Abstract: 6639

Preclinical characterization and clinical biomarker studies with RP3, a novel oncolytic immunotherapy expressing a fusogenic glycoprotein, an anti-CTLA-4 antibody, CD40L and 4-1BBL

Praveen Bommareddy[1], Kevin Harrington[2], Victoria Roulstone[2], Joan Kyula[2], Aaron Clack[1], Linta Kuncheria[1], Sylwia Jones[1], Aditya Bansali[1], Alireza Kalbasi[1], Robert Coffin[1], Mark Middleton[3]

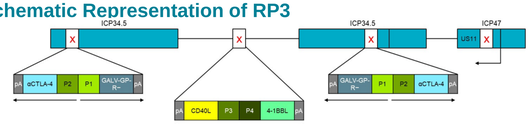
[1]Replimune Inc., Woburn MA; [2]The Institute of Cancer Research and The Royal Marsden NHS Foundation Trust, London UK; [3]University of Oxford, Oxford UK

Background

- RP3 is a selectively replication-competent HSV-based tumor-directed oncolytic immunotherapy (TDOI) that expresses the GALV-GP-R- fusogenic glycoprotein, an anti-CTLA-4 antibody-like molecule, CD40L, and 4-1BBL
- RP3 is currently being assessed in an open-label, multicenter, Phase 1 clinical trial as a single agent and in combination with nivolumab in patients with advanced solid tumors (NCT04735978)
- Here we present pre-clinical and initial clinical biomarker data from the ongoing Phase 1 clinical trial of RP3 as single agent and combined with nivolumab

Methods

Schematic Representation of RP3



αCTLA-4, anti–cytotoxic T-lymphocyte antigen 4; 4-1BBL, 4-1BB ligand; CD40L, cluster of differentiation 40 ligand; GALV-GP-R−, gibbon ape leukemia virus glycoprotein with the R sequence deleted; ICP, infected cell protein; P, promoter; pA, polyAsignal; US11, unique short 11; X, denotes inactivation of viral protein.

Pre-clinical

- Bilateral mouse A20 lymphoma tumors were grown in Balb/c mice. Viruses encoding either anti-CTLA-4, CD40L, 4-1BBL or OX40L were injected with 5 × 10^6 pfu/50 µl or with vehicle and tumor diameters then followed (Ref 1)
- A375 tumors were grown in both flanks of CD-1 nude mice and injected with 1x10^7pfu/50 µL of RP3 or a control virus which does not express either CD40-L or 4-1BBL (Virus 24). Tumors were harvested at day 3 and immunohistochemistry for CD40L, 4-1BBL, and HSV-1 antigens was performed

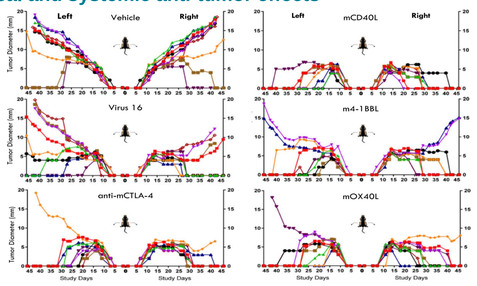
Clinical

- For the preliminary clinical biomarker studies, tumor biopsy and blood samples (PBMCs) were collected from patients at screening and at Day 43. Prior to Day 43 sample collection, patients have received either 3 doses of RP3 (monotherapy) or 3 doses of RP3 and 2 doses of nivolumab, with nivolumab starting with the second dose of RP3 (combination therapy).
  - Tumor samples were analyzed by IHC for CD8 (SP57 clone, Ventana) and PD-L1 (PD-L1 IHC 28-8 pharmDx; Agilent)
  - Gene expression analysis was conducted using the NanoString IO360-panel. The tumor inflammation signature score (TIS) was also calculated using the 18-gene signature previously identified as a prognostic indicator for response to pembrolizumab [3]
  - Systemic anti-tumor immunity was assessed using PBMCs by sequencing the CDR3 regions of human TCRβ chains using the immunoSEQ assay

Results

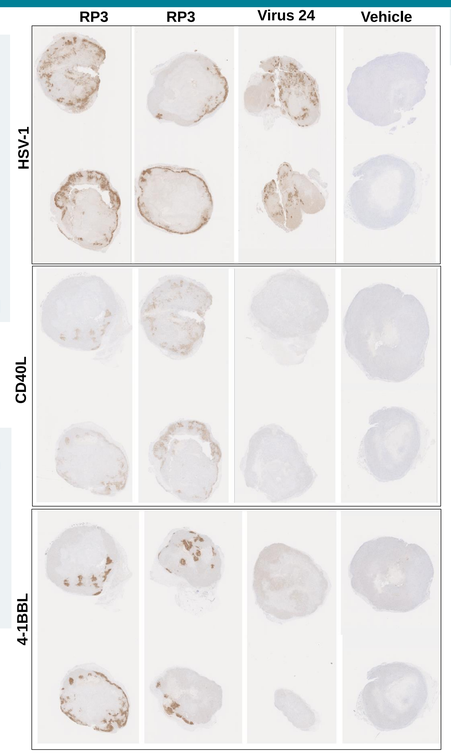
Expression of anti-CTLA-4 and co-stimulatory ligands enhance local and systemic anti-tumor effects

Individual tumor growth curves of A20 tumor bearing mice treated with the indicated virus; injected tumors (right) and contralateral uninjected tumors (left)

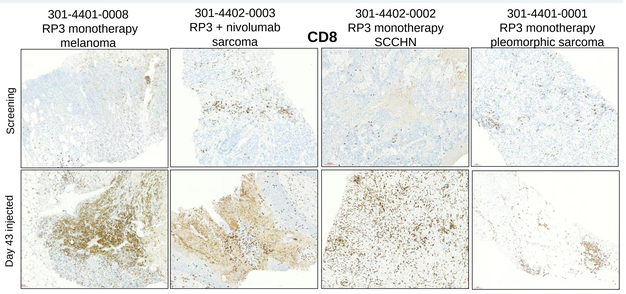


Mouse xenograft tumors treated with RP3 show widespread HSV antigen expression surrounding extensive areas of necrosis, demonstrating efficient RP3 replication, tumor killing and intratumoral spread

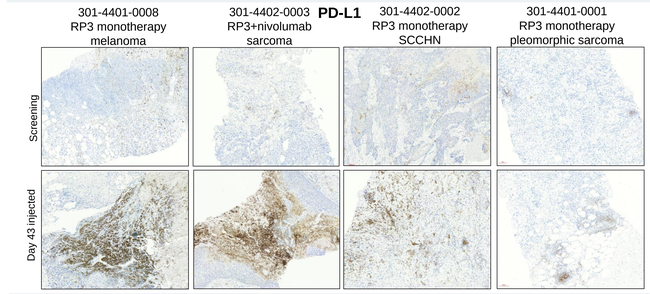
Mouse xenograft tumors treated with RP3 show widespread expression of CD40L and 4-1BBL overlapping with areas of HSV antigen expression



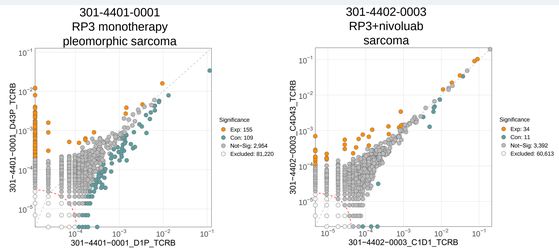
CD8+ T cell infiltration increased in patients treated with RP3 alone or combined with nivolumab



PD-L1 expression increased in patients treated with RP3 alone or combined with nivolumab



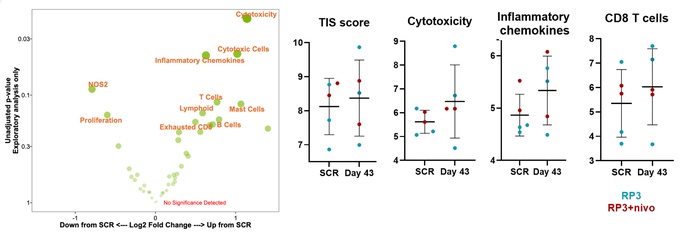
T cell receptor sequencing from patients demonstrates expansion of pre-existing and generation of new T-cell clones following treatment with RP3 and RP3 combined with nivolumab



Significantly expanded clones are shown in yellow with new ones present on the y-axis, and the blue dots are significantly contracted clones. Most clones expanded in pt 4401-001 treated with RP3 monotherapy appeared to be newly generated

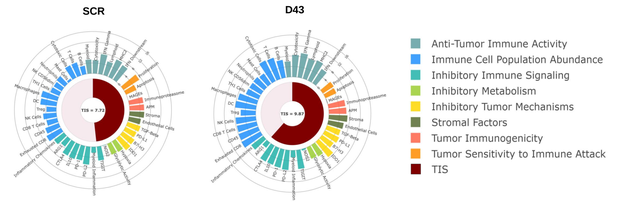
Treatment with RP3 alone or combined with nivolumab increases the expression of genes associated with immune activation

Changes in gene signatures from baseline to D43



Increase in mean TIS score was observed at D43 compared to screening, these changes are accompanied by increases in genes signatures implicated in cytotoxicity, inflammatory chemokines and CD8 T cell activity, all indicative of increased immune activation.

An example of gene expression signatures expression levels at baseline and at day 43 in an individual patient treated with RP3 monotherapy (301-4401-0001; pleomorphic sarcoma)



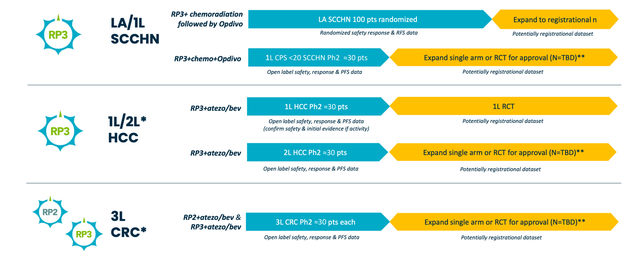
Conclusions

- Expression of each of anti-CTLA-4, OX40L, CD40L, and 4-1BBL increased both local and systemic anti-tumor effects in mouse A20 tumors
- Robust and widespread expression of CD40L and 4-1BBL, which overlapped with areas of HSV antigen expression, was demonstrated in mouse A375 tumors treated with RP3
- Biomarker analyses from patients treated with RP3 and RP3 combined with nivolumab demonstrated robust increase in PD-L1 expression and CD8+ T cell infiltration
- T cell receptor sequencing from patient samples demonstrated expansion of pre-existing and the generation of new T cell clones following treatment with RP3 and RP3 combined with nivolumab
- Gene expression analysis from patient tumor biopsies demonstrated increased expression of genes associated with innate and adaptive immune activation and in genes known to be associated with responsiveness to anti-PD-1 therapy.
- Overall, the data demonstrates that treatment with RP3 and RP3 combined with nivolumab broadly increased immune activation in patients.

Next stage development of RP3

Phase 2 studies with RP3

Expected to initiate around mid-2023



*Potential fast to market opportunities **Pending FDA agreement

Note: Replimune has clinical trial collaboration & supply agreements with BMS & Roche for the supply of Opdivo and atezo/bev in its clinical trial programs with RP2/3

Acknowledgements:

The authors would like to thank the patients for their participation in the trial. The authors would also like to acknowledge the contributions of Amanda Snook, Moran Mishal, and John McRae.

Reference:

1. Thomas S, et al. *J Immunother Cancer.* 2019;7(1):214.

Study Sponsor:

The study is sponsored by Replimune Inc, Woburn MA, USA.

Presented at the 2023 AACR meeting, April 14-19, 2023; Orlando, FL, USA