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

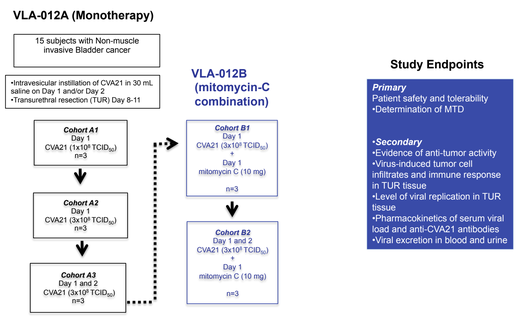
HS Pandha[1], N Annels[1], G Simpson[1], A Iqbal[1], D Mansfield[2], SS Sandhu[1], AA Melcher[3], KJ Harrington[2], B Davies[4], G Au[4], M Grose[4], H Mostafid[1] and D Shafren[4]

[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 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 NIMBC cancer cells . Combining CVA21 with either radiotherapy or chemotherapy (mitocycin C) synergistically enhances cytotoxicity *in vitro* in bladder cancer cell lines. Low (non-cytotoxic) doses of mitocycin C enhances CVA21 viral replication and oncolysis by increasing expression levels of ICAM-1 on bladder cancer cells . In this two stage Phase I study, patients with NMIBC received 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 investigated the tolerance of multiple escalating intravesicular doses of CVA21 in 16 first-line NMIBC cancer patients.

Study Design



Results

Cohort	Patient Identification Code	CVA21 Dose ($TCID_{50}$) Day 1	CVA21 Dose ($TCID_{50}$) Day 2	Mitomycin C Dose Day 1 only	Age	Gender	Pathology Finding at TUR
A1	01-B001	1×10^8	-	-	62	F	G1 pTa papillary urothelial carcinoma
	01-B003	1×10^8	-	-	62	M	G2 pTa transitional cell carcinoma
	01-B004	1×10^8	-	-	77	F	G3 transitional cell carcinoma with sarcomatoid component, at least pT3
A2	01-B005	3×10^8	-	-	83	M	G3 pTa low grade papillary transitional cell carcinoma
	01-B006	3×10^8	-	-	63	M	G3 pT1 papillary bladder cancer
	01-B007	3×10^8	-	-	50	M	G3 pTa papillary
A3	01-B008	3×10^8	3×10^8	-	67	M	no malignant cells present
	01-B009	3×10^8	3×10^8	-	58	M	G1 Ta papillary transitional epithelium
	01-B010	3×10^8	3×10^8	-	77	M	G3 pTa transitional cell carcinoma
B1	01-B011	1×10^8	1×10^8	10 mg	73	M	G2 (high and low grade) pTa transitional cell carcinoma
	01-B012	1×10^8	1×10^8	10 mg	68	M	G2 pTa transitional cell carcinoma
	01-B013	1×10^8	1×10^8	10 mg	51	F	G1 pTa transitional cell carcinoma
B2	01-B015	3×10^8	3×10^8	10 mg	67	M	G3 pTa papillary transitional cell carcinoma with focal CIS
	01-B016	3×10^8	3×10^8	10 mg	61	M	G3 pTa transitional cell carcinoma
	01-B017	3×10^8	3×10^8	10 mg	56	M	G2 pTa transitional cell carcinoma

Table 1. Patients and treatment Characteristics

		Related to CAVATAK n(%)				
MeDRA System Organ Class	MeDRA Preferred Term	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Gastrointestinal disorders	Abdominal distension	1 (1%)	-	-	-	-
	Nausea	1 (1%)	-	-	-	-
General disorders/administration site conditions	Chills	1 (1%)	-	-	-	-

Table 2. Product-related Adverse events

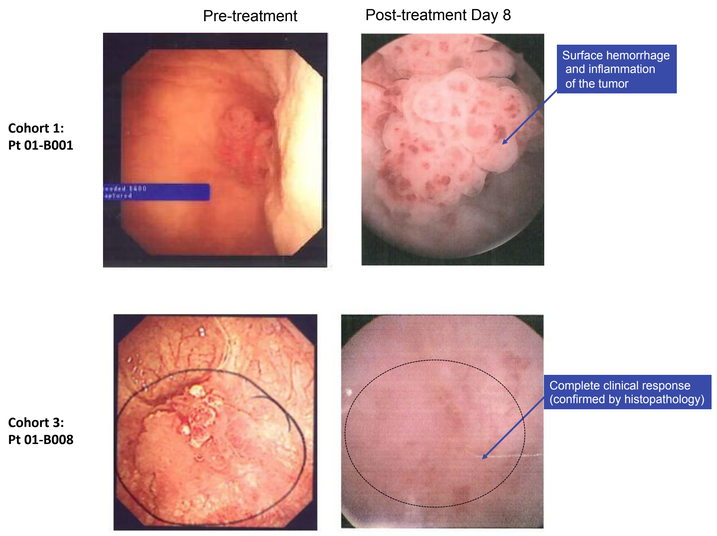
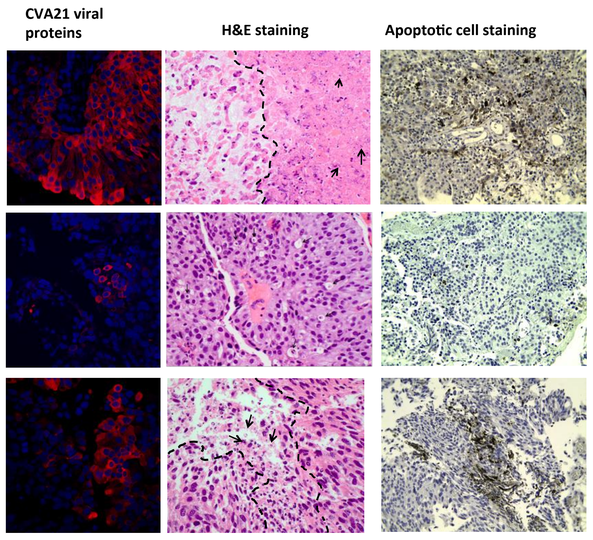


Figure 1. Tumor Response: Pre and post treatment cystoscopy



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

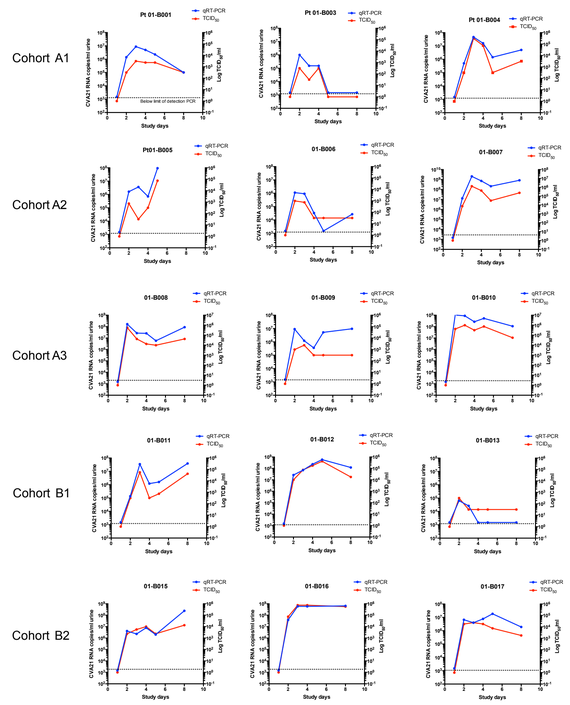


Figure 3. Levels of CVA21 viral RNA and live virus in patient urine following intravesicular CVA21 administration

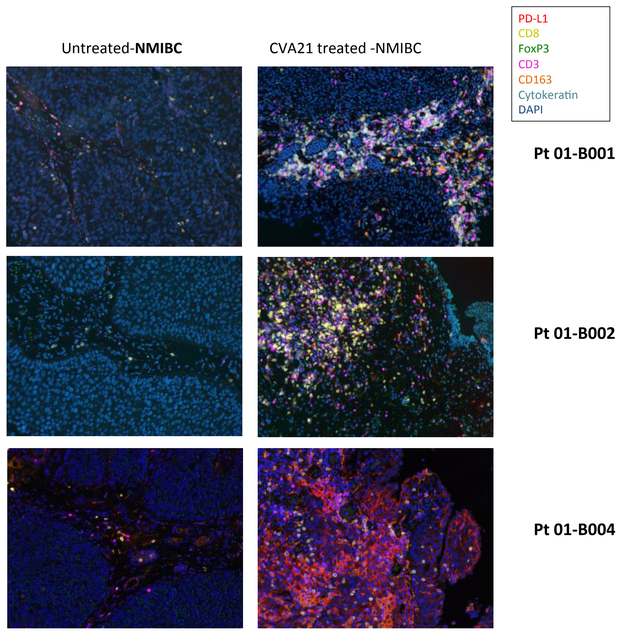
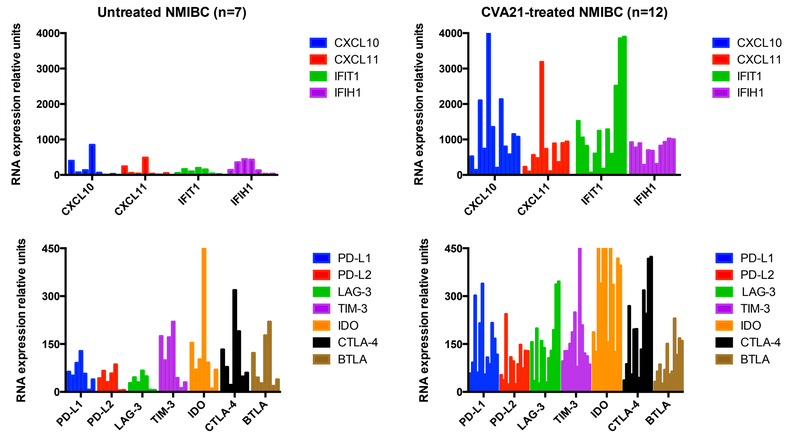


Figure 4. Coxsackievirus A21-induces immune cell infiltration in the micro-environment of NMIBC tissue assessed by multi-spectral IHC analysis

Results

Interferon-induced genes CXCL10, CXCL11 and viral RNA response genes IFIH1, IFIT1

Immune checkpoint inhibitory molecules



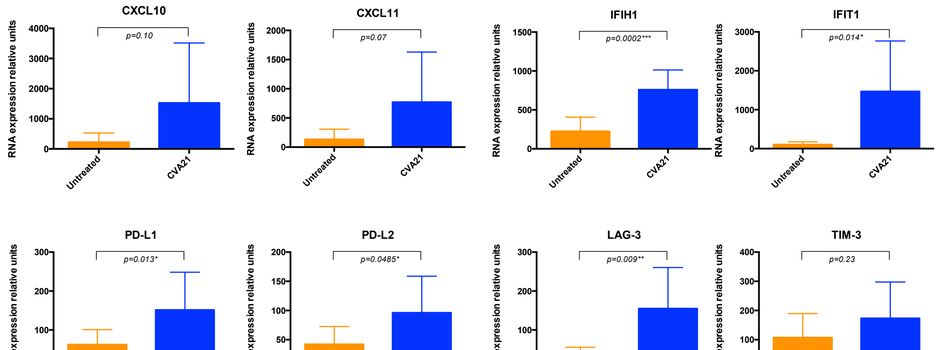


Figure 5. Coxsackievirus A21 injection up-regulates interferon-induced genes and immune checkpoint molecules within the micro-environment of NMIBC tissue (*NanoString analysis Pan Cancer Immune Profiling) Panel*)

Conclusions

- 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 (***Figure 1***), viral mediated cell apoptosis ***(Figure 2)***, viral replication (infectious virus increases in urine***, Figure 3***) 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 (***Figure 4***).
- CVA21 treatment up-regulated a number of interferon-response and immune checkpoint inhibitory genes in NMIBC biopsy tissue, including CXCL10, CXCL11, PD-L1, PD-L2, IDO and LAG-3 (***Figure 5***).
- 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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