2.6.2 PHARMACOLOGY WRITTEN SUMMARY

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1 Brief Summary

KN035 is a novel Programmed death-ligand 1(PD-L1) antagonist. In a non-clinical development program, pharmacology studies were conducted to study the *in vitro* binding activity and affinity of KN035 to targets and *in vivo* anti-tumor activities. *In vitro* studies include binding of KN035 to human, Cynomolgus monkeys, and murinePD-L1, and other checkpoint proteins, such as B7/CD28 family (PD-L2, hB7H4, hCD28, hB7H3, hICOS). 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 the Fc region of KN035 toFcγRs and FcRn receptors was tested, respectively. The antibody-dependent cell-mediated cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC) activities have also been studied. The *in vivo* studies include the anti-tumor activity of KN035 in xenografted animal models generated by transforming mixtures of A375/PD-L1 and human PBMCs. In these studies, Durvalumab (an anti-PD-L1 antibody 2.41H90P, under development by AZ/Medimmune) was used as the control. Noteworthy findings were listed in the Table 1.

2 Primary Pharmacodynamics

Table 1 Summary of Primary Pharmacology of KN035

Study Number	Study Title	Test System	Noteworthy Findings
RDR-KN035-QC-2016-010	*In Vitro* 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	*In Vitro* Binding Affinity of KN035 and Durvalumab to hPD-L1	ELISA	The binding affinities of KN035 to hPD-L1-muFc were 1.5 times higher than that of Durvalumab.
RDR-KN035-PD-2015-049	In Vitro Binding of KN035 to PD-L1 on cell surface	Flow cytometry	The $EC_{50}$of KN035 binding to A375/hPD-L1 was0.68ug/ml; KN035 does not bind to PD-L2, B7H4, CD28, B7H3, ICOS; KN035 binds to PHA activated monkey PBMCs; KN035does not bind to mouse PD-L1.
RDR-KN035-QC-2015-018	ELISA Binding Assay 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 cytometry	KN035 blocks PD-1-muFc and 293T- hPD-L1 binding, IC_{50}: 0.25 ug/ml; KN035blocks PD-L1-muFc and Jurkat-hPD-1binding,

Study Number	Study Title	Test System	Noteworthy Findings
			IC_{50}: 13.42ug/ml.
RDR-KN035-QC-2016-006	Test Report of PD-L1/CD80 Blockade by KN035	ELISA	KN035 blocks PD-L1/CD80binding, IC_{50}: 102.7ng/ml.
RDR-KN035-QC-2015-016	Binding affinity of KN035 to Fcγ Receptors	ELISA	Binding affinity of KN035to FcγRIIa and FcγRIIIa ismuch lower than that of wtFc.
RDR-KN035-PD-2015-057	Binding activity of KN035 to U937 (FcγRI)	Flow cytometry	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 KN035torhFcRnwas (4.93±0.81)E-07M. The KD of KN015(wild-type Fc) is 5.50E-07M.
RDR-KN035-PD-2015-050	ADCC activity assay of KN035	Raji-PD-L1/human PBMC	No ADCC activity
RDR-KN035-PD-2015-051	CDC activity assay of KN035	Raji-PD-L1	No CDC activity
DF-YX-KN01	KN035 MLR Assay	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.
RDR-KN035-PD-2016-006	Activating effect of KN035 on T cells in Jurkat T/Raji-PD-L1co-culture system	Co-culture 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 KN035is stronger than that of Durvalumab.
RDR-KN035-PD-2015-015	Anti-tumor efficacy of KN035on 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/humanPBMCat 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 2.41H90P in NOD-SCID xenograft model of A375-hPD-L1/human PBMC		At dose of 1mg/kg, KN035 showed similar anti-tumor efficacy compared with Durvalumab. While the anti-tumor efficacy of KN035 was much stronger than that of Durvalumab at 0.3mg/kg and

Study Number	Study Title	Test System	Noteworthy Findings
			0.1mg/kg.

2.1 *In Vitro* Pharmacology

2.1.1 *In Vitro* Binding AffinitytoPDL1-Chis (RDR-KN035-QC-2016-010)

The aim of this study was to assess binding affinity of KN035 to PDL1-Chis on Fortebio'sOctet K2 platform.KN035was diluted and prepare dosing a biosensor with a concentration of 10ug/ml. PD-L1-Chis solution was diluted and prepared with a concentration of 50nM, 25nM, 12.5nM, 6.25nM, and 3.125nM respectively. After incubation, disassociation, recovery, and neutralization procedures, Kinetics Constants (KD) of binding was measured. The average KD value of KN035 from three different lots binding tohPD-L1 were determined at (2.86±0.23) E-09M. The results are shown in Table 2.

Table 2 In Vitro Binding Affinity of KN035 to PDL1-Chis

Samples	KD (M)	kdis (1/s)	kon (1/Ms)
141219DS	3.05E-09	6.74E-04	2.21E+05
141230DS	2.93E-09	6.65E-04	2.27E+05
150107DS	2.60E-09	6.64E-04	2.56E+05

2.1.2 *In Vitro* Binding Affinities of KN035and Durvalumab to PD-L1-muFc (RDR-KN035-QC-2015-017)

The aim of this study was to study and compare binding affinities of KN035 and Durvalumab to PD-L1-muFcby ELISA assay. A ninety-six well plate was coated with PD-L1-muFc (mouse Fc).Various concentrations ofKN035 or Durvalumab was added. HRP labeled sheep anti-human Fc antibody was used for detecting of the detained KN035 or Durvalumab. After being developed by TMB, the plate was read at a wavelength of450/650nm in the microplate reader.

KN035 DS/DP of different batches all have concentration-dependent bindings to hPD-L1 with EC50 of 56.570～68.692ng/mL. The EC_{50}valuesof KN035 and Durvalumab to PD-L1 were determined at 62.275 ng/ml and 90.082 ng/ml, respectively. This result suggested that the binding affinity of KN035 to PD-L1 was 1.45 times higher than that of Durvalumab. The binding curve of KN035 and Durvalumab is shown in Figure 1.

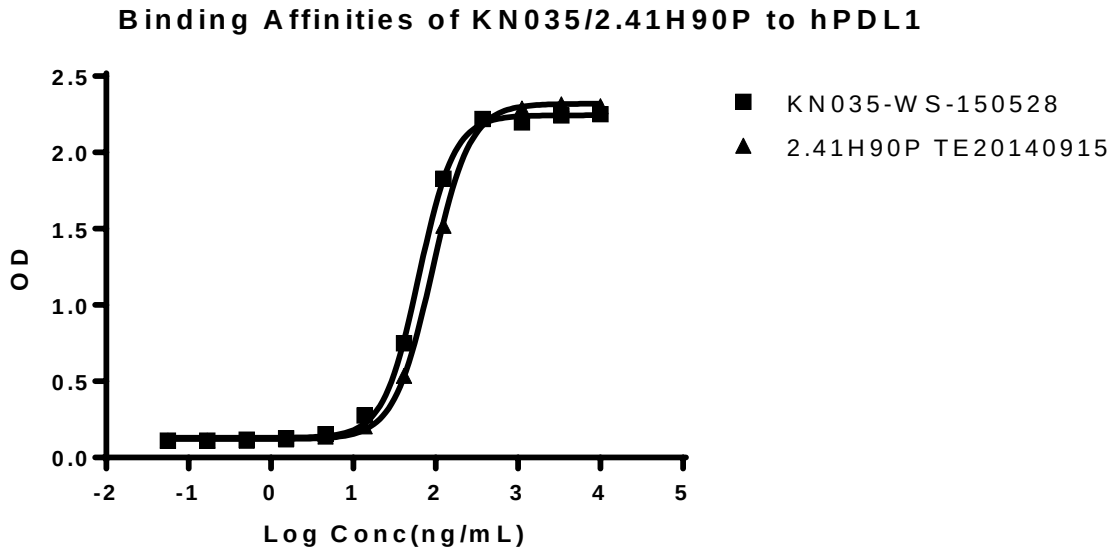


Figure 1 The Binding Curve of hPD-L1 with KN035 and Durvalumab

2.1.3 *In Vitro* Binding of KN035 toA375-hPDL1 Cell (RDR-KN035-PD-2015-049)

The aim of this study was to assess the binding activity of KN035 to A375/hPD-L1cells by flow cytometry. KN035 was diluted and prepared at concentration levels of 0, 0.0014, 0.04, 0.012, 0.037, 0.111, 0.333, 1, and 3μg/ml. A375-hPDL1 cell density was adjusted to 5×10^6cells/mL. After the binding and washing procedures, samples were loaded to flow cytometry in order to measure the Median Fluorescence Intensity (MFI).

Study results (Figure 2) demonstrated thatKN035 bound toA375-hPD-L1 in a concentration-dependent manner with anEC_{50}valueof 0.68ug/ml.

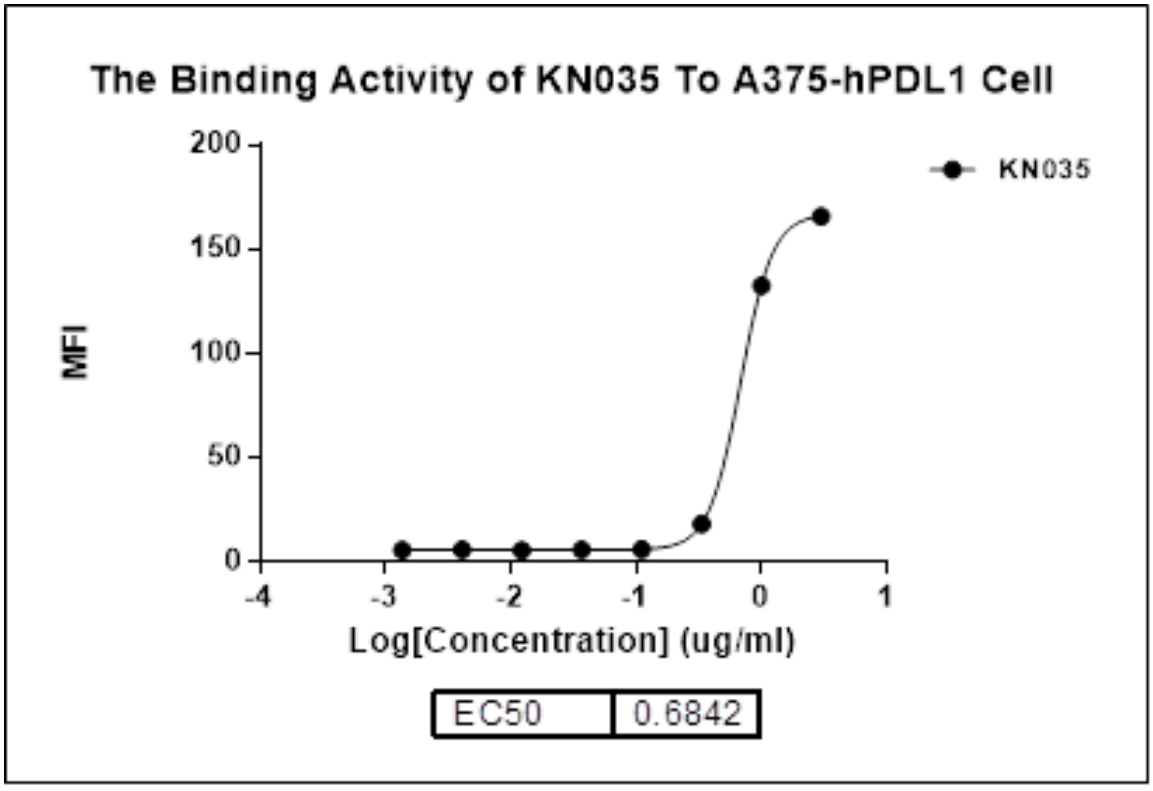


Figure 2 In Vitro Binding of KN035 to A375-hPDL1 Cell

2.1.4 *In Vitro* Binding of KN035toPeripheral Blood Mononuclear Cell (PBMC) from Cynomolgus Monkey (RDR-KN035-PD-2015-049)

The aim of this study was to evaluate the binding activity of KN035 to activated monkey PBMCs using flow cytometry. KN035 was prepared at a concentration of 10μg/ml. Monkey PBMCs was prepared in a 5μg/mL PHA+10%FBS+RPMI-1640 buffer. After the binding and washing procedures, samples were loaded to flow cytometry to measure the Median Fluorescence Intensity (MFI).

The study results (Figure 3) demonstrated that the binding activity of KN035 to PHA-activated monkey PBMCs was significantly stronger than that of non-activated PBMCs.

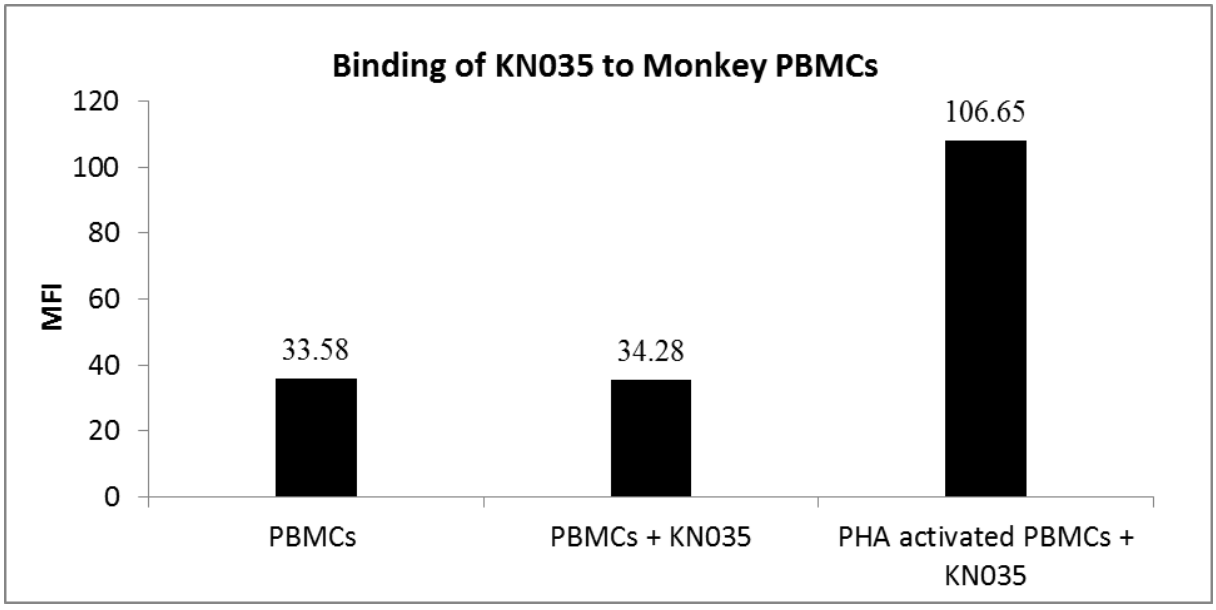


Figure 3 *In Vitro* Binding Activity of KN035 toCynomolgus Monkey PBMCs

2.1.5 *In Vitro* Binding of KN035to Mouse PD-L1 (RDR-KN035-PD-2015-049)

The aim of this study was to assess the binding of KN035 to mouse PD-L1 (mPD-L1) cells by flow cytometry. KN035 was prepared using a concentration of 10μg/ml. The mouse PD-L1gene was used to transform 293T cells to create 293T-mPDL1 cells.After binding and washing, samples were loaded to flow cytometry for analysis. Study results shown in Figure 4 demonstrated that there was no detectable binding of KN035 to mouse PD-L1.

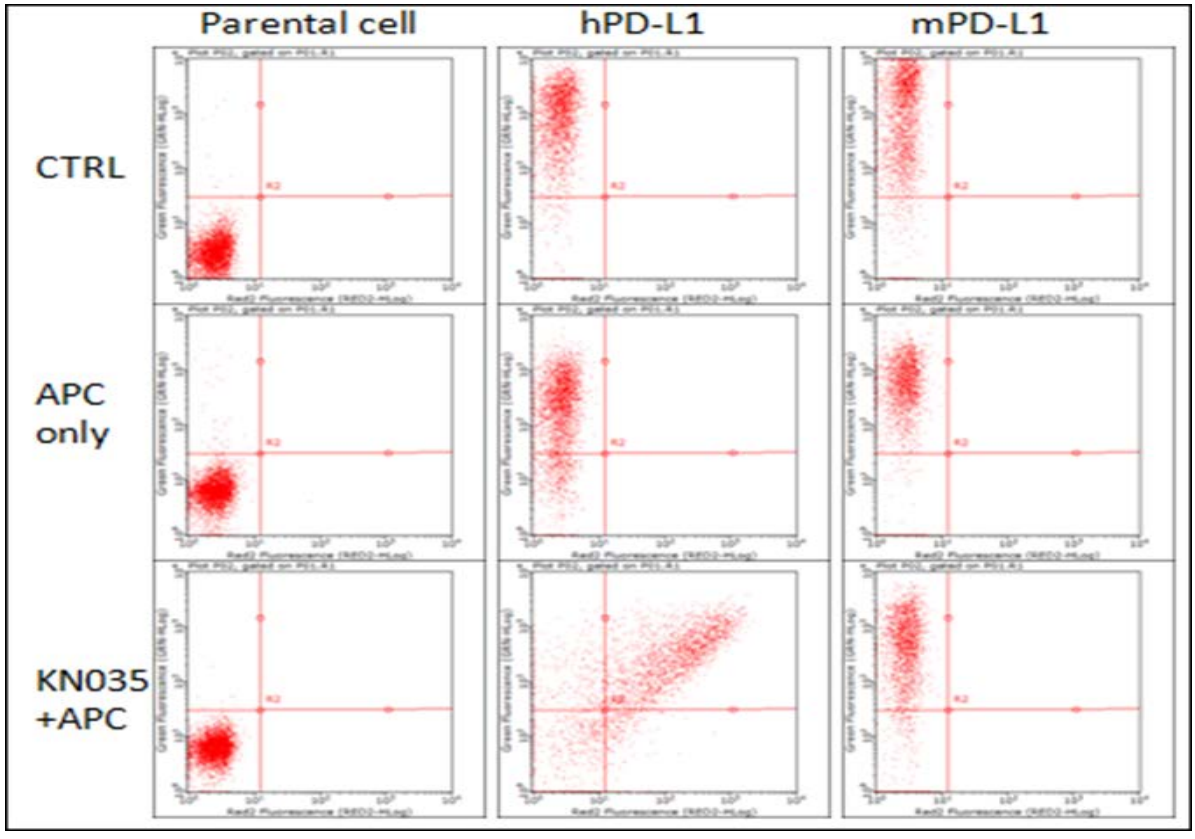
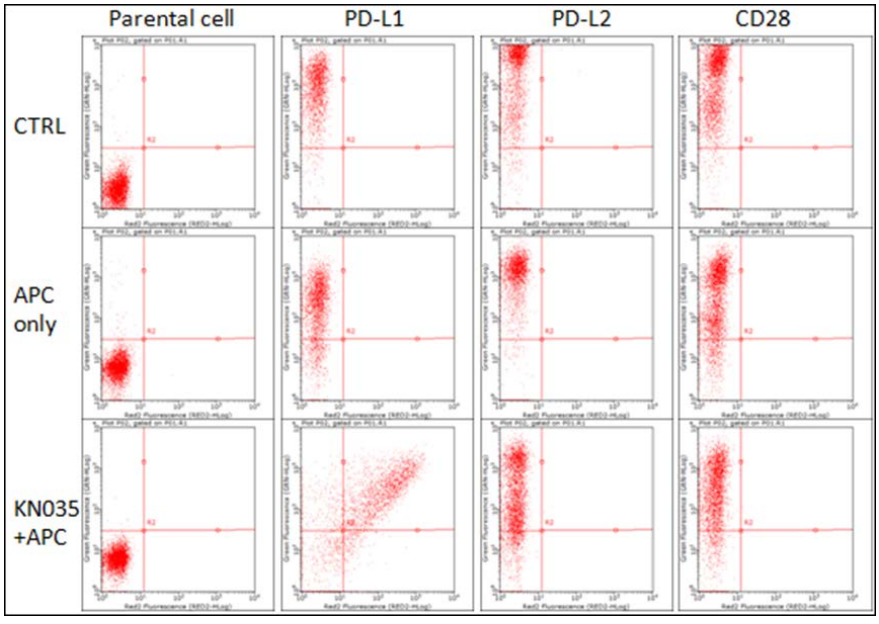
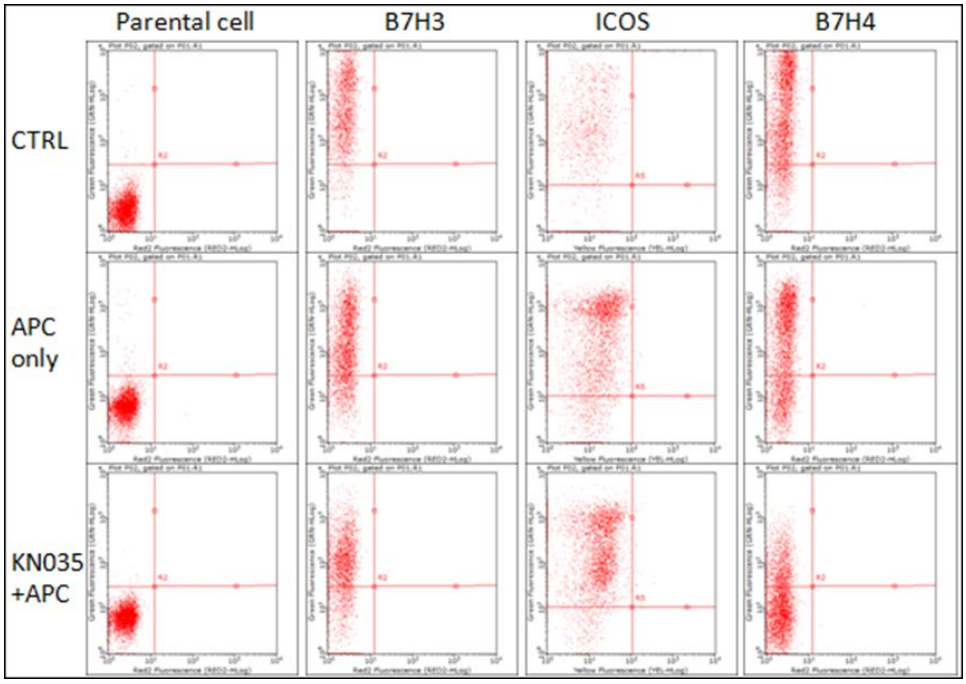


Figure 4 In Vitro Binding of KN035 toMouse PD-L1

2.1.6 *In Vitro* Binding of KN035to B7/CD28 Superfamily Proteins (RDR-KN035-PD-2015-049)

The aim of this study was to test the binding of KN035 toB7/CD28 superfamily proteins, PD-L2, B7H4, CD28, B7H3, and ICOS.KN035 was prepared at a concentration level of 10μg/ml.hPD-L2, hB7H4, hCD28, hB7H3, and hICOS genes were used to transform 293T cells. After the binding and washing procedure, samples were loaded to flow cytometry for analysis. Study results shown in Figure 5 demonstrated that there was no detectable binding activity of KN035 to hPD-L2, hB7H4, hCD28, hB7H3 or hICOS.





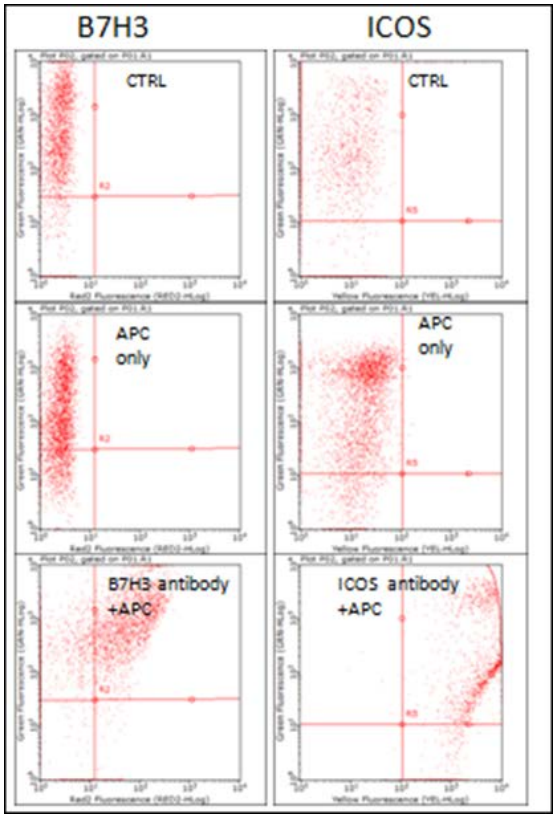
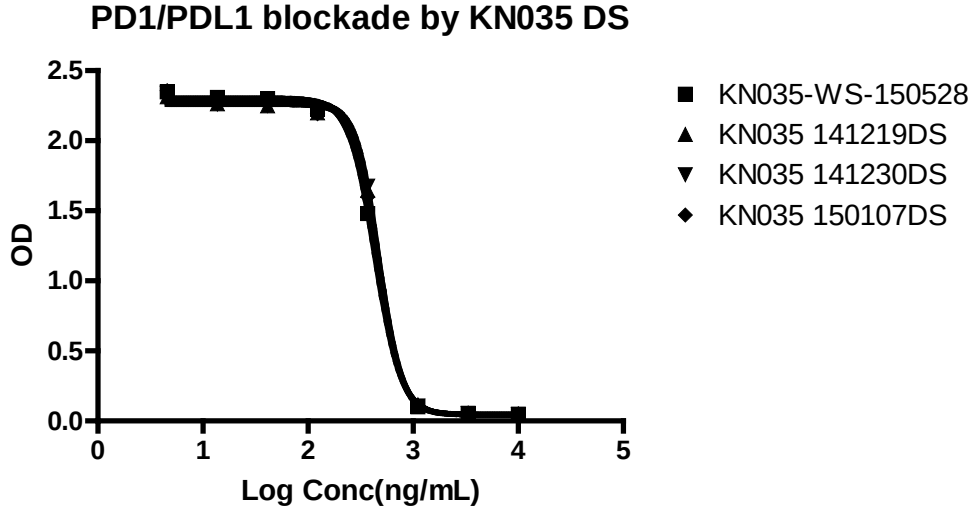


Figure 5 In Vitro Binding of KN035 to hPD-L2, hCD28, hB7H3, Bh7H4, and hICOS

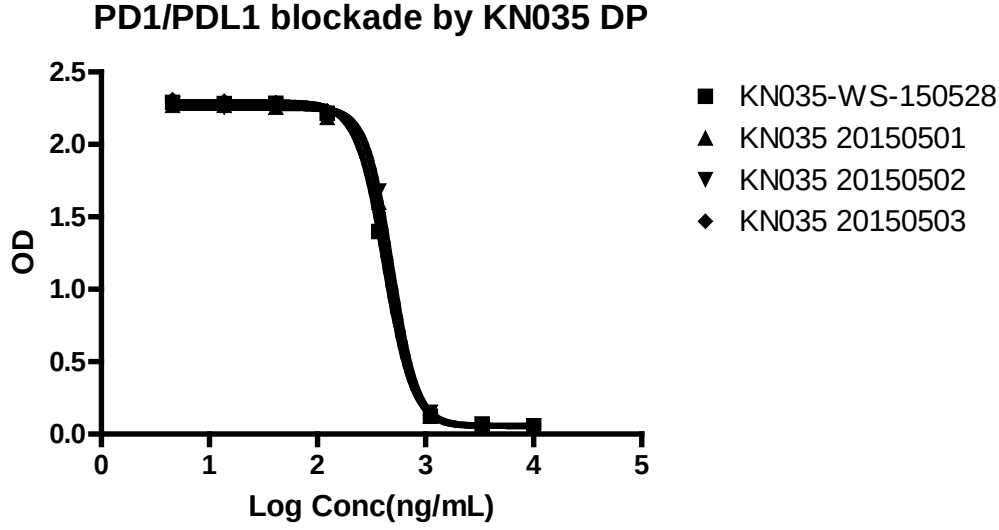
2.1.7 Blocking of PD-L1Binding to PD-1 by KN035 (RDR-KN035-QC-2015-018)

The aim of this study was to evaluate how KN035 blocks PD-L1from binding to PD-1. A ninety-six well plate was coated with 5ug/ml of PDL1-Fc. A PD1-muFcat concentration of 10ug/ml and other various concentrations of KN035were added. HRP labeled Goatanti-mouse IgG1 antibody was used for detection. After being developed by TMB, the plate was read at a wavelength of 450/650nm in the microplate reader.

Study results shown in Figure 6demonstrated that KN035 blocked PD-L1-Fc binding to PD-1-muFcin a concentration-dependent manner. The values of IC_{50}were determined at 419-488 ng/ml (six different production lots).



A



B

Figure 6 Blocking of PD-L1 Binding to PD-1 by KN035

A.-DS: drug substance
B. -DP: drug production

2.1.8 Blocking of PD-1 Binding to PD-L1 by KN035 (RDR-KN035-PD-2015-056)

The aim of this study was to evaluate how KN035blocks PD-L1from binding to PD-1via flow cytometry analysis.

In the first study, Biotin-PD-1-muFc (mouse Fc, 2ug/ml) and different concentrations of KN035 were added tohPD-L1 expression 293T cells (293T-hPD-L1). In the second study, PD-L1-muFc and KN035 were added to PD-1 expression Jurkat T cells

. PE-labeled anti-mouse IgG was used for detection.

Study results shown in Figure 7 and Figure 8demonstrated thatKN035 blocked the PD-1 binding to cell expressed PD-L1 (PD-1-muFc binding to 293T-hPD-L1) in a concentration-dependent manner. The IC_{50}value was determined at 0.25ug/ml. In the meantime, the blocking effect of KN035 on PD-L1 binding to PD-1 was also tested (PD-L1-muFc binding to Jurkat-hPD-1). The results also showed that KN035 blocked PD-1 from binding to PD-L1 in a concentration-dependent manner. The IC_{50}value was determined at 13.42ug/ml.

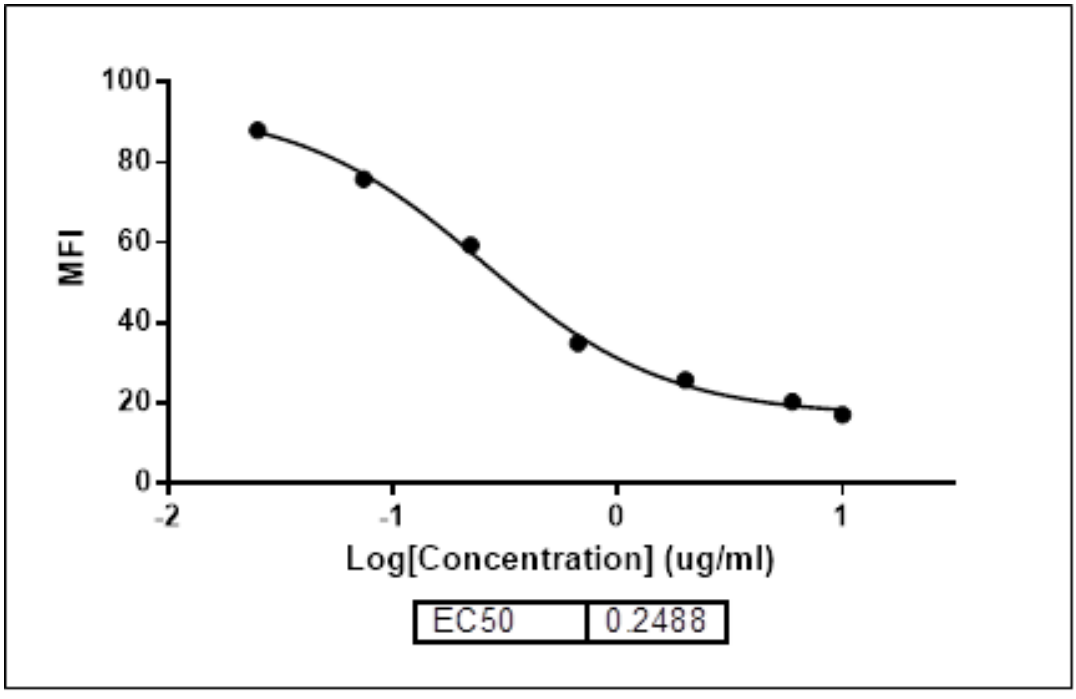


Figure 7 Blocking of PD-1-muFc Binding to 293T-hPD-L1

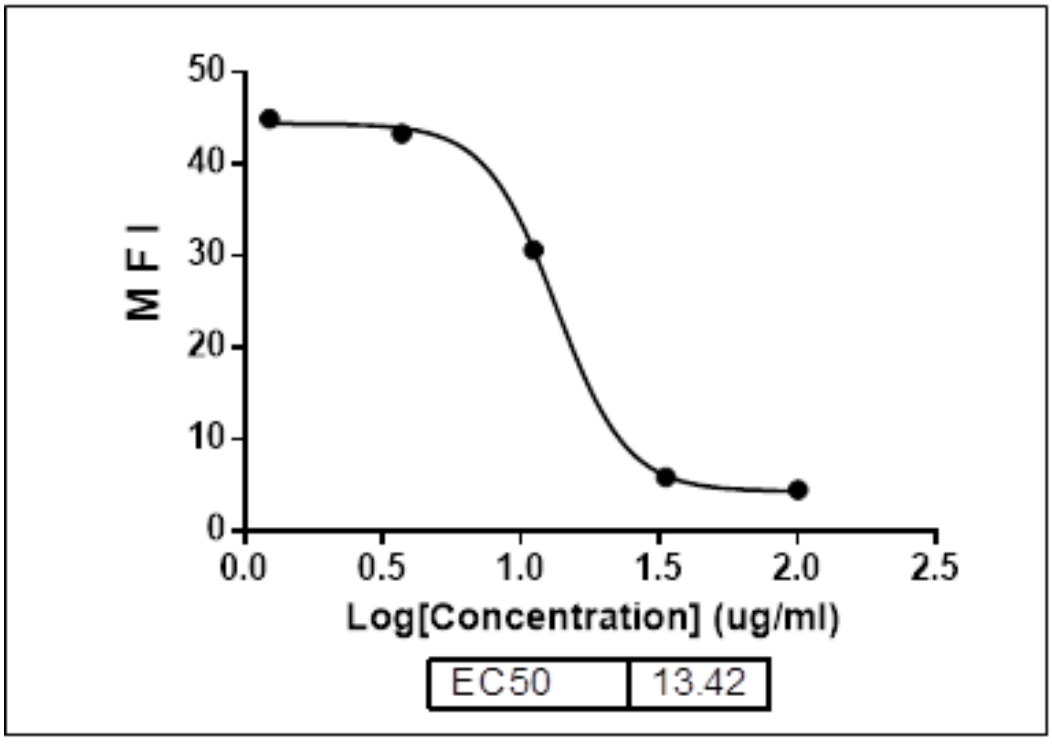


Figure 8 The Inhibition of KN035 on PD-L1-muFc and Jurkat-hPD-1 Binding

2.1.9 BlockingPD-L1Binding to CD80 by KN035 (RDR-KN035-QC-2016-006)

The aim of this study was to assess how KN035blocksPD-L1from binding to CD80. A ninety-six well plate was coated over night with 3ug/ml of PDL1-Fc and blocked for 2 hrs. Then shCD80-Fc-biotin (100ug/ml) and various concentrations of KN035 were added. After a 2-hourincubation period, Streptavidin-Peroxidase (1:1000)was added and incubated for 1.5 hrs. After being developed by TMB, the plate was read at the wavelength of 450/650nm in microplate reader.

The study results shown in Figure 9suggested that KN035 blocks the binding of PD-L1 to CD80 in a concentration-dependent manner. The IC_{50}valuewas determined at 102.7ng/ml.

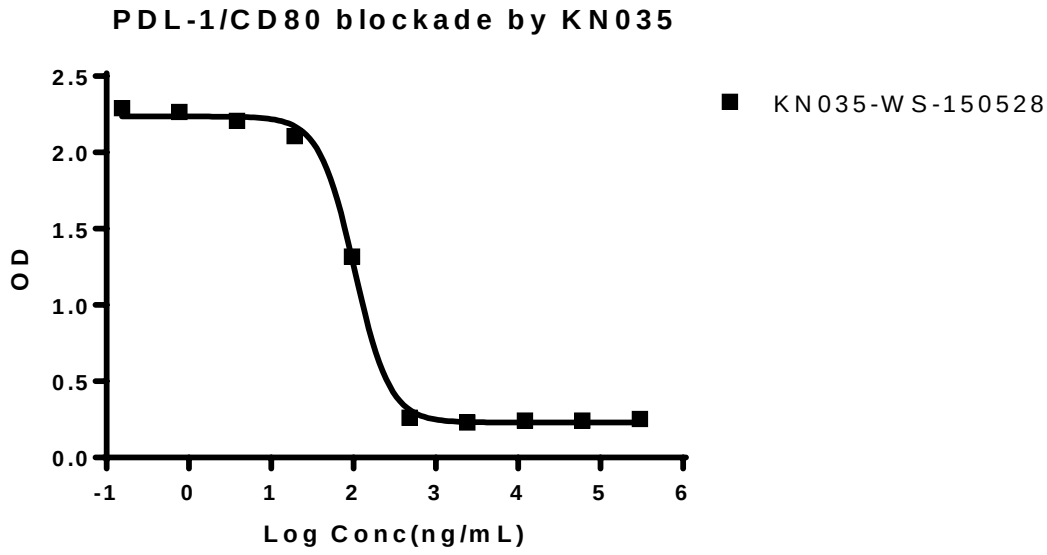


Figure 9 Blocking PD-L1 Binding to CD80 by KN035

2.1.10 *In Vitro* Binding Affinity of KN035 to Fcγ receptors

2.1.10.1 *In Vitro* Binding Affinity of KN035toFcγRIIIa176V-Chis (CD16) (RDR-KN035-QC-2015-016)

The aim of this study was to examine the *in vitro* binding affinity of KN035 to FcγRIIIusing ELISA and compare it with the wild-type Fc.

A ninety-six well plate was coated with KN035 and a control antibody (wild-typeFc), respectively. Different concentrations of FcγRIIIa176V-Chis (CD16) were added. HRP-labeled mouse anti-His antibody were used for detection. After being developed by TMB, the plate was read at the wavelength of 450/650nm in the microplate reader. The four-parameter curve fitting method was used to generate the response curve.

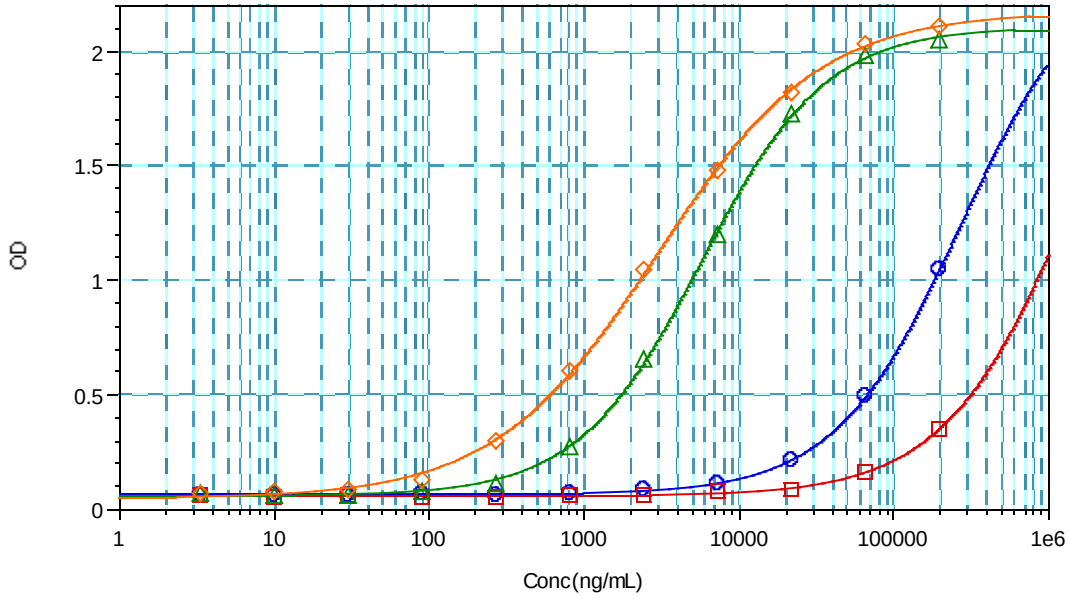
The study results shown in Figure 10 suggested that KN035 showed the binding affinity to FcγRIIIa176V-Chis (CD16)in a concentration-dependent manner. However, compared with the control antibody (wild-type Fc), the binding affinity was much lower. The EC_{50}valuesof KN035 and the control antibody (wild-type Fc) were determined at 1.62×10^{6}ng/ml and 2.96×10^{3}ng/ml, respectively.

2.1.10.2 *In Vitro* Binding Affinity of KN035 to FcγRIIa-Chis (CD32) (RDR-KN035-QC-2015-016)

The aim of this study was to examine the *in vitro* binding affinity of KN035 to FcγRII using ELISA and compare it with the wild-type Fc.

A ninety-six well plate was coated with KN035 and a control antibody (wtFc), respectively. Various concentrations of FcγRIIa-Chis (CD32)were added. HRP-labeled mouse anti-His antibody were used for detection. After being developed by TMB, the plate was read at the wavelength of 450/650nm in the microplate reader. The four-parameter curve fitting method was used to generate the response curve.

The results suggested KN035 showed the binding affinity to FcγRIIa-Chis (CD32)in a concentration dependent manner. However, the binding affinity is much lower compared with the control antibody (wild-type Fc). The EC_{50}values of the KN035 and control antibody (wild-type Fc) were 2.72×10^{5}ng/ml and 5.78×10^{3}ng/ml, respectively. The test results are shown in Figure 10.



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
Plot#1 (FcGRIIa-Chis(KN035): Concentration vs Mea...	0.0622	1.07	2.72e+05	2.41	1
Plot#2 (FcGRIIIa176V-Chis(KN035): Concentration v...	0.0552	1.02	1.62e+06	2.82	0.998
Plot#3 (FcGRIIa-Chis(wild-type Fc): Concentration v...	0.0565	1.09	5.78e+03	2.1	1
Plot#4 (FcGRIIIa176V-Chis(wild-type Fc): Concentr...	0.0452	0.829	2.96e+03	2.17	1

Weighting: Fixed

Figure 10 The Binding Affinities of KN035 and Control Antibody (wild-type Fc) to Fcγ Receptors (CD16 and CD32)

2.1.10.3 *In Vitro* Binding Affinity of KN035 to FcγRI (CD64) (RDR-KN035-PD-2015-057)

The aim of this study was to examine the *in vitro* binding affinity of KN035 to FcγRIand compare it with the wild-type Fc.

U937 cell line expressing FcγRI (CD64)and FcγRII (CD32)was used in this study. Prepare 1ml of fresh cultured U937 cells at a density of 2×10^5; add 20 μg/ml of FcγRIantibody; incubate on ice for 1 hour. Then add 4ug/ml of Biotin-labeled KN035 or control antibody (wild-type Fc) (KN002, an anti-TNFa antibody). After incubating for 30 minutes on ice, wash away free unbounded proteins and add Streptavidin PE. Samples were loaded to flow cytometry to measure the Median Fluorescence Intensity (MFI)..

The study results shown in Figure 11suggested that the control antibody (wild-type Fc) (KN002) binds to U937 cells, whereas KN035 does not. The binding of the control antibody (wild-type Fc) (KN002)to U937 cells can be blocked partially by adding FcγRIantibody.

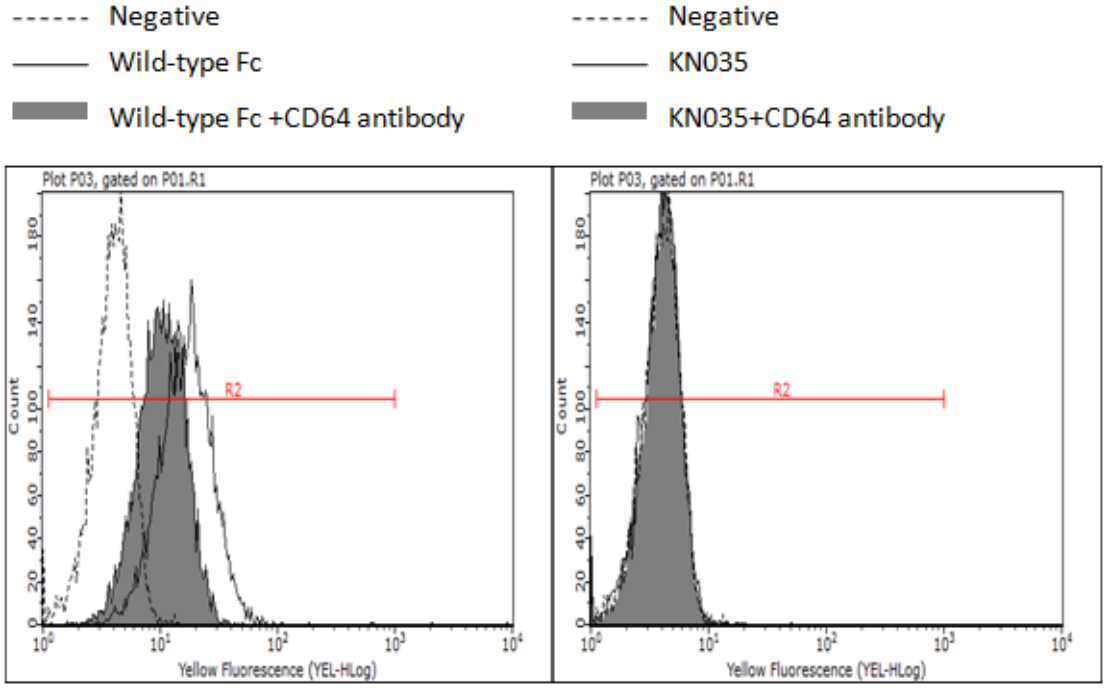


Figure 11 *In Vitro* Binding Affinity of KN035 to FcγRI (CD64)

2.1.11 *In Vitro* Binding Affinity of KN035 to Neonatal Fc receptor (FcRn) (RDR-KN035-QC-2016-011)

The purpose of this study was to assess the binding affinity of KN035 to rhFcRn.

FortebioBLI (Bio-Layer Interferometry) was used to detect the affinities of KN035 and the control antibodyKN015(wild-type Fc, FSH-Fc fusion protein) to rhFcRn. KN035-biotin or KN015-biotin was diluted to 10ug/mL. Then the samples were fixed on a SA biosensor. The rhFcRn was diluted to levels of 200nM, 100nM, 50nM, 25nM, and 12.5nM.After fixed for 100s,the samples were bound for 60s and disassociated for 30s. The fixation buffer was$PBST_{20}$ (0.05%, v/v), and with of pH7.4. The rhFcRn dilution buffer was 50mM PB, 150mM NaCl (pH6.0).

The results showed the mean KD value of KN035 to rhFcRn was (4.93±0.81) E-07M.The KD of KN015 to rhFcRn was 5.50E-07 (Table 3).

Table 3 Affinities of KN035 and KN015toFcRn

Samples	KD (M)	Kon (1/Ms)	Kdis (1/s)
KN035 141219DS	5.55E-07	1.88E+05	1.04E-01
KN035 141230DS	5.22E-07	2.02E+05	1.06E-01
KN035 150107DS	4.02E-07	2.39E+05	9.63E-02
Wild type Fc (KN015)	5.50E-07	2.13E+05	1.17E-01

2.1.12 Stimulation of IFN-γ secretion by T Cells in Mixed Leucocyte Reaction (MLR) (DF-YX-KN01)

The purpose of this study was to assess and compare the activating effects of KN035 and 2.41H90P (Duralumab) on CD4+ cells that were stimulated by allogeneic antigens in MLR.

MLR: Inoculate 10^4 of collected matured DC into each well of a 96-well plate, stand for 2-4 hrs., then add 10^5 of MACS collected CD4+ cells and various amounts of KN035 or Duralumab to each well, and cultivate the cells for 5 days at 37^{o}C; collect the culture supernatant to measure the amount of IFN-γ in each well.

The study results shown in Figure 12 suggested that KN035 can stimulate the secretion of IFN-γ in MLR. Furthermore, the stimulation effect of KN035 on the IFN-γ secretion was slightly better than that of Duralumab.

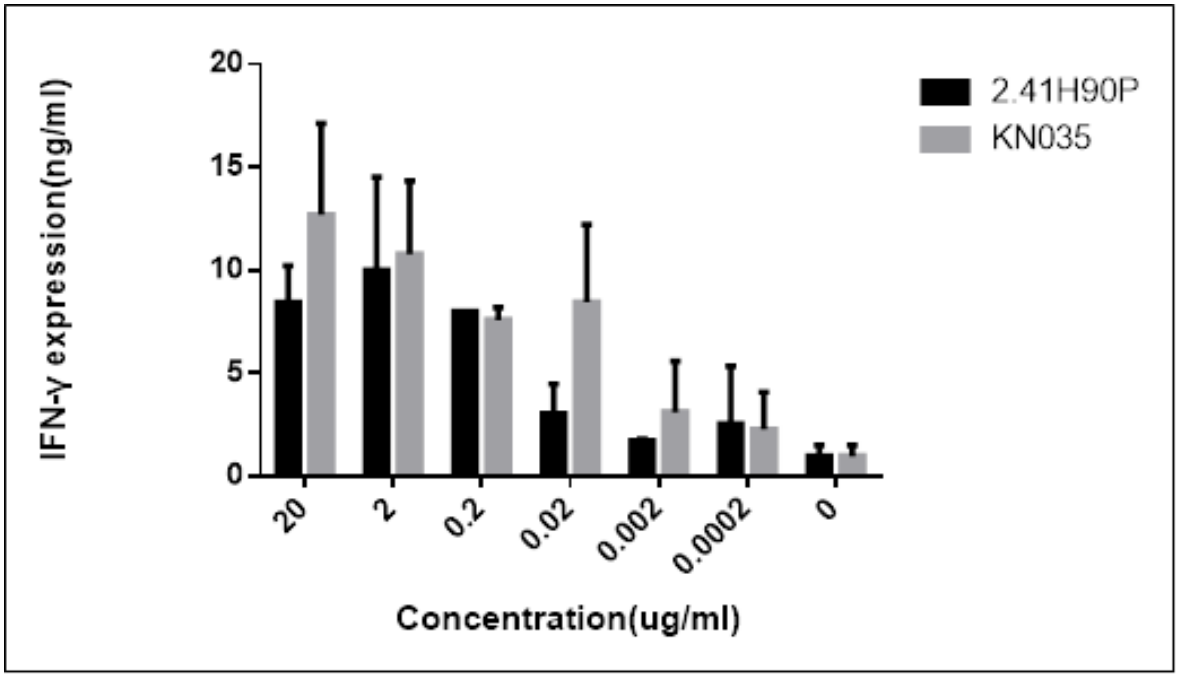


Figure 12 KN035/Duralumab Stimulating the Secretion of IFN-γ inCD4+ T cells

2.1.13 Activating Effect of KN035 on T Cells in Jurkat T/Raji-PD-L1Co-culture System (RDR-KN035-PD-2016-006)

The purpose of this study was to evaluate and compare the activating effects of KN035 and 2.41H90P (Duralumab) on T cells in a Jurkat T/Raji-PD-L1co-culture system.

50ul Jurkat T cells (3×10^6 cells/ml) and 50ulRaji-PD-L1 cells (1.5×10^6 cells/ml) were mixed together;50ul variable amount of KN035 or Durvalumab were added at the same time. After incubation for 24 hrs, the IL-2 amount was detected in the cultures.

The results showed the secretion of IL-2 was enhanced proportionally by adding 8pM～5nM of KN035 or Duralumab into the JurkatT/Raji-hPD-L1 co-culture system. Furthermore, under the same conditions, KN035 has a slightly better performance in activating Jurkat T cells compared with Duralumab. The results are shown in Figure 13.

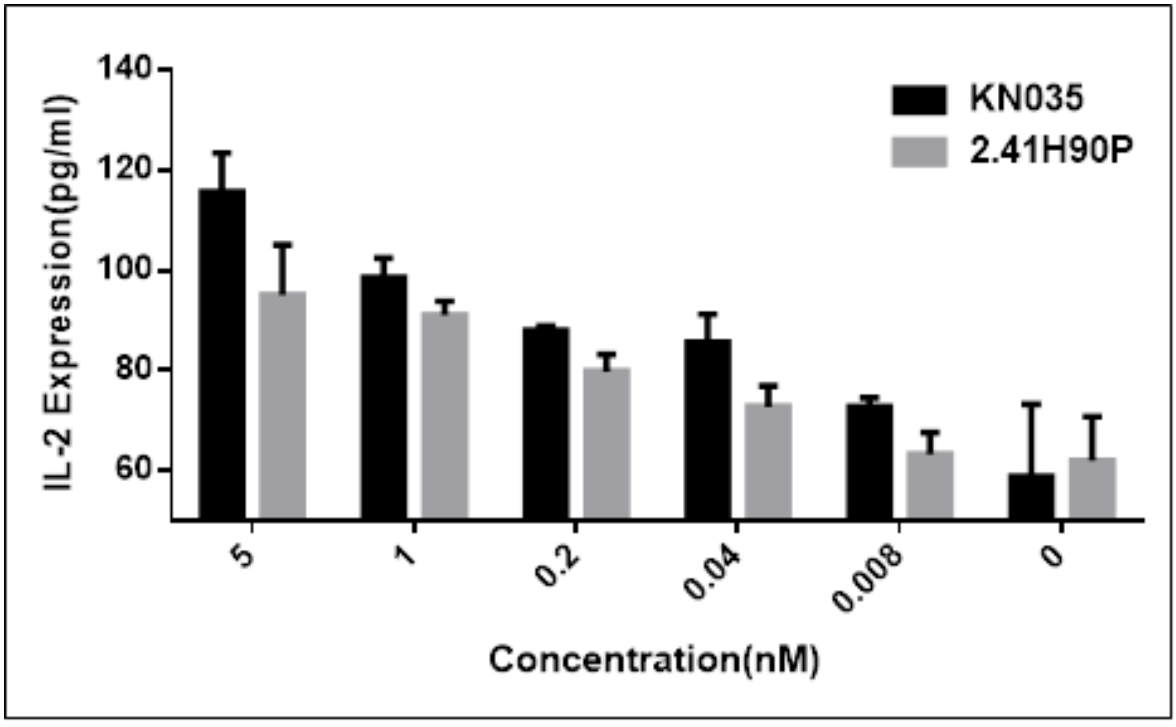


Figure 13 KN035/2.41H90P-promotedIL-2 expression increaseinJurkat T Cells

2.1.14 Complement Dependent Cytotoxicity (CDC) Activity of KN035 (RDR-KN035-PD-2015-051)

The aim of this study was to evaluate the complement dependent cytotoxicity activity of KN035.

Raji-hPD-L1 cell line was used as the target cells. Rituxanwas used as the positive control. The Cynomolgus monkey serum was used as a complementary elements supplier.

The study results shown in Figure 14 present the CDC activities of KN035 were at a baseline level (-6.85%～1.02%)in the concentration range which is from 0.02 to 20ug/ml, whereas the CDC activities of the positive control (Rituxan)increased from-4.29% to 89.75% with an obvious concentration-dependent manner.

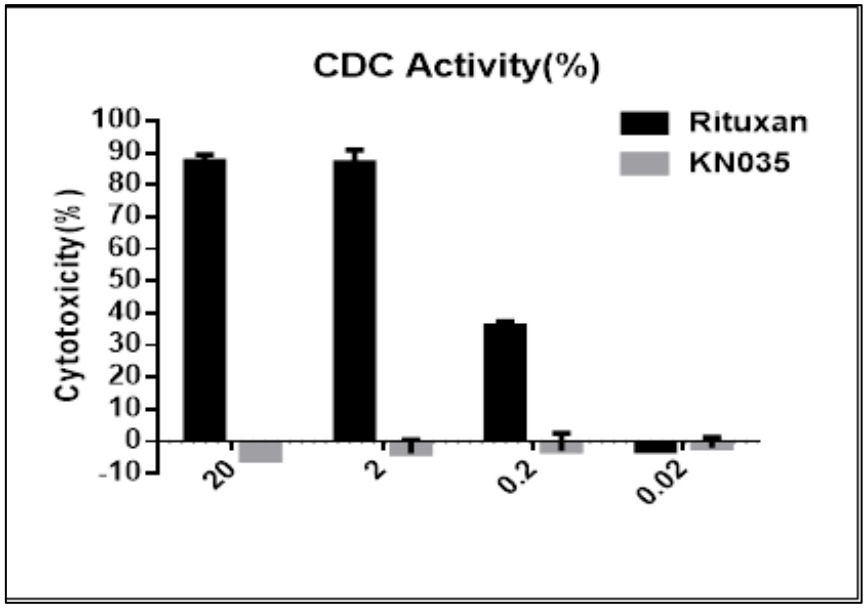


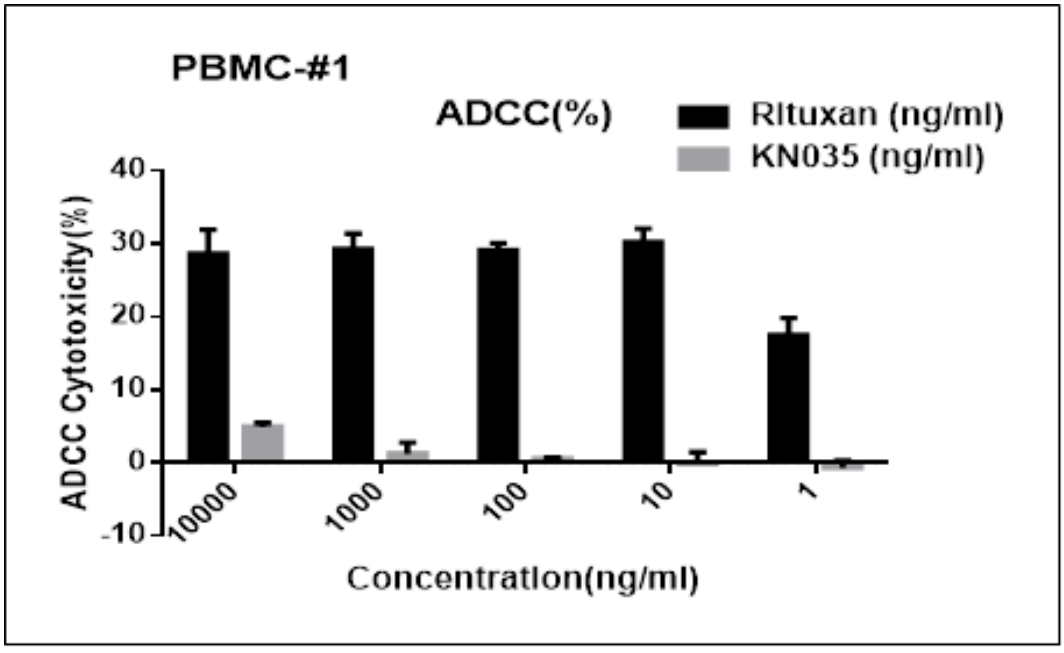
Figure 14 CDC Activity Test of KN035 and Rituxan

2.1.15 Antibody-dependent Cell-mediated Cytotoxicity (ADCC) Activity of KN035 (RDR-KN035-PD-2015-050)

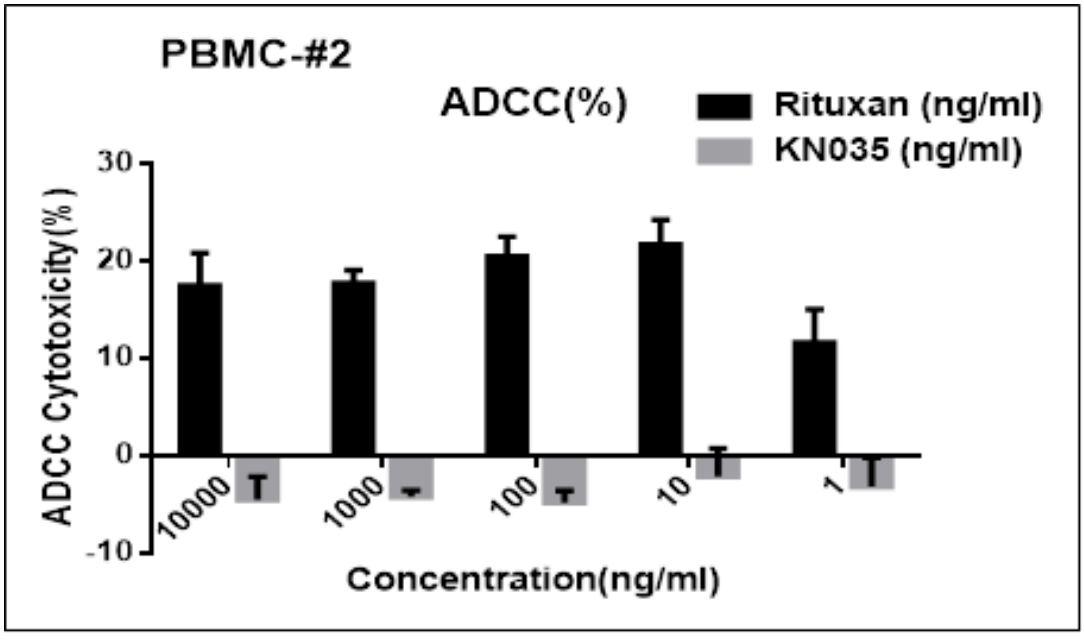
The aim of this study was to evaluate the antibody-dependent cell-mediated cytotoxicity activity of KN035.

In the study, Raji-hPD-L1 cell line was used as target cells. After incubating for 24 hours, the IL-2 activated PBMCs from different donors were used as the effective cells. Rituxan was used as the positive control.

The ADCC activities of KN035 were very close to baseline level (-1.46～5.30%, PBMC#1; -7.16～1.13%, PBMC#2)in the concentration range which is from 1ng/ml to 10ug/ml. These results indicated that KN035 has no ADCC activities. In contrast, the ADCC activities of the positive control (Rituxan)were14.77～32.39% and 8.97～23.38%, respectively, with the same sets of PBMC#1 and PBMC#2. The results are shown in Figure 15.



A



B

Figure 15 ADCC Activity Test of KN035 and Rituxan

A: PBMC-#1 ； B: PBMC-#2

2.2 *In Vivo* Pharmacology

2.2.1 Evaluation of the Anti-tumor Efficacy of KN035 in Xenograft Animal Model Transferred with Mixed A375-PD-L1 and Human PBMCs

*Effectiveness of Different Doses (*RDR-KN035-PD-2015-015*):*

The objective of this study was to evaluate the anti-tumor efficacy of KN035 at different doses in a xenograft mouse model.

Human melanoma cell line A375, which was stable-transfected with human PD-L1 (A375-hPD-L1), was mixed with human PBMCs at 4:1, and were subcutaneously inoculated to NOD-SCID mice. After 4 hours, the mice received KN035 by intraperitoneal injection once weekly at doses of 0.1, 0.3, 1, 3, and 10mg/kg for four weeks. The Tumor volume and tumor inhibition rate were evaluated.

The results suggested thatKN035 has significant anti-tumor effects at the dosed of 0.1, 0.3, 1, 3, 10mg/kg. Furthermore, it did not show an obvious dose-dependent effect. The results are shown in Figure 16.

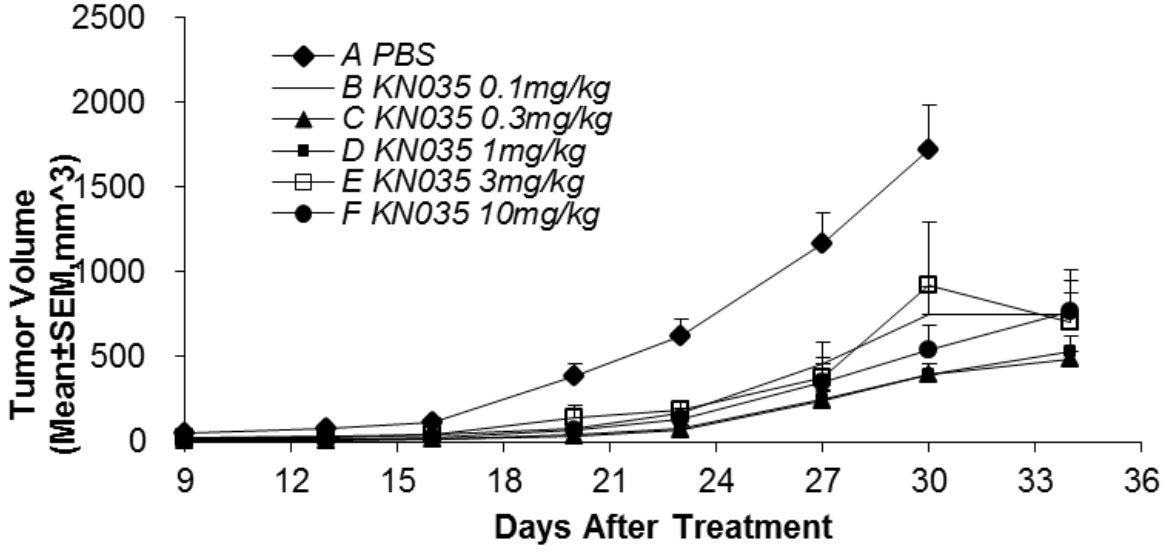


Figure 16 The Anti-tumor Activity of KN035 at Different Doses in A375-hPD-L1/ PBMC Xenograft Mouse Model

*Effectiveness of Different Dosing Frequencies (*RDR-KN035-PD-2015-023*):*

The objective of this study was to evaluate the anti-tumor efficacy of KN035 across dosing frequencies in a xenograft mouse model.

Human melanoma cell line A375, which was stable transfected with human PD-L1 (A375-hPD-L1), was mixed with human PBMCs at 4:1, and were subcutaneously inoculated to NOD-SCID mice. After 4 hours, the mice received KN035 by IP injection at 0.3mg/kg. The dosing frequencies were set as follows: 1) single injection (D1); 2)two injections (D1, D4); 3) three injections (D1, D4, D7); 4) four injections (D1, D4, D7, D10).

The results showed KN035 had obvious inhibitions on tumor growth in A375-hPD-L1/human PBMCs xenograft mouse model with every dosing frequency during the observed period. The test results are shown in Figure 17.

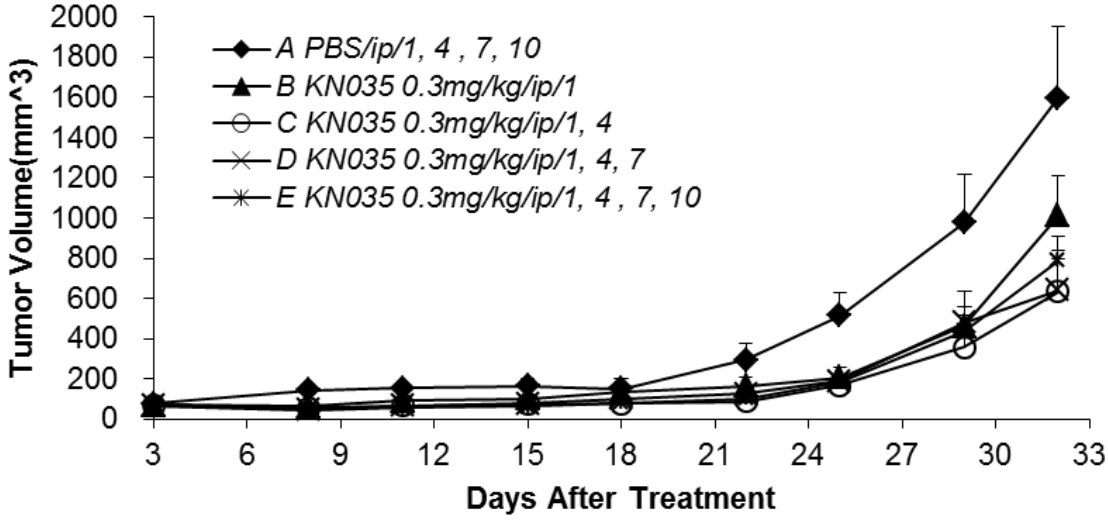


Figure 17 Inhibition of KN035 on Tumor Growth in A375-hPD-L1/ PBMC Xenograft Mouse Model with Different Dosing Frequencies

*Comparison of the Anti-tumor Efficacy between KN035 and Durvalumab (*RDR-KN035-PD-2016-005*):*

The objective of this study was to compare the anti-tumor efficacy between KN035 and Durvalumabin a xenograft mouse model.

Human melanoma cell line A375, which was stable transfected with human PD-L1 (A375-hPD-L1), was mixed with human PBMCs at 4:1, and were subcutaneously inoculated to NOD-SCID mice. After 4 hours, the mice received KN035 or Durvalumab(control) by intraperitoneal injection twice weekly at doses of 0.1, 0.3 and 1mg/kg for 7 consecutive administrations, respectively. The anti-tumor efficacy was evaluated at 8, 12, 15, 19, 22, and 26 days post-dosing.

The results showed the anti-tumor efficacy was similar between KN035 and Durvalumab at 1mg/kg, while the anti-tumor efficacy of KN035 was much stronger than that of Durvalumab at 0.3mg/kg and 0.1mg/kg. The results are shown in Figure 18.

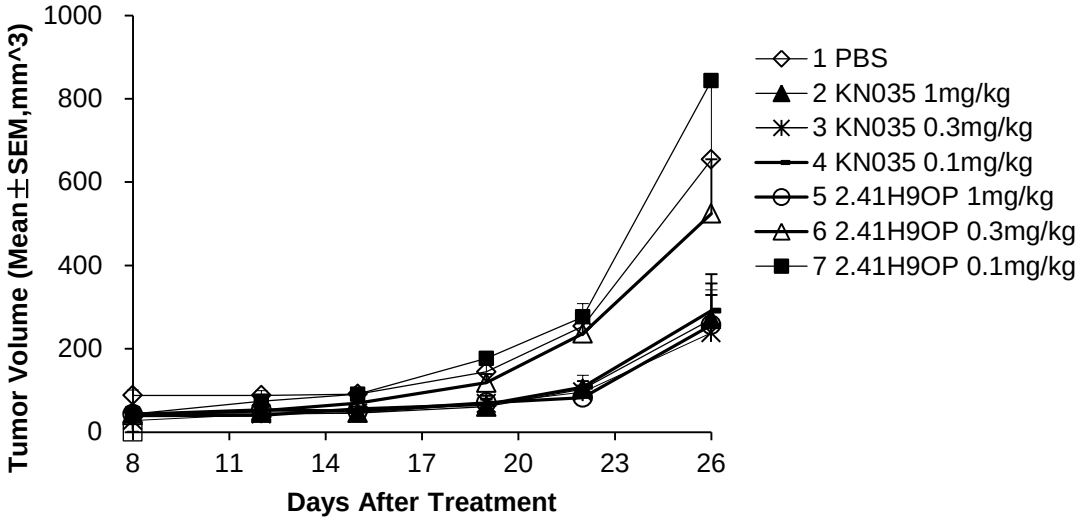


Figure 18 Comparison of the Anti-tumor Efficacy ofKN035 and Durvalumab in A375-hPD-L1/ PBMC Xenograft Animal Model

3 Secondary Pharmacodynamics

No studies conducted.

4 Safety Pharmacology

Safety pharmacology studies of KN035 were incorporated into a 4-week repeat-dose toxicity study in Cynomolgus monkeys (see Module 2.6.3 section 4). Forty animals (5/gender/group) were assigned to one control and three treatment groups. Four groups of study animals received weekly subcutaneous injections at doses of 0 (vehicle), 5, 30, and 150 mg/kg of KN035 for four weeks. Body temperature, blood pressure, and ECG were measured prior to dosing, and 2, 6, 24, and 48 hours after first dosing. No treatment-related changes of measurements were observed, suggesting that there is no adverse effect on the cardiovascular system. The clinical observation results also suggested that KN035 has no adverse effect on the central nervous and the respiratory systems in Cynomolgus monkeys.

5 Pharmacodynamic Drug Interactions

No studies on pharmacodynamic drug interactions were performed.

6 Discussion and Conclusions

KN035 is one of the antibodies that target PD-1/PD-L1 pathways for cancer immunotherapy. The difference between KN035 and other anti PD-1/PD-L1 drugs (under investigation or on market) is that KN035 is composed of the human Fc fragment linked to the CDR region of a humanized single domain antibody cloned from a camel. Structurally it just consists of two identical chains, not four chains like in human or rat antibodies. This structure of KN035 enables many advantages: (1)smaller molecules make it easier to penetrate the cancer tissue; (2)it makes it easier to be absorbed through the capillary system so that it can be administered through subcutaneous injection, not through IV; (3)it is more stable and easier to produce and handle compared with the first generation of PD-1/PD-L1 pathway blocking antibodies. KN035 can bind to human PD-L1 specifically. The binding affinity to hPD-L1 is 2.86nM.The ELISA method detected the EC_{50} of KN035 binding to PD-L1 was 62.275ng/ml. FACS detected EC_{50} of KN035 binding to A375-hPDL1 cells was 0.68ug/ml. Compared with Durvalumab, the binding affinity of KN035 to hPD-L1 was 1.45 times higher. KN035 does not bind to mouse PD-L1, whereas it binds to activated monkey PBMCs. KN035 does not have cross reactions with other B7/CD28 superfamily members such as PD-L2, B7H4, CD28, B7H3, and ICOS.

KN035 can block the binding of PD-1-muFc to PD-L1-Fc.The $IC_{50}$was 419-488ng/ml. It also blocks the binding ofPD-1 to 293T-hPD-L1, and the binding of PD-L1 to Jurkat-hPD-1. The values of IC_{50}were 0.25ug/ml and 13.42ug/ml, respectively. PD-L1 is a member of the B7/CD28 protein family, mainly expressed on APCs. Except with the activated T cells expressing PD-1, it can also interact with the activated T cells and APCs expressing CD80 to deliver the inhibitory signals to induce and maintain peripheral T cell tolerance [1]. The binding affinity ofPD-L1 to CD80 is 1.7uM (SPR), between that of CD28 (4uM) and that of CTLA-4 (0.2uM), which is slightly lower than the binding of PD-L1 to PD-1 (0.5uM).The combination sites between PD-L1 and CD80 are partly overlapped with the binding sites between PD-L1 and PD-1 [2] . These results suggested that the PD-L1 inhibitor may not only block PD-L1/PD-1 interactions, but also block the interactions between PD-L1 and CD80. The ELISA assays results showed that KN035 can block the interaction of PD-L1 with CD80. The $IC_{50}$was 102.7ng/ml. Therefore, KN035 can inhibit the PD-L1/PD-1 and PD-L1/CD80 pathways to block the negative regulation immune signals to T cells and promote the T cell activation. So far the clinically studied anti PD-L1 antibodies, such as Atezolizumab(Roche) and Durvalumab (AZ/Medimmune), all have the property to block the interaction of PD-L1 with CD80.

We used a human T cell activation system to verify whether the inhibitory activity of KN035can play a role in the antagonism of the function of PD-L1 *in vitro*. The results showed KN035 can activate CD4 positive T cells among MLR to secrete IFN-γ. Likewise, KN035 can promote the activation of Jurkat T cells to stimulate the secretion of IL-2in the Raji-hPD-L1 and Jurkat T mixed culture system. These two systems all suggested that KN035 can activate T cell activation by blocking the function of PD-L1. The activating ability of KN035 is slightly stronger than that of Durvalumab at the same concentration in the MLR system. Furthermore, compared with Durvalumab, KN035 showed slightly better performance inactivating T cells at unsaturated low molar responding ranges in a Raji-PD-L1 mixed culture system.

Since KN035 does not bind to mouse PD-L1, to evaluate the T cell activation mediated tumor killing effect of KN035, we developed an allogeneic tumor model by co-transplantation of human immune cells / tumor cell, through inoculation of mixed human PD-L1 expression tumor cells (A375-PD-L1) and human PBMCs into the subcutaneous tissue of NOD/SCID mice. KN035 can obviously reduce the size of the tumor. In the meantime, at the dose level of 0.3mg/kg, even at a single dose treatment, KN035 has showed the effect of tumor inhibition. These results are consistent with the results Durvalumab claimed in the patent (Patent No.: 2013/0034559 US). Parallel comparative research results showed that KN035 has a significant anti-tumor efficacy at lower doses, and the inhibitory effect of KN035 was better than Durvalumab under the condition of equal dose.

In addition to the expression in tumor cells, PD-L1 also has expressions in the non-hematopoietic cells, such as the cornea, vascular epithelial cells, placenta, as well as the activated T cells, B cells, and dendritic cells etc. [3,4]. Therefore, the Fc region of KN035 has been mutated to eliminate ADCC and CDC for reducing the potential side effect. The binding affinity of KN035 toFcγRs (CD64, CD32 and CD16) was significantly lower than that of wild-type Fc. But it did not lose its affinity to FcRn. Using Raji-PD-L1 as the target cells, KN035 showed no CDC and ADCC activities. Thus the clinical risks related to the Fc mediated of KN035 will be eliminated.

Based on the above results, it can be concluded thatKN035 can specifically bind to hPD-L1, block PD-1/PD-L1 interactions, and activate the functions of cancer antigen-specific T cells of cancer clearance.

7 Reference

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