Name of Sponsor/Company: Astellas Pharma Global Development, Inc.		
Name of Finished Product: Not Applicable		
Name of Active Ingredient: ASP0113		

SYNOPSIS

Title of Study:

A Phase 1, Single-Blind, Parallel-Group, Pharmacokinetic and Immunogenicity Study with ASP0113 in CMV-Seropositive and CMV-Seronegative Healthy Subjects and CMV-Seronegative Dialysis Patients

Investigators/Coordinating Investigator:

[redacted]

Study Centers:

[redacted] US)

[redacted] US)

[redacted] US)

[redacted] US)

Publication Based on the Study:

Not applicable

Study Period:

Part 1: 4Q2013 to 1Q2014

Part 2: 2Q2014 to 2Q2016

Study Initiation Date (Date of First Evaluation):

Part 1: 30 Dec 2013

Part 2: 01 May 2014

Study Completion Date (Date of Last Evaluation):

Part 1: 04 Mar 2014

Part 2: 10 May 2016

Phase of Development:

Phase 1

Objectives:

Primary Objectives:

- To determine whether ASP0113 plasmids could be detected in plasma after intramuscular injections as measured by polymerase chain reaction (PCR) and, if so, to characterize their pharmacokinetics.
- To determine whether cytomegalovirus (CMV)-seropositive and CMV-seronegative healthy subjects mounted an immune response following repeated ASP0113 intramuscular injections on a 0-, 5-, 9-, and 25-week vaccination schedule as measured by phosphoprotein 65 (pp 65)-specific T-cell assay and glycoprotein B (gB) antibody assays 2 weeks following the third injection.
- To determine whether seronegative dialysis patients mounted an immune response following repeated ASP0113 intramuscular injections compared to approximately age-matched seronegative healthy subjects as measured by pp 65-specific T-cell assays and gB antibody assays 2 weeks following the third injection.

Secondary Objectives:

- To determine whether seronegative dialysis patients mounted a recall response of similar or greater magnitude at 6 months compared to their 3-month response.
- To evaluate the safety and tolerability of ASP0113 in healthy subjects and dialysis patients.

Exploratory Objectives:

- To determine if pp 65 could be detected in white blood cells (WBCs) after injection.
- To determine whether a pharmacokinetic-pharmacodynamic relationship between ASP0113 plasmid concentrations and immune response could be established.
- To determine whether positive results from the QuantiFERON T-cell CMV assay correlated with the detection of interferon-gamma (IFN-γ)-expressing pp 65-specific T-cells as measured by flow cytometry with intracellular cytokine staining (ICS).
- To determine whether any individual-specific covariates were predictive of whether a subject was a responder or nonresponder.

Methodology:

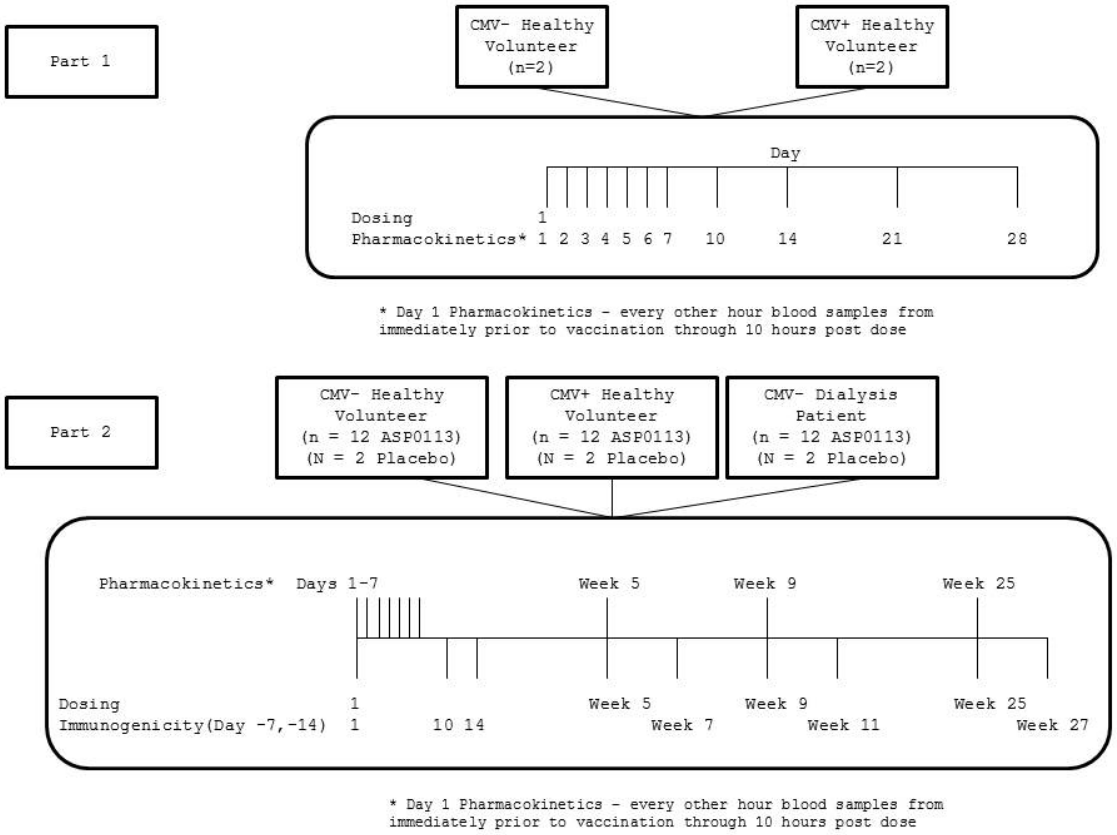
This study consisted of 2 parts.

Part 1 was a single center pilot study in 2 CMV-seronegative and 2 CMV-seropositive healthy subjects to obtain pilot pharmacokinetic data of ASP0113 plasmids following 5-mg single-dose intramuscular injection to optimize pharmacokinetic sample collection times in part 2.

Part 2 was a multi-center, single-blind, and parallel-group study. A total of 42 subjects were planned to be randomized (14 CMV-seronegative healthy subjects, 14 CMV-seropositive healthy subjects and 14 CMV-seronegative dialysis patients).

The main components of the study are presented below [Figure 1]:

Figure 1 Flow Chart of Planned Study Conduct



CMV: cytomegalovirus

Safety was assessed throughout the study. Local and systemic reactogenicity was assessed through 1 hour after injection for 7 days or until resolution after each injection.

An informal interim analysis was conducted between parts 1 and 2 of the study. An informal interim analysis of the primary endpoints was done after the week 11 visit, but did not include a statistical comparison.

Number of Subjects (Planned, Enrolled and Analyzed):

Part 1 was a pilot study and no formal sample size calculations had been performed. The planned sample total of 42 subjects (14 per arm [12 on active treatment and 2 on placebo]) in part 2 was based on precedent set by other pharmacokinetic/pharmacodynamic studies and not based on statistical considerations of power).

For part 1, a total of 36 subjects provided written informed consent and 32 subjects were screen failures. Subsequently, 4 subjects were enrolled into this part of the study. For part 2, a total of 203 subjects provided written informed consent and 159 subjects were screen failures. Subsequently, 44 subjects were enrolled into this part of the study.

All subjects in parts 1 and 2 of the study were included in the safety analysis set (SAF) and pharmacodynamic analysis set.

Diagnosis and Main Criteria for Inclusion:

CMV-seronegative and CMV-seropositive healthy subjects (18 to 65 years of age, inclusive [at screening], with a body mass index (BMI) within the range of 18.5 to 35.0 kg/m^2, inclusive) and CMV-seronegative dialysis patients (18 to 70 years of age, inclusive [at screening], with a BMI within the range of 18.5 to 40.0 kg/m^2, inclusive) who provided written informed consent and to whom all of the inclusion and none of the exclusion criteria applied, were eligible for inclusion in this study. Use of any prescribed or nonprescribed drugs, alternative and complementary medications, except for vitamins, contraceptives, hormone replacement therapy and occasional acetaminophen (to a maximum of 2 g/day), within 14 days prior to first injection with ASP0113 was prohibited. Vaccination with killed vaccines (including e.g., influenza and pneumococcal), or allergy treatment with antigen injections within 15 days prior to day -1 and vaccination with live attenuated vaccines within 30 days prior to day -1 were also prohibited.

Test Product, Dose and Mode of Administration, Batch Numbers:

ASP0113

ASP0113 was supplied by the sponsor as a frozen solution in single-dose 2-mL vials containing 1.3 mL of 5 mg/mL ASP0113. ASP0113 was a milky white suspension at room temperature, and clear at temperatures below the cloud point of CRL1005 (4° to 7°C).

Lot number: [redacted]

Initial retest date: Aug 2014

Updated retest date: Feb 2015

Duration of Treatment (or Duration of Study, if applicable):

Subjects in part 1 were immunized on day 1 and plasma samples for plasmid detection by vector-specific PCR assay were obtained until 10 hours after injection on day 1 and daily for days 2 to 7, and on days 10, 14, 21 and 28 post injection. Samples for pp 65 protein detection in WBCs by pp 65 antigenemia assay were obtained at day 1 prior to injection, and at days 3, 7, 10, 14, 21 and 28 or early termination.

Subjects randomized to the active treatment arms in part 2 were to receive 4 intramuscular injections of ASP0113 on day 1 (randomization) and at weeks 5, 9 and 25 while subjects randomized to the placebo arms were to receive placebo intramuscular injections on the same days as in the active treatment arm.

Reference Product, Dose and Mode of Administration, Batch Numbers:

Placebo

Placebo and single blinding were utilized to minimize bias and provide reference data (i.e., data from placebo treated subjects), which aided in the interpretation of results. Placebo was supplied by the sponsor in 2-mL vials containing phosphate buffered saline. It was a clear colorless liquid.

Batch number: [redacted]

Retest date: 28 Feb 2015

Criteria for Evaluation:

Safety

The following safety parameters were assessed:

- Adverse events
- Vital signs (oral temperature, pulse and sitting blood pressure)
- Clinical laboratory assessments (hematology, serum chemistry and urinalysis)
- Local and systemic reactogenicity

Pharmacokinetics

The following endpoints were calculated:

Part 1:

- ASP0113 plasmids pharmacokinetics in plasma, including AUC_{last}, AUC_{inf}, C_{last}, C_{max}, t_{max}, CL/F, V_z/F and $t_{½}$.
- pp 65 protein in WBCs; percent positive at every time point; percent positive at any time point postbaseline.

Part 2:

- ASP0113 plasmids pharmacokinetics in plasma, including AUC_{inf}, C_{max}, t_{max}, CL/F, V_z/F and $t_{½}$.
- pp 65 protein in WBCs; percent positive at every time point; percent positive at any time point.

Immunogenicity Assessment

The following endpoints were calculated (assessments were performed using the interim data at the completion of 2 months plus 2 weeks and also at the end of study completion):

- Frequency of CMV-seronegative healthy subjects who seroconvert as evidenced by acquisition of anti-gB antibodies at any time point after injection; magnitude and duration of anti-gB response in responders.
- Frequency of CMV-seronegative dialysis patients who seroconvert as evidenced by acquisition of anti-gB antibodies at any time point after injection; magnitude and duration of anti-gB response in responders.
- Frequency of CMV-seropositive healthy subjects with an increase in anti-gB antibody levels at any time point after injection; magnitude and duration of anti-gB response in responders.
- Frequency of CMV-seronegative healthy subjects with pp 65 T-cell response at any time point; magnitude and duration of pp 65 T-cell response in responders.
- Frequency of CMV-seronegative dialysis patients with pp 65 T-cell response at any time point after injection; magnitude and duration of pp 65 T-cell response in responders.
- Frequency of CMV-seropositive healthy subjects with a change in the pp 65 T-cell response at any time point after injection; magnitude and duration of pp 65 response in responders.
- Correlation between the number of IFN-γ-producing T-cells by flow cytometry with ICS and the results of the QuantiFERON T-cell CMV assay.

Statistical Methods:

Pharmacokinetics

Concentrations and pharmacokinetic parameters were summarized using descriptive statistics. SD, t_{max}, and coefficient of variation (CV), were not described if they were regarded as discrete. Concentrations below the limit of quantification (BLQ) were treated as zero in the estimation of mean concentrations. In case at least half of individual data at a given time point were BLQ, SD and CV were not calculated (and error bars were not presented on graphs). If 1 or more value(s) were BLQ, the geometric mean was not calculated.

Individual subject plasma concentrations for ASP0113 plasmids were listed and summarized for each time point by cohort. Standard graphics, including mean plasma drug concentration time profiles, overlay "spaghetti plots" and individual subject drug concentration time profiles, were produced for ASP0113 plasmids.

Individual subject's result of pp 65 protein detection in WBCs are listed, the percent of subjects with positive pp 65 at any time point was assessed and the exact 95% confidence interval (CI) was calculated for each cohort.

Part 1

ASP0113 plasmids pharmacokinetics was characterized using noncompartmental methods. Parameters included AUC_{last}, AUC_{inf}, C_{last}, C_{max}, t_{max}. CL/F, V_z/F and $t_{½}$ were calculated.

Part 2

ASP0113 plasmids pharmacokinetics was characterized in an exploratory manner using a compartmental modeling approach within a nonlinear mixed effects model framework, i.e., population pharmacokinetics. From the empirical Bayes estimates of each individual's pharmacokinetic parameters, secondary parameters including AUC_{inf}, C_{max}, t_{max}, CL/F, V_z/F and $t_{½}$ and accumulation were calculated.

Standard plots of mean and individual subject ASP0113 plasmids by time are also provided.

No additional analyses were done.

The plasma pharmacokinetic parameters were calculated using Phoenix®, Certara, St. Louis, US. Noncompartmental methods were used to derive the values of the pharmacokinetic parameters.

Pharmacodynamics

Definition of Baseline:

Each individual's baseline T- and B-cell response was calculated using responses from days -14, -7 and immediately prior to the first injection. The following assessments were done, if applicable.

T-cell Responses in Isolated Peripheral Blood Mononuclear Cells:

The percent of subjects and the exact 95% CI for the $CD4^+$ T-cell responders, as assayed by flow cytometry with ICS at each assessment time are provided by cohort for the active treatments and were enumerated by cohort for the placebo groups.

Similar results are provided for the $CD8^+$ and $CD3^+$ T-cell responders. For the purpose of assessment, the minimal definition for a responder was a seronegative individual whose quantifiable T-cell response was at least twice above the background of unstimulated cells and > 0.01% of the total $CD4^+$ or $CD8^+/CD3^+$ T-cells. More stringent requirements for a responder may have been used if the current criteria were too liberal.

In order to assess the peak magnitude of the vaccine-induced T-cell response, the mean and 95% CI of the largest change from baseline at any time point from both responders and across all subjects on the active treatment were calculated in each cohort. The largest change from baseline at any time point for the 2 subjects on placebo in each cohort was listed. The corresponding median time of peak response was also calculated for the active treatment group.

In order to assess the 3-month vaccine-induced T-cell response, the change from baseline 2 weeks after the third injection in both responders and across all subjects was calculated in each cohort for both the active treatment group and the placebo group. This was 1 of the primary endpoints.

In order to assess the durability of the vaccine-induced T-cell response, the change from baseline response immediately prior to last injection in both responders and across all subjects was calculated in each cohort for both the active treatment group and placebo.

In order to assess the generation of the vaccine-induced T-cell response, the change from baseline response 2 weeks after the last injection in both responders and across all subjects was calculated in each cohort for both the active treatment group and the placebo group.

QuantiFERON T-cell Cytomegalovirus Assay:

The concordance between IFN-γ-expressing T-cells from the release assay after stimulation T-cell CMV assay and from flow cytometry with ICS were calculated. Subjects were classified as positive, negative, or indeterminate per QuantiFERON manufacturer guidelines.

The results of the QuantiFERON T-cell CMV assay were compared to those obtained using flow cytometry with ICS. The sponsor had the additional option to substitute other assays to be used.

B-cell Responders:

The percent of subjects and the exact 95% CI for the anti-gB antibody responders at each assessment time are provided by cohort for the active treatments and were enumerated by cohort for the placebo groups. A B-cell responder was defined as an individual with a quantifiable increase in anti-gB antibody levels above baseline of at least 3-times the measurement error of the assay.

In order to assess the peak magnitude of the vaccine-induced anti-gB antibody response, the mean and 95% CI of the largest change from baseline at any time point from both responders and across all subjects on the active treatment were calculated in each cohort.

The largest change from baseline at any time point for the 2 subjects on placebo in each cohort is listed. The corresponding median time of peak response was also calculated for the active treatment group.

In order to assess the 3-month vaccine-induced anti-gB antibody response, the change from baseline response 2 weeks after the third injection in both responders and across all subjects was calculated in each cohort for both the active treatment group and the placebo group. This was 1 of the primary endpoints.

In order to assess the durability of the vaccine-induced anti-gB response, the change from baseline response in the last injection in both responders and across all subjects was calculated in each cohort for both the active treatment group and the placebo group.

In order to assess the generation of the vaccine-induced anti-gB response, the change from baseline response 2 weeks after the last injection in both responders and across all subjects was calculated in each cohort for both the active treatment group and the placebo group.

Phosphoprotein 65 in White Blood Cells:

In order to assess whether pp 65 produced from plasmids was detected in WBCs, the percent of subjects with positive pp 65 at any time point was assessed and the exact 95% CI was calculated for each cohort.

Responders Versus Nonresponders:

Exploratory analyses may have been conducted to determine whether any individual-specific covariates were predictive of response.

Pharmacokinetic-Pharmacodynamic Correlations:

Pharmacokinetic-pharmacodynamic modeling was used in an exploratory manner to correlate ASP0113 plasmids pharmacokinetics to immune response.

Safety

A detailed description of the statistical methods used to summarize the safety parameters evaluated in this study can be found in the statistical analysis plan.

All safety and tolerability data for the SAF were summarized by treatment group, unless specified otherwise. The local and systemic reactogenicity assessment results are displayed in the listings.

Summary of Results/Conclusions:

Subject disposition and analysis sets for parts 1 and 2 of the study can be found in Table 1 and Table 2, respectively.

Demographics and baseline characteristics of CMV-seronegative and CMV-seropositive healthy subjects and CMV-seronegative dialysis patients can be found in Table 3 and Table 4.

Pharmacokinetic Results:

- Part 1 of the study was used as a pilot to determine the appropriate sampling times to assess ASP0113 plasmid pharmacokinetics. The results showed that plasmid profiles were essentially cleared within a week of dosing. Hence, sampling in part 2 of the study was limited to 144 hours after the first dose.
- For part 2, ASP0113 plasmids were detected in plasma after intramuscular injection as measured by PCR. The profiles were consistent in all 3 treatment groups with no evidence of distinct pharmacokinetics in patients on dialysis.
- Upon administration 2 peaks were observed in most patients, a rapid peak occurring within 10 hours of dosing and another later peak occurring 24 to 48 hours after dosing. These double peaks are consistent with a combination of venous and lymphatic absorption seen with intramuscular and subcutaneous administration of large biologics like monoclonal antibodies. The early peak was likely due to venous absorption from the site of administration into plasma caused by local tissue damage during injection. The later peak was likely due to lymphatic absorption from the site of injection which is known to be considerably slower than venous absorption.

- Plasmid concentrations rapidly declined with a half-life of ~ 6 to 7 hours. Within 144 hours, plasmid copies reached the lower limit of quantification (LLOQ, 100 copies/mL). The short plasmid half-life was not surprising given the ubiquitous nature of nucleases meant to metabolize DNA that escapes from cells. There was little evidence of accumulation with multiple dosing as predose concentrations were below the LLOQ on weeks 5, 9 and 25 for most subjects. Only 2 subjects showed predose quantifiable concentrations with repeated dosing.

Pharmacodynamic Results:

- pp 65 antigen in WBCs was not detected in any samples from any subjects at any time points.
- Seronegative healthy subjects and dialysis patients had no detectable anti-gB antibody in their samples at any time point and showed no response to ASP0113. All seropositive healthy subjects had detectable anti-gB antibody at baseline and throughout the study but showed no statistical increase in gB enzyme-linked immunosorbent assay output. Although there was evidence that there may be a weak positive response in seropositive healthy subjects at weeks 25 and 27, there were too few subjects to state this definitely.
- Analyses with flow cytometry using ICS showed this assay to be too insensitive to detect changes in T-cell activity with only a brief peptide stimulation. No samples showed any measurable T-cell response in any treatment group.
- For the measurement of pp 65 IFN-γ in peripheral blood mononuclear cells by enzyme-linked immunospot assay (ELISPOT), a total of 4 of 11 seronegative healthy subjects treated with ASP0113 showed a positive change from baseline and were classified as responders. One subject showed a sustained positive response starting from week 9, while the others showed a positive response at only a single time point. No subjects in the seronegative dialysis group showed a positive response nor did any placebo treated subjects. Seropositive healthy subjects were not analyzed using this assay.
- Cultured ELISPOT proved to be the most sensitive method at detecting central memory T-cell responses. A total of 8 of 12 seronegative healthy subjects treated with ASP0113 showed a positive change from baseline and were classified as responders. A total of 3, 6, and 8 of 12 seronegative healthy subjects treated with ASP0113 had a positive response at weeks 9, 11 and 27, respectively. Two seronegative healthy subjects showed a sustained positive response starting from week 9. The other subjects showed a positive response at only 1 or 2 time points. Interestingly, 1 seronegative healthy subject who was administered placebo showed a very weak response at week 27 that was nonetheless classified as a positive responder. Seropositive healthy subjects were not analyzed using this assay.
- A total of 4 of 12 seronegative dialysis patients treated with ASP0113 showed a positive change from baseline and were classified as responders. A total of 1, 1 and 4 of 12 seronegative dialysis patients treated with ASP0113 had a positive response at weeks 9, 11 and 27, respectively. One patient had a sustained response starting from week 9. All other patients had a positive response at week 27 only. None of the placebo treated seronegative dialysis patients showed a positive response. In comparing the magnitude of response in seronegative healthy subjects to seronegative dialysis patients it was readily apparent that the response to ASP0113 treatment was much greater in healthy subjects than dialysis patients.
- A total of 3 of 12 seronegative healthy subjects treated with ASP0113 showed a positive change from baseline and were classified as responders. A total of 1, 1, 2 and 3 of 12 seronegative healthy subjects treated with ASP0113 had a positive response at weeks 9, 11, 25 and 27, respectively. Of these, only 1 subject had a sustained response from week 9; all others had a positive response at only 1 or 2 time

points. No placebo treated seronegative healthy subjects showed a positive response. No seropositive healthy subjects demonstrated a positive response at any time point.

- For measurement of CMV peptide stimulated T-cells in whole blood using the QuantiFERON assay, a total of 3 of 12 seronegative dialysis patients treated with ASP0113 showed a positive change from baseline and were classified as responders. A total of 1, 2 and 3 of 12 seronegative dialysis patients treated with ASP0113 had a positive response at weeks 11, 25 and 27, respectively. No seronegative dialysis patients had a sustained response; all responses were for only 1 or 2 time points. Compared to the magnitude of response in seropositive healthy subjects, the response to ASP0113 in seronegative subjects was significantly less.
- Two subjects stood out as having a consistent response across assays. The 1 subject was a seronegative healthy subject that showed a sustained response with the ELISPOT, cultured ELISPOT, and QuantiFERON assays. The other subject was a seronegative dialysis patient that had a sustained response in the cultured ELISPOT, and a positive response at weeks 11 and 25 with the QuantiFERON assay. Of those seronegative healthy subjects that showed a positive response on the QuantiFERON assay at 1 time point, they also showed a positive response on the ELISPOT assay. The same was true of dialysis patients.

Safety Results:

- TEAEs were reported for 3 (75.0%) subjects in part 1. TEAEs reported were fatigue, injection site pain and myalgia Table 6. These TEAEs were considered by the investigator to be probably or possibly related to the study drug.
- For part 2, the frequency of TEAEs reported for subjects who received ASP0113 was higher in all treatment groups than for subjects who received placebo Table 7.
- For part 2, the most commonly reported TEAE in all treatment groups was injection site pain. The TEAEs were considered by the investigator to be possibly or probably related to the study drug Table 8.
- There were no deaths, SAEs or TEAEs leading to permanent discontinuation of the study or of the study drug reported in part 1 and 1 TEAE leading to permanent discontinuation of treatment and the study reported for a subject who received placebo in part 2 Table 5 and Table 7.
- In part 1, no clinically significant changes were observed in any of the clinical laboratory values or vital signs.
- In part 2, 2 subjects presented clinically relevant changes in the clinical laboratory analyses. These TEAEs were considered by the investigator to be not serious and not related to the study drug. However, 1 of the subjects discontinued treatment and was withdrawn from the study as decided by the physician. No clinically significant changes were observed in any of the vital signs values.
- Almost all of the local and systemic reactogenicity events were mild in severity and were mainly reported for subjects who received active treatment.

CONCLUSIONS:

The plasmid that was measured was most likely degraded plasmid in that it was inactive but of sufficient size to react to the primers used in the PCR assay. First, the PCR assay did not measure intact plasmid but measured an amplicon from the kanamycin resistance gene part of the plasmid. Challenges exists if the pharmacokinetic parameters for the plasmid are interpreted similarly to a small molecule, potentially leading to bias, particularly as it applies to noncompartmental estimates of CL/F and V_z/F. Noncompartmental analyses assume that drug is cleared solely from the central compartment. For a plasmid, this was not likely due to metabolism occurring

from the peripheral compartment by nucleases. Hence, the noncompartmental estimates of clearance and volume of distribution calculated in this study and reported in the appendices may not reflect real values.

In the phase 3 study, patients are assessed for CMV viremia using local laboratories that may be using assays which could potentially cross-react with plasma ASP0113 plasmid fragments. The current study demonstrates that it takes 1 week for ASP0113 to be sufficiently cleared from the body to be nondetectable by PCR. Hence, a positive viremia test using 1 of these assays within 1 week of ASP0113 dosing could be due to remaining ASP0113 after injection, in which case physicians should re-assess for viremia after the 1 week window period.

The data indicate that seronegative subjects mounted an immune response to ASP0113 treatment which was observed only after repeated administration and not in all subjects. The data also suggest that seropositive healthy subjects did not mount an immune response to ASP0113 but, given their positive baseline response, changes in immune response would be difficult to detect. The immune response in seronegative subjects appeared to be limited to T-cell activity as no B-cell response was observed in any subject in any treatment group. Further, the magnitude of central memory T-cell response was greater in CMV-seronegative healthy subjects compared to CMV-seronegative dialysis patients, which is consistent with the reduced immune function noted in the latter group.

A positive T-cell response was seen in the majority of seronegative healthy subjects in at least 1 time point, whereas only a minority of seronegative dialysis patients appeared to mount a T-cell response, suggesting that ASP0113 preferentially generated antigen-specific central memory T-cells rather than antigen-specific effector memory T-cells. The responses in the majority of seronegative subjects were transient. Only 1 seronegative healthy subject and 1 seronegative dialysis patient mounted a sustained response from week 9 to the end of study. Further, the responses seen in seronegative healthy subjects were greater than seronegative dialysis patients. Whether this was due to impaired T-cell activity in dialysis patients, presence of concomitant medications in dialysis patients, like prednisone, that suppress immune function, or whether dialysis patients do not respond to mitogen stimulation in the ELISPOT assay the same way as healthy subjects is unclear.

Some of the assays suggest that the peak immune response occurred at week 27 since week 27 responses appear higher than week 11 responses. But given the sample size in the study this conclusion is speculative.

There did not appear to be a correlation between ASP0113 plasmid pharmacokinetics and immune response because the pharmacokinetic variability in the study was relatively small and homogenous and not all seronegative subjects responded to ASP0113 treatment. No specific covariates appeared to be predictive of whether a seronegative subject would or would not respond to ASP0113. Prior CMV seropositivity does suggest that an immune response will not be enhanced after ASP01113 treatment, but the presence of a positive baseline makes this difficult to confirm.

There did appear to be a concordance between results from the QuantiFERON and cultured ELISPOT assays even though the assay platforms were quite different. Those subjects that showed a response in the QuantiFERON assay also showed a response in the cultured ELISPOT assay, but not universally in the opposite direction. Further, the seronegative healthy subject showing a sustained QuantiFERON response from week 9 also showed a sustained response with cultured ELISPOT from week 9. But the other seronegative healthy subject showing a sustained response with cultured ELISPOT did not even show up as a responder with the QuantiFERON assay. These results suggest that QuantiFERON is not as sensitive as cultured ELISPOT.

Multiple doses of ASP0113 were safe and well-tolerated in healthy subjects and in dialysis patients. Subjects receiving ASP0113 experienced injection site pain and tenderness.

Date of Report:

25 Oct 2016

Table 1 Subject Disposition and Analysis Sets – Part 1 (All Enrolled Subjects)

Analysis Set	CMV^- Healthy Subjects (n = 2) n (%)	CMV^+ Healthy Subjects (n = 2) n (%)	Total (n = 4) n (%)
Enrolled	2 (100)	2 (100)	4 (100)
Safety Analysis Set†	2 (100)	2 (100)	4 (100)
Pharmacodynamic Analysis Set‡	2 (100)	2 (100)	4 (100)
Treatment/Study Discontinuation	0	0	0

CMV^-: cytomegalovirus-seronegative; CMV^+: cytomegalovirus-seropositive.

† All enrolled subjects who took at least 1 dose of study drug.

‡ Subjects from the safety analysis set for whom sufficient pharmacodynamic measurements were collected.

Source: End-of-Text Tables 12.1.1.2.1 and 12.1.1.3.1

Table 2 Subject Disposition and Analysis Sets – Part 2 (All Randomized Subjects)

Analysis Set	CMV$^-$ Healthy Subjects		CMV$^+$ Healthy Subjects		CMV$^-$ Dialysis Patients		Total (n = 44) n (%)
	Placebo (n = 3) n (%)	ASP0113 (n = 12) n (%)	Placebo (n = 2) n (%)	ASP0113 (n = 13) n (%)	Placebo (n = 2) n (%)	ASP0113 (n = 12) n (%)	
Randomized	3 (100)	12 (100)	2 (100)	13 (100)	2 (100)	12 (100)	44 (100)
Safety Analysis Set†	3 (100)	12 (100)	2 (100)	13 (100)	2 (100)	12 (100)	44 (100)
Pharmacodynamic Analysis Set‡	3 (100)	12 (100)	2 (100)	13 (100)	2 (100)	12 (100)	44 (100)
Treatment Discontinuation	1 (33.3)	0	0	1 (7.7)	0	0	2 (4.5)
Study Discontinuation	1 (33.3)	0	0	1 (7.7)	0	0	2 (4.5)

CMV$^-$: cytomegalovirus-seronegative; CMV$^+$: cytomegalovirus-seropositive.

† All enrolled subjects who took at least 1 dose of study drug.

‡ Subjects from the safety analysis set for whom sufficient pharmacodynamic measurements were collected.

Source: End-of-Text Tables 12.1.1.2.2, 12.1.1.3.2 and 12.1.1.3.3

Table 3 Summary of Demographics and Baseline Characteristics – Part 1 (Safety Analysis Set)

Parameter Category/ Statistics	CMV^- Healthy Subjects (n = 2)	CMV^+ Healthy Subjects (n = 2)	Total (n = 4)
Sex, n (%)			
Male	2 (100)	0	2 (50.0)
Female	0	2 (100)	2 (50.0)
Ethnicity, n (%)			
Hispanic or Latino	0	0	0
Not Hispanic or Latino	2 (100)	2 (100)	4 (100)
Race, n (%)			
White	0	0	0
Black or African American	2 (100)	2 (100)	4 (100)
Asian	0	0	0
American Indian/Alaska Native	0	0	0
Native Hawaiian/Pacific Islander	0	0	0
Other	0	0	0
Age, years			
Mean (SD)	37.0 (14.14)	36.5 (19.09)	36.8 (13.72)
Median	37.0	36.5	37.0
Min – Max	27 – 47	23 – 50	23 – 50
Weight (kg)			
Mean (SD)	94.15 (7.283)	70.40 (0.141)	82.28 (14.343)
Median	94.15	70.40	79.75
Min – Max	89.0 – 99.3	70.3 – 70.5	70.3 – 99.3
Height (cm)			
Mean (SD)	179.5 (3.54)	170.0 (5.66)	174.8 (6.70)
Median	179.5	170.0	175.5
Min – Max	177 – 182	166 – 174	166 – 182
BMI (kg/m^2)			
Mean (SD)	29.20 (1.131)	24.40 (1.697)	26.80 (3.011)
Median	29.20	24.40	27.00
Min – Max	28.4 – 30.0	23.2 – 25.6	23.2 – 30.0

All subjects who received at least 1 dose of study medication (safety analysis set).

BMI: body mass index (weight [kg]/height^2 [m^2]); CMV^-: cytomegalovirus-seronegative; CMV^+: cytomegalovirus-seropositive; Max: maximum; Min: minimum.

Source: End-of-Text Table 12.1.2.1.1

Table 4 Summary of Demographics and Baseline Characteristics – Part 2 (Safety Analysis Set)

Parameter Category/ Statistics	CMV^- Healthy Subjects		CMV^+ Healthy Subjects		CMV^- Dialysis Patients		Total (n = 44)
	Placebo (n = 3)	ASP0113 (n = 12)	Placebo (n = 2)	ASP0113 (n = 13)	Placebo (n = 2)	ASP0113 (n = 12)	
Sex, n (%)							
Male	1 (33.3)	9 (75.0)	2 (100)	8 (61.5)	2 (100)	10 (83.3)	32 (72.7)
Female	2 (66.7)	3 (25.0)	0	5 (38.5)	0	2 (16.7)	12 (27.3)
Ethnicity, n (%)							
Hispanic or Latino	1 (33.3)	0	0	3 (23.1)	0	1 (8.3)	5 (11.4)
Not Hispanic or Latino	2 (66.7)	12 (100)	2 (100)	10 (76.9)	2 (100)	11 (91.7)	39 (88.6)
Race, n (%)							
White	2 (66.7)	5 (41.7)	0	4 (30.8)	0	2 (16.7)	13 (29.5)
Black or African American	1 (33.3)	7 (58.3)	2 (100)	9 (69.2)	2 (100)	10 (83.3)	31 (70.5)
Asian	0	0	0	0	0	0	0
American Indian/Alaska Native	0	0	0	0	0	0	0
Native Hawaiian/Pacific Islander	0	0	0	0	0	0	0
Other	0	0	0	0	0	0	0
Age, years							
Mean (SD)	32.3 (0.58)	39.7 (14.42)	35.5 (12.02)	40.8 (13.89)	44.5 (3.54)	44.1 (9.34)	40.7 (11.97)
Median	32.0	45.0	35.5	40.0	44.5	46.5	42.0
Min – Max	32 – 33	22 – 57	27 – 44	22 – 63	42 – 47	28 – 55	22 – 63
Weight (kg)							
Mean (SD)	88.87 (17.328)	80.50 (14.285)	103.80 (25.314)	81.66 (17.277)	116.25 (41.931)	97.28 (22.785)	88.68 (20.935)
Median	80.40	79.45	103.80	81.00	116.25	98.85	85.90
Min – Max	77.4 – 108.8	55.7 – 106.0	85.9 – 121.7	51.3 – 112.8	86.6 – 145.9	59.1 – 144.5	51.3 – 145.9
Height (cm)							
Mean (SD)	174.3 (9.07)	175.3 (11.67)	182.0 (9.90)	171.1 (10.52)	192.1 (15.70)	174.9 (11.76)	175.0 (11.56)
Median	173.0	172.5	182.0	174.0	192.1	175.8	174.5
Min – Max	166 – 184	160 – 195	175 – 189	152 – 185	181 – 203	150 – 191	150 – 203
BMI (kg/m^2)							
Mean (SD)	29.03 (2.723)	26.11 (3.276)	31.05 (4.313)	27.64 (3.768)	30.85 (6.293)	31.48 (4.956)	28.67 (4.421)
Median	28.10	26.05	31.05	27.40	30.85	31.30	27.80
Min – Max	26.9 – 32.1	21.8 – 32.0	28.0 – 34.1	20.5 – 34.1	26.4 – 35.3	24.3 – 39.8	20.5 – 39.8

All subjects who received at least 1 dose of study medication (safety analysis set).

BMI: body mass index (weight [kg]/height^2 [m^2]); CMV^-: cytomegalovirus-seronegative; CMV^+: cytomegalovirus-seropositive; Max: maximum; Min: minimum.

Source: End-of-Text Table 12.1.2.1.2

Table 5 Overview of Treatment-emergent Adverse Events – Part 1 (Safety Analysis Set)

	CMV$^-$ Healthy Subjects (n = 2) n (%) E	CMV$^+$ Healthy Subjects (n = 2) n (%) E	Total (n = 4) n (%) E
Any TEAE	2 (100) 4	1 (50.0) 1	3 (75.0) 5
Drug-related † TEAEs	2 (100) 4	1 (50.0) 1	3 (75.0) 5
Deaths	0	0	0
Serious TEAEs ‡	0	0	0
Drug-related † Serious TEAEs ‡	0	0	0
TEAEs Leading to Discontinuation of Study Drug	0	0	0
Drug-related † TEAEs Leading to Discontinuation of Study Drug	0	0	0

All subjects who received at least 1 dose of study medication (safety analysis set).

A TEAE was defined as an adverse event that started or worsened in severity after dose of study drug through end of study.

CMV$^-$: cytomegalovirus-seronegative; CMV$^+$: cytomegalovirus-seropositive; E: number of events; TEAE: treatment-emergent adverse event.

† Possible or probable, as assessed by the investigator, or records where relationship was missing.

‡ Included serious adverse events upgraded by the sponsor based on review of the sponsor's list of Always Serious terms, if any upgrade was done.

Source: End-of-Text Table 12.6.1.1.1

Table 6 Treatment-emergent Adverse Events by Cohort – Part 1 (Safety Analysis Set)

MedDRA v16.0 System Organ Class Preferred Term	CMV$^-$ Healthy Subjects (n = 2) n (%)	CMV$^+$ Healthy Subjects (n = 2) n (%)	Total (n = 4) n (%)
Overall	**2 (100)**	**1 (50.0)**	**3 (75.0)**
General Disorders and Administration Site Conditions	**2 (100)**	**1 (50.0)**	**3 (75.0)**
Fatigue	2 (100)	0	2 (50.0)
Injection site pain	1 (50.0)	1 (50.0)	2 (50.0)
Musculoskeletal and Connective Tissue Disorders	**1 (50.0)**	**0**	**1 (25.0)**
Myalgia	1 (50.0)	0	1 (25.0)

All subjects who received at least 1 dose of study medication (safety analysis set).

A treatment-emergent adverse event was defined as an adverse event that started or worsened in severity after dose of study drug through end of study.

CMV$^-$: cytomegalovirus-seronegative; CMV$^+$: cytomegalovirus-seropositive.

Source: End-of-Text Table 12.6.1.2.1

Table 7 Overview of Treatment-emergent Adverse Events – Part 2 (Safety Analysis Set)

	CMV$^-$ Healthy Subjects		CMV$^+$ Healthy Subjects		CMV$^-$ Dialysis Patients	
	Placebo (n = 3) n (%) E	**ASP0113 (n = 12) n (%) E**	**Placebo (n = 2) n (%) E**	**ASP0113 (n = 13) n (%) E**	**Placebo (n = 2) n (%) E**	**ASP0113 (n = 12) n (%) E**
Any TEAE	1 (33.3) 1	10 (83.3) 29	0	9 (69.2) 22	1 (50.0) 8	10 (83.3) 22
Drug-related † TEAEs	0	9 (75.0) 24	0	9 (69.2) 21	0	9 (75.0) 20
Deaths	0	0	0	0	0	0
Serious TEAEs ‡	0	0	0	0	0	0
Drug-related † Serious TEAEs ‡	0	0	0	0	0	0
TEAEs Leading to Discontinuation of Study Drug	1 (33.3) 1	0				
Drug-related † TEAEs Leading to Discontinuation of Study Drug	0	0				

All subjects who received at least 1 dose of study medication (safety analysis set).

A TEAE was defined as an adverse event that started or worsened in severity after dose of study drug through end of study.

CMV$^-$: cytomegalovirus-seronegative; CMV$^+$: cytomegalovirus-seropositive; E: number of events; TEAE: treatment-emergent adverse event.

† Possible or probable, as assessed by the investigator, or records where relationship was missing.

‡ Included serious adverse events upgraded by the sponsor based on review of the sponsor's list of Always Serious terms, if any upgrade was done.

Source: End-of-Text Table 12.6.1.1.2

Table 8 Treatment-emergent Adverse Events by Cohort – Part 2 (Safety Analysis Set)

MedDRA v16.0 System Organ Class Preferred Term	CMV^- Healthy Subjects		CMV^+ Healthy Subjects		CMV^- Dialysis Patients	
	Placebo (n = 3) n (%)	ASP0113 (n = 12) n (%)	Placebo (n = 2) n (%)	ASP0113 (n = 13) n (%)	Placebo (n = 2) n (%)	ASP0113 (n = 12) n (%)
Overall	**1 (33.3)**	**10 (83.3)**	**0**	**9 (69.2)**	**1 (50.0)**	**10 (83.3)**
General Disorders and Administration Site Conditions	**0**	**9 (75.0)**	**0**	**9 (69.2)**	**1 (50.0)**	**8 (66.7)**
Fatigue	0	2 (16.7)	0	4 (30.8)	1 (50.0)	0
Injection site erythema	0	0	0	0	0	1 (8.3)
Injection site pain	0	9 (75.0)	0	8 (61.5)	0	7 (58.3)
Vessel puncture site pain	0	1 (8.3)	0	0	0	0
Vessel puncture site anaesthesia	0	1 (8.3)	0	0	0	0
Vessel puncture site haematoma	0	1 (8.3)	0	1 (7.7)	0	0
Hepatobiliary Disorders	**1 (33.3)**	**0**	**0**	**0**	**0**	**0**
Hyperbilirubinaemia	1 (33.3)	0	0	0	0	0
Infections and Infestations	**0**	**1 (8.3)**	**0**	**0**	**1 (50.0)**	**0**
Upper respiratory tract infection	0	1 (8.3)	0	0	0	0
Urinary tract infection	0	0	0	0	1 (50.0)	0
Injury, Poisoning and Procedural Complications	**0**	**0**	**0**	**0**	**0**	**1 (8.3)**
Ligament sprain	0	0	0	0	0	1 (8.3)
Investigations	**0**	**0**	**0**	**0**	**1 (50.0)**	**0**
Blood creatine phosphokinase increased	0	0	0	0	1 (50.0)	0
Musculoskeletal and Connective Tissue Disorders	**0**	**0**	**0**	**1 (7.7)**	**0**	**4 (33.3)**
Musculoskeletal chest pain	0	0	0	0	0	1 (8.3)
Myalgia	0	0	0	1 (7.7)	0	3 (25.0)
Nervous System Disorders	**0**	**1 (8.3)**	**0**	**0**	**0**	**0**
Presyncope	0	1 (8.3)	0	0	0	0
Psychiatric Disorders	**0**	**0**	**0**	**0**	**1 (50.0)**	**0**
Libido decreased	0	0	0	0	1 (50.0)	0
Reproductive System and Breast Disorders	**0**	**0**	**0**	**0**	**1 (50.0)**	**0**
Erectile dysfunction	0	0	0	0	1 (50.0)	0

All subjects who received at least 1 dose of study medication (safety analysis set).

A treatment-emergent adverse event was defined as an adverse event that started or worsened in severity after dose of study drug through end of study.

CMV^-: cytomegalovirus-seronegative; CMV^+: cytomegalovirus-seropositive.

Source: End-of-Text Table 12.6.1.2.2