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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	1.59×10^5	9.72×10^{-4}	6.11×10^{-9}	11.9
Human PD-1-mFc	3.17×10^5	1.99×10^{-4}	6.28×10^{-10}	58.0
Monkey PD-1-mmH	1.36×10^5	1.01×10^{-3}	7.43×10^{-9}	11.4
Monkey PD-1-mFc	3.14×10^5	1.64×10^{-4}	5.20×10^{-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

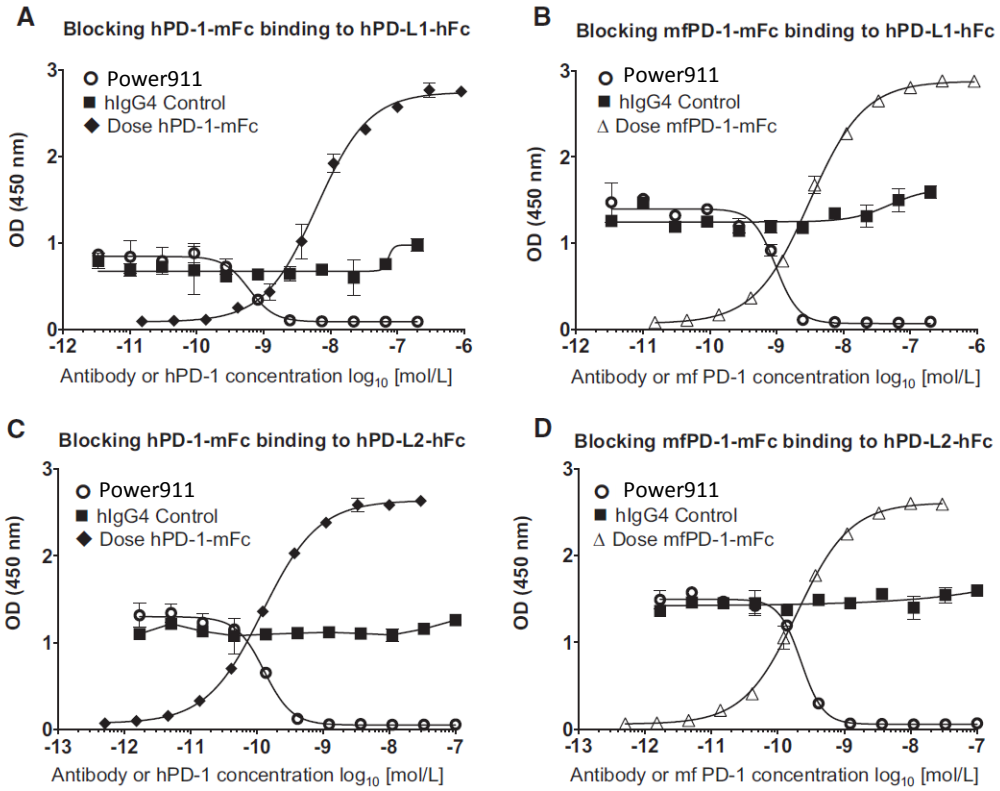

Figure 1.
Power911 inhibits binding of human and monkey PD-1 to human PD-L1 and PD-L2. A and B, Binding of 1.5 nmol/L hPD-1-mFc (A) or 2.0 nmol/L mfPD-1-mFc (B) to plate-coated hPD-L1-hFc in the presence of increasing concentration of Power911 or an isotype control antibody. C and D, Binding of 0.1 nmol/L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

- 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).

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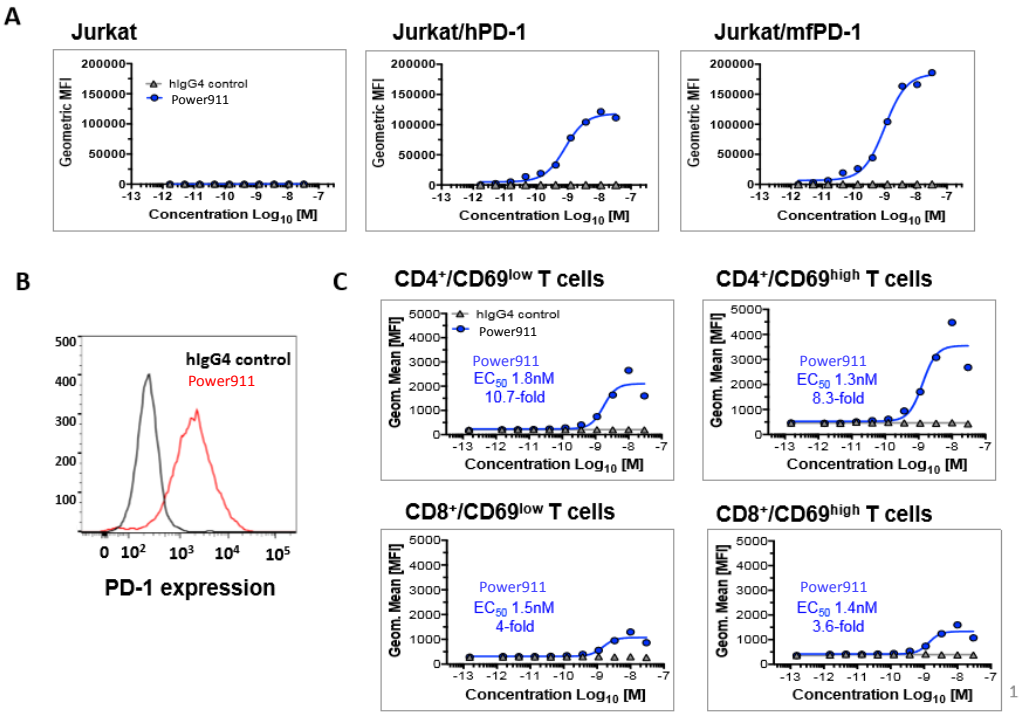
Affinity & Specificity

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.

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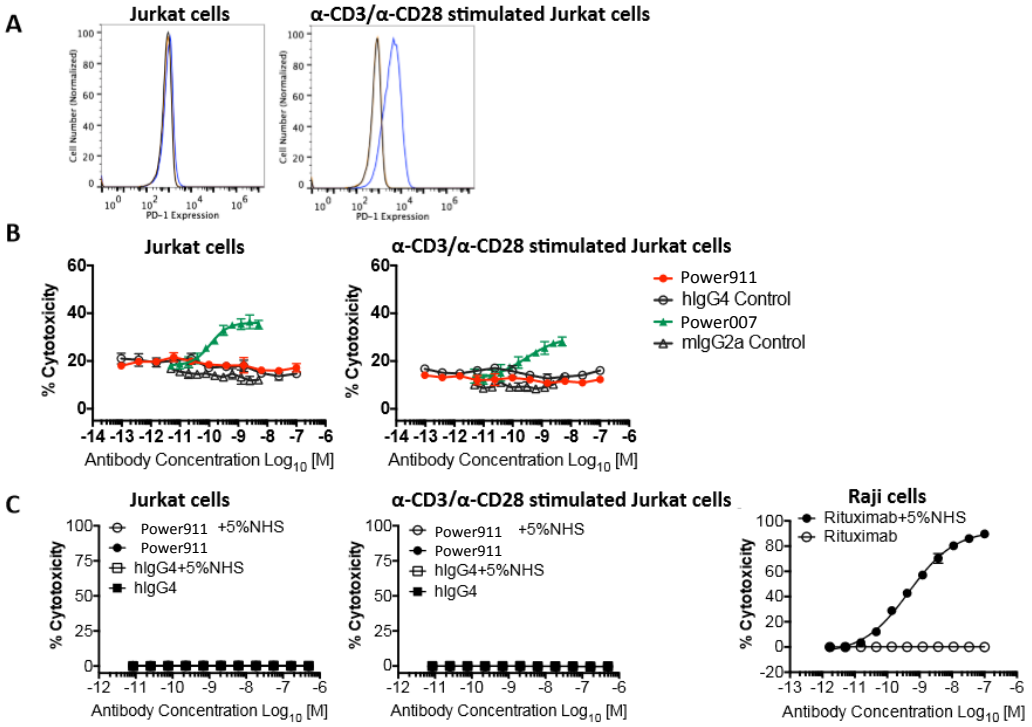
Power911: No ADCC/CDC induce

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.

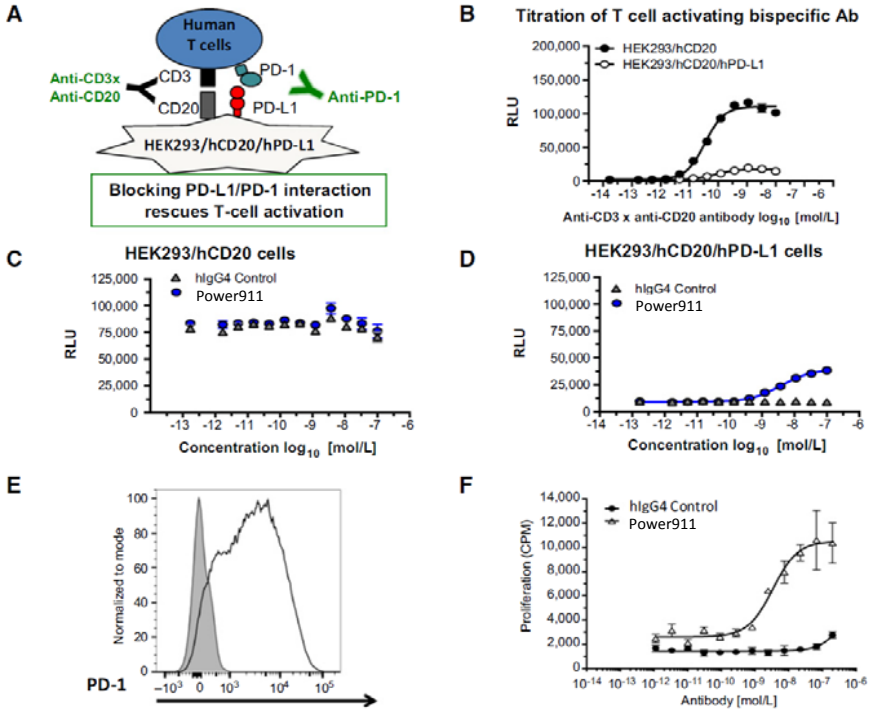
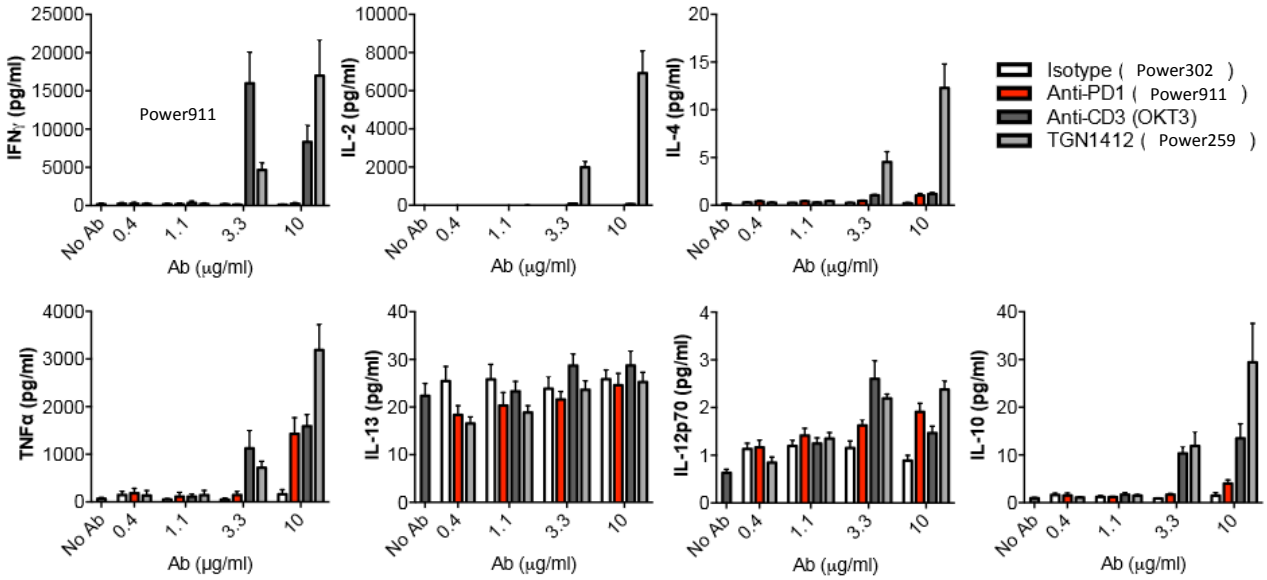


Figure 2.
Power911 blocks PD-1/PD-L1 inhibitory signaling in a T cells/engineered APC bioassay. A, Bioassay schematic: To evaluate PD-1/PD-L1 inhibition, engineered Jurkat/AP-1-Luc/hPD-1 cells or preactivated primary human CD4t T cells were incubated with HEK293/hCD20/hPD-L1–engineered APC in the presence 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.

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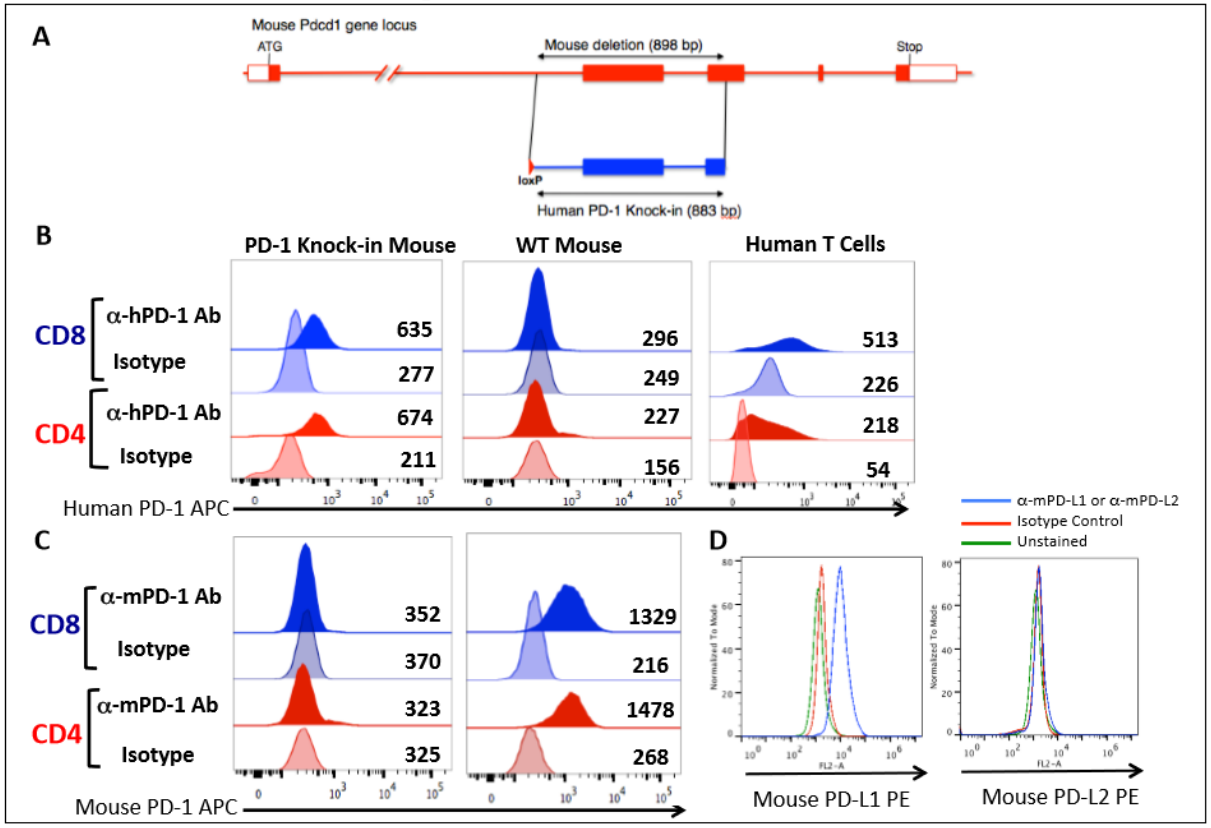
Functional replacement of mouse PD-1 with human homologue

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.

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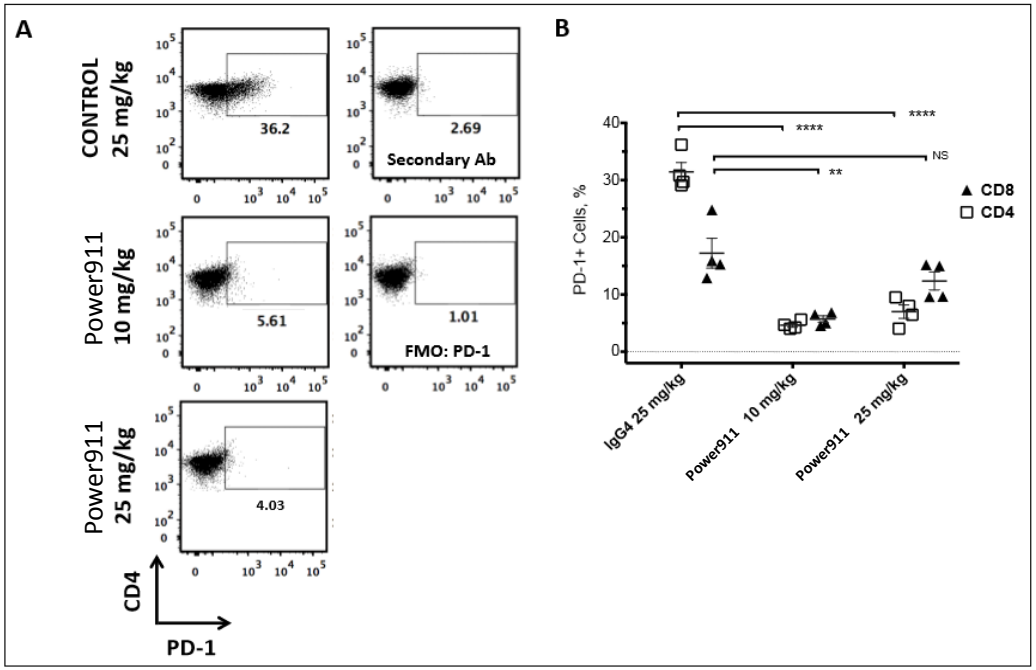
Power911 binds PD-1 in human PD-1 knock-in mice and inhibits tumor growth

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

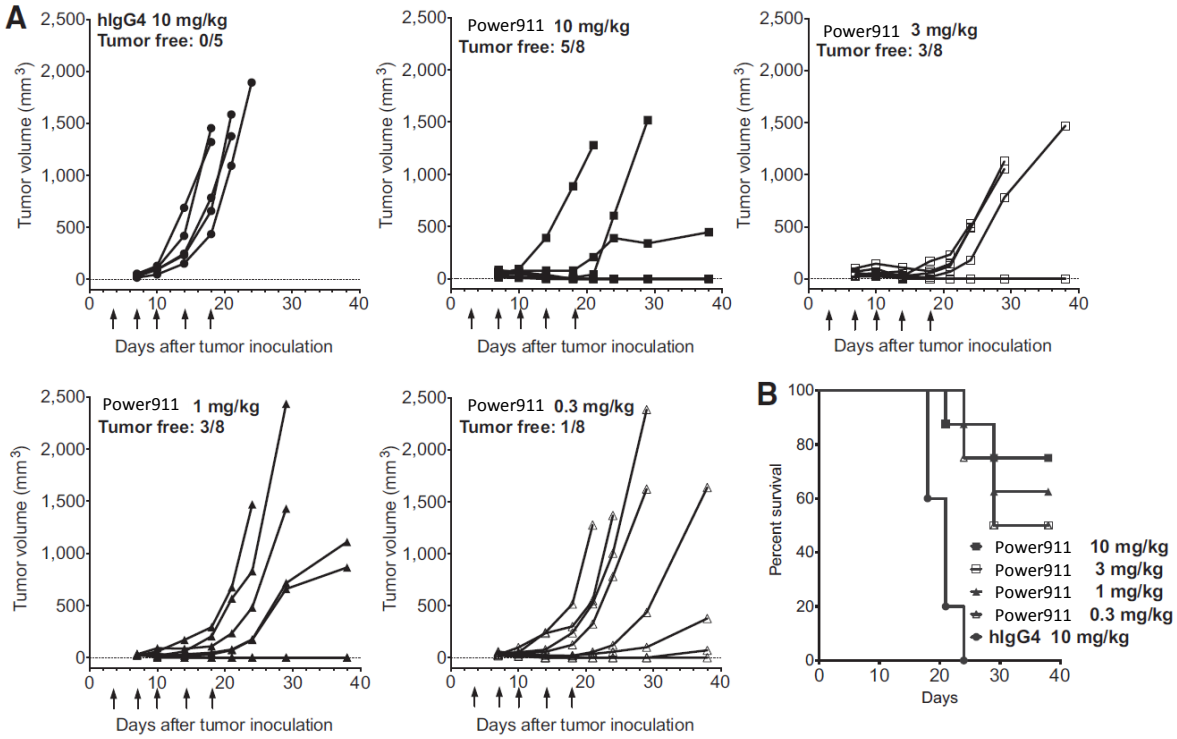


A, PD-1 expression on splenic CD4⁺ T cells from MC38.Ova tumor bearing mice treated with the indicated doses of Power911 or isotype control antibody on day 0 (at tumor volumes 100-120 mm^3), day 3, and day 6. Splenocytes were analyzed on day 10.

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

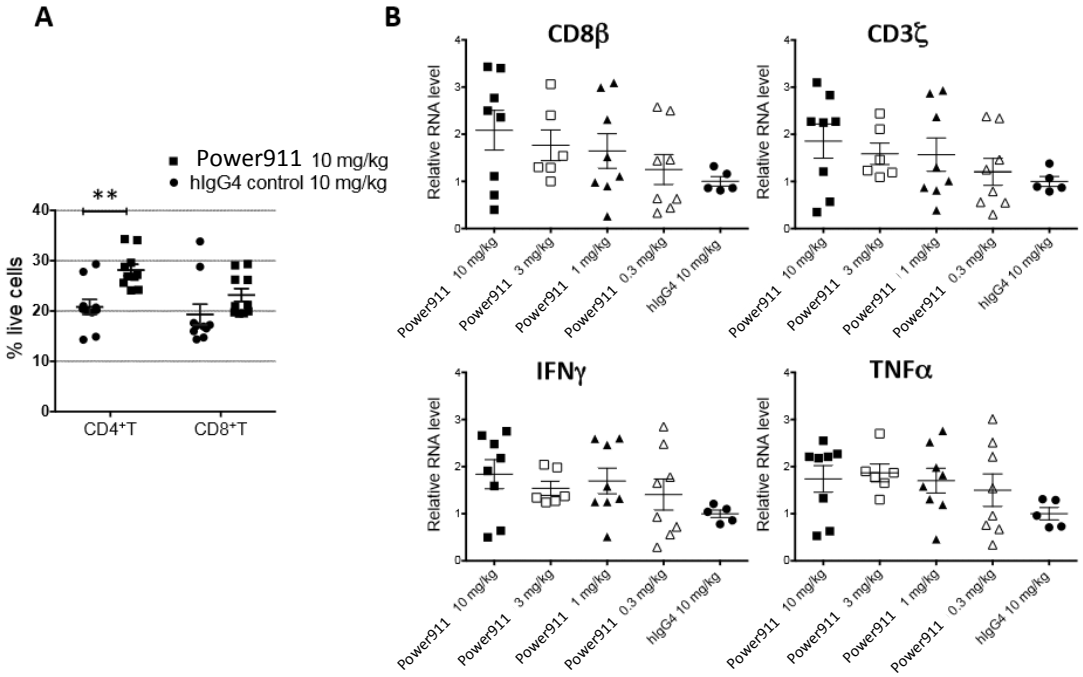


- In the minimal tumor model, human PD-1 knock-in mice engrafted withMC38.Ova cells were treated with Power911 doses ranging from 0.3 mg/kg to 10 mg/kg, starting on day 3, before the predicted appearance of measurable tumors.
- Power911 showed potent dose-dependent tumor growth inhibition, and at 10 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 (5×10^5 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*.

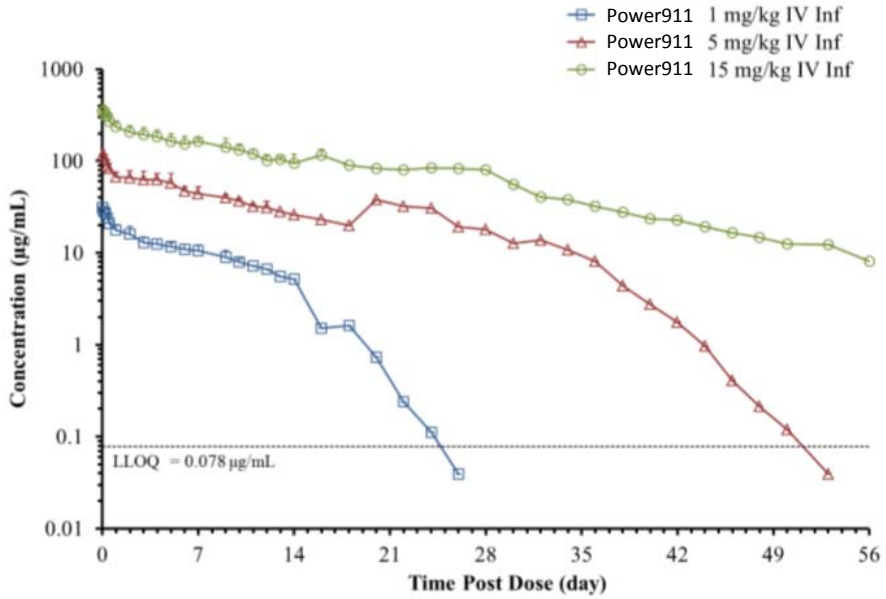


- Flow cytometric analysis of draining lymph nodes revealed an increased frequency of $CD4^+$ and $CD8^+$ T cells in mice treated with Power911 (Supplementary Fig. S6A).
- Taqman analysis of spleens revealed increased transcript levels for CD8, CD3, IFNg, and TNFα in Power911-treated mice, suggesting 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. 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. 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. 2 mg/kg: Retching, dry/flaking skin, red feces 10 mg/kg: Decreased activity, pink skin, pale gums/face 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) 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 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. 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 Dose proportionality: Cmax and AUCtau generally increased dose proportionally Accumulation: Yes, based on Cmax and AUCtau (≤4-fold and ≤5-fold on Day 176 and Day 92 compared to Day 1, respectively) Sex differences: No significant differences 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) 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 AUC_{last} *(Day 13; M/F; mean*; day·µg/mL*)*: 10 mg/kg: Not calculated / 13900 50 mg/kg: 38300 / 66500 *Dose proportionality:* C_{max} and AUC_{tau} generally increased dose proportionally *Accumulation:* Yes, based on C_{max} (≤2.4-fold) and AUC_{tau} (≤3.5-fold) after the 13th dose compared to the 1st dose *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

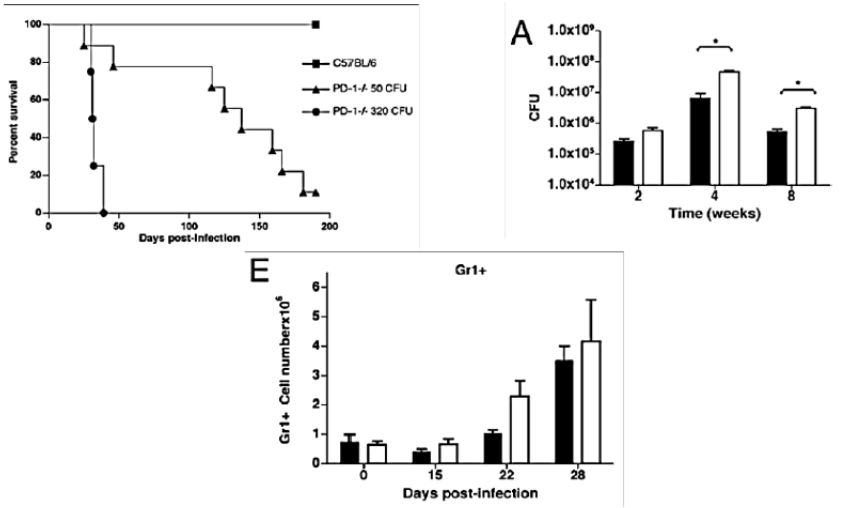
ADA-impacted concentrations were excluded from TK calculations
AUC_{tau}: Area under the concentration-time curve calculated during the dosing interval; N/C: Not Applicable; NC: Not Calculated

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.

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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**

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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

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Back-Up

Power911 clinical development pathway

Power911 information (USPI)

Code name	Power911
Applicant	USKAKA
Formulation(s)	Injection: 350mg/7mL (50mg/mL) solution in a single-dose vial
Dosing Regimen	350 mg as an intravenous infusion over 30 minutes every 3 weeks
Applicant proposed indication(s)/population(s)	Treatment of patients with metastatic cutaneous squamous cell carcinoma or patients with locally advanced cutaneous squamous cell carcinoma who are not candidates for surgery
Recommended Indication(s)/population(s)	Power911 is indicated for the treatment of patients with metastatic cutaneous squamous cell carcinoma (CSCC) or locally advanced CSCC who are not candidates for curative surgery or curative radiation
Supporting studies	Study 1423 (NCT02383212) and 1540 (NCT02760498)

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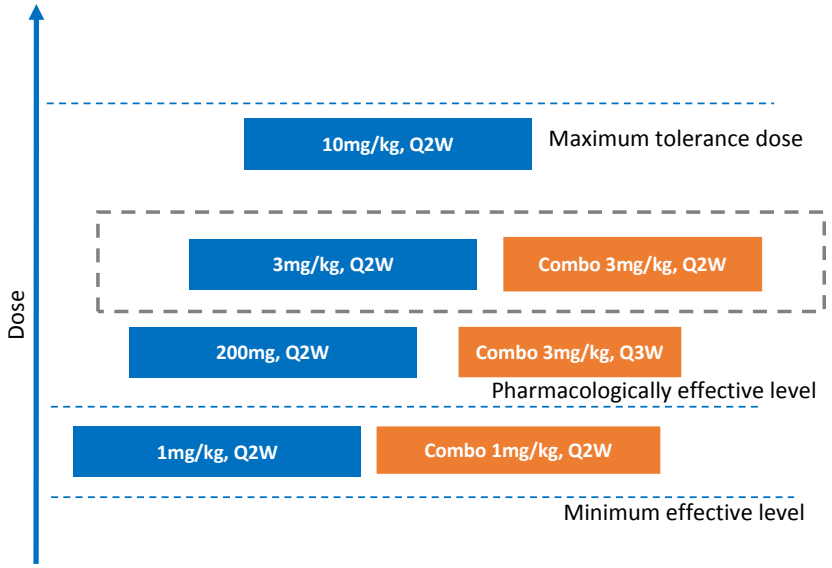
Phase I/II Study Design and preliminary result

Phase 1 Dose finding & Safety Confirmation (Part 1)



Phase 1 Dose expansion (Part 2)

Cohort 1, N=26
locally advanced (13) or metastatic cutaneous (13) squamous-cell carcinoma
3mg/kg Q2w for up to 48 weeks,
ORR assessment every 8 weeks
ORR 50% (13/26, 95%CI 30-70%)

Cohort 2
Cohort 3
Cohort 4
Cohort 20

- **no DLTs**
- **ORR 50% (30 ,70)**
- **Drug related adverse events ≥ grade 3**
  - **transaminase elevation (n = 2)**
  - **anemia (n=1)**
  - **paraneoplastic encephalitis (n=1)**

Phase 2 Single-Arm Confirmatory

Cohort 1, N=59, 3mg/kg Q2W × 96 wks. metastatic CSCC

Cohort 2, N=23, 3mg/kg Q2W × 96 wks. Local advanced CSCC

Cohort 3, N=49, 350mg Q3W × 96 wks. metastatic CSCC

- **ORR 47% (34 ,61)**
- **Among patients who had a response, the duration of response exceeded 6 months in 57%, and 82% continued to have a response and to receive Power911 at the time of data cutoff.**

Other cohort expansion includes: NSCLC, head and neck cancer, breast cancer, advanced solid tumors in patients previously treated with another anti-PD-1/PDL1 antibody, CSCC, colorectal with microsatellite instability (MSI), endometrial with MSI, prostate with MSI, other advanced solid tumors with MSI, hepatocellular carcinoma, advanced solid tumors refractory to firstline therapy in which treatment with carboplatin and/or docetaxel is clinically inappropriate, previously untreated NSCLC, GBM, and solid tumors in patients infected with HIV.

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Study of Power911 (Anti-PD-1) in Patients With Advanced Malignancies

Study 1423 (NCT02383212)

• *Primary Outcome Measures*

1. Incidence of Treatment Emergent Adverse Events (TEAEs) [Time Frame: Change from baseline to week 48]
2. Primary safety variables include incidence and severity of TEAEs, abnormal laboratory findings and number of participants with dose limiting toxicities (DLTs)
3. Incidence of abnormal laboratory findings [Time Frame: Change from baseline to week 48]
4. Number of participants with dose limiting toxicities (DLTs) [Time Frame: Change from baseline to 28 days after first dose of Power911]

• *Secondary Outcome Measures*

1. Response Evaluation Criteria in Solid Tumors (RECIST) as measured by Computed Tomography (CT) or Magnetic Resonance Imaging (MRI) [Time Frame: Change from baseline to week 48]
2. Immune-Related Response Criteria (irRC) applied to RECIST measurements [Time Frame: Change from baseline to week 48]

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Study of Power911 (Anti-PD-1) in Patients With Advanced Malignancies

Study 1423 (NCT02383212)

- ***Key Inclusion***
  1. Histologically or cytologically confirmed diagnosis of malignancy with demonstrated progression of a solid tumor (non-lymphoma) with no alternative standard-of-care therapeutic option (certain exceptions may apply).
  2. At least 1 measurable lesion according to Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 criteria for response assessment (certain exceptions may apply)
  3. Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1

- ***Key Exclusion***
  - Ongoing or recent (within 5 years) evidence of significant autoimmune disease that required treatment with systemic immunosuppressive treatments, which may suggest risk for immune-related adverse events (irAEs). The following are not exclusionary: vitiligo, childhood asthma that has resolved, residual hypothyroidism that required only hormone replacement or psoriasis that does not require systemic treatment.
  - Prior treatment with an agent that blocks the programmed death-1/ programmed death-ligand 1 (PD-1/PD-L1 pathway) (certain exceptions may apply)
  - Prior treatment with other immune modulating agents within fewer than 4 weeks prior to the first dose of Power911. Examples of immune modulating agents include blockers of CTLA-4, 4-1BB (CD137), OX-40, therapeutic vaccines, or cytokine treatments.
  - Untreated brain metastasis(es) that may be considered active. Patients with previously treated brain metastases may participate provided they are stable (i.e., without evidence of progression by imaging for at least 6 weeks prior to the first dose of study treatment, and any neurologic symptoms have returned to baseline), and there is no evidence of new or enlarging brain metastases, and the patient does not require any systemic corticosteroids for management of brain metastases within 4 weeks prior to the first dose of Power911 (certain exceptions may apply).
  - Immunosuppressive corticosteroid doses (>10 mg prednisone daily or equivalent) within 4 weeks prior to the first dose of Power911

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Study of Power911 in Patients With Advanced Cutaneous Squamous Cell Carcinoma Study 1540 (NCT02760498)

• *Primary Outcome Measures*

1. Overall Response Rate [Time Frame: 96 weeks] Group 1 and Group 3: RECIST version 1.1 will be used to determine ORR. Group 2: Clinical response criteria will be used to determine ORR

• *Secondary Outcome Measures*

1. Investigator Assessments of Overall Response Rate [Time Frame: Up to 30 months]
2. Duration of response [Time Frame: Up to 30 months]
3. PFS (progression-free survival) [Time Frame: Up to 30 months]
4. Overall Survival [Time Frame: Up to 30 months]
5. Complete Response (CR) Rate [Time Frame: Up to 30 months]
6. Change in scores of patient reported outcomes on EORTC QLQ-C30 [Time Frame: Up to 30 months]
7. Incidence of Treatment Emergent Adverse Events (TEAEs) [Time Frame: Up to 30 months]
8. Power911 PK: Concentration at end-of-infusion (Ceoi) [Time Frame: Up to 24 months]
9. Power911 PK: Pre-infusion concentration (trough) [Time Frame: Up to 24 months]
10. Power911 PK: Time of end-of-infusion (teoi) [Time Frame: Up to 24 months]
11. Anti-Power911 antibodies [Time Frame: Up to 24 months]

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Study of Power911 (Anti-PD-1) in Patients With Advanced Malignancies

Study 1423 (NCT02383212)

- ***Key Inclusion***

1. At least 1 measurable lesion
2. Eastern Cooperative Oncology Group (ECOG) performance status ≤1
3. Adequate bone marrow function
4. Adequate renal function
5. Adequate hepatic function
6. Archived or newly obtained tumor material
7. Patients must consent to undergo biopsies of externally visible CSCC lesions (Group 2 only)
8. Surgical or radiological treatment of lesions contraindicated

- ***Key Exclusion***

1. Ongoing or recent (within 5 years) evidence of significant autoimmune disease that required treatment with systemic immunosuppressive treatments, which may suggest risk for immune-related adverse events
2. Prior treatment with an agent that blocks the PD-1/PD-L1pathway
3. Prior treatment with a BRAF inhibitor
4. Prior treatment with other immune-modulating agents within fewer than 4 weeks prior to the first dose of Power911, or associated with immune-mediated adverse events that were ≥ grade 1 within 90 days prior to the first dose of Power911, or associated with toxicity that resulted in discontinuation of the immune-modulating agent. Examples of immune-modulating agents include therapeutic vaccines, cytokine treatments, or agents that target cytotoxic T-lymphocyte antigen 4 (CTLA-4), 4-1BB (CD137), or OX-40.
5. Untreated brain metastasis(es) that may be considered active
6. Immunosuppressive corticosteroid doses (>10 mg prednisone daily or equivalent) within 4 weeks prior to the first dose of Power911
7. Infection with human immunodeficiency virus (HIV) and/or chronic/active infection with hepatitis B virus or hepatitis C virus
8. History of pneumonitis within the last 5 years
9. Allergic reactions or acute hypersensitivity reaction attributed to antibody treatments
10. Known allergy to doxycycline or tetracycline
11. Patients with a history of solid organ transplant
12. Any medical co-morbidity, physical examination finding, or metabolic dysfunction, or clinical laboratory abnormality that renders the patient unsuitable
13. Prior treatment with idelalisib

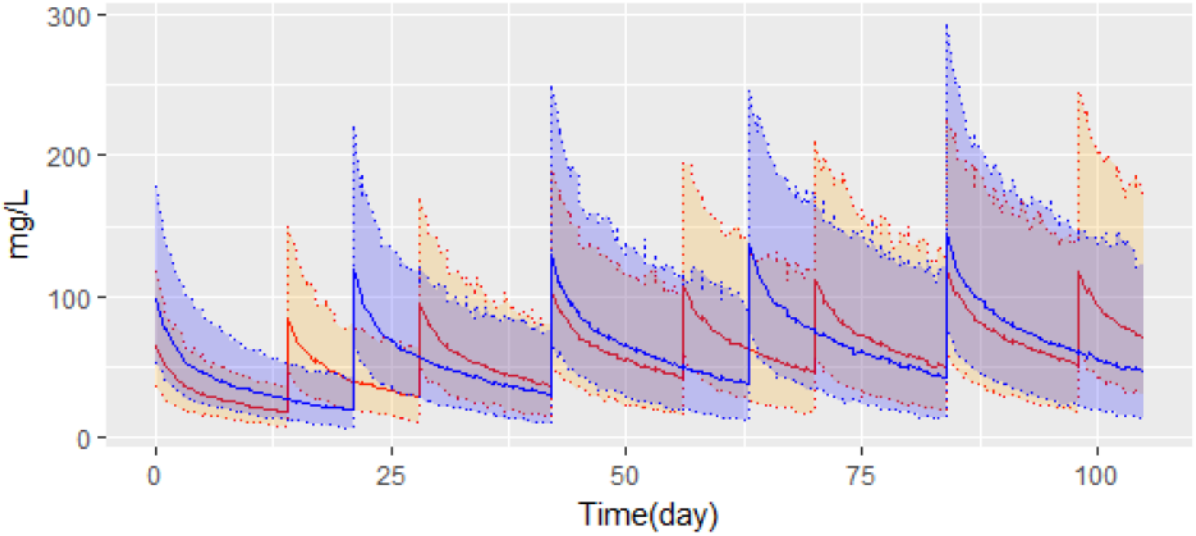
Statistical Consideration

- ORR

- The sample size for each expansion cohort in Phase 1 Study was determined separately. A total of 10 and 20 patients were planned to be enrolled in the metastatic CSCC and locally advanced CSCC cohorts, respectively. In Amendment 3, 45 metastatic CSCC and 70 locally advanced CSCC patients were planned to be enrolled, but expansion of the cohorts was halted early for the enrollment of Phase 2 Study. The primary analysis of ORR was analyzed using the exact binomial confidence interval (CI) using the Clopper-Pearson method.
- The sample size calculation in Phase 2 study was based on the primary endpoint of ORR. For the metastatic CSCC group, a total of 53 patients were needed for an ORR of 15% to be excluded using the lower limit of a two-sided 95% CI. For the locally advanced CSCC group, a total of 76 patients were needed for an ORR of 25% to be excluded using the lower limit of a two-sided 95% CI. The primary analysis of ORR was analyzed using the exact binomial CI using the Clopper- Pearson method.
- The primary analysis was summarized together and by group using pooled data from Phase 1 study and Phase 2 study.

Phase I/II Study Design and preliminary result

Figure 12. Comparison of Model Simulated 350 mg Q3W (Blue) vs. 3mg/kg Q2W (Red)



Model simulated 350 mg Q3W dosing regimen was conducted. Blue line indicated the median of 350 mg Q3W and blue shade is 95% quantile of 350 mg Q3W. Red line and orange shade indicated the median and 95% quantile of 3 mg/kg dosing regimen.

Dose selection rationale

PK: linear PK from 1 to 10 mg/kg Q2W. The 350 mg Q3W regimen resulted in similar steady-state exposure compared to the 3 mg/kg Q2W regimen (C_{trough})

PD: The preliminary efficacy results demonstrate that 350 mg Q3W is an active dose.

Safety: no meaningful exposure-response relationships identified for any explored safety variables (irAEs of all grades and irAEs of Grade ≥3). The safety data of Power911 350 mg IV Q3W is comparable to that of Power911 3 mg/kg IV Q2W.

Phase I/II Study Design and preliminary result

Trial Identity	Trial Design	Regimen/ schedule/ route	Key Study Endpoints	Treatment Duration/ Follow Up	No. of patients enrolled	Study Population	No. of Centers/ Countries
Power 911 Phase 2	Multicenter, nonrandomized, multicohort study in patients with advanced CSCC	Groups 1 and 2: 3 mg/kg Q2W Group 3 (flat dose): 350 mg Q3W	BOR by central review per RECIST 1.1 -DOR -PFS -OS -CR rate -TTR -DCR -Quality of life	Treatment until completion of 96 weeks (Grp 1, 2) or 54 weeks (Grp 3), or until unacceptable toxicity, withdrawal of consent, death Post-treatment follow-up: approximately 6.4 months	137 Group 1: 59 Group 2: 55 Group 3: 23	Advanced CSCC -Groups 1 & 3: mCSCC -Group 2: laCSCC	45 sites in U.S., Australia, Germany
Power 911 Phase 1	Multicenter, first-in-human, dose-escalation with expansion phase consisting of several disease-specific cohorts	Doses evaluated: -1 mg/kg Q2W -3 mg/kg Q2W -10 mg/kg Q2W -3 mg/kg Q3W -200 mg Q2W Single agent or in combination with cyclophosphamide or radiation	-Safety -PK BOR per RECIST 1.1 -DOR -PFS -OS -TTR DCR	Treatment until completion of 48 weeks or until unacceptable toxicity, withdrawal of consent, death Post-treatment follow-up: approximately 5.5 months	397 [includes mCSCC =16 laCSCC =10]	Metastatic or locally advanced solid tumors with no available curative therapy. Multiple disease-specific expansion cohorts including mCSCC (cohort 7) and aCSCC (cohort 8)	50 sites in North America, the EU, and Asia-Pacific

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Integrated safety summary

- Among the 534 patients treated with Power911 across the two trials, there was at least one Power911-related fatal AE in Pool 1 and at least three other Power911-related fatal AEs in Pool 3 leading to a treatment-related death rate of 0.7%.
- Power911 may have also played an indirect role in other fatal AEs. Among the 163 patients with CSCC treated with Power911, 28% experienced at least one treatment-emergent SAE including 8% who experienced a Power911-related SAE. The majority of treatment-related SAEs were immune-mediated.
- AEs leading to permanent discontinuation occurred in 5% of patients with CSCC. The most common adverse reactions reported in at least 20% of patients with CSCC were fatigue, rash and diarrhea. The most common Grade 3-4 adverse reactions (>2%) were cellulitis, sepsis, hypertension, pneumonia, musculoskeletal pain, skin infection, urinary tract infection and fatigue. Similar incidences of nonfatal AE categories were observed in the larger Pool 3.
- Serious risks of Power911 are similar to those of other monoclonal antibodies acting in the PD -1/PD-L1 pathway including serious and fatal imARs and IRRs. Among 534 patients treated with Power911, 17% (92/534) experienced at least one imAR during treatment. Four percent of patients required permanent discontinuation and 7% required temporary interruption of Power911 for imARs.
- ImARs were rarely fatal and generally manageable with corticosteroid administration. IRRs occurred in 9% of patients in Pool 3, and all were Grade 1-2 except for one patient who experienced one Grade 3 IRR. Two patients required permanent discontinuation of Power911 due to an IRR. IRRs were manageable with temporary interruptions, infusion rate reductions and administration of symptomatic treatments including antihistamines and corticosteroids.
- Overall, the safety of Power911 is consistent with the expected toxicity profile of immunologically-mediated anticancer therapies. The safety data submitted in the BLA do not change the favorable benefit-risk assessment for Power911 for the treatment of patients with advanced CSCC.

Benefit-Risk Assessment

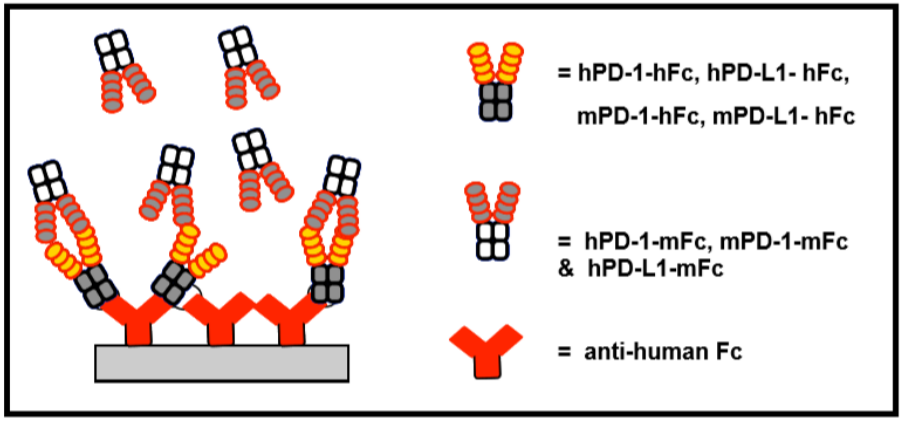
Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• CSCC has an estimated annual incidence of 700,000 cases in the US. • Second most common skin cancer • Precise incidences of overall disease, high-risk disease, and survival outcomes are not available as these cancers are grouped with other nonmelanoma skin cancers for reporting purposes • Most cases are localized and amenable to curative resection, approximately 8% of patients will experience a local recurrence, 5% will develop nodal metastases, and an estimated 2% will die from their disease • CSCC causes significant functional morbidities and cosmetic deformities as tumors commonly arise in the head and neck region and invade blood vessels, nerves, and vital organs • Patients with recurrence of disease after primary tumor resection develop local or distant metastases 25% of the time • Ten-year survival rates are less than 20% for patients with regional lymph-node involvement • Patients with distant metastases have a median survival time estimated to be less than two years	The population treated in the studies supporting this application represent patients having a serious and life-threatening disease
Current Treatment Options	• Standard of care for locoregional CSCC is complete surgical resection • For nodal involvement, a regional lymph node dissection is recommended • Adjuvant radiation therapy is utilized in most cases • There are case reports of various epithelial growth factor receptor (EGFR) inhibitors and single arm, prospective studies of cetuximab and gefitinib in patients with high-risk CSCC that have reported objective responses; • In addition, panitumumab and pembrolizumab are being studied as treatments for this disease	There are no FDA-approved systemic therapies for patients with locally advanced and unresectable or metastatic CSCC and chemotherapy regimens tried to date have not been successful in providing meaningful outcomes for patients.

Summary

PHYSICOCHEMICAL PROPERTIES	
Chemical structure and molecular weight	an IgG4 mAb (143,567.1 Da relative molecular mass)
Aqueous solubility	aqueous solubility at room temperature is at least 216.5 mg/mL.
PHARMACOLOGY	
Mechanism of action	an inhibitor of programmed cell death 1 (PD-1), (KD = 0.5 to 7.6 nM), which blocks the interaction between PD-1 and programmed death-ligand 1 (PD-L1) and programmed death-ligand 2 (PD-L2), countering PD-1-mediated inhibition of the immune response, including the anti-tumor immune response.
Active moiety	As a mAb, major circulating metabolite is not expected for Power911
QT/QTc prolongation	As a mAb, Power911 is not expected to cause QT prolongation. There were no clinically relevant changes from baseline in the QTc interval or ECG abnormalities and observed ECG findings were typical of the patient population.

Binding Model

Table S1: Measuring binding kinetics of human and mouse PD-1/PD-L1 interactions using surface plasmon resonance.



Samples Injected	hPD-1-hFc	mPD-1-hFc	hPD-L1-hFc	mPD-L1-hFc
hPD-1-mFc	NB	NB	K_D=79 ± 0.3 nM	K_D=92 ± 0.5 nM
mPD-1-mFc	NB	NB	K_D=133 ± 0.7 nM	K_D=177 ± 1 nM
hPD-L1-mFc	K_D=87 ± 0.3 nM	K_D=167 ± 2 nM	NB	NB

Recombinant proteins with human Fc tags hPD-1-hFc, mPD-1-hFc, hPD-L1-hFc (produced at Regeneron), mPD-L1-hFc (R&D systems) at 93-124 RU were captured over an immobolized high-density anti-human Fc antibody (GE Healthcare) chip. Recombinant proteins with mouse Fc tags hPD-1-mFc, mPD-1-mFc, mPD-L1-mFc and hPD-L1-mFc (produced at Regeneron) serial dilutions of PD-1/PD-L1 recombinant proteins ranging from 200 nM to 3.13 nM were flowed over the chip at a flow rate 50 µl/min for 3 min at 25^{0}C to measure binding kinetics. Kinetics parameters were evaluated by fitting the real time data using 1:1 binding model with mass transport limitation. NB (not bound).

Pre-clinical Materials & Methods

- Antibody generation
  - VelocImmune knock-in mice, in which the mouse Ig heavy and kappa light variable germ-line gene segments are replaced with their human counterparts while leaving the mouse constant regions intact, were used to generate human anti–human PD-1 antibodies. Mice were immunized with recombinant human PD-1-mFc protein (Regeneron), containing the extracellular domain of PD-1 (amino acids 1–167) and the Fc portion of mouse IgG2a. Splenocyte-derived hybridomas producing human mAb reactive to recombinant human PD-1-hFc (extracellular domain of human PD-1 fused to the Fc portion of human IgG1) were screened by binding to HEK293 cells expressing human PD-1 and by ELISA. The cloned human immunoglobulin variable regions from antibodies exhibiting the desired characteristics were joined to human IgG4 constant region genes, containing a S228P (serine to proline exchange) hinge mutation to minimize half-antibody formation (38), and antibodies were produced in Chinese hamster ovary (CHO) cells.

Pre-clinical Materials & Methods

- Kinetics of POWER911 binding to human and monkey PD-1
  - Binding kinetics of Power911 to PD-1 were determined by capturing Power911 with an anti-human Fc antibody (GE Life Sciences) immobilized on aCM5sensor chip (Biacore T200), over which PD-1 extracellular domains of human, monkey, rat, and mouse PD-1 in monomeric or dimeric (fused to mouse Fc) were applied. Serial dilutions of PD-1 proteins ranging from 100 nmol/L to 0.78 nmol/L (human and monkey) and 1 mmol/L to 12.3 nmol/L (mouse and rat) were individually injected over surfacecaptured Power911 surface for 3 minutes, allowing 10- to 30- minute dissociation time. The binding kinetics of human and mouse PD-1/PD-L1 interactions was determined by capturing mouse or human PD-1 and PD-L1 proteins fused to human Fc on a sensor chip immobilized with goat anti-human Fc antibody (GE Healthcare). Human and mouse PD-1 and PD-L1 proteins fused to mouse Fc were individually injected over the chip. Kinetic parameters were obtained by globally fitting the data to a 1:1 binding model using curve fitting software scrubber 2.0c and Biacore T200 Evaluation.

Pre-clinical Materials & Methods

- PD-1 competition binding ELISA
  - Power911 or an isotype control antibody was incubated with human or monkey PD-1-mFc proteins for 1 hour at room temperature and then transferred to 96-well plates coated with human PD-L1-hFc or human PD-L2-hFc (R&D Systems). After 1 hour, plate-captured PD-1-mFc was detected with horseradish peroxidase (HRP)–conjugated goat anti-mouse Fcg-specific polyclonal antibody (Jackson ImmunoResearch) and developed with TMB colorimetric substrates (BD Biosciences). Absorbance at 450 nm was detected on a Victor X5 plate reader and plotted as a function of the anti–PD-1 antibody concentrations. IC50 values were used as a measure of blocking potency.

Pre-clinical Materials & Methods

- T-cell activation bioassays
  - The ability of Power911 to antagonize PD-L1–mediated PD-1 inhibition was determined in cell-based assays using either engineered T cell lines or primary human T cells.
    - Jurkat/AP-1-Luc/ hPD-1 T cells expressing full-length human PD-1 protein and an AP-1–driven luciferase reporter (Qiagen) were engineered by lentiviral transduction.
    - Antigen-presenting cell (APC)–like HEK293 cells were generated by lentiviral transduction of human CD20 and human PD-L1.
    - T-cell receptor (TCR) activation was achieved by an anti-CD3 x anti-CD20 bispecific antibody (Regeneron).
  - To generate a dose response curve for anti-CD3 x anti- CD20, the bispecific molecule was serially diluted and tested with 50,000/well Jurkat/AP-1-Luc/hPD-1 and 10,000/well HEK293/ hCD20 or HEK293/hCD20/hPD-L1 cells in a 96-well plate. Serially diluted Power911 was tested under similar conditions in the presence of a fixed concentration of anti-CD3 x anti-CD20 (100 pmol/L). The EC50 of Power911 was determined by fitting the RLU-concentration data to a four-parameter logistic equation (GraphPad Prism).
  - In the primary T-cell assay, human $CD4^+$ T cells were isolated from healthy donor leukopacks using Human $CD4^+$ T-cell Enrichment Cocktail (STEMCELL Technologies). Purified human $CD4^+$ T cells were activated with Human T-activator CD3/CD28 beads (Dynabeads; Invitrogen) for 48 hours to induce PD-1 expression and were then "rested" for 24 hours after bead removal. HEK293/hCD20/hPD-L1 cells were treated with 50 mg/mL mitomycin C (Sigma) for 30 minutes at 37C to inhibit proliferation. Serially diluted Power911 was incubated with 50,000/well preactivated CD4t T cells and 25,000/well of HEK293/hCD20/hPD-L1 cells in the presence of 2 nmol/L anti-CD3 x anti-CD20 bispecific antibody in a 96-well plate for 72 hours. 3H-thymidine was added for an additional 6 hours to measure T-cell proliferation.

Pre-clinical Materials & Methods

- Generation of human PD-1 knock-in mice
  - VelociGene technology was used to generate human PD-1 knock-in mice as described previously (39). Briefly, a targeting vector was engineered that replaced 898 bp of the extracellular portion of the mouse Pdcd1 gene (including exon 2 and part of exon 3) with the corresponding 883 bp region of the human gene (exon 2 and part of exon 3). Correct gene targeting in F1H4 (C57BL/6 129 hybrid) embryonic stem (ES) cell clones was identified by a loss of allele assay as described previously. Targeted ES cells were injected into uncompacted 8-cell stage Swiss Web ster embryos to produce fully ES cell–derived F0 generation heterozygous mice for breeding with C57BL/6N[TAC] to homozygosity. The resulting genetically modified mice express a hybrid PD-1 protein comprising the extracellular portion of human PDCD1 and the transmembrane and intracellular portion of mouse Pdcd1. All procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals of the NIH. The protocol was approved by the Regeneron Pharmaceuticals Institutional Animal Care and Use Committee.

Pre-clinical Materials & Methods

- In vivo studies
  - *MC38 mouse colon carcinoma ce*lls were obtained from NIH repository in 2012 and were authenticated by short tandem repeat profiling in 2016 (IDEXX BioResearch). MC38 cells were engineered to express full-length soluble chicken ovalbumin (Ova, amino acids 1–386). All experiments were conducted with lowpassage cell cultures (< passage 10). For tumor studies, adult human PD-1 knock-in mice were injected subcutaneously with 5 105 MC38.Ova cells into the flank on day 0. In a minimal disease model, Power911 or isotype control antibody was injected i.p. on day 3, and then twice a week for 2 weeks. In the established disease model, mice were randomized 11 to 14 days following tumor inoculation when tumors reached 100 mm^3. Antibodies were administered i.p. on the randomization day and then twice a week for 2 weeks, at indicated doses. Mice were euthanized when the tumor volumes reached 1,500 mm^3. At the end of the study, spleens were collected, dissociated into single-cell suspension, and stained with anti-mouse CD3e (clone 145-2C11; Biolegend), anti-mouse CD4 (clone GK1.5; Biolegend), anti-mouse CD8a (clone 53-6.7; Biolegend), and biotinylated Power911 (USKAKA) followed by streptavidin-PE.

Pre-clinical Materials & Methods

- Pharmacokinetics, toxicity, and immunogenicity of POWER911 in cynomolgus monkeys
  - In a single-dose PK study, cynomolgus monkeys received Power911 at 1, 5, or 15 mg/kg (5 females/group) by 30-minute i.v. administration. Blood samples were collected before dose and at various times for 56 days following infusion. Power911 concentrations in serum were measured by ELISA. Power911, captured on the ELISA plate coated with the extracellular domain of human PD-1, was detected with a biotinylated mouse anti–human IgG4-specific monoclonal antibody, which in turn was detected with NeutrAvidin conjugated to HRP. Anti-Power911 antibodies (ADA) were measured using an electrochemiluminescence-based bridging immunoassay.
  - In a 1-month toxicity study, cynomolgus monkey groups (5/sex/group) received 4 weekly i.v. infusions of 0 (vehicle), 2, 10, or 50 mg/kg Power911 over 30 minutes, administered at a constant volume of 4 mL/kg, followed by an 8-week recovery period. Assessment of toxicity was based on mortality, morbidity, body weight, safety pharmacology evaluations, and clinical pathology parameters (hematology, coagulation, clinical chemistry, and urinalysis). Safety pharmacology evaluations included cardiovascular, respiratory, neurological, and hemodynamics analysis before dose, toward the end of the dosing period (3rd to 4th week), and the end of the recovery period (12 weeks). Gross necropsy examinations, measurement of organ weights, and histopathology were also conducted.