CAVATAK™ (Coxsackievirus A21) displays potent oncolytic activity in BRAF^{V600E} mutant melanoma cells resistant to selective BRAF kinase inhibitors

D.R Shafren[1,2], M. Farrelly[3], A.J Croft[3], B. Davies[2], J. Stewart[2], R. Ingham[2], M. Y. Quah[1], G. Au[1,2], and P. Hersey[3]

[1] The Picornaviral Research Unit, The School of Biomedical Sciences, Faculty of Health, University of Newcastle, Newcastle, NSW 2300, Australia;
[2] Viralytics Ltd., Suite 1B, 55-63 Grandview St., Pymble NSW 2073, Australia;
[3] Department of Oncology, University of Newcastle, David Madison Building, Newcastle, NSW 2300, Australia

Background

CAVATAK™ is a naturally occurring Picornavirus, which induces mild upper respiratory symptoms during natural infection of humans. CAVATAK™ also displays potent oncolytic activity against both *in vitro* cultures of human cancer cells and against *in vivo* xenografts of human cancers in mouse models of melanoma, prostate cancer, breast cancer and multiple myeloma, all which exhibit high surface intercellular adhesion molecule-1 (ICAM-1) expression. The cellular targeting receptor for CAVATAK™ is ICAM-1. CAVATAK™ is currently in Phase II clinical evaluation in patients with late stage melanoma. Vemurafenib (PLX4032) is a potent inhibitor of a mutant form of BRAF (BRAF^{V600E}) that is found in approximately 50% of late stage melanoma patients. In Phase III clinical studies Vemurafenib significantly increases progression free and overall survival in previously untreated late stage melanoma patients. Despite this significant anti-melanoma activity, the disease of a majority of treated patients eventually become resistant to the activity of Vemurafenib.

Materials and Methods

Cells in vitro

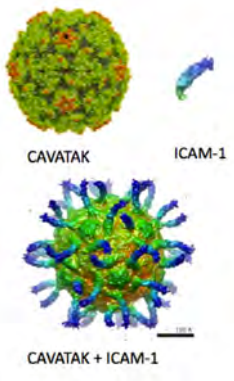
In the present study, two *in vitro* melanoma cell cultures expressing the BRAF^{V600E} mutation were generated by sequential propagation in the presence of increasing concentrations of PLX4720 (1.0mM -10mM) until drug resistant cell populations were selected (CV and RMU) .

Cells in vivo

Cell cultures were generated directly from tumour biopsies of late stage melanoma patients taken prior to treatment and following significant clinical disease progression while on Vemurafenib treatment (JR, JL and EH).

Results and Conclusions

Dose–ranging challenge of mutant BRAF melanoma *in vitro* cultures with CAVATAK™ yielded comparable levels of specific viral mediated oncolysis as evidenced by viral replication (qPCR) and cell lysis analysis. Furthermore, in studies on cell cultures generated directly from tumour biopsies of late stage melanoma patients taken prior to treatment and following significant clinical disease progression while on Vemurafenib treatment CAVATAK™ displayed comparable levels of potent oncolytic activity independent of whether the cell cultures were generated from biopsies taken prior to or following progression on Vemurafenib treatment. Overall, these preliminary findings suggest that systematic melanoma treatment regimes including both Vemurafenib and CAVATAK™ may have favourable clinical outcomes and as such deserve further evaluation.



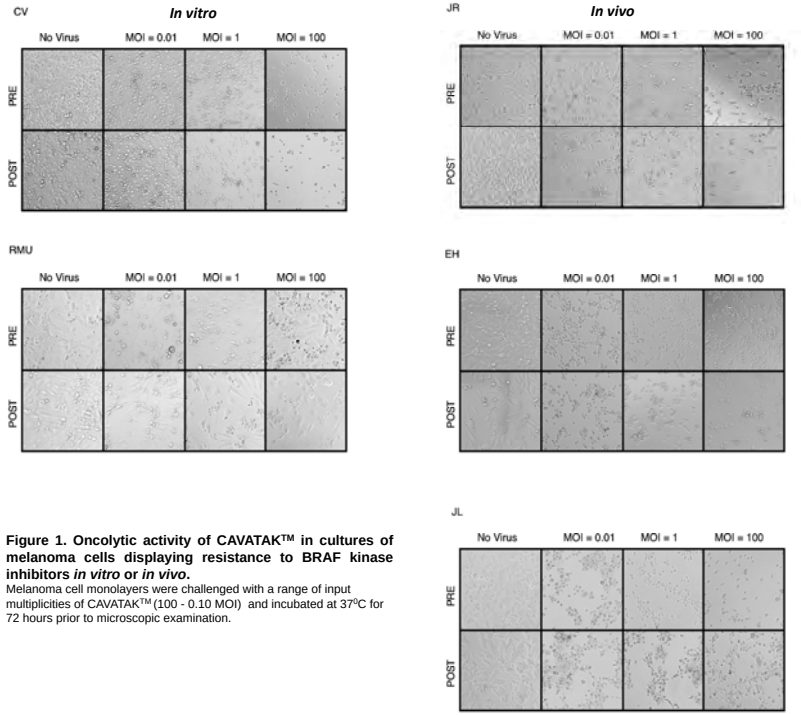


Figure 1. Oncolytic activity of CAVATAK™ in cultures of melanoma cells displaying resistance to BRAF kinase inhibitors *in vitro* or *in vivo*.
Melanoma cell monolayers were challenged with a range of input multiplicities of CAVATAK™ (100 - 0.10 MOI) and incubated at 37^{0}C for 72 hours prior to microscopic examination.

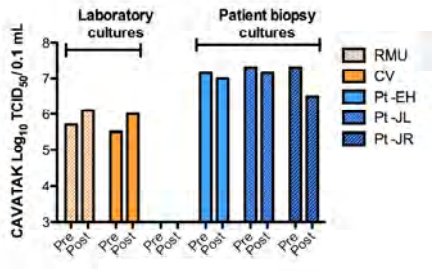


Figure 2. Quantitation of the oncolytic activity of CAVATAK™ in cultures of melanoma cells displaying resistance to BRAF kinase inhibitors *in vitro* or *in vivo*.
Melanoma cell monolayers were challenged with 10-fold dilutions of a CAVATAK™ preparation and incubated at 37^{0}C for 72 hours prior to microscopic examination for the presence of detectable viral-induced cytopathic effect. The infectious titires (TCID$_{50}$/ml) were calculated using the method of Barber.

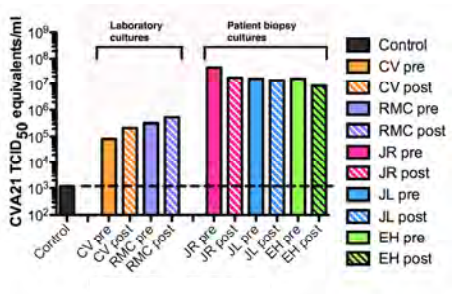


Figure 3. Replication of CAVATAK™ in cultures of melanoma cells displaying resistance to BRAF kinase inhibitors *in vitro* or *in vivo*.
Melanoma cell monolayers were challenged with an input multiplicity of ~ 1.0 MOI of CAVATAK™ and incubated at 37^{0}C for 72 hours prior to assessment of the levels of viral replication by qPCR specific for CAVATAK™ RNA.