Development & characterization of a new oncolytic immunotherapy platform based on herpes simplex virus type 1

Abstract #1470

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Overview

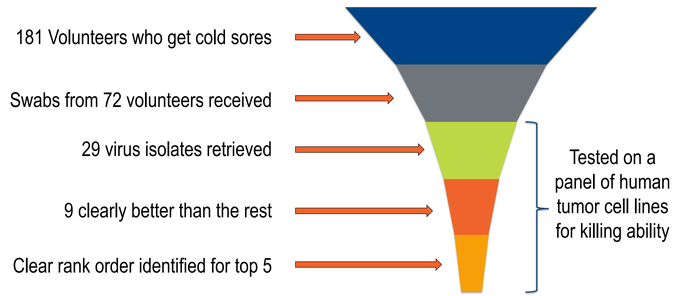
Background:

- Oncolytic viruses preferentially replicate in tumors as compared to normal tissue & promote immunogenic cell death & the induction of systemic anti-tumor immunity
- Oncolytic destruction of tumors may provide the optimal & most practical means by which to provide the patient-specific anti-tumor immune response which is required, but often missing, for the activity of PD1/L1 targeted drugs
- HSV-1 was chosen as the virus species for development because it broadly infects human tumor cells, is highly lytic, kills mainly by necrosis, its large genome provides the capacity for the insertion of multiple, potentially therapeutic, exogenous genes & as it is proven to be clinically effective for cancer treatment
- In this study we sought to further optimize HSV-1 based oncolytic immunotherapy through multiple approaches to maximize: (i) the extent of tumor cell killing,(ii) the immunogenicity of tumor cell death; and (iii) the induction of systemic anti-tumor immune responses

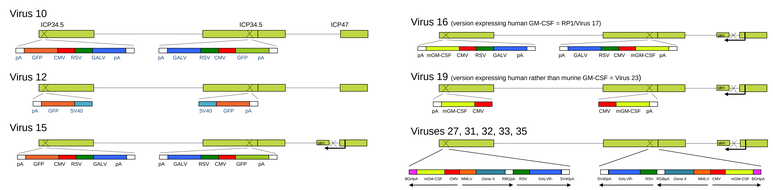
Methods & Results:

- Twenty nine new clinical strains of HSV-1 isolated from cold sores from otherwise healthy volunteers were screened across a panel of human tumor cell lines to identify the most potent strain, with considerable variation between strains being observed
- ICP34.5 & ICP47 were deleted to provide tumor selectivity & the virus armed with a gene encoding a constitutively fusogenic version of the membrane glycoprotein from gibbon ape leukemia virus (GALV-GP R-) to increase tumor cell killing, virus spread & the immunogenicity of cell death
- The ability of GALV-GP R- to enhance cell killing & immunogenic cell death were assessed
- This virus was further armed with genes encoding proteins expected to act at the site of immune response initiation in tumors & draining lymph nodes to augment the induction of an anti-tumor immune response
  - The proteins expressed were GM-CSF, an anti-CTLA-4 antibody and/or immune costimulatory pathway activating ligands
  - These were expected to have increased activity & reduced toxicity as compared to systemically administered approaches
- Anti-tumor effects were assessed in immune competent & immune deficient models & in combination with anti-PD1 therapy
- Enhanced activity was demonstrated associated with each of the approaches taken to improve the anti-tumor effect

Identification of the strain of HSV-1 for development

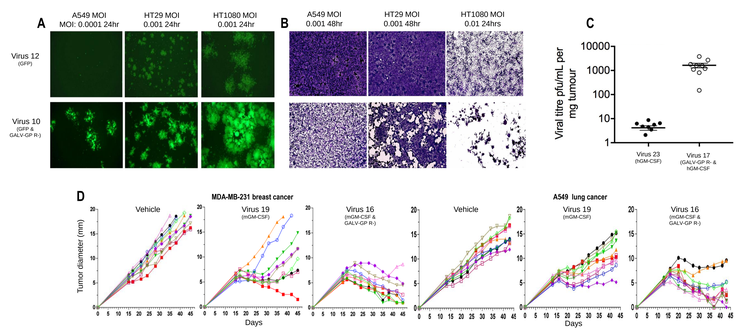


Viruses constructed & used in the study



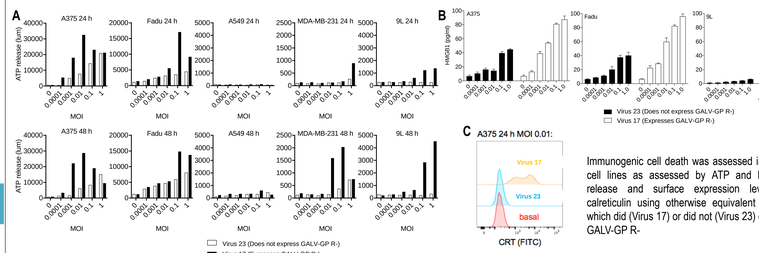
Gene X: Virus 27 = anti-mCTLA-4, Virus 31 (RP2) =anti-hCTLA-4, Virus 32 = mCD40L, Virus 33 = m4-1BBL, Virus 35 = mOX40L

Effects of GALV-GP R- in vitro and in xenograft tumour models



(A) Plaque morphology of tumor cell lines infected with Virus 12 (GFP) or Virus 10 (GFP & GALV-GP R-) **(B)** Cell killing effects of Virus 12 & Virus 10 assessed by crystal violet staining **(C)** Greatly increased viral titres in A375 human melanoma tumors harvested 4 days after the last injection of $5x10^4$ pfu 3x Q2D of Virus 17 (GALV-GP R- & hGM-CSF) as compared to Virus 23 (hGM-CSF) **(D)** Individual tumor growth curves from mice treated with either vehicle, Virus 19 (mGM-CSF) or Virus 16 (mGM-CSF and GALV-GP R-) in A549 human lung cancer tumors and MDA-MB-231 human breast cancer tumors. Tumors were injected 3x Q2D with either vehicle or $5x10^3$ pfu of the indicated virus, starting tumor diameter >5mm

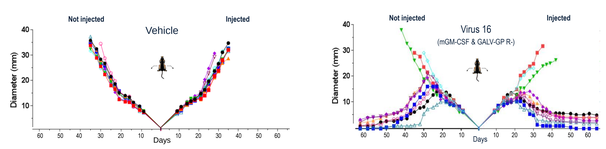
GALV-GP R- increases immunogenic cell death



Immunogenic cell death was assessed in tumor cell lines as assessed by ATP and HMBG1 release and surface expression levels of calreticulin using otherwise equivalent viruses which did (Virus 17) or did not (Virus 23) express GALV-GP R-

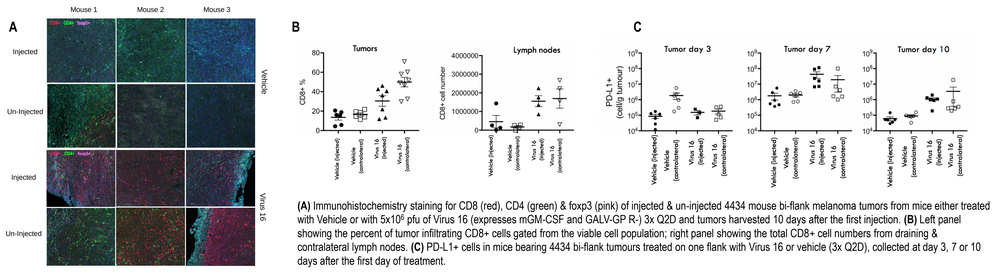
(A) Levels of ATP release were measured by luminescence in a panel of cell lines infected at the indicated MOI at 24h & 48h post infection; **(B)** ELISA used to measure HMGB1 in cell-free supernatants of cells treated for 48 h at the indicated MOI; **(C)** Histogram showing the expression levels of surface calreticulin in A375 cells treated at MOI 0.01 for 24 h. By each of the assays conducted, GALV-GP R- expression was shown to substantially increase immunogenic cell death

Efficacy in large bi-lateral tumors in immune competent rats



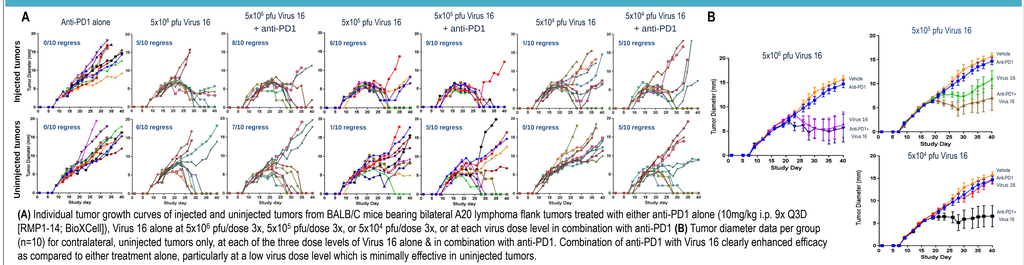
GALV-GP binds to the constitutive mammalian Pit-1 receptor, but is not compatible with the murine version, whereas it is compatible in rats. While rat tumor cells are of relatively low infectability with HSV-1, systemic effects were therefore assessed in rats using bilateral 9L tumors. Rats also allow effects in larger tumors to be assessed. Vehicle or $5x10^5$ pfu of Virus 16 (GALV-GP R- & mGM-CSF) were injected 5x Q2D from d10 into right tumors. Growth curves for individual tumors are shown. Lines of the same color are the left & right tumors from the same animal.

Virus treatment increases CD8 T cell levels & PDL1 expression in tumors



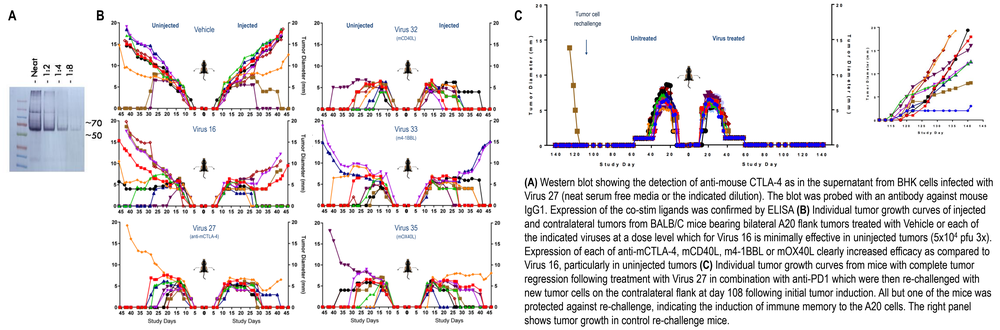
(A) Immunohistochemistry staining for CD8 (red), CD4 (green) & foxp3 (pink) of injected & un-injected 4434 mouse bi-flank melanoma tumors from mice either treated with Vehicle or with $5x10^6$ pfu of Virus 16 (expresses mGM-CSF and GALV-GP R-) 3x Q2D and tumors harvested 10 days after the first injection. **(B)** Left panel showing the percent of tumor infiltrating CD8+ cells gated from the viable cell population; right panel showing the total CD8+ cell numbers from draining & contralateral lymph nodes. **(C)** PD-L1+ cells in mice bearing 4434 bi-flank tumours treated on one flank with Virus 16 or vehicle (3x Q2D), collected at day 3, 7 or 10 days after the first day of treatment.

Synergy with anti-PD1 therapy



(A) Individual tumor growth curves of injected and uninjected tumors from BALB/C mice bearing bilateral A20 lymphoma flank tumors treated with either anti-PD1 alone (10mg/kg i.p. 9x Q3D [RMP1-14; BioXCell]), Virus 16 alone at $5x10^6$ pfu/dose 3x, $5x10^5$ pfu/dose 3x, or $5x10^4$ pfu/dose 3x, or at each virus dose level in combination with anti-PD1 **(B)** Tumor diameter data per group (n=10) for contralateral, uninjected tumors only, at each of the three dose levels of Virus 16 alone & in combination with anti-PD1. Combination of anti-PD1 with Virus 16 clearly enhanced efficacy as compared to either treatment alone, particularly at a low virus dose level which is minimally effective in uninjected tumors.

Expression of anti-CTLA-4 & immune co-stimulatory pathway activating ligands



(A) Western blot showing the detection of anti-mouse CTLA-4 as in the supernatant from BHK cells infected with Virus 27 (neat serum free media or the indicated dilution). The blot was probed with an antibody against mouse IgG1. Expression of the co-stim ligands was confirmed by ELISA **(B)** Individual tumor growth curves of injected and contralateral tumors from BALB/C mice bearing bilateral A20 flank tumors treated with Vehicle or each of the indicated viruses at a dose level which for Virus 16 is minimally effective in uninjected tumors ($5x10^4$ pfu 3x). Expression of each of anti-mCTLA-4, mCD40L, m4-1BBL or mOX40L clearly increased efficacy as compared to Virus 16, particularly in uninjected tumors **(C)** Individual tumor growth curves from mice with complete tumor regression following treatment with Virus 27 in combination with anti-PD1 which were then re-challenged with new tumor cells on the contralateral flank at day 108 following initial tumor induction. All but one of the mice was protected against re-challenge, indicating the induction of immune memory to the A20 cells. The right panel shows tumor growth in control re-challenge mice.

Summary & Conclusions

- The new fusion-enhanced oncolytic immuno-gene therapy platform presented provides a potent and versatile approach to developing new therapies intended to maximally activate a patient's immune system against their own cancer
- These viruses are expected to be most effective in combination with other anti-cancer agents, in particular PD1/L1-targeted immune checkpoint blockade
- The first virus from this program, RP1 (Virus 17; expressing GALV-GP R- and hGM-CSF), is currently being tested in a ≈150 patient phase 1/2 clinical trial alone & in combination with nivolumab in four solid tumor types (NCT03767348)
- A 240 patient, registration directed, randomized controlled phase 2 clinical trial of RP1 in combination with cemiplimab compared to cemiplimab alone in cutaneous squamous cell carcinoma is scheduled to initiate in the first half of 2019
- RP2 (Virus 31; expressing GALV-GP R-, hGM-CSF & anti-hCTLA-4) is on track to initiate a phase 1 clinical trial alone and in combination with anti-PD1 therapy in the first half of 2019
- RP3 (expresses GALV-GP R-, anti-hCTLA-4, hCD40L & h4-1BBL) is scheduled to initiate a phase 1 clinical trial alone & in combination with anti-PD1 therapy in the first half of 2020