

## 2.6.3 PHARMACOLOGY TABULATED SUMMARY

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1 Pharmacology Overview

| Overview                              |                        |   | Test Article: KN035  |                  |                                       |                                       |
|---------------------------------------|------------------------|---|--|------------------|---------------------------------------|---------------------------------------|
| Type of Study / Description           | Test System            | Method of Administration  | GLP Compliance   | Testing Facility | Study Number                          |                                       |
| Primary Pharmacodynamics              |                        |   |  |                  |                                       |                                       |
|                                       | Binding to hPD-L1 test | Bio-Layer Interferometry  | KN035 (10ug/mL) was fixed on ProteinA biosensor, dilute PD-L1-Chis to variable concentrations, fix for 120s, incubate for 300s, disassociate for 600s, recover for 10s, neutralize for 10s | No               | Alphamab                              | <a href="#">RDR-KN035-QC-2016-010</a> |
|                                       |                        | ELISA   | coat 96 well platewithhPD-L1-muFc, add variable KN035and Durvalumab, use Goat Anti-human IgG(Fc specific)antibody to test binding activity   | No               | Alphamab                              | <a href="#">RDR-KN035-QC-2015-017</a> |
|                                       |                        | Flow cytometer  | Incubate A375-hPD-L1cell with variable KN035, add FITC-labeled rabbit anti human IgG, FACs for detection   | No               | Alphamab                              | <a href="#">RDR-KN035-PD-2015-049</a> |
| Binding to checkpoint family proteins | Flow cytometer         | 293Tcells transformed with hPD-L2, hB7H4, hCD28, hB7H3 and hICOS, respectively; add 10ug/ml of KN035; use APC-labeled anti human IgG; test with FACs                              | No   | Alphamab         | <a href="#">RDR-KN035-PD-2015-049</a> |                                       |
| Binding to monkey PD-L1 test          | Flow cytometer         | Monkey PBMC was stimulated with PHA(5ug/ml) for 48h, add KN035(10ug/ml) and ice incubate for 1h, wash3 times with PBS, add FITC-labeled rabbit anti human IgG, FACs for detection | No   | Alphamab         | <a href="#">RDR-KN035-PD-2015-049</a> |                                       |

| Overview                                |                |  | Test Article: KN035 |                  |                                       |
|---|----------------|--|---------------------|------------------|---------------------------------------|
| Type of Study / Description             | Test System    | Method of Administration   | GLP Compliance      | Testing Facility | Study Number                          |
| Binding to mouse PD-L1 test             | Flow cytometer | 293T cells transformed with mouse PD-L1, culture for 48h, collect cells, add 10ug/ml KN035, incubate for 30min, add APC-labeled anti-human IgG Fc Antibody, FACs for detection | No                  | Alphamab         | <a href="#">RDR-KN035-PD-2015-049</a> |
| PD-1/PD-L1 binding block test           | ELISA          | 96-well plate coated with hPD-L1-Fc, PD1-muFc (10ug/ml) and various concentration of KN035 were added, use 1:3000diluted Goat anti-Mouse IgG1 Secondary Antibody for detection | No                  | Alphamab         | <a href="#">RDR-KN035-QC-2015-018</a> |
|   | Flow cytometer | Transform 293T hPD-L1, add PD-1-muFc, add gradient diluted KN035, detect with f(ab')-anti-mouse IgG-PE   | No                  | Alphamab         | <a href="#">RDR-KN035-PD-2015-056</a> |
|   | Flow cytometer | Jurkat-PD-1 cells and biotin labeled PD-L1-muFc incubated on ice, add gradient diluted KN035, on ice for 1h, Streptavidin PE for detection                                     | No                  | Alphamab         | <a href="#">RDR-KN035-PD-2015-056</a> |
| Block PD-L1/CD80 binding test           | ELISA          | 96-well plate coated with PD-L1-Fc, add 100ug/ml shCD80-Fc-biotin and variable KN035, 1:1000 diluted Streptavidin-Peroxidase for detection                                     | No                  | Alphamab         | <a href="#">RDR-KN035-QC-2016-006</a> |
| Binding Affinity to FcγRIII/FcγRII test | ELISA          | Use control antibody(wild type Fc) and KN035 to coat plates, add 100ul gradient diluted FcγRII a-Chis and FcγRIIIa176V-Chis protein, THE™ His Tag Antibody[HRP] for detection  | No                  | Alphamab         | <a href="#">RDR-KN035-QC-2015-016</a> |
| Binding Affinity to FcγRI test          | Flow cytometer | U937cells, add 20ug/ml CD64 antibody, on ice for 1h, add 4ug/ml Biotin-KN035 or Biotin-KN002(wtFc), SA-PE for detection  | No                  | Alphamab         | <a href="#">RDR-KN035-PD-2015-057</a> |

| Overview                                   |   |   | Test Article: KN035 |                  |                                       |
|--|---|---|---------------------|------------------|---------------------------------------|
| Type of Study / Description                | Test System   | Method of Administration  | GLP Compliance      | Testing Facility | Study Number                          |
| Bind Affinity to FcRn test                 | Bio-Layer Interferometry  | Dilute KN035-biotin/KN015-biotin to 10ug/mL, fix on SA biosensor, dilute rhFcRn to variable concentrations, fix for 100s, bind for 60s, disassociate for 30s  | No                  | Alphamab         | <a href="#">RDR-KN035-QC-2016-011</a> |
| ADCC                                       | Raji-PD-L1  | Use Raji-hPD-L1 as target cells, newly isolate 2xPBMCs as effect cells, use IL-2 to activate for 24h, add KN035(1ng/ml~10ug/ml) to react for 6h, quantitate LDH activity in supernatant to determine ADCC activity  | No                  | Alphamab         | <a href="#">RDR-KN035-PD-2015-050</a> |
| CDC  | Raji-PD-L1  | Use Raji-hPD-L1 as target cells, serum of Cynomolgus monkey to supply complementary elements, add KN035 (0.02~20ug/ml), react for 2h, use CCK-8 method to test target cell activity   | No                  | Alphamab         | <a href="#">RDR-KN035-PD-2015-051</a> |
| T cell activation test                     | CD4+ T cell/allogenic DC cell   | Inoculate 10 <sup>4</sup> of <i>in vitro</i> matured DC cells in each well of 96-well plate, culture for 2-4h, add MACS purified CD4+ T cells at 10 <sup>5</sup> /well with variable concentration of KN035 or Durvalumab, 37°C incubate for 5 days, measure IFN-γ product in supernatant | No                  | Dingfu Bio       | <a href="#">DF-YX-KN01</a>            |
|  | Coculture Raji-PD-L1 cells and Jurkat T cells                                     | Mix Jurkat T cells (3×10 <sup>6</sup> cells/ml) and Raji-PD-L1 (3×10 <sup>6</sup> cells/ml), add KN035 or Durvalumab, incubate for 24hrs, then measure IL-2 production  | No                  | Alphamab         | <a href="#">RDR-KN035-PD-2016-006</a> |
| Anti-tumor effect with dose level response | NOD-SCID mouse, A375-PD-L1/human PBMC mixture xenograft animal model, IP delivery | Mix A375-hPD-L1 and human PBMCs at 4:1 ratio, inoculate into subdermal tissue of NOD-SCID mouse, 4hrs later, inject different doses(0.1, 0.3, 1, 3, 10mg/kg) of KN035 through IP, one dose per week for 4 weeks, measure tumor size two times every week.                                 | No                  | Alphamab         | <a href="#">RDR-KN035-PD-2015-015</a> |

| Overview   |                   |  | Test Article: KN035 |                  |                                       |
|--|-------------------|--|---------------------|------------------|---------------------------------------|
| Type of Study / Description                                    | Test System       | Method of Administration   | GLP Compliance      | Testing Facility | Study Number                          |
| Anti-tumor effect with dosing frequency response               |                   | Mix A375-hPD-L1 with human PBMCs at 4: 1 ratio and inoculate into subdermal of NOD-SCID mouse, 4hrs later, inject effective dose of KN035 (0.3mg/kg) at different frequency of: 1) single dose (D1), 2) 2 doses (D1, D4), 3) 3 doses (D1, D4, D7), 4) 4 doses (D1, D4, D7, D10) through IP, measure tumor size two times every week for 4 weeks. | No                  | Alphamab         | <a href="#">RDR-KN035-PD-2015-023</a> |
| Comparison of anti-tumor efficacy between KN035 and Durvalumab |                   | Mix A375-hPDL1 and human PBMCs at 4: 1 ratio and inoculate into subdermal of NOD-SCID mouse, 4hrs later, inject KN035 or 2.41H9OP into separate group of animals though IP at doses of 0.1,0.3 and 1mg/kg. Give same dose two times per week on same dose group, 7 administrations in total. Measure tumor growth every half week.               | No                  | Alphamab         | <a href="#">RDR-KN035-PD-2016-005</a> |
| <b>Safety Pharmacology</b>                                     |                   |  |                     |                  |                                       |
| Cardiovascular   | Cynomolgus monkey | hypodermic injection   | Yes                 | NCSED            | <a href="#">2015033-1*</a>            |
| <b>Pharmacodynamic Drug Interactions:</b> No studies conducted |                   |  |                     |                  |                                       |

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

2 Primary Pharmacodynamics

Test Article: KN035

Primary Pharmacodynamics – *In vitro*

| Type of Study                         | Test System              | Method of Administration  | Noteworthy Findings   | Location in CTD (Report Number)     |
|---------------------------------------|--------------------------|---|---|-------------------------------------|
| Binding to hPD-L1 test                | Bio-Layer Interferometry | KN035(10ug/mL) fixed on ProteinA biosensor, dilute PD-L1-Chis to variable concentrations, fix for 120s, bind for 300s, disassociate for 600s, recover for 10s, neutralize for 10s | Mean KD of KN035 (three product lots): (2.86±0.23)E-09M                           | M 4.2.1.1.1 (RDR-KN035-QC-2016-010) |
| Binding to hPD-L1 test                | ELISA                    | 96-well plate coated with hPD-L1-muFc, add variable KN035and Durvalumab, use Goat Anti-human IgG (Fc specific)antibody to test binding activity                                   | The affinity of KN035 to hPD-L1-muFc was 1.5 times higher than that of Durvalumab | M 4.2.1.1.2 (RDR-KN035-QC-2015-017) |
| Binding to hPD-L1 test                | Flow cytometer           | Incubate A375-hPD-L1cells with variableKN035, add FITC-labeled rabbit anti human IgG, FACs for detection  | The EC <sub>50</sub> value was 0.68ug/ml  | M 4.2.1.1.3 (RDR-KN035-PD-2015-049) |
| Binding to checkpoint family proteins | Flow cytometer           | 293Tcells transformed with hPD-L2、hB7H4, hCD28, hB7H3 and hICOS, respectively; add 10ug/ml of KN035, use APC-labeled anti human IgG, test with FACs                               | KN035 does not bind PD-L2, B7H4, CD28, B7H3 and ICOS                              | M 4.2.1.1.3 (RDR-KN035-PD-2015-049) |
| Binding to monkey PD-L1 test          | Flow cytometer           | Monkey PBMC stimulated with PHA (5ug/ml) for 48h, add KN035 (10ug/ml), ice incubate for 1h, wash3 times with PBS, add FITC-labeled rabbit anti human IgG, FACs for detection      | KN035 binds to PHA activated monkey PBMCs   | M 4.2.1.1.3 (RDR-KN035-PD-2015-049) |
| Binding to mouse PD-L1 test           | Flow cytometer           | 293 cell transformed with mouse PD-L1, culture for 48h, collect cells, add 10ug/ml KN035, incubate for 30min, add APC-labeled anti-human IgG Fc Antibody, FACs for detection      | KN035does not bind to mouse PD-L1   | M 4.2.1.1.3 (RDR-KN035-PD-2015-049) |

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Test Article: KN035

Primary Pharmacodynamics – *In vitro*

| Type of Study                           | Test System              | Method of Administration   | Noteworthy Findings  | Location in CTD (Report Number)      |
|---|--------------------------|--|--|--------------------------------------|
| PD-1/PD-L1 binding block test           | ELISA                    | 96-well plate coated with hPD-L1-Fc, PD1-muFc (10ug/ml) and various concentration of KN035 were added, use 1:3000diluted Goat anti-Mouse IgG1 Secondary Antibody for detection | mean IC <sub>50</sub> of KN035 (6 product lots): 419-488ng/mL  | M 4.2.1.1.4<br>RDR-KN035-QC-2015-018 |
| PD-1/PD-L1 binding block test           | Flow cytometer           | Transform 293T/hPDL1, add PD-1-muFc, add gradient diluted KN035, detect with f(ab’)-anti-mouse IgG-PE  | KN035 blocks PD-1-muFc/293T-hPD-L1 binding, IC <sub>50</sub> : 0.25 ug/ml  | M 4.2.1.1.5<br>RDR-KN035-PD-2015-056 |
| PD-1/PD-L1 binding block test           | Flow cytometer           | Jurkat-PD-1 cell and biotin labeled PD-L1-muFc incubated on ice, add gradient diluted KN035, on ice for 1h, Streptavidin PE for detection                                      | KN035blocks PD-L1-muFc/Jurkat-hPD-1binding, IC <sub>50</sub> : 13.42ug/ml  | M 4.2.1.1.5<br>RDR-KN035-PD-2015-056 |
| Block PD-L1/CD80 binding test           | ELISA                    | 96-well plate coated with PDL1-Fc, add 100ug/ml shCD80-Fc-biotin and variable KN035, 1:1000 diluted Streptavidin-Peroxidase for detection                                      | KN035 blocks PDL1/CD80binding, IC <sub>50</sub> : 102.7ng/ml   | M 4.2.1.1.6<br>RDR-KN035-QC-2016-006 |
| Binding affinity to FcγRIII/FcγRII test | ELISA                    | Use wild type Fc and KN035 (Fc mutated)coat plates, add 100ul gradient diluted FcγR II a-Chis and FcγRIIIa176V-Chis protein, THE™ His Tag Antibody[HRP] for detection          | The binding affinity of KN035 binding with to FcγR II a-Chis or FcγRIIIa176V-Chis are much lower than that of wtFc | M 4.2.1.1.7<br>RDR-KN035-QC-2015-016 |
| Binding affinity to FcγRI test          | Flow cytometer           | U937cells, add 20ug/ml CD64 antibody, on ice for 1h, add 4ug/ml Biotin-KN035 or Biotin-KN002(wFc), SA-PE for detection   | KN035 does not bind to U937(CD64)  | M 4.2.1.1.8<br>RDR-KN035-PD-2015-057 |
| Binding affinity to FcRn test           | Bio-Layer Interferometry | Dilute KN035-biotin/KN015-biotin to 10ug/mL, fix on SA biosensor, dilute rhFcRn to variable concentrations, fix for 100s, bind for 60s, disassociate for 30s                   | Mean KD of KN035 (3 product lots) was (4.93±0.81)E-07M, while KD of wtFc was 5.50E-07M                             | M 4.2.1.1.9<br>RDR-KN035-QC-2016-011 |

Test Article: KN035

Primary Pharmacodynamics – *In vitro*

| Type of Study          | Test System                                    | Method of Administration  | Noteworthy Findings   | Location in CTD (Report Number)                                       |
|------------------------|--|---|---|---|
| ADCC                   | Raji-PD-L1                                     | Use Raji-hPD-L1 as target cells, newly isolate 2xPBMCs as effect cells, use IL-2 to activate for 24h, add KN035 (1ng/ml~10ug/ml), react for 6h, quantitate LDH activity in supernatant to determine ADCC activity   | No ADCC activity  | <a href="#">M 4.2.1.1.10</a><br><a href="#">RDR-KN035-PD-2015-050</a> |
| CDC                    | Raji-PD-L1                                     | Use Raji-hPD-L1as target cells, serum of Cynomolgus monkey to supply complimentary elements, add KN035 (0.02~20ug/ml), react for 2h, use CCK-8 method to test target cell activity  | No CDC activity   | <a href="#">M 4.2.1.1.11</a><br><a href="#">RDR-KN035-PD-2015-051</a> |
| T cell activation test | MLR  | Inoculate 10 <sup>4</sup> of <i>in vitro</i> maturated DC cells in each well of 96-well plate, culture for 2-4h, add MACS purified CD4+ T cell at 10 <sup>5</sup> /well with variable concentration of KN035 or Durvalumab, 37° C incubate for 5 days, measure IFN-γ product in supernatant | KN035 stimulates CD4+ T cell to secrete IFN-γ with dose response, the stimulation effect is stronger than Durvalumab        | <a href="#">M 4.2.1.1.12</a><br><a href="#">DF-YX-KN01</a>            |
| T cell activation test | Co-culture Raji-PD-L1 cells and Jurkat T cells | Mix Jurkat T cells (3×10 <sup>6</sup> cells/ml) and Raji-PD-L1 (3×10 <sup>6</sup> cells/ml), add KN035 or Durvalumab, incubate for 24hrs, then measure IL-2 production  | KN035 stimulates Jurkat T cells to secrete IL-2 with dose level response, the activation effect is stronger than Durvalumab | <a href="#">M 4.2.1.1.13</a><br><a href="#">RDR-KN035-PD-2016-006</a> |



Test Article: KN035

**Primary Pharmacodynamics –*In vivo***

| Type of Study  | Test System   | Method of Administration   | Doses   | Noteworthy Findings  | Location in CTD (Report Number)   |
|--|---|--|---|--|---|
| Anti-tumor effect with dose level response                     | NOD-SCID mouse, A375-PD-L1/human PBMC mixture xenograft animal model, IP delivery | Mix A375-hPD-L1 and human PBMCs at 4: 1 ratio, inoculate into subdermal tissue of NOD-SCID mouse, 4hrs later, inject different doses of KN035 through IP, one dose per week for 4 weeks, monitor tumor size every half week.   | 0.1, 0.3, 1, 3, 10mg/kg, once a week, for 4 weeks | KN035 inhibits tumor growth in this animal model without dose level relevance  | <a href="#">M 4.2.1.1.14</a><br><a href="#">(RDR-KN035-PD-2015-015)</a> |
| Anti-tumor effect with dosing frequency response               |   | Mix A375-hPD-L1 with human PBMCs at 4: 1 ratio and inoculate into subdermal of NOD-SCID mouse, 4hrs later, inject effective dose of KN035 at different frequency of: 1) single dose(D1), 2) 2 doses (D1, D4), 3) 3 doses (D1, D4, D7), 4) 4 doses (D1, D4, D7, D10) through IP, measure tumor size two times every week for 4 weeks. | 0.3mg/kg  | KN035 inhibits tumor growth at every dosing frequency in this animal model   | <a href="#">M 4.2.1.1.15</a><br><a href="#">RDR-KN035-PD-2015-023</a>   |
| Comparison of anti-tumor efficacy between KN035 and Durvalumab |   | Mix A375-hPD-L1 and human PBMCs at 4: 1 ratio and inoculate into subdermal of NOD-SCID mouse, 4hrs later, inject variable doses of KN035 or 2.41H9OP into separate group of animals through IP. Give same dose two times per week on same dose group. Measure tumor growth every half week.  | 0.1, 0.3 and 1mg/kg; 7 administrations in total   | At dose of 1mg/kg, KN035 showed same anti-tumor efficacy compared with Durvalumab, whereas at the test doses of 0.3 and 0.1mg/kg, KN035 has better performance | <a href="#">M 4.2.1.1.16</a><br><a href="#">RDR-KN035-PD-2016-005</a>   |

**3        Secondary Pharmacodynamics**

No studies conducted.

4      **Safety Pharmacology**

| Safety Pharmacology                               |                   |                          |  |                          |   | Test Article: KN035 |                            |
|---|-------------------|--------------------------|--|--------------------------|---|---------------------|----------------------------|
| Type of Study                                     | Species/ Strain   | Method of Administration | Doses (mg/kg)  | Gender and No. per Group | Noteworthy findings   | GLP Compliance      | Report Number              |
| Cardiovascular system, CNS and respiratory system | Cynomolgus monkey | Subcutaneous injection   | 0, 5, 30, 150 weekly for four weeks (5 subcutaneous injections in total), with a 4-week recovery phase | 5/sex/group              | No treatment-related findings in electrocardiogram parameters, CNS and respiratory system | Yes                 | <a href="#">2015033-1*</a> |

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

## **5 Pharmacodynamic Drug Interactions**

No studies conducted.