Abstract: 9534

Biosafety analysis from the skin cancer cohorts in the IGNYTE clinical trial of RP1

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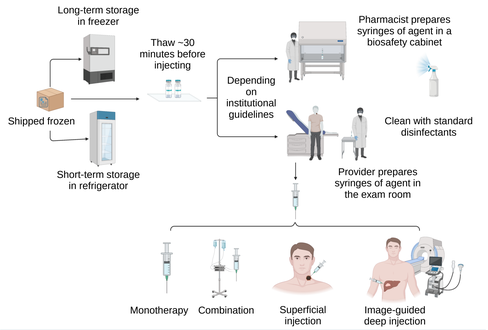
Background

- Vusolimogene oderparepvec (RP1) is a genetically modified herpes simplex virus type 1 (HSV-1)–based oncolytic immunotherapy that **selectively replicates in and lyses tumors**.[1,2] **Like HSV-1, RP1 is not airborne and retains sensitivity to acyclovir**
- IGNYTE is a phase 1/2, open-label, multicenter, dose-escalation and dose-expansion trial (NCT03767348) evaluating the safety and efficacy of RP1 in combination with the anti–PD-1 antibody nivolumab in a range of tumor types[3]
- **RP1 is administered intratumorally via injection into superficial lesions or deeper tumors using image guidance**. Handling is shown in **Figure 1**. As the field of oncolytic immunotherapy grows, understanding the safety and care considerations for this class of agents is essential to patient care

Objective

To report the biodistribution and shedding patterns of RP1 from patients (N = 278) enrolled in the phase 2 skin cancer cohorts from the IGNYTE trial

Figure 1. Handling of HSV-1 oncolytic immunotherapies[4]



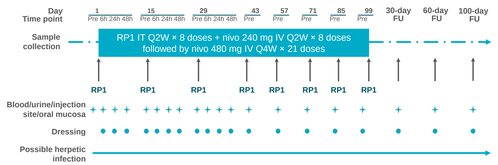
During the preparation and administration of RP1, providers should take universal safety precautions (eg, wear a gown, safety glasses, and gloves). Injection sites should be covered with occlusive dressings for 48 hours, as necessary. Investigational use only.

HSV-1, herpes simplex virus type 1.

Methods

Sample collection schema for RP1

Figure 2. Sample type and collection schedule

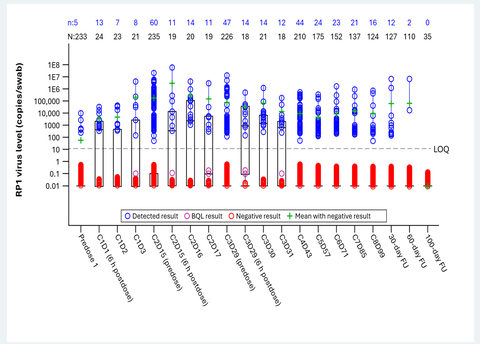


FU, follow-up; h, hours; IT, intratumorally; IV, intravenously; nivo, nivolumab; Pre, predose; Q2W, every 2 weeks; Q4W, every 4 weeks.

Blood, urine, and swabs from the surface of injection sites, the exterior of occlusive dressings, oral mucosa, and any areas of suspected herpetic infection were collected throughout the study (**Figure 2**). The presence of RP1 DNA was assessed using a RP1-specific quantitative PCR (qPCR) assay. As the **presence of DNA does not equate to live/infectious RP1**, DNA-positive swab samples were further tested for live RP1 in a validated 50% tissue culture infectious dose ($TCID_{50}$) assay.

Results

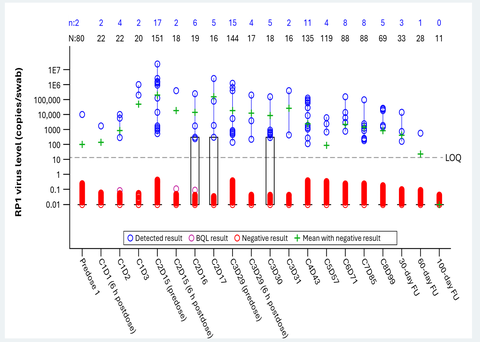
Figure 3. RP1 DNA levels at the site of injection



BQL, below quantitation limit; C, cycle; D, day; FU, follow-up; h, hours; LOQ, limit of quantification; N, number of patients tested; n, number of patients with DNA virus level equal to or above the lower LOQ.

Injection site: RP1 DNA was detected on the surface of injection sites in 112/266 (42.1%) patients and in 358/1947 (18.4%) samples. Most samples positive for RP1 DNA were collected during treatment doses 1 through 8 (**Figure 3**). **Of 314 RP1 DNA–positive samples further tested by $TCID_{50}$ assay, 4 (1.3%) were positive for live RP1.**

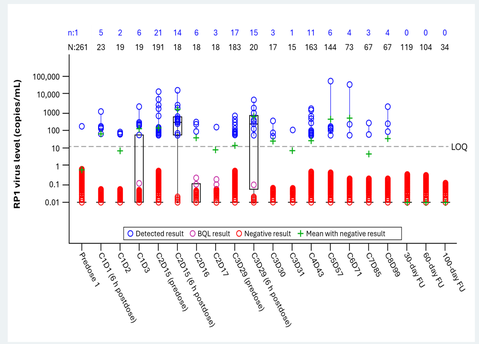
Figure 4. RP1 DNA levels from the exterior of injection-site dressings



BQL, below quantitation limit; C, cycle; D, day; FU, follow-up; h, hours; LOQ, limit of quantification; N, number of patients tested; n, number of patients with DNA virus level equal to or above the lower LOQ.

Exterior dressings: The incidence of RP1 DNA detection on injection-site dressing exterior samples (9.5%) was lower than that from injection-site samples (18.4%). Generally, lower copy numbers of RP1 DNA were detected in injection-site dressing samples compared with injection-site samples (**Figures 3 and 4**). **All RP1 DNA–positive dressing samples tested negative for live RP1 via $TCID_{50}$.**

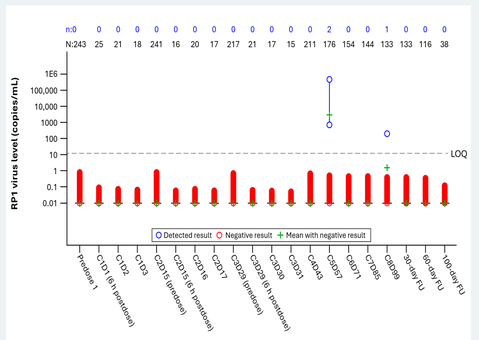
Figure 5. RP1 DNA levels in blood



BQL, below quantitation limit; C, cycle; D, day; FU, follow-up; h, hours; LOQ, limit of quantification; N, number of patients tested; n, number of patients with DNA virus level equal to or above the lower LOQ.

Blood: RP1 DNA was detected in the blood of 53/274 (19.3%) patients and in 122/1573 (7.8%) samples during treatment; the highest levels were detected shortly (within 6 hours) after injection. A minority of patients showed continued presence of RP1 DNA at the time of the next injection 15 days later, with kinetics indicative of RP1 replication in tumors (**Figure 5**).

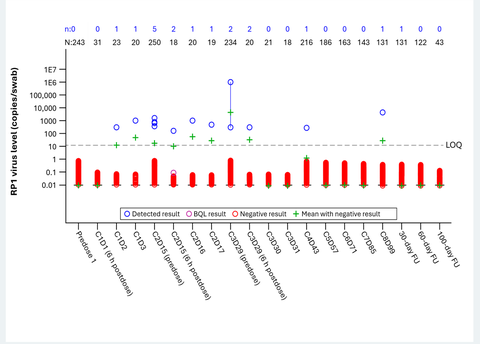
Figure 6. RP1 DNA levels in urine



C, cycle; D, day; FU, follow-up; h, hours; LOQ, limit of quantification; N, number of patients tested; n, number of patients with DNA virus level equal to or above the lower LOQ.

Urine: RP1 DNA was very rarely detected and only at low copy numbers in urine samples: 2/273 (0.7%) patients and 3/1976 (0.2%) samples tested positive (**Figure 6**). All urine samples that tested positive for RP1 DNA tested negative at the next scheduled visit (15 days later).

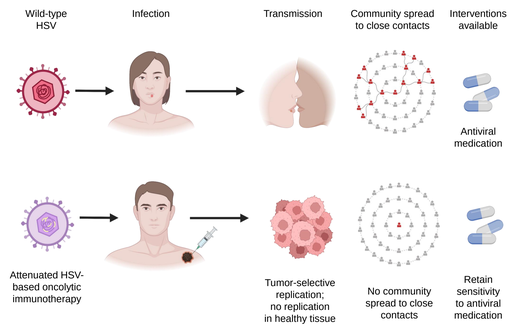
Figure 7. RP1 DNA levels from oral mucosa/saliva



BQL, below quantitation limit; C, cycle; D, day; FU, follow-up; h, hours; LOQ, limit of quantification; N, number of patients tested; n, number of patients with DNA virus level equal to or above the lower LOQ.

Oral mucosa/saliva: RP1 DNA was rarely detected and at low copy numbers in 16/272 (5.9%) patients and in 18/2052 (0.9%) samples. Most samples positive for RP1 DNA were collected during the first 3 doses of RP1 (**Figure 7**). RP1 DNA was detected below quantitation levels in 1/131 (0.8%) oral mucosa samples collected during the 30-day follow-up visit; the sample did not undergo $TCID_{50}$ testing, but detection of live RP1 would be unlikely due to the low levels of RP1 DNA. RP1 DNA remained undetectable in all tested samples at the 60-day and 100-day follow-up visits.

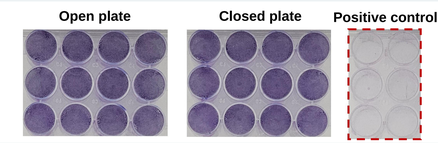
Figure 8. Differences between wild-type HSV and HSV-based oncolytic immunotherapies[4]



Wild-type HSV may replicate and transmit to others, potentially resulting in community spread. Attenuated HSV-based oncolytic immunotherapies are limited to replication within tumors and do not show evidence of transmission to close contacts.

HSV, herpes simplex virus.

Figure 9. RP1 syringe-filling testing



Two 12-well plates seeded with BHK cells were placed side by side inside of a biosafety cabinet. The lid was removed from the first plate to expose all wells (open plate), while the lid remained on the second plate (closed plate) to serve as a negative control. The biosafety cabinet was switched OFF, and a syringe was filled with 1 mL of RP1 directly above the plates. The syringe was held in the same area for 2 minutes to allow any potential droplet to settle. A lid was then placed on the open plate, and both plates were returned to the incubator. For the positive control, virus was deliberately released from the syringe into 6 wells of a third plate (red outline).
BHK cell, baby hamster kidney cell.

RP1 syringe-filling testing: Like HSV-1, RP1 is not airborne. To evaluate the potential for RP1 spill during syringe filling, syringes were filled with 1 mL of RP1 (10^7 PFU) directly over 12-well plates seeded with BHK cells to allow any droplets of virus to fall and inoculate the cells directly. No differences were observed between the open plate and closed plate (negative control), with no RP1 detected, demonstrating minimal potential for spill during syringe filling (**Figure 9**).

Table 1. Surface disinfection

Disinfectant	Steel	Epoxy	Polypropylene
PBS	Positive control	Positive control	Positive control
Decon 90	**No RP1 detected**	**No RP1 detected**	**No RP1 detected**
70% Isopropanol	**No RP1 detected**	**No RP1 detected**	**No RP1 detected**

RP1 (50 µL of 10^7 PFU/mL) was pipetted onto the surface (steel, epoxy, or polypropylene), then a disinfectant (Decon 90 or 70% isopropanol) or control (PBS) was sprayed directly onto the "spill" at the start of the measured contact time. The spill area (defined as the main liquid area that was formed, containing virus and disinfectant) was swabbed thoroughly (4 vertical and 4 horizontal swipes through the wet area). The swab was then placed into 1 mL VTM. BHK cell monolayers were inoculated, in duplicate, with 100 µL of the VTM sample. The area was wiped completely dry using lint-free clean room wipes, and a second swab (100 µL of PBS pipetted onto the now dried spill area and swabbed again as previously described) was placed into 1 mL of serum-free media. BHK cell monolayers were inoculated with 600 µL of the serum-free media sample.
BHK, baby hamster kidney; PBS, phosphate-buffered saline; PFU, plaque-forming units; VTM, viral transport medium.

RP1 was completely neutralized within 30 seconds of contact time on all 3 tested surfaces using standard disinfectants and cleaning procedures (Table 1).

Conclusions

- **The presence of RP1 DNA does not indicate live/infective RP1**
- The highest incidence of RP1 DNA was detected at the injection site, likely due to the expected replication of RP1 within the tumor, with minimal detection of live RP1, mostly up to 48 hours post injection
- A lower incidence and lower levels of RP1 DNA (10-fold) were detected from the dressing samples, with no live RP1 detected, demonstrating that the dressing prevents dissemination of RP1
- In blood, RP1 DNA was mainly detected for up to 48 hours with reducing levels thereafter
- There was negligible detection of RP1 DNA in urine (0.2%) or mucosa samples (0.9%)
- **There were no systemic herpetic infections in patients or reports of HSV-1 infections in close contacts**
- Collectively these data demonstrate that the likelihood of transmission of RP1 to patients' close contacts or into the external environment is minimal, with no transmission having been reported to date
- **RP1 is completely neutralized using standard disinfectants within 30 seconds of contact, confirming that standard disinfection procedures are sufficient for RP1 clean-up**

All tested samples with detectable RP1 DNA at follow-up visits were confirmed to be negative for live RP1.
Additionally, 8 samples were collected from 7 patients from different areas of suspected herpetic infection; none tested positive for live RP1.

The IGNYTE study is currently recruiting patients. To learn more about enrolling your patient, contact clinicaltrials@replimune.com or +1 (781) 222 9570.

Additional information can be obtained by visiting ClinicalTrials.gov (NCT03767348).

Contact: wiseth@ucmail.uc.edu

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Study Sponsor:

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