

SUPPLEMENTARY FILES

SUPPLEMENTARY FIGURE LEGENDS

S1. VISTA sequence homology and reported interaction residues with binding partners (VSIG3, LRIG1 and PSGL-1).

A. VISTA sequence alignment between mouse, rats, cynomolgus monkey and humans from MultAlign and visualized via ESPript. B. VISTA homology model showing VSIG3, PSGL-1 and LRIG1 epitope residues, reported to be involved in binding human VISTA.

S2. Murine anti-VISTA antibody, V4P, binds to VISTA with high affinity and specificity and demonstrates Fc-independent inhibition of tumor growth in vivo

A. Binding specificity of V4P by ELISA using human B7 family antigens as indicated. Data shown are mean n=2 measurements and error bars are SEM. **(B-C)** Cell derived xenograft models of C54BL/6 mice subcutaneously implanted with EL4 (B) and BALB/c mice, subcutaneously implanted with CT26 (C). Mice were randomized and dosed with concentrations of test articles at indicated time-points. Tumor volumes were measured twice a week. Each data point represents the mean tumor volume +/- SEM of n=6 mice.

S3. HMBD-002, an IgG4 monoclonal antibody, does not bind to FcγRIII or C1q that are required for Fc-mediated ADCC and CDC activity.

A. Binding of HMBD-002 to FcγRIII and B.C1q proteins as assessed by ELISA. Data shown are mean of n=3 measurements and error bars are SEM.

S4. HMBD-002 binds to a unique epitope on VISTA with very high binding affinity across a range of physiological pH.

A. HMBD-002 epitope binning, using examples of human VISTA antibodies (VSTB112, IGN175A) and B. mouse anti-VISTA antibodies (13F3, MH5A) in-tandem method was used to

test competitive binding of antibody pairs to VISTA by biolayer interferometry and signals were aligned to baseline. C-F. Surface plasma resonance (Biacore) binding kinetics of HMBD-002 to VISTA orthologs; human, mouse, rat and cyno VISTA. G. Binding affinity of HMBD-002 across pH range 5.5-7.5. Data shown are mean of n=3 measurements and error bars are SEM.

Figure S5. VISTA interacts with VSIG3, LRIG1, PSGL-1 and HMBD-002 inhibits the VISTA-LRIG1 interaction but does not inhibit the VISTA-PSGL-1 interaction.

A and B. Binding of VISTA with its binding partner VSIG3 (A) and LRIG1 (B) by ELISA. Data shown are mean of n=3 measurements and error bars are SEM. C. Inhibition of VISTA:LRIG1 binding, analyzed by competition ELISA. Data shown are mean of n=3 measurements and error bars are SEM. D. Binding of VISTA to its putative binding partner PSGL-1 at pH6 and pH7 by ELISA. Data shown are mean of n=3 measurements and error bars are SEM. E. Inhibition of VISTA:PSG-L-1 binding at pH6, analyzed by competition ELISA. Data shown are mean of n=3 measurements and error bars are SEM.

S6. HMBD-002 remodels the immune milieu toward an enhanced pro-inflammatory Th1/Th17 immune response in an allogenic Mixed Lymphocyte Reaction. A. Cytokine levels measured at 96 hrs by Luminex, from the supernatant of an allogenic mixed lymphocyte reaction. All concentrations of HMBD-002 and anti PD-1 antibody, Pembrolizumab (annotated as Pembro) in $\mu\text{g/ml}$. Data was normalized to isotype control. Data shown are mean of n=10 and error bars are SEM. B. KEGG pathway analysis using WebGestalt from bulk RNA-sequencing of MLR samples (n=10). Data was normalized to IgG4 isotype control. C. Enrichment of transcript levels in genes associated with type-I and Type-II interferon genes from bulk RNA-sequencing of MLR samples (n=10). Data represented as mean \pm SD.

S7. HMBD-002 exhibited acceptable pharmacokinetic and safety profiles in multiple pre-clinical toxicology species.

Serum concentration of HMBD-002 in **(A)** Balb/c tumor-bearing and non-tumor bearing mice (n=3), **(B)** Sprague Dawley rats (n=6) and **(C)** cynomolgus monkey (n=2). HMBD-002 was administered at the indicated concentration via intraperitoneal injection in mice, IV bolus injection into the tail vein in rats, and IV bolus injection into the peripheral vein in monkeys **(D-G)** Cytokine release assay showing representative cytokine levels of IL-2 and IL-6 using whole blood (D and E) and PBMC (F and G) treated with indicated test articles and concentrations. HMBD-002 is referred to in the figure as V4C26.hIgG4 in D-G. Data shown are mean of n=10 measurements and each point represents an independent donor.

SUPPLEMENTARY METHODS

Antibody isolation

Six- to eight-week-old female BALB/c mice were repeatedly immunized with custom immunogens. Twenty-four hours after the final immunization, total splenocytes were isolated and fused with the myeloma cell line P3X63.Ag8.653 (ATCC) using ClonaCell-HY Hybridoma Cloning Kit, in accordance with the manufacturer's instructions (Stemcell Technologies). After 7 to 10 days, single hybridoma clones were isolated and antibody-producing hybridomas were selected by screening supernatants for antigen binding using ELISA and flow cytometry. Variable regions of selected clones were amplified (SMARTer RACE 5'/3' Kit, Clontech), sequenced and cloned into expression vector and expressed in mammalian cells for in vitro functional testing. One clone, V4P, was selected for development and subsequently humanized and further affinity matured into the final antibody HMBD-002.

Hybridoma production

Approximately 6-week-old female BALB/c mice were obtained from InVivos (Singapore). Animals were housed under specific pathogen-free conditions and were treated in compliance with the Institutional Animal Care and Use Committee (IACUC) guidelines. Using our proprietary immunization protocol (mAbHits), mice were immunized with proprietary mixtures of custom immunogens. Total splenocytes were isolated and fused with the myeloma cell line P3X63.Ag8.653 (ATCC, USA) using polyethylene glycol (PEG) and ClonaCell-HY Hybridoma Cloning Kit (Stemcell Technologies, Canada), according to manufacturer's instructions. Monoclonal hybridomas were selected and supernatants from

the resulting clones were screened by enzyme linked immunosorbent assay (ELISA) and fluorescent activated cell sorting (FACS).

Antibody variable region cloning and sequencing

Total RNA was extracted from hybridoma cells using TRIzol reagent (Life Technologies, Inc., USA) according to the manufacturer's instructions. Double-stranded cDNA was synthesized using SMARTer RACE 5'/3' Kit (Clontech). The race-ready cDNAs were amplified using SeqAmp DNA Polymerase (Clontech™) and primer mixes. The resulting variable heavy (VH) and light (VL) amplicons were cloned into pJET1.2/blunt vector using CloneJET PCR Cloning Kit (Thermo Scientific) and purified PCR amplicons were sequenced through AITBiotech Pte. Ltd. (Singapore). The sequencing data was analysed using the international IMGT (ImMunoGeneTics) information system[1] to characterize the individual complementarity-determining regions (CDRs) and framework sequences.

Humanization and affinity maturation of mouse antibody

Humanization of the variable regions of the mouse sequence was performed by CDR grafting into human framework sequences and backmutation of residues in canonical positions to preserve antigen binding. The humanised antibody was further affinity matured against mouse VISTA to increase the cross-species affinity by random mutagenesis using yeast scFv surface display. Briefly, ScFv bearing the parental VH and VL in a single chain was amplified by mutagenesis PCR (Agilent Technologies GeneMorph II Random Mutagenesis Kit) for library creation, the resulting library was further amplified and cloned into yeast expression vector pCTcon2 (Addgene) for yeast surface expression by electroporation. The transfected cells were screened by mouse VISTA binding using FACS and high binder cell was sorted by BD FACS Aria II cell sorter. Selected high binder clones were enriched by 2 additional rounds

of sorting. Cells were plated in SDCAA to isolate single clones followed by DNA extraction and sequencing. The final sequence of HMBD-002 was selected from among the optimized variants based on its developability characteristics as well as in vitro physicochemical and functional properties.

Cell lines

All cell lines were purchased from ATCC and cultured as recommended. Cells were maintained in culture medium supplemented with 10% FBS and 1% Pen/Strep (ThermoFisher) and cultured at 37°C, in 5% CO₂ incubators. Prior to use, fresh vials of cells were thawed and passaged 2-5 times. And Mycoplasma testing was performed by PCR using DreamTaq Green PCR master mix (ThermoFisher #K1081) according to manufacturer's instruction. Mycoplasma (*M. orale*) positive control template (Agilent Mycosensor assay kit) and the following primers were used:

Fwd. 5' GGGAGCAAACAGGATTAGATACCCT 3'

Rev. 5' TGCACCATCTGTCACTCTGTAAACCTC 3'

Stable cell line generation

CHO-k1 cells at an optimal density of 1×10^6 cell/ml were electroporated with 5 µg of linearized IgG expression plasmid using 4D-Nucleofactor kit (Lonza) according to manufacturer's protocol. Electroporated cells were cultured in static cell incubator in a 6-well plate containing 2 ml growth medium for 24 hr. Subsequently, medium was exchanged to selection medium containing 250 nM Methotrexate (Sigma) and 200 µg/ml Zeocin (InvivoGen). Cells were spun down and re-suspended in fresh selection medium and

re-seeded to a density of 5×10^5 cell/ml once per week. Selection was completed when 95% viability was restored. Cells were transferred to shaker-incubator.

Antibody production and purification

Antibodies were produced by cultivation of stable cells in Fed-Batch mode in a shaker-incubator and subsequently purified from culture supernatants by affinity, size exclusion or mixed modal anion exchange followed by a final anion exchange chromatography. Antibody purity was assessed by size exclusion chromatography and SDS-PAGE.

VISTA sequence analysis and visualization

Multiple sequence alignments were performed with MultAlign[2] and visualized with EPScript[3]. 3D models were generated using UCSF Chimera[4].

sc-RNA-seq analysis of 68k PBMC dataset

To determine the cell types in which VISTA transcripts were most highly expressed, a publicly available 68k PBMC dataset was retrieved from the 10X Genomics website and processed using Seurat v3.2[5]. Cells with more than 6% mitochondrial transcripts, or less than 200 distinct transcripts were excluded. Data was normalized using default parameters and variable features identified using the VST method with 20,000 features. Data was subsequently scaled and then a PCA decomposition run with 15 principal components. A UMAP projection was carried out using default parameters and the FindClusters function run with a resolution of 0.8. To label the clusters the PBMC3k dataset[6] from the SeuratData package was used as a reference to map cell identities.

Epitope mapping by hydrogen-deuterium exchange mass spectrometry (HDXMS)

Epitope mapping by HDXMS was conducted using his-tagged human VISTA (residues 33-194, Sino Biological #13482-H08H) and HMBD-002 following procedures described previously[7]. Briefly, free VISTA was diluted in deuterated PBS with final deuterium oxide (D₂O) concentration at 90%. For VISTA/HMBD-002 complex, VISTA and HMBD-002 were mixed in 2:1 ratio and incubated for 15 mins at 25°C prior to deuterium labelling. Deuterium labelling reactions were carried out at 25°C for 1-, 10-, 30- and 100-min time points. Samples were then subjected to pepsin proteolytic cleavage followed by separation on an ACQUITY C18 column (1.0 x 100 mm) by nanoACQUITY UPLC (Waters) and detection by Synapt G2-Si mass spectrometer (Waters), operated in HDMS^E mode. Peptide identification and deuterium uptake monitoring were respectively performed using Protein Lynx Global Server 3.0.1 and DynamX 3.0 (Waters). Deuterium uptake for the peptides were calculated as differences in masses of the centroids of deuterated and undeuterated samples and reported as an average of triplicate measurements.

Epitope Binning

For epitope binning, human VISTA-His, or mouse VISTA-his recombinant protein (Sino Biological Inc.) in PBS was immobilized to Anti-Penta His sensor (HIS1K, Molecular Device) on an Octet QK384 (Molecular Device) instrument, for 5 mins. Sensors were briefly washed in PBS for 30s before loading 400 nM saturating antibody in PBS for 10 mins at a shake speed of 1,000 rpm. Subsequently, biosensors were washed for 2 min before immersing in 400 nM competing antibody in PBS for 7.5 mins at a shake speed of 1,000 rpm. Binding events which are correlated to change in wavelength (nm shift) reported from sensorgram, are monitored at the detector in real time.

Cross-species antibody binding affinity measurement

The affinity of HMBD-002 was determined by Surface Plasmon Resonance (SPR) using a Biacore. The assay was performed using a CM5 sensor chip (Cytiva #29104988). A Biacore HIS capture kit (GE Healthcare #28-9950-56) was employed to immobilize human, cynomolgus monkey, rat and mouse VISTA-HIS on-chip surface or left alone for background signal correction. Briefly, VISTA-HIS was captured for a contact time of 60 s at a flow rate of 5 μ l/min. HMBD-002 was flowed in two-fold serial dilutions from 50E-9 M to 390E-12 M for human, cynomolgus monkey and rat VISTA and from 12.5E-9 M to 390E-12 M for mouse VISTA, at a flow rate of 30 μ l/min for 90 sec association and 3000 sec dissociation at RT. The obtained sensograms were analyzed using Biacore T200 software and KD was calculated by fitting to a 1:1 binding kinetics model.

Antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity

96 well plates were coated with either 1 μ g/well of human C1q protein or 0.5 μ g/well of human CD16a in PBS at 4°C. Post overnight incubation, plates were washed thrice and blocked for 1 hr with blocking buffer at room temperature. Plates were incubated with HMBD-002 or isotype control for 1 hr at room temperature and incubated with 1:7000 dilution of HRP-conjugated anti-human Fc antibody for 1 hr at room temperature. Colorimetric reactions were developed using standard protocol as described for ELISA above.

VISTA-LRIG1 Inhibition Assay

VISTA-LRIG1 binding was confirmed using recombinant human VISTA-Fc protein (R&D #7126-B7) or irrelevant antigen (Human recombinant CD47 protein, Sinobiological #112283-HCCH) with standard ELISA method. For inhibition assay, 384-well plates were coated with 5 μ g/ml of human VISTA-Fc recombinant protein (R&D #7126-B7) diluted in PBS for 24 hrs at 4°C. After blocking for 2 hrs with 1% BSA at room temperature, plates were

incubated with either HMBD-002 or isotype control (Biolegend #403702) for 60 mins at room temperature. After 60 mins, recombinant human LRIG1.HIS at 1 µg/ml (EC₅₀ of VISTA-LRIG1 binding) was added for 2 hrs. Post incubation, plates were washed three times with TBST and incubated with anti-HIS HRP (Abcam #ab1187) antibody for 1 hr at room temperature followed by three further washes. Colorimetric reactions were developed using standard protocol as described for ELISA above.

VISTA-PSGL-1 Inhibition Assay

VISTA-PSGL-1 binding was confirmed using recombinant human VISTA-HIS protein (Acro Biosystems #B75-H52H0) or irrelevant antigen (Rat recombinant HER3-HIS protein, Elabscience #PKSR030358) with standard ELISA method. For the inhibition assay, 384-well plates were coated with 5 µg/ml of human VISTA-HIS recombinant protein (Acro Biosystems #B75-H52H0) diluted in PBS for 24 hrs at 4°C. After blocking for 2 hrs with 1% BSA (pH6) at room temperature, plates were incubated with either HMBD-002 or isotype control for 60 mins at room temperature in 1% BSA (pH6). After 60 mins, biotinylated recombinant human PSGL-1-Fc (R&D #3345-PS) (Recombinant Human PSGL-1-Fc was biotinylated using Invitrogen kit #21455) at 9.5E-8 M (EC₈₀ of VISTA-PSGL-1 binding) was added for 2 hrs. Post incubation, plates were washed three times with TBST (pH6) and incubated with streptavidin HRP (R&D #DY998) antibody for 1 hr at room temperature followed by three further washes. Colorimetric reactions were developed using standard protocol as described for ELISA above.

Bulk RNA-seq

10 samples per condition (V4C26, V9, anti-PD1, and IgG4) were taken from the MLR assay 96 hours after treatment. These were prepared for RNA-seq using the NEBNext Ultra II RNA library preparation kit (NEB #E7770S) and run on an Illumina NovaSeq S4 flowcell with v1.5

chemistry. Adaptor-trimmed and filtered data was evaluated using FASTQC and MultiQC[8] and reads aligned to the GRC37 human reference transcriptome using Kallisto v0.46[9]. Data was imported to R using TxImport. Normalized gene counts and gene-level differential expression was obtained using DESeq2[10]. Pathway activity was estimated using the SPEED2[11] package using default parameters.

CT26 tumor profiling

Single cell suspensions were generated by digestion of finely cut tumor tissue with 0.1 mg/ml of DNase I (Sigma #11284932001) and 1 mg/ml Collagenase (Sigma #1108866001) for 45 minutes at 37°C. Fc receptors were blocked with Human TruStain FcX (BioLegend #422302). Cells were stained with fluorophore conjugated antibodies for markers of immune cells (Table S5), washed and resuspended in buffer (1xPBS + 0.5% BSA + 1mM EDTA). Frequency of immune cell populations was determined via flow cytometry. Data was acquired on MACSQuant 10 (Milteny #130-096-343) and analyzed using FlowLogic software V7.

CT26 antigen recall assay

Single cell tumor suspensions were generated as described earlier. TILs (CD45+) or T cells (CD4+/CD8+) were enriched from the cell suspension using CD45 MicroBeads (Milteny #130-110-618) or CD4/CD8 MicroBeads (Milteny #130-116-480) as per manufacturer's protocol. CT26 cells (T: target cells), seeded 24 hrs prior, were co-cultured with enriched TILs or T cells (E: effector cells) from tumors for 72 hours at effector to target (E:T) cell ratios as indicated. All conditions were in triplicates. Cell viability was determined by CellTiter Glo (Promega#G7571). IFN- γ levels in supernatants were determined by ELISA (Invitrogen#BMS606). Lysis was calculated as: % Lysis = $((T - E:T)/T)*100$.

Pharmacokinetics

Pharmacokinetic profile of HMBD-002 was evaluated in male and female Balb/c mice, Sprague Dawley rats and cynomolgus monkeys. HMBD-002 was administered in a single dose at the indicated concentration via IP injection in mice and IV bolus injection into the tail vein in rats. For cynomolgus monkeys, HMBD-002 was administered either once at doses of 1, 10, or 100 mg/kg or twice one week apart (Days 1 and 8) at a dose of 10 mg/kg/week by IV bolus injection into the peripheral vein. Blood was drawn at different timepoints post dosing and antibody concentration in the serum was quantified by ELISA. The parameters for the pharmacokinetic analysis were derived from a non-compartmental model: maximum concentration (C_{max}), AUC (0-336hr), AUC (0-infinity), half-life (t_{1/2}), clearance (CL) and volume of distribution at steady state (V_{ss}).

SUPPLEMENTARY TABLES

Table S1: Top low energy template interfaces predicted by PRISM, utilizing the VISTA and PD-L1 structures (PDB IDs 60IL, 3BIK respectively)

| PRISM Pseudo energy Score | Template |
|---------------------------|----------|
| -56.25 | 2dpfCD |
| -34.28 | 3b76AB |

Table S2: Reconstitution composition for CD34+ humanized mice.

| CDX Model | Mouse ID# | %hCD45 | % Relative to hCD45 | | | | |
|-----------|-----------|--------|---------------------|----------------|----------------|-------|-------|
| | | | hB cells | hCD14 Monocyte | Total hT cells | hCD4T | hCD8T |
| HCT15 | 1532 | 13.4% | 54.0% | 0.4% | 39.6% | 26.8% | 9.6% |
| | 1533 | 13.9% | 73.4% | 1.6% | 16.8% | 13.5% | 3.0% |
| | 1534 | 16.1% | 71.4% | 2.0% | 18.1% | 13.6% | 3.7% |
| | 1535 | 14.1% | 64.4% | 0.9% | 22.7% | 16.6% | 5.7% |
| | 1536 | 17.6% | 57.1% | 1.9% | 35.4% | 27.6% | 7.5% |
| | 1537 | 15.4% | 64.3% | 2.9% | 23.6% | 17.6% | 6.0% |
| | 1538 | 11.3% | 48.2% | 1.8% | 43.1% | 25.9% | 15.7% |
| | 1539 | 14.6% | 35.2% | 3.3% | 50.5% | 41.8% | 7.4% |
| | 1540 | 12.0% | 68.2% | 2.4% | 16.3% | 11.9% | 3.9% |
| | 1541 | 26.2% | 47.8% | 0.7% | 45.7% | 34.1% | 11.3% |
| A549 | 1544 | 23.9% | 54.8% | 2.3% | 35.3% | 29.7% | 5.4% |

| | | | | | | | |
|--|------|-------|-------|------|-------|-------|-------|
| | 1545 | 23.9% | 53.7% | 4.1% | 36.0% | 26.8% | 8.8% |
| | 1546 | 18.7% | 63.9% | 1.1% | 29.1% | 21.3% | 7.0% |
| | 1547 | 15.2% | 30.7% | 6.8% | 55.3% | 40.5% | 14.2% |
| | 1548 | 31.6% | 50.5% | 1.6% | 39.4% | 29.0% | 9.7% |
| | 1549 | 42.8% | 56.1% | 3.9% | 30.9% | 22.1% | 7.5% |
| | 1550 | 44.2% | 56.5% | 2.9% | 29.9% | 22.2% | 6.8% |
| | 1551 | 19.6% | 41.0% | 3.2% | 50.5% | 41.6% | 8.5% |
| | 1552 | 23.8% | 58.2% | 1.4% | 32.7% | 24.8% | 7.3% |
| | 1553 | 17.9% | 45.5% | 3.3% | 43.2% | 32.7% | 9.8% |

Table S3: Clinical observations, food consumption, body weight, and clinical pathology findings during single dose intravenous tolerability study in Sprague-Dawley rats.

| Endpoints Evaluated | Results |
|-----------------------|---|
| Clinical Observations | <ul style="list-style-type: none"> • No test article-related clinical signs observed |
| Food Consumption | <ul style="list-style-type: none"> • Variations observed in food consumption of animals were of minimal magnitude and lacked a dose-response relationship • No test article-related changes observed in food consumption of female rats • Decreased food consumption observed for male rats in 1 mg/kg/dose group, however, relationship to test article unlikely due to lack of dose-response relationship, and absence of similar observation in females |
| Body Weight | <ul style="list-style-type: none"> • Body weight variations observed in animals were of minimal magnitude and lacked a dose-response relationship • No test article-related changes observed in body weights of female rats • 15% change in body weight observed for male rats in 1 mg/kg/dose group, however, relationship to test article was unlikely due to lack of dose-response |

| | |
|--|--|
| | relationship, and absence of similar observation in females |
| <p>Hematology:</p> <p>Leukocyte counts, Erythrocyte count, Hemoglobin, Hematocrit, Mean corpuscular volume, Mean corpuscular hemoglobin concentration, Reticulocyte count, RBC distribution width, Platelet count, Mean platelet volume, absolute and percent WBC Differential (Neutrophils, Eosinophils, Basophils, Monocytes, Lymphocytes), absolute Large unstained cells, percent Large unstained cells and percent Reticulocyte count</p> | <ul style="list-style-type: none"> • No test article-related changes observed in hematology parameters • All differences observed were considered incidental due to lack of a dose-response relationship, minimal magnitude and/or the individual values were within the historical control data range |
| <p>Serum Chemistry:</p> <p>Alanine Aminotransferase, Aspartate Aminotransferase,</p> | <ul style="list-style-type: none"> • No test article-related changes observed in serum chemistry • All differences observed were considered incidental due to lack of a dose-response relationship, minimal |

| | |
|---|--|
| Total Protein, Albumin, Globulin, Albumin/Globulin Ratio, Alkaline Phosphatase, γ -glutamyltransferase, Glucose, Urea, Creatinine, Calcium, Phosphorus, Total Cholesterol, Triglycerides, Total Bilirubin, Sodium, Potassium, Chloride, Creatine Kinase and C-reactive protein | magnitude and/or the individual values were within the historical control data range |
|---|--|

Table S4: Clinical observations, food consumption, body weight, and clinical pathology findings during single dose intravenous tolerability study in cynomolgus monkeys.

| Endpoints Evaluated | Results |
|--|--|
| Clinical Observations | <ul style="list-style-type: none"> • No test article-related clinical signs observed |
| Food Consumption | <ul style="list-style-type: none"> • No test article-related changes observed in food consumption |
| Body Weight | <ul style="list-style-type: none"> • No test article-related changes observed in body weights |
| Hematology: Leukocyte counts, Erythrocyte count, Hemoglobin, Hematocrit, Mean corpuscular volume, Mean corpuscular hemoglobin concentration, Reticulocyte count, RBC distribution width, Platelet count, Mean platelet volume, absolute and percent | <ul style="list-style-type: none"> • No test article-related changes observed in hematology parameters • All differences observed were considered incidental due to lack of a dose-response relationship, minimal magnitude and/or the individual values were within the historical control data range |

| | |
|--|--|
| <p>WBC Differential (Neutrophils, Eosinophils, Basophils, Monocytes, Lymphocytes), absolute Large unstained cells, percent Large unstained cells and percent Reticulocyte count</p> | |
| <p>Serum Chemistry:</p> <p>Alanine Aminotransferase, Aspartate Aminotransferase, Total Protein, Albumin, Globulin, Albumin/Globulin Ratio, Alkaline Phosphatase, γ-glutamyltransferase, Glucose, Urea, Creatinine, Calcium, Phosphorus, Total Cholesterol, Triglycerides, Total Bilirubin, Sodium, Potassium, Chloride, Creatine Kinase and C-reactive protein</p> | <ul style="list-style-type: none"> ● No test article-related changes observed in serum chemistry ● All differences observed were considered incidental due to lack of a dose-response relationship, minimal magnitude and/or the individual values were within the historical control data range |

Table S5: List of antibodies.

| Antibody | Company and Catlog |
|--------------------|--|
| Anti-mouse MHCI | Invitrogen #48-5321-82 |
| Anti-mouse Gr1 | BioLegend #108437 |
| Anti-mouse CD19 | BioLegend #152408 |
| Anti-mouse CD3 | BioLegend #100219, BioLegend #100236 |
| Anti-mouse CD11b | BioLegend #101211, BioLegend #101206, Milteny #130-113-243 |
| Anti-mouse CD45 | BioLegend #103154 |
| Anti-mouse Ly-6C | BioLegend #128006 |
| Anti-mouse Ly-6G | BioLegend #127618 |
| Anti-mouse F4/80 | BioLegend #123109 |
| Anti-mouse CD11c | BioLegend #117310 |
| Anti-mouse CD44 | BioLegend #103020 |
| Anti-mouse CD4 | BioLegend #100406 |
| Anti-mouse CD8 | BioLegend #100708 |
| Anti-mouse CD25 | BioLegend #102061 |
| Anti-mouse CD206 | BioLegend #141720 |
| Anti-mouse PD1 | BioLegend #135241 |
| Human CD45 | Biologend # 304006 |
| Mouse CD45 | Biologend #103108 |
| Rat CD45 | Biologend # 202205 |
| Anti NHP CD45 | BD Pharmingen # 557803 |
| Human/Rhesus CD11b | Biologend # 301306 |
| Mouse CD11b | Biologend # 101208 |
| Rat CD11b | Biologend # 201807 |

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