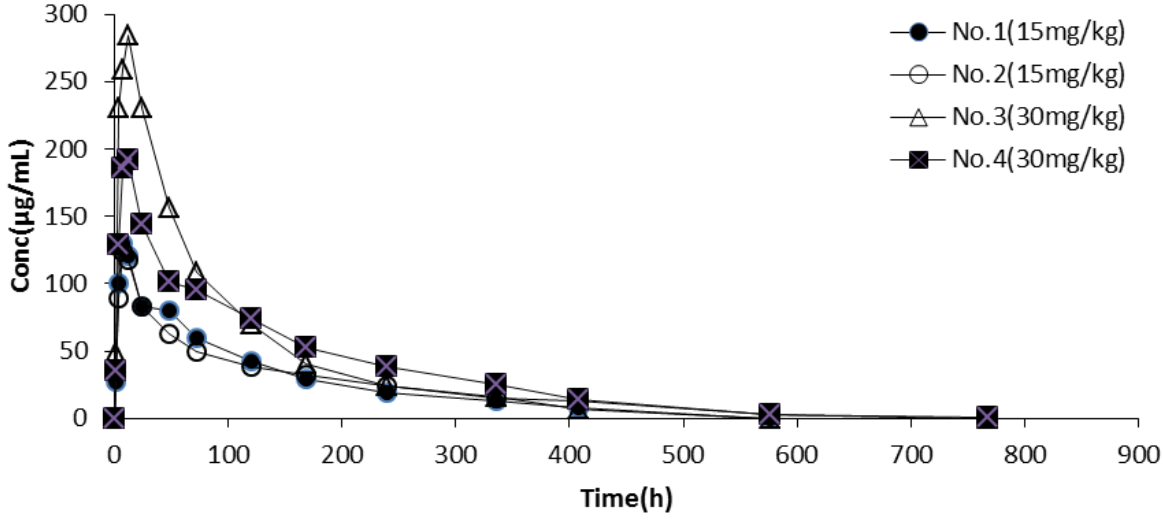

Figure 2 Concentration-time Curve of KN035 in Cynomolgus Monkey Serum (subcutaneous injection).

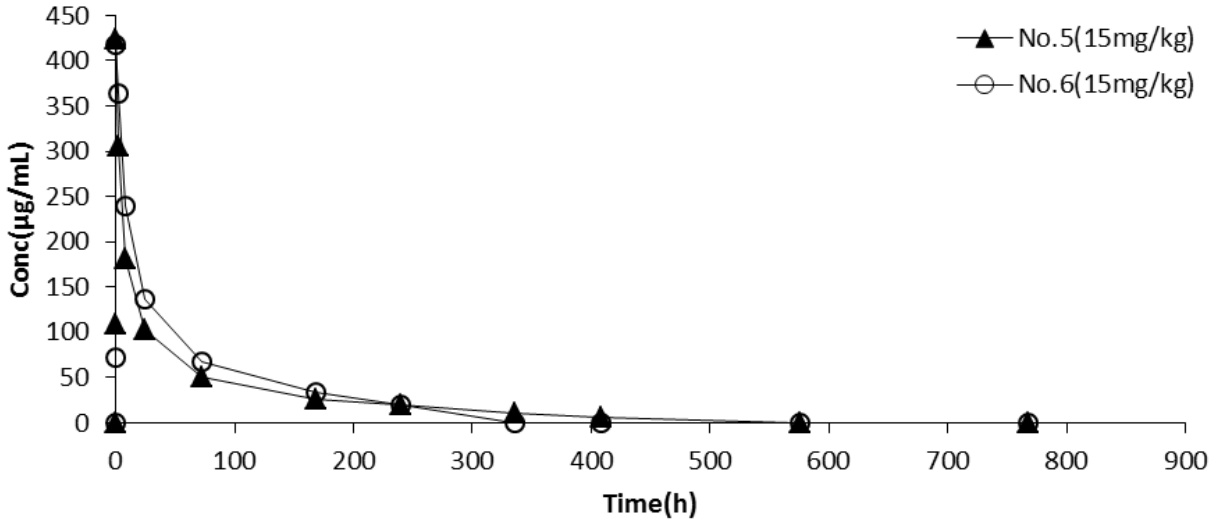

Figure 3 Concentration-time Curve of KN035 in Cynomolgus Monkey Serum (intravenous injection)

The half-life of KN035 was 29.75-82.86h in Cynomolgus monkeys after a single subcutaneous dose at 15 and 30mg/kg. The C_{max} levels were increased with dose increments.

3.3 Single-Dose Pharmacokinetic Study in Cynomolgus Monkeys (N2015042)

The aim of this study was to assess pharmacokinetic parameters following a single-dose application of KN035 and to evaluate the bioavailability following a single subcutaneous injection of the drug.

Twenty-four Cynomolgus monkeys, weighing at 2-5kg, were randomly assigned into four groups (3/gender/group). Animals in Groups 1-3 received single subcutaneous dose of KN035 at 5, 15, and 50mg/kg, respectively. Animals in Group 4 received singleintravenousdoseofKN035 at 15mg/kg. Blood samples of Group 1-3 were collected at 10-60 min before administration, and then at 1, 4, 8, 12, 24, and 48 hours post-injection, and then at 3, 5, 7, 9, 12, 15, 17, 23, 28, 32, and 37dayspost-injection. For Group 4, blood samples were collected at 10-60min before dosing, and 5 min, 30min, 1h, 2h, 5h, 8h, 12h, 24h, 48h, 3d, 5d, 7d, 9d, 12d, 15d, 17d, 23d, 28d, 32d, and 37dpost-dosing. Validated ELISA was used for the analysis of plasma drug concentration. The test limit of this method was 0.75ng/ml.

Plasma drug concentrations of KN035 were shown in Figure 4. For more detailed data, refer to Module 2.6.5 section 3.

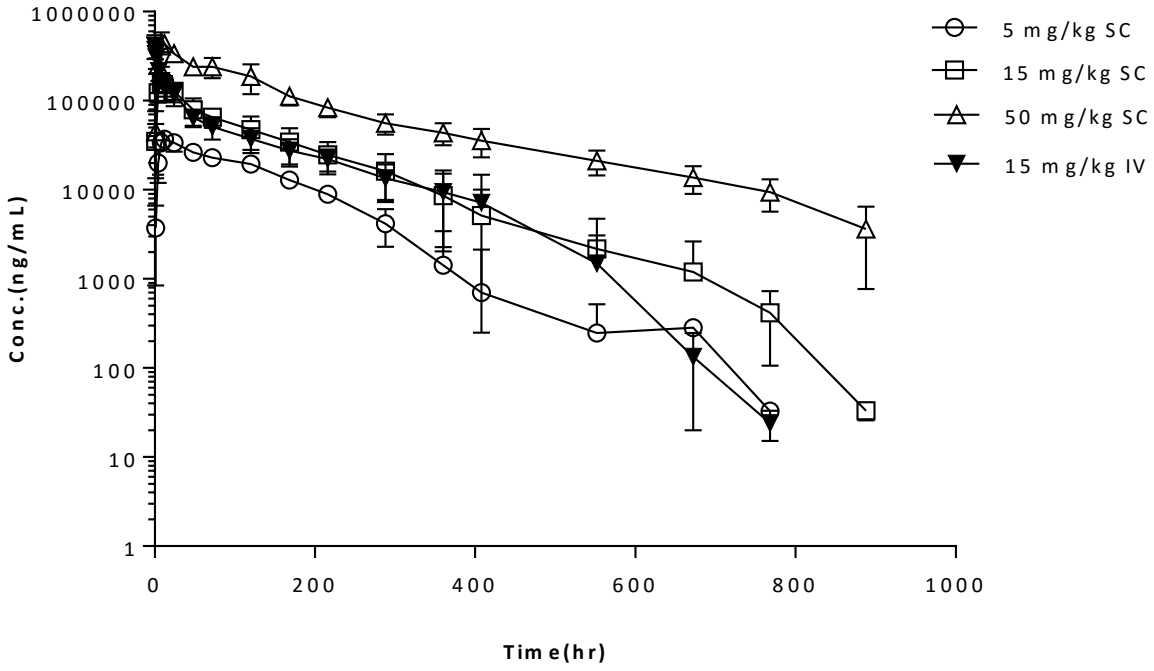

Figure 4 Concentration-time Curve of KN035 in Cynomolgus Monkey Serum (Mean±SD, n=6)

Following a single subcutaneous dose at 5, 15, and 50mg/kg, the C_{max} and $AUC_{(0-t)}$ levels of KN035 increased with dose increments in Cynomolgus monkeys. When the dose ratio was 1:3:10, the averaged C_{max} ratio was 1:4.13:12.53, and the averaged $AUC_{(0-t)}$ ratio was 1:3.24:12.01, correspondingly. The distribution and metabolism of KN035 in Cynomolgus monkeys exhibited a typical linear dynamic characteristic and without differences between males and females. The absolute bioavailability was 104.47% by subcutaneous injection of KN035 at single-dose of 15mg/kg. Following a single dose of 5, 15, and 50mg/kg by subcutaneous administration, the half-life of KN035 were 31.30±24.85h, 26.48±13.59h, and 155.81±29.33h, respectively.

3.4 Repeat-Dose Pharmacokinetic Study in Cynomolgus Monkeys (2015033-2)

The aim of this study was to investigate the pharmacokinetic profiles of KN035 after the first and 5th dose in Cynomolgus monkeys.

Forty Cynomolgus monkeys, weight of 2.3-3.8kg, were randomly assigned into four groups (5/gender/group). The animals received repeated doses of KN035 by subcutaneous injection at 0(control), 5, 30, 150 mg/kg/week for four weeks, and five injections in total. The animals recovered for 4 weeks. After the first injection, blood samples were collected at 0, 2, 6, 24, 48, and 96hours post-administration. After other injections, blood samples were collected at 0, 2, 6,

24, 48, 96 hours post dosing, and then at 7, 10, 14, and 28days post dosing. The validated ELISA method was adopted for drug quantitation in serum.

Plasma drug concentrations of KN035 in different dosing groups were shown in Figure 5 and Figure 6. For more detailed data refer to Module 2.6.5 section 4.

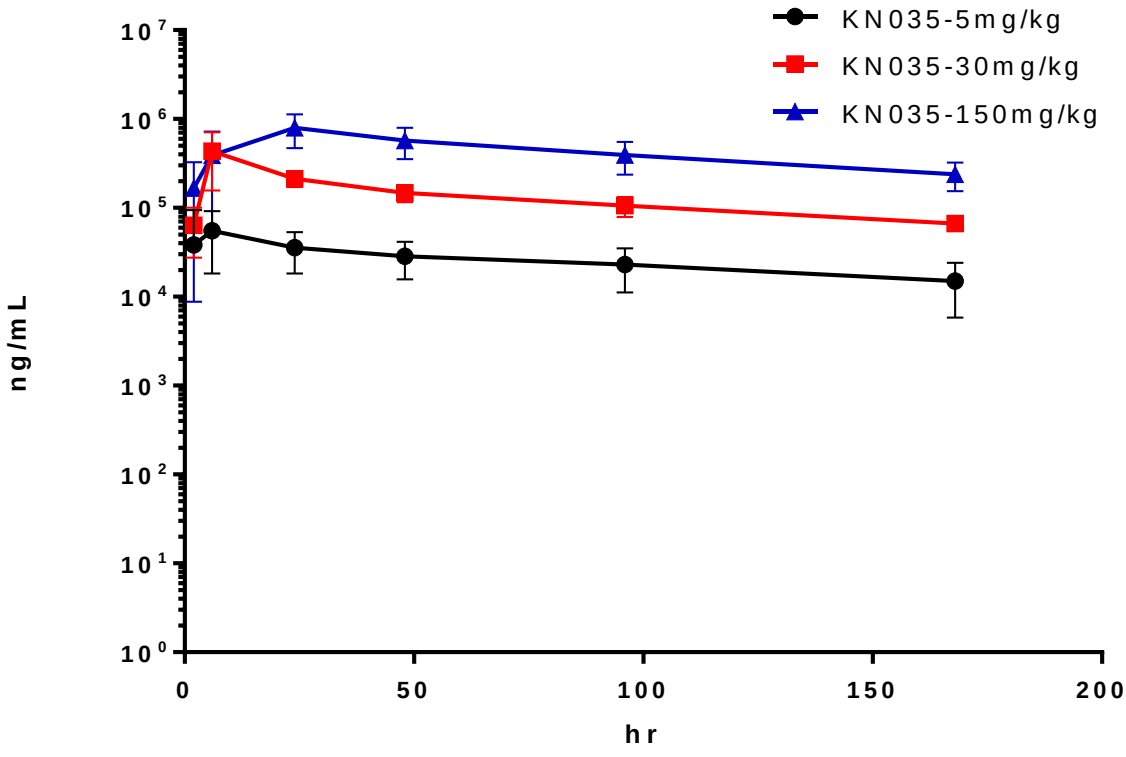

Figure 5 Average KN035 Serum Concentration-time Profiles –the 1st Dose (Mean ±SD, n=10)

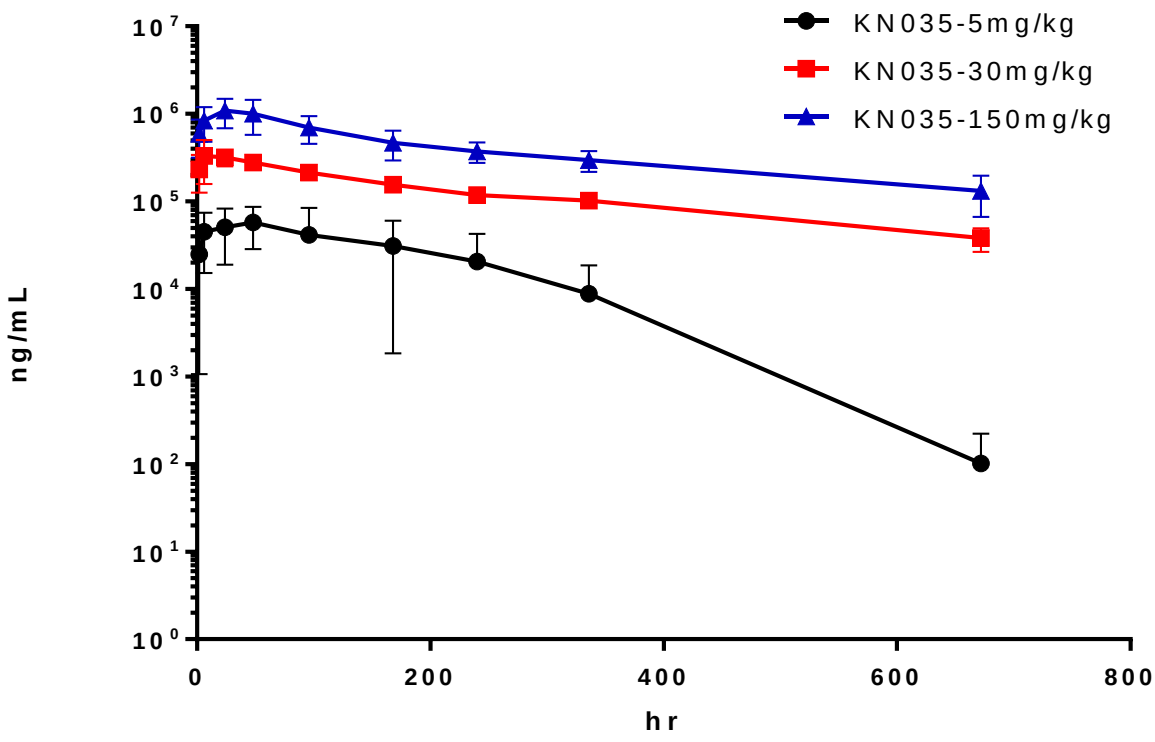

Figure 6 Average KN035 Serum Concentration-time Profiles – the 5th Dose (Mean ±SD, n=10)

Following the first dose, the mean levels of C_{max} and AUC $_{(0-t)}$were increased with dose elevation.

In the low, medium, and high-dose males, the C_{max} ratio was 1:8.42:6.42; the $AUC_{(0-t)}$ ratio was 1:4.88:8.60; after the last dose, the ratio of C_{max} and $AUC_{(0-t)}$in the low, medium, and high dose groups were 1:5.29:12.40 and 1:4.29:9.61,respectively. Between the first and last dose, the ratios of C_{max} and AUC $_{(0-t)}$ were 0.82, 0.52, 1.59 and 1.65, 1.45, 1.85, respectively. There was no significant accumulation of KN035 in male animals.

In the low, medium and high dose females, the C_{max} ratio after the first dose was 1:6.63:33.97; the $AUC_{(0-t)}$ ratio was 1:6.07:31.20; after the last dose, the ratios of the average C_{max} and $AUC_{(0-t)}$were 1:6.39:27.14 and 1:6.63:28.74, respectively. Between the first and last dose, the ratios of C_{max} and AUC $_{(0-t)}$ were 1.58, 1.52, 1.26 and 1.82, 1.99, 1.67, respectively. Also, there was no significant accumulation of KN035 in female animals.

4 Distribution

^{89}Zr labeled DFO conjugated KN035 (^{89}Zr-KN035) was used for the distribution studies. The procedures were the following: 1) inject ^{89}Zr-KN035 into tumor-bearing mice through IV; 2) apply MicroPET scan to identify and analyze the target orientation and specificity of KN035 to tumor tissue and its distribution in main organs of test animals (See Module 2.6.5 section 5.1).

In the meantime, the labeled KN035 was intravenously injected into Cynomolgus monkeys, and the live imaging protocol was used to analyze the distribution of KN035 in live animals. These steps offered real time distribution data for clinical reverences (See Module 2.6.5 section 5.2).

4.1 ^{89}Zr-KN035 Tumor Targeting and Biological Distribution in Tumor-Bearing Mice (MIRT0005-2)

The aim of this study was to investigate the *in vivo* bio-distributions of ^{89}Zr-KN035 and ^{89}Zr-2.41H9OP (Durvalumab, AZ/Medimmune) in melanoma xenografted mouse model following intravenous injection.

A375-hPD-L1 was inoculated into NOD/SCID mouse subcutaneously. When the tumor size was bigger than 100mm^3, ^{89}Zr-KN035 (10.0mg/kg) was injected through the tail vein into the transformed mice. At 1, 3, 6, 12, 24, 52， 97,and169hours post-injection, whole body MicroPET scan was conducted for the animals. PMOD software was used to analyze scan data and calculate the uptake %ID/g values of the radioactive materials in each ROI (region of interest). The tumor, heart, liver, kidney, brain, and other organs were considered as ROI, and the distributions of ^{89}Zr-labeled Durvalumab (^{89}Zr-2.41H9OP) were investigated also side by side with KN035 for comparison. The injection amount of Durvalumab was 18.4 mg/kg, same mole amount as KN035.

The results showed that following the injection of ^{89}Zr-KN035 and ^{89}Zr-2.41H9OP, the uptake of radioactive by tumor increased. At all measured time points between 1 to 52 hours, the radioactive signals were higher in KN035 group than in Durvalumab group; it showed significant difference between 1 to 2.5hours.

In the liver tissue, the radioactivity decreased overtime, and the radioactive levels in the ^{89}Zr-2.41H9OP group were greater than that in the ^{89}Zr-KN035 group. There were no significant changes of radioactive uptakes in low leg bone area within 6 hours post dose. After 12 hours post dose, the drug absorption in bone tissue increased, and reached the peak at 97 hours post dosing. Drug absorption in the kidney reached the peak at 2.5 hours post-injection, and then decreased. In brain tissues, the drug absorption in both test groups decreased following the injection. The results are shown in Figure 7and Figure 8 respectively.

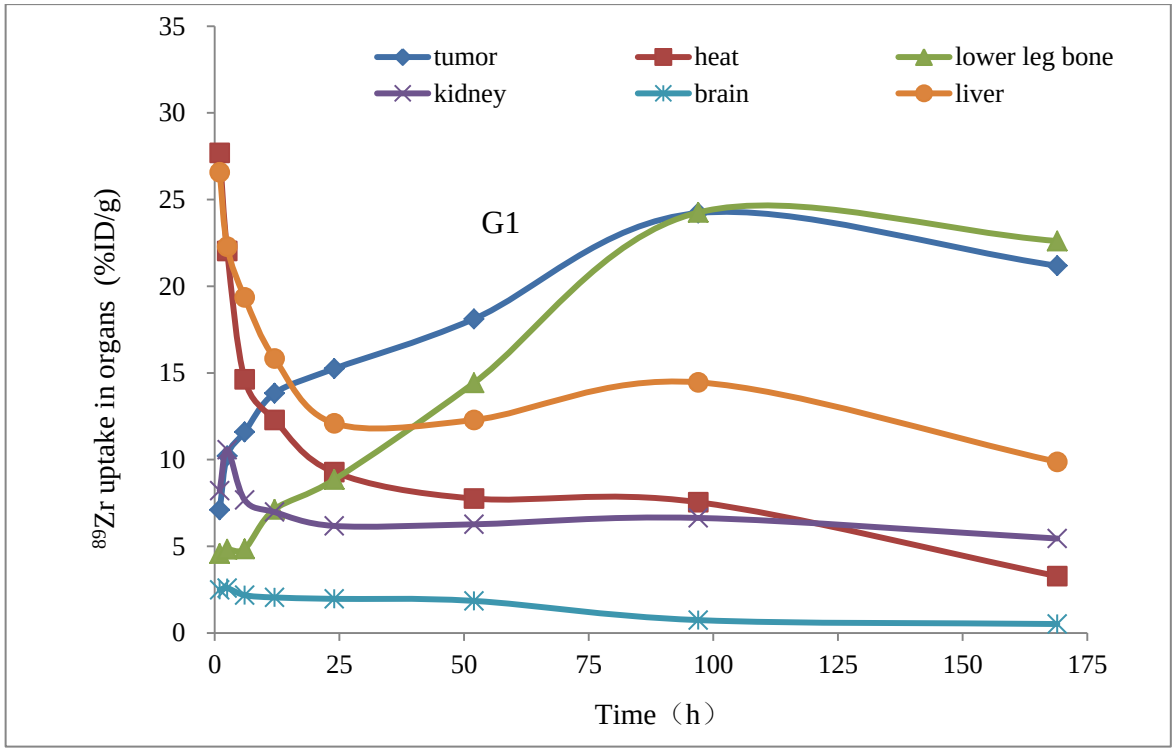

Figure 7 Variation of ^{89}Zr uptake in Tumor and Organs of Mice in Group 1with Time

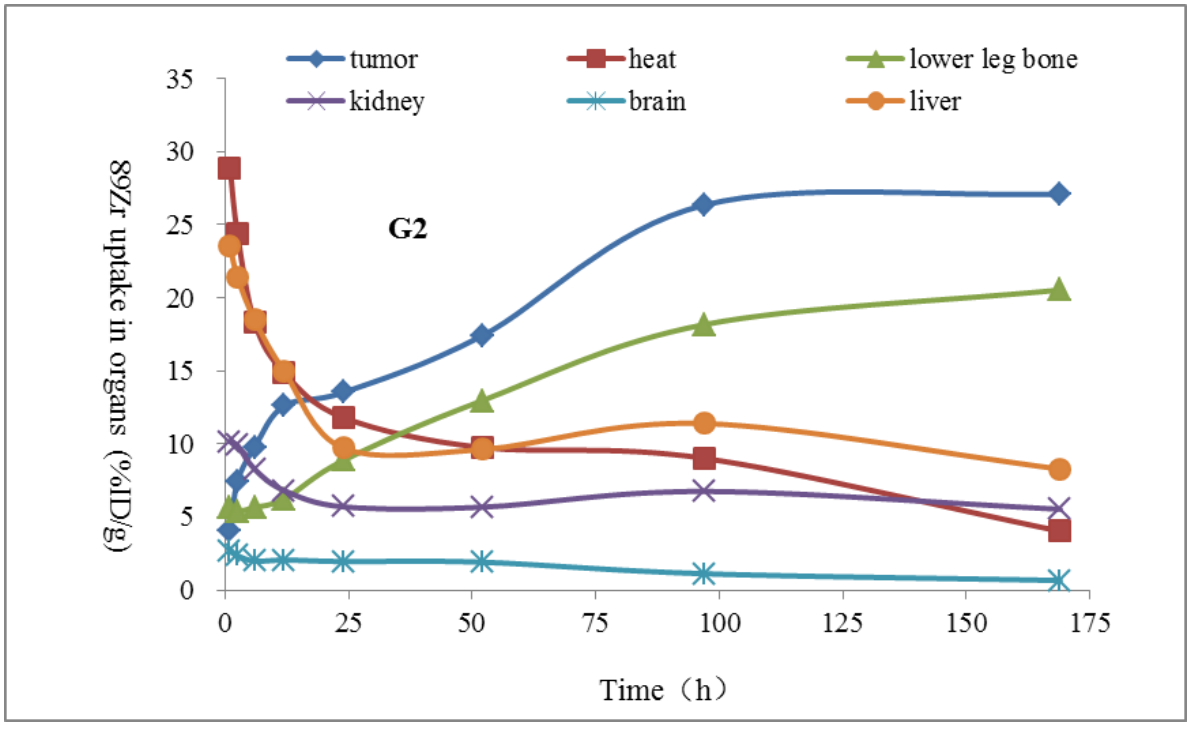

Figure 8 Variation of ^{89}Zr uptake in Tumor and Organs of Mice in Group 2 with Time

4.2 Bio-Distributions of ^{89}Zr-KN035 in Cynomolgus Monkeys (MIRT0005-1)

The aim of this study was to investigate the *in vivo* bio-distributions of ^{89}Zr-KN035 in Cynomolgus monkeys post a single dose intravenous injection.

Three Cynomolgus monkeys were injected with ^{89}Zr-KN035 1.5mCi（0.3～0.5mg/kg）through the femoral vein, and then operated with whole body PET/CT scan. The blood samples were collected after 2, 26, 49, 72, 122 and 170 hours post-dosing. The radioactive uptake SUV value was calculated through the PET/CT scan results and the analysis results of blood samples. The tissues listed in Figure 9, Figure 10, and Figure 11were considered as region of interest (ROI) and investigated extensively. Following intravenous injection of ^{89}Zr-KN035, radioactivity levels were relatively higher in the heart, liver, and kidney; radioactivity was observed in the lung tissues only in a very short period after the injection, and then went back to background level within one day. There was no radioactivity detected in the stomach, upper limb bone, brain, and muscle organs. The radioactivity in the heart reached the peak within 2hours post-injection.

In the liver tissue, the uptake of SUV between 2 to 49 hours was gradually reduced from 1.48 ± 0.16 to 1.08 ±0.18. In the kidney, after 72h post injection, the radioactivity uptake of SUV reached to its peak value of 2.13 ±0.10, and then decreased.

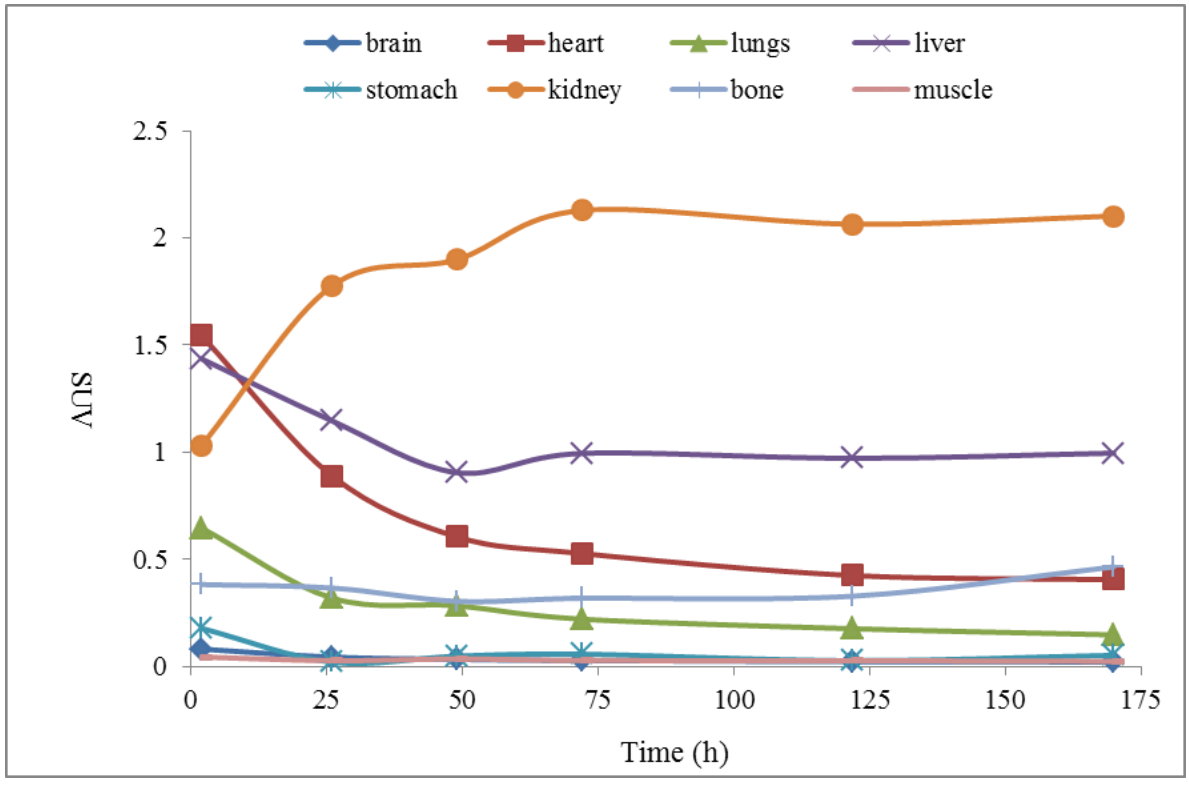

Figure 9 Variation of SUV of Different Organs of Cynomolgus monkeys (G1M01) with Time

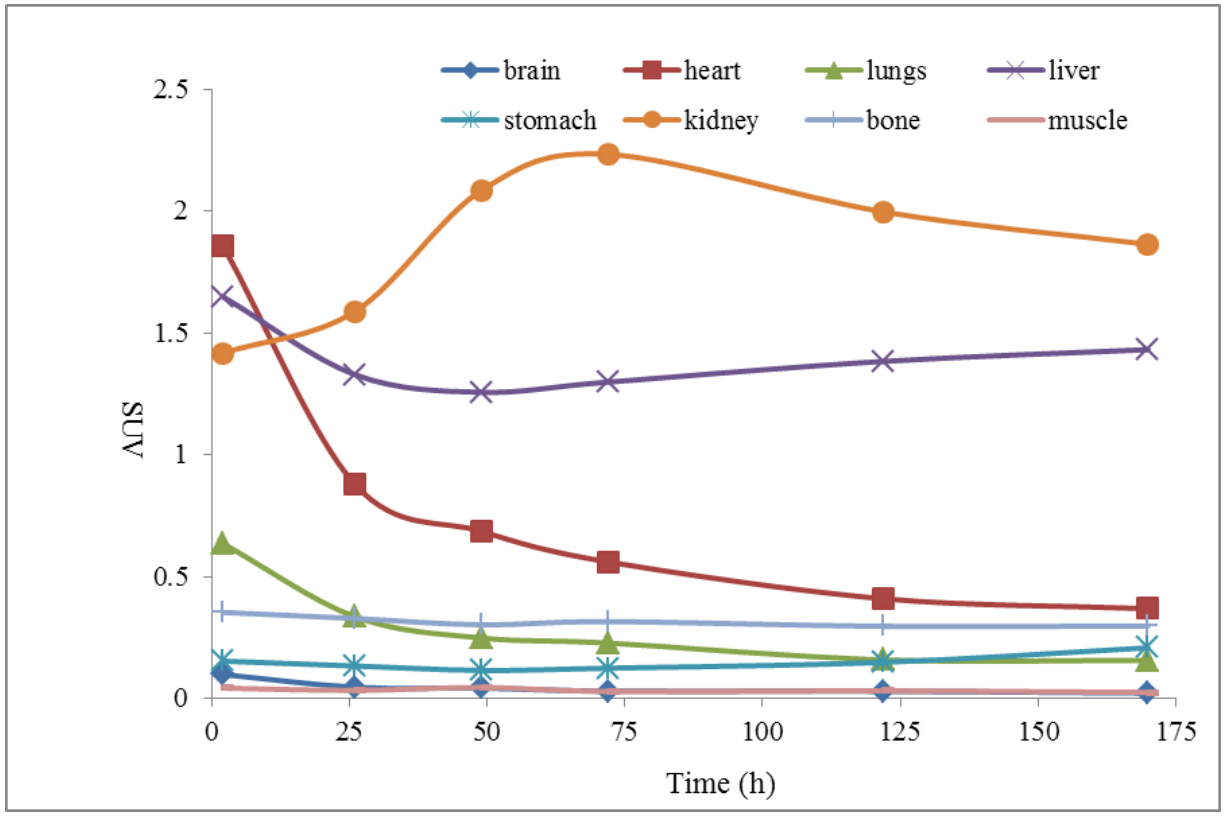

Figure 10 Variation of SUV of Different Organs of Cynomolgus monkeys (G2F01) with Time

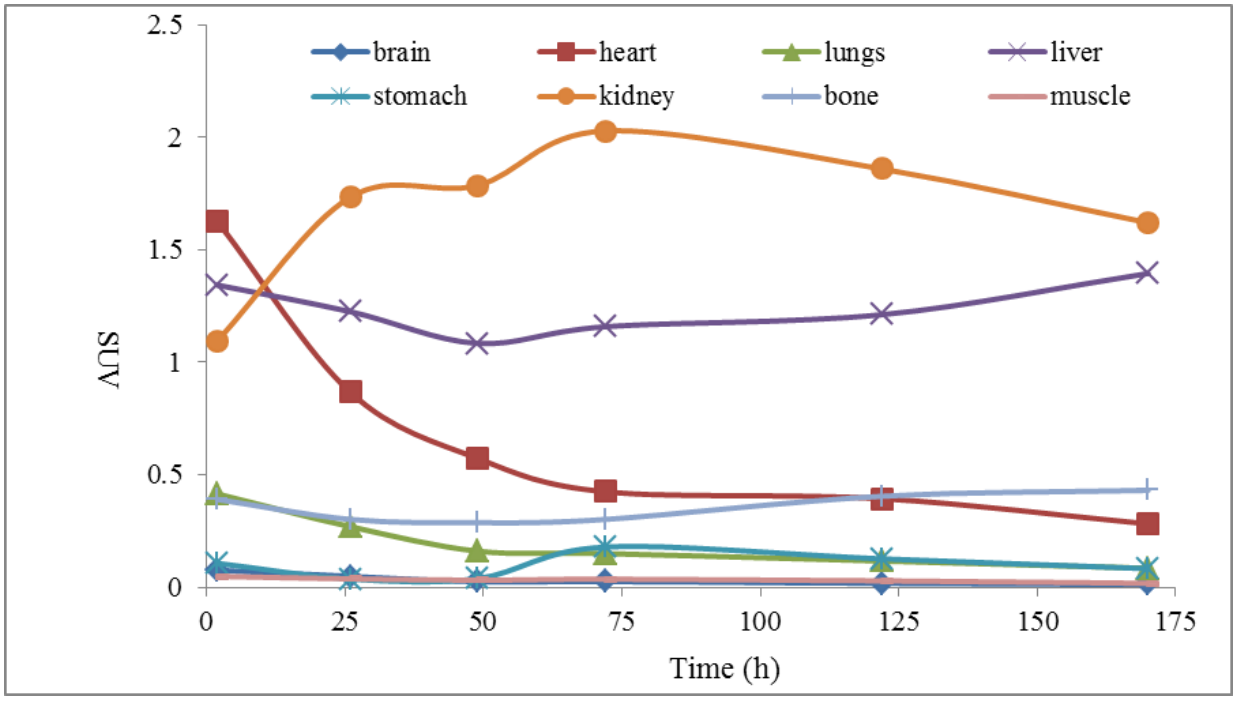

Figure 11 Variation of SUV of Different Organs of Cynomolgus monkeys (G2M02) with Time

5 Metabolism

Metabolism of KN035 was not evaluated. KN035 is a fusion Fc 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*.

6 Excretion

The excretion of KN035 was not measured. KN035 is a fusion Fc 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*.

7 Pharmacokinetic Drug Interactions

No studies conducted.

8 Other Pharmacokinetic Studies

No studies conducted.

9 Discussion and Conclusions

In the dose range of 5-50 mg/kg, the C_{max} and AUC $_{(0-t)}$ of KN035 increased with the dose. There were no significant differences between male and female animals in the main pharmacokinetic parameters. The absolute bioavailability of a single subcutaneous administration of 15mg/kg was 104.47%. In dose range of 5-150mg/kg with 5 dose administrations by one dose per week, there was no significant accumulation of KN035 in both male and female animals.

Following a single subcutaneous administration of KN035, the half-life of KN035 either in the low dose group ($t_{1/2}$=31.30±24.85h) or the middle dose group ($t_{1/2}$=26.48 + 13.59h) was significantly lower than that in the high dose group ($t_{1/2}$=155.81 + 29.33h), this may be related to the level of productions of anti-drug antibodies (ADA) in test animals.

The distributions of ^{89}Zr-KN035 in hPD-L1 positive tumor-bearing mice indicated that the target orientation of KN035 was specific. It demonstrated that the %ID/g of KN035 was even bigger than that of Durvalumab at 1 and 2.5 hours post-dosing. This suggested that the tissue penetration ability of KN035 was stronger 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. This suggested 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.Meanwhile, in the 4-week repeat dose toxicology study, the thyroid hormones levels were unaffected. Thus, this finding might be caused by none-specific stains. The *in vivo* biological distribution of ^{89}Zr-KN035 in living body of 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.