Major synergy between Coxsackievirus A21 (CAVATAK ™) and radiotherapy or chemotherapy in bladder cancer, due to up-regulation of viral receptors ICAM-1 & DAF.

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Introduction:

Between 70 and 85% of patients with bladder cancer present with exophytic disease that is confined to the epithelium or invades only the lamina propria (Kirkali et al. 2005). Non-muscle invasive bladder cancer (NMIBC) is managed by transurethral resection followed, in high risk patients, by adjuvant intravesical therapy. Intravesical therapy has been based on live bacille Calmette-Guerin (BCG) for some years, and whilst chemotherapy agents such as mitomycin C and gemcitabine have shown some efficacy, they have failed to supplant BCG (Shelley et al. 2004). Furthermore up to two thirds of these patients suffer recurrence or a new bladder tumour within five years (Sylvester et al. 2006) and approximately 15% will ultimately die from bladder cancer. As this is a clinical setting in which local live biological therapy is already well established, it presents intriguing opportunities for oncolytic virotherapy. Coxsackievirus A21 (CVA21) has recently been shown to be an efficient oncolytic agent that specifically targets and rapidly lyses human malignant melanoma, (Shafren et al. 2004; Au et al. 2005), myeloma (Au et al. 2007), prostate cancer (Berry et al. 2008) and breast cancer which express high levels of the CVA21 cellular uptake receptors both *in vitro* and *in vivo*. In a current Phase II clinical trial in late stage melanoma patients, intralesional CAVATAK treatment has demonstrated activity in both injected lesions and non-injected distant lesions, while generally being well tolerated.

Aims: To test the combination treatment of CVA21 with either radiotherapy or chemotherapy on bladder cancer cell lines. To understand possible mechanisms underlying any synergy.

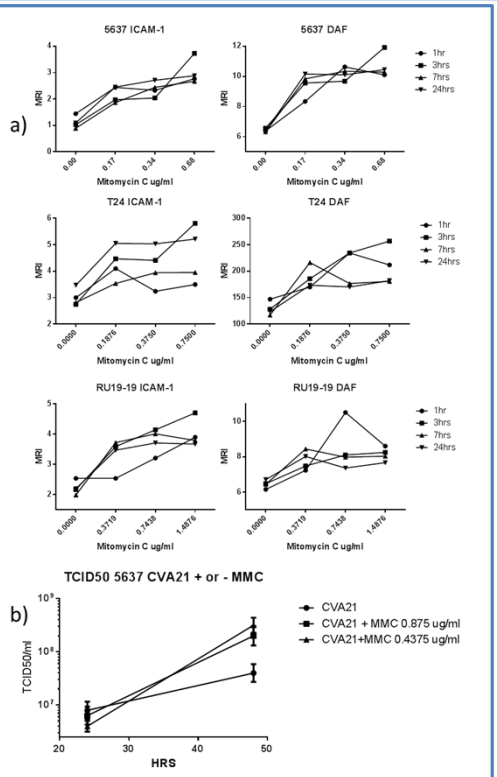
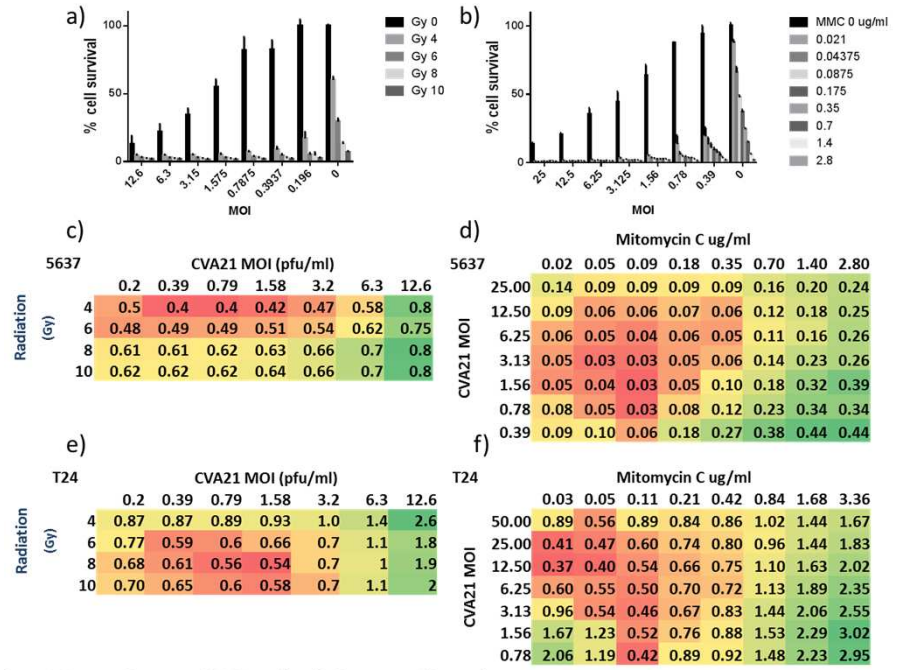


Figure 2 Up-regulation of expression of viral receptors ICAM-1 & DAF in bladder cancer cell lines after exposure to Radiotherapy or Chemotherapy which results in high viral replication :To mimic patient exposure to Mitomycin C (X0.5 fold IC50 x1, X2) we pulsed T24, Ru19-19 and 5637 cells with drug for 1, 3, 7, 24 hrs and ICAM-1 and DAF expression was measured by FACS analysis at 24 hrs. We concluded from the results that ICAM-1 and DAF expression was strongly amplified after only a short pulse (1-3hrs) of Mitomycin C on all three bladder cancer cell lines (Figure 2a). Exposure to Mitomycin C also enhanced viral replication (Figure 2b)



5637	CVA21 MOI (pfu/ml)						
Radiation (Gy)	0.2	0.39	0.79	1.58	3.2	6.3	12.6
4	0.5	0.4	0.4	0.42	0.47	0.58	0.8
6	0.48	0.49	0.49	0.51	0.54	0.62	0.75
8	0.61	0.61	0.62	0.63	0.66	0.7	0.8
10	0.62	0.62	0.62	0.64	0.66	0.7	0.8

5637	Mitomycin C ug/ml							
CVA21 MOI	0.02	0.05	0.09	0.18	0.35	0.70	1.40	2.80
25.00	0.14	0.09	0.09	0.09	0.09	0.16	0.20	0.24
12.50	0.09	0.06	0.06	0.07	0.06	0.12	0.18	0.25
6.25	0.06	0.05	0.04	0.06	0.05	0.11	0.16	0.26
3.13	0.05	0.03	0.03	0.05	0.06	0.14	0.23	0.26
1.56	0.05	0.04	0.03	0.05	0.10	0.18	0.32	0.39
0.78	0.08	0.05	0.03	0.08	0.12	0.23	0.34	0.34
0.39	0.09	0.10	0.06	0.18	0.27	0.38	0.44	0.44

T24	CVA21 MOI (pfu/ml)						
Radiation (Gy)	0.2	0.39	0.79	1.58	3.2	6.3	12.6
4	0.87	0.87	0.89	0.93	1.0	1.4	2.6
6	0.77	0.59	0.6	0.66	0.7	1.1	1.8
8	0.68	0.61	0.56	0.54	0.7	1	1.9
10	0.70	0.65	0.6	0.58	0.7	1.1	2

T24	Mitomycin C ug/ml							
CVA21 MOI	0.03	0.05	0.11	0.21	0.42	0.84	1.68	3.36
50.00	0.89	0.56	0.89	0.84	0.86	1.02	1.44	1.67
25.00	0.41	0.47	0.60	0.74	0.80	0.96	1.44	1.83
12.50	0.37	0.40	0.54	0.66	0.75	1.10	1.63	2.02
6.25	0.60	0.55	0.50	0.70	0.72	1.13	1.89	2.35
3.13	0.96	0.54	0.46	0.67	0.83	1.44	2.06	2.55
1.56	1.67	1.23	0.52	0.76	0.88	1.53	2.29	3.02
0.78	2.06	1.19	0.42	0.89	0.92	1.48	2.23	2.95

Figure 1 Synergy between CVA21 and radiotherapy or Chemotherapy : Combining CVA21 with radiotherapy has shown exceptional synergy, when 5637 cells were irradiated then 24 hours later exposed to CVA21 (Figure 1a). We have also shown enhanced synergy between Mitomycin C and CVA21 on 5637 cells (Figure 1b). Synergy was seen to a less iextent between CVA21 and other chemo agents Gemcitabine and Pirubicin (data not shown). All synergies between radiation or chemotherapy agents and CVA21 was confirmed in T24 cells (Figure 1c). For this work we have implemented a comprehensive experimental and analytical method which allows calculation of combination index values at all data points, and therefore identification of areas of high synergy across the whole response surface (Greco et al. 1995) (Figure 1c,d,e,f). **By Loewe criteria, additivity is denoted by a CI of 1, synergy by values less than 1 and more than 1 is denoted antagonistic.**

References

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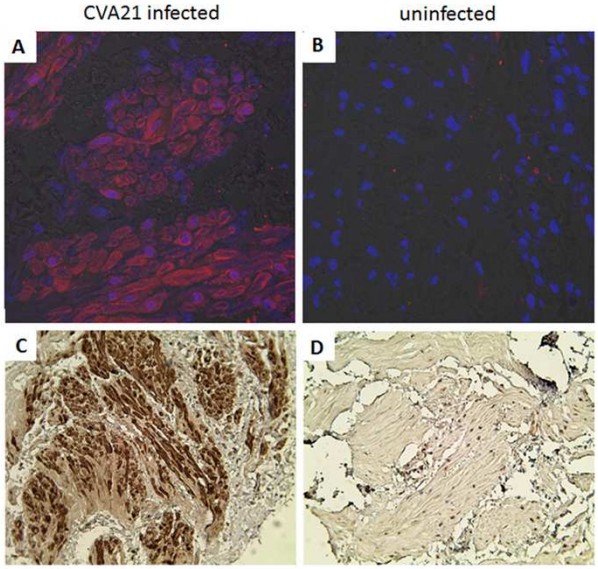

Figure 3 Ex-vivo human bladder tumour tissue is highly infectable by CVA21. Tissue pieces originating from the same human bladder tumour were either infected with CVA21 or left uninfected. Immunofluorescence and enzymatic staining for coxsackie virus was performed 48hrs post infection. Viral infections are visualized by the bright red staining in A (the blue colour shows the DAPI stained nuclei of the cells) and by the brown 3,3'-Diaminobenzidine (DAB) staining in C. No positive viral staining was observed in the uninfected bladder tumour tissues (B and D).

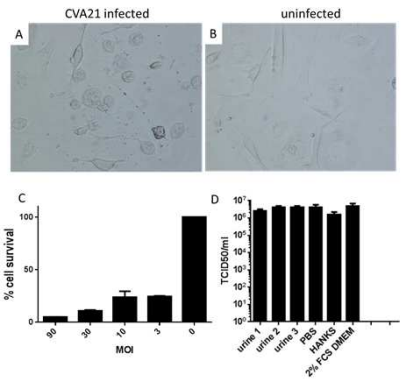

Figure 4 Patient derived bladder tumour cell line is highly infectable by CVA21. Coxsackie virus A21 is stable in human urine. Human cancer bladder tissue was disaggregated and primary tumour cells were isolated. These were tested for bladder tumour markers (Cytokeratin 7) (data not shown). Primary tumour cells were infected at varying MOIs and incubated at 37^{0}C for 72hrs then photographed and analysed by MTS assay. A) CVA21 MOI 3 B) Uninfected cells C) MTS assay. D) CVA21 (3e6 pfu) was incubated at 37^{0}C for 1hr in healthly donor urine. Resulting virus was titred by TCIID^{50} on SK-MEL-28 cells for 5days.

CONCLUSIONS:

- Combining CVA21 with either radiotherapy or chemotherapy synergistically enhances cytotoxicity in bladder cancer cell lines.
- Radiation and chemotherapy enhanced CVA21 viral replication and oncolysis, likely caused by increased expression of the viral receptors ICAM-1 and DAF.
- Ex-vivo human bladder tumour material and primary derived cell lines are highly infectable by CVA21
- These results offer strong support for translational clinical trials of CVA21 plus chemotherapy or radiotherapy that have been initiated in the clinic.