Summary of secondary and other endpoints

Viral kinetics

The concentration of virus in blood samples was measured by qualitative polymerase chain reaction (qPCR) for up to 60 days following infusion of $1x10^{10} – 1x10^{13}$ viral particles (vp). Results show that maximum concentrations (C_{max}) and area under the curve (AUC) can be controlled by adjusting the dose and rate of infusion.

Viral clearance kinetics fits to a one-phase decay with a dose independent alpha half-life of approximately 17 minutes. After the initial clearance there is a long circulating fraction that remains present for approximately 22 days. The mean concentration of virus deoxyribonucleic acid (DNA) remaining in the blood stream is dose independent and may be associated with blood cells. It is unknown if this is viable virus particles or not (PCR method detects a small fragment of DNA).

The initial clearance half-life is not significantly different on Days 1, 3 or 5. However, there is a significant increase in C_{max} on Day 5 relative to Day 1. This is consistent with the initial dose suppressing the activity of macrophage lineage cells such as Kupffer cells in the liver.

Infectious virus can be detected in the blood for up to 24 hours following dosing. These data confirm that enadenotucirev is not immediately neutralised.

At $1 x 10^{10}$ and $1x 10^{11}$ vp there is some evidence of viral replication in the subject. At higher doses, evidence of virus replication may be 'masked' by the elevated long circulating fraction.

Analysis of the clearance kinetics of both dosing schedules in Phase Ib confirmed that there were no significant difference between repeat dosing in either schedule.

Tumour response

Tumour response data were assessed using Response Evaluation Criteria in Solid Tumours (RECIST) Version 1.1 throughout the study and additionally by immune-related Response Criteria (irRC) Version 1 during Phase Ib.

Using RECIST Version 1.1, there was a single partial response as assessed by the Investigator and 16 subjects with a best overall response of stable disease. Only 5 subjects met the protocol definition of clinical benefit as assessed by the investigator. The independent assessor did not confirm the one partial response but considered 18 subjects had a best response of stable disease with 5 meeting the protocol definition of clinical benefit.

Using irRC during Phase Ib , there were no tumour responses but four of the 24 subjects had a best overall response of stable disease as assessed by both the Investigator and Independent assessor but this was only maintained for 12 weeks in one subject reaching the protocol criteria for clinical benefit.

Progression-free survival (PFS) and overall survival were very variable and ranged up to 8.6 and 37.7 months during Phase I, respectively with three subjects known to be alive when the study was terminated. In Phase Ib PFS ranged up to 7.6 months using RECIST Version 1.1 criteria and up to 12.3 months using irRC criteria. Overall survival ranged up to 16.5 months

but is understated as eight subjects were known to be alive at the time the data were censored at data cut-off.

Shedding

Enadenotucirev viral shedding was monitored to determine whether enadenotucirev is shed from a human subject and if so in what form, amount and duration. Virus was dosed by intravenous (IV) infusion on Days 1, 3 and 5. Shedding was monitored by taking rectal swabs, buccal swabs and urine for analysis of viral DNA by qPCR analysis. Measuring for viral DNA is a very sensitive technique, however it cannot discriminate between infectious virus, non-infectious virus or viral breakdown products.

Rectal shedding was detected in all but the lowest dose cohort (1×10^{10} vp), and overall in 17 of 31 subjects assessed. Very little shedding was detected in subjects dosed with $<1 \times 10^{12}$ vp, at doses greater than this there was little correlation between dose and the amount of detected viral DNA. The difference in the quantity of virus shed is greater within a dosing cohort than between cohorts and viral DNA was most commonly observed in the rectal swabs between Days 4 and 6. The maximum shed virus was 2.24×10^6 viral particles per swab (assuming this was all viral particles and not DNA fragments). Shedding was only detected in one subject (out of 22) in the quantifiable range at Day 15 and none beyond this time point.

Buccal shedding was detected in subjects from all dose levels. In general, the quantity of shedding correlated with the dose received. Viral DNA shedding was most commonly detected between Days 6 and 8. The maximum shed virus was 8.05×10^5 viral particles in a swab for a subject who received the highest dose, this dropped 10-fold by the next sampling point and was not detected for subsequent time points. No shedding was detected in the quantifiable range from Day 15 onwards.

Urine shedding was detected in subjects from all dose levels, with the majority of samples containing less virus DNA than could be quantified (<LOQ). The peak in frequency of detectable viral DNA in the urine samples was at Day 8, which is later than that observed for buccal and rectal shedding. There is a degree of correlation with shedding and dose, in that shedding was detected more frequently at higher doses.

There is little supporting evidence for long term shedding of enadenotucirev following IV administration.

Antibody response

Anti-enadenotucirev antibody concentrations were measured for subjects at various timepoints. Prior to dosing most subjects had no detectable, or very low, levels of antibodies against enadenotucirev. Following dosing, all patents showed an increase in antibody titres that plateaued around Day 20. There was no significant difference in antibody titres in relation to dose or schedule over the duration of the study. An initial neutralisation assay performed in 3 subjects confirmed that neutralising antibodies were present. However the relevance of this finding to repeat dosing is not known.

Cytokines

The concentrations of Interleukin-2 (IL-2), IL-4, IL-6, IL-10, IL-12, Interferon- gamma (IFNγ), Tumour Necrosis Factor α (TNFα) and Monocyte Chemotactic Protein 1 (MCP-1) were analysed in serum samples following IV dosing with enadenotucirev.

Following the first virus dose, transient increases in MCP-1 was observed in all subjects with the highest levels recorded at 12 hours post infusion but generally returning to baseline by 24 hours. The response was broadly dose dependent with cohorts receiving $1x10^{12}$ vp and above recording constantly higher levels than cohorts that receiving lower doses. A transient increase in IL-6 was also observed. The response was generally dose dependent; with a much greater response observed in subjects dosed with $6x10^{12}$vp or higher. Elevated TNFα and IFNγ serum concentrations were only occasionally observed in lower dose cohorts but were consistently recorded at doses above $6x10^{12}$vp.

Where elevated cytokine levels were observed in a subject following the initial dose, subsequent administrations on Days 3, and 5 resulted in a substantially lower response. Increases in IL-10 serum concentrations were delayed compared to the other cytokines, with the initial peak in IL-10 occurring four days after the first delivery and then a smaller response was observed following each subsequent administration of virus.

IL-2, IL-4 and IL-12, all remained at low levels in most of the subjects at the time points examined. These data are consistent with pre-clinical findings and previous experiences of acute responses to virus particles administered to humans.

Common adverse reactions to systemic viral delivery are of an inflammatory nature, particularly pyrexia and chills which have onset within 24 hours of dosing. The frequency of these types of events is lower after subsequent doses and appears to generally reflect the cytokine responses to treatment.

Within the limitations of this study, it is not possible to draw any specific conclusion about either individual cytokines or subjects. However, the general trend was that acute cytokine response was transient and predominantly associated with the initial dose. Subsequent doses, at any dose level, typically resulted in a less marked cytokine response.