# Simulation of Phase 1 Trial Design PD-1 mAb

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# *Let's assume: Power911*

- A fully ***human monoclonal antibody*** generated in VelocImmune mice that contains a human light chain variable domain fused to human kappa constant and a heavy chain variable regions based on IgG4 Fc format.
- High-affinity anti–PD-1 antibody that potently blocks PD-1/ PD-L1 functional interaction.
- Enhances human primary T-cell responses in vitro and inhibits the growth of syngeneic colorectal carcinomas in mice genetically engineered to express a human/mouse PD-1 chimeric receptor from the mouse locus
- Of note, human PD-1 is capable of interacting with mouse PDL1 ligand, and cell type and tissue-specific expression of PD-1 is conserved between mouse and human, this strategy allowed for the first time to evaluate the preclinical activity of a human PD-1–blocking antibody, which does not bind mouse PD-1, in vivo.

# *Summary of Power911 pre-clinical biology*

- Power911 binds to PD-1 with high affinity and specificity inhibits PD-1 binding to PD-L1 and PD-L2 ligands
- Power911 induced a dose-dependent increase in T-cell proliferation with similar average/median $EC_{50}$ in 8 tested donors.
- Power911 does not result in TCR-independent T-cell activation.
- Power911 do not mediate ADCC or CDC activity, indicating unlikely to cause the depletion of PD-1–expressing cells.
- Human PD-1 occupancy was not increased with the higher dose (25 mg/kg), a dose of 10 mg/kg appears to be sufficient to occupy human PD-1 in humanized PD-1 mice.
- Power911 showed potent dose-dependent tumor growth inhibition in human PD-1 knock-in mice engrafted withMC38.Ova cells with best efficacy at 10 mg/kg, and less efficacy at 3mg/kg and 1mg/kg.

# *Summary Power911 pre-clinical PK and Toxicity*

- Mean beta phase half-lives (t1/2 beta) were comparable across the 1, 5, and 15 mg/kg groups. (9.84±1.13 days, to 12.4±1.67 days)
- There was one mortality each after multiple injections at the 10 mg/kg and 50mg/kg dose levels attributed to pulmonary hemorrhage and edema considered to be secondary to immunogenicity.
- Power911 induced an increase in the incidence and/or severity of multi-organ mononuclear cell infiltration
- Other potential target organs included the spleen, eye (corneal hyperkeratosis), and cecum (erosion and mixed cell inflammation)
- There were no substantial drug-related effects on fertility parameters or the reproductive tract at any of the dose levels tested.
- There is concern that treatment with Power911 may increase susceptibility to tuberculosis infection and/or that infected patients may develop more severe disease.
- Toxicokinetic $T_{1/2}$ 13.5-19.3 days

# *Affinity & Specificity*

- Power911 binds to PD-1 with high affinity and specificity inhibits PD-1 binding to PD-L1 and PD-L2 ligands, and does not induce ADCC or CDC
  - 6.11nmol/L for monomeric human PD-1-mmH and 628 pmol/L for dimeric human PD-1-mFc proteins.
  - Similarly potent binding for monomeric and dimeric forms of cynomolgus monkey PD-1 recombinant proteins.
  - The 10-fold tighter binding of Power911 to dimeric versus monomeric human or monkey PD-1 proteins likely reflects avidity-driven interactions.
  - Do not bind to monomeric rat and mouse PD-1

**Table 1.** Power911 binds with high affinity to human and cynomolgus monkey PD-1

| | Biacore kinetic parameters for Power911 binding to soluble PD=1 ectodomain at 25℃ | | | |
|---|---|---|---|---|
| **Test ligand** | $k_a$ ($M^{-1}s^{-1}$) [a] | $k_d$ ($s^{-1}$) [b] | $K_D$ (M) [c] | $T_{1/2}$ (min) [d] |
| Human PD-1-mmH | $1.59 \times 10^5$ | $9.72 \times 10^{-4}$ | $6.11 \times 10^{-9}$ | 11.9 |
| Human PD-1-mFc | $3.17 \times 10^5$ | $1.99 \times 10^{-4}$ | $6.28 \times 10^{-10}$ | 58.0 |
| Monkey PD-1-mmH | $1.36 \times 10^5$ | $1.01 \times 10^{-3}$ | $7.43 \times 10^{-9}$ | 11.4 |
| Monkey PD-1-mFc | $3.14 \times 10^5$ | $1.64 \times 10^{-4}$ | $5.20 \times 10^{-10}$ | 70.6 |
| Rat PD-1-mmH | – | – | NB | NB |
| Mouse PD-1-mmH | – | – | NB | NB |

NOTE: Human or cynomolgus monkey monomeric PD-1-mmH (myc-myc-hexahistidine tag) or dimeric PD-1-mFc proteins, as well as rat or mouse monomeric PD-1-mmH, were injected across a low-density anti–hFc-captured Power911 chip surface.

Abbreviation: NB, No detectable binding under the assay conditions tested.

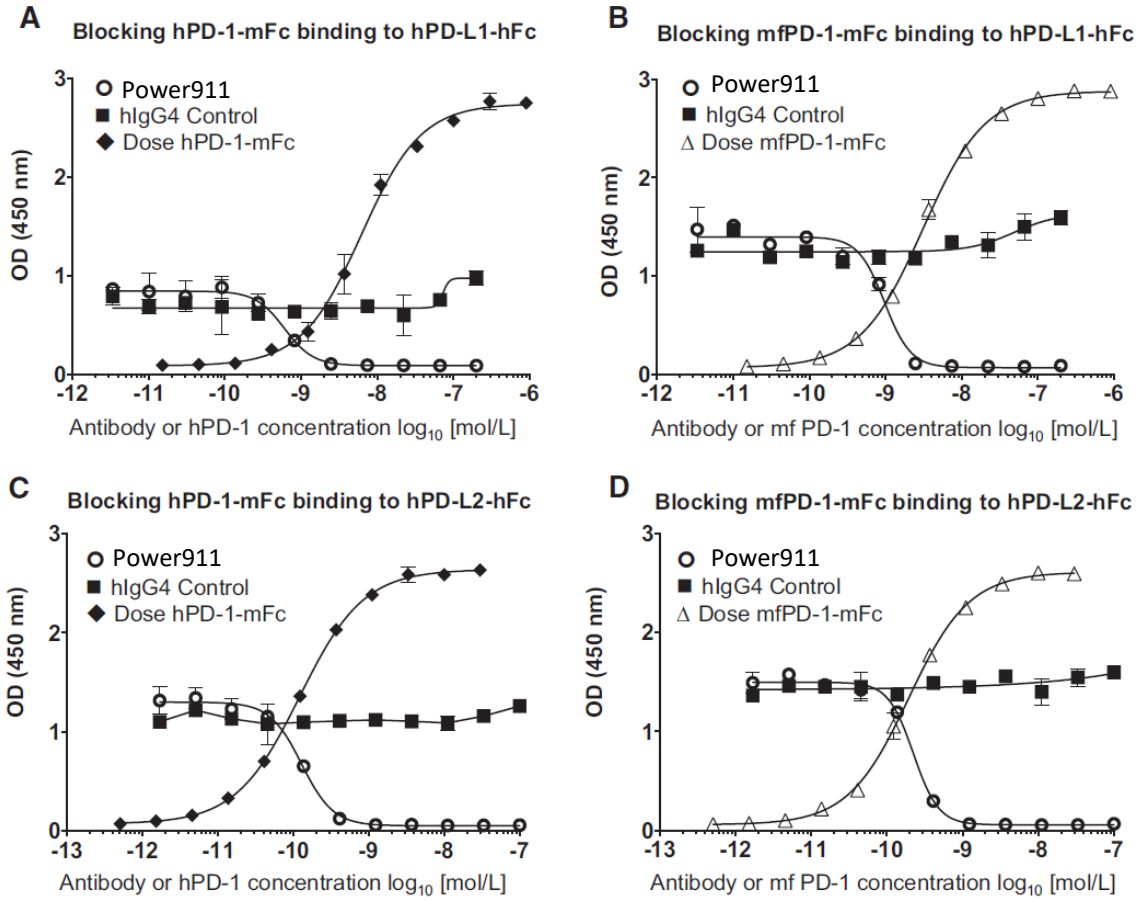
[a]Association rate constant.

[b]Dissociation rate constant.

[c]Equilibrium dissociation constant.

[d]Dissociation half-life $T_{1/2}$.

# *Affinity & Specificity*

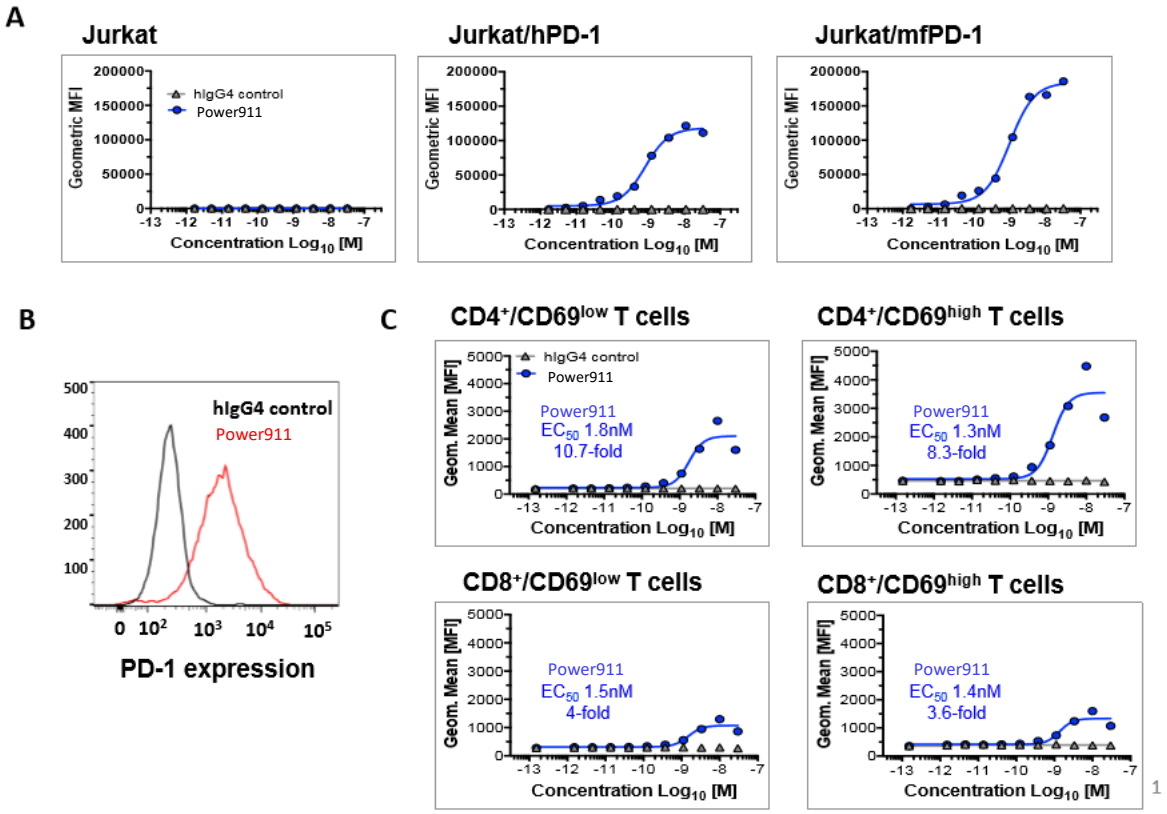



- Power911 inhibited both hPD-1-mFc and mfPD-1-mFc from binding to plate-bound hPD-L1-hFc with IC50 values of 0.60 nmol/L and 0.97 nmol/L, respectively (Fig. 1A and B).
- Similarly, Power911 prevented hPD-1-mFc and mfPD-1-mFc binding to hPD-L2-hFc with IC50 values of 0.13 nmol/L and 0.22 nmol/L, respectively (Fig. 1C and D).

**Figure 1.**
Power911 inhibits binding of human and monkey PD-1 to human PD-L1 and PD-L2. A and B, Binding of 1.5 nmol/L hPD-1-mFc (A) or 2.0 nmol/L mfPD-1-mFc (B) to plate-coated hPD-L1-hFc in the presence of increasing concentration of Power911 or an isotype control antibody. C and D, Binding of 0.1 nmol/L hPD-1-mFc (C) or 0.25 nmol/L mfPD-1-mFc (D) to plate-coated hPD-L2-hFc in the presence of increasing concentration of Power911 or an isotype control antibody. hPD-1, human PD-1; mfPD-1, cynomolgus monkey PD-1.

# *Affinity & Specificity*

**Figure S1.** Power911 binds to PD-1 on engineered Jurkat cells and on activated human and cynomolgus monkey T cells.

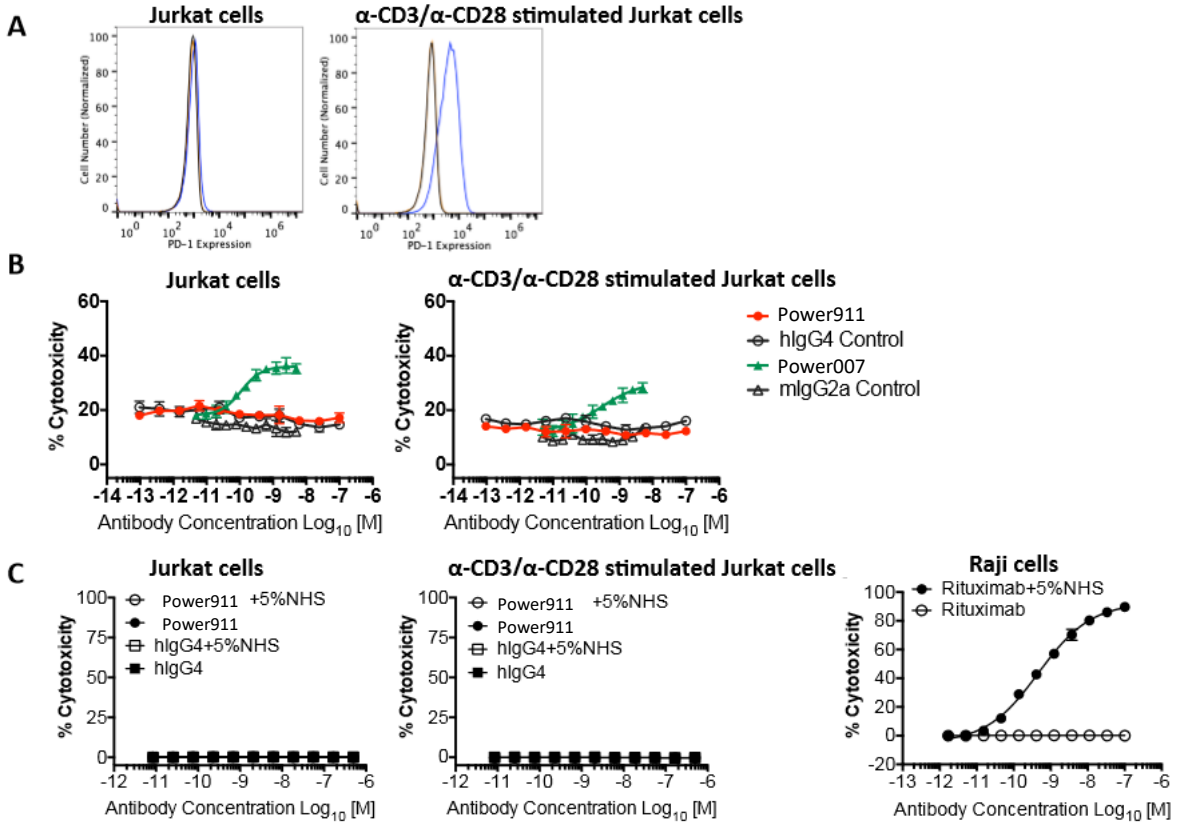



- Power911 bound to human Jurkat cells engineered to overexpress human or cynomolgus monkey PD-1 protein with a similar $EC_{50}$ values of approximately 0.8 nmol/L and 1 nmol/L, respectively (Supplementary Fig. S1A).
- Parental Jurkat cells showed minimal Power911 binding consistent with low levels of endogenous surface PD-1 expression.
- Flow cytometric analysis confirmed Power911 binding to PD-1 on activated primary human CD3þ T cells.
- Power911 bound PD-1 on activated cynomolgus monkey CD4þ and CD8þ T cells expressing either low or high level of the early activation marker CD69 with similar EC50 values ranging from 1.3 to 1.8 nmol/L.

A, Jurkat cells expressing either human or monkey PD-1 were incubated with Power911 (blue circles) or isotype control antibody (grey triangles) pre-complexed with Alexa 647 Fab anti-hIgG (Zenon® labeling kit, Molecular Probes). The x-axis indicates the antibody ($Log_{10}$) concentration and the y-axis the geometric MFI intensity of Alexa 647 cell staining.
B, purified human $CD3^+$ T cells were activated by human T-Activator CD3/CD28 beads for 48 hours and incubated with Power911. Power911 binding was detected by a secondary antibody against the human kappa chain.
C, activated primary monkey $CD4^+$ or $CD8^+$ T-cells were stained and analyzed as in A.

# *Power911: No ADCC/CDC induce*

**Figure S2.** Power911 does not induce ADCC or CDC in either unstimulated or CD3/CD28 stimulated Jurkat cells.




- Not observation of Power911-mediated ADCC or CDC activity, indicating that Power911 is unlikely to cause the depletion of PD-1–expressing cells.
- In addition, Power911 did not mediate complement-dependent cytotoxicity (CDC) in activated Jurkat cells incubated with human serum complement.

A, increased PD-1 expression on CD3/CD28 stimulated Jurkat cells. Jurkat cells remained unstimulated or were stimulated overnight with plate-coated anti-CD3 and anti-CD28 and stained with FITC-labeled anti-PD-1 antibody (blue histogram) or isotype control (black histogram).

B, unstimulated or CD3/CD28 stimulated Jurkat cells were incubated with NK92/CD16$^{176V}$ cells and increasing concentrations of Power911, hIgG4 isotype control Power302, positive control antibody Power007 (pan-HLA Class I, mIgG2a), or mIgG2a isotype control. Nonspecific cell killing was 18% (unstimulated Jurkat cells) and 12% (anti-CD3/anti-CD28 stimulated Jurkat cells) upon addition of the NK92/CD16$^{176V}$ cells to the respective target cells.

C, unstimulated or anti-CD3/anti-CD28 stimulated Jurkat cells were incubated with increasing concentrations of Power911 (circles) or an isotype control antibody (squares) with (open symbols) or without (closed symbols) 5% Normal Human Serum (NHS). Neither Power911 nor Power302 exhibited CDC against either target cell line. As a positive control, Raji target cells were incubated with 5% NHS and Rituximab (anti-CD20 hIgG1), which exhibited dose-dependent CDC activity.

# *In vitro result*
# *PD-1/PD-L1 inhibitory signals and T-cell activation in vitro*

- The ability of Power911 to enhance T-cell function was investigated using either engineered Jurkat T cells or preactivated primary human T cells, in combination with HEK293 APC-like cells engineered to express human CD20 with or without human PD-L1.
- Power911 induced a dose-dependent increase in T-cell proliferation with similar average and median $EC_{50}$ in 8 tested donors.
- In cell-based bioassays performed in the presence of suboptimal TCR engagement, nanomolar concentrations of Power911 effectively blocked PD-1/PD-L1 interactions and thereby increased TCR signaling in engineered Jurkat T cells and increased proliferation of primary activated human T cells.

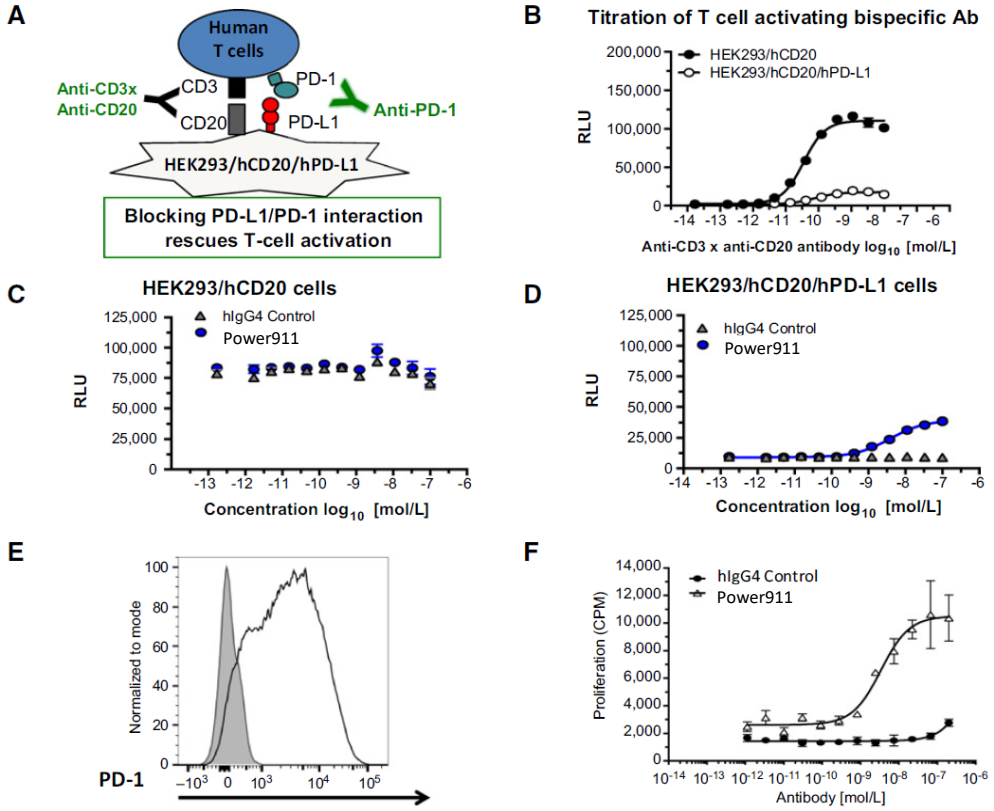
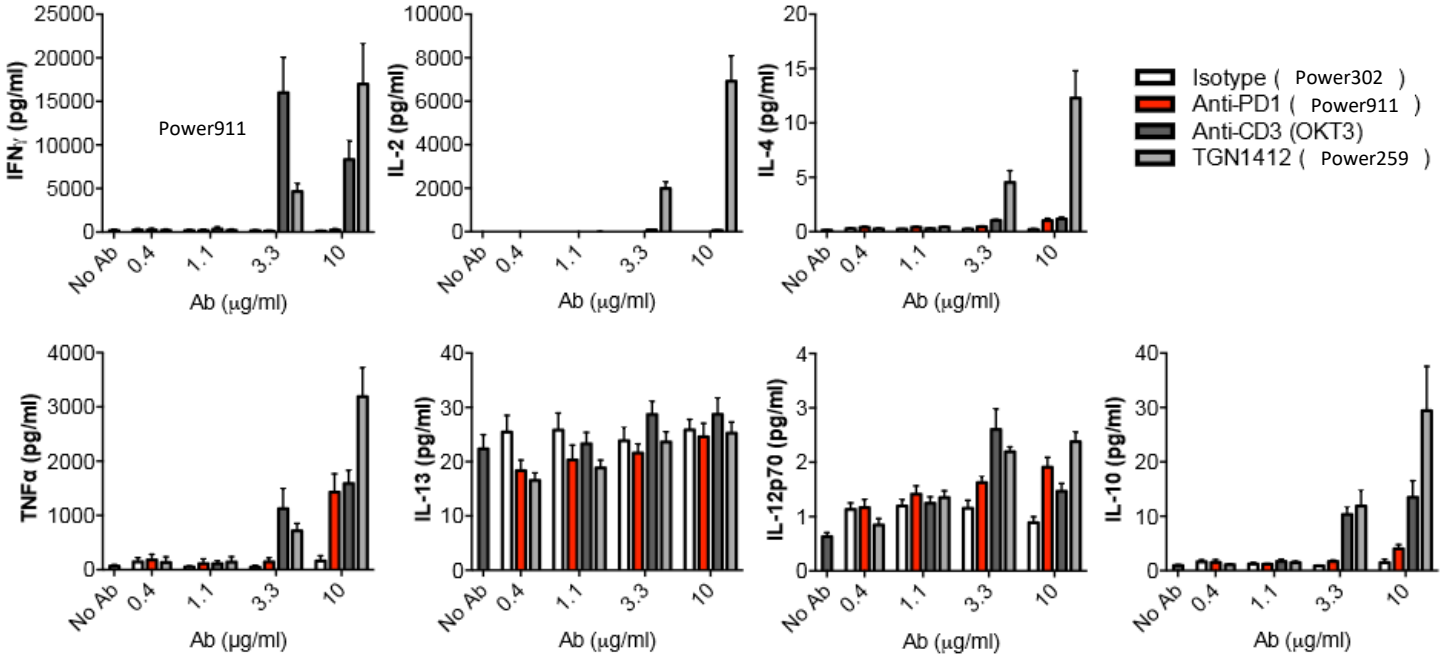



**Figure 2.**
Power911 blocks PD-1/PD-L1 inhibitory signaling in a T cells/engineered APC bioassay. A, Bioassay schematic: To evaluate PD-1/PD-L1 inhibition, engineered Jurkat/AP-1-Luc/hPD-1 cells or preactivated primary human CD4t T cells were incubated with HEK293/hCD20/hPD-L1–engineered APC in the presence of an anti-CD3 x anti-CD20 bispecific antibody. B, Jurkat /AP-1-Luc/PD-1 cells activation by anti-CD3 x anti-CD20 bispecific antibody in the presence of HEK293/hCD20 or HEK293/hCD20/hPD-L1 cells. C and D, Power911 rescues PD-1 inhibition in Jurkat/AP-1-Luc/hPD-1 cells in the presence of 100 pmol/L anti-CD3 x anti-CD20 bispecific Ab and HEK293/hCD20/hPD-L1 (D), but not HEK293/hCD20 cells (C). The x axis indicates concentration of antibodies (Log10), and the y axis indicates the emitted light by the luciferase reaction expressed in RLU. E, staining for PD-1 expression on preactivated primary CD4t T cells with anti–PD-1 APC (clone EH12.247, black line) or isotype control antibody APC (gray filled line). F, Power911 rescues CD4t T-cell proliferation inhibited by HEK293/hCD20/hPD-L1 cells.

# *Power911 does not result in TCR-independent T-cell activation*

**Figure S3.** Cytokine release following human PBMC exposure to immobilized Power911 , Power259 (TGN1412) and anti-CD3 (OKT3) antibodies.

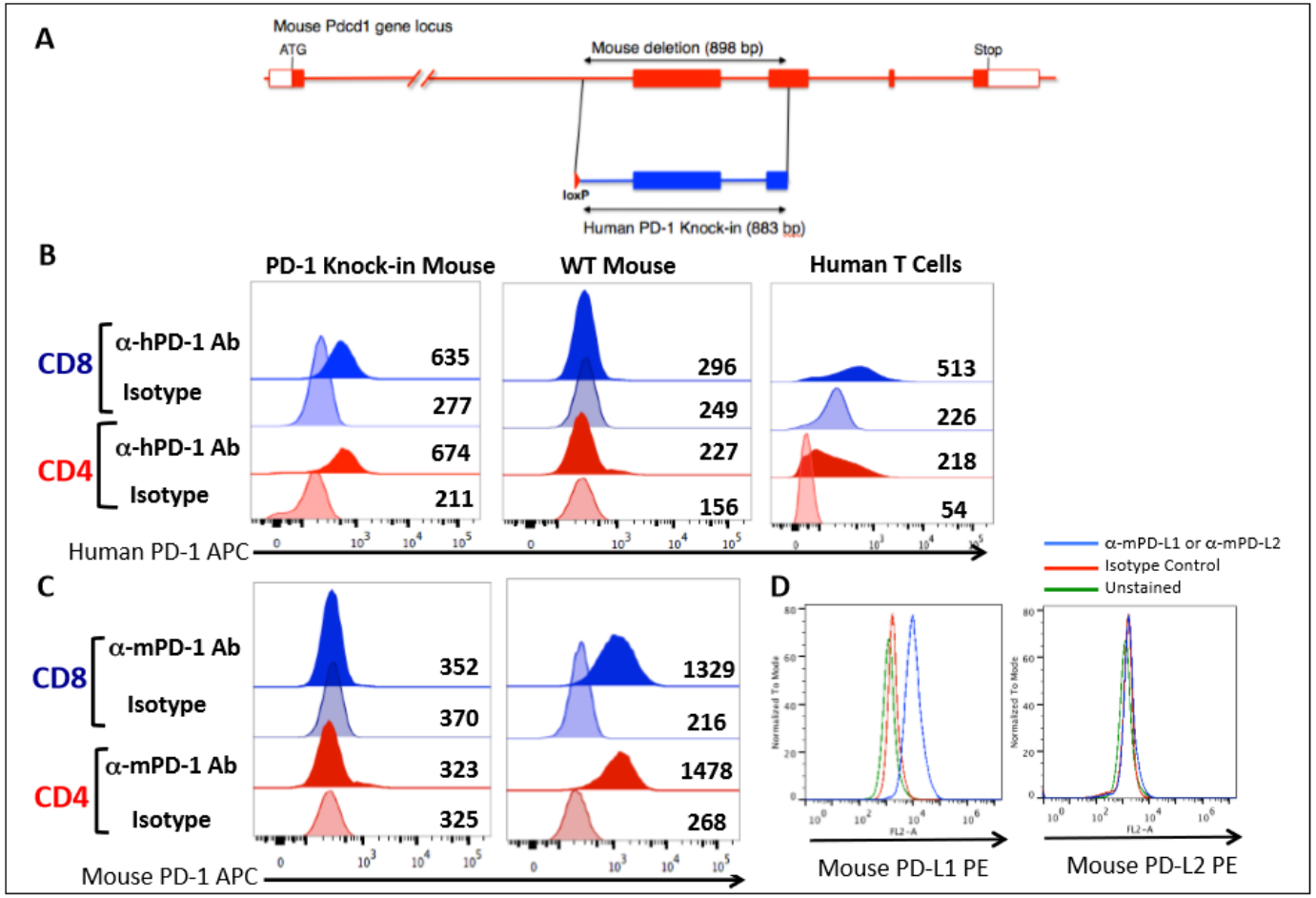



- There was no significant release of inflammatory cytokines by human PBMCs cultured in plates with immobilized Power911.
- Power911 does not result in TCR-independent T-cell activation.

Freshly isolated PBMCs (125,000 cells/well) were incubated for 18 hours with immobilized antibodies. Power911, Power259 (TGN1412, positive control), anti-CD3 (OKT3 clone, positive control) and Power302 (hIgG4 isotype control) were immobilized by air-drying as previously described. PBMCs culture supernatants were collected and cytokines measured using MSD Multi-Spot Assay System Proinflammatory Panel 1 (human) kit (Meso Scale Discovery). The values (pg/ml) for IL-2, IFNg, TNFα, IL-12p70, IL-4, IL-10 and IL-13 are mean ± S.E.M. of 12 donors.

# *Functional replacement of mouse PD-1 with human homologue*

**Figure S4.** Expression of human PD-1 on activated T cells from human PD-1 knock-in mice and expression of PD-L1 on MC38.Ova cells.




Collectively, the chimeric PD-1 protein containing a human ectodomain is functional and human PD-1 knock-in mice can be used to evaluate Power911 in vivo.

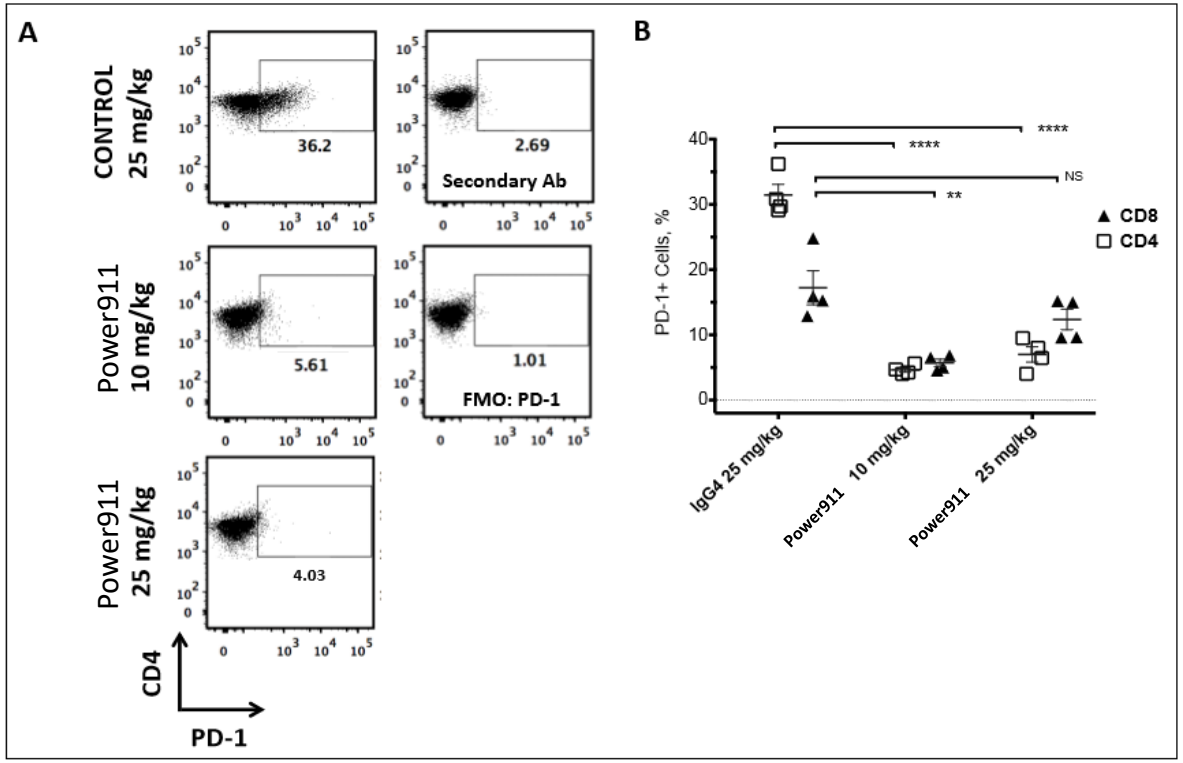
A, schematic illustrating the replacement of the mouse extracellular PD-1 domain with the human homologue in human PD-1 knock-in mice.

B-C, Splenocytes from C57BL/6 mice homozygous for human PD-1 knock-in allele or wild type (WT) C57BL/6 control mice were stimulated with anti-CD3 (1 μg/ml) and anti-CD28 (1 μg/ml) antibodies for 72 hrs, and subsequently stained with LIVE/DEAD Fixable Aqua stain (Invitrogen), and antibodies to mouse CD8, mouse CD4, mouse PD-1 (clone J43, eBioscience), human PD-1 (clone MIH4, BD Biosciences), or isotype control antibodies. B, human PD-1 is detected on stimulated $CD8^+$ and $CD4^+$ T cells from human PD-1 knock-in mice (left panel), but not from WT mice (middle panel). Human $CD8^+$ and $CD4^+$ T cells were used as positive controls for PD-1 expression (right panel). $CD8^+$ and $CD4^+$ T cells purified from peripheral blood were activated using CD3/CD28 Dynabeads® (Invitrogen) and stained for human CD8, human CD4 and human PD-1. C, mouse PD-1 protein is expressed on stimulated $CD8^+$ and $CD4^+$ T cells from WT mice (right panel), but not from human PD-1 knock-in mice (left panel). Numbers indicate geometric MFI intensity of APC cell staining.

D, MC38.Ova cells express mouse PD-L1 (left panel), but not mouse PD-L2 (right panel). MC38.Ova cells were stained with LIVE/DEAD Fixable Aqua stain (Invitrogen), and antibodies to mouse PD-L1 (clone 10F.9G2, Biolegend), mouse PD-L2 (clone TY25, Biolegend), or isotype control antibodies.

# *Power911 binds PD-1 in human PD-1 knock-in mice and inhibits tumor growth*

**Figure S5.** Power911 binds to PD-1 on T cells in tumor-bearing human PD-1 knock-in mice.

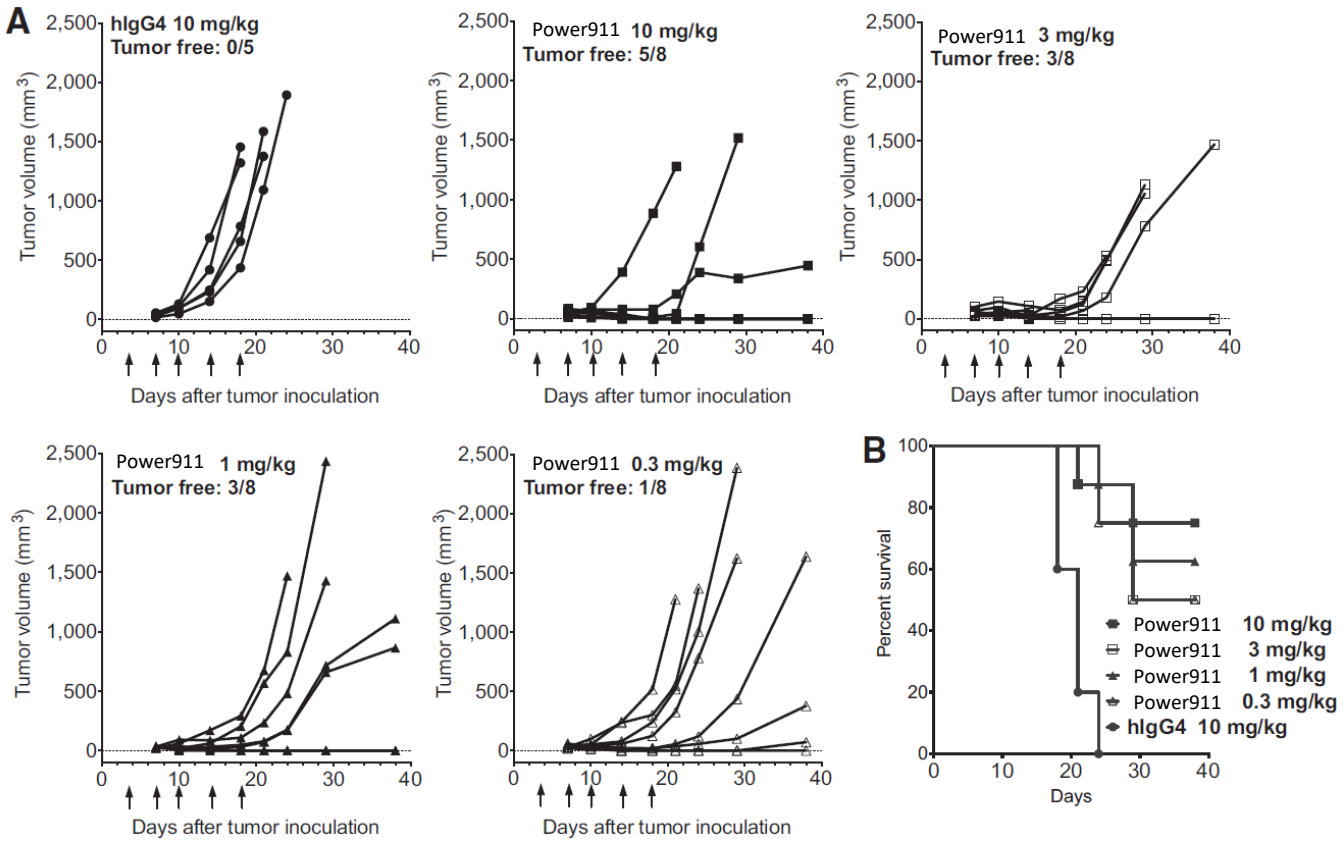



A, PD-1 expression on splenic $CD4^+$ T cells from MC38.Ova tumor bearing mice treated with the indicated doses of Power911 or isotype control antibody on day 0 (at tumor volumes 100-120 $mm^3$), day 3, and day 6. Splenocytes were analyzed on day 10.

B, percentage of PD-1-expressing $CD4^+$ and $CD8^+$ T cells in treated animals (n=4/group). **, $p<0.05$; ****, $p<0.0005$ (unpaired t-test).

- In mice that received Power911 at 25 mg/kg or 10 mg/kg, respectively, the frequency of PD-$1_t$ cells was reduced to 7.0% and 4.6% for $CD4_t$ T cells, and 12.4% and 5.7% for $CD8_t$ T cells, suggesting that human PD-1 binding sites were occupied by Power911 antibodies in vivo.
- Because the human PD-1 occupancy was not increased with the higher dose (25 mg/kg), a dose of 10 mg/kg appears to be sufficient to occupy human PD-1 in humanized PD-1 mice.

# *Power911 binds PD-1 in human PD-1 knock-in mice and inhibits tumor growth*

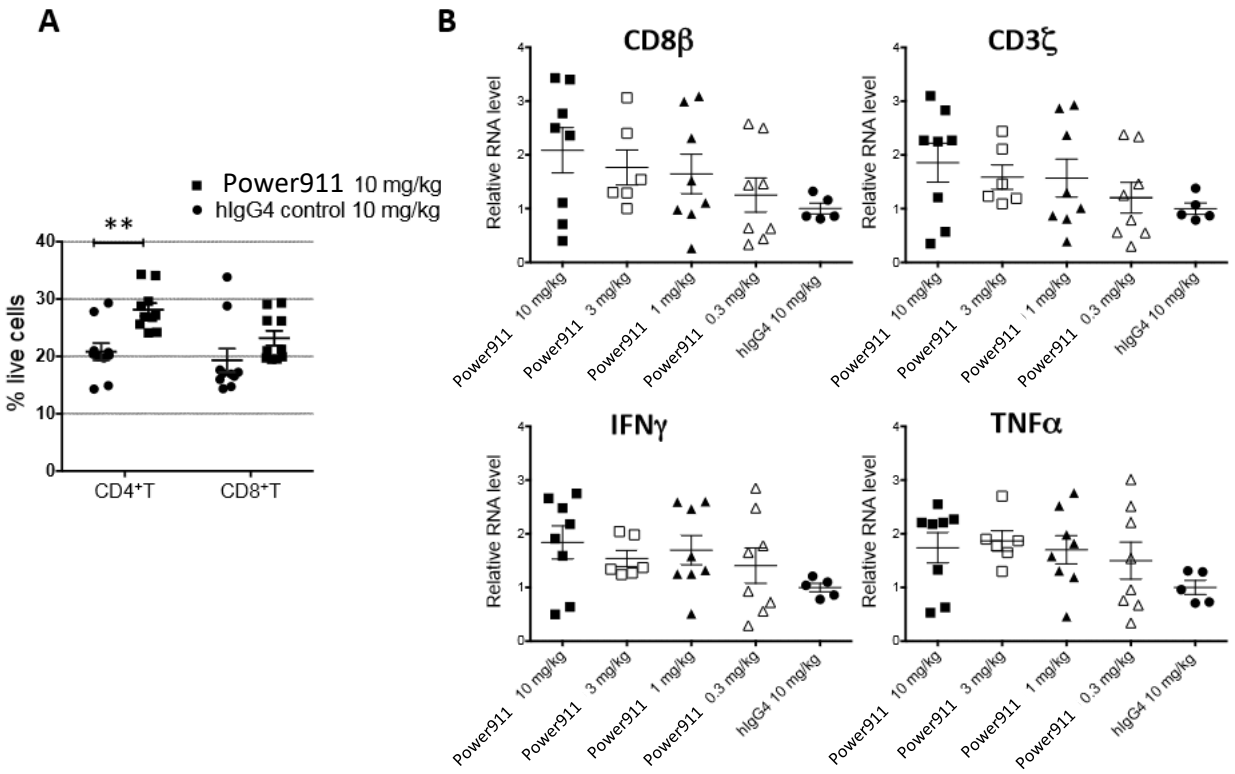



- In the minimal tumor model, human PD-1 knock-in mice engrafted withMC38.Ova cells were treated with Power911 doses ranging from 0.3 mg/kg to 10 mg/kg, starting on day 3, before the predicted appearance of measurable tumors.
- Power911 showed potent dose-dependent tumor growth inhibition, and at 10 mg/kg, 5/8 mice were tumor free, whereas none of the isotype control–treated animals were tumor-free (Fig. 3A). At 3 mg/kg and 1 mg/kg, Power911 was slightly less efficacious, with 3/8 tumor-free mice at the end of the study on day 38.
- All Power911-treated groups showed prolonged survival ($P < 0.00001$), most evident at 10 mg/kg dose (Fig. 3B).

**Figure 3.**
Power911 therapy inhibits tumor growth and improves survival of tumor-bearing human PD-1 knock-in mice. A, growth kinetics of MC38.Ova tumors in a minimal disease model. Mice were engrafted s.c. into the flank with MC38.Ova cells ($5\times10^5$ cells/mouse) on day 0. Mice were treated i.p. with Power911 (10 mg/kg, 3 mg/kg, 1 mg/kg, or 0.3 mg/kg; n=8/group) or isotype control antibody (10 mg/kg; n=5) on days 3, 7, 10, 14, and 18, and tumor volumes were monitored until day 38. Mice were euthanized at maximum allowed tumor burden. The number of tumor-free animals on day 38 is shown for each treatment group. B, Kaplan–Meier survival curves of mice treated with Power911 or control antibody. A log-rank (Mantel–Cox) test revealed that Power911 antibodies significantly prolonged mouse survival ($P < 0.00001$).

# *Power911 therapy enhances adaptive immune responses in vivo*

**Figure S6. Power911** therapy enhances adaptive immune responses *in vivo*.

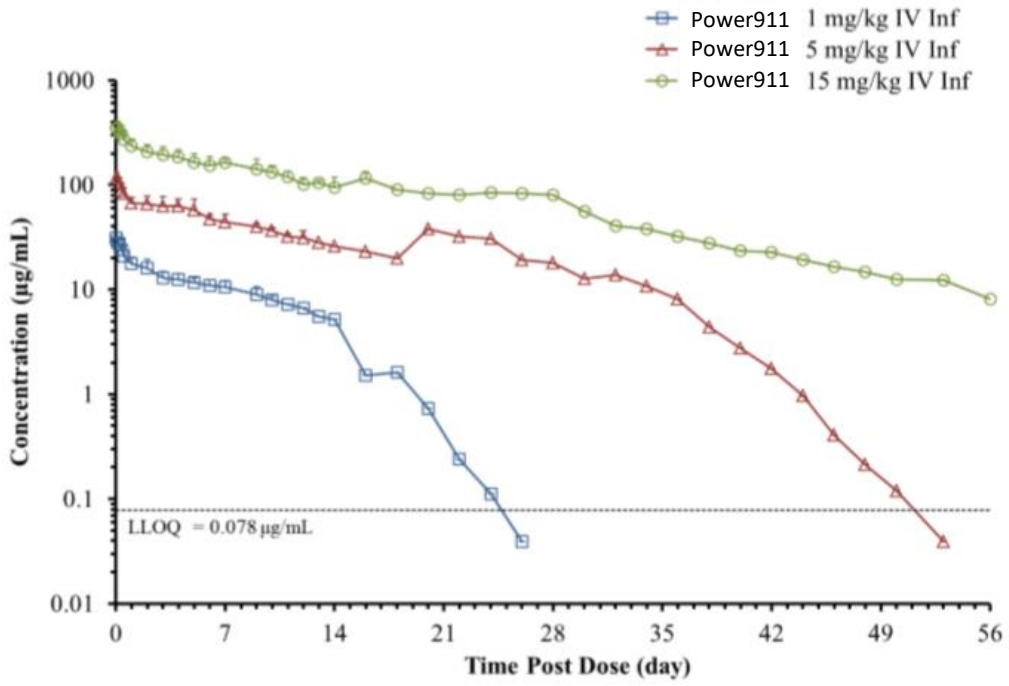



- Flow cytometric analysis of draining lymph nodes revealed an increased frequency of $CD4^+$ and $CD8^+$ T cells in mice treated with Power911 (Supplementary Fig. S6A).
- Taqman analysis of spleens revealed increased transcript levels for CD8, CD3, IFNg, and TNFα in Power911-treated mice, suggesting an increase in $CD8^+$ effector T cells and effector function (Supplementary Fig. S6B).
- These results further validate that PD-1 signaling is intact in human PD-1 knock-in mice and confirm the immune-enhancing function of Power911 in vivo.

A, Power911 promotes T cells expansion in draining lymph nodes of tumor-bearing human PD-1 knock-in mice. Mice were engrafted with MC38.Ova cells ($5x10^5$ cells/mouse) s.c. into the flank on day 0, and injected i.p. with Power911 (10 mg/kg, n=10) or isotype control antibody (10 mg/kg, n=10) on days 3, 7, 10, 14, and 18. On day 20, cells isolated from draining lymph nodes were stained with LIVE/DEAD Fixable Aqua stain (Invitrogen), and antibodies to mouse CD4 (clone GK1.5) and mouse CD8 (clone 53-6.7). Data are shown as percentage of live cells and error bars represent S.E.M. **, $p<0.01$ (unpaired t-test).
B, Power911 promotes CD8+ T cells expansion in spleens of tumor-bearing human PD-1 knock-in mice. Mice were treated as in A and spleens were collected on day 38 (Figure 4). Spleen RNA was analyzed using Taqman real-time PCR with probes specific for mouse CD8b (probe: AGCAGCTCTGCCCTCAT, forward primer: GCTCTGGCTGGTCTTCAGTATG, reverse primer: TTGCCGTATGGTTGGTTTGAAC), mouse CD3z (Mm00446171_m1, Applied Biosystems), mouse IFNg (Mm01168134_m1, Applied Biosystems) a (Mm00443260_g1, Applied Biosystems). The graph depicts relative levels of CD8b, CD3z, IFNg, a RNA (normalized to mouse cyclophilin RNA) in the Power911 treatment group compared to the isotype control group (assigned a value of 1.0). Error bars represent S.D. Panels A and B show representative results of two independent experiments.

# *Concentration-time profiles of Power911*

**Figure S7:** Concentration-time profile of **Power911** in serum following a single intravenous infusion to cynomolgus monkeys.




- A single-dose PK study in cynomolgus monkeys provided a Power911 PK profile that can support clinical testing.
- The concentration–time profiles of Power911 were characterized by an initial brief distribution phase, followed by a linear beta elimination phase and a terminal target–mediated elimination phase.
- Following IV infusion, the terminal target–mediated elimination phase of the concentration–time profile of Power911 was evident at Power911 serum concentrations below approximately 5 to 20 mg/mL in the 1 and 5 mg/kg groups (Supplementary Fig. S7).
- The target-mediated elimination phase was not observed in the 15 mg/kg group that led to Power911 serum concentrations greater than 20 mg/mL throughout the 56-day study duration (Supplementary Fig. S7).

# *PK parameters*

**Table 2.** Mean PK parameters of Power911 in serum of cynomolgus monkeys following a single i.v. infusion

| Parameter | Units | Dose of Power911 | | |
|---|---|---|---|---|
| | | 1 mg/kg | 5 mg/kg | 15 mg/kg |
| $C_{max}$ | µg/mL | 33.3 ± 1.91 | 121 ± 10.2 | 355 ± 64.7 |
| $AUC_{last}$ | day x µg/mL | 168 | 1,100 | 3,950 |
| $AUC_{last}$/dose | day x kg x µg/mL/mg | 168 | 220 | 263 |
| $t_{1/2\ terminal}$ | day | 1.19 | 2.02 | 9.85 |
| $t_{1/2\ beta}$ | day | 9.84 ± 1.13 | 10.9 ± 3.82 | 12.4 ± 1.67 |
| CL | mL/day/kg | 5.99 | 4.56 | 3.68 |

NOTE: $N = 5$/group. $AUC_{last}$, $AUC_{last}$/dose, $t_{1/2\ terminal}$, and CL were estimated based on PK profiles of animals not affected by ADA; $n = 2$, 1, and 2 for the 1, 5, and 15 mg/kg dose groups, respectively. Concentrations considered to be outliers were excluded for one animal in each of the 1 and 15 mg/kg dose groups (≤ 2 timepoints/animal). Values for $C_{max}$ and $t_{1/2\ beta}$ are mean ± SD. Abbreviations: $C_{max}$, maximum drug concentration observed in serum; $AUC_{last}$, area under the concentration–time curve from time zero to the last measurable concentration; $t_{1/2\ terminal}$, half-life estimated by the observed terminal phase of the concentration–time curve; $t_{1/2\ beta}$, half-life estimated by the observed beta phase of the concentration–time curve.

- Mean beta phase half-lives (t1/2 beta) were comparable across the 1, 5, and 15 mg/kg groups.
- The mean AUClast values were 168, 1,100, and 3,950 day·g/mL following i.v. infusion of 1, 5, and 15 mg/kg Power911, respectively.
- The corresponding dose-normalized mean AUClast values (AUC last/dose) of 168, 220, and 263 day · mg/mL per mg/kg indicated a greater than dose-proportional increase across the dose levels.
- Consistent with this finding, mean total body clearance (CL) was dose-dependent and decreased with increasing dose.
- Mean terminal half-lives (t 1/2 terminal) of 1.19 and 2.02 days in the 1 and 5 mg/kg dose groups, respectively, were shorter relative to 9.85 days in the 15 mg/kg group.
- Anti-Power911 antibodies were observed in all animals by 28 days after dose, which resulted in accelerated elimination of Power911 from the serum of 67% of the animals across all of the dose levels (3/5, 4/5, and 3/5 animals in the 1, 5, and 15 mg/kg groups, respectively). These Power911 concentrations affected by ADA were excluded from the PK analysis.

# *General Toxicity*

- Study title/ number: A 26-Week Intravenous Toxicology Study in Cynomolgus Monkeys with a 12-Week Recovery Period/ Power911-TX-14153
- 0, 2, 10, and 50 mg/kg once weekly for 26 weeks
- **Key Study Findings**
  - There was one mortality each after multiple injections at the 10 mg/kg and 50mg/kg dose levels attributed to pulmonary hemorrhage and edema considered to be secondary to immunogenicity. The high dose preterm decedent also exhibited histologic hemorrhage and/or edema in the kidney, skin, urinary bladder, cecum, stomach, and liver.
  - Power911 induced an increase in the incidence and/or severity of multi-organ mononuclear cell infiltration
  - Other potential target organs included the spleen, eye (corneal hyperkeratosis), and cecum (erosion and mixed cell inflammation)

# *Toxicity-26 weeks*

| Parameters | Major Findings |
|---|---|
| Mortality | There were five mortalities; three (2 control and one MD monkey) were not drug-related. The other two are described below.<br>10 mg/kg: 1 MD female (#3503) exhibiting lethargy, weakness, dyspnea, decreased activity, dilated pupils, pale gums, and vomitus was euthanized on Day 36 (dosing day) after the 6th dose. Exhibited fluid accumulation in the lung correlating histologically with hemorrhage and edema. ADA were detected on Day 36 correlating with reduced Power911 exposure. The cause of death was considered pulmonary hemorrhage and edema secondary to immunogenicity.<br>50 mg/kg: 1 HD male (#4006) exhibiting decreased activity, loss of consciousness, lying on side, reduced appetite, decreased respiratory rate, and uncoordination was found dead on Day 94 two days after the 14th dose on Day 92. ADA were detected on Day 86+ correlating with reduced Power911 exposure. The cause of death was considered pulmonary hemorrhage and edema secondary to immunogenicity. |
| Clinical Signs | See clinical signs in preterm decedents above. There was a dose-related increase in the incidence of red skin beginning at the LD, including facial redness beginning by Day 22.<br>2 mg/kg: Retching, dry/flaking skin, red feces<br>10 mg/kg: Decreased activity, pink skin, pale gums/face<br>50 mg/kg: Decreased activity (no dose response), prepuce swelling, liquid/mucoid feces, vomitus |
| Clinical Chemistry | Statistically significant 38% increase in mean bilirubin on Day 92 in HD females. |
| Gross Pathology | 2 mg/kg: Dark red focus in stomach (fundus) in 1 male monkey (#2001)<br>50 mg/kg: Enlarged spleen in 1 female monkey (#4502) |
| Organ Weights | Increase in spleen weight (absolute and relative to body and brain weight) in 1 HD female monkey (#4502) up to 314%. |
| Histopathology<br>Adequate battery: Yes | there was an increased incidence and/or severity of multi-organ (including the brain) mononuclear cell infiltration up to mild at the HD. |
| Immunophenotyping | Unremarkable; no drug-related effects on absolute or percent T lymphocytes, T-cytotoxic lymphocytes, T-helper lymphocytes, monocytes, B-lymphocytes, or natural killer cells |

# *Toxicity*

| Parameters | Major Findings |
|---|---|
| Reversibility | There was a statistically significant transient decrease (≤25% ) in mean heart rate at an ECG timepoint scheduled within one week of the recovery necropsy in monkeys dosed with ≥2 mg/kg Power911 compared to controls; this decrease was observed 4 hrs. into the 24 hr. monitoring period and showed evidence of recovery.<br>In general, all findings trended towards recovery or were similar to controls except for the recovery cohort findings in Table 4, some of which developed during the recovery period. Minimal mononuclear cell infiltration was still present in the brain (MDHD), esophagus (HD), salivary gland (LD-HD), kidney (LD-HD), and urinary bladder (LD-MD) and also developed in the optic nerve in one HD monkey during the recovery period. |
| Immunogenicity | ADAs were detected in 19/36 (53%) monkeys dosed with Power911 including 11/12 (92%), 4/12 (33%), and 4/12 (33%) monkeys dosed with 2, 10, and 50 mg/kg Power911, respectively. ADAs generally resulted in lower Power911 concentrations. Given the drug tolerance limit (DTL) of ~1284 µg/mL in the validated ADA assay, ADA formation at the HD was likely masked by high Power911 concentrations. |
| Toxicokinetic | T1/2: 13.5-19.3 days; Tmax: ~0.583 hours<br>Dose proportionality: Cmax and AUCtau generally increased dose proportionally<br>Accumulation: Yes, based on Cmax and AUCtau (≤4-fold and ≤5-fold on Day 176 and Day 92 compared to Day 1, respectively)<br>Sex differences: No significant differences<br>On Day 176, AUCtau was only calculated in 2 and 3 monkeys dosed with 10 mg/kg and 50 mg/kg Power911, respectively, due to ADAs |

# *General Toxicity*

- Study title/ number: 4-Week Intravenous Toxicology Study in Cynomolgus Monkeys with an 8-Week Recovery Period / Power911-TX-14059
- Administered intravenously once weekly at dose levels of 2, 10, or 50 mg/kg for 4 weeks to cynomolgus monkeys.
- **Key Study Findings**
  - Power911 was administered intravenously once weekly at dose levels of 2, 10, or 50 mg/kg for 4 weeks to cynomolgus monkeys. There were no mortalities.
  - ADAs were detected in 23/30 (77%) monkeys dosed with Power911 including 10/10 (100%), 7/10 (70%), and 6/10 (60%) monkeys dosed with 2, 10, and 50 mg/kg Power911, respectively. There was evidence of immune complex deposition, and histologic findings were seen in the adrenal gland, spleen, liver, mandibular lymph node, and axillary lymph node.
  - As determined by flow cytometry for Ki67, Power911 induced dose-independent increases in the absolute counts of proliferating T lymphocytes, T-helper lymphocytes, and T-cytotoxic lymphocytes on Day 9 which generally showed evidence of recovery by Day 50. There were no Power911-related increases in the proliferation of isolated PBMCs activated ex vivo or in C1q-CIC or C3d-CIC formation.

# *General Toxicity*

- Study title/ number: A 13-Week Intravenous Toxicology Fertility Assessment Study in Sexually Mature Cynomolgus Monkeys With a 12-Week Recovery Period/ Power911- TX-15151
- 0, 10, and 50 mg/kg once weekly for 13 weeks
- **Key Study Findings**
  - There was a slight increase in the severity of tubular hypoplasia/atrophy in the testis of one low dose recovery cohort male monkey compared to controls
  - One low dose female monkey exhibited an extended menstrual cycle during the dosing phase compared to the pre-dosing phase and one high dose female monkey exhibited amenorrhea in the dosing phase. These menstrual cycle irregularities did not correlate with any histologic findings and are not clearly drug-related.
  - There were no substantial drug-related effects on fertility parameters or the reproductive tract at any of the dose levels tested.

# *Toxicity-13 weeks*

**Observations and Results: changes from control**

| Parameters | Major findings |
|---|---|
| **Mortality** | None |
| **Clinical Signs** | One LD male monkey (#I13508) exhibited ecchymotic hemorrhage in the scrotum and legs on Day 44, which was attributed to a possible antigen/antibody reaction. Black feces were observed at the HD on Day 90. |
| **Body Weights** | Unremarkable |
| **Hematology** | 10 mg/kg: -15% neutrophils (male and female)<br>50 mg/kg: -26% (male) and -43%* (female) neutrophils |
| **Clinical Chemistry** | Unremarkable |
| **Urinalysis** | Unremarkable |
| **Gross Pathology** | Unremarkable |
| **Organ Weights** | Statistically significant increase in mean lung weight (absolute and relative to body and brain weight) in HD male monkeys up to 44% |
| **Parameters** | **Major findings** |
| **Menstrual Cycle analysis** | One LD female (#I13526) exhibited one long menstrual cycle in the dosing phase (83 days) compared to two shorter cycles in the pre-dosing phase (mean = 36 days). One HD female (#I13531) exhibited menstrual bleeding during the first half of the pre-dose phase followed by amenorrhea during the dosing phase. Recovery was not assessed in this animal. These menstrual cycle irregularities did not correlate with any histologic findings in female reproductive organs. Given the lack of dose dependence and histologic correlates, as well as the high variability of the menstrual cycle data, these findings were not considered to be drug-related. |
| **Semen analysis** | Unremarkable. There were no drug-related findings in semen sample weight, sperm density/morphology, total sperm count, or percent sperm motility. |
| **Testicular measurements** | Unremarkable |
| **Reversibility** | Findings trended towards recovery or were similar to controls except for the recovery cohort findings shown in the histopathology table above, some of which developed during the recovery period. There was an increase in the severity of tubular hypoplasia/atrophy in the testis of one LD recovery cohort monkey (marked) compared to controls in the dosing phase (up to moderate). |

| | |
|---|---|
| **Immunogenicity** | ADAs were detected in 8/24 (33%) monkeys dosed with REGN2810 including 6/12 and 2/12 monkeys dosed with 10 and 50 mg/kg REGN2810, respectively. ADA formation generally resulted in lower REGN2810 concentrations, was more prevalent in male monkeys, and was likely masked at the HD by high REGN2810 concentrations. |
| **Toxicokinetics** | $T_{1/2}$: 12.2-17.7 days; $T_{max}$: ~0.583 hours<br>$AUC_{last}$ *(Day 13; M/F; mean*; day·µg/mL*)*:<br>10 mg/kg: Not calculated / 13900<br>50 mg/kg: 38300 / 66500<br>*Dose proportionality:* $C_{max}$ and $AUC_{tau}$ generally increased dose proportionally<br>*Accumulation:* Yes, based on $C_{max}$ (≤2.4-fold) and $AUC_{tau}$ (≤3.5-fold) after the 13th dose compared to the 1st dose<br>*Sex differences:* No significant differences |

| Dose # | Dose (mg/kg) | $C_{max}$ (µg/mL) | | $AUC_{tau}$ (day·µg/mL) | | $T_{1/2}$ (days) | |
|---|---|---|---|---|---|---|---|
| | | M | F | M | F | M | F |
| 1 | 10 | 335 | 263 | 1270 | 904 | NC | NC |
| | 50 | 1510 | 1330 | 5350 | 4280 | NC | NC |
| 7 | 10 | 551 | 525 | 2680 | 2620 | NC | NC |
| | 50 | 3240 | 2650 | 16200 | 13200 | NC | NC |
| 13 | 10 | 745 | 569 | 4080 | 2920 | NC | 17.7 |
| | 50 | 3680 | 2850 | 18700 | 13600 | 12.2 | 17.1 |

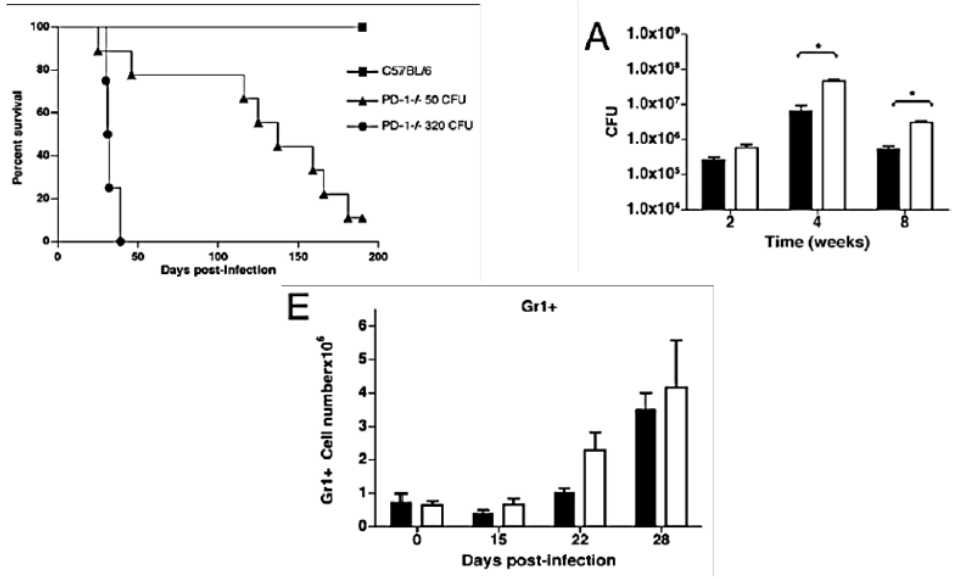
ADA-impacted concentrations were excluded from TK calculations
$AUC_{tau}$: Area under the concentration-time curve calculated during the dosing interval; N/C: Not Applicable; NC: Not Calculated

LD: low dose (10 mg/kg); HD: high dose (50 mg/kg); -: indicates reduction in parameters compared to control; +: indicates increase in parameters compared to control; *, P ≤ 0.05 vs. controls; ADA: anti-drug antibody

# *Literature based assessment of potential for effects on infection*

- While loss of PD-1 function enhances clearance of some tumors and viral infections, it increases susceptibility to certain other pathogens such as tuberculosis in some animal models.
- PD-1 appears to be required to control infection and the inflammatory responses in the lungs of mice infected with M. tuberculosis (Lazar-Molnar, et al., 2010); however, the pathogenesis of this observation has not been clearly-defined. In particular, it is unclear whether the decreased survival reflects rampant bacterial growth resulting from an inability to mount appropriate antibacterial responses and/or whether it is a failure to downregulate the immune reaction that leads to massive tissue destruction and organ failure.
- These data suggest that there is concern that treatment with Power911 may increase susceptibility to tuberculosis infection and/or that infected patients may develop more severe disease.
- The potential for increased toxicity in the presence of Power911 may also be a concern following viral infection. In mouse models of LCMV infection, the absence of PD-1 pathway signaling resulted in fatal CD8+ T cell-mediated pathology due to killing of virally infected endothelial cells, systemic vascular leakage, and ultimately cardiovascular collapse (Frebel et al. 2012; Mueller et al. 2010). Similarly, PD-L1 deficient mice died early after chronic systemic LCMV infection (Barber et al. 2006).

**Figure 8: Decreased Survival, Increased Bacterial Proliferation and Increased Inflammation in PD-1-deficient Mice Infected with M. tuberculosis**




(Figures derived from: Lazar-Molnar, et al. 2010)

# *Summary*

- In conclusion, the studies presented in this presentation demonstrate that Power911 is a potent PD-1 inhibitor both in vitro and in vivo.
- Clinical efficacy of PD-1–blocking antibodies for cancer immunotherapy has reaffirmed the ability of PD-1/PD-L1 axis blockade to yield significant benefits in patients by unleashing the cytotoxic function of tumor-specific T cells. Preclinical and clinical evidence for the enhanced benefit of PD-1 inhibitors in combination with other agents continues to grow.
- Taken together, the preclinical data support PD-1 blockade with Power911 to be a promising foundation for combination cancer immunotherapy.

# Dose selection consideration

**Table 3: Conversion of Animal Doses to Human Equivalent Doses Based on Body Surface Area**

| Species | Reference Body Weight (kg) | Working Weight Range[a] (kg) | Body Surface Area ($m^2$) | To Convert Dose in mg/kg to Dose in $mg/m^2$ Multiply by $k_m$ | To Convert Animal Dose in mg/kg to HED[b] in mg/kg, Either | |
|---|---|---|---|---|---|---|
| | | | | | Divide Animal Dose By | Multiply Animal Dose By |
| Human | 60 | --- | 1.62 | 37 | --- | --- |
| Child[c] | 20 | --- | 0.80 | 25 | --- | --- |
| Mouse | 0.020 | 0.011-0.034 | 0.007 | 3 | 12.3 | 0.081 |
| Hamster | 0.080 | 0.047-0.157 | 0.016 | 5 | 7.4 | 0.135 |
| Rat | 0.150 | 0.080-0.270 | 0.025 | 6 | 6.2 | 0.162 |
| Ferret | 0.300 | 0.160-0.540 | 0.043 | 7 | 5.3 | 0.189 |
| Guinea pig | 0.400 | 0.208-0.700 | 0.05 | 8 | 4.6 | 0.216 |
| Rabbit | 1.8 | 0.9-3.0 | 0.15 | 12 | 3.1 | 0.324 |
| Dog | 10 | 5-17 | 0.50 | 20 | 1.8 | 0.541 |
| Primates: | | | | | | |
| Monkeys[d] | 3 | 1.4-4.9 | 0.25 | 12 | 3.1 | 0.324 |
| Marmoset | 0.350 | 0.140-0.720 | 0.06 | 6 | 6.2 | 0.162 |
| Squirrel monkey | 0.600 | 0.290-0.970 | 0.09 | 7 | 5.3 | 0.189 |
| Baboon | 12 | 7-23 | 0.60 | 20 | 1.8 | 0.541 |
| Micro-pig | 20 | 10-33 | 0.74 | 27 | 1.4 | 0.730 |
| Mini-pig | 40 | 25-64 | 1.14 | 35 | 1.1 | 0.946 |

[a] For animal weights within the specified ranges, the HED for a 60 kg human calculated using the standard $k_m$ value will not vary more than ±20 percent from the HED calculated using a $k_m$ value based on the exact animal weight.
[b] Assumes 60 kg human. For species not listed or for weights outside the standard ranges, human equivalent dose can be calculated from the formula: HED = animal dose in mg/kg x (animal weight in kg/human weight in kg)$^{0.33}$.
[c] The $k_m$ value is provided for reference only since healthy children will rarely be volunteers for phase 1 trials.
[d] For example, cynomolgus, rhesus, and stumptail.

- https://www.fda.gov/downloads/Drugs/Guidances/UCM078932.pdf%23search=%27guidekines+for+industry+sfe+starting%27

## Guidance for Industry

### Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers

1. **Toxicity study shows HNSTD 50mg/kg in monkey**
2. **Best efficacy observed in vivo study with 10mg/kg, less with 1mg/kg and 3mg/kg**

# *Study Objectives*

**Primary Objective**

- Are there any considerations for patient population for this compound?
    - Biomarker?
    - Special patient population based on the compound preclinical properties

**Secondary Objectives**

-
-
-
-

**Exploratory Objectives**

-

# *Study Design*

- Starting dose
- Dose escalation schema and dose range
- Dosing schedule, e.g., QD, BID, etc.
- Inclusion/Exclusion criteria
  - Any special considerations
    - biomarker requirements?
    - tumor biopsies?
    - organ function based on preclinical toxicology findings
    - exclusion of certain co-mobilities based on preclinical toxicology findings
    - drug-drug interaction risks
- Special toxicity monitoring based on preclinical toxicology findings