**Supplementary Material**

**Supplementary methods**

**Comparative binding of ALT-803 to mouse, human, and monkey IL-15 receptors**

Simian blood samples from 3 female cynomolgus monkeys were purchased from Sierra for Medical Science (Whittier, CA). Human blood samples were collected from 3 healthy donors. PBMCs were isolated from the blood using Histopaque (Sigma, St. Louis, MO). Mouse splenocytes were obtained from C57BL/6 mice. PBMCs and splenocytes were stained on ice with 0-200 µg/mL FITC-labelled ALT-803 and antibodies against CD8 and NK cell markers (NK1.1 for murine cells; CD335 (NKp46) for human and simian cells) and then analyzed on a FACSVerse flow cytometer with FACSuite software (BD Biosciences).

**Supplementary Table S1. Antibodies used in immune cell characterization by flow cytometry**

|  |  |  |
| --- | --- | --- |
| **Human PBMCs –**  **In vitro cultures** | **Cynomolgus monkey PBMCs – Toxicology study** | **Cynomolgus monkey blood cells – Immune cell phenotype study** |
| PE-Cy7- and FITC-conjugated anti-CD8a (HIT8a) 1 | FITC-conjugated anti-CD8 (M-T477) 3 | PE-Cy7-conjugated anti-CD16 (3G8) 3 |
| PE-conjugated anti-CD335 (NKp46) (9E2) 1 | PE-conjugated ant-CD335 (BAB281) 2 | Alexa Fluor 700-conjugated anti-CD3 (SP34-2) 3 |
| FITC-conjugated anti-CD19 (HIB19) 1 | FITC-conjugated anti-CD4 (RPA- T8) 3 | APC-Cy7-conjugated anti-CD20 (2H7) 3 |
| Brilliant Violet 510-, PE- and FITC-conjugated anti-CD4 (OKT4) 1 | PE-conjugated anti-CD20 (2H7) 3 | Pacific Blue-conjugated anti-CD8 (RPA-T8) 3 |
| Human Treg Kit (Alexa Fluor 488 anti- FOXP3/PE-Cy5 anti- CD4/PE anti-CD25 PE) 1 |  | PerCP-Cy5.5-conjugated anti-CD56 (B159) 3 |
| PE-conjugated anti-CD335 (NKp46) (BAB281) 2 |  | APC-conjugated anti-CD45 (D058-1283) 3 |
| APC-conjugated anti-CD25 (BC96) 1 |  | FITC-conjugated anti-Ki-67 (B56) 3 |
| Brilliant Violet 421-conjugated anti-CD16 (3G8) 1 |  | AmCyan-conjugated anti-CD4 (SK3) 3 |
| FITC-conjugated anti-granzyme B (GB11) 1 |  | V450-conjugated anti-CD45RA (5H9) 3 |
| FITC-conjugated anti-perforin (dG9) 1 |  | biotin-conjugated anti-CCR7 (3D12) 3 |
|  |  | PE-conjugated Streptavidin 3 |
|  |  | FITC-conjugated anti-CD95 (DX2) 3 |
|  |  | PerCP-Cy5.5-conjugated anti-Ki-67 (B56) 3 |
|  |  | PE-Cy5-conjugated anti-CD8 (B9.11) 3 |
|  |  | PE-Texas Red-conjugated anti-CD28 (28.2) 2 |

1 Biolegend

2 Beckman Coulter

3 BD Bioscience

**Supplementary Table S2. PK parameters of ALT-803 administered to cynomolgus monkeys**

|  |  |  |
| --- | --- | --- |
|  | **0.03 mg/kg\*** | **0.10 mg/kg\*** |
| **Terminal t1/2 (h)** | 7.18 ± 1.76 | 7.97 ± 1.29 |
| **Cmax (ng/mL)** | 583 ± 138 | 2792 ± 614 |
| **Cmax (nM)** | 6.31 ± 1.49 | 30.22 ± 6.64 |
| **Vss (mL/kg)** | 54.5 ± 14.7 | 37.56 ± 9.1 |
| **Cl (mL/hr)** | 6.00 ± 1.68 | 3.02 ± 0.67 |
| **AUCINF (hr\*ng/mL)** | 5939 ± 1517 | 31828 ± 7507 |
| **AUCINF/dose (hr\*ng/mL/ng/kg)** | 0.20 ± 0.05 | 0.32 ± 0.08 |
| **r2** | 0.97 ± 0.05 | 0.97 ± 0.03 |
| **\***mean ± SD |  |  |

**Supplementary Table S3. Representative clinical characteristics of cynomolgus monkeys following 4 weekly doses of ALT-803 administration (study day 26)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter a** | **PBS** | **0.03 mg/kg**  **ALT-803** | **0.10 mg/kg**  **ALT-803** |
| Body weight (kg) **b** | 2.22 ± 0.15 | 2.19 ± 0.13 | 2.10 ± 0.24 |
| Kidney (g) **b** | 8.28 ± 0.62 | 8.45 ± 0.25 | 8.93 ± 0.3 |
| Liver (g) **b** | 41.4 ± 4.4 | 43.2 ± 2.4 | 43.8 ± 2.2 |
| Lung (g) **b** | 11.1 ± 0.9 | 13.9 ± 1.5 | 10.4 ± 1.4 |
| Spleen (g) **b** | 2.66 ± 0.27 | 3.46 ± 0.52 | 3.81 ± 0.61 |
| RBC count (106/μL) **c** | 6.61 ± 0.32 | 6.29 ± 0.38 | 6.89 ± 0.41 |
| Platelets (103/μL) **c** | 345 ± 34 | 421 ± 36 | 371 ± 41 |
| Hemoglobin (g/L) **c** | 151 ± 7 | 148 ± 5 | 155 ± 6 |
| ALT **d** (U/L) **c** | 36.3 ± 3.9 | 39.2 ± 5.2 | 28.3 ± 3.1 |
| AST **d** (U/L) **c** | 32.8 ± 1.9 | 34.5 ± 2.3 | 27.6 ± 1.7 |
| Albumin (g/L) **c** | 44.6 ± 1.3 | 41.5 ± 0.8 | 38.5 ± 1.2\*\* |
| BUN **d** (mmol/L) **c** | 6.99 ± 0.34 | 7.48 ± 0.38 | 6.58 ± 0.27 |
| Creatitine (μmol/L) **c** | 67.3 ± 2.76 | 73.5 ± 4.9 | 66.9 ± 2.7 |
| Anti-ALT-803 Ab  (% incidence [concentration range]) **e** | 0% | 30%  [34 – 237 ng/mL] | 50%  [36 – 527 ng/mL] |

a WBC and leukocyte subset results shown in Fig. 7.

b mean ± SE, n = 6/group.

c mean ± SE, n = 10/group.

d Alanine aminotransferase, ALT; aspartate aminotransferase, AST; blood urea nitrogen, BUN.

e Samples analyzed were collected SD 22 and 36, n = 10/group.

**Supplementary Table S4. Incidence of major histopathologic findings in cynomolgus monkeys following 4 weekly doses of ALT-803 administration (study day 26)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Organ/Tissue** | **PBS** | **0.03 mg/kg**  **ALT-803** | **0.10 mg/kg**  **ALT-803** |
| Bone marrow | 6 / 6 a | 5 / 6 a | 6 / 6 a |
| Kidney | 1 / 6 b | 1 / 6 b | 4 / 6 b |
| Liver | 1 / 6 b,c | 3 / 6 b,c | 5 / 6 b,c |
| Lung | 1 / 6 b | 4 / 6 b | 2 / 6 b |
| Lymph nodes | 2 / 6 a | 1 / 6 a | 1 / 6 a |
| Salivary gland | 0 / 6 | 0 / 6c | 1 / 6 b,c |
| Spleen | 0 / 6 | 0 / 6 | 0 / 6 |
| Thymus | 0 / 5 | 0 / 6 | 0 / 6 |

a Hyperplasia

b Inflammation

c Necrosis

**Supplementary Figure and Figure Legend**

**Supplementary Figure S1.**

Comparative binding of ALT-803 to IL-15 receptors on mouse, cynomolgus monkey, and human immune cells. NK cell (triangles) and CD8+ T cell (circles) populations were stained with the indicated concentration of ALT-803-FITC and analyzed for mean fluorescence intensity (MFI) by flow cytometry. The symbols represent MFI ± SE for 3-4 samples.

