A Phase I clinical trial of a genetically modified and imageable oncolytic vaccinia virus GL-ONC1 with clinical green fluorescent protein (GFP) imaging

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Background

GLV-1h68/GL-ONC1 is a genetically engineered live vaccinia virus attenuated by insertion of the *ruc-gfp* (a luciferase and green fluorescent protein fusion gene), beta-galactosidase (LacZ) and beta-glucuronidase (gusA) reporter genes into the *F14.5L, J2R* (thymidine kinase) and *A56R* (hemagglutinin) loci respectively. See fig. 1.

Strategy of mechanism:

1. Replicates only within the cytoplasm of the cancer cells therefore DNA is not integrated into the host chromosomes.

2. Deletion of thymidine gene leads to dependence of virus on cellular thymidine kinase expression, which is constitutively expressed at high levels in the majority of cancer cells.

3. Direct infection of cancer cells results in cell lysis and death.

4. Adaptive and innate immune response are harnessed to fight cancer.

5. Diagnostic proteins are produced so tumour regression can be supervised. See fig.2.

Fig. 1. Loci of inserted genes

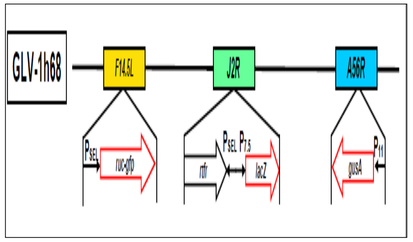
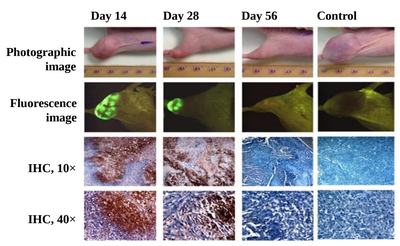


Fig. 2. IHC/GFP imaging (animals)



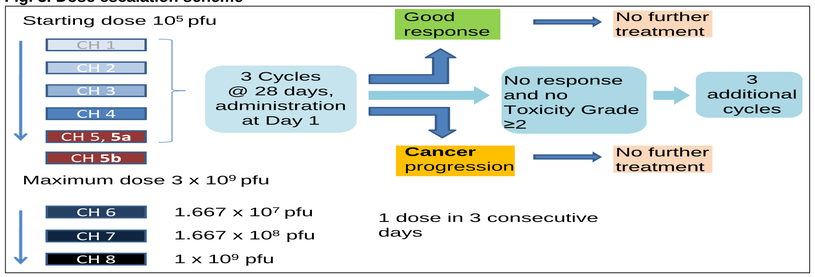
Methodology

• Open-label, dose-escalating, non randomised, single centre phase 1 study with three sub-sites.

• **Primary objective:** Determine the safety profile of the GL-ONC1 when administered intravenously to subjects with advanced solid tumours.

• **Secondary objectives:** Detection of virus delivery by PCR,VPA & IHC, neutralizing antibody response, evaluation of viral delivery by imaging of GFP expression and recommendation of dose/schedule for future trials.

Fig. 3. Dose escalation scheme



Results

Table 1. Patient characteristics

Age, years		
Median	60	
Range	39-73	
Gender	*N*	%
Male	18	75
Female	6	25
Tumor type		
Melanoma	6	25
Head and Neck	6	25
Colorectal	5	21
Parotid	2	9
Oesophagus	1	4
Thyroid	1	4
Myxoid chondrosarcoma	1	4
Non-small Cell Lung Cancer	1	4
Renal cell carcinoma	1	4

Fig. 4. GFP imaging of Vaccinia virus induced rash

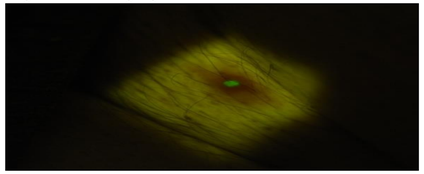
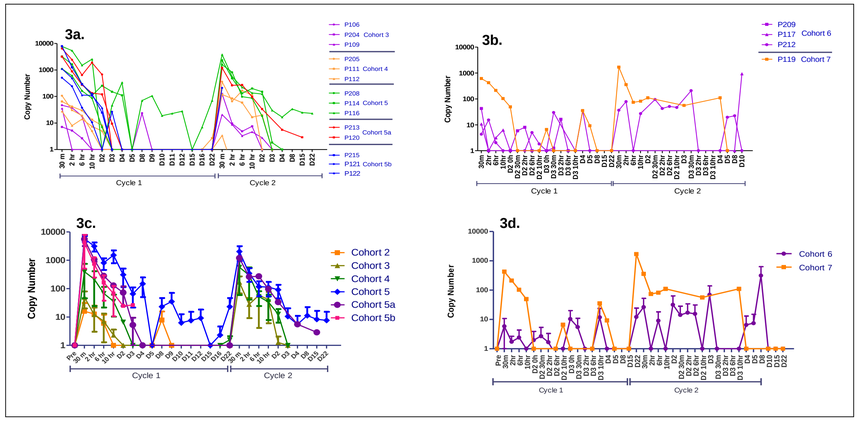


Table 3a, 3b, 3c and 3d. Virus disposition in the blood



•One dose limiting toxicity has been observed in 24 pts. This pt received treatment at the 1x10^9 pfu dose level and developed a short-lived grade 3 rise in aspartate transaminase after a single infusion. The CEA dropped from 11331 to 4530 after the single infusion and CT was stable by RECIST.

•A rash comprising of vaccinia pustules appeared in two pts during cycle 1 and resolved without treatment at the end of cycle 1. It was positive for GL-ONC1 viral plaque assay (VPA) and GFP imaging expression. See fig. 4 for the first time GFP imaging in a human being.

• In one pt with SCC of base of tongue (1x10^7 pfu) a submental biopsy showed positive IHC after four cycles of treatment described as “very focal moderate true positivity mainly around the tumour islands and occasionally within the tumour islands.” See fig 5.

• There was an increase in Nab in all except one pt. VPA of blood, urine, stool and sputum were negative for viral shedding in all except one pt.

•Best response was stable disease at 34 weeks for one pt, 24 weeks in two pts and 8-12 weeks observed in five pts. The stable disease was seen in CRC (n: 2), head & neck (n: 2), chondrosarcoma (n: 1), parotid (n: 1), thyroid (n: 1) and esophageal (n: 1).

Table 2. Total adverse events reported

	Gr. 1 (Mild)	Gr. 2 (Moderate)	Gr. 3 (Severe)
Pyrexia	7	5	2
Musculoskeletal pain	4	3	0
Fatigue	4	3	0
Nausea	4	1	0
Maculopapular rash	3	1	0
Vomiting	2	2	0
Rigors	2	0	0
Flu-like symptoms	2	0	0
Extremity tenderness	2	0	0
Hyperhidrosis	1	0	0
Leg stiffness	0	1	0
Malaise	0	1	0
Rhinorrhea	2	0	0
Tachycardia	2	0	0
Thrombocytopenia	0	2	0
Lymphopenia	0	1	1
Hypotension	1	0	0
Diarrhoea	1	0	0
Phlebitis (forearm)	1	0	0
Seborrhoe	1	0	0
Oedema (neck lesion)	0	1	0
Thrombocytosis	0	1	0
Hyperbilirubinemia	0	1	0
Rise in CK	0	1	0
Arterial blood clot	0	0	1
Rise in AST	0	0	1

Table 4. Mean Antibody Response to GL-ONC1

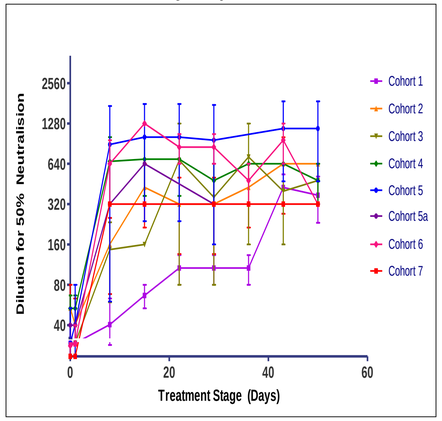
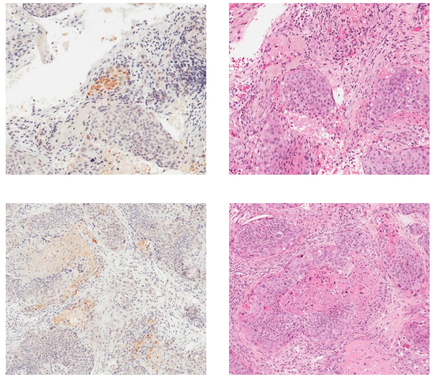


Fig. 5. Positive IHC at C4D15 biopsy from SCC tongue



Conclusion

•GL-ONC1 is well tolerated with minimal toxicity and preliminary evidence of anti-tumour activity .

Acknowledgements

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• ClinicalTrials.gov Identifier #: NCT00794131

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