Phase I/II CANON study: Oncolytic immunotherapy for the treatment of Non-Muscle Invasive Bladder Cancer using intravesical CAVATAK (Coxsackievirus A21)

HS Pandha[1], N Annels[1], G Simpson[1], A Iqbal[1], M Arif[1] , D Mansfield[2], SS Sandhu[1], AA Melcher[3], KJ Harrington[2], B Davies[4], G Au[4], M Grose[4], H Mostafid[1] and D Shafren[4]

[1]. Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK; [2]. Targeted Therapy Group, Institute of Cancer Research, London, UK ;[3]. Leeds Cancer Research UK Clinical Centre, University of Leeds, Leeds, UK; [4]. Viralytics Limited, Sydney, Australia.

Introduction

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 bladder tumor (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-1 (ICAM-1)-targeted immunotherapeutic virus. Surface ICAM-1 is up-regulated 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 enhance cytotoxicity *in vitro* in bladder cancer cell lines. Low (non-cytotoxic) doses of mitomycin-C enhances CVA21 viral replication and oncolysis 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 neoadjuvant CVA21 or low dose mitomycin-C plus CVA21 prior to routine surgical removal (TURBT). We present preliminary data from the Phase I/II CANON (***CA***VATAK in ***NON***-Muscle invasive bladder cancer: NCT02316171**)** study which is investigating the tolerance of multiple escalating intravesicular doses of CVA21 in approximately 30 first-line NMIBC cancer patients.

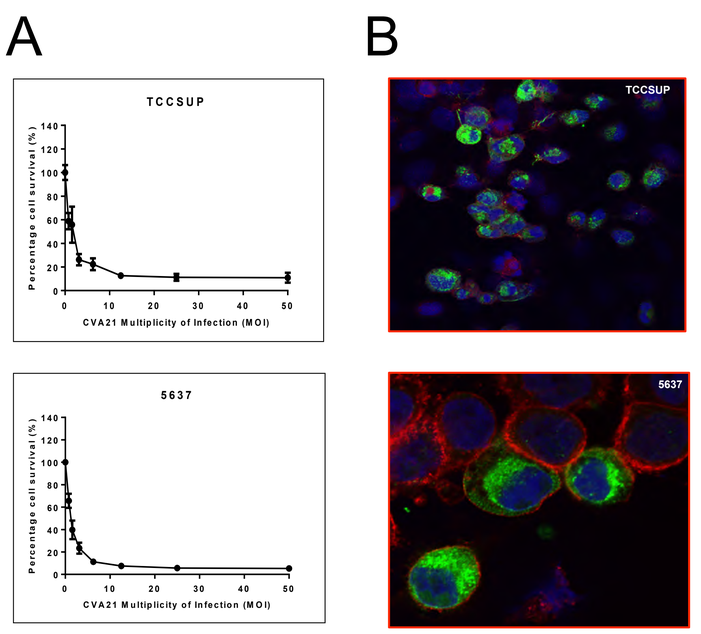


Figure 1. Human bladder cancer cells are highly susceptible to lytic infection by CVA21. (A) Monolayer cultures of human bladder cancer cells were challenged with increasing multiplicities of CVA21 and assessed for cell survival at 72h post-infection, with live cells being quantified by MTS assay. **(B)** Confocal images of human bladder cancer cell lines 24h post CVA21 infection (Green: CVA21 viral proteins, red: wheat germ agglutinin, blue: TO-PRO-3).

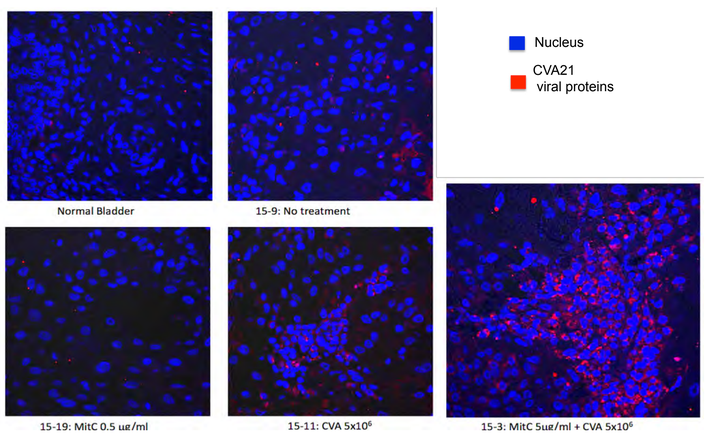
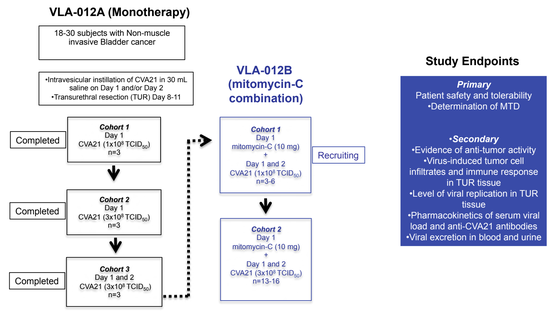


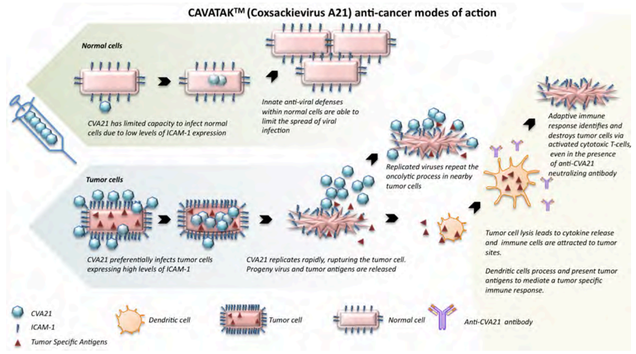
Figure 2. Enhanced CVA21 replication in combination with mitomycin C in *ex-vivo* human bladder tumor. Tissue tumor sections from a patient with NMIBC cut with a vibrating microtome were challenged with CVA21 (5 x 10^6 $TCID_{50}$) in the presence or absence of mitomycin C (5μg/ml) Immunofluorescence staining for CVA21 viral protein was performed 48hrs post infection. Viral infections are visualized by the bright red staining with the blue colour indicating DAPI stained nuclei of the cells

Study Design

VLA-012 (CANON study): Phase I intravesicular ***CA***VATAK in subjects ***NON***-muscle invasive Bladder cancer



Mode of Action



Preliminary Data

Table 1. Patients and treatment Characteristics

Cohort	Patient Identification Code	CVA21 Dose ($TCID_{50}$) Day 1	CVA21 Dose ($TCID_{50}$) Day 2	Mitomycin C Dose (Day 1 only)	Gender	ECOG at Screening	Tumour Assessment at TUR-BT
A1	01-B001	1 x 10^8	-	none	Female	0	G2 (high grade) papillary urothelial carcinoma
	01-B003	1 x 10^8	-	none	Male	0	pTa G2 TCC
	01-B004	1 x 10^8	-	none	Female	0	G3 TCC with sarcomatoid component, at least pT3
A2	01-B005	3 x 10^8	-	none	Male	0	G2 low grade papillary
	01-B006	3 x 10^8	-	none	Male	0	pT1 G3 papillary
	01-B007	3 x 10^8	-	none	Male	0	Papillary TCC
A3	01-B008	3 x 10^8	3 x 10^8	none	Male	0	(not reported yet)
	01-B009	3 x 10^8	3 x 10^8	none	Male	0	Ta G1 TCC
	01-B010	3 x 10^8	3 x 10^8	none	Male	0	pTa G3 TCC
B1	01-B011	1 x 10^8	1 x 10^8	10 mg	Male	0	(not reported yet)
B2	01-B012	1 x 10^8	1 x 10^8	10 mg	Male	0	(not reported yet)

Table 2. Product-related Adverse events*

AE Terminology	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Tight feeling, left side of abdomen	1 (10%)	-	-	-	-
Shivers/cold feeling	1 (10%)	-	-	-	-

Current treatment-related adverse events [n (% of patients)] for patients in Cohorts 1-3.

Figure 3. Tumor Response: Pre and post treatment cystoscopy

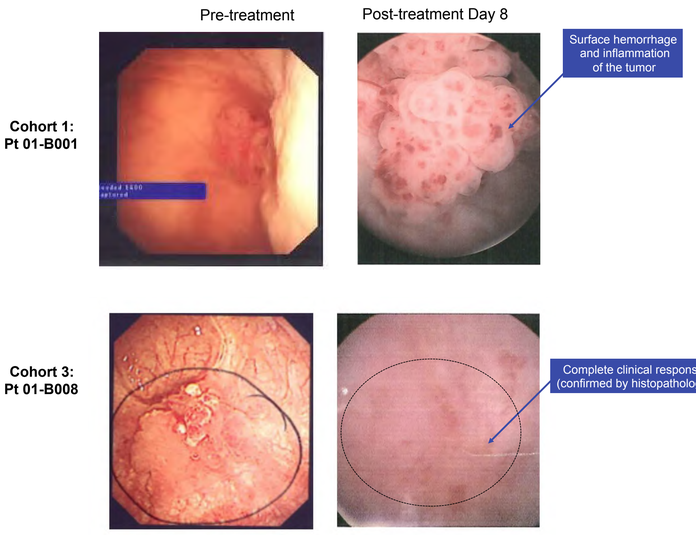
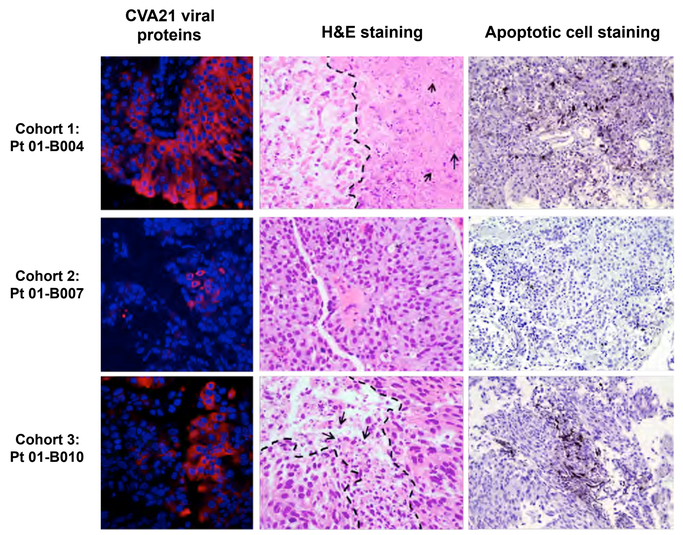
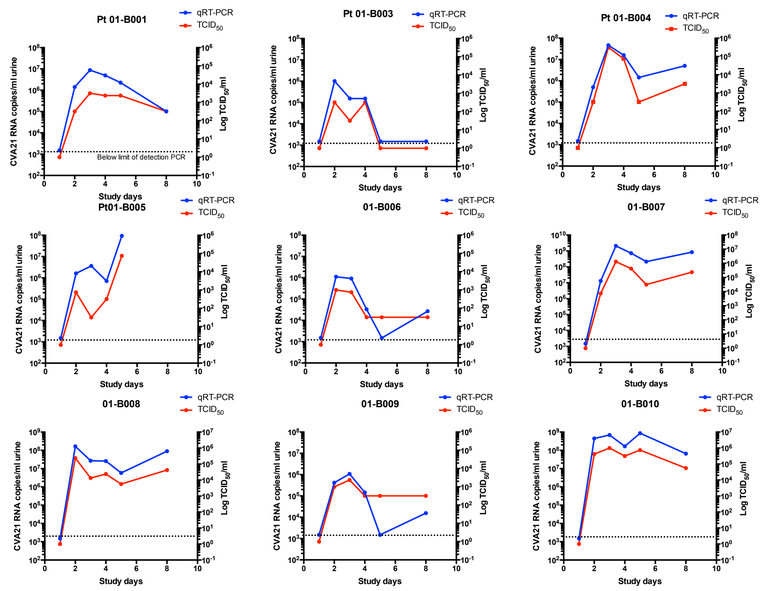


Figure 4. Levels of CVA21 cytoplasmic replication and viral-induced apoptosis in transurethral resection tissue



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.

Figure 5. Levels of CVA21 viral RNA and live virus in patient urine following intravesicular CVA21 administration



Conclusions

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

Future Directions

- Immune infiltrate characterization to define the CVA21-induced local mucosal immune induction
- Phase II neo-adjuvant study with patients administered CVA21 via the intravesicular route prior to TURBT against patients with TURBT alone. Recurrence-free survival as primary endpoint.
- Phase Ib/II study with patients administered CVA21 in combination with immune checkpoint blockade.

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NHS Royal Surrey County Hospital NHS Foundation Trust

Leeds Institute of Molecular Medicine

The Institute of Cancer Research

Viralytics Developers of Oncolytic Immunotherapies