Clinical biomarker studies with two fusion-enhanced versions of oncolytic HSV (RP1 and RP2) alone and in combination with nivolumab in cancer patients indicate potent immune activation

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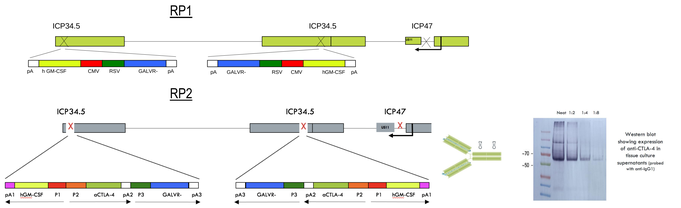
Overview

Background

- RP1 and RP2 are novel, enhanced potency oncolytic versions of HSV1 engineered to express human GM-CSF and the gibbon ape leukemia virus membrane R- glycoprotein (GALV-GP R-), providing constitutive fusion activity and increased immunogenic cell death.
- In addition to GALV-GP R- and GM-CSF, RP2 further expresses an anti-CTLA-4 antibody-like molecule.
- Murine versions of RP1 and RP2 exhibit potent systemic (i.e. abscopal) effects in rodents, and synergy in combination with anti-mouse-PD-1 in mice (Thomas et al JITC 2019).
- RP1 and RP2 are currently being evaluated in clinical trials in a range of solid tumors alone and combined with anti-PD1 therapy, where deep and durable responses have been demonstrated, including following single agent therapy with RP2 and also including in patients having previously failed prior anti-PD1 or combined anti-PD1/anti-CTLA-4 therapy, and (SITC 2020).
- Here we present ongoing biomarker data from a Phase 1/2 clinical trial of RP1 combined with nivolumab (NCT03767348) and from a clinical trial with RP2 alone and combined with nivolumab (NCT04336241).



Objectives

- To assess the effects of RP1/RP2 in combination with nivolumab and RP2 monotherapy in tumor biopsies and peripheral blood mononuclear cell (PBMC) samples collected from patients enrolled into the NCT03767348 and NCT04336241 clinical trails.
- In these clinical trials RP1 or RP2 were dosed every two weeks by intratumoral injection (up to 10ml depending on tumor size of between $1x10^5$-$1x10^7$ PFU/mL for up to 8 doses, with nivolumab given at standard clinical doses starting from the second dose of RP1/2 for combination therapy

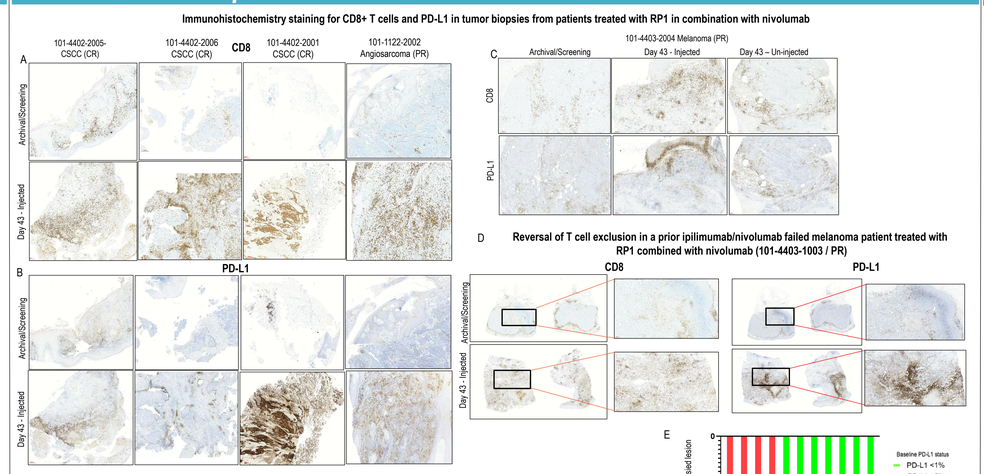
Methods

- Tumor biopsies (injected with RP1/2 - protocol mandated, un-injected with RP1/2 – optional) and PBMCs were collected at screening and at D43 following the first dose of RP1/2, after combination therapy with nivolumab for RP1 and RP2 or following single agent treatment for RP2.
- Immunohistochemistry (IHC) was performed for CD8 (SP57 clone, Ventana) and for PD-L1 (PD-L1 IHC 28-8 pharmDx by Agilent)
- Gene expression was analyzed using NanoString to assess effects on a range of genes using a custom code set consisting of a bespoke 40 gene panel for RP1 (see Table 1) or using the IO360™ panel (Nanostring) for RP2. This incorporates 48 potentially predictive Research Use Only (RUO) biological signatures, including the 18-gene Tumor Inflammation Signature score (TIS) known to be associated with response to PD-1/PD-L1 inhibitors as previously described (Ayers et al. JCI 2017).
- Immunosequencing of the CDR3 regions of human TCRβ chains was performed using the immunoSEQ Assay (Adaptive Biotechnologies, Seattle, WA).
  - Extracted genomic DNA was amplified in a bias-controlled multiplex PCR, followed by high-throughput sequencing (Robins, H. S. et al. 2009).
  - Two quantitative components were used to assess diversity of productive sequences and compare samples.
  - First, Simpson Clonality was used to describe how evenly rearrangements are distributed amongst a set of T cells.
  - Second, sample richness was calculated as the number of unique productive rearrangements in a sample after computationally down sampling to a common number of T cells.
  - Lastly, the number of expanded clones relative to screening was calculated according to a binomial distribution framework.

Table 1 – The genes included in the bespoke 40 gene panel

IFN-γ biology	T/NK cells		Antigen presenting cells	Anti-viral		Autophagy	Housekeeping	
CCL5	TIGIT	HLA-E	PSMB10	CGAS	TLR3	STK11IP	ABCF1	TBP
CXCL9	CD8a	NKG7	HLA-DQA1	DDX58	TLR7		G6PD	TBC1D10B
CD27	LAG3		HLA-DRB1	IRF3	TLR7/8		NRDE2	UBB
CXCR6	CD274		CMKLR1	IRF7	TLR9		QAZ1	
IDO1	PDCD1LG2			PKR	MDA5		POLR2	
STAT1	CD276			RIG-1	STING		SDHA	

Increases in CD8+ T cell infiltration and PD-L1 expression are frequently observed in patients treated with RP1 in combination with nivolumab



Biopsies from 52 patients were taken and included in this analysis, with representative biopsies shown

Immuno-histochemistry for CD8 and PD-L1 expression (A-C)

- IHC images showing CD8 and PD-L1 staining and best overall RECIST response.
- Increases in CD8 and PD-L1 expression were observed across tumor types, including in patients having failed prior anti-PD1 therapy +/- anti-CTLA-4.
- Conversion of immune deserted/CD8 and PD-L1 low to an immune inflamed phenotype was frequently observed.
- In most cases the biopsies collected at D43 contained largely necrotic tumor tissue which limited the ability to perform quantitative analysis.

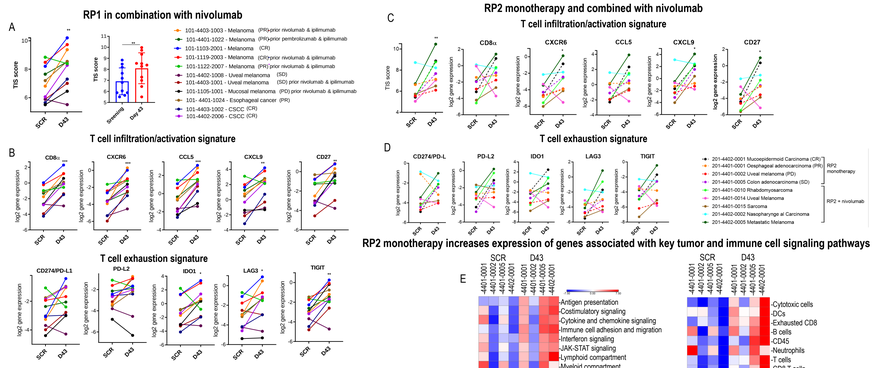
Reversal of T cell exclusion (D)

- CD8+ T cells and PD-L1 expression are restricted to tumor margins at baseline.
- IHC of the Day 43 biopsy demonstrates a striking influx of CD8+ T cells, indicative of a reversal of baseline T cell exclusion.
- A corresponding increase in PD-L1 is also observed.

Correlation of response with baseline PD-L1 status (E):

Preliminary analysis demonstrated no correlation between tumor response and baseline PD-L1 expression levels, i.e. injected tumor and overall (RECIST) tumor regression was observed in tumors with both baseline low (<1%) as well as med-high (>5%) baseline PD-L1 expression.

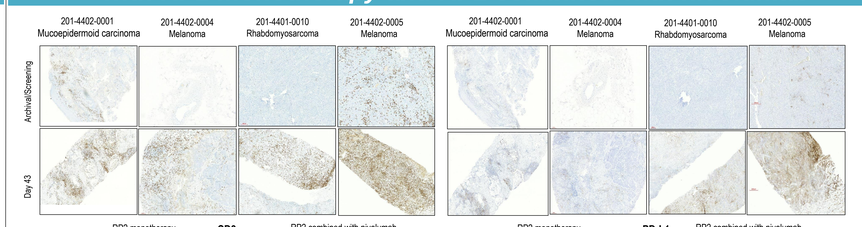
Increased expression of genes associated with immune activation following treatment with RP1/RP2 combined with nivolumab or RP2 monotherapy



Increase in TIS score, T cell infiltration & immune exhaustion signature

- Data for all patients for which analysis has been conducted so far are shown, N=11 for RP1+nivolumab, and N=9 (RP2 monotherapy N = 4, RP2+nivolumab N = 5). Statistical differences between groups were measured by two-tailed Student's t test. *P < 0.05, **P < 0.01, ***P < 0.001.
- RP1/RP2 combined with nivolumab or RP2 alone increased the TIS score/the expression of genes implicated in T cell infiltration/activation and genes previously reported to be associated with responsiveness to anti-PD1 therapy, particularly CD8, CXCL9, CD27 and TIGIT (A-D).
- IO 360 panel analysis following RP2 monotherapy also demonstrated increased expression of genes associated with key tumor and immune cell signaling pathways indicative of conversion of the tumor microenvironment (TME) from "cold" to a "hot (E).

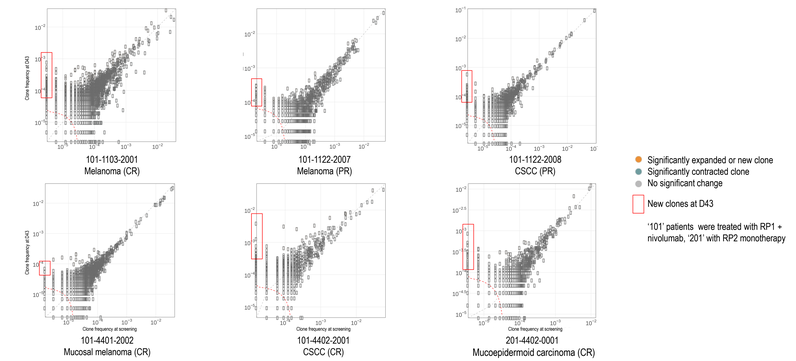
Increase in CD8+ T cells and PD-L1 are observed in patients treated with RP2 monotherapy and RP2 combined with nivolumab



Immuno-histochemistry for CD8+ T cells & PD-L1

- Robust increase in CD8+ T cell infiltration and PD-L1 expression was observed in biopsies collected at Day 43 following both RP2 monotherapy and RP2 in combination with nivolumab.

Expansion & generation of T cell clones following treatment with RP1 combined with nivolumab or RP2 monotherapy



Increase in peripheral T cell diversity and emergence of new T cell clones

- TCR sequencing of PBMCs revealed expansions of T cell clones post RP1 in combination with nivolumab and RP2 monotherapy.
- Many of the expanded clones (range 20-80%) are newly detected at Day 43, suggesting that treatment not only expanded existing T cell clones but generated new T cell clones.
- A particularly striking expansion of T cell clones (n=170) was observed for melanoma pt (101-1103-2001) with an ongoing complete response.
- RP2 monotherapy led to expansion of 43 T cell clones with the majority (60%) being newly detected at Day 43 in a mucoepidermoid carcinoma patient (201-4402-0001) with an ongoing complete response.

Summary & conclusions

- Immunohistochemistry for CD8 and PD-L1 from paired tumor biopsies demonstrated robust and increased infiltration of CD8+ T cells and PD-L1 expression, both after combined treatment with RP1 and nivolumab and after single agent RP2 across different tumor types, including reversal of T cell exclusion following prior combined treatment with ipilimumab and nivolumab in melanoma.
- Gene expression analysis demonstrated a significant increase in the expression levels of genes associated with innate and adaptive immune activation and genes previously reported to be associated with responsiveness to anti-PD1 therapy, particularly CD8, CXCL9, CD27 and TIGIT, as well as consistently increased TIS.
- Increased CD8+ T cell infiltration as well as robust changes in key tumor and immune cell signaling pathway genes with RP2 monotherapy were observed, indicating the ability of RP2 monotherapy to convert an immunologically inert to an immune inflamed phenotype.
- T cell receptor sequencing indicated the expansion of existing T cell clones and generation of new T cell clones.
- Increase in CD8+ T cell infiltration and PD-L1 expression in un-injected lesions, coupled with changes in TCR clonal expansion in PBMC samples suggest systemic immune activation.
- Additional assays and analyses which have yet to be conducted include tumor mutation burden, RNA sequencing and also further studies with a particular focus on uninjected tumors.
- Overall, the biomarker data generated to date indicates that both RP1 and RP2 are able to increase immune activation in patients with cancer across a range of different tumor types, consistent with the intended mechanism of action for RP1 and RP2, and consistent with the clinical efficacy data generated to date.