Phase I/II clinical trial of a genetically modified and oncolytic vaccinia virus GL-ONC1 in patients with unresectable, chemotherapy-resistant peritoneal carcinomatosis

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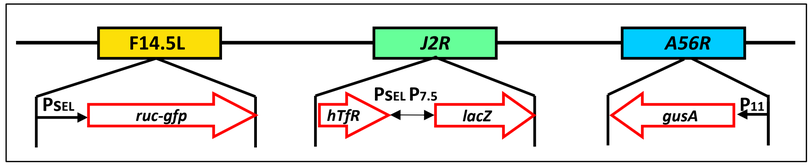
Background

GL-ONC1 is a genetically engineered vaccinia virus attenuated by insertion of the RUC-GFP (Renilla luciferase and Aequorea green fluorescent protein fusion gene), ß-galactosidase (ß-gal; lacZ gene) and ß-glucuronidase (ß-gluc; gusA gene) reporter genes into the F14.5L, J2R (thymidine kinase, TK) and A56R (hemagglutinin, HA) loci, respectively (see Fig. 1).

Outline of mechanism:

1. Replicates only within the cytoplasm of cancer cells; therefore, the viral DNA is not integrated into the host chromosomes (important safety aspect).
2. Deletion of the viral thymidine kinase gene leads to dependence of GL-ONC1 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. Innate and adaptive immune responses are harnessed to fight cancer.
5. Reporter proteins such as ß-glucuronidase can be used directly to monitor the process of oncolysis.

Fig. 1. Schematic represeantation of the GL-ONC1 construct

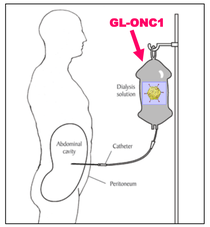


Central Features of Tuebingen Clinical Virotherapy Trial

- Open-label, dose-escalating, non-randomised, phase I/II study (NCT01443260).
- **Primary study objective** is to determine the safety profile of GL-ONC1, an attenuated vaccinia virus, when administered to patients with peritoneal carcinomatosis via intraperitoneal infusion employing an indwelling catheter.
- **Secondary study objectives** include (i) determination of a recommended dose (RD) and schedule for the phase II portion of this study as well as for future investigations; (ii) sampling of evidence of anti-tumor activity; (iii) detection of virus in body fluids; (iv) comparative analysis of viral delivery to tumor and normal cells; (v) detection of virus encoded reporter proteins in body fluids; (vi) evaluation of anti-vaccinia virus immune response (e.g., antibody responses).

Fig. 2. Phase I dose escalation scheme

Cohort	Dose*	Number of treatment days at each cycle (on days 1 (C1D1), 29 (C2D1), 57 (C3D1), 85 (C4D1))	Study specific procedures
+1	1×10^7 pfu**	1	- Final volume of preparation is 500 mL, infused within 10 min - Tumor imaging: • pre-study: PET-CT • at mid-term (after C2): CT • post-treatment (after C4): PET-CT
+2	1×10^8 pfu	1	
+3	1×10^9 pfu	1	
+4	3×10^9 pfu	1	
+5	5×10^9 pfu	1	



* Dosage independent of body weight; intermediate dose levels may be evaluated if indicated;
** pfu: plaque forming units (equivalent of viral infectious dosage)

Results

Fig. 3. Detection of infectious GL-ONC1 particles (study patient 401)

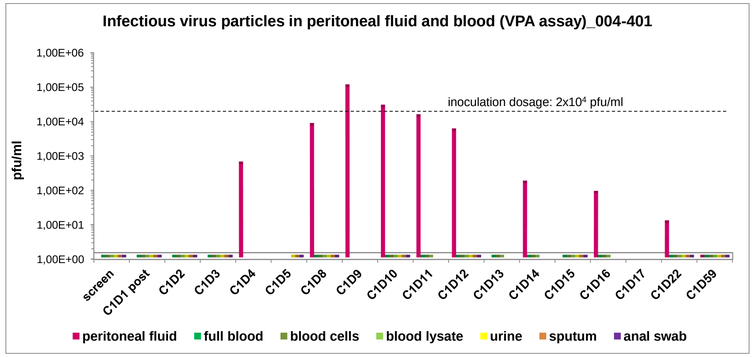


Fig. 4. Direct monitoring of oncolysis by determining release of ß-gluc (study patient 401)

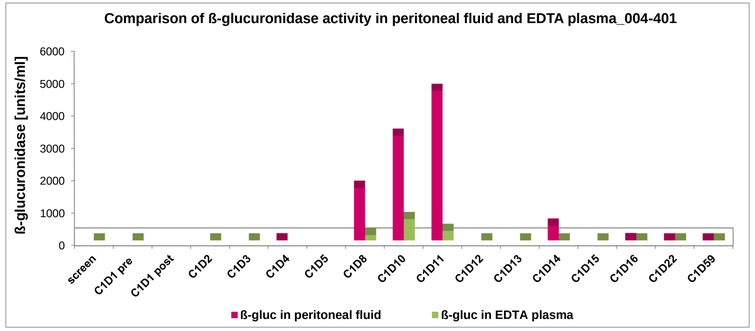


Fig. 5. Imaging response parameters (study patient 401)

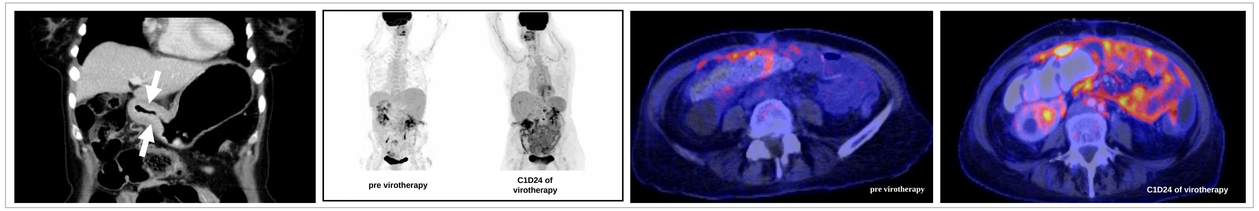


Fig. 6. IHC analysis of peritoneal fluid (study patient 401)

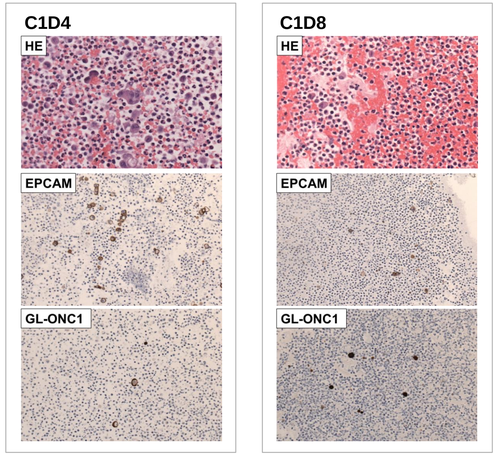
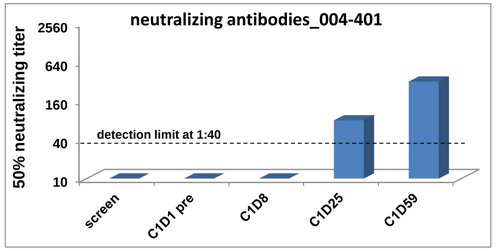


Fig. 7. Antibody response to GL-ONC1 (study patient 401)



- GL-ONC1 is well-tolerated after intraperitoneal administration.
- Inflammatory response: 3 day period of fever (C1D7 - C1D9) with a max. of 39.0°C, leucocyte count max. of 14.580/µl (C1D9); CRP max. of 32.5 mg/dl (C1D10).
- Typical signs of GL-ONC1 induced viral peritonitis occurred in parallel to the fever period going along with transient symptoms such as increased abdominal pain, nausea, vomiting, and fatigue.
- No virus-specific impairment of organ functions (heart, kidneys, liver, pancreas, hematopoiesis, skin, brain, lungs); no signs in patient´s serum indicating significant organ toxicity (heart, kidneys, liver, pancreas, hemato-poiesis).
- Cytological analysis demonstrates tumor cell colonization.
- Patient inherent (*in situ*) production of GL-ONC1 pro-geny viral particles has taken place as demonstrated by VPA analysis, being at least factor 15 higher than the input virus dosage.
- Prolonged *in situ* production of progeny virus has taken place; however, on C1D59, no longer infectious GL-ONC1 particles were detected in the peritoneal fluid.
- Detection of β-glucuronidase release into peritoneal fluid and blood plasma provides direct evidence of virus mediated oncolysis.
- Neutralizing antibodies are not detectable on C1D1 and C1D8 (no prior vaccination). The next scheduled measurement was on C1D25; at this time point neutralizing antibodies could be detected with a further increase over time.
- Cohorts 1 and 2 have been completed without DLT. Accordingly, enrollment to cohort 3 starts in June 2013.

Conclusion

- GL-ONC1 administered intraperitoneally is well-tolerated without any signs of relevant organ toxicity.
- Early results provide evidence for tumor colonization, effective patient-inherent production of progeny virus particles over a prolonged period of time as well as virus mediated oncolysis.