

Combined Coxsackievirus A21 Oncolytic Virotherapy and Chemotherapy for the Treatment of Malignant Melanoma



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Abstract

Recent growth in the field of oncolytic virotherapy has led to an increased number of clinical trials and acceptance of tumour selective viruses as a promising cancer treatment strategy. The current clinical approach is for the combination of oncolytic virotherapy with mainstream therapies such as chemotherapy. Our lab has demonstrated that the picornavirus Coxsackievirus A21 (CVA21), has the inherent capacity to preferentially infect and destroy malignant cells bearing the viral receptor intercellular adhesion molecule-1 (ICAM-1). Clinically relevant anti-cancer drugs such as dacarbazine or paclitaxel and carboplatin were tested in combination with CVA21 on five melanoma cell lines. Two pre-clinical mouse melanoma models, an immunodeficient xenograft model and a syngeneic immunocompetent model were established to evaluate the safety and efficacy aspects of the combination therapy.

Methods

Cells:

Sk-Mel-28, Sk-Mel-RM, ME4405, MV3, B16-ICAM-1. All cells were maintained in DMEM with 10% FBS at 37°C with 5% CO₂.

Virus:

Coxsackievirus A21 Kuykendall strain (CAVATAK™) [Viralytics Ltd]

Chemotherapy:

Dacarbazine, Paclitaxel and Carboplatin were obtained from the Department of Clinical Toxicology & Pharmacology (Calvary Mater Newcastle, Newcastle, Australia). Dacarbazine was reconstituted to a concentration of 10 mg/mL by adding 19.7 mL of sterile water for injections. Paclitaxel and carboplatin have been manufactured to its soluble form.

CVA21- and drug- induced cell death and synergistic interactions:

Confluent monolayers of cells were inoculated with serial dilutions of CVA21, dacarbazine (DTIC) or paclitaxel + carboplatin (P + C) and incubated at 37 °C/5% CO₂ for 72 h. Dose-response curves were generated and concentrations killing 50% of cells (EC₅₀) were determined using untreated cells as a control. Virus/drug ratios were kept constant based on the EC₅₀ value calculated previously. Combination indexes values (CI) were calculated to determine whether the combination was synergistic or antagonistic. Cell viability for all treatments was assessed using the MTT assay.

In vivo tumour growth

Sk-Mel-28 cells transfected with the luciferase gene (Sk-Mel-28 luc), were subcutaneously implanted (2 × 10⁵ cells in 100 µL) on the flanks of female SCID mice. Mice received a single intratumoural injection of either PBS or CVA21 (1 × 10⁸ TCID₅₀), DTIC (8 mg/kg) single I.P. injection, paclitaxel (15 mg/kg) + carboplatin (50 mg/kg) injection single I.P., or virus in combination with DTIC or P + C. Mice received a second dose of chemotherapy intraperitoneally on Day 10. Tumour development was monitored on a weekly basis using the Xenogen IVIS100 bioluminescent live imaging system and measured physically using digital callipers twice weekly. This model was repeated in a immune-competent model using B16-ICAM-1 murine cells transplanted on C57/BL6 female mice.

Results

1. Oncolytic effect and chemosensitivity of melanoma cell lines

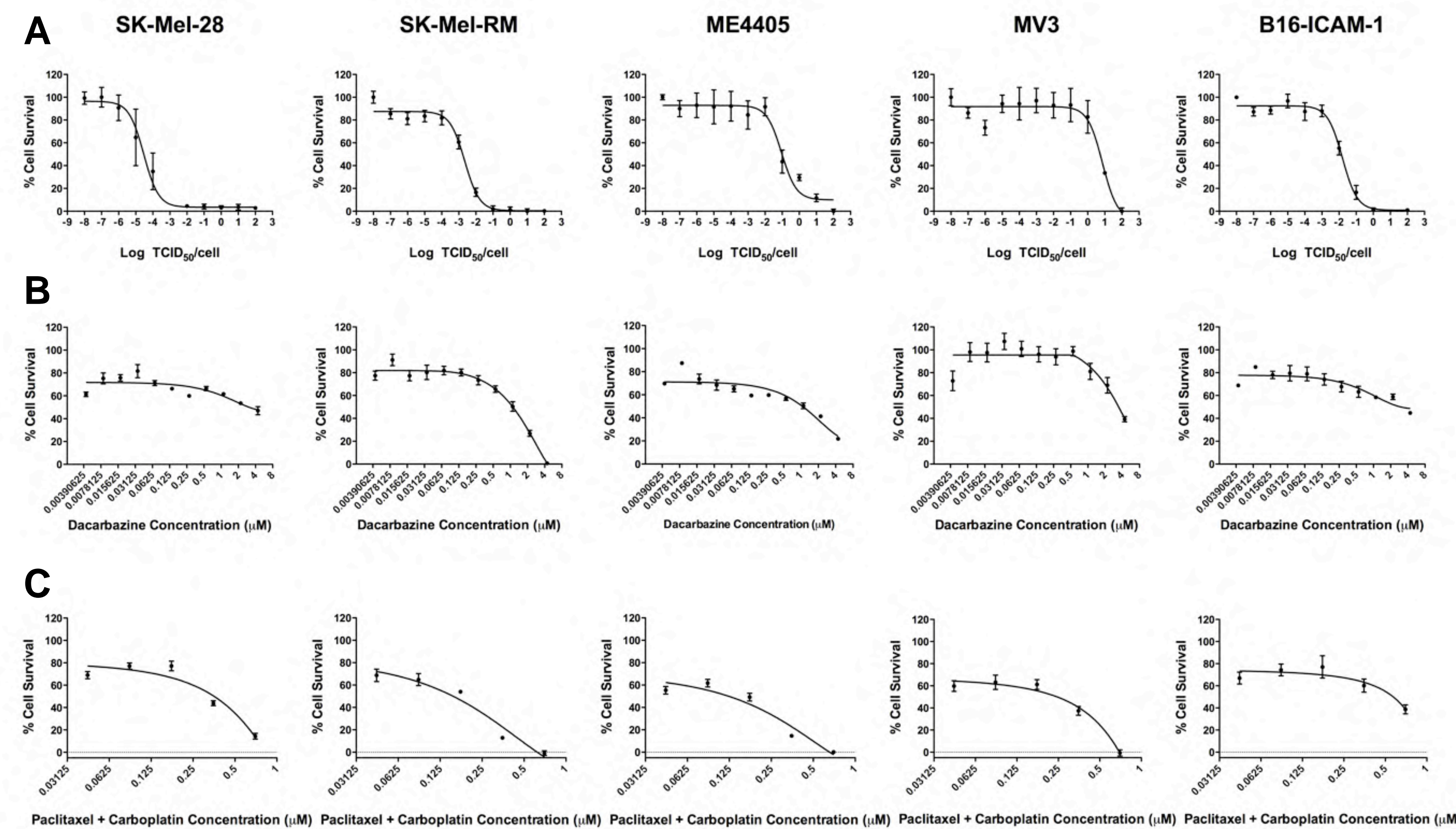


Figure 1. Dose response curves of 4 human cell lines Sk-Mel-28, Sk-Mel-RM, ME4405, MV3 and, one murine cell line B16-ICAM-1 cells treated serial dilutions of CVA21, DTIC and P + C, 72 h post treatment. (A) Oncolytic effect of CVA21 in melanoma cell lines. CVA21 dose is defined as multiplicity of infection (MOI) expressed as tissue culture infectious dose per cell (TCID₅₀/cell). (B) Chemosensitivity of melanoma cell lines to DTIC. DTIC concentrations were expressed in µM. (C) Chemosensitivity of melanoma cell lines to paclitaxel + carboplatin. P+C concentrations were expressed in µM.

2. Combination effects of CVA21 and chemotherapeutics in melanoma cell lines

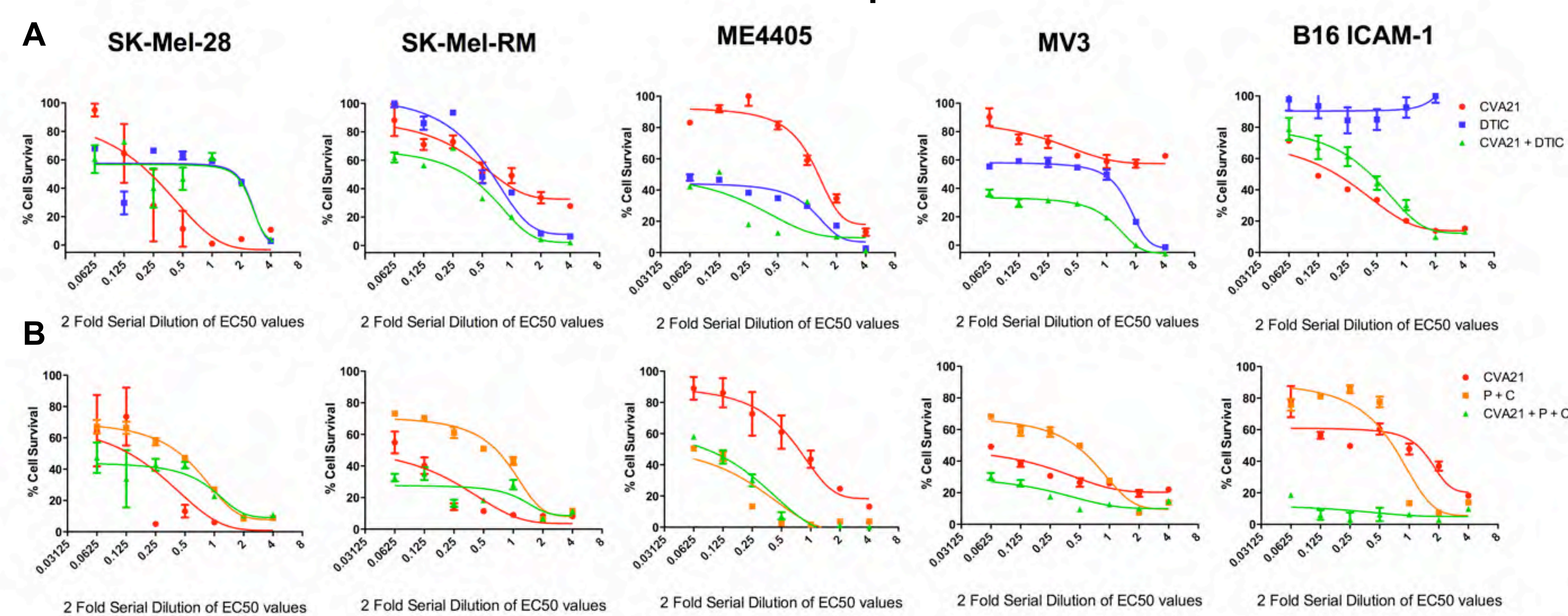


Figure 2. Dose response curves of CVA21 in combination with chemotherapy in melanoma cells. (A) The effects of combining CVA21 with DTIC. The following combination resulted in enhanced killing of Sk-Mel-RM, ME4405 and MV3 cells. (B) The combination effects of CVA21 with paclitaxel + carboplatin. The following combination was generally synergistic on all cell lines at low concentrations except for the Sk-Mel-28 cell line. Enhanced cell death was clearly seen in the MV3 and B16-ICAM-1 cells.

Conclusions

In vitro, the co-treatment of CVA21 with either dacarbazine or paclitaxel + carboplatin was found to be synergistic in most melanoma cell lines.

Using Chou-Talalay's drug combination analysis to generate combination index values, high synergism was observed in two relatively CVA21 insensitive melanoma cell line when treated in combination with paclitaxel + carboplatin.

In vivo, the co-administration of anti-neoplastic drugs did not inhibit the oncolytic activity of CVA21 in an immune-deficient setting as tumour regression were observed in animals treated with CVA21 alone and CVA21 in combination with chemotherapy.

Furthermore, in the presence of an intact host immune system, the co-treatment of CVA21 with chemotherapeutics could significantly inhibit tumour growth by day 28.

3. Combination index value (CI) for CVA21-chemotherapeutic combination regimens

Drugs & Cell Lines	Ratio	Combination Index Value			Dm ¹	m ²	r ³
		EC50	EC75	EC90			
Paclitaxel + Carboplatin							
SK-Mel-28	1:1	1.600	3.244	6.593	0.090	0.527	0.839
SK-Mel-RM	1:1	0.312	1.060	3.713	0.011	0.381	0.765
ME4405	1:1	0.320	0.500	0.727	0.006	0.316	0.651
MV3	1:1	0.058	0.134	0.886	0.001	0.282	0.773
B16-ICAM-1	1:1	6.20E-08	1.70E-05	0.006	1.40E-08	0.155	0.826
Dacarbazine							
SK-Mel-28	1:1	2.759	5.247	11.601	0.317	0.633	0.704
SK-Mel-RM	1:1	0.598	0.535	0.534	0.194	1.119	0.932
ME4405	1:1	0.660	0.589	0.562	0.073	0.805	0.824
MV3	1:1	0.680	0.592	0.533	0.139	3.073	0.788
B16-ICAM-1	1:1	8.085	35.265	190.855	0.321	0.862	0.971

Figure 3. Combination index (CI) values of each cell line treated with CVA21 and chemotherapy. Drug concentrations were maintained at a constant ratio to each other. CI values > 1, = 1, < 1 indicates, antagonism, additive effect and synergism respectively. ¹Dm: The median-effect dose, usually depicted at the EC₅₀ value of the dose effect curve. ²M value: a measurement of sigmoidicity of the dose-effect curve; m = 1, > 1, < 1 indicates hyperbolic, sigmoidal, and negative sigmoidal respectively. ³r value: correlation coefficient; r = 1 perfect conformity, poor r value may be a result of biological variability.

4. Evaluation of CVA21-chemotherapy treatment in an immune-deficient and immune-competent setting

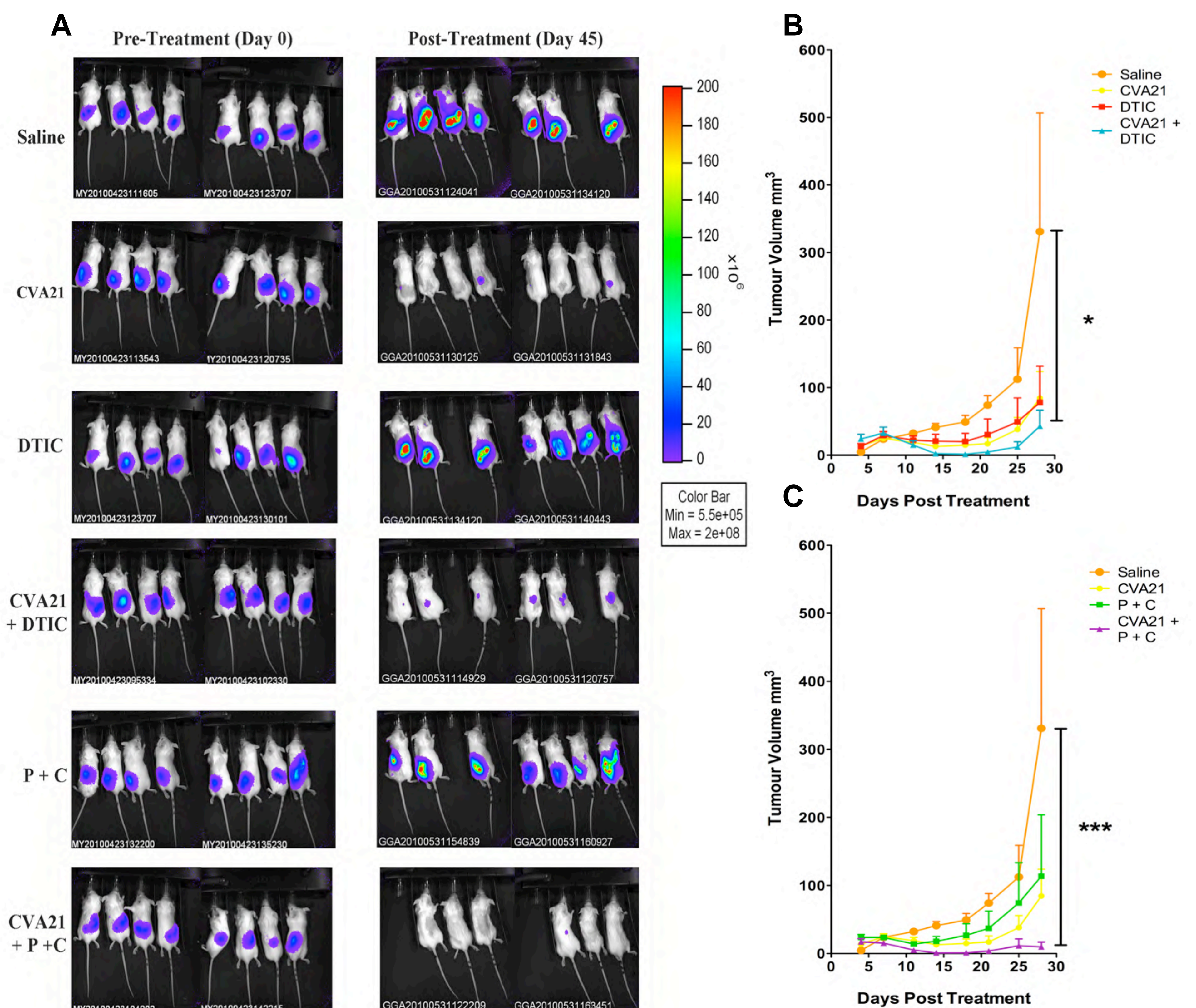


Figure 4. Oncolytic activity of CVA21 in combination with chemotherapy in a model of melanoma. (A) Immune-deficient model: Bioluminescent imaging of Sk-Mel-28 luciferase expressing tumours pre-treatment (Day 0) and 45 days post treatment. Significant regression was observed in mice treated with CVA21, or in combination with chemotherapy. (B & C) Immune-competent model: Significant tumour growth inhibition was noted 28 days post treatment in animals that were administered the combination therapy. All tumour volumes are expressed as the average tumour burdens ± SEM (standard error of mean, n = 8). * denotes statistical significance.