CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125514Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Keytruda (pembrolizumab)

Date: August 22, 2014
To: File for BLA 125514
From: John K. Leighton, PhD, DABT
Acting Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting review for Keytruda conducted by Dr. Weis, and secondary memorandum and labeling provided by Dr. Helms. I concur with Dr. Helms' conclusion that Keytruda may be approved and the recommendation for a postmarketing commitment to conduct a study to further characterize the effect of pembrolizumab on the immune memory response.

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/s/

JOHN K LEIGHTON
08/22/2014

MEMORANDUM

Date: August 5, 2014
From: Whitney S. Helms, Ph.D.
Pharmacology Supervisor
Division of Hematology Oncology Toxicology for Division of Oncology Products 2
To: File for BLA # 125514
KEYTRUDA (pembrolizumab)
Re: Approvability of Pharmacology and Toxicology

On February 27, 2014 Merck completed the submission of biological license application (BLA) 125514 for pembrolizumab for the treatment of patients with unresectable or metastatic melanoma whose disease has progressed on or after treatment with ipilimumab. Non-clinical studies examining the pharmacology and toxicology of pembrolizumab provided to support BLA 125514 were reviewed in detail by Shawna L. Weis, Ph.D. The findings of these studies are summarized in the "Executive Summary" of the BLA review and reflected in the product label.

Pembrolizumab is a fully human IgG4 monoclonal antibody targeting programmed cell death 1 (PD-1). Merck submitted pharmacology studies demonstrating that pembrolizumab is able to bind PD-1 from both humans and cynomolgus monkeys and to prevent the interaction of PD-1 with its ligands, PD-L1 and PD-L2. This interaction plays an important role in the maintenance of self-tolerance and the prevention of unnecessary tissue damage following immune activation after acute infection by downregulating the immune response. Blocking this signaling pathway thus enhances immune reactivity and, by extension, can serve to enhance tumor immunosurveillance and the anti-tumor immune response.

General toxicology studies of pembrolizumab administered weekly for 1 or 6 months were conducted in cynomolgus monkeys to investigate the safety of the antibody. Ex vivo studies using blood from treated animals suggested that receptor saturation of PD-1 and saturation of the pharmacodynamic activity of the receptor occurred at all dose levels used in these studies. In addition, in both monkey studies, exposure of pembrolizumab at all dose levels tested exceeded that measured in humans at the intended clinical dose and schedule of 2 mg/kg once every 3 weeks. Toxicities noted in both of these studies were limited to mild increases in monocytic and lymphocytic infiltration of tissues. These changes are consistent with the pharmacologic activity of the antibody: blocking signaling through the immunoinhibitory PD-1 pathway. While monkeys did not develop any clear signs of the major toxicity associated with the use of pembrolizumab clinically, development of various autoimmune pathologies, the generalized lymphocytic infiltration does suggest a milder precursor of these types of toxicities. Given pembrolizumab's mechanism of action, the possibility exists that greater exposure to environmental influences in treated monkeys would result in a more serious inflammatory pattern closer to that seen clinically.

The requirement for reproductive toxicity studies was discussed by the Applicant prior to submission of the BLA. In accordance with principles outlined in ICH guidances S9 and S6, the Agency agreed that an assessment of reproductive toxicology based on non-product specific

literature might be acceptable in the case of this antibody. The Applicant provided literature describing investigations of the effects of disruption of PD-1 signaling in mouse models of allogeneic pregnancy. Based on the information provided, inhibition of this signaling pathway, either through administration of an anti-PD-L1 antibody or through genetic disruption, leads to increases in loss of allogeneic pregnancy. Thus, the use of an antibody inhibiting this pathway during pregnancy represents a risk to the fetus. Pembrolizumab is, therefore, not recommended for use during pregnancy unless the benefits to the mother outweigh the risks to a fetus. In mouse PD-1 knockout models, no clear physical malformations have been reported, however, late onset of or potentiation of autoimmune disorders have been associated with the loss of PD-1 signaling. Blocking PD-1 signaling during development may, therefore, result in alterations in the developing immune system. Dedicated nonclinical studies investigating the effects of pembrolizumab on embryofetal development are not warranted at this time to support its use in the intended patient population.

Consistent with the ICH S6 guidance, genetic toxicology studies were not conducted or required for pembrolizumab. Carcinogenicity studies were not required to support the licensing application for a product to treat advanced human cancer and are neither planned nor expected as post-marketing requirements at this time.

Ex vivo studies using human PBMCs demonstrated increased antigen responsiveness in the presence of pembrolizumab. Increases were noted not only following primary immune activation, but also in response to a previously recognized antigen (tetanus toxoid). The Applicant did not, however, investigate the potential for an exaggerated recall response to an antigen in either the presence or absence of pembrolizumab following a primary response in the presence of anti-PD-1. Understanding this potential may be useful in advising patients on the safety of vaccination during treatment with pembrolizumab, and an additional pharmacology study has been requested as a post-marketing commitment to better define this potential.

Finally, there are published reports showing that the absence of PD-1 signaling can result in detrimental alterations in the immune response to pathogens. Notably, infection of PD-1 deficient mice with tuberculosis was associated with a decrease in survival compared to wild type animals[1]. Similarly, decreases in survival have been reported in mouse models of lymphocytic choriomeningitis virus (LCMV) infection, though the etiology of these decreases is different between the types of infection[2-3]. Following the communication of concerns about the use of pembrolizumab in cases of chronic infection, the Applicant provided additional data late in the review cycle on the effects of pembrolizumab administration on chimpanzees infected with hepatitis B virus. Though this study was not requested by the FDA and does not appear to have been specifically conducted to address this question, the data from this study do show significant increases in liver enzymes in infected monkeys following administration of the antibody without clear effects on viral load. Overall the data is consistent with the potential for increased pembrolizumab-mediated toxicity following administration of the drug to virally-infected patients. Systematically collected clinical data from patients with chronic viral infections is limited. Data from these animal models of infection are recommended for inclusion in Section 13.2 of the label.

Recommendations: I concur with the conclusion of Dr. Weis that the pharmacology and toxicology data support the approval of BLA 125514 for Keytruda for the treatment of patients with unresectable or metastatic melanoma whose disease has progressed on or after treatment with ipilimumab. There are no outstanding nonclinical issues that would prevent the approval of Keytruda for the treatment of the intended patient population. A post-marketing commitment to further investigate the effects of pembrolizumab on the recall response has been requested.

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[1] Lazar-Molnar, et al., 2010, Programmed death-1 (PD-1)–deficient mice are extraordinarily sensitive to tuberculosis, PNAS, 107(30):13402-13407.

[2] Frebel, H., et. al., 2012, Programmed death 1 protects from fatal circulatory failure during systemic virus infection of mice. J. Exp. Med., 209(13): 2485-2499

[3] Mueller, S.N., et. al., 2010, PD-L1 has distinct functions in hematopoietic and nonhematopoietic cells in regulating T cell responses during chronic infection in mice, J. Clin. Invest., 120: 2508-2515.

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/s/

WHITNEY S HELMS
08/05/2014

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number:	BLA 125514
Supporting document/s:	001
Applicant's letter date:	28 February 2014
CDER stamp date:	28 February 2014
Product:	Pembrolizumab (KEYTRUDA)
Indication:	Malignant or metastatic melanoma in patients who have been previously treated with ipilimumab
Applicant:	Merck Sharpe and Dohme Corp.
Review Division:	DHOT / DOP2
Reviewer:	Shawna L. Weis, PhD
Supervisor/Team Leader:	Whitney S. Helms, PhD
Division Director:	John K. Leighton, PhD, DABT (DHOT) / Patricia Keegan, MD (DOP2)
Project Manager:	Sharon Sickafuse

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of BLA 125514 are owned by Merck Sharpe and Dohme or are data for which Merck Sharpe and Dohme has obtained a written right of reference. Any information or data necessary for approval of BLA 125514 that Merck Sharpe and Dohme does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of BLA 125514.

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1 Executive Summary

1.1 Introduction

Pembrolizumab is a monoclonal antibody that targets programmed cell death 1 (PD-1 receptor). PD-1 is a negative regulator of the immune response, which limits the response following initial activation. Engagement of PD-1 with its ligands, PD-L1 and PD-L2 limits T cell proliferation and cytokine production, and appears to be especially important in the maintenance of self-tolerance. Inhibition of PD-1 signaling is intended to enhance tumor immunosurveillance and anti-tumor immune responses. The Applicant has submitted the current BLA to support the use of pembrolizumab for the treatment of patients with malignant or metastatic melanoma, who have previously undergone treatment with ipilimumab.

1.2 Brief Discussion of Nonclinical Findings

Pembrolizumab is a monoclonal antibody of the $IgG_{4κ}$ subtype. Its binding affinities to PD-1 derived from human and cynomolgus monkeys were comparable (~2X difference), however, the antibody did not bind to rodent or dog PD-1. The cynomolgus monkey was therefore considered the most toxicologically-relevant model for this molecule. The pharmacological potency (IC_{50}s) as measured by inhibition of the interaction between PD-1 and its known receptors, PD-L1 and PD-L2, was also comparable between humans and monkeys (within the range of experimental variability). Consistent with its $IgG_{4κ}$ framework, pembrolizumab does not possess effector activity as measured by lack of binding to C1q and CD64; thus, it is not considered likely to clear bound T cells in vivo. Pembrolizumab was evaluated in a cytokine release assay there was no evidence of cytokine release, as assessed by IL-2 production following culture of hPBMCs in pembrolizumab-immobilized (air-dried) plates.

Pembrolizumab was able to block the binding of both PDL1 and PDL2 to PD-1 in in vitro assays using either human or cynomolgus monkey proteins. Consistent with this activity, pembrolizumab increased antigen-stimulated IL-2 when cultured with human or cynomolgus peripheral blood mononuclear cells (PBMCs) stimulated with staphylococcus enterotoxin B (SEB), relative to levels produced when PBMCs were stimulated with SEB alone. Similarly, pembrolizumab increased cytokine production in cultures of tetanus-toxoid-stimulated PBMCs from donors who had recently received the tetanus vaccine. Finally, administration of a murine anti-PD-1 antibody alone or in combination with other chemotherapeutic agents reduced tumor growth and increased survival in mice implanted with syngeneic tumor cell lines.

Pembrolizumab was evaluated in two repeat-dose toxicology studies in the cynomolgus monkey. There were no severe toxicities and no patterns of histopathological change that were suggestive of specific target organ toxicity in either study. Of note, there were considerably fewer background findings in the 6-month study than there were in the 1-month study, and the pattern of changes noted in treated animals (most of which were

described as cellular infiltration) was generally consistent with a heightened pattern of immune surveillance, as expected from the pro-inflammatory mechanism of action of the drug. The pharmacodynamic activity of pembrolizumab was demonstrated using SEB- stimulated T cell assay using whole blood from treated monkeys to examine the potential for potentiation of IL-2 production in the presence of the antibody. Pharmacodynamic saturation, as measured by the inability of additional pembrolizumab to enhance in vitro SEB-induced IL-2 production in cells from treated animals, was demonstrated in both monkey studies through the end of the dosing interval even at the lowest dose of administration.

In patients that receive pembrolizumab, autoimmune diseases are the most common adverse events, and are likely secondary to the pharmacological actions of the drug. The relationship between PD-1 pathway deficiency and most of the major autoimmune diseases is supported by data obtained in PD-1-deficient animal models and from human epidemiology studies of patients lacking PD-1. In humans, deficiency in PD-1 or its ligands has been associated systemic lupus erythematosis, rheumatoid arthritis, Hashimoto's thyroiditis, and Addison's disease, among others. Similarly, in clinical trials, pembrolizumab administration was associated with immune-related autoimmune diseases, including pneumonitis, colitis, hepatitis and thyroid disorders.

Pembrolizumab was not evaluated in a dedicated embryofetal toxicity study. In the reproductive toxicity assessment provided by the Applicant, data on adverse pregnancy outcomes in PD-1-deficient mice and in mice treated with PD-L1-neutralizing antibodies suggest that PD-1 pathway inhibition is abortifacient, as dams carrying hemi-allogeneic fetuses exhibited fetal loss in a manner consistent with immunological rejection. This observation is in keeping with the known role of PD-1 in promoting maternal tolerance to fetal antigens at the placental/fetal interface and in draining uterine lymph nodes of pregnant dams.

While antibody penetration during pregnancy is low, some monoclonal antibodies have been demonstrated to cross the placenta at sufficient levels during the period of organogenesis to induce malformations in the developing fetus. That IgG_4 monoclonal antibodies are generally capable of crossing the placental barrier is known; however, whether they do so at sufficient levels during the period of organogenesis to induce any potentially pharmacologically-mediated malformations is unclear. There are no reports of fetal malformations associated with PD-1 deficiency in mice; however, the risk of malformations does not appear to have been fully characterized in the scientific literature. Lack of PD-1 signaling has been associated with an increased risk of developing autoimmune phenotypes over time, though whether adverse immunological outcomes could arise as a result of transient PD-1 deficiency in the embryonic and early postnatal period, is unknown. Because many autoimmune adverse events occurring in patients receiving treatment with anti-PD-1 monoclonal antibodies are poorly reversible, there is some concern that autoimmune diseases, should they arise, may not be reversible. In light of the predicted abortifacient risk and a teratogenic risk that has not been fully characterized, pembrolizumab Pregnancy Category D is recommended.

Because of its known mechanism of action in limiting the immune response, there is concern that blocking PD-1 activity may exacerbate response to recall antigen stimulation. The Applicant evaluated pembrolizumab in cultures of peripheral blood cells derived from healthy volunteers that had been recently vaccinated with tetanus toxoid. When cells were re-stimulated with tetanus toxoid in culture there was an ~ 2-5X increase in the amount of IFNγ produced in pembrolizumab-treated cultures (Pembrolizumab + TT) relative to control-treated cultures (TT alone). Because pembrolizumab may also increase the numbers of circulating and tissue responder-cells, patients who are vaccinated or re-vaccinated while undergoing treatment with pembrolizumab may experience an enhanced immune response resulting in vaccine-associated toxicity.

In addition to dis-inhibiting the immune response, there is data to suggest that blockade of PD-1 can skew the lymphocyte population toward a TH1 phenotype (Amaranth, et al., 2011) and also that it may lead to inappropriate containment of chronic infections such as tuberculosis. Data in TB-infected mice suggest that PD-1 deficiency is associated with a markedly reduced survival relative to wild-type mice. These data are of concern for the treatment of patients with chronic diseases such as TB. The Applicant has indicated that patients with chronic infections such as TB and hepatitis were excluded from clinical trials. Because of these outstanding concerns about long-term consequences following use of a PD-1 inhibitor on immune response and chronic infection, additional studies to investigate the pharmacology of pembrolizumab in these situations may be requested as post-marketing requirements.

1.3 Recommendations

1.3.1 Approvability

Pembrolizumab is approvable from the nonclinical perspective for treatment of malignant or metastatic melanoma in patients who have been previously treated with ipilimumab.

1.3.2 Additional Non Clinical Recommendations

Treatment with pembrolizumab may result in enhanced immune-mediated toxicity following vaccination and recall responses. To investigate this potential, characterize the magnitude, kinetics, and resolution of the immune response following repeated vaccination (recall challenge) in anti-PD-1-treated versus control-treated animals. Measure the effect of PD-1 inhibition on the magnitude of the primary (1st vaccination) and recall (2nd vaccination) antibody responses to antigen challenge (e.g. tetanus toxoid or KLH). Evaluate the effect of PD-1 inhibition on the primary immune response once steady state plasma levels have been achieved. In half of the animals, continue dosing main study animals and reassess the magnitude of the recall response after a suitable period of continued dosing. In the other half, discontinue dosing and assess the magnitude of the response after the terminal phase. Monitor the magnitude of the recall response at least twice weekly in both subsets. Monitor clinical signs and body weight throughout the study. For animals that die on-study or are euthanized in extremis,

histological examination of major organs is suggested to characterize potential vaccine-induced toxicities.

- 1.3.3 Labeling

A separate labelling review will be provided.

2 Drug Information

2.1 Drug

CAS Registry Number (Optional)

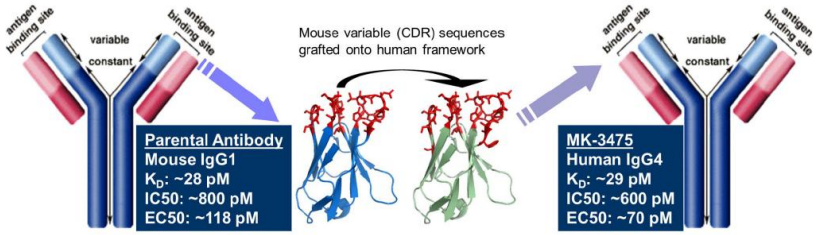
Generic Name
Pembrolizumab

Code Names
- MK-3475
- SCH 900475

Molecular Formula/Molecular Weight
MK-3475 is a humanized anti-PD-1 mAb of the $IgG_4\kappa$ (b) (4) (b) (4). The theoretical molecular weight of the polypeptide is 146.3 kDa.

Structure or Biochemical Description

Figure 1: Structure and derivation of the MK-3475 IgG4 framework from a murine IgG1 parental antibody



c.f. Merck's public presentation at the Pediatric ODAC on 05 November 2013 (http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/OncologicDrugsAdvisoryCommittee/UCM375645.pdf)

Pharmacologic Class
Pembrolizumab is a monoclonal antibody that blocks the activity of programmed cell-death 1 (PD-1).

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 110,080

2.3 Drug Formulation

MK-3475 is formulated as an aqueous solution stored under refrigerated (2-8°C) conditions at a concentration of (b) (4) in (b) (4) histidine buffer, pH (b) (4) and a fully formulated aqueous solution stored (b) (4) (b) (4)

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

None known

2.6 Proposed Clinical Population and Dosing Regimen

Malignant or metastatic melanoma in patients who have been previously treated with ipilimumab

2.7 Regulatory Background

MK-3475 (SCH 900475) was granted breakthrough status on 17 July 2013. The BLA was filed as a rolling submission, beginning with the nonclinical section, which was filed on 26 November 2013. MK-3475 has been evaluated under several INDs, as indicated in Table 1. IND 110080 is considered the primary IND for this BLA.

Table 1: MK-3475 Regulatory History

06 February 2014	IND (b) (4)
24 October 2013	Pre-BLA meeting between DOP2 and the applicant
03 October 2013	Proprietary name (Keytruda) granted
23 August 2013	IND- (b) (4)
02 August 2013	IND (b) (4)
19 April 2013	Response to Applicant re: adequacy of scientific data to support pregnancy labeling
17 January 2013	Breakthrough designation request granted
07 January 2011	IND 110080 for melanoma

3 Studies Submitted

3.1 Studies Reviewed

PRIMARY PHARMACODYNAMICS

- Assessment of functional cross-reactivity of humanized antihuman PD-1 monoclonal antibody SCH 900475 to PD-1 from various species (SN 09536)
- Biophysical characterization of humanized anti-human PD-1 monoclonal antibodies – binding and ligand blockade (SN 09544)

- SEB-induced T-cell IL-2 production as a translational biomarker for PD-1 blockade using SCH 900475/(b) (4) 307488-0 (SN09545)
- Assessment of anti-tumor activity of anti-mouse PD-1 antibody, J43, in syngeneic mouse tumor models (SN 09546)
- Source data supporting translational modeling & simulation analyses (PD-007)
- Ex vivo biological activity of humanized anti-human PD-1 monoclonal antibody SCH 900475. (b) (4): 2010 November (SN 10121)
- Pharmaceutical Sciences Technical Report (SN 10122)
- Generation and characterization of anti-mouse PD-1 antibody DX400 and murinized DX400 (PD-008)

METHOD VALIDATION:

- Quantitation of SCH 900475 drug levels in cynomolgus monkey serum using an electrochemiluminescence (ECL) assay on the MSD sector imager 6000 analyzer (DM27759)
- Quantitation of anti-SCH 900475 antibodies in cynomolgus monkey serum using an electrochemiluminescence (ECL) assay on the MSD-sector imager 6000 analyzer (DM27757)
- Detection of anti- SCH 900475 neutralizing antibodies in cynomolgus monkey serum using an electrochemiluminescence (ECL l) assay on the MSD sector imager 6000 analyzer (DM27756)
- Mk-3475: transfer validation of a quantitative ECL immunoassay using meso scale discovery's sector imager 2400 for the detection of MK-3475 concentrations in cynomolgus monkey serum (AR3607)
- MK-3475: validation of an immunoassay using meso scale discovery's sector imager 2400 for the detection of anti-MK-3475 antibodies in cynomolgus monkey serum

PHARMACOKINETICS/ADME:

- Non-GLP pharmacokinetics, pharmacodynamics and tolerability of SCH900475/(b) (4) 307488-0 following a single intravenous dose administration in female cynomolgus monkeys
- Lack of Fab-arm (Half Molecule) Exchange in MK-3475 (Anti-PD1) (PK007)

TOXICOLOGY:

- One-month intravenous (bolus) toxicity and toxicokinetic study of SCH900475 in cynomolgus monkeys with a four-month recovery period (08396)
- MK-3475 Six-Month Intravenous Toxicity Study in Cynomolgus Monkeys With a 4-Month Treatment Free Period (tt# 11-1084)

TISSUE CROSS-REACTIVITY:

- Cross-reactivity study of SCH 900475 with normal cynomolgus monkey tissues (IM1749)
- Cross-reactivity study of SCH 900475 with normal human tissues (IM1748)

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

None

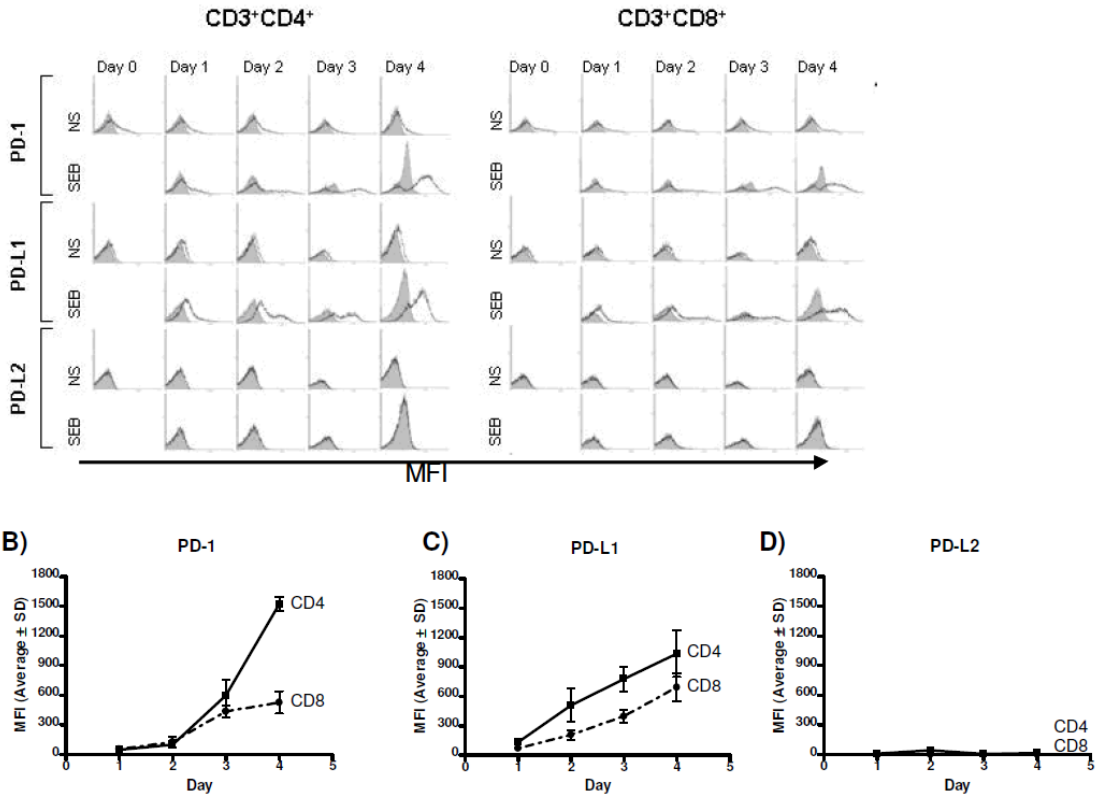
4 Pharmacology

4.1 Primary Pharmacology

MK-3475 is an inhibitory IgG_{4k} monoclonal antibody directed against human PD-1 that binds with high affinity (29 pM) and antagonizes the interaction between PD-1 and both of its known ligands (PD-L1 and PD-L2) with IC_{50}s of between 500 pM and 1 nM. PD-1 is involved in the maintenance of self-tolerance. Expressed on activated lymphocytes, PD-1 binds to its ligands, PD-L1, expressed on an array of peripheral tissues, and PD-L2, which is expressed primarily on macrophages and dendritic cells. Engagement of PD-1 signaling blunts T cell proliferation and cytokine responses.

The Applicant evaluated the expression pattern of PD-1 and its ligand following superantigen (staphylococcus enterotoxin B; SEB)-stimulation of isolated peripheral blood lymphocytes in culture. As indicated in Applicant-Figure 2, stimulation of lymphocytes with SEB enhances the surface expression PD-1 and PD-L1 in cultured lymphocytes in a time-dependent fashion, consistent with its known role in limiting the duration and/or magnitude of the inflammatory response.

Figure 2: Surface expression of PD-1, PD-L1 and PD-L2 on CD4+ and CD8+ T cells following SEB stimulation



MK-3475 was constructed [redacted](b) (4)

Inhibition of PD-1 in murine xenograft models was associated with tumor shrinkage.

Toxicological Species Specificity

The selected toxicological species was the cynomolgus monkey. Relevance of the cynomolgus monkey for prediction of potential human toxicity was demonstrated by amino acid sequence homology, binding affinity, and comparison of pharmacodynamic target inhibition in monkeys and humans. As illustrated in Applicant-Figure 3, the species bearing the greatest amino acid sequence homology to humans were the rhesus and cynomolgus monkeys. The Applicant states that the amino acid sequence identity was 96% with monkeys (both rhesus and cynomolgus), 72% with dogs and 66 and 62% with rats and mice, respectively.

Figure 3: Amino acid sequence alignment for the PD-1 extracellular binding domain in humans, monkeys, dogs and rodents

```
                10        20        30        40        50        60        70
Human   LDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCR
Rhesus  LESPDRPWNPPTFSPALLLVTEGDNATFTCSFSNASESFVLNWYRMSPSNQTDKLAAFPEDRSQPGRDCR
Cyno    LESPDRPWNPPTFSPALLLVTEGDNATFTCSFSNASESFVLNWYRMSPSNQTDKLAAFPEDRSQPGRDCR
Dog     LDSPDRPWSPLTFSPAQLTVQEGENATFTCSLADIPDSFVLNWYRLSPRNQTDKLAAFQEDRIEPGRDRR
Rat     LEVLNKPWRPLTFSPTWLTVSEGANATFTCSFSNWSEDLKLNWYRLSPSNQTEKQAAFCNGYSQPVRDAR
Mouse   LEVPNGPWRSLTFYPAWLTVSEGANATFTCSLSNWSEDLMLNWNRLSPSNQTEKQAAFCNGLSQPVQDAR

                80        90       100       110       120       130
Human   FRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPS
Rhesus  FRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPS
Cyno    FRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPS
Dog     FRVMRLPNGRDFHMSIVAARLNDSGIYLCGAIYLPPNTQINESPRAELSVTERTLEPPTQSPS
Rat     FQIVQLPNGHDFHMNILDARRNDSGIYLCGAISLPPKAQIKESPGAELVVTERILETPTRYPR
Mouse   FQITQLPNRHDFHMNILDTRRNDSGIYLCGAISLHPKAKIEESPGAELVVTERILETSTRYPS
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The ability of MK-3475 to bind cell-associated PD-1 was assessed using CHO cells that were stably transfected with either human or macaque *PD-1* using both flow cytometry and a cell-based ELISA method. As illustrated in Applicant-Figure 4 (flow cytometry of PD-1 expressing CHO cells), and Applicant -Figure 5 (ELISA), MK-3475 (h409A11) bound both the human and cynomolgus monkey isoforms of PD-1 with similar affinity.

Figure 4: binding affinity of MK-3475 toward human, cyno and mouse PD-1

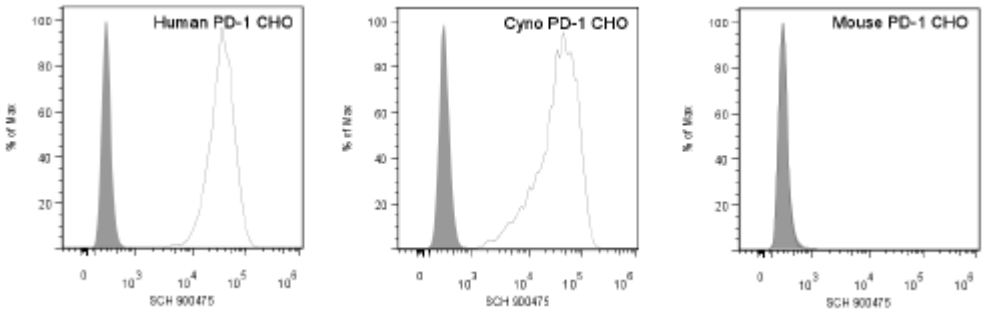
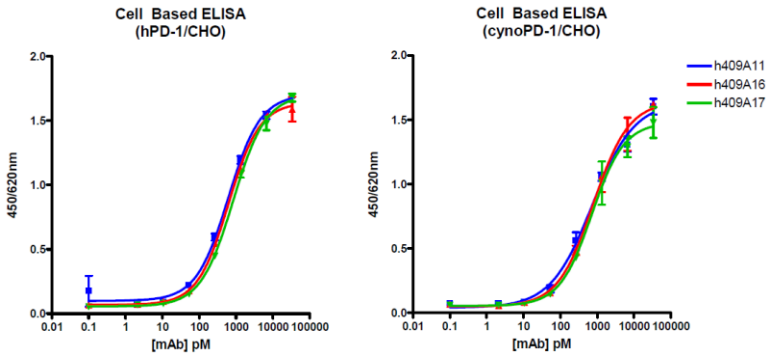
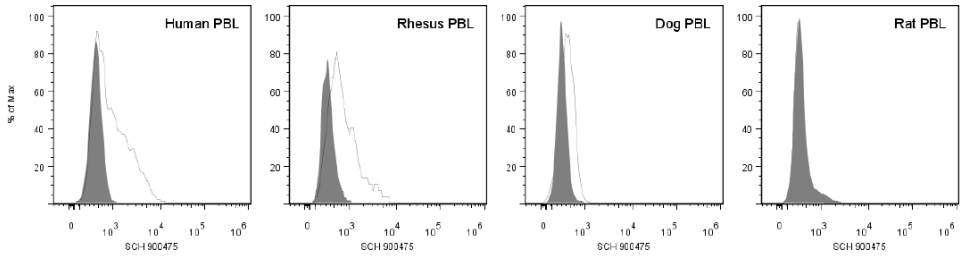


Figure 5: Ability of MK-3475 to bind cell-associated PD-1 derived from humans and cynos



As illustrated in Applicant-Figure 6, MK-3475 does not bind sufficiently to dog or rat peripheral blood lymphocytes (PBLs) to permit meaningful toxicological evaluation in either species.

Figure 6: Species Specificity of Pembrolizumab



The Applicant also evaluated the ability of MK-3475 to block PD-1 interaction with its ligands in transfected PD-1-expressing CHO (human or cyno PD-1). In this assay, cells were pre-incubated with fluorescently-labeled ligands then mixed with unlabeled antibodies. As illustrated in Applicant-Figure 7, MK-3475 (h409A11) inhibited interaction of PD-1 with labeled PD-L1 and PD-L2 in a concentration-dependent fashion. Other antibodies tested (h409A16 and h409A17) were commercially available comparator anti-PD-1 antibodies produced by (b) (4) A summary of the half-maximal inhibitory MK-3475 concentrations (IC_{50}) for inhibition PD-1 binding to PD-L1 and PD-L2 derived from both cynomolgus monkeys and humans, is provided in Table 2.

Figure 7: Inhibition of ligand binding in CHO cells stably transfected with either human PD-1 or cyno PD-1

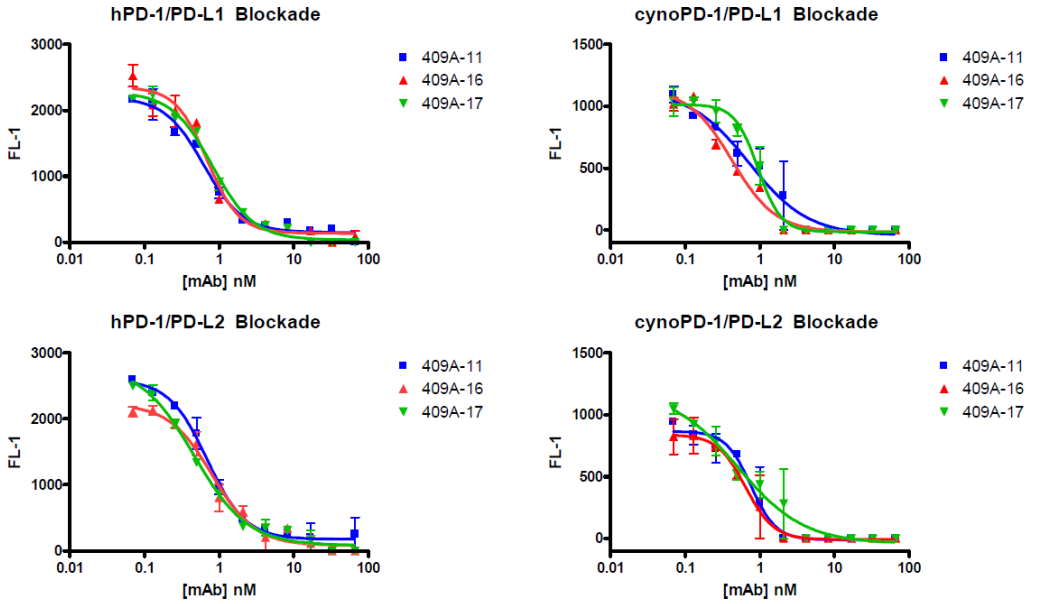


Table 2: Inhibitory concentrations (IC_{50}; pM) for blockade of human or cyno PD-1 binding to PD-L1 and PD-L2 on CHO cells

hPD-1/CHO		Cyno PD-1/CHO	
PD-L1	**PD-L2**	**PD-L1**	**PD-L2**
625±130	695±360	721±150	762±200

To demonstrate pharmacodynamic activity in animals and to develop a biomarker to predict potential patient responses, the Applicant developed a cell-based assay in which whole cynomolgus monkey or human blood was pre-incubated with MK-3475 and subsequently exposed to the super-antigen, staphylococcus enterotoxin B (SEB) to stimulate cytokine release. Consistent with its role in mediating self-tolerance, inhibition of PD-1 by MK-3475 enhanced SEB-stimulated cytokine production in both humans and cynomolgus monkeys. As illustrated in Applicant-Figure 9, MK-3475 (i.e. 900475) potentiated IL-2 secretion by approximately 2X following SEB-exposure of whole monkey blood (Applicant-Figure 8; *see also* Section 5.1, PK/ADME). A similar degree of potentiation (2-4X) was observed when MK-3475-treated whole human blood was exposed to SEB, compared to levels observed when cultures were exposed to nonspecific IgG_4 isotype control antibody (Applicant-Figure 8). The magnitude of this effect was comparable when blood from patients with advanced melanoma was compared with that of healthy volunteers, suggesting that there is no further potentiation of cytokine induction in the context of advanced cancer (Applicant-Figure 10).

Figure 8: Potentiation of IL-2 production by MK-3475 in cynomolgus monkey blood following incubation with SEB

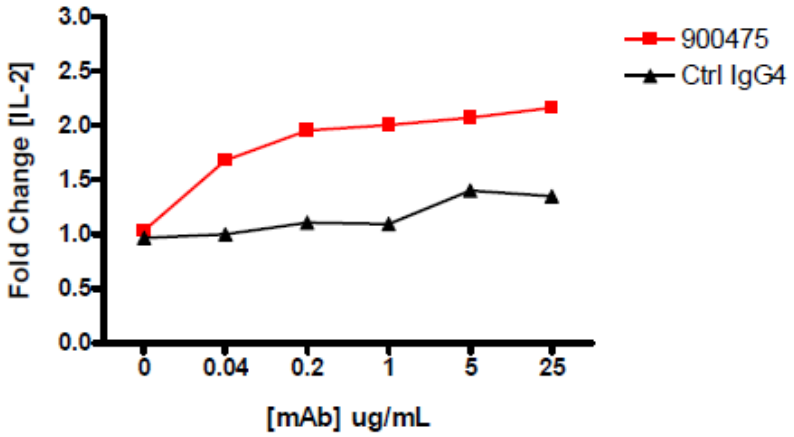


Figure 9: Enhanced IL-2 production following SEB-stimulation of healthy human whole blood

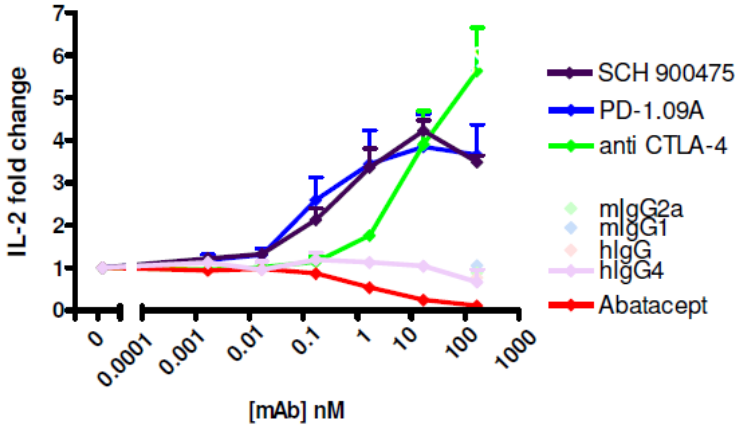
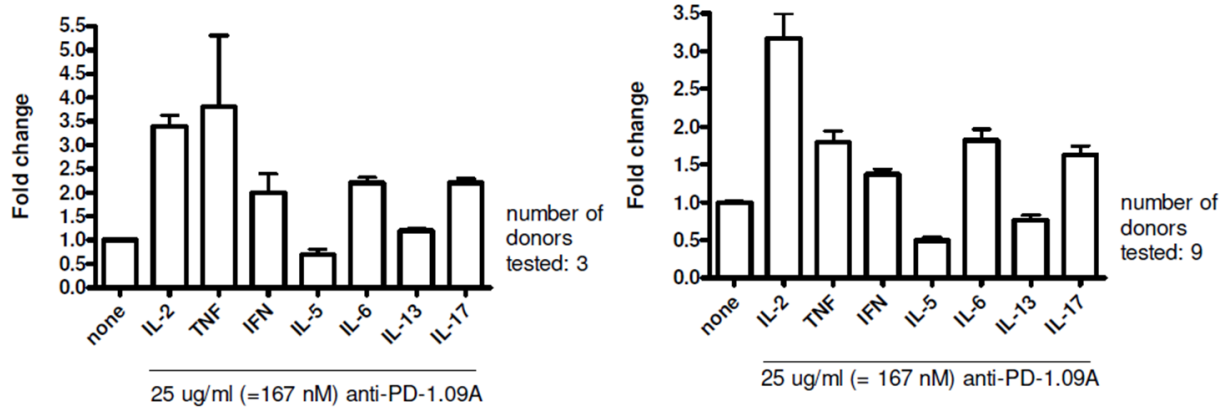


Figure 10: MK-3475 potentiation of IL2 following SEB-stimulation of whole blood from healthy donors (L) and patients with advanced melanoma (R)



Effector Activity:

Because IgG_4 antibodies do not activate complement, and exhibit reduced binding to low-affinity Fc-γ receptors, the IgG_4 framework was selected to ensure that binding of MK-3475 did not mediate clearance of tumor-reactive T-cells. As illustrated in Applicant-Figure 11 and Applicant-Figure 12, respectively, MK-3475 did not mediate effector functions, as assessed by binding to C1q and CD64, both of which are considered surrogates for potential antibody dependent cell cytotoxicity (ADCC) activity. Both assays were ELISA-based, using drug-bound plates and either biotin-labeled anti-target mAbs (i.e., C1q or CD64) with streptavidin-HRP for detection, or directly-labeled anti-target mAbs.

The results demonstrated that binding of MK-3745 to C1q (Applicant-Figure 11) and CD64 (Applicant-Figure 12) was significantly lower than that of the IgG1 nonspecific control, and was comparable to nonspecific IgG4 control antibody. Taken together, these data suggest that MK-3475 is unlikely to elicit ADCC, consistent with its IgG4 framework.

Figure 11: Binding of two lots of MK-3475 to C1q vs. control IgG4 and control IgG1

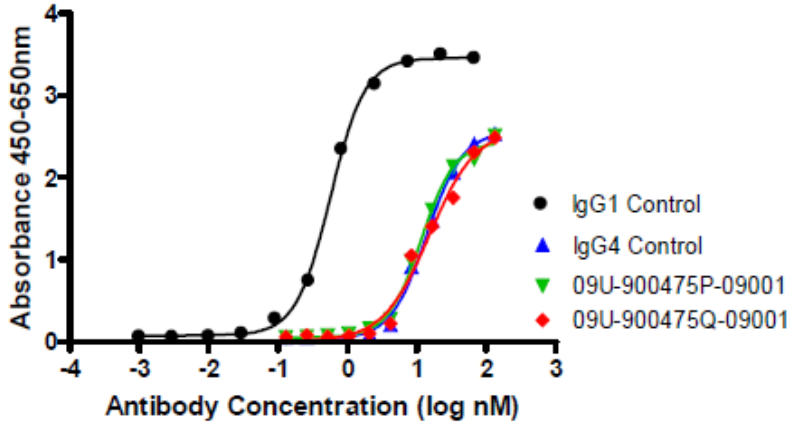
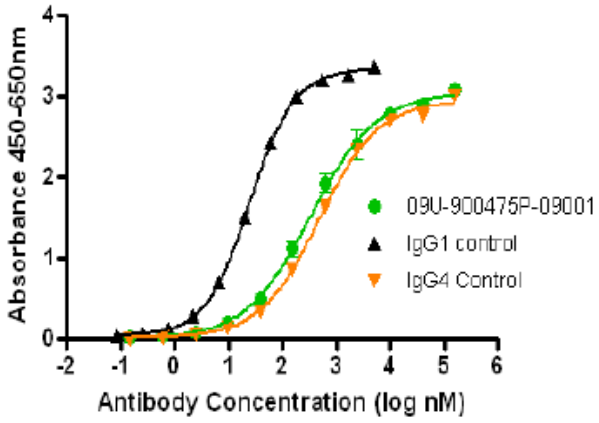


Figure 12: Binding of MK-3475 to CD64 vs. control IgG4 and control IgG1



Arm Exchange:
Due to the increased susceptibility of the IgG_4 hinge region to disulfide bond reduction, heavy chain separation and random-re-association occurs to a greater extent with IgG_4 antibodies than with other antibody subtypes (Figure 13).

Figure 13: Schematic of IgG4 arm-exchange

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The resulting products often possess bifunctional binding properties; however because each arm is monovalent for a given antigen, the resulting antibodies possess different antigen-binding properties (i.e. pharmacodynamics) from the parents, as well as potential differences in kinetics and disposition. (b) (4)

(b) (4) The Applicant evaluated the ability of MK-3475 to arm exchange using size exclusion chromatography (SEC-HPLC) and fluorescence resonance energy transfer (FRET), which measures the change in fluorescence wavelength emitted when two different fluorochromes are brought into close proximity. The FRET principle is illustrated in Applicant-Figure 14. The close proximity of arms labeled with Dy488 (488 nm) and Dy594 (594 nm) leads to emission of light at a wavelength of 815 nm (L panel vs. R panel). As indicated in Applicant-Figure 15 compared with the wildtype anti-PD-1 antibody, MK-3475 did not engage in arm exchange with the anti-PCSK9 wild-type IgG4 mAb**.**

Figure 14: The use of FRET to visualize IgG4 arm exchange

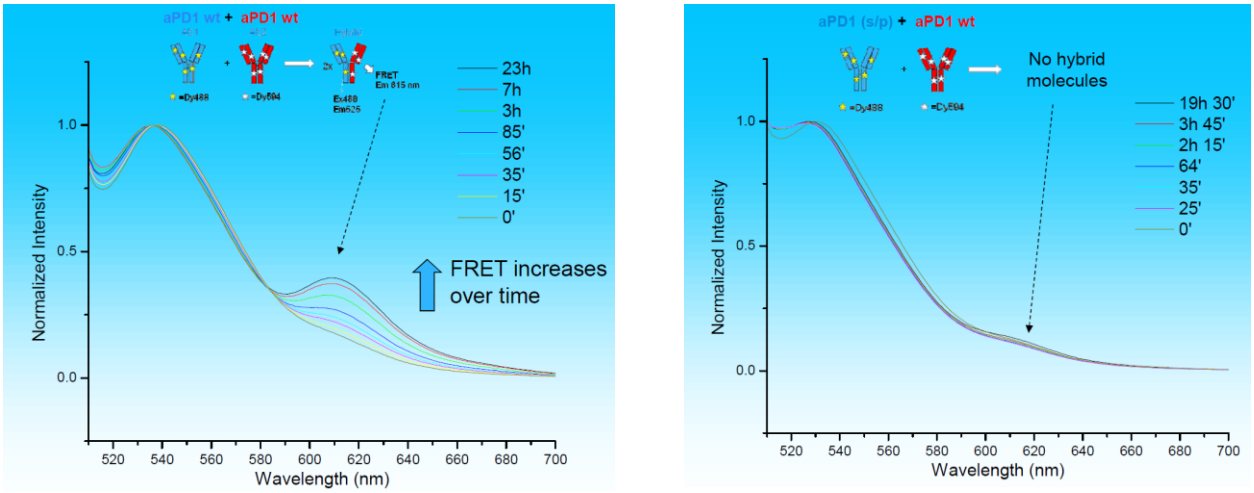
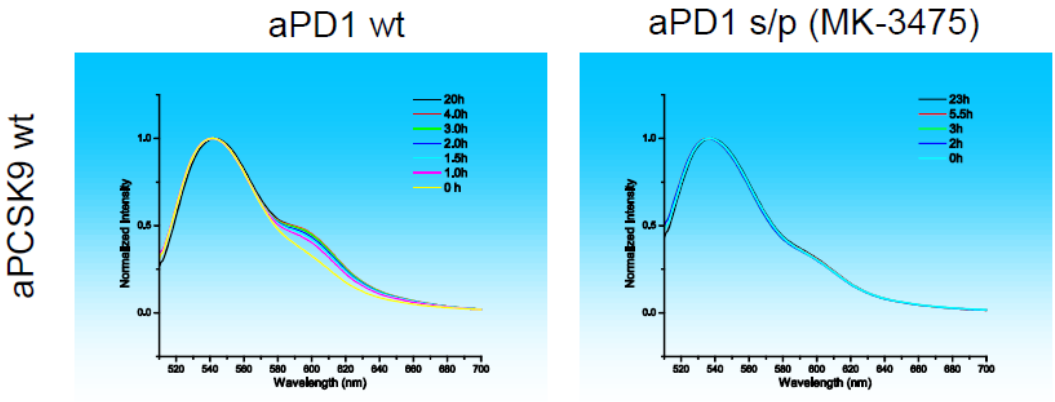


Figure 15: FRET assay with MK-3475 in the arm-exchange assay



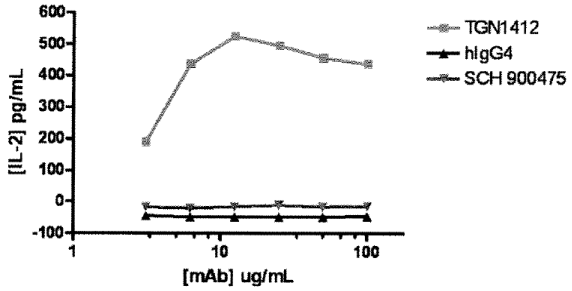
Similarly, when evaluated by SEC-HPLC (Applicant-**Error! Reference source not found.**), no intermediate peak (Panel A), (b) (4) was observed when MK-3475 was assessed in this assay (Panel B).

Figure 16: SEC assay confirming (b) (4) formation in MK-3475 arm-exchange assay

(b) (4)

Potential for Superagonist Activity:
The Applicant evaluated the potential for MK-3475 to stimulate cellular proliferation and cytokine release in an in vitro assay using heparinized whole blood from healthy volunteers. As illustrated in Applicant-Figure 17, neither control hIgG$_4$ nor MK-3475 elicited IL-2 release from healthy donor blood in this assay. Values obtained in this assay format were compared to those produced following stimulation with immobilized TGN-1412, a superagonist.

Figure 17: IL-2 release following exposure of whole human blood to immobilized MK-3475 or TGN-1412



In Report SN095045, the Applicant also evaluated cytokine levels in SEB-stimulated (0.1µg/mL) blood cultures (up to 4 days) from healthy human donors and patients with melanoma and prostate cancer. The Applicant stated that they used the Bio-Plex/Luminex human cytokine 27-plex panel kit from BioRad to assess the following cytokines: eotaxin, FGF basic, IL-1β, IL-1Ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF bb, RANTES,TNF-α, and VEGF. Under the conditions of the assay, no stimulation was observed for the following cytokines: IL-13, eotaxin, FGF, IL-1β, IL-1Ra, IL-4, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-15, G-CSF, GM-CSF, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF, RANTES, and VEGF. As illustrated in Applicant-Figure 18, increased IL-2, TNFα, IFNγ, IL-6 and IL-17 were observed. The levels produced were similar with both MK-3475 (SCH900475) and a comparator anti-human-PD-1 antibody (PD1.09A; mouse-anti-human PD-1). In comparison, IL-5 production was decreased in this assay.

Figure 18: Anti-PD-1-potentiated cytokine release following SEB-stimulation of PBMCs from healthy volunteers or cancer patients

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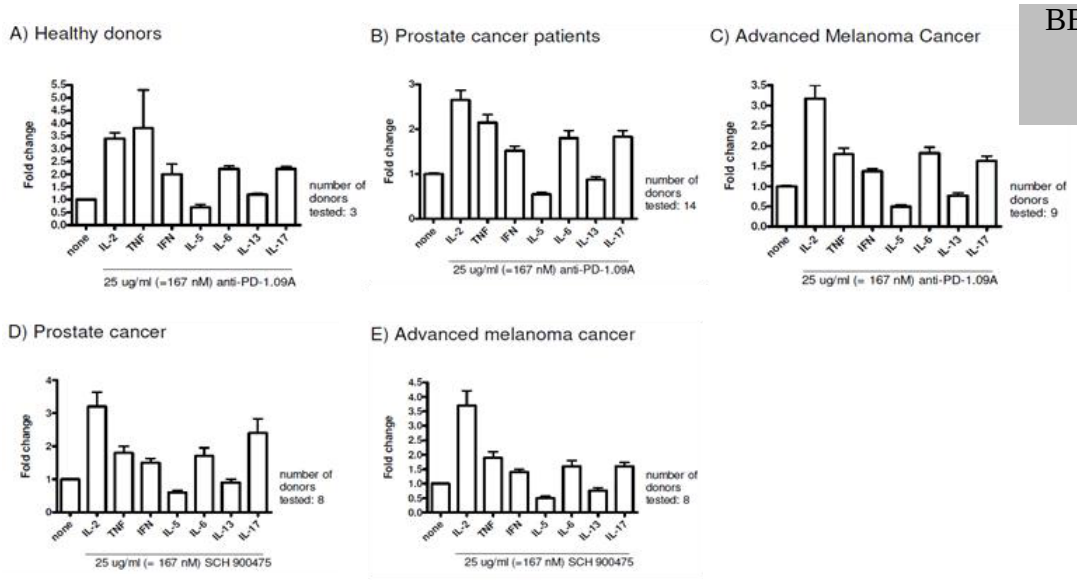
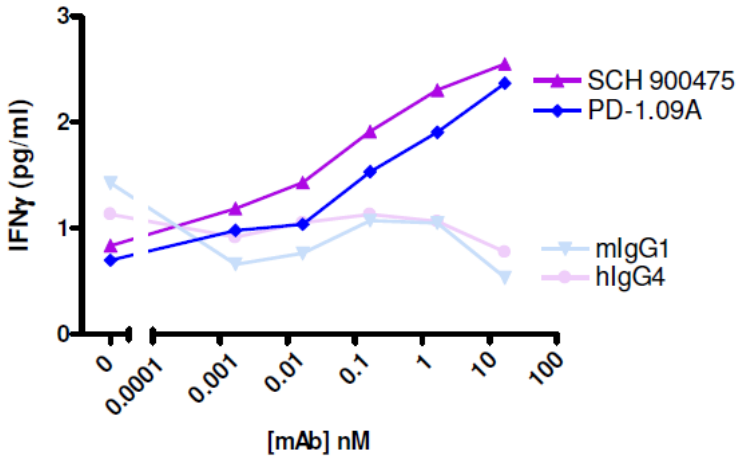


In vitro immunological re-challenge assay:
Because of the potential to increase cytokine responses following stimulation of activated T cells, the Applicant also evaluated the effect of MK-3475 exposure on release of IL-2 from healthy human donors who had been recently re-vaccinated with tetanus toxoid (TT) to assess the effect of PD-1 inhibition on the magnitude of the recall response. In this assay, cryopreserved PBMCs from subjects who had been re-vaccinated within a year of the date of collection were thawed and cultured in the presence of MK-3475 and TT (1µg/mL). Isolated PBMCs were pre-incubated with either MK-3475 or isotype control antibody (0.0001-100 nM) for 30 minutes to 1 hour prior to addition of TT. Supernatants were harvested for cytokine levels following a 7-day post-TT incubation period.

As illustrated in Applicant-Figure 19, in vitro production of IFNγ was increased approximately 2X in the presence of anti-PD-1 antibody vs. isotype control (nonspecific) antibody. This increase was similar to increases seen following primary stimulation cells with SEB in the presence of MK-3475. Because MK-3475 dis-inhibits proliferation of activated T cells, it is expected that over time, the number of potential responder cells may increase in individuals treated with MK-3475. This effect could become physiologically significant if the number of responder cells increases greatly; thus, there is concern that the magnitude of the re-challenge response may increase in proportion to size of the memory T cell population.

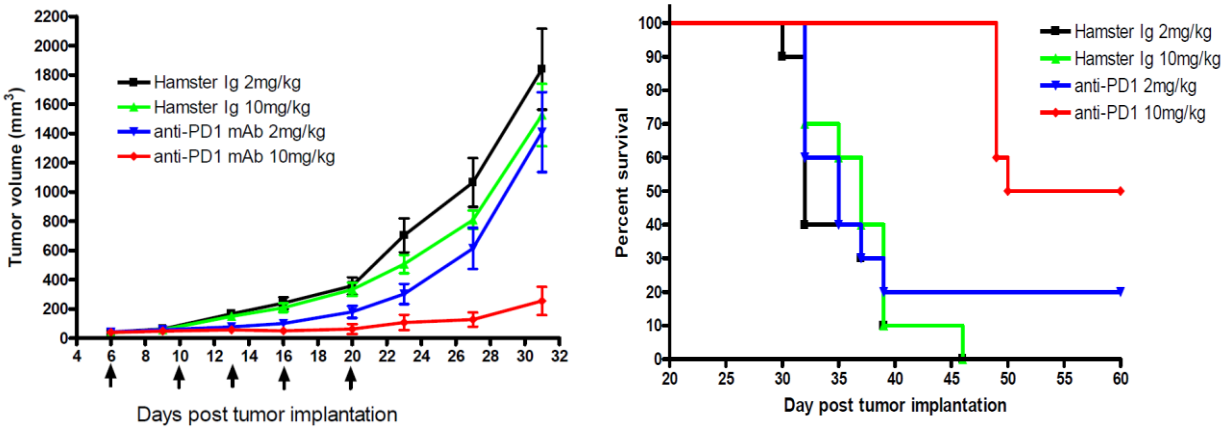
Figure 19: Enhancement of antigen-specific T-cell IFNγ production in hPBMCs from healthy volunteers who were recently re-vaccinated with TT



In Vivo Efficacy:
The potential for PD-1 inhibition to suppress tumor growth was assessed in murine tumor models of colon adenocarcinoma using a murine surrogate molecule (anti-mouse-PD-1 mAb). Administration of hamster-anti-mouse-PD-1 demonstrated anti-tumor activity against subcutaneously implanted tumors when administered alone or in combination with other chemotherapeutic agents.

In the first study, female C57Bl/6 mice were subcutaneously implanted with $1X10^6$ MC38 colon adenocarcinoma cells 6 days prior to initiation of treatment with either control hamster Ig or hamster anti-mouse PD-1 mAb. As illustrated in Applicant-Figure 20, anti-mouse-PD-1 antibody suppressed tumor growth in a dose-dependent manner and, at 10 mg/kg, prolonged survival when administered on an every 3 day schedule.

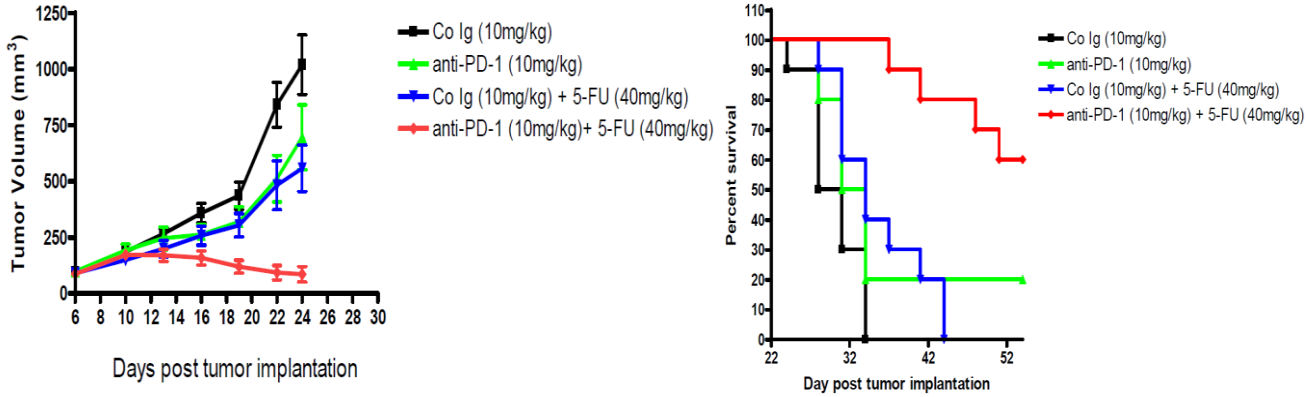
Figure 20: Anti-tumor response in a syngeneic mouse tumor model of M38 colon adenocarcinoma when treated with anti-mouse-PD-1 mAb



In a similar set of experiments, tumor-bearing mice were treated with a combination of anti-mouse-PD-1 plus either 5-fluorouricil (5-FU; Applicant-Figure 21) or gemcitabine (Applicant-Figure 22).

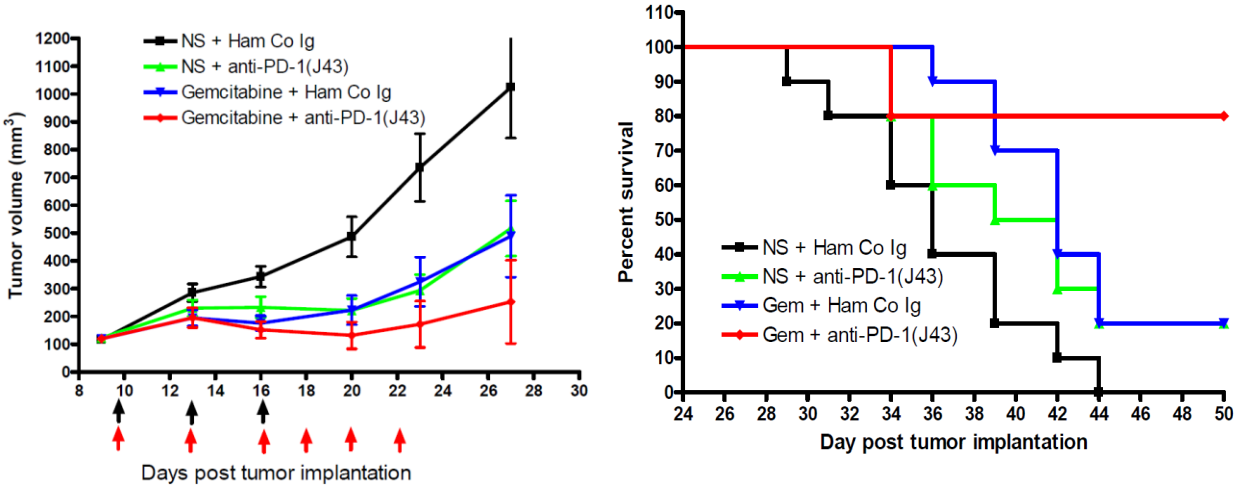
In a study of 5-FU plus anti-mouse-PD-1 mAb, C57Bl/6 female mice were implanted subcutaneously with $1X10^6$ MC38 colon adenocarcinoma cells 7 days prior to IP administration of control hamster IgG or anti-mouse PD-1 at doses of 10 mg/kg in combination with either control saline or 40 mg/kg 5-FU on Days 7, 10, 13, 16 and 19. As illustrated in Applicant-Figure 21, treatment with anti-mouse-PD-1 plus 5-FU exhibited greater anti-tumor activity than treatment with anti-mouse-PD-1 alone, and at the termination of the study, over 60% of animals in the combination treatment group survived beyond 50 days with no evidence of tumor relapse, though it was unclear from the report whether complete necropsies were performed to assess for metastatic spread beyond the local site.

Figure 21: Anti-tumor response in a syngeneic mouse tumor model of M38 colon adenocarcinoma when treated with mouse-anti-PD-1 mAb plus 5-FU



In a study of gemcitabine plus anti-mouse-PD-1 mAb, C57Bl/6 female mice were implanted subcutaneously with $1X10^6$ MC38 colon adenocarcinoma cells 10 days prior to administration of IP injections of either control hamster IgG or anti-mouse PD-1. Animals received either control saline or 40 mg/kg gemcitabine plus anti-mouse-PD-1 on Days 10, 13, and 16, at which time chemotherapy was stopped and the antibody injections continued on Days 18, 20, and 22. As illustrated in Applicant-Figure 22, treatment with anti-PD-1 plus gemcitabine suppressed tumor growth to a greater degree than anti-PD-1 alone (the response to which was comparable to gemcitabine plus control mAb). At the termination of the study, the Applicant states that there was no evidence of tumor relapse in the 80% of surviving animals in the gemcitabine/anti-PD-1 treated group.

Figure 22: Anti-tumor response in a syngeneic mouse tumor model of M38 colon adenocarcinoma when treated with mouse-anti-PD-1 mAb plus gemcitabine



Tissue samples from these in vivo studies, as well as tissues from similar rat xenograft studies were used to assess the relationship between dose and target occupancy, both in the vascular compartment and in tumor tissues. Receptor occupancy (RO) was assessed by staining blood or tumor cells from treated animals with fluorescently-labeled anti-drug antibody and expressing the mean fluorescence intensity as a percentage of the maximum fluorescence intensity achieved when samples were incubated ex vivo with an excess of drug.

In peripheral blood cells, there was no apparent dose-response in mean receptor occupancy across the range of doses tested, suggesting that saturation of target occurred at even the lowest dose of 0.1 mg/kg (Table 3). In tumor tissue, maximal binding was observed at doses ≥ 1.4 mg/kg of anti-rat-PD-1 mAb (Table 4).

Table 3: Mean receptor occupancy in rat peripheral blood following treatment with anti-rat-PD-1 mAb

	0.1 mg/kg	0.4 mg/kg	1.4 mg/kg	5 mg/kg
Day 1	253	167	101.6	111.6
Day 4	65.2	85.2	96.8	96.8
Day 5	152.8	118	142.6	141.1

Table 4: Mean receptor occupancy in rat tumor tissue following treatment with anti-rat-PD-1 mAb

	0.1 mg/kg	0.4 mg/kg	1.4 mg/kg	5 mg/kg
Day 1	22.2	32.2	54.2	41.8
Day 4	9.8	43	61.4	65.4
Day 5	33.8	41	51.6	57

Effects of MK-3475 on hepatitis B (HBV) Viral Load

The Applicant also performed an exploratory study to evaluate the effects of MK-3475 on safety, tolerability, and viral load in hepatitis B-infected chimpanzees. The purpose of this study is unclear, as CDER does not require or recommend conducting studies in chimpanzees, nor was the study clearly related to the proposed indication. The Applicant states that chimpanzees were not purposely infected with hepatitis B for use in this study.

Four, HBV-infected chimpanzees received once-weekly escalating IV doses according to the design described in Table 5. Blood sampling for pharmacokinetics, anti-drug antibody, and clinical pathology were collected on Study Days -14, -7, 0, 3, 7, 10, 14, 17, 21, 24, 28, 31, 35, 42, 49 and 56. An additional set of blood samples was collected on Day 63 from animal A2A004 to monitor the ALT/AST flare. Viral load was also assessed. Cytokine levels were evaluated in whole blood following stimulation with peptides derived from HBV polymerase (designated as Pol1, Pol2, Pol3, Pol4), capsid, and HBV surface antigen (sAg). Liver biopsies were collected on Days 1 and 28.

Table 5: Dose escalation schema used in the rising-dose study in the chimpanzee

	Study Day	Dose Level
First Dose	0	1 mg/kg
Second Dose	7	2 mg/kg
Third Dose	14	5 mg/kg
Fourth Dose	21	10 mg/kg
Fifth Dose	28	10 mg/kg

All animals survived and were returned to the holding colony at the termination of the study. There were no effects of treatment on blood pressure or body weight, and no changes in the limited hematology (hematocrit) or clinical chemistry parameters (bilirubin, triglycerides) measured. The pharmacokinetics of MK-3475 was also

evaluated in the chimpanzee. Exposure was highly similar between all the animals at each dose level, and there were no observations of ADA formation (data not shown).

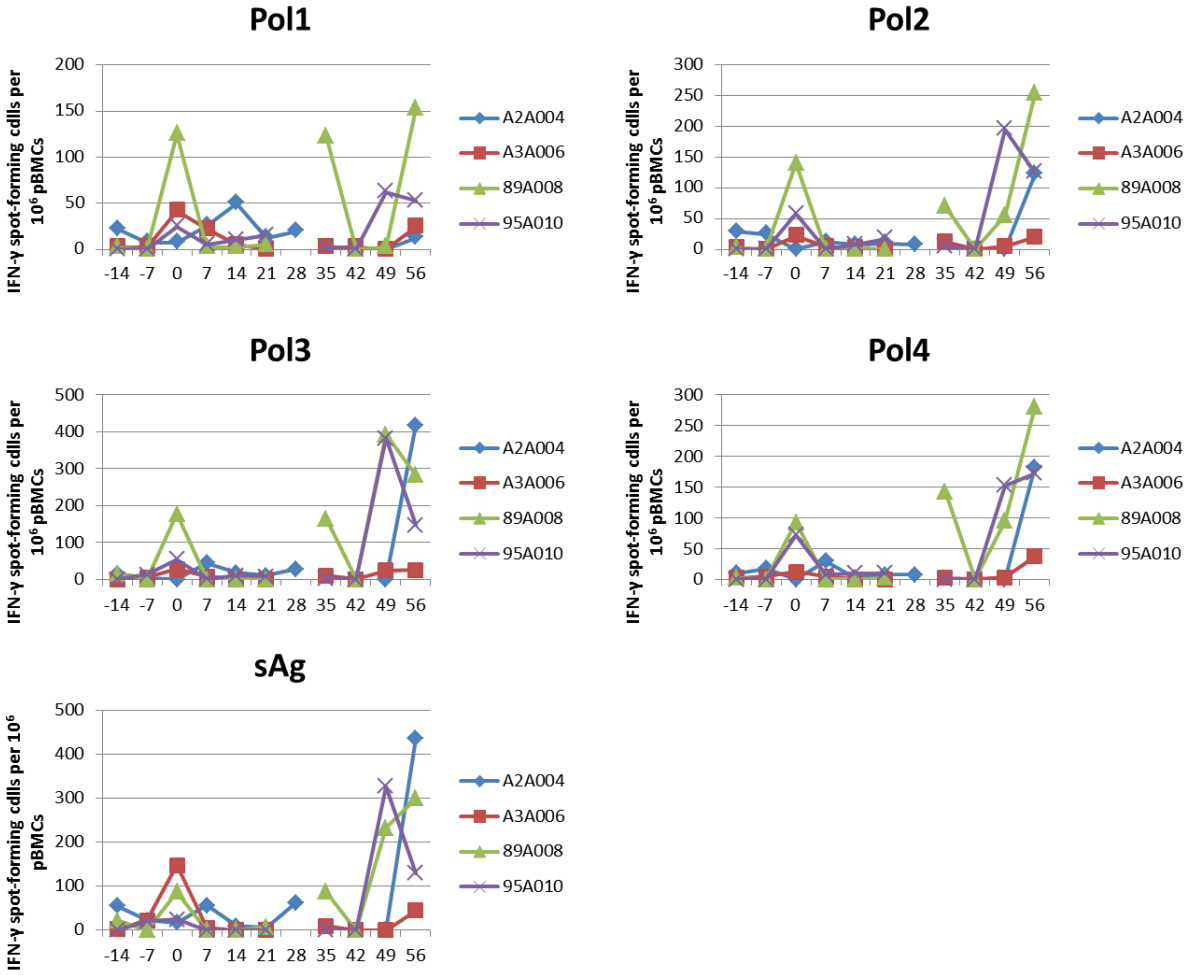
While the data do not suggest an impact of MK-3475 level on the viral burden (as measured by Log Copies/mL (Applicant-Table 6), the data do suggest that in the context of HBV, MK-3475 may increase immune-mediated destruction of infected cells in the liver. This is an effect that is expected by virtue of its pro-inflammatory mechanism of action, and its interpretation is supported by data in models of viral injury. Infection of PD-1 deficient mice with murine hepatitis virus leads to a massive inflammatory response, accompanied by marked increases in liver function enzymes (e.g. ALT; Chen et al., 2011) relative to those observed in strain-matched wild-type controls.

Table 6: Efficacy as measured by viral load (Log copies/mL)

Day	Animal Number			
	A3A006	A2A004	89A008	95A010
-14	> 8.0	> 8.0	> 8.0	4.8
-7	> 8.0	> 8.0	> 8.0	4.3
0 (First dose)	> 8.0	> 8.0	> 8.0	4.1
3	> 8.0	> 8.0	> 8.0	4.2
7 (Second dose)	> 8.0	> 8.0	> 8.0	4.1
10	> 8.0	> 8.0	> 8.0	4.1
14 (Third dose)	> 8.0	> 8.0	> 8.0	4.5
17	> 8.0	> 8.0	> 8.0	3.7
21 (Fourth dose)	> 8.0	> 8.0	> 8.0	4.7
24	> 8.0	> 8.0	> 8.0	4.7
28 (Fifth dose)	> 8.0	> 8.0	> 8.0	4.6
31	> 8.0	> 8.0	> 8.0	4.6
35	> 8.0	> 8.0	> 8.0	4.6
42	> 8.0	> 8.0	> 8.0	4.5
49	> 8.0	> 8.0	> 8.0	4.5
56	> 8.0	> 8.0	> 8.0	4.3
63	NA	> 8.0	NA	NA

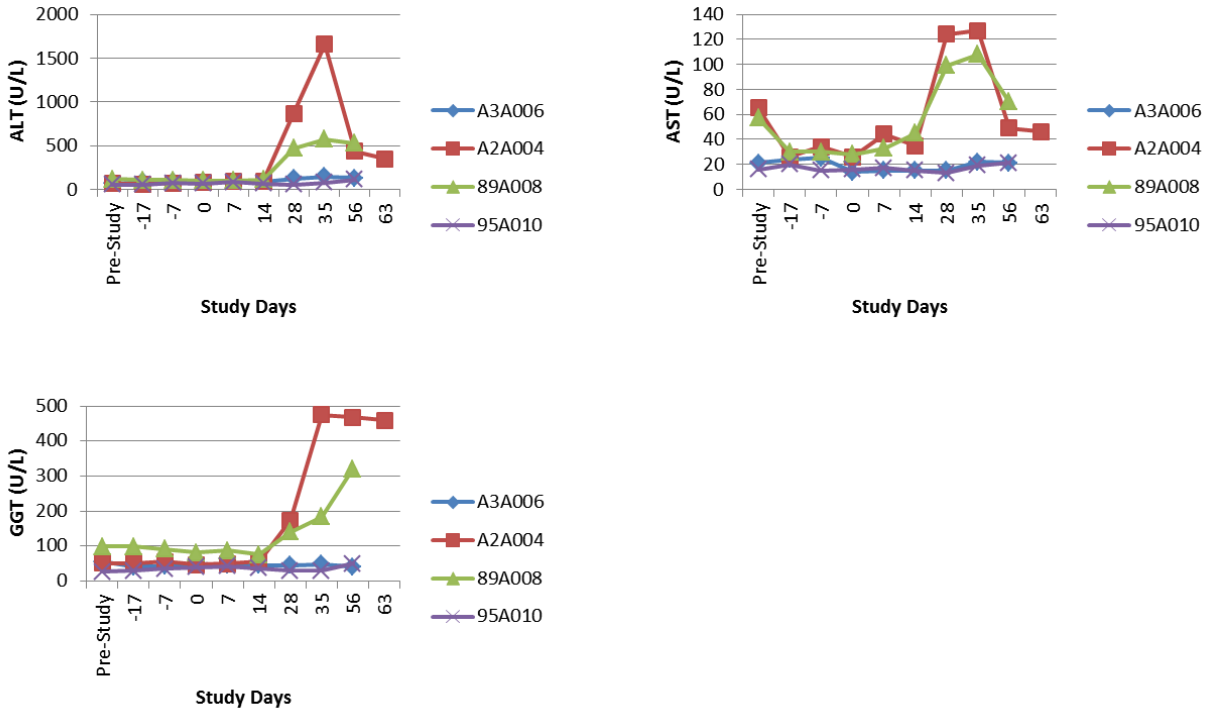
While IFN-γ production in whole chimpanzee blood stimulated with HBV antigens was highly variable, larger responses were generally noted during the post-dose washout phase (Figure 23).

Figure 23:IFN-γ production following HBV antigen stimulation in whole chimpanzee blood



In two of the four chimpanzees, liver enzymes (LFTs) were markedly increased following administration of the second high dose (10 mg/kg) of MK-3475. The Applicant attributes this in part to procedural events (biopsy); however, given the pharmacology of the drug, a relationship to treatment is plausible. Animal A2A004 exhibited the highest IFNγ response to sAg (Figure 23) and the most pronounced increases in GGT, ALT and/or AST (Figure 24). This animal also exhibited an IFNγ response in the liver biopsy assay. Out of the four biopsies collected, this was the only animal that exhibited a detectable response (data not shown).

Figure 24: Liver enzyme measurements in MK-3475-treated chimpanzees



4.3 Safety Pharmacology

Safety pharmacology endpoints were incorporated into general toxicology studies.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

5.1.1 Non-GLP pharmacokinetics, pharmacodynamics and tolerability of SCH900475/(b) (4) 307488-0 following a single intravenous dose administration in female cynomolgus monkeys

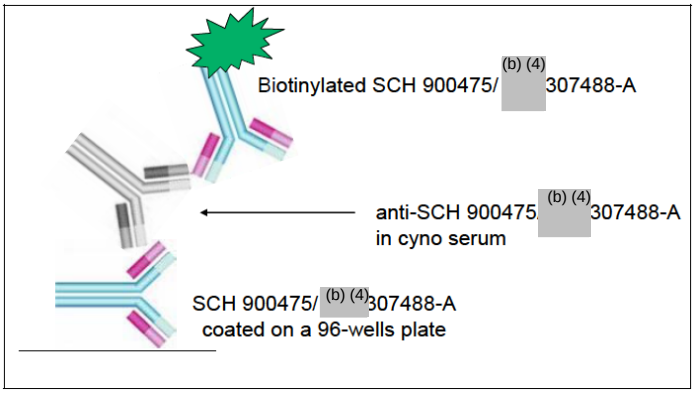
The purpose of this study was to characterize the pharmacokinetics of MK-3475 in female cynomolgus monkeys following single IV doses of 0.3, 3, or 30 mg/kg. In addition, changes in body weight, clinical signs, and changes in clinical pathology parameters were assessed. Blood samples were used to develop an investigational biomarker of anti-PD-1 activity that might predict therapeutic response in patients with advanced cancer. Baseline samples were collected on Study Days -21, -14, -7, and post-dose samples were collected on Study Days 1, 2, 3, 4, and 7, then weekly thereafter (Days 14, 21, 28, 35, 42, 40, 56, 63, 70, 77 and 84). Clinical pathology samples were collected on Days -21, -14, 7, 1, 7, 28, 56, and 84. PBMCs were collected predose (Days -17 and -7), and on postodse Days 7, 28, and 84.

Peak (C_{max}) and overall MK-3475 exposures (AUC_{∞}) were generally dose-proportional over the 100-fold dose-range evaluated. Half-life estimates ranged from 3.9 to greater than 10 days (Applicant-Table 7). A high incidence of anti-drug antibody (ADA) formation was observed. By Day 21, all animals in the low-dose group were ADA-positive, and by Day 63, all but one of the animals in the high dose group were ADA positive. Given the method described in Applicant-Figure 25, it is likely that study drug interfered with the detection of ADA in this assay, and that many of the animals determined to have been ADA negative prior to Study Day 63 were in fact positive earlier, as ADA molecules that were bivalently bound to study drug would have been unavailable to bind immobilized MK-3475.

Table 7: PK parameters following single IV dosing of MK-3475 (SCH 900475) in female cynomolgus monkeys

	0.3 mg/kg SCH 900475 Average ± sem *(monkey 11116, 12412,5294)* ***With ADA***	3 mg/kg SCH 900475 Average ± sem *(monkey 11250,11258,1382)* ***With ADA***	30 mg/kg SCH 900475 Average ± sem *(monkey 10304,11252,12082)* ***With/ without ADA***	30 mg/kg SCH 900475 *(monkey 12082)* ***Without ADA***
Corr	-0.968 ± 0.011	-0.933 ± 0.043	-0.0961 ± 0.008	-0.9711
Cmax (mg/L)	15.3 ± 4.3	117.7 ± 5.2	1265 ± 73	1188
AUC_extrap (%)	19.5 ± 6.7	4.0 ± 1.3	21.0 ± 11.2	1.2
AUC_last (day/mg/L)	41.0 ± 2.9	700.0 ± 76.5	6374 ± 767	7616
AUC_inf (day/mg/L	51.1 ± 1.5	729.3 ± 79.5	8124 ± 416	7725
T ½ _Terminal (day[s])	3.9 ± 0.7	5.9 ± 1.6	10.6 ± 0.4	10.4
$T_{1/2}$–VSS / Cl (days)	3.7 ± 0.8	6.3 ± 1.4	10.2 ± 0.2	10.7
CL (mL/day/kg)	5.7 ± 0.2	4.2 ± 0.4	3.7 ± 0.1	3.9
Vss (mL/kg)	30.9 ± 6.0	36.8 ± 4.6	54.8 ± 5.7	60.0

Figure 25: Anti-drug antibody ELISA method



The ability of MK-3475 to potentiate cytokine release even in the presence of a superagonist was noted in early development studies with this compound. (b) (4)

(b) (4)

Applicant-Figure 26, summarizes IL2-potentiation data obtained in a PK-PD study of MK-3475 in the monkey. In this study, whole blood was collected from all animals prior to dose administration, and at varying post-dose timepoints to assess the effect of a single dose of MK-3475 on SEB-induced IL-2 production. For this study, samples were divided into two cultures: those incubated in the presence of SEB alone, and those incubated in the presence of SEB+MK-3475. For each animal at each timepoint, the amount of IL-2 produced in the presence of MK-3475 was normalized to the amount produced in the presence of SEB alone.

As illustrated in Figure 26, MK-3475 potentiated production of IL-2 in all dose groups; however, following dose administration, further potentiation of IL-2 release was lost in animals that received MK-3475 (presumably as a result of receptor saturation). Further, loss of potentiation was observed across all dose levels of MK-3475, suggesting that pharmacodynamic saturation occurred even at the lowest dose tested (0.3 mg/kg). The ability to potentiate further IL-2 production was restored to control levels by approximately Day 60, which the Applicant attributes to drug-clearance over the 90-day washout interval (Applicant-Figure 26). This explanation is supported by the pharmacokinetic clearance data provided in Applicant-Figure 27.

Figure 26: PD biomarker analysis of SEB-stimulated IL-2 production in monkey PBMCs

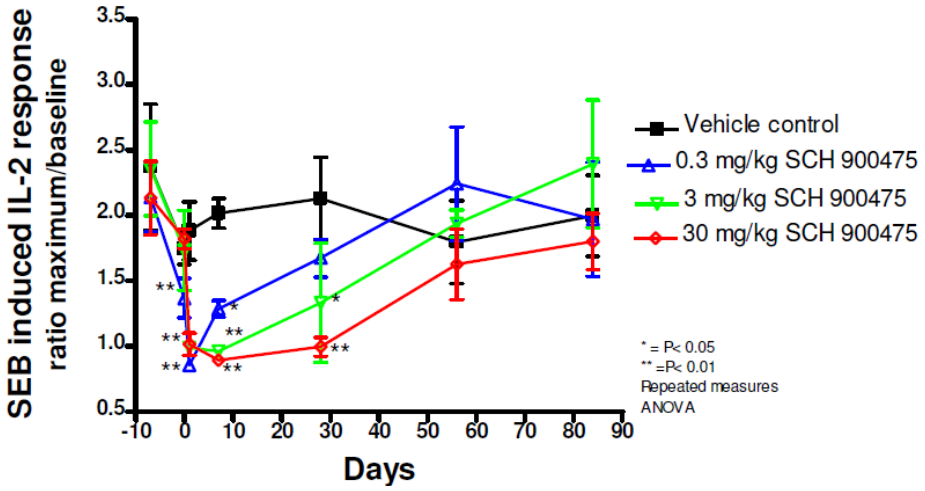
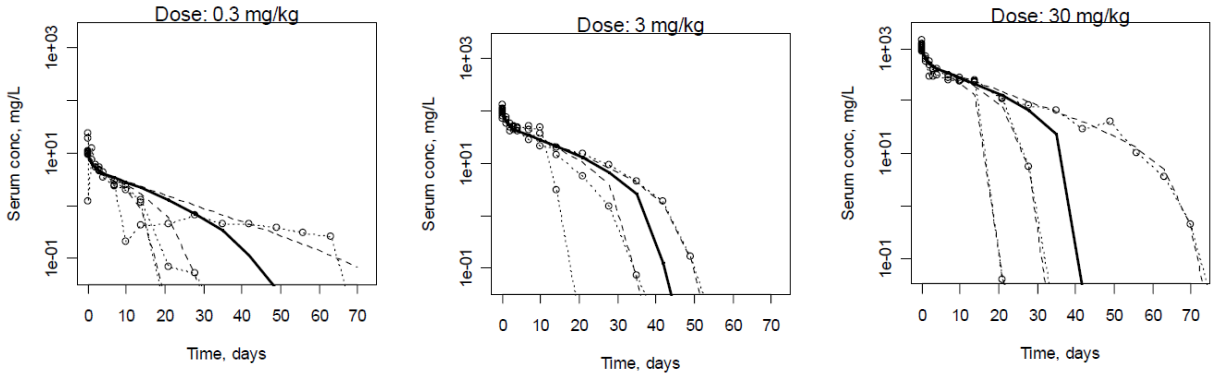


Figure 27: Individual concentration-time profiles of MK-3475 in female cynomolgus monkeys following a single IV dose



6 General Toxicology

6.2 Repeat-Dose Toxicity

6.2.1 One-month intravenous (bolus) toxicity and toxicokinetic study of SCH 900475 in cynomolgus monkeys with a four-month Recovery period

Key Study Findings

There were no preterm deaths and no treatment-related toxicities observed. Sporadic, non-dose-related changes in clinical pathology parameters were observed; however, these were of equivocal relationship to MK-3475 treatment. A diffuse pattern of immune cell infiltration was observed histologically in treated animals; however, in general these were not considered dose-limiting, though they may be toxicologically significant as they may hallmark the immunologically-mediated adverse events that are observed in patients treated with MK-3475 (*e.g.* renal, endocrine, dermal and pulmonary auto-immune conditions).

Methods

Doses:	6, 40, 200 mg/kg/dose
Frequency of dosing:	Weekly (5 total doses)
Route of administration:	IV bolus
Dose volume:	0.24, 1.6 and 8 mL/kg for low, mid and high dose levels, respectively
Formulation/Vehicle:	10 mM histidine pH 5.5, 7% (w/v) sucrose, 0.02% (w/v) polysorbate 80
Species/Strain:	Macaca fascicularis
Number/Sex/Group:	4/Sex (main study) + 2/Sex (Recovery cohorts, all groups)
Age:	36-39 months (male)

	24-51 months (female)
Weight:	3.0-4.6 kg (male)
	2.5-3.9 kg (female)
Satellite groups:	None
Unique study design:	Staphylococcus enterotoxin B (SEB)-stimulated IL-2 measurement from isolated blood samples (pharmacodynamic activity)
Deviation from study protocol:	Peer review was not performed as indicated per protocol

Observations and Results

Mortality

There were no preterm deaths that were ascribed to the study drug. One low-dose female was euthanized on Study Day 1 following a fracture of the right radius; however, the injury was not attributable to treatment. This animal was subsequently replaced with female #3505.

Clinical Signs

Alopecia, soft/loose stool, skin lesions

Body Weights

Unremarkable; the differences in body weight in high dose females reflect lower mean baseline values.

Figure 28: Male mean body weights in the 4-week repeat-dose toxicity study in the cynomolgus monkey

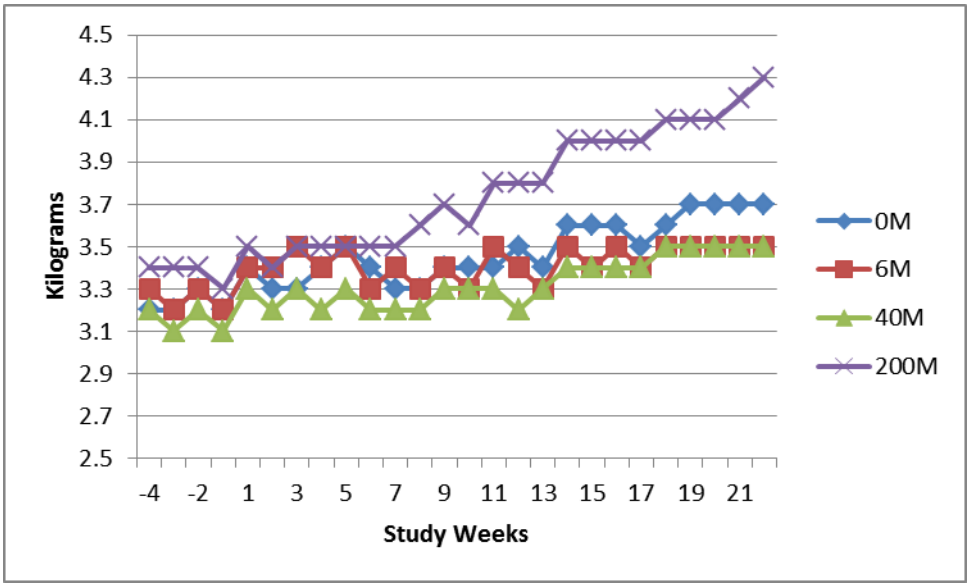
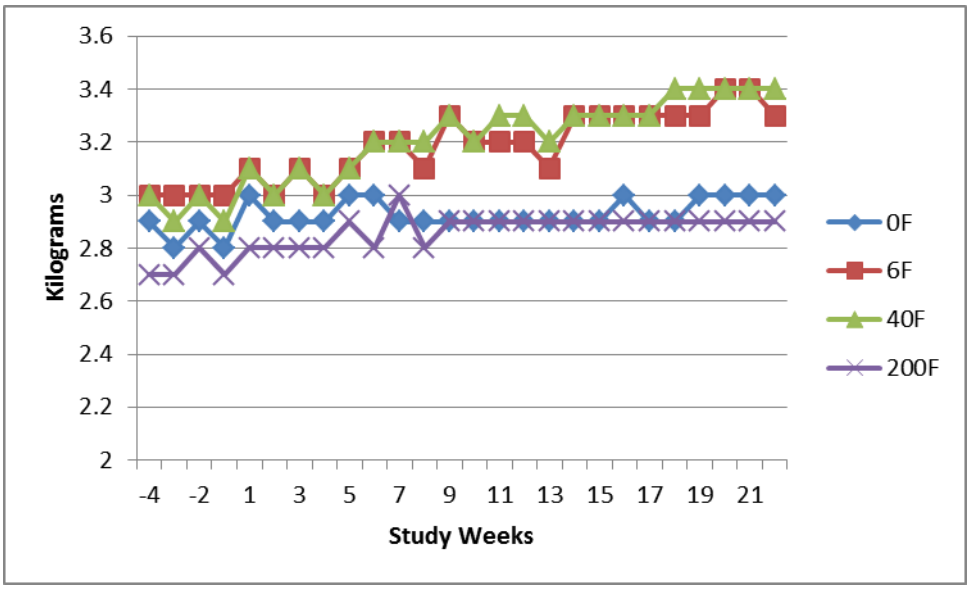


Figure 29: Female mean body weights in the 4-week repeat-dose toxicity study in the cynomolgus monkey



Feed Consumption

Unremarkable

Ophthalmoscopy

Unremarkable

ECG

Unremarkable

Hematology

Unremarkable. Mean hematology parameter values are presented in Table 8. While individual variations in hematology parameters vs. pretest and/or concurrent control data were occasionally noted; these are considered to be unrelated to treatment.

Table 8: Mean hematology parameters measured in the 4-week cyno study

	Week	1M	2M	3M	4M	1F	2F	3F	3F
RBC (10^6/µL)	-3.0	7.1	6.7	6.5	7.0	6.4	6.5	6.9	6.7
	-1.0	7.0	6.5	6.3	6.9	6.4	6.4	6.7	6.5
	4.0	6.8	6.5	6.1	6.7	6.1	6.3	6.7	6.2
	22.0	7.0	6.8	6.2	6.8	6.3	6.6	7.0	6.4
HGB (g/dL)	-3.0	13.4	13.4	13.5	14.1	12.9	13.2	13.7	13.7
	-1.0	13.0	13.0	12.9	13.7	12.7	12.7	13.2	13.3
	4.0	12.6	12.8	12.5	13.1	12.2	12.4	12.9	12.6
	22.0	13.2	14.1	12.9	13.6	12.9	12.3	13.7	12.3
HCT (%)	-3.0	46.3	45.1	45.3	48.1	44.4	44.4	46.9	46.7
	-1.0	45.9	43.8	44.0	47.4	44.4	43.6	45.0	45.9
	4.0	45.3	43.5	43.6	45.6	43.2	43.2	45.7	45.0
	22.0	45.9	47.2	43.8	47.5	44.2	42.3	47.7	42.6

	Week	1M	2M	3M	4M	1F	2F	3F	3F
MCV (fL)	-3.0	65.9	67.3	69.8	68.5	69.6	68.7	67.8	70.3
	-1.0	65.8	67.1	70.2	68.3	69.6	68.8	66.9	70.7
	4.0	66.9	67.2	70.9	68.0	70.5	69.1	68.7	72.4
	22.0	66.3	69.1	70.8	69.8	70.0	64.7	68.1	67.2
MCH (pg)	-3.0	19.0	20.0	20.8	20.1	20.2	20.4	19.9	20.7
	-1.0	18.7	19.9	20.5	19.8	20.0	20.0	19.6	20.4
	4.0	18.6	19.7	20.2	19.5	20.0	19.8	19.4	20.3
	22.0	19.1	20.5	20.8	20.1	20.4	18.8	19.6	19.3
MCHC (g/dL)	-3.0	28.9	29.8	29.8	29.3	29.0	29.7	29.3	29.4
	-1.0	28.4	29.7	29.3	29.0	28.7	29.1	29.3	28.9
	4.0	27.7	29.3	28.6	28.6	28.3	28.7	28.3	28.1
	22.0	28.8	29.8	29.3	28.7	29.2	29.0	28.7	28.7
RET(%)	-3.0	0.5	0.4	0.4	0.5	0.5	0.5	0.7	0.6
	-1.0	0.7	0.5	0.7	0.5	0.7	0.6	0.9	0.7
	4.0	1.0	1.0	1.1	1.1	1.2	1.3	1.6	1.4
	22.0	0.4	0.5	0.6	0.4	0.3	0.3	1.1	0.8
ARET (1000/µL)	-3.0	31.1	29.6	26.8	33.3	33.0	31.1	51.2	37.5
	-1.0	46.6	32.2	43.5	35.0	41.4	40.5	61.5	46.3
	4.0	66.5	63.0	66.5	73.0	73.1	80.1	103.0	87.0
	22.0	27.7	31.8	36.2	26.6	17.9	21.4	72.7	48.5
PLT (10^3/µL)	-3.0	420.2	468.0	495.0	422.5	464.7	436.0	451.7	407.8
	-1.0	420.5	432.7	489.8	421.8	428.3	424.0	404.5	407.8
	4.0	424.2	435.7	493.0	405.7	418.0	421.0	422.5	418.3
	22.0	379.5	426.0	465.0	454.0	464.5	399.0	450.0	374.0
MPV (fL)	-3.0	9.0	8.7	8.6	9.2	9.3	9.6	10.0	9.3
	-1.0	9.0	8.9	8.8	9.0	9.2	9.4	9.7	9.4
	4.0	9.1	8.6	8.6	8.6	9.9	9.4	9.5	9.1
	22.0	9.2	9.4	8.8	9.2	9.9	9.1	9.1	9.0
RDW (%)	-3.0	14.5	14.4	14.1	13.4	14.8	14.2	15.1	14.5
	-1.0	14.3	14.2	13.9	13.1	14.3	13.9	15.0	14.2
	4.0	14.6	13.7	13.5	13.3	14.1	14.2	14.8	14.0
	22.0	14.3	13.1	13.7	13.4	14.3	13.8	14.2	13.8
WBC (10^3/µL)	-3.0	10.9	11.0	11.4	11.1	11.2	10.5	15.4	10.8
	-1.0	12.7	10.8	15.3	15.6	13.0	12.0	14.0	11.2
	4.0	10.7	12.9	11.3	10.1	10.8	10.0	13.2	9.9
	22.0	10.0	10.4	8.8	9.3	11.9	14.6	10.7	11.1
LUC (10^3/µL)	-3.0	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.1
	-1.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	4.0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	22.0	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.1
LYM (10^3/µL)	-3.0	5.8	5.4	4.8	6.6	4.4	4.7	6.4	5.4
	-1.0	5.5	5.7	5.1	6.8	4.8	4.8	4.7	5.5
	4.0	6.2	6.8	5.5	5.5	5.2	5.4	7.1	5.5
	22.0	5.6	6.7	6.0	5.9	7.4	5.6	6.9	5.1

	Week	1M	2M	3M	4M	1F	2F	3F	3F
NEUT (10^3/µL)	-3.0	4.2	5.0	5.0	3.9	6.0	5.0	8.2	4.6
	-1.0	6.3	4.4	4.4	8.0	7.6	6.5	8.8	5.0
	4.0	3.4	5.1	5.1	3.8	4.6	3.5	5.1	3.4
	22.0	3.3	2.8	2.8	2.7	3.6	7.8	3.2	5.2
EOS (10^3/µL)	-3.0	0.3	0.1	0.2	0.1	0.3	0.3	0.1	0.2
	-1.0	0.3	0.1	0.3	0.1	0.2	0.3	0.1	0.2
	4.0	0.4	0.2	0.4	0.2	0.3	0.5	0.3	0.3
	22.0	0.5	0.2	0.3	0.1	0.2	0.6	0.1	0.3
MONO (10^3/µL)	-3.0	0.4	0.4	0.3	0.4	0.4	0.4	0.4	0.4
	-1.0	0.4	0.4	0.4	0.5	0.3	0.3	0.4	0.4
	4.0	0.5	0.6	0.3	0.4	0.5	0.4	0.5	0.5
	22.0	0.5	0.6	0.4	0.4	0.4	0.5	0.4	0.3
BASO (10^3/µL)	-3.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	-1.0	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0
	4.0	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1
	22.0	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1
LUC (%)	-3.0	1.5	1.2	1.0	1.2	1.1	1.0	1.0	1.1
	-1.0	0.9	1.0	0.8	0.8	0.7	0.7	0.6	0.9
	4.0	1.4	1.8	1.4	2.1	1.4	1.6	1.4	1.7
	22.0	1.7	1.2	1.4	1.1	1.5	0.7	1.1	1.1
LYM (%)	-3.0	53.6	53.3	43.6	61.9	43.9	46.5	42.8	50.9
	-1.0	44.5	55.0	37.1	48.1	39.5	41.9	35.2	48.5
	4.0	58.4	53.2	48.3	54.4	48.2	54.2	56.5	56.2
	22.0	55.6	63.4	70.0	64.3	62.6	40.1	64.3	46.0
NEUT (%)	-3.0	37.8	40.7	50.7	32.2	49.5	45.8	52.5	42.0
	-1.0	49.0	39.1	57.3	46.7	54.8	52.1	60.8	44.6
	4.0	31.7	38.3	43.7	36.9	42.5	34.9	36.2	33.7
	22.0	33.4	27.7	21.4	29.1	30.6	51.9	29.8	46.8
EOS (%)	-3.0	2.9	0.5	1.8	0.6	1.9	2.7	0.6	1.5
	-1.0	2.0	1.0	1.8	0.7	1.9	2.5	0.4	2.1
	4.0	3.6	1.5	3.3	2.1	3.3	4.8	1.8	2.6
	22.0	4.0	2.0	2.9	0.8	1.3	3.7	0.9	2.7
MONO (%)	-3.0	3.5	3.5	2.4	3.5	3.1	3.4	2.7	3.9
	-1.0	3.3	3.5	2.8	3.2	2.8	2.5	2.7	3.5
	4.0	4.6	4.7	3.0	4.2	4.3	4.2	3.7	4.8
	22.0	4.7	5.3	3.9	4.4	3.3	3.4	3.5	3.1
BASO (%)	-3.0	0.6	0.7	0.6	0.6	0.6	0.6	0.6	0.6
	-1.0	0.4	0.4	0.3	0.5	0.3	0.3	0.3	0.4
	4.0	0.5	0.6	0.4	0.4	0.4	0.4	0.4	1.4
	22.0	0.5	0.6	0.5	0.6	0.7	0.5	0.6	0.5

Clinical Chemistry

Unremarkable. A summary of the mean clinical chemistry parameter values are presented in Table 9. While individual differences in serum chemistry parameters vs.

pretest and/or concurrent control data were considered occasionally noted, they are considered unrelated to treatment.

Note that clinical chemistry was collected during Week 1 for female #1505 that underwent unscheduled euthanasia due to a radial fracture on Day 1.

Table 9: Mean Clinical Chemistry Values (1 month)

	Week	1M	2M	3M	4M	1F	2F	3F	4F
GLUCOSE (mg/dL)	-3	69.5	72.0	72.3	77.5	59.2	70.9	90.7	76.0
	-1	81.8	74.5	83.3	89.8	71.5	75.4	85.8	87.5
	1	NC	NC	NC	NC	NC	77.0	NC	NC
	4	67.5	64.8	74.0	64.0	73.3	65.0	81.7	66.7
	22	87.0	98.0	78.5	95.5	111.5	111.0	94.0	67.0
BUN (mg/dL)	-3	20.7	16.2	17.5	17.3	17.3	16.6	20.0	17.0
	-1	19.5	16.5	17.5	17.5	16.3	15.6	18.5	15.8
	1	NC	NC	NC	NC	NC	13.0	NC	NC
	4	18.3	16.2	16.7	15.5	16.8	15.5	17.0	16.2
	22	21.5	16.5	19.0	18.5	19.5	23.5	18.5	19.0
CREAT (mg/dL)	-3	1.0	1.0	1.0	1.0	0.9	0.9	1.1	0.9
	-1	1.0	1.0	0.9	0.9	0.9	0.8	0.9	0.9
	1	NC	NC	NC	NC	NC	0.7	NC	NC
	4	1.0	0.9	0.9	0.9	0.9	0.8	0.9	0.9
	22	1.0	1.0	0.9	1.0	0.8	0.9	0.9	0.8
ALT (IU/L)	-3	64.2	58.2	61.3	45.2	52.3	76.4	49.0	54.5
	-1	49.3	48.3	58.3	45.2	46.7	49.6	47.3	55.0
	1	NC	NC	NC	NC	NC	53.0	NC	NC
	4	59.8	54.8	53.2	51.8	50.7	63.8	57.8	54.0
	22	54.0	45.0	41.5	38.0	76.0	64.5	65.5	56.5
AST (IU/L)	-3	52.3	54.0	57.0	56.5	54.3	48.3	61.2	52.0
	-1	51.8	60.0	56.8	55.8	54.5	51.6	57.3	52.3
	1	NC	NC	NC	NC	NC	67.0	NC	NC
	4	75.2	55.7	53.0	52.7	54.0	55.5	62.0	49.2
	22	46.0	60.0	42.5	57.0	55.5	59.0	59.5	40.0
AP (IU/L)	-3	862.2	666.0	914.8	920.3	551.8	652.0	642.7	653.0
	-1	830.2	592.5	789.2	803.3	491.3	545.4	548.3	569.3
	1	NC	NC	NC	NC	NC	454.0	NC	NC
	4	836.7	591.7	810.2	851.0	493.8	509.3	561.2	549.0
	22	890.5	730.5	884.5	783.0	580.0	438.0	591.0	545.0
GGT (IU/L)	-3	185.7	160.5	188.2	217.2	117.7	140.6	125.5	133.3
	-1	180.2	148.2	186.8	202.7	113.3	124.9	115.3	129.5
	1	NC	NC	NC	NC	NC	140.0	NC	NC
	4	182.0	153.5	199.0	199.7	115.3	117.8	124.5	125.5
	22	188.0	196.0	193.5	264.0	145.0	109.0	129.0	129.5

	Week	1M	2M	3M	4M	1F	2F	3F	4F
TBILI (mg/dL)	-3	0.4	0.4	0.5	0.4	0.5	0.4	0.5	0.4
	-1	0.4	0.3	0.3	0.4	0.4	0.3	0.5	0.4
	1	NC	NC	NC	NC	NC	0.3	NC	NC
	4	0.5	0.5	0.5	0.5	0.5	0.5	0.6	0.5
	22	0.4	0.4	0.3	0.4	0.4	0.4	0.4	0.3
TP (g/dL)	-3	7.9	7.9	8.3	7.9	7.9	7.7	8.1	7.7
	-1	7.7	7.6	7.9	7.6	7.9	7.5	7.7	7.5
	1	NC	NC	NC	NC	NC	6.6	NC	NC
	4	7.6	7.5	7.9	8.0	7.8	7.6	8.1	7.6
	22	7.1	7.7	7.7	7.2	7.7	7.1	7.7	7.0
ALBUMIN (g/dL)	-3	4.8	4.9	5.0	4.9	4.7	4.7	4.9	4.7
	-1	4.8	4.8	4.9	4.8	4.7	4.7	4.9	4.6
	1	NC	NC	NC	NC	NC	3.8	NC	NC
	4	4.8	4.7	4.9	4.7	4.7	4.6	4.9	4.6
	22	4.4	4.8	4.6	4.7	4.4	4.3	4.8	4.2
GLOB (g/dL)	-3	3.1	3.0	3.3	3.0	3.3	3.0	3.2	3.0
	-1	3.0	2.8	3.0	2.8	3.2	2.9	2.9	2.9
	1	NC	NC	NC	NC	NC	2.8	NC	NC
	4	2.8	2.8	3.0	3.3	3.2	3.0	3.2	3.0
	22	2.7	2.9	3.1	2.6	3.3	2.8	2.9	2.8
A/G	-3	1.6	1.7	1.5	1.6	1.5	1.6	1.6	1.6
	-1	1.6	1.7	1.6	1.7	1.5	1.7	1.7	1.6
	1	NC	NC	NC	NC	NC	1.4	NC	NC
	4	1.7	1.7	1.7	1.4	1.5	1.6	1.6	1.6
	22	1.6	1.6	1.5	1.8	1.3	1.6	1.7	1.5
CHOL (mg/dL)	-3	129.8	120.2	119.2	121.3	119.8	121.3	114.3	115.3
	-1	118.5	112.3	110.7	108.2	122.8	108.7	107.3	114.0
	1	NC	NC	NC	NC	NC	86.0	NC	NC
	4	128.7	112.3	119.8	115.7	128.0	117.7	115.7	117.2
	22	108.0	111.5	97.5	86.0	90.0	90.0	92.5	94.5
TRIG (mg/dL)	-3	44.0	46.0	41.7	29.7	36.5	31.1	30.0	35.7
	-1	63.8	52.0	58.5	44.3	38.8	39.6	32.5	33.5
	1	NC	NC	NC	NC	NC	53.0	NC	NC
	4	46.7	41.8	44.8	30.7	37.3	33.2	33.7	35.5
	22	44.5	33.0	64.0	35.5	85.0	87.5	48.0	63.5
CALCIUM (mg/L)	-3	10.2	10.3	10.1	10.1	9.9	10.2	10.2	10.1
	-1	10.2	10.1	10.2	10.1	9.9	10.1	10.0	10.0
	1	NC	NC	NC	NC	NC	9.7	NC	NC
	4	10.0	10.0	10.1	9.9	9.9	10.0	10.1	10.1
	22	9.9	10.1	10.1	10.2	10.1	10.0	10.0	10.1
PHOS (mg/dL)	-3	6.4	5.7	5.7	6.2	6.1	5.0	6.5	5.5
	-1	5.1	4.9	4.9	5.1	5.5	4.6	5.4	5.6
	1	NC	NC	NC	NC	NC	4.6	NC	NC
	4	7.5	6.4	6.4	6.9	6.3	5.6	7.1	6.1
	22	6.0	7.0	7.0	5.9	5.7	4.6	6.2	4.0

	Week	1M	2M	3M	4M	1F	2F	3F	4F
SODIUM (mEq/L)	-3	148.3	147.8	148.2	147.7	147.3	146.9	149.5	147.7
	-1	147.3	147.2	148.0	147.8	146.7	147.3	147.5	148.0
	1	NC	NC	NC	NC	NC	152.0	NC	NC
	4	148.5	145.8	148.5	146.2	147.5	146.0	149.2	146.8
	22	145.5	146.5	148.0	147.0	146.5	146.0	149.0	147.0
POTASSIUM (mEq/L)	-3	4.8	4.8	4.4	4.8	4.8	4.5	5.2	4.6
	-1	4.6	4.3	4.4	4.4	4.1	4.4	4.6	4.3
	1	NC	NC	NC	NC	NC	4.8	NC	NC
	4	4.7	4.5	4.4	4.3	4.9	4.3	4.7	4.6
	22	4.6	5.0	4.5	4.7	4.9	4.5	5.0	3.9
CHLORIDE (mEq/L)	-3	108.0	107.7	108.3	109.0	108.7	109.0	111.0	110.2
	-1	106.7	106.0	107.2	106.8	105.5	107.0	106.5	105.8
	1	NC	NC	NC	NC	NC	114.0	NC	NC
	4	108.0	105.7	107.0	105.0	109.5	107.0	108.3	107.5
	22	111.0	109.5	108.5	107.5	113.0	110.0	110.5	110.5

NC = not collected (per protocol)

Urinalysis

Unremarkable

Gross Pathology

The following gross observations were noted:

- Focal discoloration of the stomach mucosa in one HDF
- Focal discoloration of the lung in one LDM
- Ovary finding of small ovary (unilateral) and discoloration, focal, unilateral in one HDF
- Ovarian cyst unilateral in one HDF

Organ Weights

The following organ weight changes, as assessed by changes in group mean differences from concurrent control-mean values, appeared to exhibit a dose-related pattern. Except for the increase in spleen weight, which is expected by virtue of the pharmacodynamic action of the drug, the toxicological significance, if any, is unclear.

Main Study:

- Increased spleen, decreased thyroid weights in males and females
- Increased thymus and uterus in females.

Recovery:

- Increased adrenal, lung, spleen and thymus in males
- Increased spleen and thymus in females
- Decreased thyroid weights in males
- Markedly increased thyroid in females, particularly in high dose females (↑72%)
- Decreased heart in females

Histopathology

Adequate Battery
Yes
Peer Review
A peer review was planned per protocol; however, no peer review was conducted. The peer review does not appear to have been amended out of the protocol; therefore the absence of a peer review is presumably a protocol deviation.

Histological Findings
A general pattern of increased immune cell infiltration was observed in multiple tissues, which is consistent with the mechanism of action for this drug. That the pattern exhibited no particular relationship with dose is likely the result of target saturation at the lowest dose level. The enhancement of immune cell infiltration may be related to the pattern of immunologically-mediated adverse reactions observed in humans; however, the mechanism by which PD-1 inhibition promotes these adverse events is unclear in light of their apparently idiosyncratic nature. In this study, all histological findings were of minimal to mild severity with the exception of the broken bone that led to the preterm euthanasia of the low dose female on Study Day 1. That event was not related to treatment with the study drug.

Table 10: Histological findings of potential or equivocal relationship to treatment

Sex	**MALE**				**FEMALE**			
Dose (mg/kg)	0	6	40	200	0	6	40	200
Tissue, Histological Description								
Adrenal, mononuclear cell infiltration, focal/multifocal							1	1
--thrombus						1		
--mineralization, focal/multifocal							1	
--hypertrophy, zona fasciculata		1						
--depletion, cytoplasmic, zona fasciculata				1		1		
Bone, radius, fracture						1‡†		
Bone, hemorrhage, periosteal, focal/multifocal			1R					
Bone marrow, lymphoid nodule								1
--aggregate(s) mononuclear		1R						1
Brain, mononuclear cell infiltration, focal/multifocal	1+1R	2+1R	4+1R	2+2R	1	1	2	2
--gliosis, focal/multifocal			1					1
Colon, herniated, crypt, focal**				1				
Esophagus, mononuclear cell infiltration, focal/multifocal			1					
--bacteria						2		
Eye, mononuclear cell infiltration, focal/multifocal	2+1R	1		1R		1R		1+1R
--degeneration, retina, peripheral		1R						

Sex	MALE				FEMALE			
Dose (mg/kg)	0	6	40	200	0	6	40	200
Tissue, Histological Description								
Heart, mononuclear cell infiltration, focal/multifocal	2	1	1	3		3	2	2+1R
Injection site, hemorrhage, subcutis**	2	1	1	2	4	2	2	3
Kidney, mononuclar cell infiltration, focal/multifocal	1+1R	2	2	1+1R	1	2	2+1R	3+1R
Lacrimal gland, mononuclear cell infiltration, focal/multifocal**	1+1R	4+1R	4	4+2R	4+2R	3+2R	4+1R	4+1R
Liver, mononuclear cell infiltration, periportal, focal/multifocal	1	3	1	1	2	1	2	
--vacuolation, diffuse						1		
--vacuolation, hepatocellular, focal**			1			1+1R		
Lung, mixed inflammatory cell infiltration, focal/multifocal, hemorrhagic						1		
--mononuclear cell infiltration, pleural, focal/multifocal				1				
--Hemorrhage, focal/multifocal		1						
Lymph nodes, pigment accumulation	4+1R	3+2R	3	2+2R	3+1R	4+2R	4+2R	4+1R
--hemorrhage		1						1
Lymph node, mandibular, histiocytosis, subcapsular					4	1	1	
Lymph nodes, mesenteric, hemorrhage, focal/multifocal		1R						1R
Lymph node, mandibular, hyperplasia, subcapsular						1	1	
Nerve, peripheral, mononuclear cell infiltration, focal/multifocal	1					1		
Ovary, cyst**	--	--	--	--		1	3	1
--mineralization, follicular	--	--	--	--		2	1R	
--involution, corpora lutea, unilateral								1
Pancreas, mononuclear cell infiltration, focal/multifocal	1R					1		1R
Parathyroid, mononuclear cell infiltration, focal/multifocal**	1R		2		1	2	1	
--cyst								1
Pituitary, cyst**						1+2R		
Prostate, mononuclear cell infiltration, focal/multifocal	1		1	1+1R	--	--	--	--
Sleketal muscle, monocytolysis, focal/multifocal						1		
--hemorrhage						1		
Skin, herniated, adipose tissue							1	
--hemorrhage, subcutis**	3	1	1	1	2	1	1	
--granulation tissue, focal/multifocal							1	
Salivary glands, mononuclear cell infiltration, focal/multifocal**	4+2R	3	3	2+1R	4+2R	2+1R	2+2R	3+1R

Sex	MALE				FEMALE			
Dose (mg/kg)	0	6	40	200	0	6	40	200
Tissue, Histological Description								
Spleen, macrophage accumulation, focal/multifocal				1				
--enlarged periarterial lymphoid sheath				2				
--enlarged lymphoid marginal zone						1		
Stomach, mononuclear cell infiltration, focal/multifocal	1R		1					
--atrophy, fundus								1
--atrophy, pyloric						1		
Testes, ectasia, focal/multifocal, tubular		1		1R				
--atrophy, segmental, unilateral				1R				
Thymus, hypocellularity		1		1			1	1
--cyst**	2	1	1R	2	1R	1	1	
Thyroid, ectopic tissue, thymic	1+1R	1		1R	1	3	1+1R	1
--mononuclear cell infiltration, focal/multifocal					1	1	1+1R	2
--degeneration, follicular, focal/multifocal				1	1			2
--cyst**	2	3	1	1	2	1+2R		1
Trachea, mononuclear cell infiltration, focal/multifocal			1	1				2+2R
--protozoa				1				
Uterus, accumulation/infiltration, perivascular, focal/multifocal	--	--	--	--			1	
Vagina, mucification	--	--	--	--		1		

**denotes increasing severity with dose; R = Recovery cohort; ‡ denotes highest severity (severe) noted in some animals; † denotes preterm decedent

Special Evaluation

SEB-stimulated IL-2 was measured from whole blood collected on Days -11, -5, 0, 28, 56, 98, 126, and 154 of this study. As described previously in this review, the assay was performed by incubating whole blood with exogenous MK-3475 then stimulating with SEB for 3 days prior to analysis.

Data from this analysis indicates the following:

(1) In untreated animals and/or predose samples, incubation with MK-3475 plus SEB led to an increase in IL-2 production that was approximately 2-5X higher than that observed when samples were incubated with SEB alone

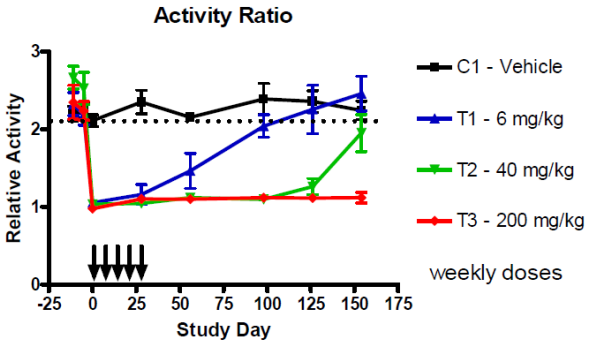
(2) When samples from MK-3475-treated animals, collected at 24 hours post-dose on Day 1, were incubated with in vitro MK-3475, there was no increase in IL-2 over baseline-SEB-treated (control-treated; without in vitro MK-3475) samples, indicating saturation of response even at the lowest dose level of 6 mg/kg/dose.

(3) Similar results were obtained on Days 1 and 28, despite a reduction in measurable drug levels due to ADA formation in some animals.

(4) The pharmacodynamic effect (saturation of IL-2 production) was observed throughout the recovery interval in high dose animals, but decreased in a time-

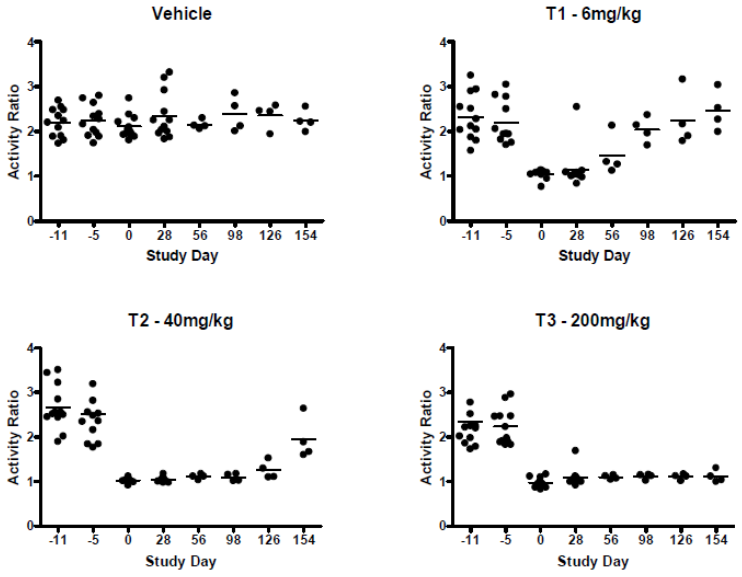
and dose-dependent manner in mid- and low-dose groups, returning to control levels by Days 98 and 154, respectively.

Figure 30: Timecourse of average SEB-induced IL-2 expression in monkeys



Individual animal IL-2 induction data are presented in Applicant-Figure 31. These data demonstrate a high degree of concordance among individuals within each dose cohort, and a timecourse that suggests decreasing pharmacodynamic activity with increasing duration of post-dose washout. Note that in samples from high dose animals, suppression of IL-2 potentiation by MK-3475 in this assay is maintained, suggesting that, at high dose levels, the PD-1 receptor remained fully saturated for the duration of the recovery interval.

Figure 31: Individual IL-2 activity estimates by dose group



Applicant Figure 32 through Figure 37 depict the individual animal pharmacodynamic response in the context of each animal's drug level and the timecourse of ADA onset. Arrows in these figures represent administration of the antibody. Particularly in low-dose animals, onset of ADA was associated with a decrease in pharmacodynamic activity and/or plasma drug levels, which suggests that the ADA was neutralizing in these

animals. Note that there was no demonstrable ADA formation in high dose animals; however, given the assay format, it is likely that excess unbound drug present in study samples interfered with the detection of anti-drug antibodies in these samples. That the pharmacodynamic activity of MK-3475 remained intact for all animals in the study suggests that any ADA formation that might have occurred was overwhelmed by excess drug (i.e. the study "dosed-through" the anti-drug antibody response).

Figure 32: Timecourse of IL-2 induction, in relation to toxicokinetic drug exposures and ADA levels in individual animals (6 mg/kg Main Study males and females)

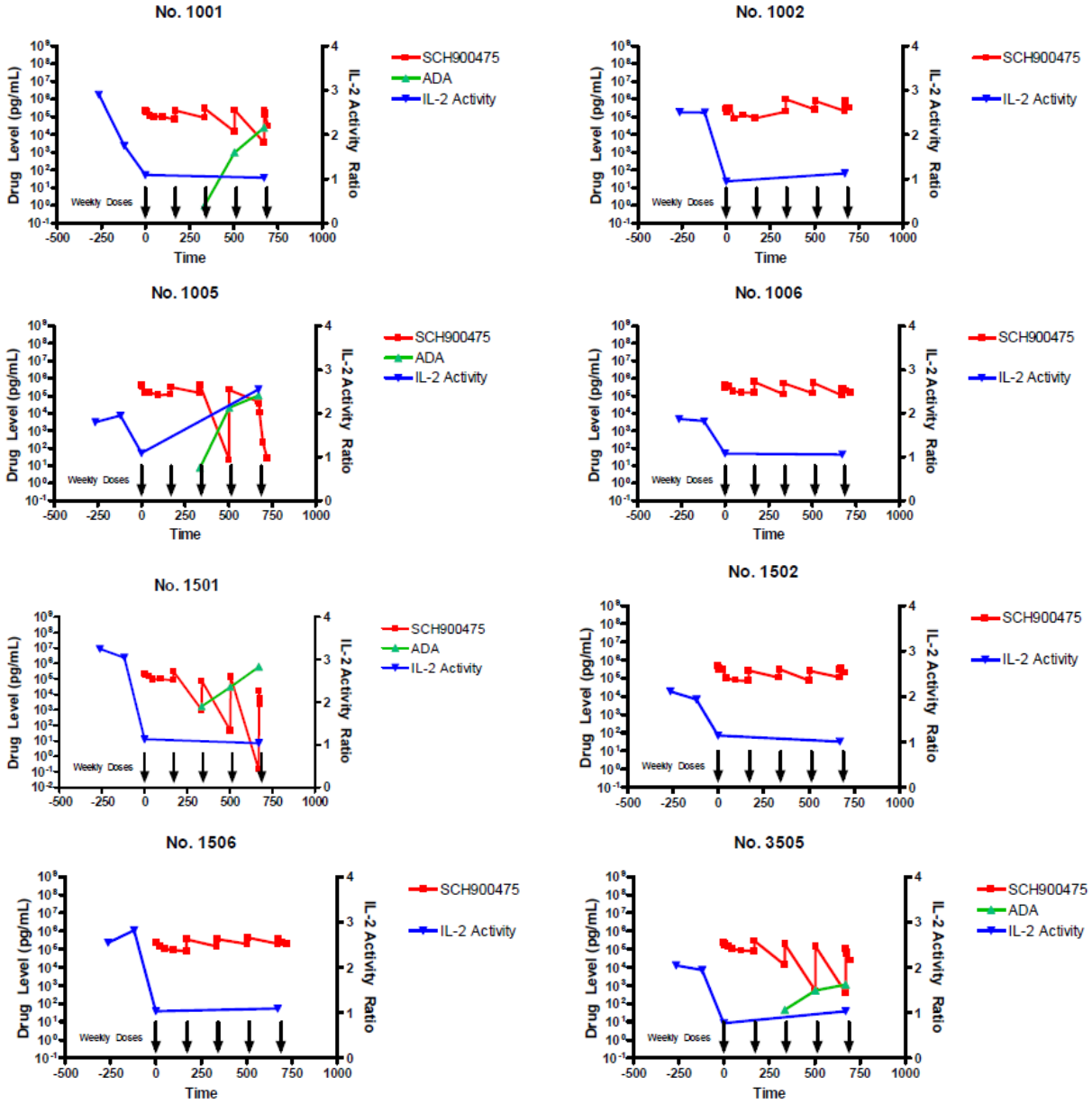


Figure 33: Timecourse of IL-2 induction, in relation to toxicokinetic drug exposures and ADA levels in individual animals (6 mg/kg Recovery Cohort males and females)

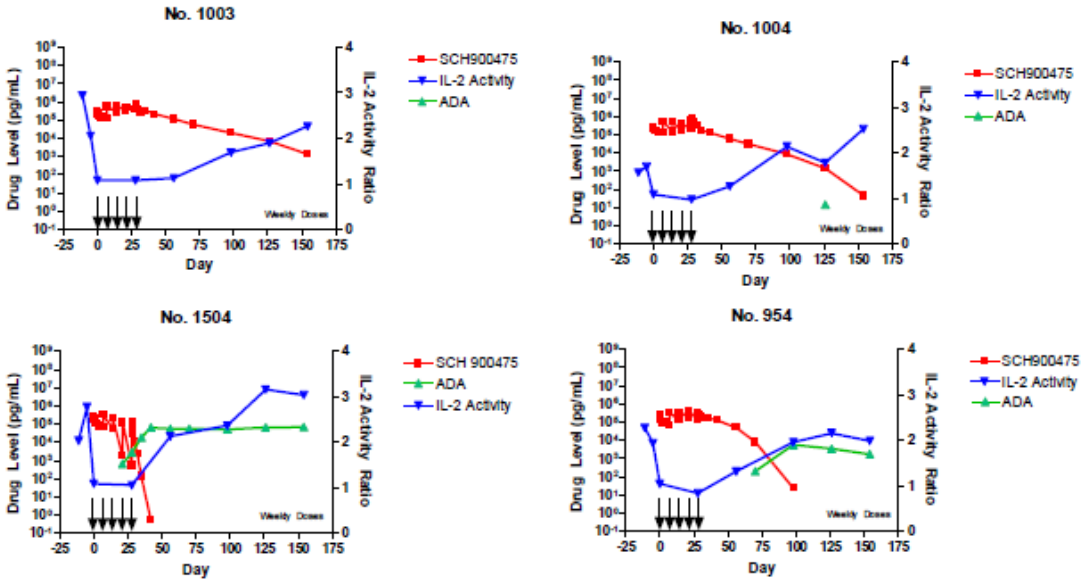


Figure 34: Timecourse of IL-2 induction, in relation to toxicokinetic drug exposures and ADA levels in individual animals (40 mg/kg Main Study males and females)

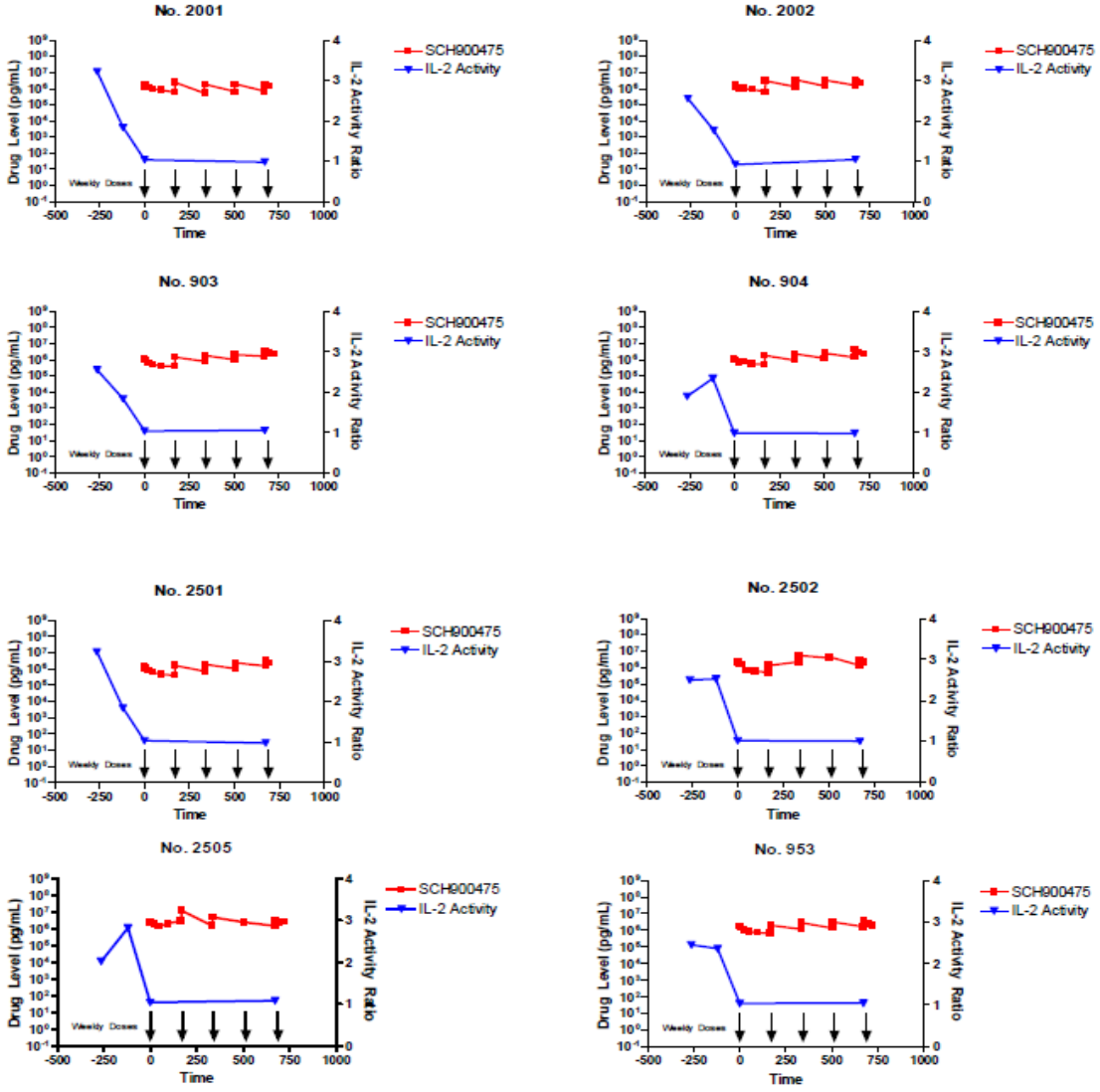


Figure 35: Timecourse of IL-2 induction, in relation to toxicokinetic drug exposures and ADA levels in individual animals (40 mg/kg Recovery Cohort males and females)

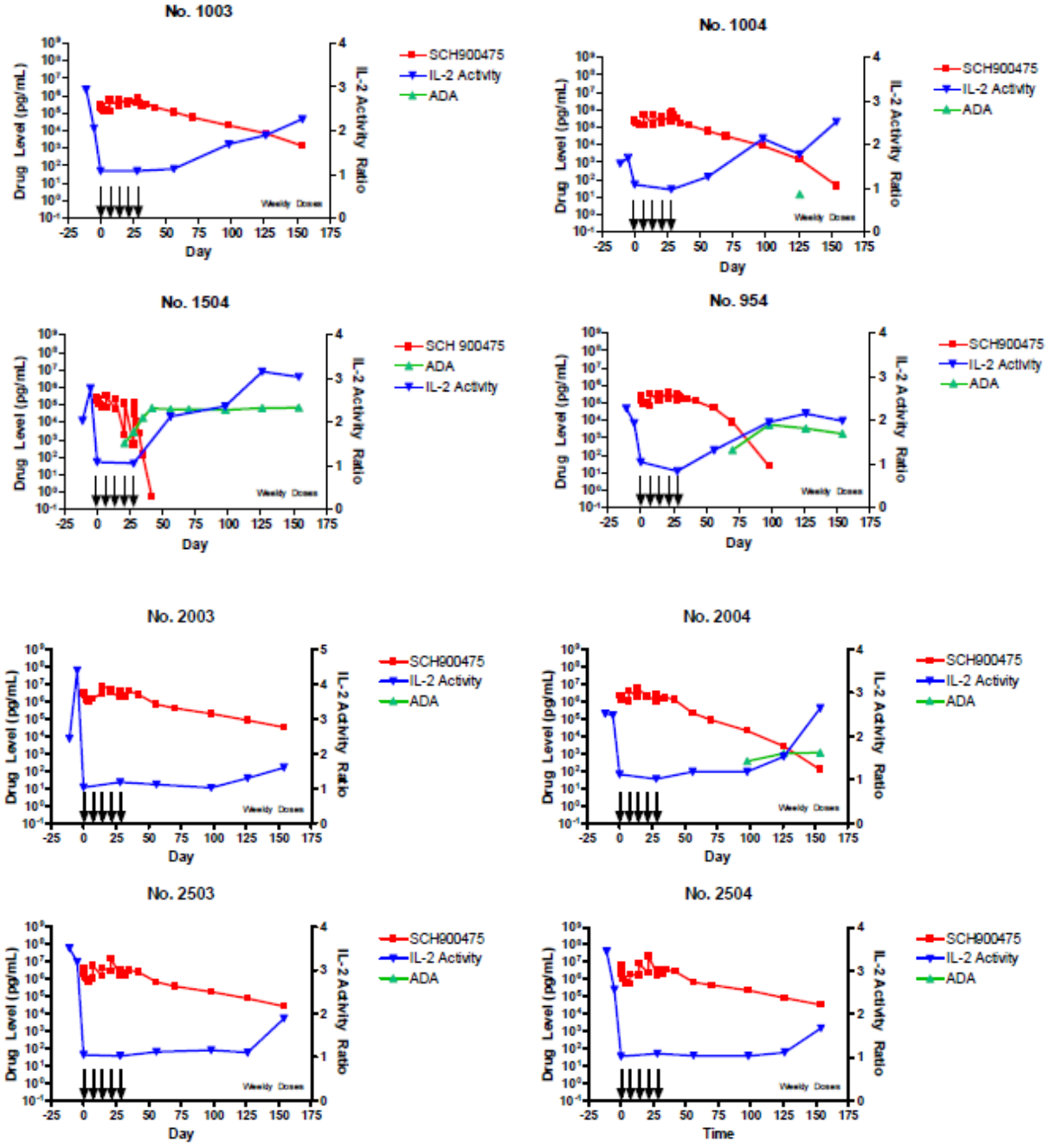


Figure 36: Timecourse of IL-2 induction, in relation to toxicokinetic drug exposures and ADA levels in individual animals (200 mg/kg Main Study males and females)

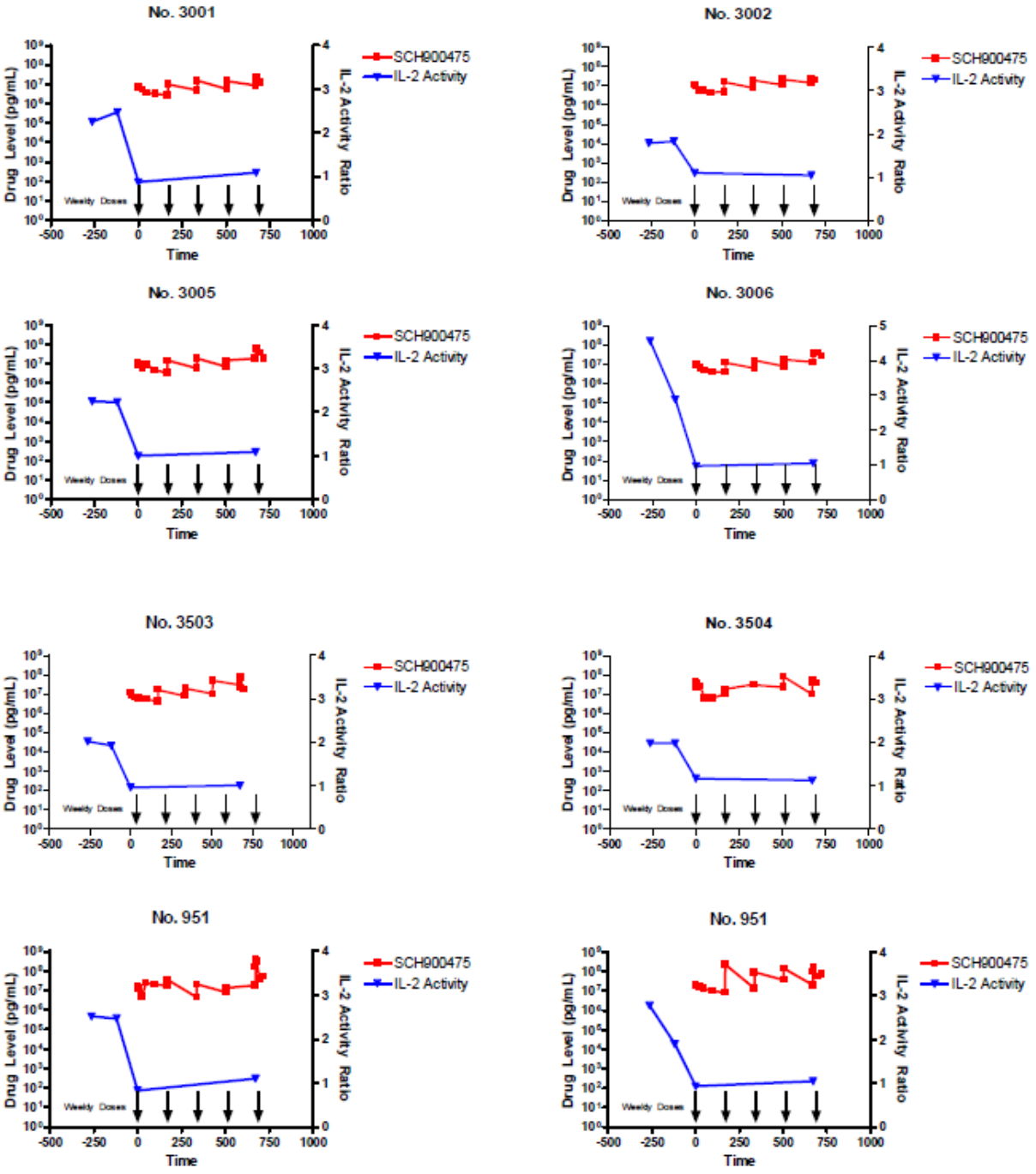
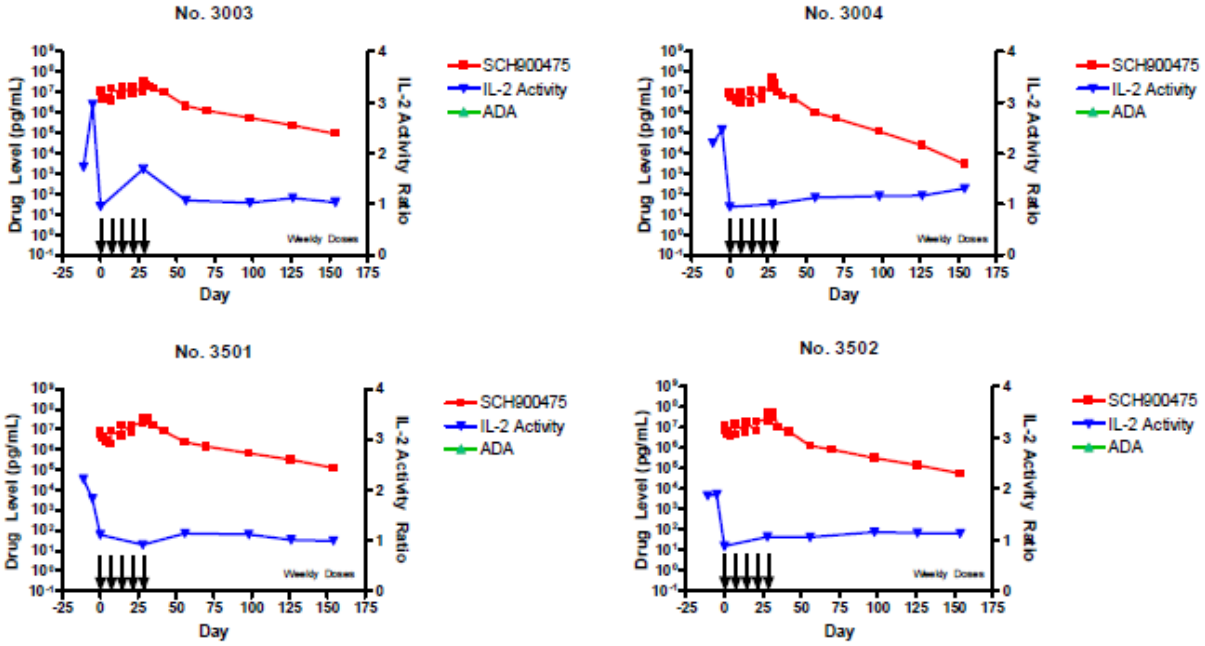


Figure 37: Timecourse of IL-2 induction, in relation to toxicokinetic drug exposures and ADA levels in individual animals (200 mg/kg Recovery Cohort males and females)



Toxicokinetics

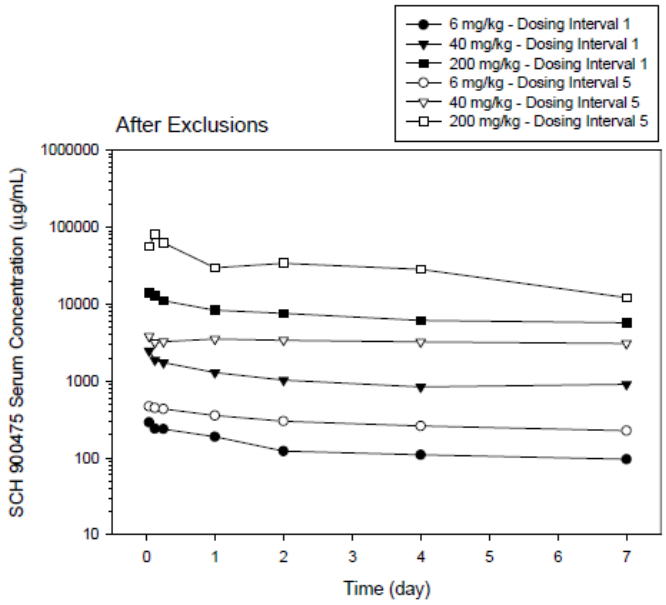
As illustrated in Applicant-Table 11 and Applicant-Figure 38, exposures were generally proportional over the dose range on Day 1. There was no evidence of accumulation at the low dose; however, accumulation ratios of between 2 and 5.6X (C_{max}) or 3X (AUC_{0-7d}) were observed at the mid- and high-dose levels over the 5-week dosing interval, presumably reflecting saturation of clearance. ADA was detected in 7/10 animals at the low dose level and in one animal at the 40 mg/kg dose level. The Applicant states that ADA was associated with an increase in plasma exposure; therefore, the extent to which ADA impacted the toxicokinetic parameter estimates for the 5th dosing interval, is unclear.

Table 11: Mean (CV) toxicokinetic parameters in the cynomolgus monkey on Days 1 and 28 following weekly IV injection of 6, 40 or 200 mg/kg MK-3475

Dose[a,b] (mg/kg)	Dosing Interval	C_0 (µg/mL)	Cmax (µg/mL)	Tmax[c] (hr)	AUC(0-7 days) (µg·day/mL)	R[d]
6	1	322 (39)[e]	292 (32)[e]	1 (1-24)[e]	923 (23)	NA[f]
	5	338 (76)	350 (82)	2 (1-24)	1790 (73)[g]	1.78 (64)[g]
40	1	2950 (79)	2510 (57)	1 (1-168)	7240 (44)	NA
	5	4390 (81)	4770 (72)	2 (1-168)	24100 (76)[g]	3.12 (93)[g]
200	1	14800 (67)	14800 (66)	1 (1-48)	49700 (61)	NA
	5	58900 (72)	86200 (125)	3 (1-24)	170000 (30)[g]	5.58 (44)[g]

a: SCH 900475
b: N = 12/dose group unless otherwise noted
c: Median (Minimum - Maximum)
d: R = AUC(0-7 days)$_{\text{Dosing Interval 5}}$ ÷ AUC(0-7 days)$_{\text{Dosing Interval 1}}$
e: N = 13
f: NA = Not applicable
g: N = 4

Figure 38: MK-3475 concentration-time profiles in the 4-week study in the cynomolgus monkey



Note that mean values in Applicant-Table 11 reflect the combined data from males and females. The Applicant states that there was no impact of sex on exposure.

In a separate evaluation, the Applicant assessed the terminal elimination half life and mean residence time to be between 14-22 days. There was no apparent impact of dose on the estimation of half-life or mean residence time.

Dosing Solution Analysis

MK-3475 (SCH 900475) was not formulated; it was used as supplied, and was therefore characterized by its labeling and the analytical documentation supplied in the Certificate of Test Results (appended to the final report).

6.2.2 Study title: Six-Month Intravenous Toxicity Study in Cynomolgus Monkeys With a 4-Month Treatment Free Period

Study title: Six-Month Intravenous Toxicity Study in Cynomolgus Monkeys With a 4-Month Treatment Free Period

Study no.:	TT #11-1084
Study report location:	4.2.3.2
Conducting laboratory and location:	Safety Assessment and Laboratory Animal Resources Merck Research Laboratories West Point, Pennsylvania 19486 U.S.A.
Date of study initiation:	15 August 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MK-3475, Batch: 09U-900475P-09001,

Key Study Findings

There were no adverse findings observed in this study.

Doses:	6, 40, 200 mg/kg/dose
Frequency of dosing:	Q2Wk
Route of administration:	Bolus IV injection via the saphenous vein
Dose volume:	0.24 mL/kg (6 mg/kg dose level); 1.6 mL/kg (40 mg/kg dose level); 8 mL/kg for control and high dose levels
Formulation/Vehicle:	10 mM histidine, 7% (w/v) sucrose, 0.02% polysorbate 80, pH 5.5
Species/Strain:	Macaca fascicularis
Number/Sex/Group:	5/sex/group
Age:	1-3 years
Weight:	2.1-3.4 (females) / 2.5-3.8 (males)
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	On Study Day 1, one low dose animal inadvertently received 25 mg/kg rather than 6 mg/kg. This animal was excluded from TK Analysis. This deviation was unlikely to have affected overall study interpretation. There were no other reported deviations.

Observations and Results

Mortality

There were no preterm deaths

Clinical Signs

There was an increase in the incidences of alopecia, scabs, and liquid/unformed feces in treated males and/or females, relative to controls.

Body Weights

Unremarkable

Figure 39: Female body weight measurements in the 6m monkey study

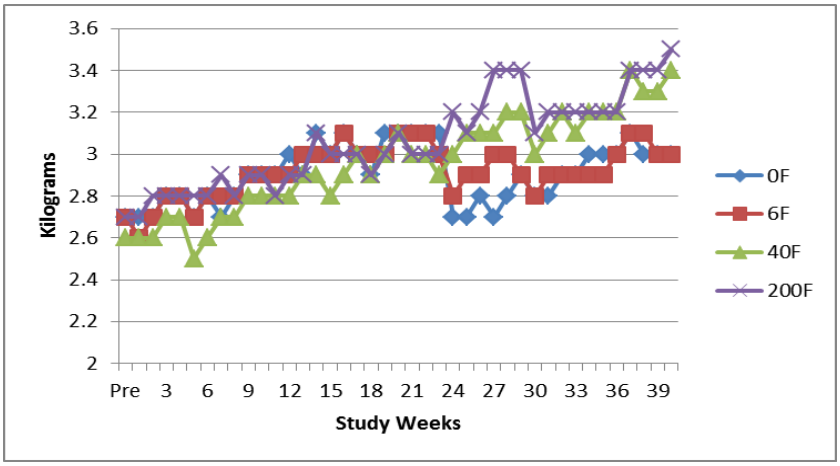
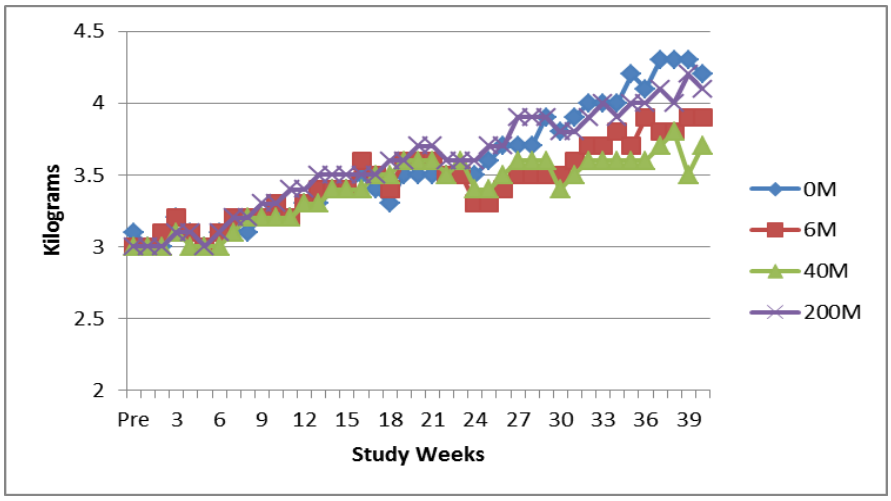


Figure 40: Male body weight measurements in the 6m monkey study



Feed Consumption

Unremarkable

Ophthalmoscopy

Potential increases in the incidence of iris hyperpigmentation and lens opacity were observed in treated males and females relative to controls at the end of the dosing phase (Table 12). These are of equivocal relationship to treatment.
Findings of iris hyperpigmentation were observed in the 1-month study as well; however as the finding was also noted pre-dose, it was considered unrelated to treatment.

Table 12: Potential MK3475-related ophthalmic changes in the 6m monkey study

	Iris hyperpigmentation		lens opacity	
Group	**Pre-Dose**	**Day 156**	**Pre-Dose**	**Day 156**
1F	0	1	1	1
1M	0	0	1	2
2F	0	0	0	1
2M	0	1	2	2
3F	0	0	1	1
3M	0	0	2	3
4F	0	0	3	4
4M	0	1	2	4

ECG

Unremarkable

Parameter		1F	1M	2F	2M	3F	3M	4F	4M
	Pre	219	236	249	237	234	229	250	236
	Wk 13	229	238	247	240	240	222	238	229
Heart Rate (Beats/Min)	**Wk 23**	234	239	245	231	237	218	239	231
	Pre	0.28	0.26	0.24	0.26	0.26	0.27	0.24	0.26
	Wk 13	0.26	0.26	0.25	0.25	0.25	0.28	0.25	0.27
RR Interval (sec)	**Wk 23**	0.26	0.25	0.25	0.26	0.25	0.27	0.25	0.27
	Pre	0.07	0.07	0.07	0.07	0.07	0.07	0.06	0.07
	Wk 13	0.07	0.07	0.06	0.07	0.06	0.07	0.06	0.07
PR Interval (sec)	**Wk 23**	0.07	0.06	0.06	0.06	0.07	0.08	0.06	0.07
	Pre	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
	Wk 13	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
PRS Interval (sec)	**Wk 23**	0.03	0.04	0.04	0.04	0.04	0.04	0.04	0.04
	Pre	0.18	0.18	0.17	0.17	0.18	0.17	0.18	0.17
	Wk 13	0.17	0.17	0.16	0.17	0.16	0.18	0.17	0.17
QT Interval (sec)	**Wk 23**	0.16	0.17	0.16	0.17	0.17	0.17	0.17	0.17
	Pre	0.34	0.35	0.35	0.34	0.35	0.34	0.36	0.33
	Wk 13	0.33	0.34	0.33	0.33	0.31	0.34	0.33	0.34
QTc Interval (sec)	**Wk 23**	0.31	0.34	0.32	0.32	0.34	0.32	0.34	0.33

Hematology

There were no statistically-significant treatment-related changes in any hematology parameter evaluated. Spontaneous variations were observed in individual animal hematological parameters that exceeded control and/or predose ranges; however, the changes did not appear to exhibit a relationship to dose or duration of exposure. Because pharmacodynamic saturation occurred at all dose levels, and because toxicity

for this drug is expected to be an extension of the pharmacodynamic response, non-dose-related changes in hematology parameters cannot be excluded from treatment; however, their low frequency and low magnitude of severity implies that they are of little toxicological significance. Group mean hematology parameters in females and males are summarized in Table 13 and Table 14, respectively.

Individual variations were noted in a number of hematology parameters of treated animals vs. concurrent control ranges. One high dose female exhibited reduced red cell parameters (reduced RBCs, HGB, HCT but not the corresponding red cell indices) during Study Week 11 which had normalized by Study Week 21, at which time the animal's reticulocytes were demonstrably elevated, suggesting an ongoing regenerative response to the previous red cell reduction.

In addition, there were sporadic decreases in coagulation times (PT, APTT) and/or increased fibrinogen in some treated males and females relative to control and baseline values during weeks 11 and/or 21; however these were mild, exhibited no intra-individual or inter-group pattern and no dose-response in the magnitude or incidence of the observation. The effects on fibrinogen and/or coagulation parameters are expected due to the drug's pro-inflammatory mechanism of action and the lack of dose response is likely the result of pathway saturation at the lowest dose tested.

Table 13: Group mean hematology values for females in the 6 month toxicology study

	Timepoint	1F	2F	3F	4F
RBC	Pre	6.39	6.76	6.64	6.59
	1	5.81	5.98	6	5.68
	11	5.91	6.07	6.21	5.7
	21	6.01	6.17	6.21	5.95
	40	6.56	6.6	6.99	6.96
RET%	Pre	0.8	0.8	0.8	0.8
	1	1	0.8	1	1.3
	11	0.8	0.7	0.6	1.2
	21	0.5	0.5	0.6	1.2
	40	0.4	0.7	0.6	0.7
ARET	Pre	50.4	52.1	52.6	56.4
	1	56.1	49.5	55	76
	11	45.7	40.2	39	64.5
	21	27.2	30.2	35.8	72
	40	28.4	47.3	38.5	46.2
Hgb	Pre	12.9	13.5	13.6	13.4
	1	11.6	11.9	12.4	11.5
	11	11.7	11.8	12.4	11.1
	21	11.7	12	12.3	11.5
	40	13	13.5	14.2	13.6

	Timepoint	1F	2F	3F	4F
Hct	Pre	45.5	47.7	47.3	46.1
	1	40.6	41	42.1	39.9
	11	39.9	40.8	41.2	38.7
	21	41.9	43.1	44	42.3
	40	45.3	46.5	48.4	47
MCV	Pre	71.4	70.6	71.3	70
	1	70	68.6	70.1	70.4
	11	67.6	67.3	66.2	68
	21	69.8	69.9	70.8	71.2
	40	69.2	70.4	69.4	67.7
MCH	Pre	20.1	20	20.5	20.3
	1	20.1	20.1	20.6	20.2
	11	19.8	19.5	20	19.6
	21	19.5	19.4	19.8	19.4
	40	19.9	20.4	20.3	19.6
MCHC	Pre	28.2	28.3	28.8	29
	1	28.7	29.2	29.4	28.8
	11	29.2	29.1	30.2	28.8
	21	28	27.8	28	27.3
	40	28.8	29	29.3	29
PT	Pre	10.8	10.9	10.6	10.7
	1	10.4	10.4	10.5	10.4
	11	10.2	10	9.9	10.2
	21	10.1	9.9	9.9	10.2
	40	10.9	10.6	10.7	11.3
APTT	Pre	19.2	20.2	20.2	19.3
	1	18.2	19.1	18.9	18.8
	11	17.7	19.7	18.3	18.4
	21	17.6	19.6	19.4	19
	40	19.2	20.1	19.8	21.7
Fib	Pre	192	192.4	226.9	198.4
	1	274.5	246.7	299.2	264
	11	247.9	254.4	249.5	247.8
	21	263.8	271.7	295.3	247.3
	40	195.5	253	200.1	159.8
MPV	Pre	9.5	9.2	9	9.4
	1	8.8	9	9.3	9.3
	11	9.3	9	8.7	9
	21	8.7	8.6	8.8	9.1
	40	9	9.3	9.2	8.6
APLT	Pre	364	432	406	379
	1	404	424	402	409
	11	426	462	466	444
	21	453	471	499	476
	40	459	429	515	475

	Timepoint	1F	2F	3F	4F
WBC	Pre	7.46	9.17	9.44	8.8
	1	9.08	8.33	11.65	8.31
	11	7.52	10.2	12.31	8.91
	21	11.21	11.06	14.46	12.46
	40	6.58	8.81	10.92	7.75
NEUT%	Pre	51.1	41.2	45.2	46.2
	1	44.3	44.2	53.7	44.1
	11	42.9	48.7	55.9	53.7
	21	47.7	48.7	53	53
	40	46.9	50.5	53.1	49.7
ANEUT	Pre	3.85	4.01	4.41	4.25
	1	4.05	3.73	6.63	3.6
	11	3.22	5.09	7.29	4.78
	21	5.62	5.68	7.75	6.71
	40	3.08	4.45	5.85	4.02
LYM%	Pre	43.5	51.7	46.9	46.1
	1	48.8	47.6	38	48.3
	11	51.6	44.2	38.3	42
	21	46.7	44.1	39.7	40.9
	40	47.9	42.8	39.6	43.7
ALYM	Pre	3.21	4.5	4.31	3.92
	1	4.41	3.91	4.08	4.1
	11	3.89	4.39	4.36	3.76
	21	5.02	4.57	5.67	5.04
	40	3.16	3.77	4.26	3.18
LUC%	Pre	1	1	1.5	1.1
	1	1	1.4	1.3	1.5
	11	1.3	1.6	1.4	1.1
	21	0.9	1	1	1.1
	40	0.8	0.9	0.9	1.2
ALUC	Pre	0.08	0.09	0.14	0.09
	1	0.09	0.12	0.15	0.13
	11	0.1	0.17	0.16	0.09
	21	0.09	0.12	0.14	0.13
	40	0.05	0.08	0.1	0.1
EOS%	Pre	0.7	1.4	1.2	2.2
	1	1.1	1.6	1.8	1.4
	11	0.9	1.3	1.7	1
	21	0.7	1.5	2.1	1.5
	40	0.8	1.5	2.5	2.1
AEOS	Pre	0.05	0.13	0.12	0.17
	1	0.1	0.12	0.21	0.11
	11	0.06	0.14	0.19	0.09
	21	0.07	0.17	0.3	0.17
	40	0.05	0.13	0.28	0.18

	Timepoint	1F	2F	3F	4F
MONO%	Pre	3.3	4.1	4.8	4
	1	4.5	4.8	4.7	3.9
	11	2.9	3.8	2.4	1.9
	21	3.7	4.2	3.9	3.2
	40	3.7	4.1	3.7	3.1
AMONO	Pre	0.24	0.39	0.42	0.32
	1	0.4	0.42	0.52	0.32
	11	0.22	0.38	0.26	0.16
	21	0.38	0.48	0.55	0.37
	40	0.24	0.37	0.4	0.26
BASO%	Pre	0.4	0.5	0.4	0.5
	1	0.3	0.5	0.5	0.7
	11	0.4	0.3	0.3	0.3
	21	0.3	0.3	0.4	0.3
	40	0.1	0.3	0.3	0.3
ABASO	Pre	0.03	0.05	0.04	0.04
	1	0.03	0.04	0.05	0.06
	11	0.03	0.04	0.04	0.02
	21	0.03	0.04	0.05	0.04
	40	0.01	0.03	0.03	0.03
RDW%	Pre	14.7	14.6	14.9	14.1
	1	14.2	14	14.3	13.6
	11	14.9	14.2	14.7	13.9
	21	14	13.5	13.7	13.9
	40	14.6	13.5	13.3	13.2

Table 14: Group mean hematology values for males in the 6 month toxicology study

	Timepoint	1M	2M	3M	4M
RBC	Pre	6.84	6.77	6.84	6.93
	1	6.25	6.05	6.11	6.19
	11	6.49	6.55	6.63	6.56
	21	6.71	6.55	6.63	6.51
	40	6.71	7.29	6.5	7.01
RETIC%	Pre	0.6	1	0.8	0.5
	1	0.8	1.1	0.9	0.6
	11	0.6	0.8	0.6	0.5
	21	0.4	0.4	0.4	0.4
	40	0.6	0.7	2.3	0.5
ARET	Pre	40.3	64.5	54	36.1
	1	51.8	65	52	38
	11	40	51	38.8	30
	21	26.8	25.5	26.4	22.9
	40	37.4	49.9	132	31.3

	Timepoint	1M	2M	3M	4M
HGB	Pre	13.2	13.5	13.3	13.2
	1	12	12.1	11.9	11.8
	11	12.3	12.8	12.6	12.3
	21	12.5	12.6	12.5	11.9
	40	13.1	14.5	11.9	13.5
HCT	Pre	47	48.2	47.3	46.2
	1	42.4	41.8	41	40.5
	11	42	44.2	43.6	41.1
	21	47.1	45	45.7	43.3
	40	46.8	52.3	43.8	47.3
MCV	Pre	68.8	71.2	69.2	66.8
	1	68	69.2	67.1	65.4
	11	64.9	67.4	65.8	62.9
	21	70.4	68.7	68.9	66.5
	40	69.8	71.7	67.3	67.7
MCH	Pre	19.3	20	19.4	19.1
	1	19.3	19.9	19.5	19.1
	11	18.9	19.5	19.1	18.8
	21	18.7	19.3	18.9	18.3
	40	19.5	19.8	18.1	19.3
MCHC	Pre	28.1	28.1	28.1	28.6
	1	28.4	28.8	29	29.2
	11	29.1	28.9	29	29.8
	21	26.6	28.1	27.5	27.6
	40	28	27.6	26.9	28.5
PT	Pre	10.8	11	11.2	10.6
	1	10.1	10.6	10.6	10.3
	11	9.7	10.2	10.3	10.1
	21	10.1	10.2	10.5	10.1
	40	10.8	10.9	12.2	11.1
APTT	Pre	20.1	19.9	20.5	19.8
	1	18.6	18.6	18.8	19
	11	18.2	18.5	18.8	19.2
	21	19.4	18.5	19.4	19.6
	40	21.6	21.4	24.9	21.7
FIB	Pre	200.6	200.1	207.8	208.3
	1	311.8	249.8	293.3	283.9
	11	311.3	266.9	248.9	254.1
	21	297.6	272	255.1	271.7
	40	241.4	212.9	127.3	212.3
MPV	Pre	9.1	9.7	9.5	8.5
	1	9	8.4	9.4	8.4
	11	9.3	9.2	9.4	8.7
	21	9.7	8.9	9	8.8
	40	10.8	9	12.3	8.4

	Timepoint	1M	2M	3M	4M
APLAT	Pre	368	331	348	396
	1	403	378	382	424
	11	380	382	396	418
	21	400	382	431	415
	40	369	405	417	424
WBC	Pre	10.08	8.35	8.68	7.31
	1	11.89	7.39	9.71	7.68
	11	10.12	7.96	10.07	8.58
	21	11.03	10.45	11.53	10.7
	40	12.94	11.66	12.88	11.44
NEUT%	Pre	51.7	54.5	39.2	47.1
	1	47.7	45.5	37.1	42.4
	11	43	42.6	37.5	47.1
	21	36.4	47.8	32.5	43.6
	40	41.1	25.2	26.7	23.9
ANEUT	Pre	5.4	4.54	3.66	3.47
	1	5.66	3.34	3.67	3.22
	11	4.42	3.35	3.83	3.99
	21	4.02	4.89	3.74	4.62
	40	5.81	2.9	3.45	2.72
LYMP%	Pre	42.8	39.6	51.9	47
	1	45.6	46.3	51.6	48.4
	11	50.6	49.5	54.4	46.3
	21	56.3	44.8	57.3	48.2
	40	52.1	64.8	64.5	64.6
ALYMP	Pre	4.13	3.32	4.26	3.41
	1	5.42	3.43	4.93	3.74
	11	5.08	3.97	5.4	4.01
	21	6.22	4.77	6.59	5.21
	40	6.31	7.56	8.26	7.41
LUC%	Pre	1.1	1.2	1.2	1.4
	1	1.2	1.5	1.5	1.6
	11	1.9	2.2	1.8	2
	21	1.3	1.3	1.3	1.3
	40	1.2	1.5	1.8	1.9
ALUC	Pre	0.11	0.1	0.09	0.1
	1	0.14	0.11	0.14	0.13
	11	0.19	0.18	0.18	0.17
	21	0.14	0.14	0.15	0.14
	40	0.15	0.17	0.21	0.22
EOS%	Pre	0.7	0.8	2.8	0.9
	1	1	1.4	3.2	2.7
	11	1.2	1.5	2.7	1.9
	21	1.8	1.6	3.7	3.2
	40	1.5	2.8	2	5.6

	Timepoint	1M	2M	3M	4M
AEOS	Pre	0.07	0.07	0.26	0.07
	1	0.13	0.11	0.33	0.22
	11	0.1	0.13	0.29	0.18
	21	0.2	0.18	0.44	0.34
	40	0.15	0.36	0.31	0.63
MONO%	Pre	3.5	3.4	4.6	3.3
	1	4.1	5	6.1	4.4
	11	2.9	3.7	3.3	2.4
	21	3.8	4.1	4.8	3.4
	40	3.9	5.4	4.7	3.8
AMONO	Pre	0.34	0.29	0.38	0.23
	1	0.49	0.38	0.59	0.34
	11	0.28	0.3	0.33	0.2
	21	0.41	0.43	0.56	0.36
	40	0.5	0.63	0.6	0.44
BASO%	Pre	0.3	0.5	0.4	0.3
	1	0.4	0.4	0.5	0.5
	11	0.5	0.4	0.4	0.3
	21	0.4	0.4	0.4	0.3
	40	0.3	0.4	0.5	0.3
ABASO	Pre	0.03	0.04	0.03	0.02
	1	0.04	0.03	0.04	0.03
	11	0.04	0.03	0.04	0.03
	21	0.05	0.04	0.05	0.03
	40	0.04	0.05	0.06	0.04
RDW	Pre	15.8	14.8	14.4	14.5
	1	15.1	14.2	14.1	13.7
	11	15.7	14.5	14.1	14.6
	21	15.2	13.8	13.9	13.9
	40	15.6	13.5	15.1	14.1

Clinical Chemistry

There were no statistically-significant treatment-related changes in any clinical chemistry parameter evaluated. Because pharmacodynamic saturation occurred at all dose levels, and because toxicity for this drug is expected to be an extension of the pharmacodynamic response, changes in clinical chemistry parameters cannot be excluded from treatment; however, their low frequency and low magnitude of severity implies that they are of little toxicological significance. A summary of group mean clinical chemistry parameters in females or males is provided in Table 15 and Table 16, respectively.

Individual variations were observed in chemistry parameters that exceeded control and/or predose ranges; however, the changes did not appear to exhibit a relationship to dose and are therefore of equivocal relationship to treatment. For example, several females in the low and mid-dose group exhibited reduced glucose levels during Weeks

11 and/or 21; however, there were no changes in glucose levels noted in high dose females. Increased serum creatinine and total protein was observed in two high dose females during weeks 11 and/or 21. The A/G ratios were decreased in individual animals across all treated female groups during weeks 11 and/or 21; however, this appears to be attributable primarily to an increase in globulin in these animals. Increased LFTs (AST and ALT) were observed in isolated animals across the low and mid-dose levels during weeks 1 and 11; however, in most cases, these resolved by Week 21 and none were observed in high dose animals. Increased calcium was observed in two low dose females (Week 1 and 21 only) and two high dose females (week 21). Increased GGT was observed in one mid-dose female (Week 1) and one high dose female (Weeks 11 and 21).

In males, decreased potassium was observed in individual animals across all treated groups during Week 21, and decreased calcium was observed in one high dose male during Weeks 1 and 11. Decreased phosphorous was observed in one animal in each treatment group during Weeks 1 (high and low dose groups) and 21 (high and mid-dose groups). Decreased triglycerides were observed in one low-, 2 mid- and three high-dose group males; however the timing of the decrease was highly variable and not clearly attributable to dose or duration of exposure.

Because pharmacodynamic saturation occurred at all dose levels, and because toxicity for this drug is expected to be an extension of the pharmacodynamic response, changes in clinical chemistry parameters cannot be excluded from treatment; however, their low frequency and low magnitude of severity implies that they are unlikely to be toxicologically significant.

Table 15: Summary of group mean clinical chemistry values for females in the 6 month toxicology study

Parameter	Study Week	1F	2F	3F	4F
GLU	Pre	61	57	62	62
	1	61	70	65	64
	11	75	54	50	55
	21	77	56	62	63
	40	59	74	63	64
BUN	Pre	17	20	17	19
	1	21	23	20	20
	11	21	22	18	20
	21	22	22	21	21
	40	21	22	17	21
CREA	Pre	0.8	0.9	0.8	0.9
	1	0.8	0.9	0.9	0.9
	11	0.9	0.9	0.7	0.9
	21	0.9	0.9	0.8	0.9
	40	0.8	0.9	0.9	0.8

Parameter	Study Week	1F	2F	3F	4F
TP	Pre	7.4	7.3	7.2	7.1
	1	7.5	7.1	7.2	7.4
	11	7.4	7.1	7.1	7.3
	21	7.6	7.4	7.3	7.8
	40	7.2	6.7	7.4	7.1
ALB	Pre	5.2	5	4.8	4.9
	1	5.2	4.9	4.8	4.9
	11	4.9	4.6	4.5	4.7
	21	5	4.7	4.5	4.8
	40	4.7	4.4	4.6	4.6
A/G	Pre	2.4	2.2	2	2.1
	1	2.3	2.2	1.9	1.9
	11	2	1.8	1.8	1.8
	21	1.9	1.8	1.7	1.6
	40	1.9	1.9	1.7	1.9
GLOB	Pre	2.2	2.3	2.4	2.3
	1	2.3	2.2	2.5	2.6
	11	2.4	2.5	2.5	2.6
	21	2.6	2.7	2.8	3
	40	2.5	2.4	2.8	2.5
AST	Pre	42	54	44	48
	1	45	61	43	51
	11	54	68	57	44
	21	55	42	47	44
	40	34	29	43	31
ALT	Pre	49	64	52	43
	1	52	75	54	55
	11	56	71	54	45
	21	53	52	62	42
	40	44	47	83	33
ALP	Pre	798	795	688	772
	1	724	719	638	664
	11	789	745	653	703
	21	662	662	580	640
	40	573	545	534	487
NA	Pre	153	154	152	151
	1	152	150	152	150
	11	154	153	152	152
	21	153	152	150	156
	40	148	151	148	151
K	Pre	5.7	6	5.6	5.4
	1	5.1	5.1	4.8	4.7
	11	5.1	5	5.4	5.2
	21	4.7	5	4.9	5
	40	4.6	4.7	4.8	4.7

Parameter	Study Week	1F	2F	3F	4F
CL	Pre	109	109	109	108
	1	107	108	108	107
	11	108	108	110	109
	21	107	106	107	107
	40	106	108	110	109
CA	Pre	11	11.2	10.7	10.8
	1	10.5	10.6	10.3	10.3
	11	10.3	10.1	10	10.3
	21	10.6	10.8	10.3	10.9
	40	10	10.5	10.2	10.1
PO4	Pre	6.9	7	7.1	7.1
	1	6.2	6.3	6.4	6.5
	11	6.7	6.6	6.7	6.6
	21	6.4	6.7	6.8	6.8
	40	6	5.8	5.8	6.4
CHOL	Pre	110	124	108	109
	1	114	122	107	111
	11	108	112	103	106
	21	113	115	100	113
	40	124	107	89	99
TRIG	Pre	71	73	71	74
	1	64	64	57	73
	11	60	65	52	71
	21	51	73	53	68
	40	54	62	43	68
TBILI	Pre	0.3	0.4	0.3	0.4
	1	0.4	0.5	0.4	0.5
	11	0.3	0.4	0.3	0.5
	21	0.3	0.3	0.2	0.3
	40	0.5	0.5	0.2	0.4
GGT	Pre	137	149	138	124
	1	123	135	133	111
	11	133	133	131	112
	21	111	121	115	109
	40	111	118	92	76

Table 16: Summary of group mean clinical chemistry values for males in the 6 month toxicology study

Parameter	Study Week	1M	2M	3M	4M
GLU	Pre	61	62	64	68
	1	60	64	64	62
	11	63	56	54	54
	21	60	64	70	67
	40	82	78	69	88

Parameter	Study Week	1M	2M	3M	4M
BUN	Pre	20	21	21	18
	1	21	24	22	20
	11	23	24	22	19
	21	22	24	24	21
	40	20	23	26	19
CREA	Pre	0.9	0.9	1	0.9
	1	0.9	0.9	0.9	0.9
	11	0.9	1	0.9	0.9
	21	0.9	0.9	0.9	0.9
	40	1	0.9	0.9	0.8
TP	Pre	7.2	7.5	7.6	7.4
	1	7.3	7.1	7.3	7.3
	11	7.3	7.3	7.3	7.2
	21	7.7	7.4	7.5	7.4
	40	7.5	7.1	6.9	7
ALB	Pre	4.9	5.2	5.1	4.9
	1	4.9	5	4.9	4.8
	11	4.7	4.9	4.9	4.5
	21	5	4.9	5	4.6
	40	4.8	4.9	4.4	4.6
A/G	Pre	2.1	2.2	2.1	2.1
	1	2	2.5	2.1	2
	11	1.9	2	2.1	1.6
	21	1.8	2	1.9	1.7
	40	1.8	2.2	1.8	1.9
GLOB	Pre	2.3	2.3	2.4	2.4
	1	2.4	2.1	2.3	2.5
	11	2.6	2.5	2.4	2.8
	21	2.8	2.6	2.6	2.8
	40	2.8	2.3	2.5	2.5
AST	Pre	49	44	41	49
	1	50	62	35	38
	11	79	48	42	47
	21	68	37	47	41
	40	45	41	62	60
ALT	Pre	59	45	42	41
	1	63	48	44	48
	11	69	44	49	41
	21	76	42	62	47
	40	60	47	127	56
ALP	Pre	817	875	742	804
	1	756	780	693	727
	11	886	880	774	792
	21	939	835	703	789
	40	893	856	1128	744

Parameter	Study Week	1M	2M	3M	4M
NA	Pre	152	156	155	154
	1	151	149	151	149
	11	152	158	154	150
	21	157	152	151	152
	40	153	153	153	149
K	Pre	5.5	5.3	5.6	5.8
	1	5	5	5	4.8
	11	5.7	5.7	5.1	5.3
	21	5.6	5	4.8	4.7
	40	4.3	4.7	4.5	4.4
CL	Pre	108	107	109	109
	1	107	106	107	107
	11	108	109	108	108
	21	107	106	105	107
	40	106	105	104	106
CA	Pre	10.7	11	11.5	10.9
	1	10.4	10.1	10.6	10.3
	11	10.4	10.4	10.5	10.2
	21	11	10.6	10.9	10.2
	40	9.9	10	10.2	9.8
PO4	Pre	7.6	7.6	7.1	7
	1	6.7	6.6	7	6.7
	11	7.7	7.6	7.1	6.8
	21	7.6	7	6.9	6.7
	40	7.5	7.1	7.4	6.3
CHOL	Pre	100	111	114	109
	1	113	113	117	108
	11	115	117	113	109
	21	116	111	113	101
	40	94	129	186	107
TRIG	Pre	80	82	53	60
	1	79	63	52	51
	11	58	73	49	45
	21	58	53	46	51
	40	37	36	69	33
TBILI	Pre	0.4	0.4	0.2	0.3
	1	0.5	0.5	0.4	0.3
	11	0.4	0.4	0.3	0.2
	21	0.4	0.3	0.3	0.2
	40	0.2	0.2	0.2	0.1
GGT	Pre	168	200	197	171
	1	167	189	186	153
	11	205	213	222	171
	21	212	197	197	165
	40	191	214	162	195

Urinalysis

Unremarkable

Gross Pathology

Unremarkable. A summary of all gross findings noted is provided in Table 17.

Table 17: Summary of gross necropsy observations

	1M	2M	3M	4M	1F	2F	3F	4F
Main Study								
Kidney, focus				1				
Ovary, cyst							1	
Ovary, nodule							1	
Injection site, discoloration	2	3	3	3	2	3	3	3
Recovery Necropsy								
Parathyroid, ↑ size						1		
Kidney, focus		1						
Ovary, discoloration							1	1
Ovary, ↑ size					1	2		
Vagina, ↑ thickness					1	1		

Organ Weights

Unremarkable. Note that for relative organ weights, the data from males and females were pooled in recovery cohorts (N = 4). For main study animals, relative organ weight data were presented by sex. There were no statistically significant differences in absolute or relative organ weights noted.

Histopathology

Adequate Battery

The Applicant originally evaluated only control and high-dose males and females in main study cohorts, and did not evaluate animals in recovery cohorts (total of 6 out of 30 treated animals and 6 out of 10 controls).
Adverse events are observed in humans following treatment with PD-1 inhibitors, and these are typically autoimmune-mediated, which is consistent with the data obtained in

PD-1-pathway-deficient animals. The spectrum of autoimmune diseases observed in humans treated with pembrolimumab is broad and apparently idiosyncratic (e.g., hypothyroidism, transaminitis, nephritis, colitis, pneumonitis, etc.); however, the aggregate of autoimmune adverse effects, many of which are dose-limiting, occur at relatively high frequencies (e.g., according to Merck's presentation to the pediatric ODAC (http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/OncologicDrugsAdvisoryCommittee/UCM375645.pdf), hypothyroidism occurred at a frequency of ~ 8%).

This unpredictable pattern of immune-mediated toxicity, particularly in light of the pharmacodynamic saturation that was demonstrated to have occurred at doses as low as 0.3 mg/kg suggests that limiting the histological assessment to animals in the high dose group is not justified and likely results in an under-representation of true rate and extent of histopathological changes that occurred in this study. This is particularly striking when comparing the histological findings observed in the 6-month study (Table 18) with those observed in the 1-month study (Table 10). As a result, the original histological evaluation was deemed inadequate to determine the pattern or extent of target organ toxicity in this study and Merck was asked to re-evaluate this data and include animals from all dose groups. This re-evaluation (Table 19) was considered adequate and the results of both evaluations are included below.

Peer Review
Yes
Histological Findings

Table 18: Histological changes of potential or equivocal relationship to MK-3475 treatment in control and high dose animals

Sex	**MALE**				**FEMALE**			
Dose (mg/kg)	0	6	40	200	0	6	40	200
Tissue, Histological Description								
Injection site, hemorrhage --inflammation	3 3	--	--	3 3	2 1	--	--	3
Pancreas, inflammation, focal **Thyroid**, cyst		-- --	-- --		 1	-- --	-- --	1

Table 19: Histological changes of potential or equivocal relationship to MK-3475 treatment in all groups including recovery cohort animals

Sex	**MALE**				**FEMALE**			
Dose (mg/kg)	0	6	40	200	0	6	40	200
Tissue, Histological Description								
Epididymides, cellular infiltration, focal			1R					

Sex	MALE				FEMALE			
Dose (mg/kg)	0	6	40	200	0	6	40	200
Esophagus, cellular infiltration			1R	1		1	1	
Heart, cellular infiltration, focal						1R		
Injection site, hemorrhage	2	2	2	3	3	3	1	3
--inflammation	1		2		3	2	1	3
Kidney, cellular infiltration, increased			1					
Large intestine, serosa, cellular infiltration, perivascular				1R				
Liver, cellular infiltration, increased			1R					
Lymph node, lymphoid follicle, increased amount			1R					
Ovary, cyst						1		
Pancreas, inflammation, focal				1				1R
Parathyroid, cellular infiltration, focal		1	1R				1	
--cyst		1+1R					1	1R
Pituitary, cellular infiltration			1R					
Prostate, cellular infiltration, focal			1R					
Sleketal muscle, interstitium, cellular infiltration, focal			2			1		
Spleen, periarteriolar lymphoid sheath, increased amount			1R					
Thyroid, cellular infiltration, focal		1	1R					
--cyst	1	1	1					1R
Thymus, inflammation, focal			1R					
--cortex, depletion, lymphoid			1R					
--medulla, increased amount, lymphocytic			1R					
Vagina, inflammation						1	1R	

R = Recovery cohort

Special Evaluation

None

Toxicokinetics

Toxicokinetic sampling for exposure, pharmacodynamics and ADA was performed at various intervals throughout the study, and full profiles were collected on Days 1, 71, and 141. Exposure was essentially linear (slightly supra-proportional with dose) over the 33-fold dose range on Day 1. Exposure was generally similar on Study Days 71 and 141. Accumulation (~2X) was observed upon repeated dosing. There was evidence of an effect of immunogenicity on the plasma exposure levels, particularly in the low dose group. The terminal elimination half-life was estimated to be between 409-616 hours. There was no apparent effect of dose on the estimation of the terminal elimination half-life.

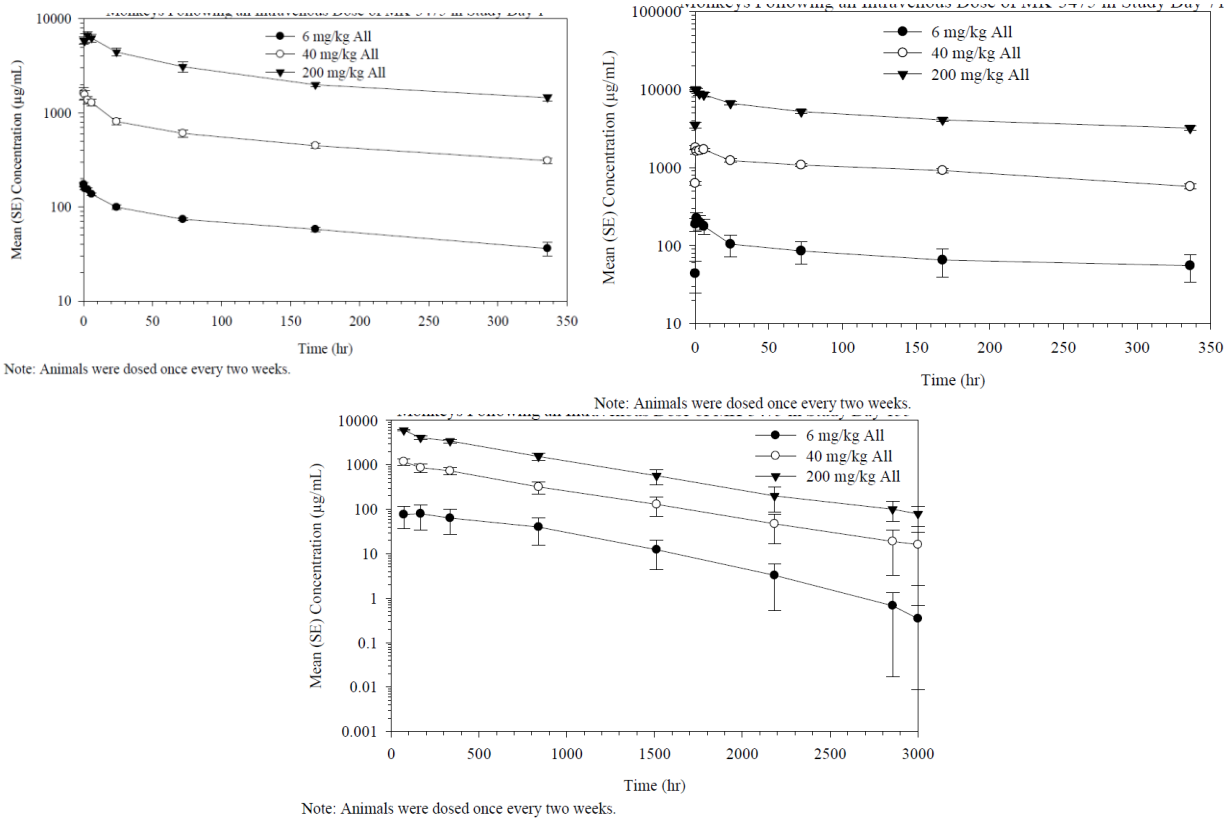
In one control animal, there was measurable drug on Study Day 1 and on Study Day 99, plasma levels exceeded the LLOQ for two control animals. In all cases, drug levels were low (<1% of concurrent values in the low dose group), suggesting sample contamination rather than misdosing. There was evidence of an effect of immunogenicity on exposure; 5/6 animals in the low dose group and 1 animal in the high dose group were ADA positive. By Study Day 43, exposure was reduced or ablated in most animals in the low dose group.

Table 20: Mean (M+F) toxicokinetics summary from the 6 month monkey study

Dose	Day	AUC_{0-336} (µg*hr/mL)	Cmax (µg/mL)	Tmax (hr)
6	1*	21,400+915	168+7.90	0.36+0.11
	71*	48,500+8320	332+67.6	2.6+1.3
	141*	59,100+1880	326+18.6	0.25+0.0
40	1	176,000+11,000	1840+216	1.6+0.59
	71	313,000+16,500	1930+93.8	2.0+0.75
	141	322,000+27,000	1890+180	1.5+0.56
200	1	848,000+60,400	7470+529	4.8+2.2
	71	1,540,000+59,100	10,500-489	0.55+0.12
	141	1,620,000+107,000	10,000+772	1.1+0.55

* = animal(s) excluded due to insufficient quantifiable time points to calculate $AUC_{0-336\ hr}$ or potential effect of MAHA on serum concentrations

Figure 41: MK-3475 Concentration-time profiles in male and female cynomolgus monkeys on Study Days, 1, 71, and 155



Incurred sample reanalysis (ISR) was performed on toxicokinetic plasma samples collected during the course of this study. The samples reanalyzed were distributed across all dose groups and from all TK sampling intervals, indicating that the Applicant attempted to evaluate the full range of achieved concentrations. The analytical method met the criteria for ISR, as the reanalyzed concentrations were within 30% of the original concentration estimates in ~73% of the samples assessed. (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM368107.pdf)

Dosing Solution Analysis

Met pre-specified acceptance criteria.

7 Genetic Toxicology

Genetic toxicology studies were not conducted with MK-3475, since, as a monoclonal antibody, it is not expected to interact directly with DNA or other chromosomal material. This is in keeping with the principles of the ICH S6 guideline for the Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals.

8 Carcinogenicity

Carcinogenicity studies were not conducted with this MK-3475, which is consistent with its use in patients with advanced malignancies (*see* the ICH S9 Guideline for the Nonclinical Evaluation of Anticancer Pharmaceuticals). If MK-3475 is developed for use in other indications, an assessment of its carcinogenic potential may be warranted; however rodent bioassays are likely inappropriate, as MK-3475 does not appear to be active in rodents.

9 Reproductive and Developmental Toxicology

The intimate contact between maternal blood cells and fetal trophoblastic cells at the maternal/placental interface and the hemi-allogeneic nature of fetal cells requires that the pregnant dam simultaneously suppress the immune response to the fetus while maintaining immunocompetence against infections. Recognition of the fetal allograft occurs directly at the placenta, as well as indirectly through traffic of fetal antigens to secondary lymphoid organs of the mother, where dendritic cells present the antigen to paternally-reactive T cells, which are then clonally deleted. This was demonstrated in a murine model in which non-transgenic females were mated with Act-mOVA males, and pregnant dams received adoptive transfers of fluorescently-labeled CD4+ or CD8+ TCR transgenic T cells specific for OVA-derived (i.e. fetal) antigens. In these studies, adoptively transferred cells were found to have proliferated in secondary lymphoid organs of the dams after a 2-day post-transfer period. In contrast, dams that were mated to WT partners (lacking OVA antigens) had no demonstrable proliferation of donor T cells (*reviewed in* Petroff, 2011).

In addition to the indirect clonal deletion of reactive anti-fetal clones, immune reactivity toward fetal antigens is directly suppressed at the maternal/placental interface through the expression of a number of immunomodulatory molecules. Murine and human trophhoblasts are equipped with an array of immunomodulatory proteins that can down-regulate the maternal immune response, including PD-L1. Within the placenta, PD-1 on decidual T cells interacts with PD-L1 on trophoblasts and induces tolerance. In mice, maternal PD-1-expressing T regulatory cells expand during pregnancy and suppress rejection of paternally-derived fetal antigens. In the absence of a functional PD-1 signaling pathway, immune-mediated rejection of the fetus occurs. Inhibition of PD-1 signaling by use of an anti-PD-L1 antibody in dams carrying allogeneic litters (CBA X B6) led to an increase in the rate of resorption (~86%) compared to that of control-treated dams carrying similarly allogeneic litters (~18%). (Guleria, et al., 2005).

On the basis of the known association between PD-1 deficiency and increased rates of abortion, FDA agreed with the Applicant's request to provide a literature assessment of the scientific data of pregnancy outcomes in PD-$1^{-/-}$ mice in lieu of conducting an embryofetal study in the monkey with MK-3475. This decision was made in part because MK-3475 is not active in any species other than the nonhuman primate. While monkeys are commonly used as a for reproductive toxicity testing, concern about high rates of abortion associated with PD-1 inhibition, in conjunction with the high rate of

spontaneous abortion in the monkey which can exceed 20% in untreated controls, suggested that such a study may be uninterpretable.

Concerns about potential teratogenicity resulting from fetal antibody exposure have not been addressed by this approach; however, data in knockout mice do not suggest an association between PD-1 deficiency and malformations in these animals. Data in PD-$1^{-/-}$ mice do, however, suggest that effects on the developing immune system are likely in fetuses from mothers treated with pembrolizumab if sufficient transplacental exposure is achieved during fetal development. While transplacental exposure to monoclonal antibodies is low during embryonic development; it is clear that for some monoclonal antibodies, sufficient exposure has been obtained to produce teratogenic effects in the offspring (*e.g.* bevacizumab and pertuzumab, both IgG1 monoclonal antibodies).

While PD-1 deficient mice appear to develop normally with no clear physical malformations, potential consequences of exposure to pembrolizumab on the developing immune system, particularly regarding the potential for development of autoimmune diseases in infants, children and adolescents cannot be excluded. In mice, PD-1 deficiency has resulted in late onset autoimmune disorders and in the potentiation of autoimmunity.

10 Special Toxicology Studies

10.1 Cross-reactivity study of SCH 900475 with normal cynomolgus monkey tissues (IM1749)

The purpose of this study was to evaluate the potential for MK-3475, an IgG4 monoclonal antibody directed against human PD-1, to bind to cryosections (10µm) of normal monkey tissues. The primary and secondary antibodies were mixed prior to application to tissue sections to avoid the need to label the primary antibody. The secondary antibody was a biotin-labeled $(ab')_2$ donkey-anti-human IgG specific for the Fcγ fragment. The concentrations of MK-3475 used were 1 and 10 µg/mL. The ratio of primary to secondary antibody was 1:1.5. Up to 3 donors/tissue were evaluated. Detection of binding was accomplished using an avidin-conjugated enzyme; the chromogen used was 3,3-diaminobenzidine.

- Assay Validity: The test article stained the positive control material (inflamed human tonsils), but produced no specific pattern of reactivity with the negative control material; thus, the study met the pre-specified criteria for a valid assay.
- MK-3475 bound strongly with stroma of the bone marrow, eye, GI tract (colon, esophagus, small intestine, stomach) kidney, lungs, ovary, pancreas, peripheral nerve, prostate, spinal cord, spleen, testis, tonsil, urinary bladder, uterus (endometrium) and uterus-cervix.
- Cytoplasmic staining of the smooth myofibers of the GI tract, lung, ovary, fallopian tube, prostate and uterus (endometrium) was observed.

- Cytoplasmic/cytoplasmic granule/plasma membrane and/or plasma membrane granule staining was observed in mononuclear leucocytes in the GI tract (colon, small intestine, stomach) kidney, lung, lymph node, salivary gland, spinal cord, testis, spleen, thymus, tonsil and ureter.
- Cytoplasmic or cytoplasmic granular staining of the epithelium was observed in the brain, cerebellum, GI tract (stomach, small intestine), parathyroid, pituitary and prostate.
- Staining of the lens of the eye was observed.
- Cytoplasmic staining of the liver, the epithelial-reticular cells (including Hassal's corpuscles) of the thymus, and oocytes of the ovary, and spindloid/dendritic cells of the ovary.
- Spermatid tails were positive for MK-3475 reactivity
- Extracellular staining of the neurohypophysis of the pituitary was observed.

10.2 Cross-reactivity study of SCH 900475 with normal human tissues

The purpose of this study was to evaluate the potential for MK-3475 to bind to cryosections (10µm) of normal human tissues from 3 normal cadaveric donors. The concentrations evaluated were 1 and 10 µg/mL. The primary and secondary antibodies were mixed prior to application to tissue sections to avoid the need to label the primary antibody. The secondary antibody was a biotin-labeled $(ab')_2$ donkey-anti-human IgG specific for the Fcγ fragment. The concentrations of MK-3475 used were 1 and 10 µg/mL. The ratio of primary to secondary antibody was 1:1.5. Up to 3 donors/tissue were evaluated. Detection of binding was accomplished using an avidin-conjugated enzyme; the chromogen used was 3,3-diaminobenzidine.

- Assay Validity: The test article reacted with positive control material (inflamed human tonsil) but not with negative control material (normal striated human skeletal muscle). The negative control article (anti-RSV-IgG4) did not react with either positive- or negative-control material. Taken together, these data suggest that the assay met the criteria for a valid assay.
- Stromal staining (granular to fibrillar) was observed in all tissues but the placenta.
- Cytoplasmic staining (diffuse or granular): was observed in the endothelium, brain (cerebrum) GI tract (colon, esophagus, stomach), kidney, liver, parathyroid, peripheral nerve, testis, thymus, tonsil, urinary bladder, uterus (body and cervix).
- Mononuclear leucocytes of the GI tract (esophagus, stomach) kidney, lymph node, spleen, thymus and tonsil Staining pattern was consistent with cytoplasmic, cytoplasmic granules, plasma membrane and/or plasma membrane granules.
- Macrophages of the ovary: staining pattern was cytoplasmic.
- Pituitary: cytoplasmic and cytoplasmic granules of the epithelium

10.3 Method validations:

The following table summarizes the bioanalytical method validations that were performed:

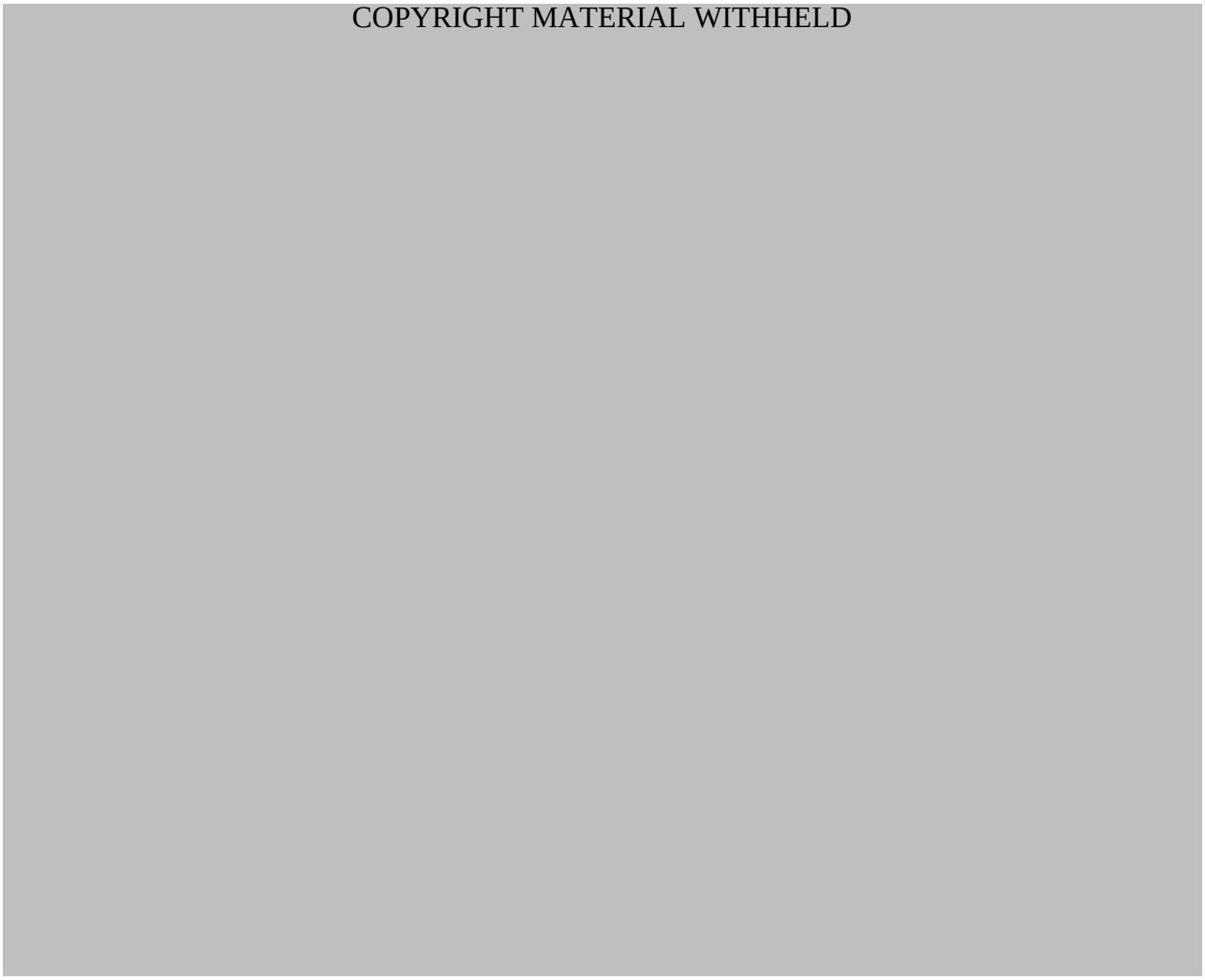
Method	Method Format	Species	Comments:
DM27759	ECL-ELISA for MK-3475	Monkey	❖ LOQ: 0.137 ng/mL ❖ Range: 0.137-100 ng/mL ❖ ADA interference observed, however unclear what portion of the molecule the ADA targets (framework or CDR) ❖ Assay format: streptavidin-coated plate, biotinylated anti-kappa Ab for capture of MK-3475
DM27757	ECL-ELISA for anti-MK-3475 (ADA)	Monkey	❖ LOQ: 7.81 ng/mL ❖ Range: 7.81-500 ng/mL ❖ Drug interference (reduced signal) at concentrations of 100-1000 ng/mL ❖ Spiked recovery in one run did not meet acceptance criteria (exceeded nominal recovery for both high and low QCs – attributed to matrix effect)
DM27756	ECL-ELISA for neutralizing ADA	Monkey	❖ Assay format: Plate was streptavidin-coated; PD-1 was biotinylated ❖ Anti-Kappa was TAGged (ruthenylated) – n.b. there was a contradiction in the report about whether it was PD-1 or anti-kappa that was biotinylated. Since this is a nAb assay, it's presumed to be the PD-1. ❖ LOD: 500 ng/mL ❖ Range: up to 6.26 µg/mL ❖ Blocking PD-1 (target) binding was demonstrated by ADA ❖ Assay was sensitive to residual drug in samples
AR3607	ECL ELISA for MK-3475	Monkey	❖ Assay format: streptavidin-coated plates + biotinulated PD-1; anti-kappa was sulfo-tagged. ❖ LOQ 0.4 ng/mL ❖ Range: 0.4-80 ng/mL
3608	ECL-ELISA for anti-MK-3475 (ADA)	Monkey	Refers to DM2257

11. Integrated Summary of Results

Pembrolizumab is an IgG_4 monoclonal antibody directed against human PD-1. PD-1 is a receptor of the immunoglobulin superfamily; its inducible expression is primarily limited to CD4+ and CD8+ T cells, NK cells, B cells, and activated monocytes (Figure 42). Expression of PD-1-ligands is also broad and inducible. The PD-L2 ligand, expressed primarily on activated dendritic cells, macrophages and mast cells, is more

restricted in its distribution than the PD-L1 ligand, which exhibits both a broad basal expression pattern and the potential for upregulation under conditions of immune stimulation.

Figure 42: Tissue distribution of PD-1 and PD-1 ligands in humans and mice



c.f. Keir, et al., 2008. *Ann. Rev. Immunol.*

Engagement of PD-1 with its ligands, PD-L1 and PD-L2, inhibits TCR-mediated T cell proliferation and cytokine production, (*discussed in* Wang, et al., 2011). Activation of PD-1 inhibits CD28 signaling through the PI3K/AKT pathway (probably through recruitment of the SHP-2 and SHP-1 phosphatases to the immune synapse) thus blocking the upregulation of pro-inflammatory mediators (e.g., IL-2 and IFNγ) and survival signals (e.g. Bcl-xl; Figure 43).

Figure 43: Summary of the PD-1 signaling pathway in activated T cells

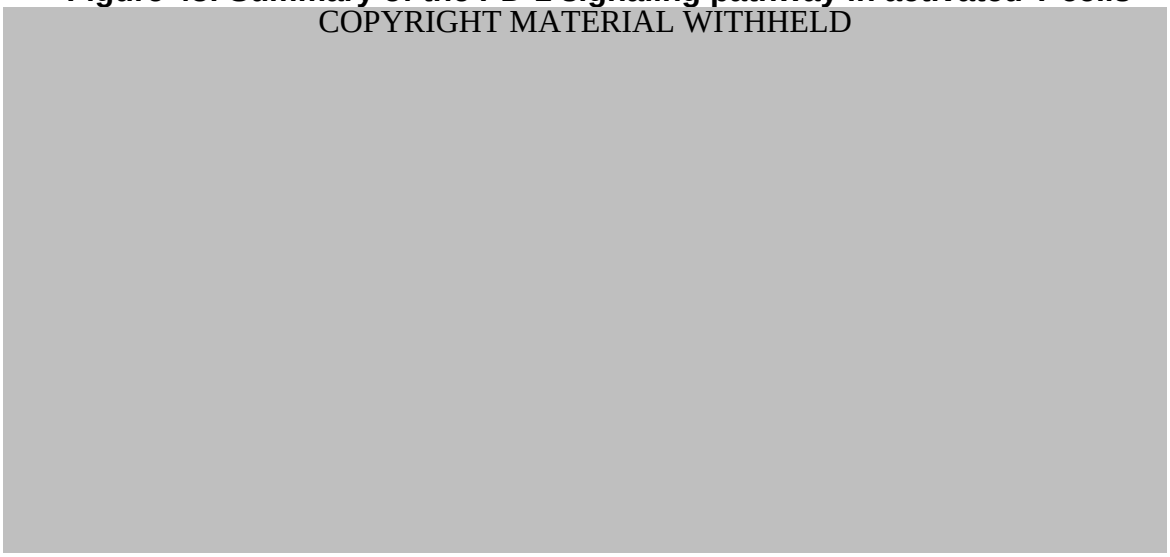
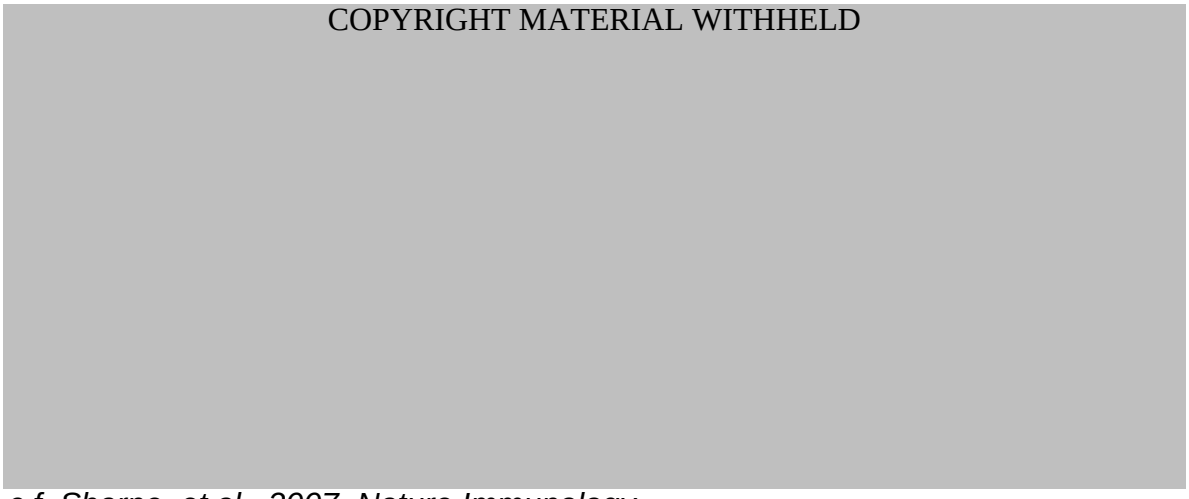


Figure c.f. Keir, ME, *et al*., 2008. *Annu. Rev. Immunol*. 2008. 26:677–704

The interaction between PD-1 and its ligands, thus, plays a role in the decision between clonal expansion of the reactive T cell population and dampening of the immune response or, more broadly, in maintaining the balance between immune activation and tolerance (Figure 44). In addition to the inhibitory effects of ligand-engagement on the PD-1-expressing T cell, there is also evidence of bidirectional signaling between the activated T cell and the APC. Engagement of PD-Ls with PD-1 has been demonstrated to modulate the function of activated dendritic cells via increasing production of cytokines such as IL-10, associated with dampening the immune response.

Figure 44: PD-1 and PD-1 ligands in tolerance and inflammation



c.f. Sharpe, et al., 2007. Nature Immunology

Direct evidence for the role of PD-1 in the maintenance of self-tolerance derives from a large body of data generated in PD-1 knockout animals, and from studies of mouse and

human autoimmunity. PD-1 is expressed in immune privileged sites such as the eye and the placenta; accordingly, loss of PD-1 activity at these sites, either by application of a PD-1 inhibitor or by functional or genetic deletion, leads to ocular graft rejection and increased rates of spontaneous abortion, respectively (*reviewed in*: Fife & Bluestone, 2008). The tissue distribution of the PD-1 ligands is widespread throughout the major organ systems of the body, including the endocrine organs (pancreas, thyroid, pituitary, adrenal), the heart, lungs, brain, and kidney (Figure 42). Accordingly, loss of expression by functional inactivation or inhibition by antibodies directed at PD-1 or its ligands is often associated with autoimmune conditions in those organs. In mice, inactivation of PD-1 or its ligands leads to a number of autoimmune conditions, including systemic lupus erythematosus (SLE), dilated cardiomyopathy, diabetes, and experimental autoimmune encephalitis (EAE). To date, over 30 single nucleotide polymorphisms (SNPs) have been identified on the human PD-1 gene that have been investigated for their linkage to autoimmune disease (reviewed in Okazaki and Honjo, 2007). One allele, PD1.3A in intron 4, which serves as a binding site for the transcription factor RUNX1, is associated with lupus nephritis, type I diabetes, and progressive multiple sclerosis (MS).

Table 21: Endogenous tissue expression pattern of PD-1 and PD-1 ligands in naive BALB/c mice

Background	PD-1	PD-L1	PD-L2
Naïve BALB/c Mice	Thymus	Thymus	Thymus
	Spleen	Spleen	
		Cardiac endothelium	
		Pancreatic islets	
		Syncyciotrophoblasts of the placenta	
		Mononuclear cells of the lamina propria of the small intestine	
		Lung macrophages	

While there appears to be an overall association between PD-1 deficiency and autoimmune disease, which autoimmune disease will manifest in a particular individual appears to be a complex function of genetic background and existing comorbidity(ies). For example, in BALB/c animals, PD-1 deficiency is associated with dilated cardiomyopathy, which is driven by anti-murine-troponin I in these animals, whereas in C57BL/6 PD-1 deficient animals, arthritis and glomerulonephritis are the predominant autoimmune diseases observed (*discussion in* Liang, et al., 2003).

There is also some evidence that disease status may affect the expression patterns of PD-1 and its ligands (Table 22; Liang, et al., 2003). In non-obese diabetic (NOD) mice, superimposition of PD-1 or PD-L1 deficiency greatly exacerbates and accelerates the onset of the diabetic phenotype. In pre-diabetic NOD mice, there is little or no expression of PD-L1 in pancreatic islet cells; however, upon attainment of the diabetic phenotype (~ 9 weeks of age), marked upregulation of PD-L1 is observed vs. that observed in healthy, naïve BALB/c mice. Absence of PD-1-in NOD mice (NOD-Pdcd1-

/-) accelerates the onset and incidence of diabetes in these animals, which is temporally associated with a massive infiltration of lymphocytes surrounding β-cells of the islets (Okazaki and Honjo, 2007), suggesting that the presence of PD-L1 on β- and islet dendritic cells performs a barrier function that blocks diabetogenic lymphocytes from penetrating the islets.

Similarly, in models of EAE, PD-1- or PD-1 ligand-deficiency increases both the rate and the severity of the disease. Taken together, these data suggest that genetic background, which may include co-segregating loci other than those immediately involved in the PD-1 signaling pathway, may modulate both the penetrance of the trait(s) as well as the specific manner in which it is manifested.

Table 22: Effect of background and disease status on the pathogenic outcomes observed in PD-1/PD-L knockout models

Background	Deficiency Model		
	PD-1	PD-L1	PD-L2
BALB/c-Pdcd1-/- mice	Dilated cardiomyopathy with anti-troponin I auto-antibody (presumed pathogenic mediator)		
C57BL/6-Pdcd1-/-	Arthritis		
	Glomerulonephritis		
Pdcd1-/- NOD mice	Infiltrating cells of pancreatic islets	Infiltrating cells of pancreatic islets	
		↑↑ Pancreatic islet cells	
Pdcd1-/- EAE (C57BL/6)	Mononuclear cells of the meninges	Mononuclear cells of the meninges	Mononuclear cells of the meninges
		Endothelium of brain surrounding areas of mononuclear infiltrates	Apparently expressed on B cell infiltrates

While PD-1 is involved in maintaining the balance between self-tolerance on the one hand, and immunopathology on the other, within this spectrum falls the need for immune-mediated destruction of pathogens and transformed cells. Evidence from virally immunosuppressed patients and from the chronic treatment of patients with immune-suppressing therapies in the treatment of autoimmune conditions has revealed that failure of the adaptive immune system to mount an effective immune response is associated with a number of pathogenic processes, including cancer and chronic infection. Immune checkpoint inhibitors such as pembrolizumab, therefore, aim to shift the balance toward immune reactivity in the treatment of cancer and chronic infection.

Pembrolizumab was demonstrated to bind to PD-1 from humans and cynomolgus monkeys to a similar extent, but did not bind to PD-1 from rodents or dogs; accordingly, the cynomolgus monkey was chosen as the toxicology model for this drug. MK-3475 did not possess effector activity, as demonstrated by lack of binding to C1q or CD64. There was no evidence of cytokine release, as assessed by IL-2 production following culture of hPBMCs in pembrolizumab-immobilized (air-dried) plates. Finally, in murine

xenograft models, pembrolizumab exhibited anti-tumor activity when administered alone and in combination with other chemotherapeutic agents.

The pharmacodynamic activity of pembrolizumab was demonstrated in an SEB-stimulated IL-2 assay using whole blood from both monkeys and humans. Cultures of whole blood incubated with the superagonist SEB ± pembrolizumab were assessed for induction of IL-2. In blood from both monkeys and humans, an approximately 2-4X increase in the production of IL-2 was observed when incubated with SEB plus pembrolizumab, relative to cultures incubated with SEB alone. The magnitude of this increase was similar in both monkeys and humans. The addition of pembrolizumab, however, had no effect on IL-2 production following SEB stimulation of blood from animals that had been dosed with pembrolizumab, suggesting pharmacodynamic saturation of the pathway in these animals.

In the monkey, pharmacodynamic saturation was demonstrated following administration of a single IV dose of 0.3 mg/kg, which is well below the doses used for toxicological evaluation. Pharmacodynamic activity was demonstrated in both monkey studies through the end of the dosing interval. In some low-dose animals, ADA onset correlated with loss of the pharmacodynamic response (i.e. in those cultures, pembrolizumab+SEB could potentiate IL-2 levels over those of SEB alone); thus it was considered that in those animals, the ADA was neutralizing.

The mechanism of pharmacodynamic neutralization is not clear, though it appears to correlate with enhanced drug clearance. Whether the ADA produced in those animals also blocked target binding (e.g. was anti-idiotypic or by some other means blocked target engagement), was unclear. In mid- and high-dose animals that were ADA-positive, no suppression of pharmacodynamic activity was observed. This may be due to the difference between the supraphysiologic doses administered in the toxicology study in relation to the titer of ADA produced by the animals (i.e., the Applicant “dosed-through” the ADA response).

Pembrolizumab was evaluated in repeat-dose toxicology studies of one- and six-months duration in the monkey. In the one-month study, animals received a total of 5 IV doses of pembrolizumab at dose levels of 0, 6, 40 and 200 mg/kg/week. There were no effects on survival, clinical signs, body weight, food consumption, cardiovascular measurements, or clinical pathology endpoints. Histopathology data obtained in the 1-month study indicated a tendency toward increased monocytic and/or lymphocytic infiltration of tissues, many of which are known autoimmune target organs in humans; however, the severity scores were generally low in magnitude.

In the 6-month study, animals received a total of 12 biweekly IV doses of pembrolizumab at dose levels of 0, 6, 40 and 200 mg/kg/dose. There were no preterm deaths, and no effects on clinical signs, body weight, food consumption, cardiovascular or clinical pathology endpoints. Histological changes in this study were similar in character to those observed in the 1-month study, and included an increased incidence of monocytic and/or lymphocytic tissue infiltration; however, the magnitude and/or

severity was similar across studies and did not exhibit a tendency toward increasing severity with increasing duration of exposure.

The Applicant offered several possible explanations of the discrepancies between the two studies, including differences in the supplier, age of animals, testing facility and evaluating pathologist(s). They stated that while all monkeys used in the study were captive-bred and originated in Mauritius, the supplier for the two studies differed, as did the age range. In the 1-month study, animals were 2-4.5 years of age at study-start, and in the 6-month study, animals were 1.5-3 years of age. The 1-month study was performed by Schering-Plough in Lafayette, NJ, whereas the 6-month study was performed by the Applicant (Merck Research Laboratories, in West Point, PA). In addition, the slides were read and peer-reviewed by different pathologists. These factors may in part explain the differences in histopathological outcomes between the two studies. The Applicant provided references in which they demonstrate that the majority of the findings are considered common background findings in this age and strain of animals.

While there is no disagreement that the lesions are of generally low severity and uncertain clinical significance to the animal at the time of the study termination, there is some concern about the relationship between these subclinical histological changes observed in animals and the pattern of toxicity observed in humans that undergo treatment with MK-3475. There was also no evidence of a dose response in the pattern or severity of histological changes in the monkey, although it should be noted that all doses produced exposures that were above the level of pharmacodynamic saturation, as demonstrated in multiple nonclinical studies.

As indicated in Table 23, which summarizes the monkey histological changes noted in the 6-month study (excluding reproductive and lymphoid organs), many of the sites of dose-limiting autoimmune toxicities observed in humans have corresponding histopathological changes (primarily cellular infiltration) in the monkey, though it should be noted that this list does not differentiate autoimmune diseases observed in patients who were previously treated with ipilimumab, a previously approved immunomodulatory antibody that targets CTLA-4. Human targets with no corresponding primate histopathology include the eye and lung; sites of histopathological findings in the monkey with no correlating human toxicities include the esophagus, and parathyroid.

Table 23: Common human autoimmune adverse reactions by site with corresponding primate histopathology, as observed in the 6-month monkey study

Common treatment-related human autoimmune AEs†	Monkey histopathology
Uveitis	--
Pneumonitis	--
--	Esophagus: cellular infiltration
--	Heart: cellular infiltration, focal
Nephritis	Kidney: cellular infiltration, increased
Colitis	Large intestine, serosa: cellular infiltration, perivascular

Common treatment-related human autoimmune AEs†	Monkey histopathology
Hepatitis	Liver: cellular infiltration, increased
--	Parathyroid: cellular infiltration, focal
	Parathyroid: cyst
Hypopituitarism, hypophysitis	Pituitary: cellular infiltration
Myositis	Skeletal muscle: interstitium, cellular infiltration, focal
Hypothroidism Hyperthyroidism	Thyroid: cellular infiltration
	Thyroid: cyst
	Thymus: inflammation, focal
†Excludes lymphoid organs (pharmacodynamic) and reproductive organs	

Dedicated embryofetal development studies to investigate the risk of reproductive toxicity following administration of pembrolizumab were not conducted. The Applicant submitted data from the scientific literature supporting the role of the PD-1 signaling pathway in the maintenance of allopregnancy as evidence that use of pembrolizumab during pregnancy carries an increased risk of embryofetal loss. The teratogenic risk for the developing fetus following treatment has not been characterized, though loss of PD-1 signaling in mice has not been associated with obvious malformations. Pertinent findings cited from studies of PD-1-deficient animal models have included increases in the incidence of late onset autoimmune disorders or earlier onset of autoimmune disorders in certain genetic backgrounds. Thus, fetal exposure to pembrolizumab may increase the risk of developing autoimmune disorders or of altering the normal immune response following exposure to pembrolizumab during postnatal development.

To what extent PD-1 is involved in the pre-programming of the immune response at birth is unclear, as is the question of whether disrupting PD-1 signaling in the prenatal and/or perinatal period will alter the appropriateness of the immune response to vaccination and pathogen challenges. Because the PD-1 pathway is involved in the conversion of TH1 cells to T-regulatory cells, inhibition of PD-1 is likely to skew the T-cell response toward a TH1 pattern (Amaranth et al., 2011); thus, higher levels of TH1 cytokines, including IFN-γ, IL-2 and TNF may occur, and may lead to a greater risk of vaccine-related adverse events as a result of amplified TH1 responses.

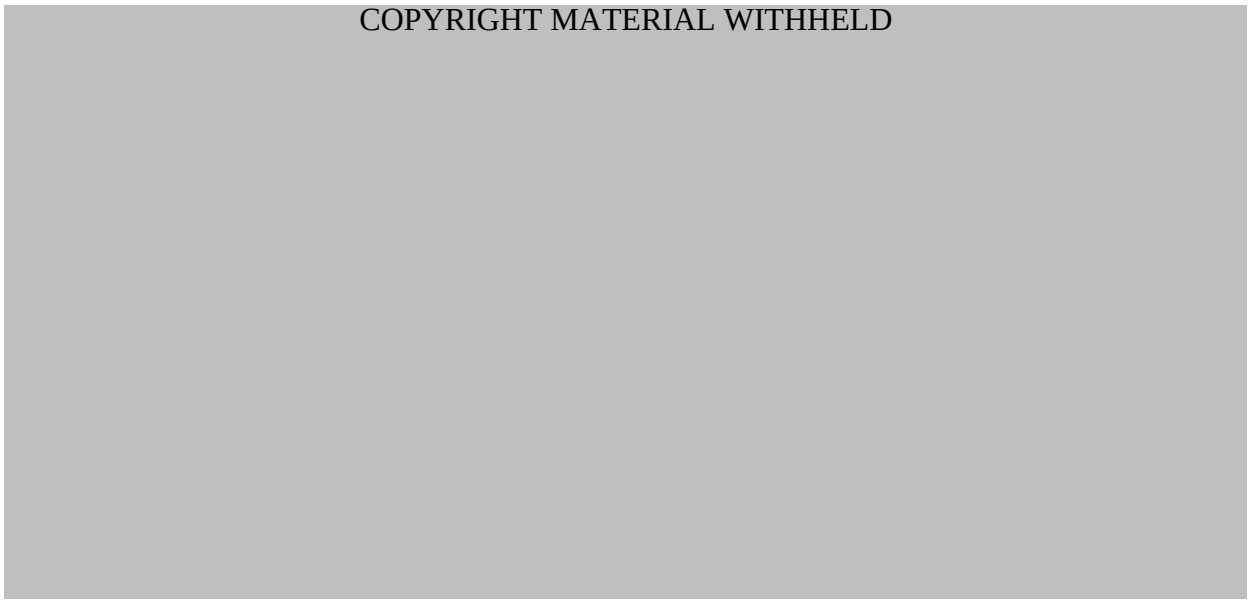
Clues about the potential for changes in vaccine-related response patterns were gleaned from an in vitro assay in which PD-1 inhibition on the recall response was assessed in hPBLs collected from patients who had undergone recent immunization with tetanus toxoid. Data from this series of experiments demonstrated that when hPBLs from recently vaccinated subjects were cultured with PD-1-blocking antibodies, the magnitude of the recall response was enhanced by about 2-5X as measured by increases in cytokine expression. Because pembrolizumab may also increase the numbers of circulating and tissue responder-cells, patients who are vaccinated or re-vaccinated while undergoing treatment with pembrolizumab may experience an enhanced vaccine-associated toxicity.

Besides the concerns about induction of autoimmunity demonstrated in clinical trials, additional concerns around the use of pembrolizumab in patients with chronic infections

may also exist. In models of chronic infection, resident T cells are rendered anergic in the context of invading pathogens, leading to increased susceptibility. In HIV-infection, T-cell anergy resulting from high levels of T-cell PD-1 expression leads to reduced viral clearance. PD-1 blockade in HIV-infected patients resulted in increased T cell proliferation, though the response was highly variable between individuals (Rosignoli et al., 2009).

Paradoxically, while loss of PD-1 function enhances clearance of some tumors and viral infections, it increases susceptibility to certain other pathogens such as tuberculosis. PD-1-deficient mice (C57BL/6) infected with *M. tuberculosis* exhibited a dramatic decrease in survival (Figure 45 upper L panel), which correlated with uncontrolled bacterial proliferation (Figure 45, upper R panel; dark bars = WT) and a larger inflammatory response in the lungs of PD-1-deficient mice compared with wild type controls (Figure 45, lower panel; dark bars = WT). Thus, 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.

Figure 45: Decreased survival, increased bacterial proliferation and increased inflammation in PD-1-deficient mice infected with M. tuberculosis

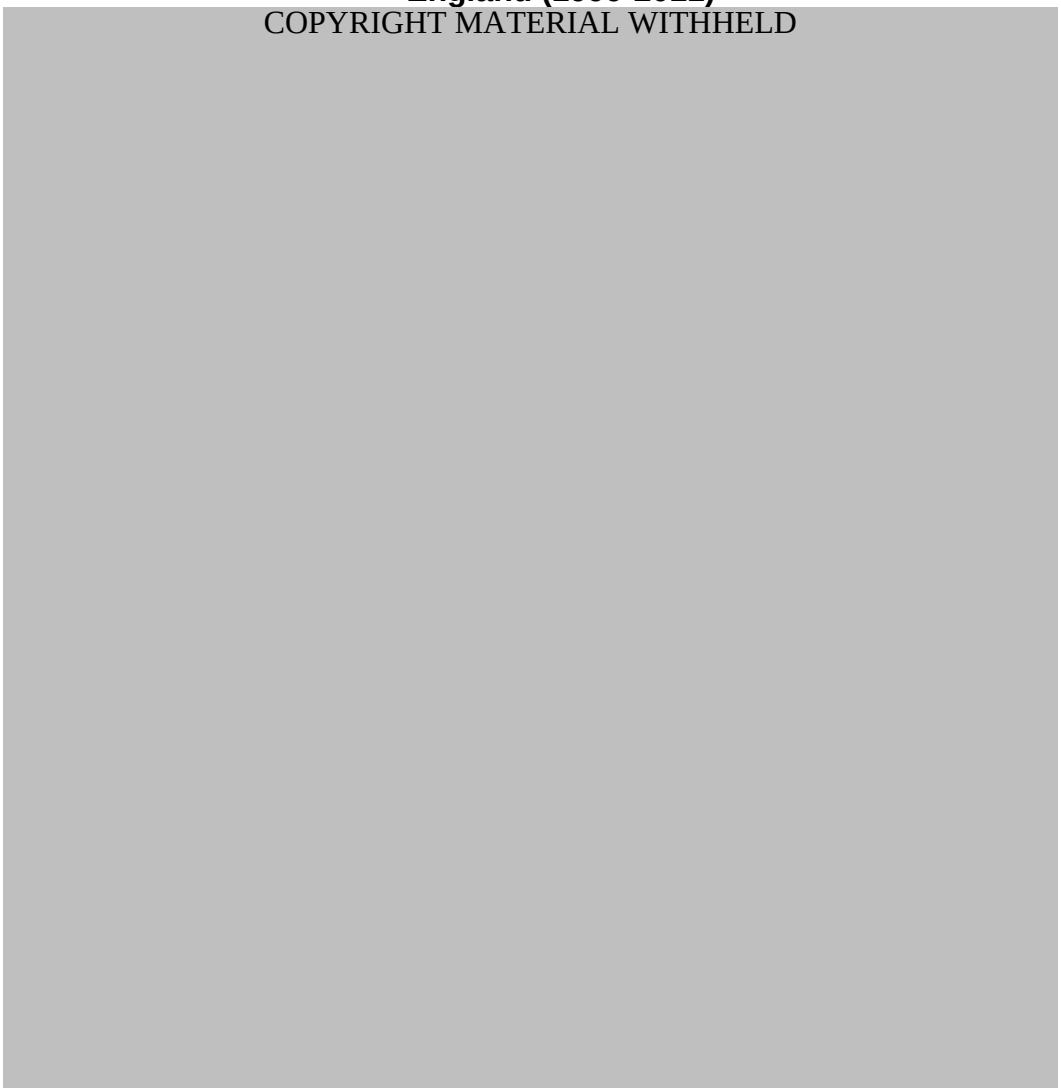


Derived from: Lazar-Molnar, et al., 2010.

These data are of special interest in light of the observed association between TB infection and autoimmune disease in humans. For over 30 years, numerous studies have speculated about an association between autoimmune disease and the risk of contracting TB. A recent study by Ramagopalan et al. examined death certificates and

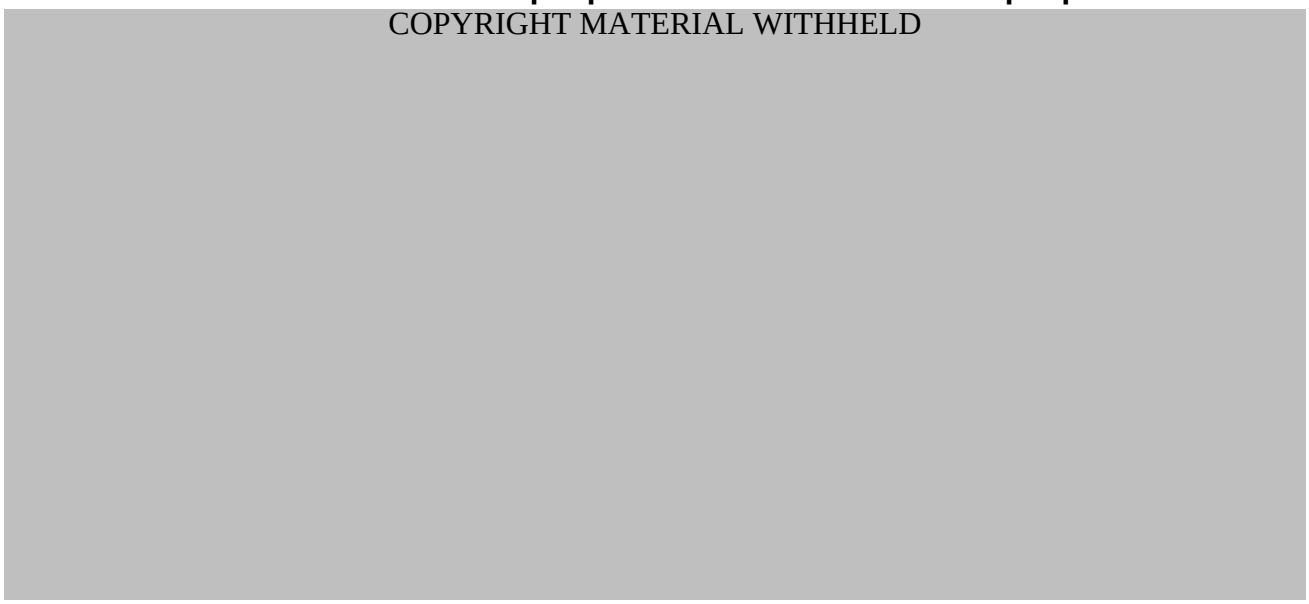
hospital admissions records for patients who died and/or were hospitalized in England between the years 1999-2011. The investigators compared the rate ratios for TB in immune-mediated disease (IMD) cohorts with that of other cohorts and found a strong association between certain IMD and the risk of TB (both pulmonary and extra-pulmonary). The relative risk of TB (Table 24) was highest in patients with Addison's disease, Goodpasture's syndrome, SLE, polymyositis, polyarteriosis nodosa, dermatomyositis, scleroderma, and autoimmune hemolytic anemia. Importantly, the risk was found to be bidirectional (i.e., an increased risk of TB was observed in patients with IMD and risk of IMD was observed in patients with TB (Table 25).

Table 24: Relative risk of TB in patients with immune-mediated diseases in England (1999-2011)



c.f. Ramagopalan et al., BMC Medicine 2013

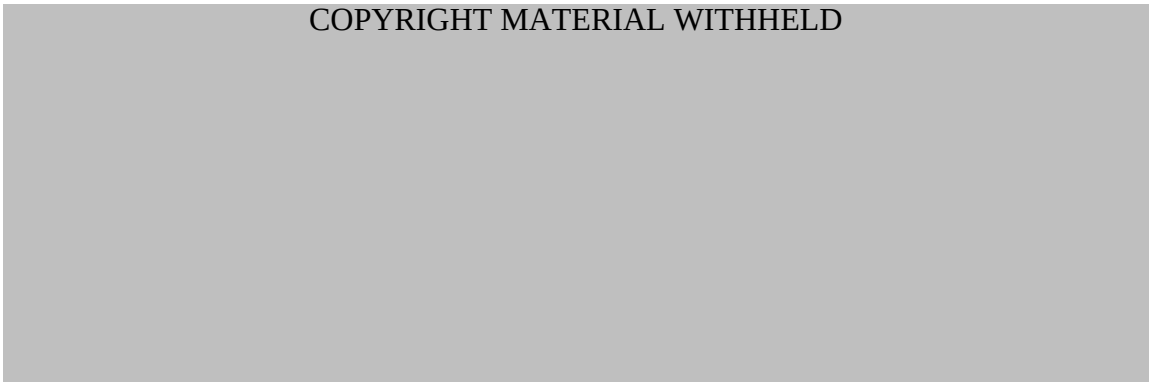
Table 25: Rate ratios for TB in people with IMD and for IMD in people with TB



c.f. Ramagopalan et al., BMC Medicine 2013

While this analysis was unable to exclude the co-varying risk of TB associated with immunosuppressive drug use in these patient populations, other studies that evaluated the risk of TB in patients receiving anti-TNF therapies found that the risk of TB was increased 4-fold in patients with RA irrespective of anti-TNF-therapy (Askling, et al., 2005; Table 26), suggesting an elevated baseline risk of TB in these patients which may be explained in part by the known association between PD-1 polymorphisms and IMD.

Table 26: Relative risk of TB in Swedish RA patients treated with or without TNF-inhibitors



c.f. Askling, et al., Arthritis and Rheumatism, 2005

Taken together - the known association between PD-1-deficiency and IMD, the exquisite sensitivity of PD-1-deficient mice to *M. tuberculosis,* and the demonstrated association between pembrolizumab administration and autoimmune disease – the data suggest that there is concern that treatment with pembrolizumab may increase susceptibility to TB infection and/or that infected patients may develop more severe

disease. The potential for increased toxicity in the presence of pembrolizumab 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 resulting in cardiovascular collapse (Frebel et. al., 2012; Mueller et. al., 2010). Consistent with this observation, are data supplied by the Applicant which point to a potential risk of liver injury when pembrolizumab is administered in the context of viral hepatitis. Administration of pembrolizumab to HBV-infected chimpanzees led to marked increases in LFTs (ALT, AST and/or GGT) in 2 of the 4 animals treated; however, whether this represents an enhanced immune-mediated destruction of infected cells is unclear, as microscopic analysis of biopsy samples collected in this study was not performed. Together these data suggest that administration of pembrolizumab to patients with acute or chronic viral infections may result in stronger immune reactions and increased toxicity compared to uninfected patients.

12. References

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/s/

SHAWNA L WEIS
07/30/2014

WHITNEY S HELMS
07/30/2014

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

BLA Number: BLA 125,514 **Applicant: Merck Sharpe and Dohme** **Stamp Date: 27 February 2014**

Drug Name: pembrolizumab **BLA Type: Breakthrough**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		No issues were identified to date. This will be further assessed during the review.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		Individual reports state compliance with US GLP regulations and deviations from GLP regulations, when they occurred, were noted and impact statements were provided.
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		The applicant provided data as requested during the initial review of the BLA. The data are adequate to support review of the application.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		To date, no impurities have been identified that require pharm/tox input.
11	Has the applicant addressed any abuse potential issues in the submission?			NA
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable.

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? __Yes_____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

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/s/

SHAWNA L WEIS
04/07/2014

WHITNEY S HELMS
04/10/2014