

ARTICLE

Maintaining therapeutic activity in the operating room: compatibility of a gamma-retroviral replicating vector with clinical materials and biofluids

Ryan Burnett¹, Carlos E. Ibañez¹, Pär L. Pettersson¹, Ching-I Chen¹, Shraddha Parab¹, Tiffany Huang¹, Joan Robbins¹, Krystof Bankiewicz², Manish Aghi², Christopher Logg³, Noriyuki Kasahara³, Dan Pertschuk¹, Harry E. Gruber¹ and Douglas J. Jolly¹

Toca 511 is a novel retroviral replicating vector, encoding a modified yeast cytosine deaminase, administered to recurrent high grade glioma patients in Phase 1 trials by stereotactic, transcranial injection into the tumor or into the walls of the resection cavity. A key issue, with little published data, is vector biocompatibility with agents likely to be encountered in a neurosurgical setting. We tested biocompatibility of Toca 511 with: delivery devices; MRI contrast agents, including ProHance supporting coinjection for real time MRI-guided intratumoral delivery; hemostatic agents; biofluids (blood and cerebrospinal fluid); potential adjuvants; and a needleless vial adapter that reduces risk of accidental needle sticks. Toca 511 is stable upon thawing at ambient temperature for at least 6 hours, allowing sufficient time for administration, and its viability is not reduced in the presence of: stainless steel and silica-based delivery devices; the potential MRI contrast agent, Feraheme; ProHance at several concentrations; the hemostatic agent SURGIFOAM; blood; cerebrospinal fluid; and the needleless vial adapter. Toca 511 is not compatible with the hemostatic agent SURGICEL or with extended exposures to titanium-based biopsy needles.

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INTRODUCTION

Toca 511 (vocimagene amiretrorepvec) is a novel retroviral replicating vector (RRV) currently in Phase I clinical trials (NCT01156584 and NCT01470794) for the treatment of recurrent high grade (Grade III and IV) gliomas (HGG).¹ Toca 511 selectively delivers an optimized yeast-derived cytosine deaminase transgene (CD) to dividing tumor cells. The RRV spreads and integrates proviral DNA copies into the genome of infected cells, leading to the expression of the CD enzyme. Subsequent administration of the orally bioavailable prodrug 5-fluorocytosine (5-FC) leads to local conversion of 5-FC to the anticancer drug, 5-fluorouracil (5-FU) in the CD-expressing tumor cells.^{2,3}

The tumor specificity of Toca 511 has been demonstrated in rodent models and was originally proposed based on both the high specificity of gamma-retroviruses for replicating cells and the defects in innate immunity that are known to occur in most tumors.⁴ As part of the current dose-escalation safety studies, Toca 511 was administered one time either by stereotactic, intratumoral injection or by injection into the walls of the surgical resection cavity after tumor resection. After waiting several weeks to allow Toca 511 to infect neighboring tumor cells, a course of 5-FC (extended-release, Toca FC) was taken orally. Since Toca 511 is a nonlytic integrating retrovirus and 5-FU kills during cell replication,^{5,6} infected tumor cells not destroyed by the first cycle of Toca FC may continue to

produce virus and infect residual tumor cells. In the current trials, cycles of viral spread by intratumoral replication (for either 3 or 7 weeks) alternate with 6–8 day courses of Toca FC to evaluate both safety and potential efficacy.

Although nonreplicative viral vectors have been investigated previously as therapeutic agents,^{7,8} there has been no published study of the compatibility or stability of viruses being investigated in the treatment of HGG with the equipment or materials used in preclinical and clinical studies. Therefore, for these first-in-human studies, it is necessary to understand how routine compounds, hemostatic agents, delivery devices, biofluids, and procedures associated with a neuro-surgical setting could impact the administration, stability, and infectivity of the Toca 511 vector.

We investigated the following areas: (i) post thaw stability of Toca 511 and the impact of dwell times with various surgical delivery devices, (ii) compatibilities with solvents, adjuvants, hemostatic agents, and biofluids that may come into contact with Toca 511 during or after surgery, (iii) biocompatibility of Toca 511 with tracking and infectivity enhancing agents, and (iv) the effect on Toca 511 of solvents frequently used in other agents (ethanol and dimethyl sulfoxide (DMSO)), and adjuvants. This report describes the results of this survey and provides guidance for the use of materials in conjunction with Toca 511 in the OR.

¹Tocagen Inc., Bunker Hill St., San Diego, CA, USA; ²Department of Neurological Surgery, University of California San Francisco (UCSF), San Francisco, CA, USA; ³Department of Medicine, University of California Los Angeles (UCLA), Los Angeles, CA, USA. Correspondence: Jolly (djolly@tocagen.com)

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RESULTS

General format of stability testing experiments

Compatibility assessments were made by incubating Toca 511 and a delivery device (needle, catheter, or cannula), MRI contrast agent, hemostatic agent, biofluid, or other solvents (or combination thereof), and then determining the resulting transduction titer (TU/ml) compared with control test articles. Usually, the compatibility assessment represents incubations over time periods that may occur in a clinical or surgical setting.

The transduction titer assay for Toca 511 depends on the time of exposure to target cells and azidothymidine (AZT) at 24 hours prevents subsequent spread

Figure 1a (open squares) shows the resulting transduction titers on PC-3 cells in 12-well plates and the rate of vector uptake. These data show a highest value of 3×10^8 TU/ml after a minimum of 6-hour exposure, and this value stays steady out to 24 hours. Therefore, a 16–24-hour exposure of Toca 511 to PC-3 cells was used in subsequent experiments. Because Toca 511 is replication competent, AZT is used to inhibit subsequent replication, after the first round of infection is complete. The determination of transduction titer of Toca 511 was investigated by incubating vector-transduced host cells with and without the addition of AZT to 40 $\mu\text{mol/l}$. Figure 1b shows the titer determinations for untreated cells, cells treated once with AZT, and cells with additions every 24 hours (4 total for 96 hours). Based on these data, the assay was performed subsequently using a single AZT administration at 20–28 hours posttransduction.

Needleless vial adapters allow sterile extraction of material from the filled vial and avoids needle sticks

The exterior of the 2 ml glass vial in which Toca 511 is supplied is not sterile and could not be handled directly by a surgeon in the operating room (OR). To extract vector from the vial in the OR, a sterile 13 mm needleless vial adaptor (West Pharmaceutical; Ref # 8070117), was aseptically placed by a surgical assistant over the Toca 511 vial, snap fitting into place. This allows the surgeon to remove the vector from the vial using a sterile syringe without having to directly contact the vial and break sterility. Volume recovery and compatibility studies indicated a loss of 0.2 ml (from a vial with 0.8 or 1.7 ml fill volume) associated with the use of the vial adaptor and no loss in transduction titer for the recovered vector. An additional benefit of the use of this vial adaptor is the reduced risk of an accidental needle stick injury. Over 70 patients have now been treated with vector extracted this way without needle sticks or patient infections attributable to this system.

Toca 511 is compatible with MRI contrast agents

Mixing MRI contrast agents with Toca 511 is potentially useful for verifying catheter placement and tracking *in vivo* delivery of Toca 511 in a tumor. Visualization in real time of the delivery of the mixture by MRI allows rapid assessment of alternative delivery devices, including convection-enhanced delivery (CED) methods^{14,15,19,20,22,23} at different possible flow rates and delivery volumes. To assess the utility of such methods, we conducted pilot studies for two potential MRI contrast strategies: mixing of gadolinium-based small molecule contrast agents with vector preparations to allow real-time

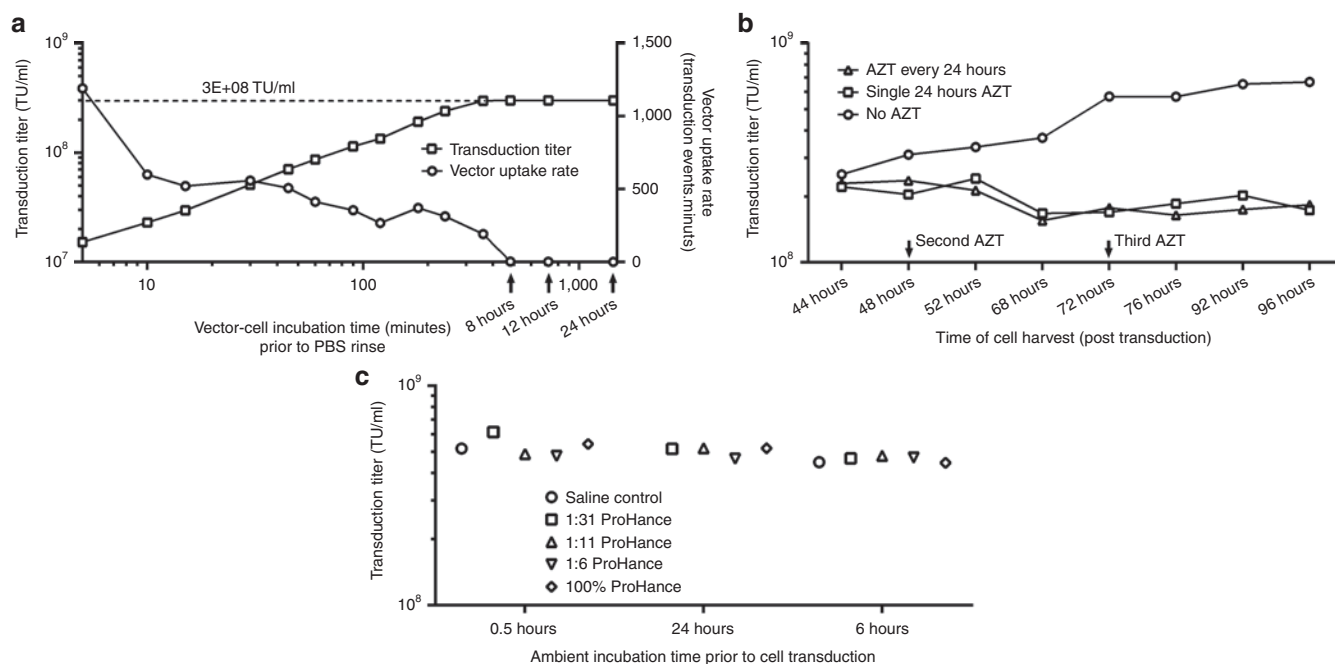


Figure 1 Optimization, characterization, and representative stability and compatibility data for the Toca 511 transduction titer assay. **(a)** Determination of appropriate time for transduction. The transduction timing was measured by determining transduction titers for Toca 511 after replacing cell culture media of transducing host cells at the indicated times (open squares, an average of three determination/time point). The vector uptake rate (open circles, second y-axis) was determined by calculating the total number of proviral copy number (by qPCR) generated with the given transduction time intervals. **(b)** AZT timing to determine single round of transduction. AZT is added to 40 $\mu\text{mol/l}$ 24 hours after transduction (single addition of AZT, open squares; multiple addition AZT, open triangles, timing as indicated) to prevent additional replication and spread of Toca 511 (determined by measuring titer). **(c)** Representative transduction titer results of an ambient temperature time-course profile of Toca 511 incubated with ProHance. ProHance (Gadoteridol Injection, 279.3 mg/ml) was diluted 1:31, 1:11, and 1:6 in saline (plus 100%, undiluted) followed by incubation at 1:17 with Toca 511 (0.53 mg/ml, 1.49 mg/ml, 2.74 mg/ml, and 16.43 mg/ml, respectively).

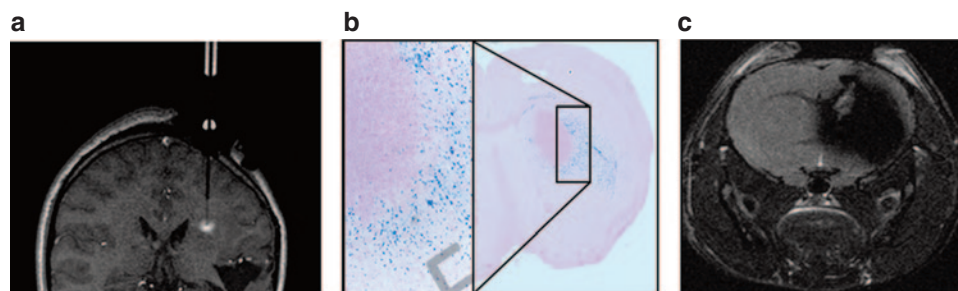


Figure 2 *In vivo* deliveries of retroviral vector with MRI contrast agents ProHance and Feraheme. **(a)** Toca 511 delivered intracranially with gadoteridol injection (0.53 mg/ml) to a human subject with glioblastoma visualized in real time with MRI during administration. **(b)** Histochemistry of a B6C3F1 mouse brain stained with Prussian blue after injection with RRV mixed with 15 μ g Feraheme showing distribution of Feraheme particles magnified in the inset. **(c)** T2 SWI MRI at 7 Tesla of brain section shown in **(b)** demonstrating strong Feraheme localization. MRI, magnetic resonance imaging; SWI, susceptibility weighted imaging.

Table 1 Compatibility of Toca 511 with MRI contrast agents

Contrast agent	Difference from CTRL (log)	Compatibility	Notes on methodology (all incubations and dwell times occur at ambient temperatures)
ProHance: study A (w/CED catheter; Polymicro Technologies)	0.089	+++	1:31 saline dilution of ProHance mixed with undiluted vector (1:17); Infused at a rate of 1.2 μ l/min. over 4 hr via laboratory syringe pump.
ProHance: study B (w/Steel Biopsy Needle (BrainLAB))	0.108 ^a	++	2:31 saline dilution of ProHance mixed with undiluted vector (1:17); slow manual infusion through the needle and collected at intervals over 6 hours.
ProHance: study C ^b	0.065	+++	1:31, 1:11, 1:6 saline dilutions of ProHance (as well as undiluted) mixed with undiluted vector (1:17) and incubated at up to 6 hours.
Feraheme ^b	-0.086	+++	1:31, 1:11, 1:6 saline dilutions of Feraheme (and undiluted) mixed with undiluted vector (1:17) and incubated at up to 6 hours.

^aControl incubations of 1:31 and 2:31 saline-diluted ProHance with Toca 511 not infused through a needle resulted in transduction titers with difference in logs from the control of -0.040 and -0.014, respectively. ^bTransduction titer difference data shown from the undiluted ProHance or Feraheme with Toca 511 1:17 mixture upon 6-hour incubation. Results from lower concentrations of ProHance as well as all earlier time points showed no significant changes in titers (data not shown). CTRL, control.

MRI visualization of vector delivery (Figure 2a) and mixing of iron nanoparticles²¹ with vector to allow real time and sustained tracking over several weeks (performed in mice, Figure 2b,c). Further details of these experiments will be published elsewhere. In preparation for these studies, we performed biocompatibility testing of Toca 511 with the MRI contrast agents ProHance (Gadoteridol Injection) and Feraheme (Ferumoxytol Injection).²¹

A 1:31 dilution of ProHance saline solution was added to Toca 511 (at 1:17) and infused at a rate of 1.2 μ l/min through a sterile fused silica CED catheter (Polymicro Technologies, Phoenix, AZ) attached to PFA Teflon tubing. Results summarized in Table 1 show no loss of transduction titers compared with controls over 4 hours at ambient temperature, indicating good compatibility. Additional studies with 2:31, 1:11, 1:6 (ProHance:saline) solutions and undiluted ProHance mixed with Toca 511 also resulted in no loss of titer over a 6 hours ambient incubation. The 2:31 sample was infused manually through a BrainLAB AG disposable biopsy needle, while collecting samples every 2 hours for titer measurement. These results supported incorporation of ProHance into our current clinical protocol; Toca 511 is combined with a gadoteridol saline solution to a final 0.53 mg/ml concentration (1:31, the lowest concentration of gadoteridol tested), and this procedure allows real time MRI *in vivo* visualization of Toca 511 placement. A representative coronal T1-weighted MRI image obtained from our clinical study using 0.53 mg/ml concentrations of gadoteridol is shown in Figure 2a.

Feraheme compatibility with Toca 511 was investigated in a similar assay matrix to ProHance. Mixing Feraheme with an RRV

can be used to track the delivery of an injectate by either histochemical staining or by MRI detection as shown in Figure 2b,c in preclinical mouse model studies. 1:31, 1:11, 1:6, straight dilutions of stock Feraheme were prepared as for ProHance in saline and combined with Toca 511 at 1:17. Table 1 summarizes the results and shows compatibility at up to the highest concentration of Feraheme.

Toca 511 is stable over 6 hours without and with ProHance. The titer of Toca 511 was monitored for ambient (18–23 °C) temperature stability over a period 6 hours to allow for delays during surgical administration. Figure 1c shows transduction titer results of Toca 511 monitored over 6 hours at ambient temperature with or without various concentration of the MRI contrast agent ProHance (Gadoteridol) upon being passed through a stainless steel biopsy needle. Table 1 shows that transduction titers did not change beyond 0.108 log of the control condition when incubated with ProHance at various concentrations.

Toca 511 is compatible with several delivery devices but not titanium needles

Compatibility of Toca 511 was assessed with stainless steel and titanium biopsy needles, a Rickham Catheter/Reservoir (polysulfone) and the Medtronic Acute Neurological Therapy Infusion System (MANTIS) investigational intracerebral delivery system. The results are shown in Table 2 and indicate complete compatibility with all

Table 2 Compatibility of Toca 511 with surgical delivery devices

Device	Difference from CTRL (log) ^a	Compatibility	Notes on methodology (all incubations and dwell times occur at ambient temperatures)
Steel Biopsy Needle (Ad-Tech)	-0.010	+++	Slow manual infusion of undiluted Toca 511 collected at intervals over 4 hours.
Neurocut Titanium Biopsy Needle (<i>in vivo</i>): study A	0.596	NC	Slow manual infusion of undiluted Toca 511 collected at intervals over 4 hours.
Neurocut Titanium Biopsy Needle (<i>in vivo</i>): study B	0.189	++	Slow manual infusion of undiluted Toca 511 collected at intervals over 2 hours.
Steel Coudé Blunt Nerve Block Needle (Epimed)	-0.086	+++	Slow manual infusion of undiluted Toca 511 collected at intervals over 2 hours.
Rickham Catheter/Reservoir (Vygon S.A., polysulfone)	-0.022	+++	Slow manual infusion of 1:50 (formulation buffer) Toca 511 collected at intervals over 2 hours.
MANTIS (Medtronic)	-0.110	+++	Undiluted Toca 511 infused at a rate of 200 µl/h over 6 hours via MiniMed Paradigm pump.
MRI-Compatible SmartFlow Cannula (MRI Interventions, silica inner lumen)	0.051	+++	Undiluted Toca 511 infused at a rate of 10 µl/min. over 4 hours via laboratory syringe pump.

MANTIS, Medtronic Acute Neurological Therapy Infusion System; NC, not compatible.

^aDifference shown is based on log of transduction titers of samples at the final collected time point vs. the analogous control.

devices except the Neurocut titanium biopsy needle, which showed a variable time dependent drop in apparent vector titer.

Toca 511 is compatible with the surgical hemostatic material SURGICEL but SURGIFOAM causes deleterious changes in pH. Toca 511 was assessed for stability in the presence of SURGICEL sterile absorbable hemostat, SURGIFOAM absorbable gelatin sponge, and Gelfoam (absorbable gelatin sponge). These hemostatic agents are often used in a surgical setting to control bleeding after tumor resection. The hemostatic agents were cut into approx. 0.5 × 0.5 cm squares (1–10 squares of SURGICEL or SURGIFOAM, and up to eight squares for Gelfoam) and incubated for 2 hours at ambient temperature with 1 ml of a 1:200 (1:50 for the Gelfoam samples) media dilution of Toca 511. SURGICEL and SURGIFOAM assessments were made by either adding the cut squares directly to the diluted vector or presoaking the squares in sterile saline prior to transferring to the diluted vector (data for the saline-soaked samples not shown; compatibility results were equivalent to the directly added samples). During the incubation with SURGICEL, a color change in the dilution media (from the phenol red, see Figure 3a) as a function of the number of squares present during the incubation, indicative of a lower pH. SURGIFOAM did not significantly change the media color. The extent of the pH change was confirmed by direct measurement (Figure 3b). The pH dropped from 7.78 for the media-diluted vector alone to 3.17 when incubated with SURGICEL (10 squares), while it only dropped to 7.19 when incubated with SURGIFOAM (10 squares) and 5.81 while incubated with Gelfoam (8 squares). For SURGICEL, titer determination of the samples revealed a sharp drop in transduction titers that was dependent on the total surface area of the SURGICEL squares incubated with the vector (Figure 3c; Table 3). Incubations with Gelfoam indicate compatibility with the smallest surface area exposure (1 square), but decreasing compatibility as a function of surface area of the agent. By contrast, incubations with the SURGIFOAM had a minimum impact on pH and measured no impact on transduction titers indicating good compatibility.

Toca 511 is compatible with cerebrospinal fluid, blood, serum, and plasma

We also assessed the impact on Toca 511 vector activity of direct contact with cerebrospinal fluid (CSF), serum, and heparin or ethylenediaminetetraacetic acid (EDTA) plasma. A 1:10 dilution of Toca 511 was incubated with increasing amounts of CSF (4.8–90.5% by volume in formulation buffer for a total final dilution of Toca 511 of 1:105) for 2 hours at ambient temperature. Table 4 shows that no significant losses of transduction titers were observed, demonstrating good biocompatibility in the presence of CSF. For blood compatibility testing, Toca 511 was spiked (100 µl into 900 µl) into fresh drawn whole blood from a healthy adult volunteer and serum or plasma with EDTA or sodium heparin prepared. All samples were centrifuged upon completion of the incubation period (20 seconds) to remove any clotted material and supernatants analyzed for transduction titers. Table 4 shows none of these matrices caused a significant loss in titer, indicating that clotted blood and blood-containing EDTA or heparin anticoagulants are compatible with Toca 511 under these conditions.

Ethanol inactivates Toca 11 more readily than DMSO

Solvents (ethanol and DMSO) common in the dissolution of small molecule drugs for *in vitro* and *in vivo* studies were investigated for effect on Toca 511 titers. The stability of Toca 511 over time with increasing concentrations of these solvents (0.5–50% by volume individually and 1–12.5% in equal proportion) was measured. These data showed that Toca 511 was not inactivated by up to 12.5% ethanol and up to 25% DMSO at time zero (*i.e.*, measured immediately after mixing) The combination of DMSO and ethanol at 12.5% each, was also not immediately inactivating (Table 4). However, after 4 hours at ambient temperature, ethanol concentrations above 5% and DMSO/ethanol mixes above 5% each reduced the vector titer by up to 25%. DMSO alone (up to 12.5%) did not decrease vector titers over 4 hours at ambient temperature.

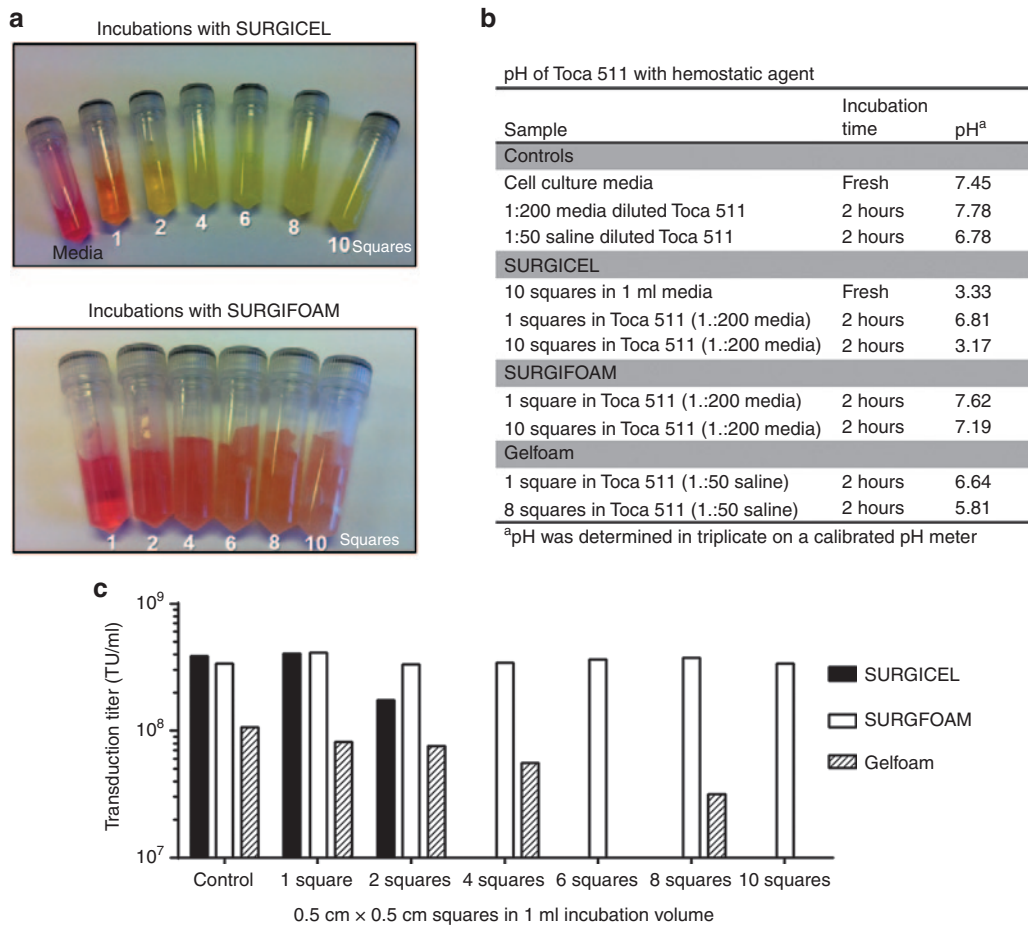


Figure 3 Compatibility assessments of Toca 511 with hemostatic agents. **(a)** pH indicator color changes from a 1 ml (1:200) dilution of Toca 511 with 1, 2, 4, 6, 8, or 10 squares (0.5 × 0.5 cm/sq) of SURGICEL or SURGI FOAM absorbable hemostatic agent. **(b)** pH measurements (Orion ROSS Micro pH Electrode (P/N 8220BNWP) of controls and the 2 hours, ambient temperature incubations (average of three determinations shown). **(c)** Transduction titer results from Toca 511/hemostat incubations. Twenty microliters of the incubation or the no-hemostat control was transferred to seeded host PC-3 cells for determination of proviral copy number after the initial round of infection (see materials and methods). Compatibility determinations (Table 3) were determined comparing incubation test articles to controls. SURGICEL became nonmeasurable for incubations greater than two squares. Gelfoam was tested for compatibility by combining saline-diluted (1:50) Toca 511 to 1, 2, 4, or 8 (0.5 × 0.5 cm) squares (6 and 10 square samples were not measured as with the other hemostatic agents) and incubated at ambient temperature for 2 hours.

Temperature-dependent gel, Lutrol F 127, does not inactivate Toca 511

Lutrol F 127 is a candidate delivery matrix for Toca 511 as it retains low viscosity at 0–4 °C and becomes highly viscous at temperatures between ambient and 37 °C. The vector-loaded gel may slow vector diffusion from the delivery area and, therefore, has the potential to enhance local delivery through slow release of the vector. Toca 511 was mixed 1:40 with a 20% aqueous Lutrol F 127 solution (19.5% final) and allowed to sit on ice along with control vector similarly diluted in phosphate-buffered saline for 60 minutes prior to transduction. In this assessment, Toca 511 retained transduction titer equivalent to controls, indicating the compatibility of Toca 511 with this potential adjuvant (Table 4).

DISCUSSION

Despite considerable activity in the use of replicating viruses for treating cancer¹⁶ and brain cancer in particular,⁹ there have been few reports of systematic investigations of biocompatibility of agents used in this context. In this report, we describe the methods and results of biocompatibility studies of Toca 511, a RRV under investigation for the treatment of recurrent high grade glioma (HGG) in two

protocols: (i) by transcranial injection into the tumor (NCT01156584) and (ii) by the injection of the resection cavity for those patients who chose a resection upon recurrence (NCT01470794). Stereotactic, transcranial injection is guided by real-time MRI visualization to accurately deliver vector to the tumor. Prior to initiating these clinical trials, compatibility testing needed to be performed to assess any negative impact on Toca 511 infectivity as a result of direct contact with different types of delivery devices, MRI contrast agents, and body fluids. Several general investigations of the stability of amphotropic retroviruses^{11–13} indicate cholesterol levels^{10,17} as well as the stability of the envelope protein complex¹⁸ may contribute to sensitivities to temperature, pH, and buffer conditions.

Toca 511 is generally stable under conditions commonly encountered during usage and/or in samples obtained for clinical laboratory tests. Exceptions include SURGI FOAM and titanium needles. In addition, we demonstrated the use of a sterile needled vial adaptor that will allow the removal of the vector without having to directly contact the vial and break sterility. Toca 511 is not affected by ProHance, a commonly used MRI contrast agent, which allows real time or near real time visualization of the vector fluid after injections into patients' brains.

Table 3 Compatibility of Toca 511 with hemostatic agents

<i>Hemostatic agent</i>	<i>Difference from CTRL (log) (surface area of hemostatic agent tested)</i>	<i>Compatibility</i>	<i>Notes on methodology (all incubations and dwell times occur at ambient temperatures)</i>
SURGICEL absorbable (oxidized cellulose-based cloth-like hemostat)	-0.107 (0.25 cm ²)	+++	1 ml of a 1:200 dilution of Toca 511 was incubated with indicated amounts of SURGICEL for 2 hours prior to determination of transduction titer.
	0.263 (0.50 cm ²)	+	
	n/d (1.00 cm ²)	NC	
	n/d (1.50 cm ²)	NC	
	n/d (2.00 cm ²)	NC	
SURGIFOAM absorbable (gelatin-based sponge-like hemostat)	-0.145 (0.25 cm ²)	+++	1 ml of a 1:200 dilution of Toca 511 was incubated with indicated amounts of SURGIFOAM for 2 hours prior to determination of transduction titer.
	-0.056 (0.50 cm ²)	+++	
	-0.066 (1.00 cm ²)	+++	
	-0.095 (1.50 cm ²)	+++	
	-0.107 (2.00 cm ²)	+++	
Gelfoam Absorbable Gelatin Sponge, USP	-0.059 (2.50 cm ²)	+++	1 ml of 1:50 saline-diluted Toca 511 was incubated with indicated amounts of Gelfoam for 2 hours prior to the determination of transduction titer.
	0.113 (0.25 cm ²)	++	
	0.150 (0.50 cm ²)	++	
	0.284 (1.00 cm ²)	+	
	0.532 (2.00 cm ²)	NC	

NC, not compatible; n/d, samples whose infectivity and proviral copy number are not detectable by the transduction titer assay method (see Materials and Methods).

In addition, Toca 511 is stable under ambient conditions for at least 6 hours after thawing. This time should be sufficient to retrieve Toca 511 from the pharmacy where it is stored frozen, transfer it to the operating theater, and administer it either by transcranial injection or into the walls of the resection cavity.

Multiple delivery devices capable of varying the infusion rate of delivery are available on the market. We found that with one exception all delivery devices tested were biocompatible with Toca 511. A titanium-based biopsy needle demonstrated a negative effect when in contact with the vector for longer dwell times (4 hours). Biocompatible devices include steel biopsy needles (BrainLAB, Ad-Tech, Racine, WI); Blunt Nerve Block needle (Epimed, Johnstown, NY); Rickham Catheter/Reservoir (Vygon S.A; Vygon, Lansdale, PA.), MANTIS investigational device; Medtronic, Minneapolis, MN), and MRI-Compatible SmartFlow Cannula (MRI Interventions, Memphis, TN).

One of the more important findings of this study is the incompatibility of Toca 511 with the hemostatic agent SURGICEL (an absorbable cloth-like material made from oxidized cellulose) commonly used to control bleeding during and after neurosurgical procedures. The study demonstrated that this agent lowers the pH of the test environment. The pH drop is believed to have had a negative effect on the infectivity of Toca 511. Based on these findings, SURGICEL is excluded from use in the current clinical trials of Toca 511. SURGIFOAM (an absorbable sponge-like agent made of gelatin), and to a lesser extent Gelfoam, are recommended as alternative hemostatic agents to control bleeding where necessary.

The current study should alleviate the concern that body fluids could negatively impact the infectivity of Toca 511. The Toca 511 vector is produced from a human cell line. Previous work demonstrated that retroviral vectors produced from human cell lines are relatively resistant to inactivation by human blood, including complement-mediated inactivation.²⁴ This report was able to support this finding as human whole blood (pre- or postclot) did not appear to have any impact on the infectivity of Toca 511. Exposure to CSF also showed complete compatibility with Toca 511 for at least 2 hours.

In summary, formulations of Toca 511 appear to have good stability characteristics capable of withstanding most operating room situations tested here, including 6 hours stability at room temperature after thawing from freezing at ≤ -65 °C. A notably incompatible product was SURGICEL, a hemostatic device that reduces pH upon contact with aqueous liquids. Otherwise Toca 511 was able to withstand exposure to a variety of physical and chemical agents used in the required neurosurgical procedures. Nevertheless, it will be valuable to continue testing any new excipients or devices when their use with Toca 511 is considered.

In conclusion, our data show that it is important to conduct studies, such as these with novel biological treatment agents, as the risk of inactivation is difficult to predict without experimental verification. This strategy eliminates possible sources of uncertainty in interpreting clinical trial results, and maintains the expected risk/benefit ratio for clinical trial subjects.

Table 4 Compatibility of Toca 511 with biofluids, solvents, and adjuvants

Fluid agent	Difference from CTRL (log)	Compatibility	Notes on methodology (all incubations and dwell times occur at ambient temperatures)
Biofluids			
Cerebrospinal fluid (CSF)	-0.061	+++	CSF was incubated from 4.8–90.5% volumetrically with a 1:10 dilution (in formulation buffer) of Toca 511 and incubated for 2 hours prior to titer measurement.
Human whole blood			
(serum preclot)	-0.009	+++	Blood collected to serum tubes was divided to preclot and postclot. Toca 511 was added at a ratio of 1:10 and mixed before clotting with “preclot” and added and mixed after clotting for 20 minutes for “postclot.” Toca 511 was added at a 1:10 ratio, mixed, and incubated for 20 minutes in blood with EDTA or heparin. All samples were spun and supernatants further diluted 1:10 in culture media prior to transduction.
(serum postclot)	-0.084	+++	
(EDTA plasma)	-0.057	+++	
(heparin plasma)	0.002	+++	
Solvents			
Ethanol (up to 5%)	0.070	+++	Toca 511 was diluted 1:20 in media prior to mixing 1:1 with solvents and diluted solvents (1:40 final for vector). Percentage shown is the % solvent (% each solvent for the 1:1 combination). Data shown represents the results of the 4 hours incubation; To determine the impact of limited exposure, time points of 30, 60, and 120 minutes were also collected and analyzed. For EtOH samples, incubations at 12.5% were fully compatible (+++) until 2 hours. For DMSO samples, incubations at 25% were compatible (+++) until 2 hours as well. For the EtOH:DMSO mixture, 12.5% was compatible (+++) at 30 minutes and dropped significantly (+) by 1 hour.
(12.5%)	0.248	++	
(≥25%)	n/d	NC	
DMSO (up to 12.5%)	-0.052	+++	
(25%)	0.137	++	
(50%)	n/d	NC	
1:1 EtOH/DMSO (1%)	0.055	+++	
(2%)	0.198	++	
(5%)	0.292	+	
(12.5%)	0.898	NC	
Adjuvant			
20% Lutrol F 127 (aqueous)	0.060	+++	Toca 511 was mixed 1:40 with 20% Lutrol and incubated 1 hour on ice to maintain low viscosity (at ambient to 37 °C, aqueous Lutrol resembles a firm colloid suspension) prior to host cell transduction.

DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; EtOH, ethanol.

MATERIALS AND METHODS

Needles and delivery devices

The following devices were tested: Stainless steel disposable biopsy needle (BrainLAB AG, Feldkirchen, Germany); stainless steel disposable biopsy needle (Ad-Tech; P/N DBN-08-19X); Neurocut titanium biopsy needle (In vivo, Hennigsdorf, Germany; Ref # 2014-03); fused silica CED catheter (Polymicro Technologies; P/N TSP 320450) coupled to PFA Teflon tubing (0.02" ID × 1/16" OD; Upchurch Scientific, IDEX, Oak Harbor, WA; P/N 1622); Coudé blunt nerve block needle (Epimed; Ref # 117-2230); 1/2CC U-100 Insulin syringe, 1-, 3-, 5-, and 10-ml BD Luer-Lok syringes and 21G (Ref # 305167) needle (BD, Franklin Lakes, NJ); Rickham Catheter/Reservoir (Vygon S.A., PC01 15 cm ventricular catheter; Vygon S.A., VR05 polysulfone Rickham Reservoir); MRI Interventions Smartflow neuro ventricular cannula (MRI Interventions; Ref # NGS-NC-02; 10 f. by 0.008" internal diameter); MANTIS, an investigational intracranial catheter and extension set, an investigational cranial anchor, an accessory kit, and an external MiniMed Paradigm pump adapted for this investigational use (Ref # 8764, 8763, 8765 and MMT-715NALN, respectively; Medtronic).

Hemostatic materials

The following materials were tested: SURGICEL sterile absorbable hemostat (oxidized cellulose-based cloth-like hemostat, Johnson and Johnson, P/N 1952); SURGIFOAM (absorbable gelatin-based sponge-like hemostat; Johnson and Johnson; P/N 1972); Gelfoam (absorbable gelatin sponge, USP; Pfizer Pharmaceutical).

Solvents, adjuvants, biofluids, and MRI contrast reagents

The following liquid agents were tested: saline (0.9% sodium chloride, injection, USP, NDC 0409-7983-02; Hospira, Lake Forest, IL); dimethyl sulfoxide (Calbiochem, Merck Millipore, Darmstadt, Germany); ethanol (Absolute for Molecular Biology, Sigma-Aldrich, St Louis, MO); ProHance (Gadoteridol Injection, 279.3 mg/ml; Bracco Diagnostics, Monroe Township, NJ); Feraheme (Ferumoxytol) injection (AMAG Pharmaceuticals, Waltham, MA); Lutrol F 127 (BASF, Ludwigshafen, Germany, article 51632903); CSF (BioReclamation, Westbury, NY; P/N HMCSE, lot # BRH45611); peripheral blood from a normal, healthy individual drawn into glass serum, EDTA, or heparin (Vacutainer, Ref. 366430, 366643 and 367871, respectively; BD) blood tubes.

To evaluate ProHance, 50 μ l of a 1 mmol/l ProHance solution in saline was added to 800 μ l of Toca 511 (1:17) for a final concentration of 0.53 mg/ml gadoteridol (referred to as 1:31). Additional studies with higher concentrations of ProHance, 1.06, 1.49, 2.74, and 16.43 mg/ml final gadoteridol (2:31, 1:11, 1:6, and undiluted dilutions, respectively) were conducted. Feraheme compatibility with Toca 511 was investigated in a similar manner to the ProHance dilution paradigm. The same 1:31, 1:11, and 1:6, straight dilutions as for ProHance were prepared in saline and combined with Toca 511 in a 1:17 ratio as described in the ProHance method above.

Toca 511

Toca 511 is a murine RRV with an amphotropic envelope and a modified yeast CD transgene expressed from an internal ribosome entry signal (IRES). Toca 511 is categorized as a Risk Group 2 (RG2) virus described in the NIH Guidelines for Research Involving Recombinant DNA Molecules. Construction, modifications, and infectious vector production has been previously described.^{1,3}

Toca 511 transduction titer

Compatibility assessments were based on transduction titer (TU/ml) determinations of vector preparations after exposure to an agent or device for various times or under a variety of conditions compared to appropriate controls. Transduction titers were determined by measuring the integrated copy number of the Toca 511 provirus after an initial round of infection of PC-3 cells (human prostate carcinoma, ATCC no. CRL-1435). Host PC-3 cells were cultured at 37 °C and 5% CO₂ in complete DMEM supplemented 10% with gamma-irradiated, heat-inactivated FBS (Hyclone, Logan, UT), 2 mmol/l Glutamax (Invitrogen, Life Technologies, Carlsbad, CA), and 10 μ mol/l sodium pyruvate (Hyclone) (also known as culture media). Twelve to eighteen hours prior to transduction, host cells were harvested, counted, and seeded at 2×10^5 cells/well in 12-well plates. On the day of transduction, compatibility assessment samples, reference standards, and controls were set up ahead of time to complete any dwell-time and/or incubation such that all transductions occurred together. Typically, 20 μ l of a 1:10–1:200 dilution (in culture media to be within linear range of transduction of the assay) of controls and test articles were used to transduce host cell wells (in triplicate) in the presence of 4 μ g/ml hexadimethrine bromide (polybrene) (Sigma-Aldrich; 1 mg/ml stock in phosphate-buffered saline sterile filtered). Cells were returned to the incubator for 20–28 hours followed by addition of Azido-3'-deoxythymidine (AZT) (Sigma-Aldrich; 10 mmol/l stock in phosphate-buffered saline sterile filtered) to 40 μ mol/l to arrest subsequent viral replication. On an additional 24 hours of incubation (44–52 hours posttransduction), cells were harvested to purify genomic DNA (gDNA) by phosphate-buffered saline rinse and trypsinization (Trypzean; Sigma-Aldrich). DNA was purified using a Promega Maxwell 16 automated purification instrument using Maxwell 16 Tissue DNA Purification Kits (Promega, Madison, WI) (AS1030). Once purified, gDNA concentrations were measured in triplicate and averaged using a Nanodrop ND-8000 (Thermo Scientific, Waltham, MA). Absolute quantitative PCR (qPCR) was performed in triplicate on each preparation using primers and probes (Integrated DNA Technologies; forward: 5'-AGC CCA CAA CCC CTC ACT C-3'; reverse: 5'-TCT CCC GAT CCC GGA CGA-3'; probe: 5'-(FAM)-CCC CAA ATG AAG GAC CCC CGC TGA CG-(BHQ)-3') to span the U3 to Psi region of the MLV backbone producing a 192-bp amplicon. QPCR was performed on a Bio-Rad CFX96 Real Time System (Bio-Rad Laboratories, Hercules, CA) using the following parameters: 95 °C for 5 minutes followed by 38 cycles of 95 °C for 15 seconds, 60 °C for 30 seconds, and channel 1 (FAM) fluorophore measurement. The proviral copy number was determined using an intraplate 7-log serial dilution plasmid standard measured in triplicate. Proviral copy numbers were used to determine the initial number of transduction events during transduction.

Statistics and compatibility determinations

Within individual assays for the compatibility assessments, transduction titers were determined from averaging a minimum of three transduction events from which the proviral copy number measurements were made from triplicate qPCR reactions within replicate transduction wells (thus transduction titer determinations are from a minimum of nine averaged qPCR proviral copy number determinations from the three replicate transductions) and converted to transduction titer as described above. Intraassay precision determinations using a Toca 511 reference vector indicate an ~20% CV corresponding to a 0.10 log difference of the transduction titers. Interassay precision measurements over >20 assays for the same reference vector indicated an ~30% CV.

Because the assay utilized is a biological assay and subject to some assay variability, compatibility differences were compared in increments of 0.25 log differences of average replicate measurements to identify changes in titer between the various conditions and time points. To assess the compatibility of Toca 511 under the conditions described in this report, we defined criteria based on the changes observed in transduction titer in increments of 0–0.1 (+++), 0.1–0.25 (++), 0.25–0.5 (+), and >0.5 (not compatible) log differences from controls.

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CONFLICT OF INTEREST

R.B., C.E.I., P.L.P., C.-I.C., S.P., T.H., J.R., D.P., H.E.G. and D.J.J. are employees and/or shareholders of Tocagen. C.L. and N.K. are consultants, shareholders, and recipients of a research grant from Tocagen.

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