Tracking of tumor cell colonization, in-patient replication, and oncolysis by GL-ONC1 employed in a phase I/II virotherapy study on patients with 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).

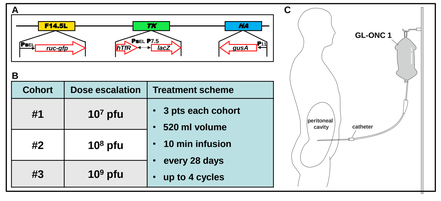
Outline of mechanism:

1. GL-ONC1 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 to monitor the process of oncolysis.

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 response).

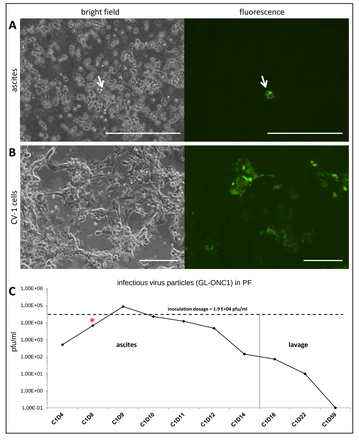
Fig. 1. Outlay of the monocentric phase I virotherapy study design



Cohort	Dose escalation	Treatment scheme
#1	10^7 pfu	• 3 pts each cohort • 520 ml volume • 10 min infusion • every 28 days • up to 4 cycles
#2	10^8 pfu	
#3	10^9 pfu	

(A) Schematic overview of the therapeutic virus GL-ONC1, a recombinant vaccinia virus (LIVP strain) encoding the marker genes *ruc-gfp* (a fusion gene of Renilla luciferase and green fluorescent protein), *lacZ* (ß-galactosidase), and *gusA* (ß-glucuronidase) inserted into F14.5L (located between F14L and F15L), thymidine kinase (TK), and hemagglutinin (HA) loci, respectively. (B) Schematic overview of the treatment scheme for the phase I trial. (C) Schematic overview of the intraperitoneal application route of the GL-ONC1 treatment. A total volume of 520 ml of virus solution is infused via a peritoneal catheter for up to four cycles.

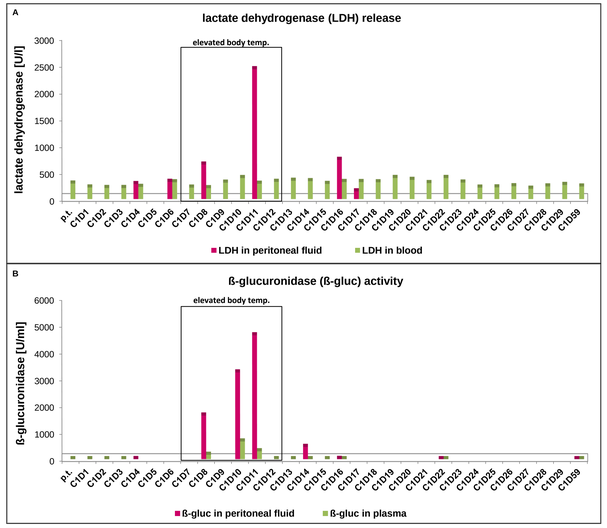
Fig. 2. Prolonged "in patient" generation of oncolytic viral particles



Time course of the "in situ" production of infectious viral particles in peritoneal fluid (PF) of patient #1: fluorescence microscopy only led to the direct detection of GL-ONC1 infected, GFP-positive cells (white arrows (A)), but also revealed numerous virus-induced plaques in a CV-1 mono-layer (B) being incubated for 24 hrs with PF taken on C1D8. (C) The concentration of infectious viral particles in PF was quantified via vaccinia virus plaque assay (VPA): on C1D9, "in patient" virus replication resulted in a virus concentration higher than applied for the initial virotherapeutic treatment (C1D1: inoculation dosis: 1.9 x 10^4 pfu/ml); furthermore, a prolonged presence of infectious viral particles was observed for at least 21 days post treatment (C1D22). *C1D8: under-estimated result of virus quanitification due to assay limitions; bars represent 200 µm.

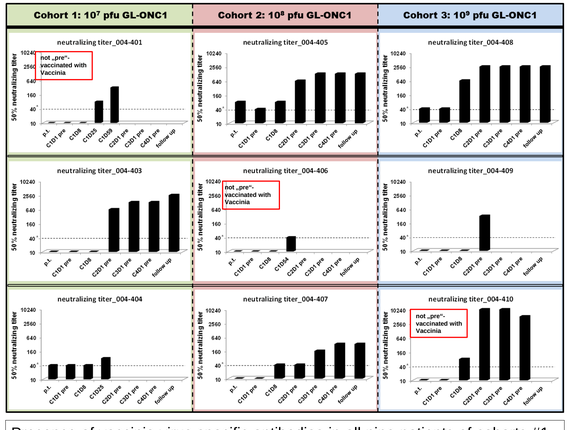
Results

Fig. 3. Quantification of the oncolytic activity (study patient 401)



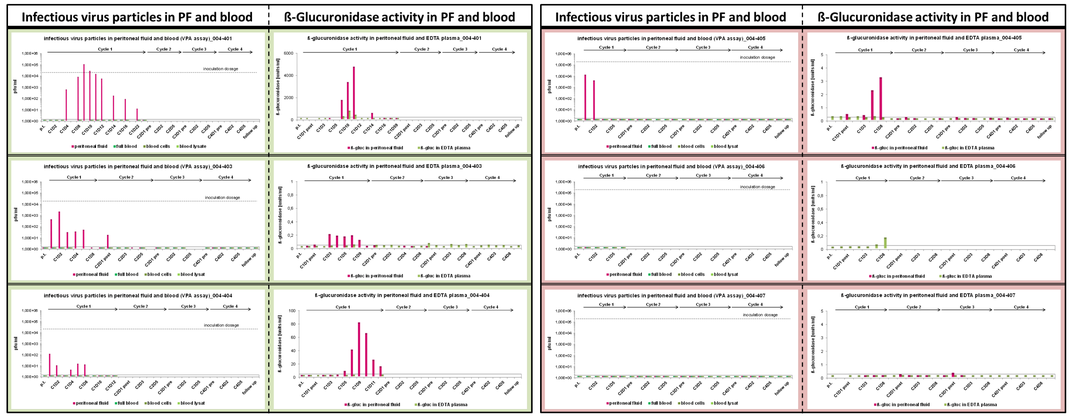
GL-ONC1 induced lytic events were determined in peritoneal fluid and blood plasma. (A) While the detection of released LDH constitutes an unspecific marker for gross cellular destruction, (B) the release of virus encoded ß-glucuronidase (ß-gluc) is dependent on a successful infection of tumor cells and a subsequent virus dependent cell lysis. Of note, in patient 401, maximum concentrations of LDH and ß-gluc were detectable in the period of elevated body temperature and reached peak values on C1D11 in peritoneal fluid. p.t.: pre-treatment.

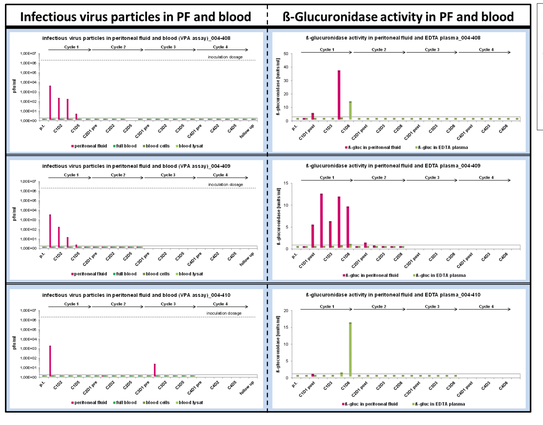
Fig. 4. Antibody response to GL-ONC1



Presence of vaccinia virus specific antibodies in all nine patients of cohorts #1 - #3 was determined pre-treatment (p.t.) and at subsequent time points during the study. Only dilutions of 1:40 or higher resulting in 50% neutralization are covered with this assay (* detection minimum). An increase of the neutralizing capacity of vaccinia virus was observed over time for all nine patients even after a single virotherapeutic treatment.

Fig. 5. Analysis of " in patient" replication and oncolysis of GL-ONC1





Comparison of detected infectious viral particles in peritoneal fluid (PF) and blood fractions with released ß-glucuronidase in PF and EDTA plasma of all three patients of each cohort (#1-#3). With a slight delay after the detection of infectious viral particles in PF, an increase of the ß-glucuronidase concentration could be observed in PF as well as to a lower extent in EDTA plasma in the majority of patients. p.t.: pre-treatment.

Summary & Conclusions

- **GL-ONC1 administered intraperitoneally is well-tolerated and does not lead to any viral shedding so far.**
- **In 8 out of 9 patients effective intraperitoneal infections and in-patient replication of GL-ONC1 (demonstrated by viral plaque assays) as well as subsequent oncolysis (demonstrated by release of GL-ONC1 encoded ß-glucuronidase) have been demonstrated.**
- **Pre-vaccination did not exert any negative impact on these processes of oncolysis.**
- **All 9 study patients developed neutralizing antibodies against Gl-ONC1.**
- **Tracking of tumor cell colonization, "in patient" replication and oncolysis yields important information on safety as well as efficiency of this virotherapeutic approach and therefore is considered as a standard also for other virotherapy studies to be performed in the future.**