# Immune response to an Oncolytic Human Picornavirus, Coxsackievirus A21 (CAVATAK™) in Patients with Late Stage Melanoma

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## Introduction

During numerous clinical evaluations of oncolytic viruses (virotherapy), the host immune system is constantly questioned as to playing a "friend or foe" role in potential anti-cancer efficacy outcomes. The development of viral-specific neutralising antibodies and activated immune cells can potentially hinder viral spread both in the systemic circulation and in the localised tumour environment, thereby reducing oncolytic activity. In contrast, activated immune cells have the capacity to destroy virally infected tumour cells and in the process, undergo stimulation from surrounding self generated tumour specific antigens leading to further potential anti-tumour activity. CAVATAK™ (Coxsackievirus A21), is a naturally occurring human common cold picornavirus that targets and destroys susceptible cells via specific capsid interactions with surface expressed virus receptors, comprising intercellular adhesion molecule-1 (ICAM-1) and decay-accelerating factor (DAF). In general, the expression of these biological targets is up-regulated on the surface of a number malignant cancer cells relative to surrounding benign tissue. CAVATAK™ exhibits a high oncolytic capacity against numerous *in vitro* cultures of human cancer cells and ICAM-1/DAF expressing *in vivo* xenografts of such cells [1,2,3,4]. Against this background, during a Phase I dose-escalation clinical trial in late stage malignant melanoma patients undertaken primarily to assess the safety and tolerability of intratumoural injections of CAVATAK™ , we assessed host immune responses following viral administration.

## Trial Design

**Objectives:**
The primary aim was to assess the efficacy and safety of two escalating intratumoural doses of CAVATAK™ in 9 patients with stage IV melanoma. A secondary objective of this study was to evaluate the host immune response to 2 intratumoural injections (administered 48 hours apart) of CAVATAK™ in terms of examining serum for levels of anti- CAVATAK™ specific neutralising antibody and a panel of immuno-regulatory cytokines.

**Methodology:**
Phase I, single centre, open-label study to assess the efficacy and safety of two escalating doses of CAVATAK™ injected intratumourally into a single lesion in 9 patients with stage IV melanoma.

**Diagnosis and main criteria for inclusion:**
Patients with stage IV metastatic melanoma with at least one subcutaneous lesion (longest diameter, 2-5 cm) aged at least 18 years who had failed or refused standard chemotherapy. The patients serum anti- CAVATAK™ specific neutralising antibody had to be less than 1:16 and a biopsy of the tumour to be injected had to express detectable cellular expression of ICAM-1and DAF ***(Table 1)***.

**Test product, dose and mode of administration:**
Doses of CAVATAK™ ($1.0 \times 10^7$ and $1.0 \times 10^8$ $TCID_{50}$) were diluted to 10% tumour volume in saline and administered into one subcutaneous metastatic nodule measuring 2 – 5 cm in diameter on two occasions (48 hours apart) for each three patients. Recruitment for the final 3 patients to be administered two injections of $1.0 \times 10^9$ $TCID_{50}$ CAVATAK™ is ongoing.

## Results and Conclusions

•CAVATAK™ administration induced generation of neutralising levels of serum specific anti-viral antibodies in 5 of 6 patients by ~day 10 post-injection, the production of which appeared not to be dose related ***(Figure 1)***;

•Preliminary data in ***Figure 2*** indicates that 1 of 3 patients in Cohort I and all patients in Cohort II displayed some elevation in serum levels of Th1 related response cytokines (IL-12, γ-IFN and GM-CSF). Overall, patients Pt03 (Cohort I) and Pt06 (Cohort II) exhibited the strongest Th1 cytokine response.

•Intratumoural delivery of CAVATAK™ induced some reduction in the volume of the injected tumour of 2 of 6 patients (Pt03, Pt06) at 24 days post CAVATAK™ administration ***(Table 4)***.

•As these reductions in the volume of the injected tumours occurred at a time when both patients possessed high levels of serum neutralising anti- CAVATAK™ antibody ***(Figure 2 )***, the inhibitory of role systemic anti-viral antibody in the localised tumour environment must be questioned.

•Interestingly, both the patients (Pt03, Pt06) that displayed higher serum levels of the Th1-related cytokines (IL-12, GM-CSF and γ-IFN, ***Figure 2***) also displayed volume reductions in the injected tumour at 24 days post CAVATAK™ administration ***(Table 4)***. Such cytokine responses may have been generated via host immune cells directly challenging CAVATAK™ infected tumour cells and/or tumour cells alone.

## References

(1) Shafren, DR., *et al. Systemic Therapy of Malignant Human Melanoma Tumors by a Common Cold-Producing Enterovirus, Coxsackievirus A21.* Clinical Cancer Research Vol. 10, 53-60, January ,2004;
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## Trial Sponsors

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**Disclosure**
Darren Shafren is a Director of Viralytics Ltd.
Darren Shafren, Susanne Johansson and Gough Au are Viralytics shareholders

| | Patient | Injection Dose ($TCID_{50}$) | Number of injections | Age | Stage of disease | Tumour volume (cm3) Pre-injection | Tumour CAVATAK receptor expression at screening | | Anti-CAVATAK neutralising antibody titre at baseline |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | ICAM-1 | DAF | |
| Cohort I | Pt01 | $10^7$ | 2 | 69 | IV | 2.72 | +++ | + | 1:4 |
| | Pt03 | $10^7$ | 2 | 65 | IV | 33.99 | ++ | ++ | <1:4 |
| | Pt04 | $10^7$ | 2 | 58 | IV | 1.95 | +/++ | ++ | <1:4 |
| Cohort II | Pt05 | $10^8$ | 2 | 57 | IV | 18.85 | ++ | + | <1:4 |
| | Pt06 | $10^8$ | 2 | 64 | IV | 28.10 | ++ | + | 1:10 |
| | Pt07 | $10^8$ | 2 | 81 | IV | 8.02 | + | ++ | <1:4 |

**Table 1. Trial patient baseline characteristics.** Tumour Volumes were calculated from ultrasound measurements using the formula for an ellipsoid: $V=4/3\pi(A/2xB/2xC/2)$, where A=tumour diameter, B=tumour width and C=tumour height. CAVATAK™ cellular receptor expression was determined by immuno-histochemistry staining. Anti- CAVATAK™ antibody levels were determined by employing standard cell-based serum neutralisation assays.

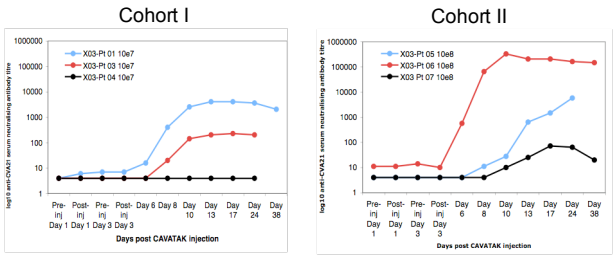


**Figure 1. Levels of serum anti- CAVATAK™ specific neutralising antibody.** Anti- CAVATAK™ antibody levels were determined by employing standard cell-based serum neutralisation assays. The positive/negative cut-off level for protective levels of Anti- CAVATAK™ antibody is suggested to lie between an antibody titre of ~1:4 and 1:16

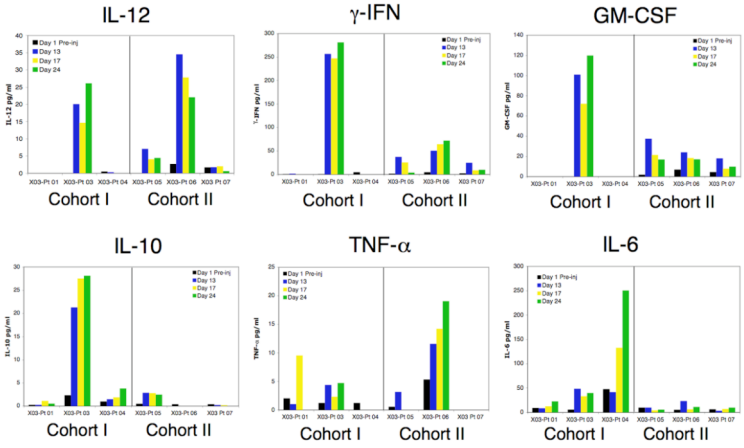


**Figure 2. Levels of serum cytokines following intratumoural administration of CAVATAK™.** Serum samples were analysed for levels of IL-1ß*, IL-6, IL-10, IL-12p70, TNF-α, IFN-γ, granulocyte macrophage colony-stimulating factor (GM-CSF) using the Bio-Plex suspension array system (Bio-Rad Laboratories, Hercules, CA). *, No significant level of IL-1ß was detected in any sample.

| | Patient | Injected tumour volume* (ultrasound) Pre-screening | Pre-injection | PI Day 13 | PI Day 24 | PI Day 52 | PI Day 87 |
|---|---|---|---|---|---|---|---|
| Cohort I | Pt01 | 57.9 | 100.0 | 145.5 | 108.1+ | N/A | N/A |
| | Pt03 | 63.2 | 100.0 | 100.0 | 70.9 | N/A | N/A |
| | Pt04 | 80.6 | 100.0 | 104.9 | 145.9 | N/A | N/A |
| Cohort II | Pt05 | 78.4 | 100.0 | 105.9 | 140.5 | 351.3 | N/A |
| | Pt06 | 93.7 | 100.0 | 111.0 | 46.8 | 103.9 | N/A |
| | Pt07 | 94.1 | 100.0 | 115.0 | 98.1 | N/A | N/A |

*, % Changes relative to tumour volume on the day of first injection ;
+, Early termination visit, Day 31;
N/A= Not available;
PI= post-injection

**Table 2. Percentage change in the injected tumour volumes following intratumoural administration of CAVATAK™.** Tumour Volumes were calculated from ultrasound measurements using the formula for an ellipsoid: $V=4/3\pi$ (A/2xB/2xC/2)Where A=tumour diameter, B=tumour width and C=tumour height.