# Investigation of Potential Oncolytic Effects of Replication-Competent Coxsackievirus A21 (CVA21) in Combination with Chemotherapy on Various Lung Carcinomas

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## Abstract

Research into oncolytic virotherapy has advanced significantly and progressed past the stage of laboratory testing to the point of large-scale human clinical trials. Coxsackievirus A21 (CVA21), a replication-competent positive-sense single stranded RNA enterovirus is one such example. CAVATAK™ is the name of a proprietary formulation of CVA21 currently being evaluated in Phase II trials in patients with malignant melanoma. We have recently investigated the feasibility of using CVA21 for the treatment of lung cancer. Clinically, the use of CVA21 in combination with standard chemotherapeutic agents may be a more effective method of destroying cancer cells than with single agents administered as mono-therapies. This prompted us to study the combinatorial effects of first-line chemotherapeutic agent docetaxel and oncolytic virus CVA21 on lung cancer cell lines. We tested the sensitivity of a range of non-small cell lung cancer (NSCLC) cells to CVA21 in combination with docetaxel.

Of the four NSCLC cell lines tested, we found that CVA21 and docetaxel were synergistic in three of the cell lines (H157, A549 and NCI-H1299) with the fourth cell line showing additive effects (NCI-H838). Viral growth curve studies of CVA21 in combination with docetaxel found that viral replication was comparable irrespective of docetaxel co-administration. A preclinical study using NCI-H1299 tumour xenografts confirmed that the administration of CVA21 as a single agent, or in combination with docetaxel was successful in controlling the progression of the disease. These studies on the combinatorial effects of docetaxel with CVA21 will further enhance our understanding in providing an effective treatment for lung cancer.

## Conclusion

- Preliminary data suggests that exposure to docetaxel increases ICAM-1 receptor expression in certain NSCLC cell lines.
- Our results showed that the highest level of cell death was induced when cells were treated with CVA21 and docetaxel simultaneously.
- In our *in vitro* studies, synergism was observed in the combined treatment of CVA21 and docetaxel in three out of four NSCLC cell lines tested.
- CVA21 replication was not inhibited in NSCLC cells treated with the maximum concentration of docetaxel attainable in human serum.
- Combination of CVA21 and docetaxel treatment *in vivo* resulted in complete tumour remission.
- Tumour shrinkage was observed in mice even with the presence of anti-CVA21 neutralising antibodies.
- CVA21 in combination with docetaxel displays potent oncolytic activity against human NSCLC cells in both *in vitro* and *in vivo* environments.
- These findings suggest that docetaxel in combination with the oncolytic immunotherapy agent CVA21 may be a promising candidate for lung cancer therapy in a clinical setting.

## Methods & Results

### #1 ICAM-1 receptor expression after docetaxel exposure on a panel of NSCLC cell lines

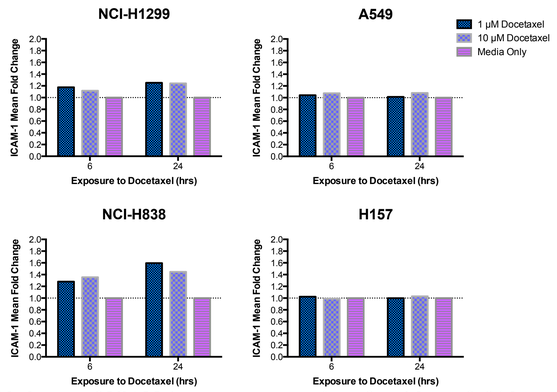


**Figure 1. Mean fold change of ICAM-1 receptor expression after 6 hour & 24 hour exposure to docetaxel.** A panel of lung cells were examined by flow cytometry for the expression of ICAM-1 receptors after being exposed to two different concentrations of docetaxel for 6 and 24 hours. Graph shows the fold change in ICAM-1 mean fluorescence following FACS analysis. In some cell lines, there was a slight increase in ICAM-1 expression following 6 and 24 hours of exposure to docetaxel.

### #3 Combinatorial effects of first-line chemotherapeutic agent, docetaxel and oncolytic virus, CVA21 on NSCLC cell lines *in vitro*

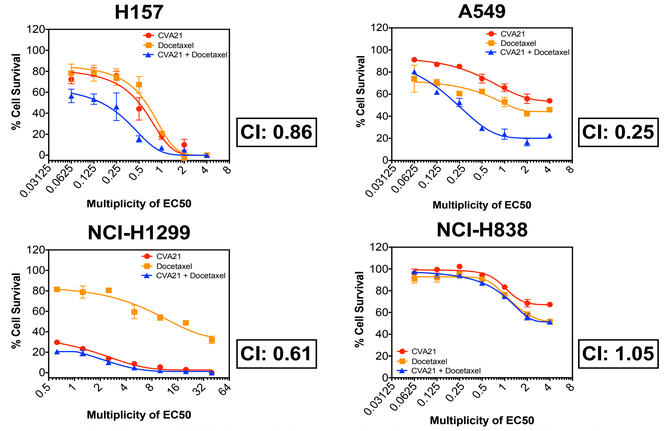


**Figure 3. Combinatorial effects of CVA21 and docetaxel in four NSCLC cell lines.** Drug concentrations were maintained at a constant ratio to each other except for cell line NCI-H1299 where CVA21 was given at a ratio of 10:1 of docetaxel. Combination index (CI) values > 1, = 1, <1 indicates antagonistic, additive and synergistic respectively. The CI values proved synergism between CVA21 and docetaxel in the cell lines H157 (CI = 0.86), NCI-H1299 (CI = 0.61) and A549 (CI = 0.25); but only nearly additive in the cell line NCI-H838 (CI = 1.05).

### #5 Combination of docetaxel and CVA21 therapy on NCI-H1299 lung cancer xenografts *in vivo*

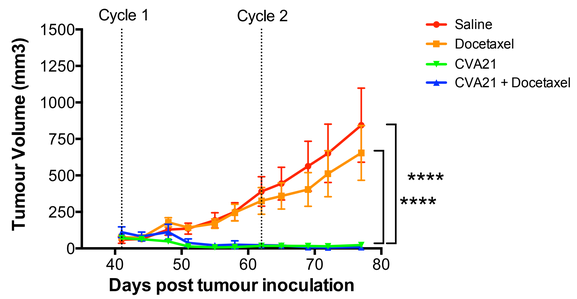


**Figure 5. Tumour volumes (mm³) following treatment with either saline; CVA21; docetaxel; or combination of CVA21 + docetaxel.** Tumour volumes in the saline and docetaxel treated groups continued to escalate for the duration of this study, while CVA21 and combination of CVA21 + docetaxel treated mice had no palpable tumours from Day 55 onwards. Based on a two-way ANOVA analysis, the decreases in tumour volume for both the CVA21 and combination of CVA21 + docetaxel treatment group were statistically significant compared to the saline and docetaxel treated groups ($P < 0.0001$ at Day 77 for CVA21 or CVA21 + docetaxel vs saline and/or docetaxel). No significant difference between CVA21 and combination of CVA21 + docetaxel treated groups.

### #2 Determination of optimum timing for the combination of CVA21 & docetaxel treatment on NSCLC cell lines

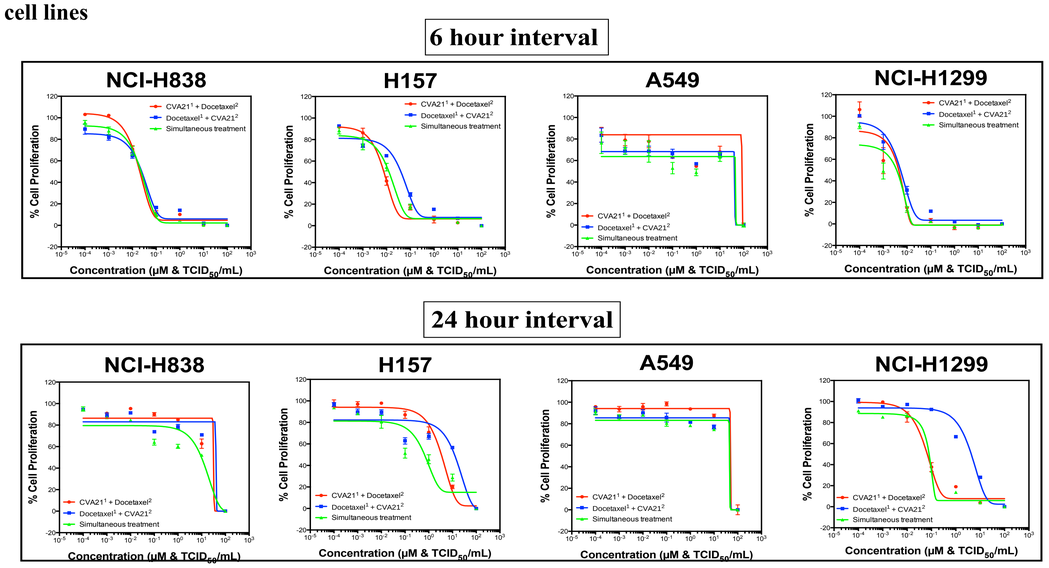


**Figure 2. Dose response curve of a panel of NSCLC cells pre-sensitized with either CVA21 or docetaxel before the administration of the other respective combination treatment agent at a 6 hour and 24 hour interval.** Analysis of results revealed that all four cell lines had a lower percentage of cell proliferation (more cells killed) when both CVA21 and docetaxel were administered simultaneously. This is followed by cells pre-sensitized with CVA21 at both time intervals with the exception of cell line A459 which showed that pre-sensitization to docetaxel led to higher levels of cell death for both the 6 hour and 24 hour time point.

### #4 Effect of docetaxel on CVA21 replication in NSCLC cell lines

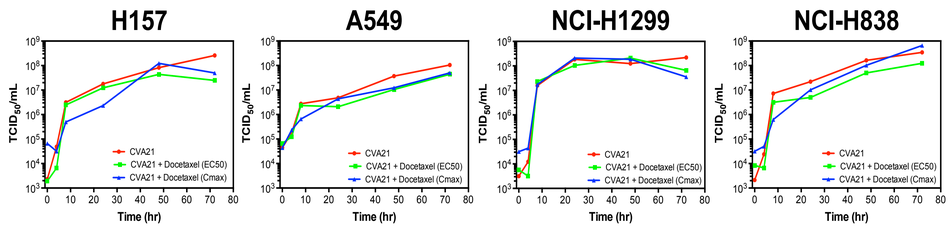


**Figure 4. Effect of docetaxel on CVA21 replication within four NSCLC cell lines.** docetaxel (EC50) represents an effective concentration value that inhibits cell proliferation by 50%. These values are dependent on individual cell lines (data not shown). While docetaxel (Cmax) is the maximal concentration of docetaxel attainable in human serum (approximately 5µM). CVA21 was able to replicate exponentially within the first 48 hours post-infection while in presence of docetaxel, with continuous replication at 72 hours post-infection in cell lines A549 and NCI-H838.

### #6 Post-treatment neutralising antibody levels to CVA21 in NCI-H1299 lung cancer xenograft mice

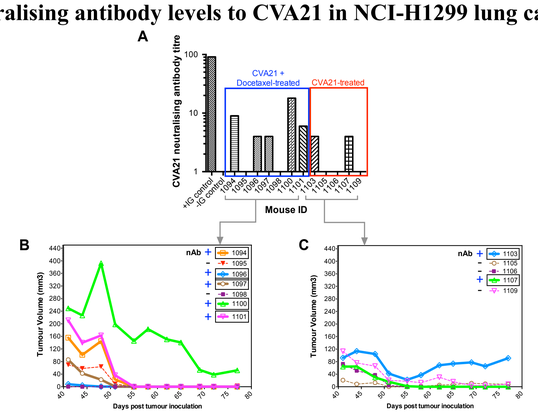


**Figure 6. Presence of neutralising antibodies (nAb) in serum following treatment with CVA21 alone; and with CVA21 in combination with docetaxel.** A) Neutralising antibody levels found in circulation following treatment with either CVA21 in combination with docetaxel or CVA21 alone. B) Individual tumour volume data of CVA21 + docetaxel treated mice. C) Individual tumour volume data of CVA21 treated mice. Animals that are positive for nAb are enclosed within a black box and labeled with a "+" symbol in the legend. In both treatment groups, clear signs of tumour regression were observed even in the presence of anti-CVA21 nAb levels.