Supplementary Information: In silico trials predict that combination strategies for enhancing vesicular stomatitis oncolytic virus are determined by tumour aggressivity

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Figs. S1 to S5

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Jenner AL, *et al. J Immunother Cancer* 2021; 9:e001387. doi: 10.1136/jitc-2020-001387

SUPPLEMENTARY RESULTS

Virtual individuals parameter distributions

Parameter sets for the *in silico* trial were drawn from normal distributions centered at the fixed values of the 4T1-VV+VSV immune model (Table TS3-TS4). The model was then simulated for each parameter set, and only those virtual individuals whose tumour growth were within two standard deviations of the standard error reported in the data from the experiments were retained in the *in silico* trial. All parameter distributions were confirmed to be normally distributed using the Shapiro-Wilk test[1,2] (Fig. S1). A histogram of the initial number of tumour cells across the cohort is provided in Fig. S2, p-values indicate the results of a Shapiro-Wilk test of normality.

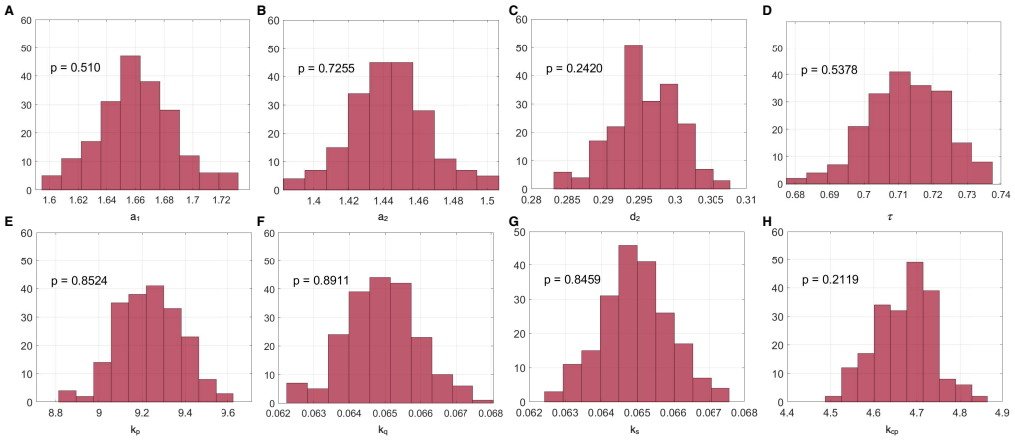

Figure S1. Distribution for the parameter values in the in silico trial. A)-H) *The distribution for the parameters* $a_1, a_2, d_2, \tau, k_p, k_q, k_s, k_{cp}$. *The p-value returned from the Shapiro-Wilk test of normality for each distribution is indicated on the corresponding plots. p-values greater than 0.05 imply that we cannot reject the null-hypothesis of no statistically significant difference between the parameter and normal distributions.*

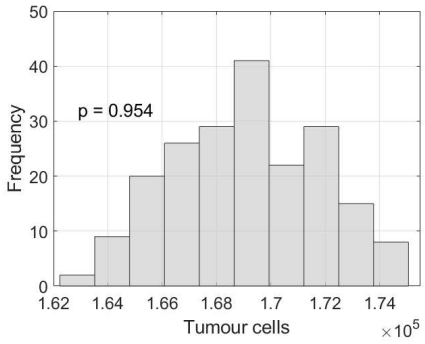

Figure S2. Distribution of the total initial number of tumour cells for the cohort (200 virtual patients) at the start of treatment (day 0). *Patient-specific initial conditions were determined by seeding each virtual patient's initial tumour with* 10^5 *cells and simulating the computational model until day 6, p-value reports the outcome of the Shapiro-Wilk test for normality.*

Sensitivity of tumour growth in 4T1-VV+VSV immune model

We investigated the sensitivity of each of the parameters fit to the 4T1 tumour growth (*4T1/IC*) in immunocompetent (IC) mice (see Technical Supplementary Information) by perturbing their values $\pm 10\%$ (Fig. S3A) and found parameters relating tumour growth to rate cells transit from interphase to active phase (a_2), and immune related parameters including the immune cell-tumour cell contact rate k_p and the maximal immune cell production rate k_{cp} to be the most locally sensitive. This suggests that a stronger immune cell response to the presence of tumour cells can result in natural tumour eradication without treatment which is consistent with the analytical study of the model[3]. Complete tumour eradication was also achieved through manipulating parameters for the cell cycle a_1 and a_2 and the death of quiescent cells d_2.

In the case of fits to 4T1 tumour growth under VV and VSV treatment (*4T1/IC-VV+VSV*), the same parameters a_2, k_p and k_{cp} were again found to be most sensitive (Fig. S3B), suggesting that the underlying tumour growth and immune involvement are major determinants of the treatment outcome, and that viral characteristics are less important. The sensitivity to a_2 is likely due to the fact that cells in the G_1 phase of the model have a constant death rate, so the longer or shorter that they spend in G_1 will impact the number of cells that survive that phase of the cell cycle and go on to reproduce.

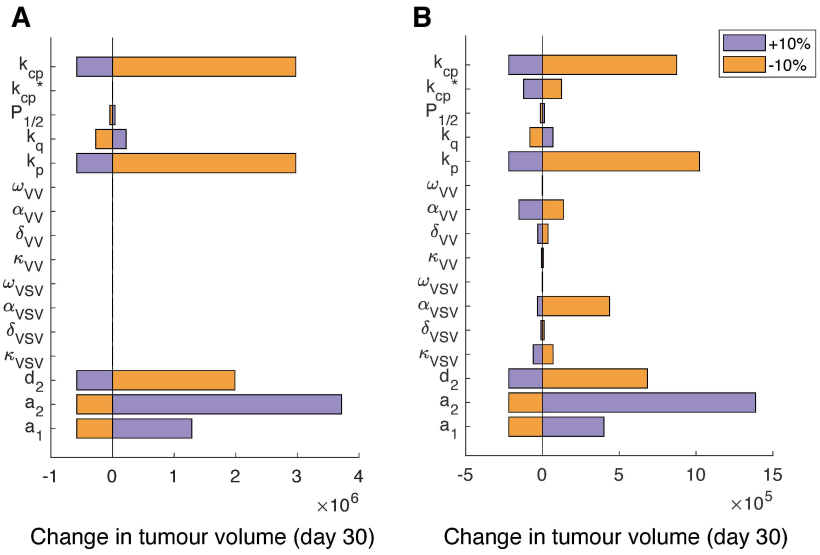

Figure S3. Parameter sensitivity results. *Tornado plot of parameter sensitivity in A) 4T1/IC model (control tumour growth) and B) the 4T1/IC-VV+VSV model (consecutive injections of VV and VSV in 4T1 tumour immunocompetent model). Each parameter was varied by* ±10% *and the resulting change in the tumour volume on day 20 was recorded. Purple bars correspond to an increase of* 10% *and orange bars correspond to a decrease of 10%.*

Tests for significance in the enhancer-multiplicity protocols

The Kolmogorov-Smirnov test, which assesses whether two arbitrary distributions are the same, was used to evaluate the significance between cohort tumour cell numbers after different protocols. The Matlab function *kstest2* was used to test the null hypothesis that the data were from the same continuous distribution (Fig. S4).

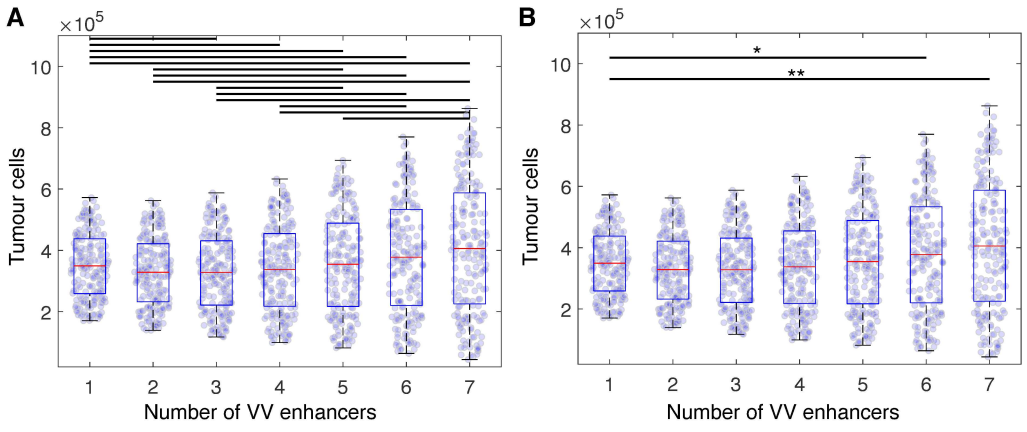

Figure S4. Significance results for the enhancer multiplicity trial. *Tumour growth for the cohort of patients was simulated for 1 to 7 enhancers with a VSV 7 days after the last enhancer. The number of tumour cells was measured for each individual in the cohort 15 days after the VSV. A) Results of the Kolmogorov-Smirnov test for significance, where the significant pairings have been noted* ($p < 0.05$). *B) Result of a two sample t-test, where the significant pairings have been noted. The bar for significance represents the two sample t-test. The test returned a significant p-value 0.005<p*<0.05 and p**<0.0005 for one enhancer and all other enhancers.*

Full results for the enhancer multiplicity investigation

The temporal dynamics for all enhancer multiplicities considered (extension of Fig. 4C in the text) and the survival for the cohort under all enhancer multiplicities considered (extension of Fig. 4D) are provided in Figs. S5A and S5B, respectively.

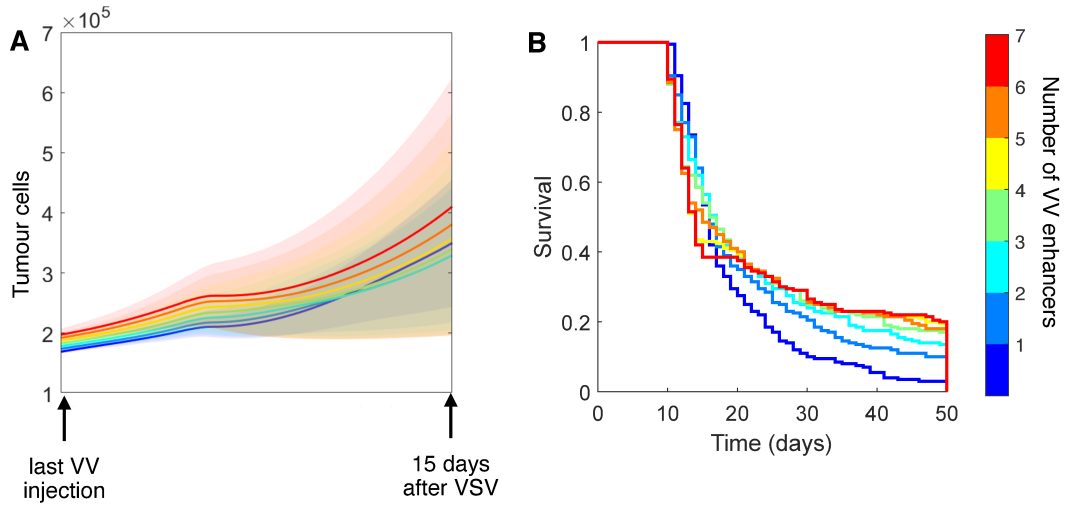

Figure S5. Influence of enhancer multiplicity on tumour growth. *Complementary results to Fig. 4 in Main Text. A) The effects of enhancer multiplicity were investigated by simulating 1-7 enhancers, with a VSV administered 7 days after the final priming dose (statistical significances found in Fig. S4A). Tumour growth was assessed 15 days after the administration of the VSV. B) Kaplan-Meier survival curves for all enhancer multiplicities.*

Tests for significance in the VSV-lag protocols

A two-sample t-test was used to determine whether there was significant difference in the mean outcome for the cohorts under different VSV lags (from 1 day to 15 days). All means were

significantly different for the VSV lag protocols with 1 enhancer. For 7 enhancer VSV lag protocols, a VSV lag of n days and $n + 1$ days was found to not be significant (Fig. S6A). On average, cohort responses were statistically significant for the 1 enhancer VSV-lag protocols (pairwise T-test, $p < 0.05$), whereas we found consecutive VSV lags of n and $n + 1$ days were not statistically significant (pairwise T-test, $p < 0.05$) for the 7 enhancer VSV-lag protocols. Comparing the 1 enhancer and 7 enhancer VSV-lag protocols we found that 1 enhancer protocols with n day VSV-lag and 7 enhancers with an $n + 2$ day VSV-lag were not significantly different (pairwise T-test, $p < 0.05$, Fig. S6B), which suggests that equivalent average responses from a 1 enhancer protocol could be obtained with a 7-enhancer protocol by extending the VSV lag by 2 ± 1 days.

Order of optimal VSV lag for the 1 enhancer protocol

For each patient, the VSV lags from 1 day to 15 days were ordered from most optimal to least optimal based on the tumour size obtained 15 days after the last VSV given only a single enhancer (Fig. S7). Most patients had an optimal VSV lag of 1 day and a least optimal VSV lag of 15 days, implying that it is best to administer the VSV as soon as possible, if only administering a single enhancer. For some patients who had low tumour growth rates r, the optimal VSV lag was 7 days. Overall, for all patients, the least optimal VSV lag was 15 days.

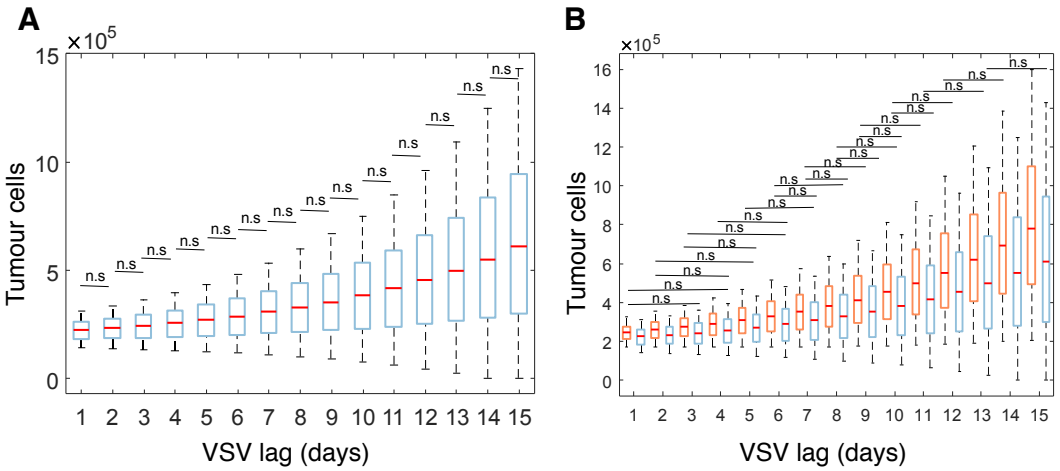

Figure S6. Significance of VSV lag. *A) Results of the two-sample t-test for significance for the 7 enhancer VSV-lag trial, where the non-significant pairings have been noted. The test returns a significant p-value <0.05.*

B) Non-significant pairings of 1 enhancer (orange) and 7-enhancer (blue) VSV-lag protocols comparisons. All other pairings were significant.

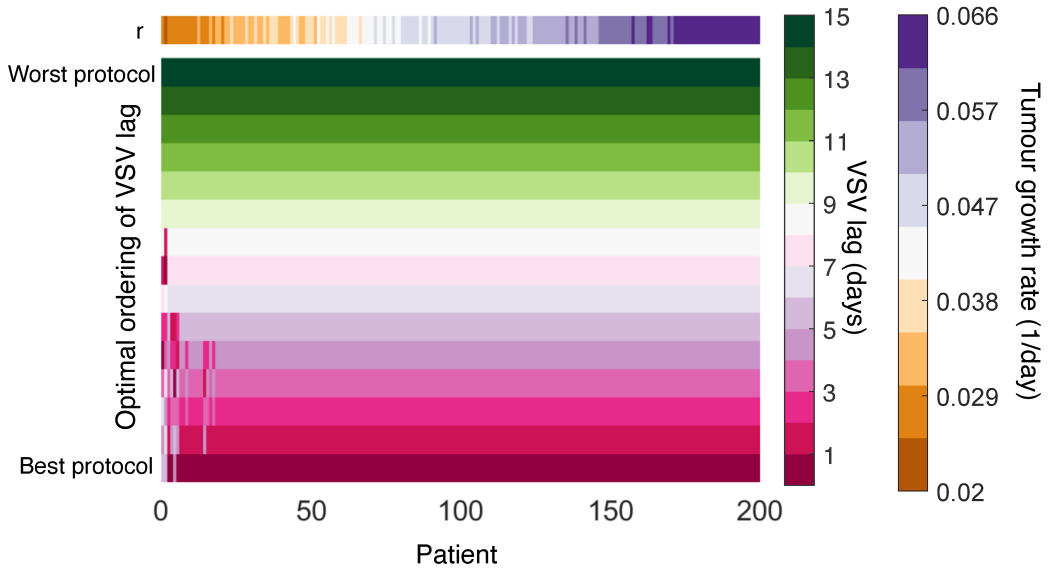

Figure S7. Optimal VSV lag for 1 enhancer protocol. *Ordering of protocols from best (bottom row) to worst (top row) for each patient based on the tumour size 15 days after the last VSV administration for 7 enhancer protocol. Corresponding tumour growth rates are plotted above (patient ordering identical based on intrinsic tumour growth rate as in Fig. 7A in the Main Text.*

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