Phase I/II CANON study
Oncolytic immunotherapy for the treatment of non-muscle invasive bladder cancer using intravesical Coxsackievirus A21

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Non-muscle invasive (superficial) bladder cancer

• High incidence, high prevalence
• Lifelong surveillance, hence high cost
• BCG immunotherapy for high grade: good response, ‘fails’ in 40% patients within 2 years
• 15% develop invasive disease
• Complex immune response
• Mitomycin C, adjuvant, low grade dose
Non-muscle invasive (superficial) bladder cancer
Coxsackievirus A21 (CVA21)

Genus: Picornaviridae

Polioviruses

Coxsackie A viruses

Coxsackie B viruses

Echoviruses

Enteroviruses

- Replicate mainly in the gut
- Single stranded naked RNA virus
- Capsid has 60 copies each of 4 proteins, VP1, VP2, VP3 and VP4, arranged with icosahedral symmetry around a positive sense genome

ICAM-1 (CD54) is primary receptor for attach/internalisation

DAF (CD55) co receptor, virus attachment
Susceptibility of bladder cancer cell lines to CVA21 infection
Expression profile of surface ICAM-1 and DAF on bladder cancer cell lines

A

T24

TCCSUP

5637

KU19-19

VMCUB

RT112

ICAM-1 positive

Whole population

ICAM-1 negative

B

ICAM-1 PGE Molecules/cell

C

Percentage Survival (%)

CVA21 MOI

ICAM-1
Synergistic effect of combination treatment with CVA21 and Mitomycin C
Mitomycin C up-regulates ICAM-1 expression.
Increasing viral replication after MMC exposure

![Graphs showing viral replication in different cell lines over time.](image-url)
Effect of low dose 0.5 ug/ml MMC on gene expression in 5637 cell line
Apoptotic cell death pathway induced by CVA21.
ICD marker induction by CVA21 and in combination

A

T24

MFI: Calreticulin

24 Hours 48 Hours 72 Hours

5637

MFI: Calreticulin

24 Hours 48 Hours 72 Hours

B

T24

HMGB1 conc (ng/ml)

24 Hours 48 Hours 72 Hours

TCCSUP

24 Hours 48 Hours 72 Hours

5637

24 Hours 48 Hours 72 Hours
CVA21-induced ICD effectively vaccinates mice
ICAM-1 expression in NMIBC
Enhanced cytotoxicity of mitomycin C and CVA21 on bladder cancer tumour slices

A

Normal bladder | Original bladder biopsy | Untreated slice
---|---|---
MitC 0.5 µg/ml | CVA21 5x10^6 | MitC 0.5 µg/ml + CVA21 5x10^6

B

-ve control: no TdT enzyme | Original Bladder biopsy | Untreated slice
---|---|---
MitC 0.5µg/ml | CVA21 5x10^6 | MitC 0.5µg/ml + CVA21 5x10^6
Immune profiling of NMIBC Versus normal bladder by nanostring
CANON study schema

Screen photo CVA21 TUR surgery Histology

D-7 D1 D2 D3 D4 D5 D8
Cohort A1
Day 1
CVA21 (1x10^8 TCID_{50})
n=3

Intravesicular instillation of CVA21 in 30 mL saline on Day 1 and/or Day 2
Transurethral resection (TUR) Day 8-11

Cohort B1
Day 1
CVA21 (3x10^8 TCID_{50}) +
Day 1
mitomycin C (10 mg)
n=3

Primary
Patient safety and tolerability
• Determination of MTD

Secondary
• Evidence of anti-tumor activity
• Virus-induced tumor cell infiltrates and immune response in TUR tissue
• Level of viral replication in TUR tissue
• Pharmacokinetics of serum viral load and anti-CVA21 antibodies
• Viral excretion in blood and urine

Cohort A2
Day 1
CVA21 (3x10^8 TCID_{50})
n=3

Cohort B2
Day 1 and 2
CVA21 (3x10^8 TCID_{50}) +
Day 1
mitomycin C (10 mg)
n=3

Cohort A3
Day 1 and 2
CVA21 (3x10^8 TCID_{50})
n=3

Study Endpoints

VLA-012B
(mitomycin-C combination)

VLA-012A (Monotherapy)

16 subjects with Non-muscle invasive Bladder cancer
<table>
<thead>
<tr>
<th>Cohort</th>
<th>Patient Identification Code</th>
<th>CVA21 Dose (TCID&lt;sub&gt;50&lt;/sub&gt;)</th>
<th>Mitomycin C Dose</th>
<th>Age</th>
<th>Gender</th>
<th>Pathology Finding at TUR</th>
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<td>1 x 10&lt;sup&gt;8&lt;/sup&gt;</td>
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<td>62</td>
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<td>G1 Ta papillary transitional epithelium</td>
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<td>10 mg</td>
<td>73</td>
<td>M</td>
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<td>10 mg</td>
<td>68</td>
<td>M</td>
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<td>10 mg</td>
<td>51</td>
<td>F</td>
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<td>10 mg</td>
<td>61</td>
<td>M</td>
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<td>3 x 10&lt;sup&gt;8&lt;/sup&gt;</td>
<td>3 x 10&lt;sup&gt;8&lt;/sup&gt;</td>
<td>10 mg</td>
<td>56</td>
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CVA21-related adverse events

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<td>Abdominal/discomfort</td>
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<td>-</td>
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<tr>
<td>Shivers/feeling cold</td>
<td>1(7%)</td>
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<td>-</td>
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<tr>
<td>Haematuria</td>
<td>1(7%)</td>
<td>-</td>
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CVA21 detection in urine

CANON trial Pt. 01B/001
1x10^8 TCID₅₀ infused on Day 1

RNA copies/ml

TCID₅₀/ml

Day
Levels of CVA21 viral RNA and live virus in patient urine after CVA21 treatment
CVA21 viral proteins

H&E staining

Apoptotic cell staining

CVA21 viral protein staining, Red=CVA21 proteins; Blue=Nucleus. H&E stain, black arrows indicate apoptotic bodies. Apoptotic cell staining, brown cells represent cleaved caspase-3 staining by IHC.
Response Data

Figure 1. Tumor Response: Pre and post treatment cystoscopy

Cohort 1:
Pt 01-B001

Pre-treatment
Post-treatment Day 8

Surface hemorrhage and inflammation of the tumor

Cohort 3:
Pt 01-B008

Complete clinical response (confirmed by histopathology)
HMGB-1 levels in urine
Urinary cytokine levels

Q-plex human cytokine Screen (16-plex, Quansys)
NMIBC untreated

Image ID

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<th>IM3-4 C</th>
<th>IM3-5 A</th>
<th>IM3-8 B</th>
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PD-L1  CD3  CD8  FoxP3  CD163  CK  DAPI
NMIBC untreated

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CVA21-treated
01-B004 patient
Complete responder

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CVA21-treated
01-B004 patient

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<td>IM3-10-B</td>
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**Immunofluorescence Staining:**
- **PD-L1**
- **CD3**
- **CD8**
- **FoxP3**
- **CD163**
- **CK**
- **DAPI**
Quantitation Multispectral imaging

12579-1 Untreated (Tumor)

14281-1 Untreated (Tumor)

Pt-B004-treated (Tumor)

Pt 01/B008 Treated (Stroma)
Conclusions and Future Directions

- Proof of concept viral targeting, replication and tumor cell death following a single or multiple intravesicular administrations of CVA21 was achieved in patients from monotherapy Cohorts A1, A2, A3 and mitomycin-C combination Cohorts B1, B2.

- Clinical activity of CVA21 demonstrated by complete tumor response, viral mediated cell apoptosis, viral replication (infectious virus increases in urine and notable signs of viral-induced tumor inflammation.

- Single agent and CVA21-combination treatments facilitated notable changes within the NMIBC tissue by inducing increases in immune cell infiltrates (CD3+CD8+) and expression of PD-L1 compared to untreated NMIBC controls.

- CVA21 mediated increases in “immunological heat” within the tumor micro-environment with regards to immune-cell infiltrates and up-regulation of immune checkpoint molecules suggest possible increased anti-tumor activity when used in combination with immune checkpoint blockade strategies.

- No evidence of systemic spread of CVA21 or development of anti-CVA21 serum neutralizing antibody.

- Intravesicular administration of CVA21 as a single agent or in combination with mitomycin-C was generally well tolerated with no Grade 2,3 or 4 product-related AE’s.

- The observed tumor targeting, viral replication is likely to provide a strong signal in generating both a strong local and systemic anti-tumor immune response.
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