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

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Introduction: Between 70 and 85% of patients with bladder cancer present with asymptomatic disease that is confined to the urothelium or invades only the lamina propria (Kirkali et al. 2000). Noninvasive invasive bladder cancer (NMIBC) is managed by transurethral resection followed, in high risk patients, by adjuvant intravesical therapy, intravesical instillation of Bacillus Calmette-Guérin (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 to two-thirds of these patients recur a new bladder tumour within five years (Sylvester et al. 2005) and approximately 15% will ultimately die from their disease. As this is a clinical trial in which local live biological therapy is already well established, it presents intriguing opportunities for oncolytic virotherapy. Coxackievirus A21 (CVA21) has recently shown to be an efficient oncolytic agent that specifically targets and rapidly lyses human malignant melanomas (Shafren et al. 2004; Au et al. 2005), myeloma (Au et al. 2007), prostate cancer (Berry et al. 2005) and breast cancer which expresses high levels of the CVA21 cellular uptake receptors both in vitro and in vivo. In addition, a Phase I clinical trial in late stage melanoma patients has recently been completed, and has demonstrated that intratumorally administered CVA21 is well tolerated in humans, and that 55.5% of patients experienced stabilization or reduction in injected tumour volumes, leading to a phase II trial in this setting. An intravesical delivery that has also come completed, 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.

Expression of ICAM-1 & DAF: The cellular uptake of coxsackievirus A21 is upregulated to be mediated by intercellular adhesion molecule-1 (ICAM-1) and decay accelerating factor (DAF, CD55) acting as a cooperative sequestration of the virus (Figure 1). Aims: To assess the cellular expression of ICAM-1 and DAF in a bladder cancer cell line panel (Figure 1). All bladder cell lines tested exhibited ICAM-1 expression except RT4 cells (Figure 1). Nearly the resistant cell lines RU19-19 and VMCU-1 (Figure 2a) also demonstrate ICAM-1 expression, suggesting that other phenotypic features of resistance may need to be explored for future patient treatment.

Synergy between CVA21 and Chemotherapy: CVA21 is an effective oncolytic virus in three bladder cancer cell lines, T24, 5637 and TCCSUP-1 with typical ED50 values of 5.8 ± 1.7 and 2.0 ± 0.2 fold respectively (Figure 2b). Combining CVA21 with the chemotherapy agents Mitomycin C and Gemcitabine has shown good synergy. Using a fixed ratio design, we found, from the 30 to the 90% to effect levels, combination index values of 0.30 - 0.55 with Mitomycin C (Figure 2c). Preliminary data using the same method has found from the 50% to the 90% to effect levels, combination index values of 0.99 ± 0.01 with Gemcitabine (Figure 2b).

Synergy between CVA21 and radiotherapy: Combining CVA21 with the radiotherapy has shown exceptional synergy. When 50% cells were irradiated (4 – 10 Gy) then 24 hours later exposed to CVA21 (multiplicity of infection 0.5X – 12.6X), clear synergy was seen (Figure 3a). Dose matrix analysis showed that combination indices reached minima of approximately 0.4 (Figure 3b). Synergy between radiation and CVA21 was confirmed in T24 cells (Figure 3c). For this work we have implemented a comprehensive experimental and analytic 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). Pharmacol Au, (2005). Int Korkis (2005). Urology 71(6): 4736-4743. Sylvester (2006). European urology 66(6 Suppl 1): 4-34.

CONCLUSIONS:
- Combining CVA21 with either radiotherapy or chemotherapy synergistically enhances cytotoxicity in bladder cancer cell line.
- Radiation and chemotherapy enhanced CVA21 oncolysis, likely by increased viral receptors ICAM-1 & DAF expression.
- These results offer strong support for translational clinical trials of CVA21 plus chemotherapy or radiotherapy.

Figure 1 Surface expression of ICAM-1 (CD54) and DAF (CD55) in bladder cell line panel.

Figure 2 a) The effect of the combination of CVA21 and chemotherapy on cell proliferation as assessed by calculating combination index (CI) values using CalcuSyn software (Biosoft).

Figure 3 Combination index (CI) values for single fraction radiation and CVA21 in bladder cancer cell lines T24 and 5637. CI values are calculated using CalcuSyn software (Biosoft). Synergy by values less than 1.