Immunomodulatory effects of a novel, enhanced potency gibbon ape leukaemia virus (GALV) fusogenic membrane glycoprotein-expressing herpes simplex virus platform with increased efficacy combined with anti PD-1 therapy

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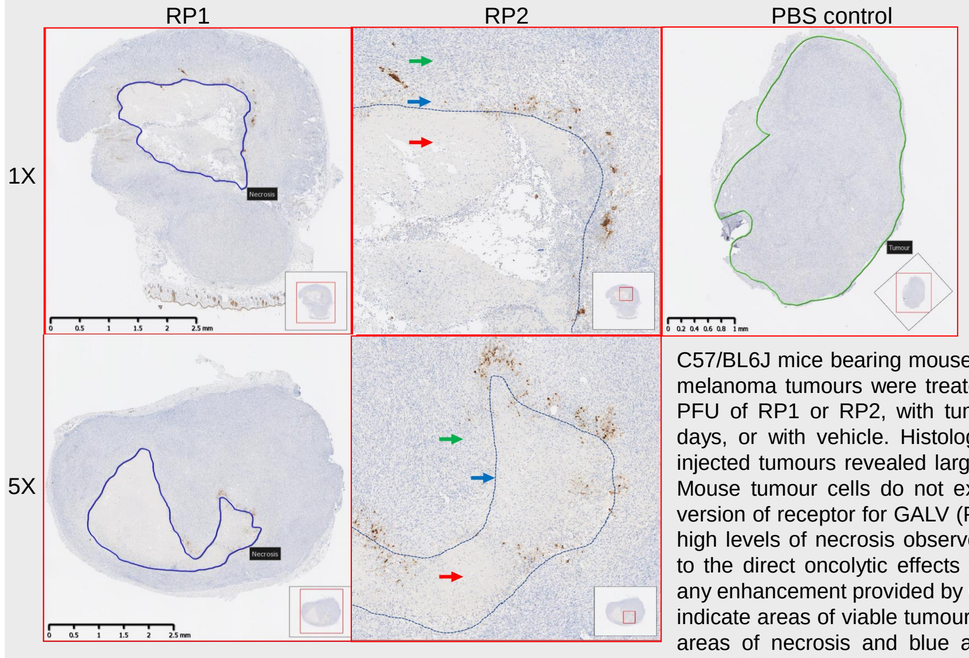
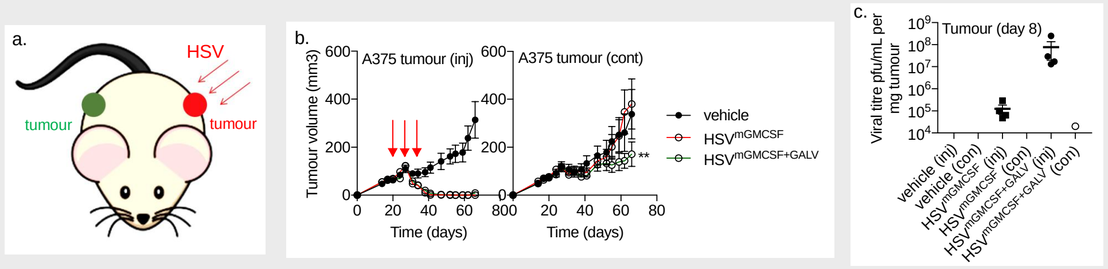
RP1 & RP2 cause large areas of necrosis in syngeneic mouse tumors, even without any effects of GALV (GALV is non-functional in murine cells)



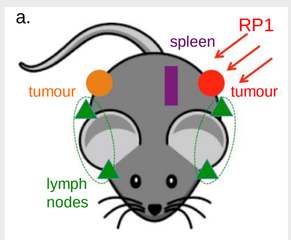
C57/BL6J mice bearing mouse 4434 BRAF mutant melanoma tumours were treated either with $5x10^5$ PFU of RP1 or RP2, with tumors harvested at 3 days, or with vehicle. Histological examination of injected tumours revealed large areas of necrosis. Mouse tumour cells do not express a compatible version of receptor for GALV (PiT1), and hence the high levels of necrosis observed are primarily due to the direct oncolytic effects of the virus, without any enhancement provided by GALV. Green arrows indicate areas of viable tumour, red arrows indicate areas of necrosis and blue arrows indicate peri-necrotic boundary. Brown staining is for HSV.

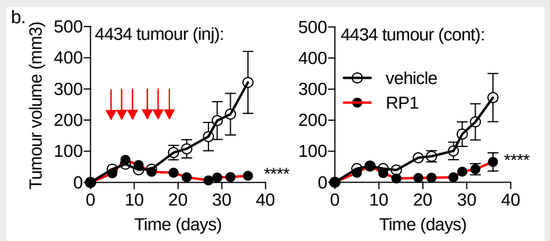
RP1 reduces both injected and contralateral tumours in nude mice, which requires GALV for the uninjected tumor effect



mRP1 ($HSV^{mGM\text{-}CSF+GALV}$) has previously been shown to provide potent abscopal effects in immune competent rodent models (mice & rats). Since a compatible version of the receptor for GALV (PiT1) is not expressed on murine cells, a CD1 nude mice model bearing human A375 bi-flank tumours was used to assess the direct effects of GALV, as well as indirect effects. One flank was injected with either 3 doses of mRP1, HSV^{GMCSF}, i.e. without the GALV insertion, or vehicle (a). Injected tumors were entirely eradicated with both viruses. In uninjected tumors the virus without GALV had no effect, whereas the virus with GALV significantly reduced tumor growth (b). No virus reached contralateral tumours (con), while the injected (inj) tumours treated with $HSV^{GMCSF+GALV}$ had a high viral yield when compared to HSV^{GMCSF} (c). *N = 10* at least per group. The contralateral anti-tumor effects seen with mRP1, but not seen with the virus without GALV, were assumed in this setting (i.e. in nude mice which lack an adaptive immune system) to result from innate immune activation, bearing in mind that virus was not present in these tumors.

RP1 increases infiltration of PD-L1+ cells within tumours and lymph nodes.

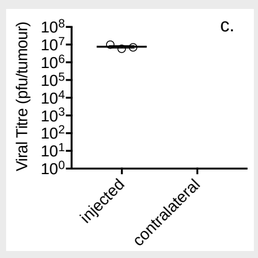


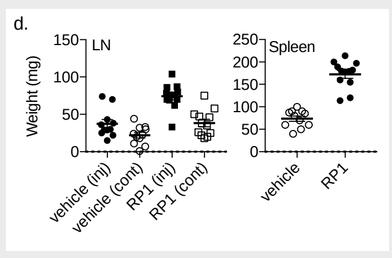


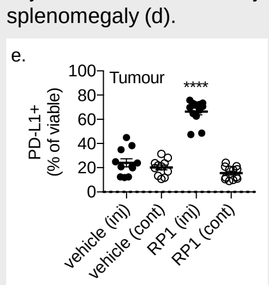
Here we assessed mRP1 in an immune competent bi-flank model using mouse BRAF mutant melanoma 4434 cells. 6 doses of 5 x 10^6 pfu RP1 were given intratumorally. Tumour volume was assessed and compared in injected (inj) and contralateral (cont) tumours.

Injected and contralateral tumours, lymph nodes and spleens were collected for analysis (a). RP1 reduced tumour burden on both injected (inj) and contralateral (cont) tumours (b).

RP1 replication was restricted to the injected (inj) tumour while no viral yield was seen in contralateral (cont) tumours (c). Furthermore, animals developed swelling in lymph nodes adjacent to RP1 injected tumours and splenomegaly (d).

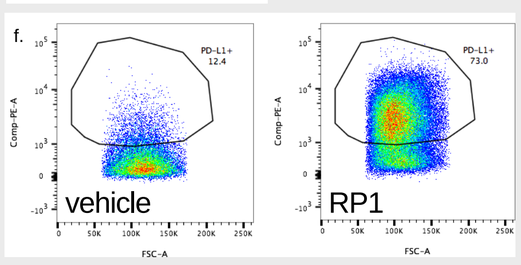


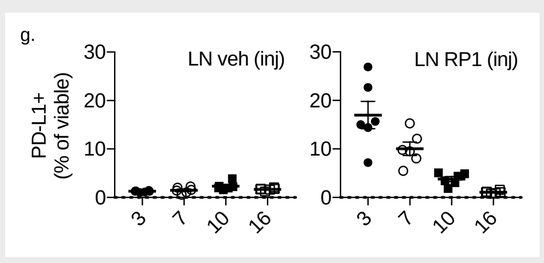




PD-L1 (programmed death ligand 1) has a major role in suppressing anti-tumor immune responses, which is induced along with anti-tumor immunity. We therefore assessed the presence of PD-L1+ cells within tumours before and following treatment. Seven days after treatment commenced, there was a significant increase in PD-L1+ tumour-infiltrating cells in injected tumours (e). Examples of the staining observed for PD-L1+ cell in injected tumour samples are shown (f). While clear anti-tumor effects were seen, in this model and in contrast to prior data, increases in PD-L1 were not seen in uninjected tumors as a % of viable cells.

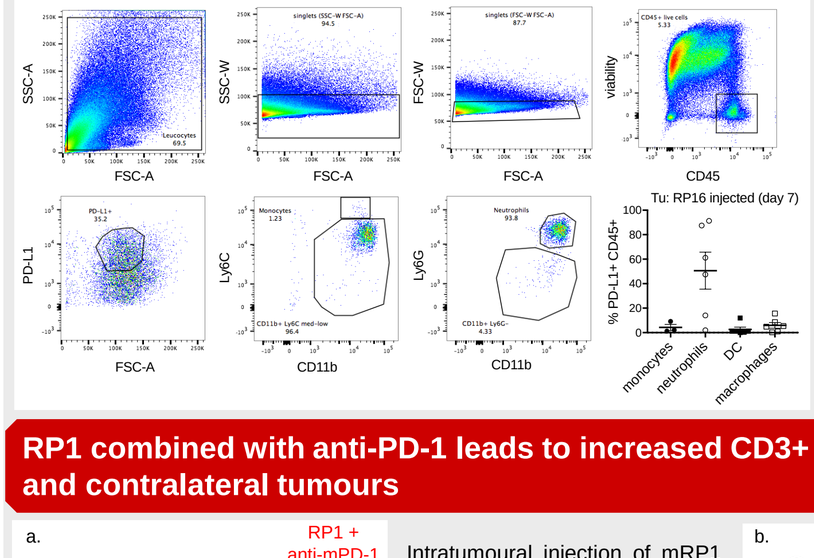
In lymph nodes draining from injected tumours, PD-L1+ cells were elevated by day 3, which then reduced over time (g).

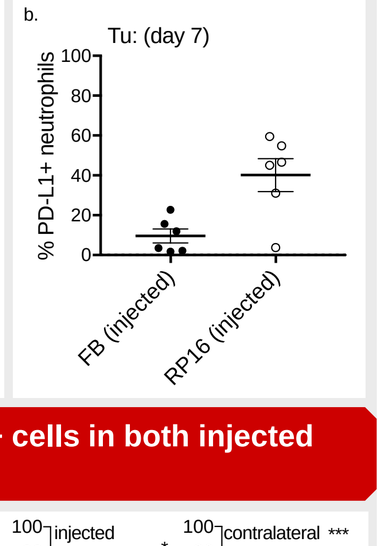




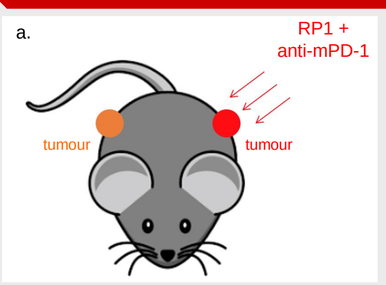
The infiltrating PD-L1+ cells in RP1-treated tumours are neutrophils.

We next assessed which cells were expressing PD-L1 within these tumours. We designed a separate myeloid panel to look for monocytes, neutrophils, DC's and macrophages. By selecting out the PD-L1+ cells from this panel, we identified this population of PD-L1+ cells as neutrophils (a). Similarly, by gating on the neutrophils, RP1 injection (vs. sham injection) elevated PD-L1+ expression was seen on these cells (b).

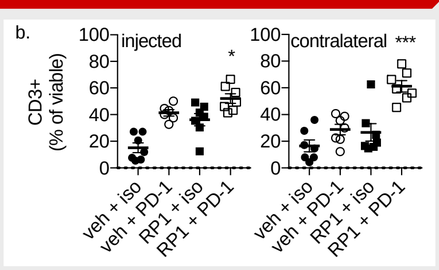




RP1 combined with anti-PD-1 leads to increased CD3+ cells in both injected and contralateral tumours



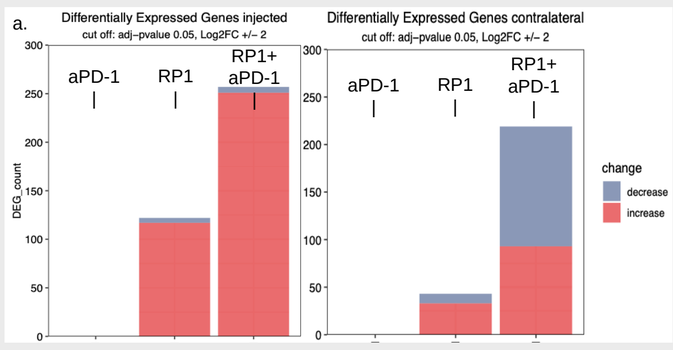
Intratumoural injection of mRP1 (3 doses, $5x10^6$ pfu) was combined with anti-mouse PD-1 (3 doses, 10mg/kg BE0146, RMP1-14 CD279, invivoMAb), or the combination (concomitantly) in C57BL/6 immune competent mice bearing bi-flank 4434 tumours (a).

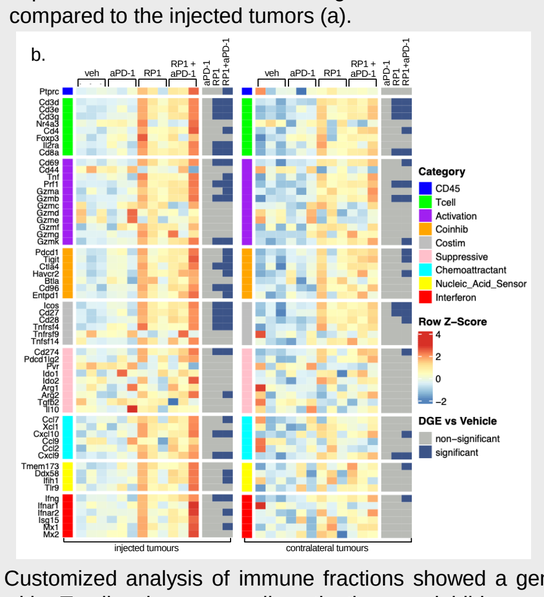


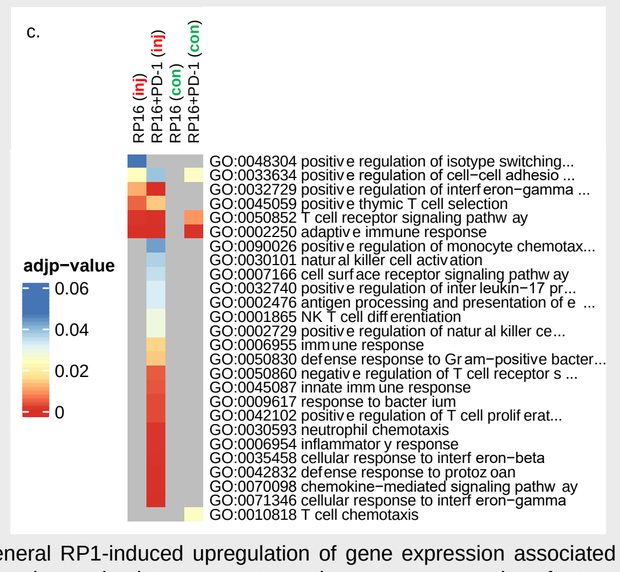
Tumours were harvested 7 days after treatment commenced for analysis of tumour immune infiltrate. This analysis revealed that tumour samples from animals that received the combination therapy had a significantly greater number of CD3+ cells within the tumour infiltrate compared with their single-agent counterparts, in both injected and contralateral tumours (b).

RNA seq following treatment with RP1 or anti-PD1 alone or in combination

Tumour samples from the same experiment as above were RNA sequenced (n=3) for analysis of differentially expressed genes. The count of upregulated and downregulated genes are shown compared with vehicle. There were very few differentially expressed genes for anti-PD1 alone (which may indicate a sub-therapeutic dose level), with an increased number with RP1 alone, which was still further increased when RP1 was combined with anti-PD1. In contralateral tumors, a similar pattern was seen, with, however, a greater number of genes for which expression was reduced following treatment compared to the injected tumors (a).







Customized analysis of immune fractions showed a general RP1-induced upregulation of gene expression associated with T-cells, immune cell activation, co-inhibitory and co-stimulatory receptors, immune suppressive factors, chemoattractants, nucleic acid sensing and interferon-associated genes. This upregulation was more frequently significant with combination therapy, indicative of anti-PD1 increasing the effect of RP1 (b). Despite a high degree of variability, analysis by topgo also revealed a greater number of differentially expressed genes within immune-related GO term pathways for the combination treated samples relative to RP1 alone (c).

Summary & Conclusions

Oncolytic viruses are an attractive treatment modality because they are self-amplifying, kill through multiple both direct and immune mechanisms and can promote anti-tumour immune responses. RP1 & RP2 are novel versions of HSV which cause tumor cell fusion through expression of the GALV protein. Histological examination of injected tumours revealed large areas of necrosis in syngeneic mouse tumours, even where GALV is not functional, with GALV enhancing both injected (prior data) and contralateral (data presented here & previously) anti-tumor effects in models where GALV is functional, including in nude mice. In nude mice, this was presumed to result from enhanced innate immune activation. The data described shows that RP1 increases PD-L1 expression, particularly on neutrophils, increases CD3 T cell infiltration in injected and contralateral tumors, and that profound effects on the gene expression profile are also seen in both injected and contralateral tumors which are consistent with potent and broad immune activation, and which is further enhanced by treatment with anti-PD1.

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Acknowledgments

We would like to thank Replimune Inc for providing us with the viruses used (mRP1 & mRP2).

mRP1 & mRP2 are enhanced potency oncolytic versions of HSV1 engineered to express murine GM-CSF and the gibbon ape leukemia virus membrane glycoprotein (GALV). In addition to GALV and mGM-CSF, RP2 further expresses an anti-mCTLA-4 antibody. RP1 & RP2 (i.e, expressing human GM-CSF & anti-human CTLA-4) are currently being evaluated in clinical trials in a range of solid tumors alone and combined with anti-PD1 therapy.

The Royal Marsden NHS Foundation Trust

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