Neutralisation effects of pleural fluids do not predict the persistence of the oncolytic HSV Seprehvir following intrapleural administration in patients with malignant pleural mesothelioma.

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Abstract

Malignant pleural mesothelioma (MPM) remains a major challenge, with limited therapeutic options. Multifocal intrapleural disease can cause disabling symptoms of pain and breathlessness, in the absence of distant metastases, so an intrapleural treatment approach is attractive. Seprehvir (HSV1716) is an engineered oncolytic herpes simplex virus type 1 deleted in the RL1 gene which encodes the virulence protein ICP34.5. Mutants lacking the RL1 gene are capable of specific replication in cancer cells and inducing anti-tumour immune responses.

Clinical studies with Seprehvir have been completed in adult high grade glioma (HGG), melanoma, squamous cell head and neck cancer, and studies are ongoing in both pediatric HGG and pediatric non-CNS solid tumors and adult MPM. In total, 91 patients have received Seprehvir and the virus is well-tolerated with no spread to surrounding normal tissue or no shedding in patients. Seprehvir selectivity for replication only in tumour cells, signals of efficacy, and immuno-stimulatory potential have been demonstrated. Of note, 47 patients with high-grade glioma received Seprehvir either intratumorally or injected into the resected tumor bed; more than 60% of treated HGG patients survived longer than the median normal survival period for their disease and, indeed, 3 have survived >10 years.

We are currently conducting a phase I/IIa trial to determine the safety and potential for efficacy of Seprehvir given intrapleurally to patients with MPM. Patients receive 1x10^{7}iu through their pleural catheter on one, two, or four occasions a week apart, in three separate patient cohorts. To date 9 patients have been treated, 3 in each cohort and Seprehvir has been well-tolerated with few adverse events in any patients. Pleural fluid samples have been collected pre- and post treatment and analysed to assess virus neutralisation capacity in titration assays and Seprehvir persistence post administration.

Pre-clinical studies with pleural fluids demonstrated variable effects on HSV titration assays from strong, to little or no, neutralisation of Seprehvir plaque formation. Surprisingly, in tissue culture cells, even strongly neutralising pleural fluids did not significantly interfere with Seprehvir replication. In patient samples from our clinical study, levels of neutralisation also varied with most demonstrating a strong inhibitory effect in titration assays. However, HSV DNA was detected in the pleural fluids of most patients and persisted in some at high levels for at least two weeks post-administration. Thus, neutralisation capacity of pleural fluids is not a determinant of oncolytic HSV replication and Seprehvir persisted and replicated in seemingly unfavorable conditions.

Pre-clinical testing

- 5 pleural fluids tested for effects on Seprehvir
- 3/5 rapidly neutralised 1x10^7 pfu in plaque assay, 1/5 had limited neutralisation effect and 1/5 had no effect (Figure 1)
- Despite neutralisation effect, pre-coating cells with pleural fluids did not significantly reduce Seprehvir output at 72 hrs (Figure 2)

Figure 1. Seprehvir (1x10^7 pfu) stability in Pleural Fluid Samples

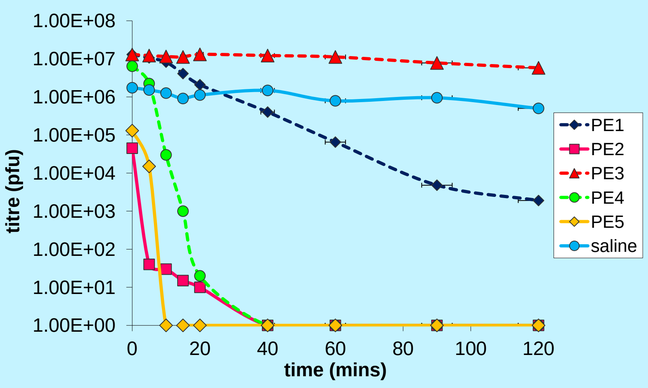
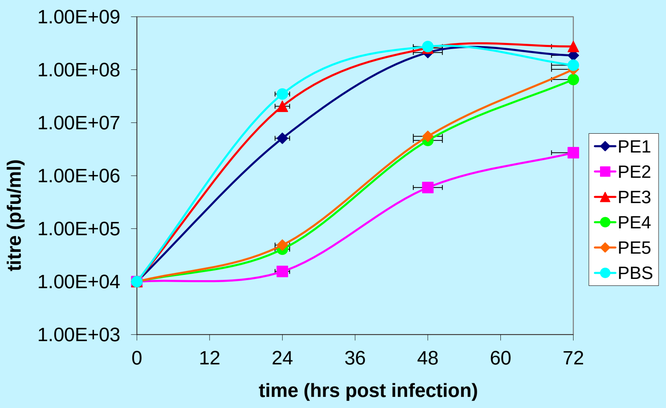
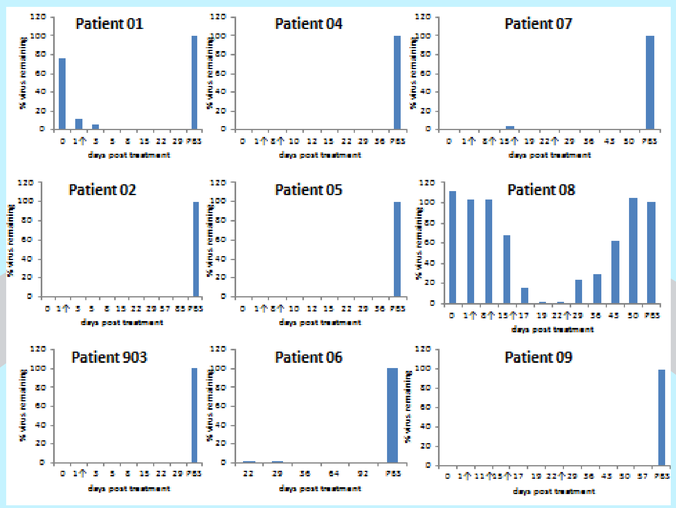


Figure 2 Seprehvir output (pfu/ml) at 24, 48 and 72 hrs after infection of BHK cells pre-coated with pleural effusions or PBS

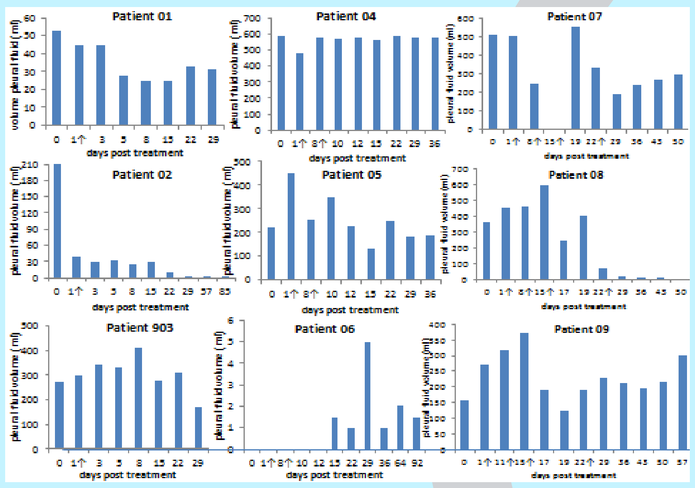


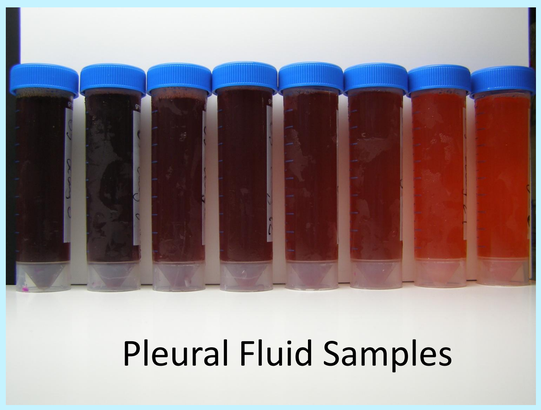
Clinical testing



Neutralisation effect on 1x10^7 pfu Seprehvir in plaque assay

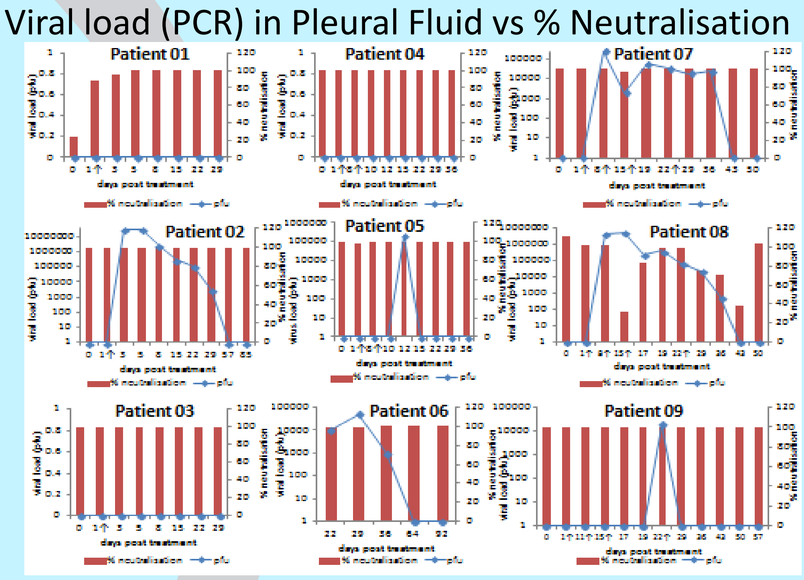
Pleural Fluid Volume



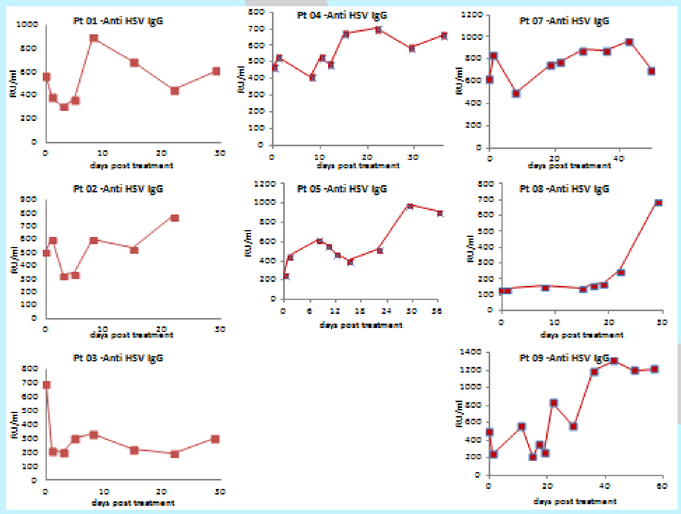


Pleural Fluid Samples

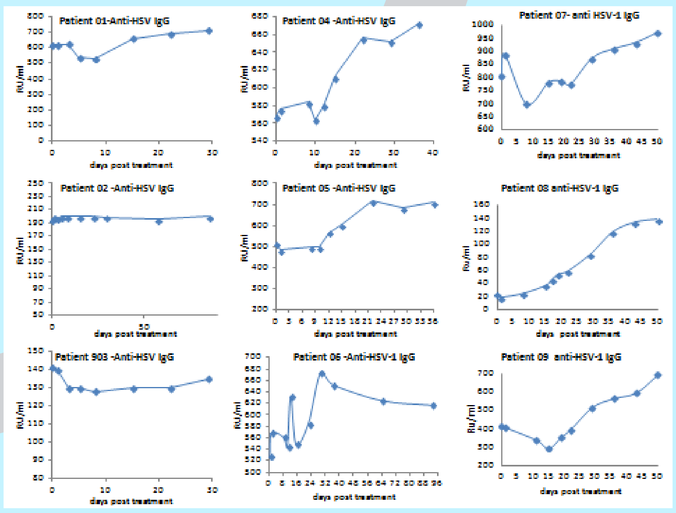
Viral load (PCR) in Pleural Fluid vs % Neutralisation



Pleural Fluid Anti-HSV IgG



Serum Anti-HSV IgG



Current Status of Trial

- Purpose – safety, tolerability and potential for efficacy
- Phase 1/2a 3+3 dose escalating (1, 2 & 4 doses) study delivering Seprehvir via existing intra-pleural catheter
- A group 2 extension phase is currently ongoing with patients receiving 4 doses of Seprehvir
- Well tolerated with few virus-related adverse events
- Potential signals of efficacy (9 patients treated, 1 PR, 5 SD, 3 PD)

	No. of doses	Number of patients	Status
Part A	1 dose of 1x10^7 i.u.	Patients 1-3	Completed
Part B: Group 1	2 doses of 1x10^7 i.u.	Patients 4-6	Completed
Part B: Group 2	4 doses of 1x10^7 i.u.	Patient 7-9	Completed

Conclusions

- Preclinical studies suggested potent neutralisation effect in some pleural fluids did not restrict Seprehvir replication potential
- In clinical study, 8/9 pleural fluids had potent neutralisation effects on Seprehvir
- 2 and 4 intrapleural doses of Seprehvir stimulated a pronounced serum anti-HSV IgG response
- Anti-HSV IgG was detectable in all pleural fluids and increased in most post Seprehvir administration
- Despite neutralisation potential of pleural fluids and presence of anti-HSV IgG, Seprehvir persistence/replication was demonstrated in 6/9 patients
- Hostile, neutralising effects of pleural fluids did not interfere with Seprehvir activity