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-GLONC1 is a genetically engineered live vaccinia virus attenuated by insertion of the nuc-gfp (a luciferase and green fluorescent protein fusion gene), beta-galactosidase (LaZ) and beta-glucuronidase (pIgA) reporter genes into the F14.5L, J2R (thymidine kinase) and AS89 (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 genome.
2. Detection 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 supressed. See fig.2.

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, neutralising antibody response evaluation of viral delivery by imaging of GFP expression and recommendation of doseschedule for future trials.

Dose Escalation Schema

- **Starting dose:** 10^5 pfu
- **Cycle 1:** 10^5 pfu
- **Cycle 2:** 10^6 pfu
- **Cycle 3:** 10^7 pfu
- **Cycle 4:** 10^8 pfu
- **Cycle 5:** 10^9 pfu
- **Cycle 6:** 10^10 pfu

- **Toxicity Grading:**
  - **No response and no toxicity (Grade 0).**
  - **Minor toxicity (Grade 1).**
  - **Moderate toxicity (Grade 2).**
  - **Severe toxicity (Grade 3).**

- **Concordance with Radiation Therapy:**
  - **HR:**
    - **Fig. 2:** IHC/GFP imaging (animals)
    - **Fig. 3:** IHC/GFP imaging (animals)

- **Time points:**
  - **Cycle 1:**
    - **D3 0h**
    - **D3 2hr**
    - **D5**
  - **Cycle 2:**
    - **D3 10 hr**
    - **D4**
    - **D5**
    - **D8**
    - **D10**
    - **D15**
    - **D16**
    - **D22**

Results

- **Table 1. Patient characteristics**

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Cycle 1</th>
<th>Cycle 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>F</td>
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- **One dose limiting toxicity has been observed in 24 pts.**
  - This pt received treatment at the 1x10^6 pfu dose level and developed a short-lived grade 3 rise in aspartate transaminase after a single injection. The CEA dropped from 11331 to 4530 after the single injection and CT was stable by RECIST.
  - A rash consisting 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 (1x10^6 pfu) a subcutaneous 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 Na 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 for 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 sarcinophagi (n = 1).

- **Table 2. Total adverse events reported**

<table>
<thead>
<tr>
<th>Event</th>
<th>Cycle 1</th>
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<tbody>
<tr>
<td>Fever</td>
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<tr>
<td>Rigors</td>
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<td>Fatigue</td>
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<td>Hypotension</td>
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<tr>
<td>Dizziness</td>
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<tr>
<td>Chills</td>
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<tr>
<td>Conjunctivitis</td>
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<td></td>
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<tr>
<td>Vomiting</td>
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<tr>
<td>Diarrhea</td>
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<tr>
<td>Gastrointestinal bleeding</td>
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<tr>
<td>Abdominal pain</td>
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<tr>
<td>Myalgia</td>
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<tr>
<td>Rash</td>
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<tr>
<td>Pruritus</td>
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<tr>
<td>Headache</td>
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<tr>
<td>Nausea</td>
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Conclusion

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

Acknowledgements

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- ClinicalTrials.gov Identifier #: NCT07094131

References