The treatment of non-muscle invasive bladder cancer (NMIBC) has not changed significantly in 25 years. Treatment with intravesical BCG (Bacillus Calmette Guérin) and chemotherapy after transurethral resection of tumour (TURBT) does not alter the natural history of the disease and has significant toxicity for patients. CAVATAK (Coxsackievirus A21, CVA21) is a novel intercellular adhesion molecule (ICAM)-1 targeted immunotherapeutic virus. Surface ICAM-1 is upregulated on a number of cancers including melanoma, non-small cell lung, prostate and in particular, bladder cancer. CVA21 displays potent oncolytic activity against monolayer cultures of NMIBC cancer cells (Figure 1). Combining CVA21 with either radiotherapy or chemotherapy (mitomycin C) synergistically enhances cytotoxicity in vitro in bladder cancer cell lines. Low (non-cytotoxic) doses of mitomycin C enhance CVA21 viral replication and cytotoxicity by increasing expression levels of ICAM-1 on bladder cancer cells (Figure 2). In this two stage Phase I study, patients with NMIBC will receive a dose of CVA21 or low dose mitomycin C plus CVA21 intravesically prior to routine surgical removal (TURBT). We present preliminary data from the Phase I/II CANON (CAVATAK in NMIBC) study which is investigating the tolerance of multiple escalating intravesical doses of CVA21 in approximately 25 transitional NMIBC cancer patients.

Figure 1. Human bladder cancer cells are highly susceptible to lytic infection by CVA21. (A) Monolayer cultures of human bladder cancer cells were challenged with increasing multiplicity of CVA21 and assessed cell survival at 72h post-infection, with live cell counts (black) compared to mock-infected controls (grey). Significant cell death occurred in all cell lines 24h post CVA21 infection (Green: CVA21 viral protein, red: wheat germ agglutinin, blue: DAPI). (B) Flow cytometry analysis of surface ICAM-1 expression in TCCSUP cells (human transitional cell carcinoma) following treatment with CVA21. ICAM-1 expression was upregulated by treatment with CVA21 compared to control cells (TCCSUP mock-infected with Mock virus).

Figure 2. Enhanced CVA21 replication in combination with mitomycin C in an in-vivo human bladder tumour. Tissue sections from a patient with bladder cancer (B006) cut at a distance of 100 microns from the tumour site and stained with haematoxylin and eosin. The absence of mitomycin C (Supplementary Figure S1) immunohistochemistry staining for CVA21 viral protein was performed 48h post-infection. Viral infections are visualized by the bright red staining with the blue colour indicating DAPI stained nuclei of the cells.

Table 1. Patients and treatment characteristics

| Cohort | Patient Identification Code | CVA21 Dose (TCID50) | Mitomycin C Dose (Day 1 only) | Gender | ECOG at Baseline | Treatment 
|---|---|---|---|---|---|---
| 1 | B013 | 3 x 10^7 | 3 x 10^6 | Male | 0 | TCC high grade papillary/ focal CIS
| 2 | B014 | 3 x 10^7 | 3 x 10^6 | Male | 0 | TCC high grade papillary/ focal CIS
| 3 | B015 | 3 x 10^7 | 3 x 10^6 | Male | 0 | TCC high grade papillary/ focal CIS

Table 2. Product-related adverse events

<table>
<thead>
<tr>
<th>AEs</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>Grade 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal liver function test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Constipation</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fatigue</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Headache</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Conclusions

- CANON Phase I trial: Proof of concept viral targeting, replication and tumour cell death following a single or multiple intravesical administrations of CVA21 was achieved in patients from monotherapy Cohorts 1, 2 and 3 and combination Cohorts 1, and 2.
- Clinical activity of CVA21 demonstrated by complete tumour response, viral replication (infectious virus increases in urine) and notable signs of viral-induced tumour inflammation.
- No evidence of systemic spread of CVA21 or development of serum neutralizing antibody.
- To date intravesical administration of CVA21 has been generally well tolerated with no Grade 3, 4, or 5 product-related AEs.
- The observed tumour targeting and viral replication is likely to provide a strong signal in generating both a strong local and systemic anti-tumour immune response.

Future Directions

- Phase II neo-adjuvant study with patients administered CVA21 via the intravesical route prior to TURBT against patients with TURBT-alone. Recurrence-free survival as primary endpoint.
- Phase IIb study with patients administered CVA21 in combination with immune checkpoint blockade.