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The Rational Design and Development of A Dual Chamber Vaginal/Rectal Microbicide Gel Formulation for HIV Prevention

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Abstract

The DuoGel™ was developed for safe and effective dual chamber administration of antiretroviral drugs to reduce the high incidence of HIV transmission during receptive vaginal and anal intercourse. The DuoGel™s containing IQP-0528, a non-nucleoside reverse transcriptase inhibitor (NNRTI), were formulated from GRAS excipients approved for vaginal and rectal administration. The DuoGel™s were evaluated based upon quantitative physicochemical and biological evaluations defined by a Target Product Profile (TPP) acceptable for vaginal and rectal application. From the two primary TPP characteristics defined to accommodate safe rectal administration three DuoGel™ formulations (IQB3000, IQB3001, and IQB3002) were developed at pH 6.00 and osmolality 400 mmol/kg. The DuoGel™s displayed no *in vitro* cellular or bacterial toxicity and no loss in viability in ectocervical and colorectal tissue. IQB3000 was removed from consideration due to reduced NNRTI delivery (~65% reduction) and IQB3001 was removed due to increase spread resulting in leakage. IQB3002 containing IQP-0528 was defined as our lead DuoGel™ formulation, possessing potent activity against HIV-1 (EC₅₀ = 10 nM). Over 12 month stability evaluations, IQB3002 maintained formulation stability. This study has identified a lead DuoGel™ formulation that will safely deliver IQP-0528 to prevent sexual HIV-1 transmission in the vagina and rectum.

Keywords

microbicides; HIV prevention; formulation; vaginal gel; rectal gel; pyrimidinedione

1. INTRODUCTION

In addition to the ongoing development of products to effectively prevent the transmission of HIV in the vagina, there is a growing appreciation of the significant need to mitigate

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rectally transmitted HIV (Beyrer *et al.*, 2012; Beyrer *et al.*, 2013; El-Sadr *et al.*, 2010). Unprotected receptive anal intercourse (RAI) is practiced by both women and men in the developing (Kalichman *et al.*, 2009; Lane *et al.*, 2006) and developed (Gorbach *et al.*, 2009) world, representing one of the highest risk sexual behaviors for HIV transmission with rates of transmission 10 to 20 times greater than unprotected vaginal intercourse (Vittinghoff *et al.*, 1999). Consequently, as microbicide products advance in the clinic, it is critical that rectally-delivered microbicides also be evaluated. Microbicides are agents applied topically to the vagina or rectum prior to sexual intercourse to prophylactically inhibit transmission of STIs, including HIV, and are currently in relatively early stages of development. Gels are the leading dosage form since minimal behavior modification is needed due to common use of lubricants by men who have sex with men (MSM) during RAI (Carballo-Díezguéz *et al.*, 2000). However, many of the regularly used lubrication products are hyperosmolar and have shown toxicity to colonic epithelial cells and tissues *in vitro* and use of these products has resulted in increased risk of pathogen acquisition (Begay *et al.*, 2011; Dezzutti *et al.*, 2012a; Gorbach *et al.*, 2012; Rebe *et al.*, 2014). This osmolality issue must be considered as a critical aspect in the design of microbicide formulations such as gels and liquid/semi-solid dosage forms. Since the practice of RAI is not limited to MSM, with reports of approximately 30% of women also engaging in RAI, there is additional rationale for development of a single microbicide formulation that is suitable for both rectal and vaginal applications (Gorbach *et al.*, 2014).

Willingness to use a product is a critical issue for microbicide development in general. It has become well accepted in the microbicide field that even without a rectal microbicide product with proven efficacy, there is a need to integrate qualitative and quantitative user sensory perception and experience (USPE) data to direct the formulation characteristics most desired by the targeted users of a product (Morrow and Ruiz, 2008). Indeed, lack of product efficacy in the VOICE and FACTS 001 trials of a vaginal gel delivering tenofovir are attributed, in large part, to poor adherence by trial participants (Marrazzo *et al.*, 2015; Rees *et al.*, 2015). To better inform microbicide development, participants in user acceptability studies have been asked to comment upon hypothetical as well as specific products, including preferred product characteristics and their willingness to use such products. Women have expressed an interest in a rectal microbicide (Exner *et al.*, 2008) and gels have been indicated as the preferred dosage form for rectal use, in volumes of up to 35 mL (Carballo-Díezguéz *et al.*, 2008; Carballo-Díezguéz *et al.*, 2007). A user acceptability study of a thiocarboxanilide (UC781)-containing rectal gel resulted in a favorable rating and high intention to use such a product among both men and women (Ventuneac *et al.*, 2010).

To date, the rectal microbicide field has focused primarily on rectal application of formulations which were created for vaginal use. However, the significant physiological differences between the vaginal and rectal compartments challenge a simple translation of a vaginal product to a rectal product. Structurally, the vagina is composed of a stratified squamous epithelium, while the rectum/lower gastrointestinal tract is covered by a simple columnar epithelium. Additionally, the length of the colon as an “open cavity,” compared to the “closed cavity” of the vagina, poses challenges for choosing dosage volume as it provides a greater surface area for infection than does the vagina. The normal vagina is

acidic (pH 4–4.5) due to the presence of lactic acid producing *Lactobacilli*, whereas the rectum has neutral to slightly alkaline pH. Microbicide gels suitable for rectal delivery have been developed (Anton *et al.*, 2012; Dezzutti *et al.*, 2012b; McGowan, 2011; McGowan and Dezzutti, 2014). In this process, early vaginal microbicides and commercial rectal lubricants were found to be hyperosmotic, and the prevailing thought is that to minimize damage to the rectal tissue, a rectal microbicide gel should be iso-osmotic and at a neutral pH (Wang *et al.*, 2011). There is evidence of a direct relationship between higher product osmolality and epithelial damage to vaginal and rectal epithelial tissue and structure (Fuchs *et al.*, 2007; Rebe *et al.*, 2014; Rohan *et al.*, 2010).

Because of the need to protect MSM populations from rectal HIV exposure, and increasing evidence that a significant proportion of women engage in RAI (often in the same sex act involving vaginal sex), there is a need to develop a microbicide product that is specifically designed for both vaginal and rectal applications (Dezzutti *et al.*, 2012b; Gorbach *et al.*, 2014). For women who engage in both vaginal and anal sex in the same sexual encounter, use of a single product that is safe for both compartments would be convenient and more likely to be used. Herein, we describe the development of a gel formulation, designed for safe and efficacious use in both the vagina and rectum, which delivers the nonnucleoside reverse transcriptase inhibitor (NNRTI) IQP-0528 as a topical anti-HIV microbicide. The data presented summarize diverse, pharmacologically relevant evaluations of candidate gels (termed “DuoGel™s”) including their rheological, *in vitro* and *ex vivo* safety and bioactivity properties. These performance analyses of prototype DuoGel™s yielded a lead candidate formulation indicated for further development towards joint vaginal and rectal use.

2. MATERIALS AND METHODS

2.1. Compound

IQP-0528 was provided by Samjin Pharmaceutical Co. (Seoul, Korea), and is licensed to ImQuest BioSciences Inc. (Frederick, MD). IQP-0528 is a nonnucleoside reverse transcriptase inhibitor with a molecular weight of 340.42 Da and an EC₅₀ of 0.0005 μM (Buckheit *et al.*, 2008; Buckheit *et al.*, 2007). It is practically insoluble in water and has a calculated Log P of 4.1 (Mahalingam *et al.*, 2011). ImQuest BioSciences currently has an open Investigational New Drug program for the clinical evaluation of a vaginal gel containing IQP-0528.

2.2. Cell Lines, Virus and Bacteria

The TZM-bl-FcRI cells were a gift from Dr. David Montefiori (Duke University, Durham, NC). The Ca Ski, ME180, HEC-1A, END1, ECT1, VK2, Caco-2 and *Lactobacillus* strains (*L. crispatus* ATCC # 33820, *L. jensenii* ATCC #25258 and *L. acidophilus* ATCC #11975) were purchased from the American Type Culture Collection (Manassas, VA). The HIV-1_{BaL} and clinical virus isolates were obtained from the NIAID AIDS Research and Reference Reagent Program (Rockville, MD) or HIV-1_{BaL} was purchased from Advanced Biotechnologies Inc. (Eldersburg, MD). Human peripheral mononuclear blood cells (PBMCs) were derived from human blood which was purchased from Biological Specialty Corporation (Colmar, PA). The cell lines were propagated as recommended; titrated stocks

of HIV-1_{BaL} and clinical virus strains were stored at -80°C prior to being used in the antiviral assays.

2.3. Vaginal and Seminal Fluid Simulants

The vaginal and seminal fluid simulants used in the HIV-1 efficacy assays in TZM-bl-FcRI cells were prepared as previously reported (Owen and Katz, 1999; Owen and Katz, 2005).

2.4. Human Tissue

Normal human ectocervical and colonic tissues were acquired from pre-menopausal women undergoing hysterectomy or persons undergoing gastrointestinal surgery for non-inflammatory conditions, respectively, under approved IRB protocols with all patient identifiers removed. Normal human ectocervical tissue was also purchased from the National Disease Registry Interchange (<http://ndriresource.org/>) through an approved protocol, and shipped overnight on ice. Polarized explant cultures were set-up in duplicate as previously described (Rohan *et al.*, 2010). Briefly, an explant was placed with the luminal side up in a transwell. The edges around the explant were sealed with Matrigel™ (BD Biosciences, San Jose, CA). The explants were maintained with the luminal surface at the air-liquid interface. The lamina propria was immersed in medium for cervical explants or rested on medium-soaked gelfoam for colonic explants. Cultures were maintained at 37°C in a 5% CO_2 atmosphere.

2.5. DuoGel™ formulation development

Working with excipients that pharmacologically were generally regarded as safe (GRAS), prototype gels were formulated in several iterations. These gels were evaluated against a defined Target Product Profile (TPP) developed by ImQuest BioSciences (Figure 1). The physicochemical characteristics of these prototype formulations included gel appearance, pH (SI Analytics Lab850), viscosity (at 1 sec^{-1} ; TA Instruments, Discovery HR-1 Rheometer), osmolality (Wescor Vapro Osmometer), and IQP-0528 content (Shimadzu Prominence HPLC). The formulations were assessed over a two-week period. Those achieving the defined TPP were retained for more detailed evaluations and further development. The TPP defined for a successful DuoGel™ formulation includes: IQP-0528 drug content of 1.0% (w/w), formulation osmolality to be $< 400\text{ mOsm/kg}$, pH in the range of 5.9 to 6.1, a formulation point viscosity ($@ 1\text{ sec}^{-1}$) of $120\text{ Pa}\cdot\text{s}$, and a demonstrated *in vitro/ex vivo* safety profile against vaginal and rectal cells and tissue and Lactobacilli.

2.6. Rheological characterization

The rheological properties of the gel formulations were characterized at 37°C by steady shear rheology using a TA Instruments model AR 1500ex rheometer with a 20 mm, 4° angle cone and plate geometry. The loading volume of undiluted gel was $150\text{ }\mu\text{L}$ with a gap distance between the geometry and Peltier plate of $108\text{ }\mu\text{m}$. Gel viscosity was then monitored in a shear rate ramp from 0.05 Pa to 250 Pa and then from 1 s^{-1} to 250 s^{-1} . Residual stresses of gels were measured, as surrogates for yield stress, by stress relaxation experiments in a Brookfield 5HB DV-III Ultra rheometer with a CPE-40 cone. The gel was initially stressed at 10 sec^{-1} for 5 min, and then relaxed for 14 min, during which time stress

was measured to determine a limiting value. Raw rheometric data were fit to the Carreau-like constitutive equation, which relates viscosity to shear rate:

$$F(t) = \frac{1}{m_0} + \frac{1}{m} \left(\frac{\tau}{m} \right)^{\frac{1-n}{n}}$$

$$\text{where } \dot{\gamma} = \tau F(\tau)$$

Here, τ is shear stress and $\dot{\gamma}$ is shear strain rate; m_0 , m and n are parameters that characterize the rheological behavior of the material. Rheological parameters computed from these analyses were input to a computational model of gel spreading along the vaginal canal (Gao *et al.*, 2015).

2.7. Osmolality evaluation

The osmolality of the gel was determined using a Vapro 5520 vapor pressure osmometer with standard calibration as per the manufacturer's instructions. The measurements were performed in triplicate (n=3).

2.8. IQP-0528 content

IQP-0528 concentrations were determined using a gradient HPLC method. IQP-0528 was analyzed using an ES Industries Sonoma C18 (150mm × 4.6 mm, 5 μm) analytical column with a Zorbax Eclipse XDB-C18 (12.5 mm × 4.6 mm, 5 μm) guard column. The mobile phases were: (A) H₂O + 0.1% TFA (v/v) and (B) ACN + 0.1% TFA (v/v). IQP-0528 was injected at 20 μL at a flow rate of 1.00 mL/min with gradient of 20% mobile phase B to 80% mobile phase B over 28 minutes. IQP-0528 was detected at a wavelength of 267 nm with an LLOQ of 7.35 nM.

2.9. IQP-0528 release and permeability

The release of active pharmaceutical ingredient (API) from the gel formulations was evaluated in a Franz Cell apparatus (PermaGear) (Sassi *et al.*, 2004). API permeated into and through three-dimensional, full thickness VEC-100 FT EpiVaginal tissue purchased from MatTek Corporation (Ashland, MA). In the donor compartment, 900 μL of undiluted gel was applied to the apical side of the tissue. Subsequently, tissue was incubated at 37°C/ 5% CO₂ for 4 hours. In separate experiments, at hours 1, 2, 4 and 6, the tissue sample was removed from the Franz cell and washed with PBS to remove excess gel formulation. The tissue was then homogenized via probe sonication in mobile phase. The solution was centrifuged and filtered, and concentration of IQP-0528 extracted from the tissue was determined via HPLC analysis. The basal tissue culture medium was collected at each time point and the concentration of IQP-0528 was also determined by HPLC analysis.

2.10. In-vitro efficacy and toxicity

2.10.1. Anti-HIV assay using Human Peripheral Blood Mononuclear Cells—

PBMC-based anti-HIV assays were performed as previously described (Watson *et al.*,

2008). Briefly, isolated PBMCs from three individual donors were obtained following Ficoll-Hypaque fractionation and were activated and induced to proliferate with PHA for 3 days and then cultured in the presence of IL-2. PBMCs from three donors were pooled together and re-suspended in tissue culture medium at a concentration of 1×10^6 cells/mL and plated in the interior wells of a 96-well round bottom microtiter plate in a volume of 50 μ L/well. Based upon IQP-0528 drug concentrations, serially diluted DuoGel™ formulations were added to the plate followed by the appropriate pre-titered strain of HIV_{US/92/727} in triplicate experiments. The culture was then incubated for 7 days at 37°C/5% CO₂. Following the incubation, supernatants were collected for analysis of virus replication by supernatant reverse transcriptase (RT) activity and cells were analyzed for viability by XTT tetrazolium dye reduction. AZT was used as an internal assay standard control. Clinical subtypes of virus that represent HIV strains found in various geographic locales throughout the world, representing clades A through G and O, were utilized.

2.10.2. Inhibition of HIV-1 in the presence of simulants in TZM-bl-FcRI cells—

Twenty-four hours prior to compound and virus addition, TZM-bl-FcRI cells diluted in DMEM supplemented with 10% FBS and antibiotics were plated in 96-well flat bottomed microtiter plates at 1.5×10^4 cells per well in a volume of 100 μ L and incubated overnight at 37°C/5% CO₂. Following the incubation, test compound was serially diluted in vaginal fluid simulant or seminal fluid simulant, which was added to the cells in a volume of 100 μ L per well. HIV-1_{BaL} was diluted to a pre-determined infectious titer in assay medium and added to the microtiter plate in a volume of 100 μ L. The cultures were incubated for 48 hours at 37°C/5% CO₂. Following the incubation, toxicity plates were evaluated by XTT staining, as previously described (Buckheit *et al.*, 1995) and efficacy plates were evaluated for virus production by chemiluminescence detection using Gal-Screen (Applied Biosystems) according to the manufacturer's instructions.

2.10.3. Evaluation of toxicity to epithelial cell lines—

ME180 (cervical epithelial), Ca Ski (cervical epithelial), HEC-1-A (uterine epithelial) Caco-2 (epithelial colorectal), ECT1 (ectocervical), END1 (endocervical) and VK2 (vaginal epithelium) cells were added to a 96-well flat-bottom plate 24 hours prior to the addition of compound and media at a density of $1 \times 10^4 - 5 \times 10^4$ cells/well (depending on the cell line) in a total volume of 100 μ L. DuoGel™ was serially diluted in half-logarithmic increments and added in a volume of 100 μ L to the seeded cells following removal of the media. One hundred microliters (100 μ L) of medium was added to each plate. The plates were incubated for 24 hours at 37°C/5% CO₂ and washed three times with RPMI-1640 (no additives). Following the incubation, 200 μ L of assay medium was then added to the plates and allowed to incubate at 37°C/5% CO₂ for 24 hours. Cellular toxicity was then evaluated using the tetrazolium dye XTT.

2.10.4. Evaluation of toxicity to human PBMCs—

Isolated PBMCs from three donors were obtained following Ficoll-Hypaque fractionation and were activated and induced to proliferate with PHA for 3 days and then cultured in the presence of IL-2. They were then pooled and resuspended in fresh tissue culture medium at a concentration of 1×10^6 cells/mL and plated in the interior wells of a 96-well round bottom microtiter plate in a volume of 50 μ L/well. One-hundred microliters (100 μ L) of medium with 2X the

concentration of compound was transferred to a round-bottom 96-well plate containing the cells. Immediately following addition of compound to the wells, 50 μ L of media was added. After 6 days in culture at 5% CO₂/37°C, cytotoxicity was evaluated using the tetrazolium dye XTT as described above.

2.10.5. Lactobacillus Toxicity Assay—In a 15 mL conical tube, 10 mL of MRS media was inoculated with a stab from a frozen glycerol stock of *Lactobacillus crispatus*, *L. jensenii*, or *L. acidophilus* and was incubated for 24 hours at 37°C in an anaerobic chamber. The overnight culture was diluted in MRS media until an absorbance of 0.06 at 670 nm was obtained. Six serial ½ log dilutions of the compound were performed and were added in a volume of 100 μ L to the plate, followed by the addition of 100 μ L of the diluted bacteria. The plates were placed in an anaerobic chamber and incubated at 37°C for 24 hours. Following the incubation the plates were read spectrophotometrically at 490 nm.

2.10.6. Epivaginal Tissue Toxicity Assay (MatTek)—Upon receipt of the epivaginal full thickness tissues, the agarose was removed from the tissue inserts and they were placed into a 6-well plate containing 2.5 mL of pre-warmed media on the basal surface only. After a 24 hour equilibration period at 37°C/5% CO₂, the media was removed from the plate and 2.5 mL of fresh, pre-warmed media was added to the basal well layer. The apical surface of the tissues was then exposed to 100 μ L of the test article in duplicate for a period of 24 hours at 37°C/5% CO₂. Following the incubation period, the tissues were washed three times with PBS to remove the test article from the tissue. Toxicity was evaluated utilizing MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The exposed tissues were stained with MTT at a concentration of 2 mg/mL for three hours at 37°C/5% CO₂. Following the incubation the tissues were placed into 4 mL of MTT solution and incubated overnight at room temperature. Two-hundred microliters of the extractant solution was removed from the tissues and optical density was evaluated at wavelengths of 570/650 nm.

2.11. Ex vivo safety testing of DuoGel™ in human ectocervical tissue and colonic tissue

A 1:5 dilution of gel in media was applied to the apical side of the explants for 18h at 37°C. As controls, explants were untreated or a 1:5 dilution of Gynol II, a 3% nonoxynol-9 gel (Personal Products Company/McNeil-PPC, Inc., Skillman, NJ), was applied apically for 24h at 37°C. The next day, explants were washed and viability was evaluated using the MTT [1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan] assay and histology.

2.12. Ex vivo efficacy of DuoGel™ in human ectocervical tissue and colonic tissue

DuoGel™s containing IQP-0528 were initially screened at a 1:5 dilution to assess product efficacy. Once the final DuoGel™ formulation was identified, 10-fold dilutions beginning with an initial 30-fold dilution were made in the appropriate medium to determine the potency of the product. In both cases, product was applied to the apical surface of polarized ectocervical and colonic explants. Medium-treated only explants served as controls. HIV-1_{BaL} was then added to the apical surface. After overnight culture at 37°C, the explants were then washed and fresh medium was replaced in the basolateral compartment. Every 3–4 days, supernatant was collected and replenished through day 21 of culture and stored at –80°C for evaluation in the HIV-1 ELISA (Perkin-Elmer, Waltham, MA). For ectocervical

explants, tissue was fixed in paraformaldehyde to perform immunohistochemistry for HIV-1 p24 antigen.

2.13. Stability of IQP-0528 DuoGel™

Stability evaluations of the three lead DuoGel™ formulations were performed over 6 months. One kilogram gel batches were prepared and placed on formal stability evaluations in 20 mL glass vials with PTFE lined caps under accelerated conditions (40°C/75%RH). The testing was performed at initiation, one, three, and six months. Gels were assessed for their stability via physical evaluation, osmolality, viscosity, dosage form pH, and API recovery. Following the 6 month stability evaluation, the final DuoGel™ formulation was identified and this formulation was carried through additional stability testing at nine and twelve months.

3. RESULTS

3.1. Iterative Formulation of DuoGel™s IQB3000, IQB3001, and IQB3002

3.1.1. DuoGel™ formulation—The DuoGel™ was developed building upon on our experience creating vaginal gels containing IQP-0528 (Ham *et al.*, 2012; Mahalingam *et al.*, 2011). Four primary characteristics were defined as the Target Product Profile (TPP) of the DuoGel™. Based on our experience with prior IQP-0528 gels along with the Tenofovir-containing gels, it has been accepted that an API dosing of 1% weight drug per weight formulation (w/w), is an appropriate amount of the antiviral agent (Ham *et al.*, 2012; Mahalingam *et al.*, 2011; Rohan *et al.*, 2010). Therefore, the DuoGel™ formulation TPP was defined at 1% (w/w) IQP-0528. All excipients included in the gel formulations here were tested for compatibility with the candidate API. The DuoGel™ formulations developed were composed of excipients either listed in the FDA Inactive Ingredients Database (<http://www.accessdata.fda.gov/scripts/cder/iig/index.cfm>) as accepted excipients in vaginal products, rectal products, or already used in over the counter products. The TPP viscosity of the DuoGel™ was initially defined at 120 Pa·s at 1 sec⁻¹ shear stress in order to provide sufficient gel coverage in the vagina and rectum without causing leakage (Gao *et al.*, 2015). The DuoGel™ TPP pH of 6.1 was based on achieving a compromise value that does not harmfully perturb ambient conditions in the vagina or rectum. The final TPP characteristic is formulation osmolality which was defined at < 400 mOsm/kg in order to approach physiological iso-osmolality. In several iterative steps, multiple prototype formulations of the DuoGel™s were manufactured and quickly evaluated against the defined TPP (Figure 1). Formulations that did not meet the TPP were removed from further development. Based on the initial formulation development, three final DuoGel™ formulations, designated IQB3000, IQB3001, and IQB3002 (Table 1), were chosen for scaled up to 1 kilogram batches for formal evaluations.

3.1.2. Rheological characterization—On a log-log scale, the viscosity of all 3 formulations decreased quasi-linearly as the shear rate decreased from 10⁻² to 100 s⁻¹ consistent with a shear thinning behavior (Figure 2). The viscosity measurements taken at a shear rate of 1 s⁻¹, which are a basic, albeit incomplete measure of the gels' spreading capabilities that is often reported (Table 1) (Ham *et al.*, 2012). These viscosities for

IQB3000, IQB3001, and IQB3002 are all below the upper limit in the TPP (120 Pa·s), and are 105.01 ± 2.48 Pa·s, 43.77 ± 1.56 Pa·s and 89.79 ± 5.97 Pa·s, respectively.

3.1.3. Spreading of the DuoGel™s—Table 2 summarizes the rheological properties determined for the three candidate DuoGel™ formulations, and also gives computational predictions of vaginal surface areas coated by 4 mL volumes of them after 120 min of spreading. These coated areas were compared to that of the “universal HEC placebo” gel used in multiple microbicide trials (Tien *et al.*, 2005). The resulting ratios ranged from 84% (IQB3002) to 120% (IQB3001). Changing gel volume to 3 mL had minimal effect on these ratios (data not shown). Note that gel spreading depends upon three distinct rheological parameters, m_0 (viscosity at zero shear rate), m (viscosity at which the shoulder of the curve begins to bend over) and n (the slope of the downward sloping curve, after the shoulder, of log-viscosity vs. log-shear rate). Gels IQB3000 and IQB3002 coat about the same area at 120 min, but this derives from different specific rheological properties. The former has a relatively high zero shear viscosity and also a yield stress. The latter has a larger zero shear viscosity, but no yield stress.

3.1.4. Osmolality evaluation—The undiluted DuoGel™ formulation exhibited measured osmolality values of 271 ± 40 mOsm/kg (IQB3000), 256 ± 33 mOsm/kg (IQB3001), and 260 ± 36 mOsm/kg (IQB3002) ($n = 3$, mean $\pm$ SD). The osmolality of all the DuoGel™s fall within the acceptable TPP and within the range of near physiological iso-osmolality.

3.1.5. In-vitro release testing—The DuoGel™ formulations were evaluated in the Franz cell apparatus through MatTek VEC-100 FT EpiVaginal tissue for 4 hours. Over the four hour application, the permeation rate of IQP-0528 into the vaginal tissue model was very consistent, with IQB3000 having a flux of 5.86 ± 0.41 $\mu\text{g}/\text{cm}^2\text{hr}$, IQB3001 18.88 ± 3.35 $\mu\text{g}/\text{cm}^2\text{hr}$, and IQB3002 22.85 ± 0.19 $\mu\text{g}/\text{cm}^2\text{hr}$ (Figure 3). From the evaluation of the release of IQP-0528 into vaginal tissue, DuoGel™ Formulation IQB3002 displayed the highest permeation of drug per volume of tissue at 65.89 ± 8.33 $\mu\text{g}/\text{cm}^3$.

3.1.6. Drug content in the basal media—The levels of IQP-0528 (Figure 4) collected from the basal medium of the tissue were measured at hours 1, 2, 4, and 6 of gel application. After a 4 hour application, the concentrations of drug in the medium were 0.026 μM for IQB3000, 0.032 μM for IQB3001, and 0.033 μM for IQB3002. Based on published data, the resulting IQP-0528 levels in the basal medium for all the DuoGel™s were greater than 100 times the EC_{50} of IQP-0528.

3.1.7. In vitro efficacy of IQP-0528-containing DuoGel™s—Gels IQB3000, IQB3001 and IQB3002 were evaluated for anti-HIV activity in human PBMCs against a representative CCR5-tropic, Clade B virus isolate. The EC_{50} values obtained for each gel were similar, ranging from 4.11 nM to 4.79 nM (Figure 5). Toxicity was evaluated in parallel, and each gel was found to be non-toxic up to the high test IQP-0528 concentration evaluated: 1.47 μM . Efficacy of the placebo gel was also evaluated in parallel and the EC_{50} was found to be less than the lowest dilution evaluated, which was 1:10.

3.1.8. *Ex vivo* evaluation of the DuoGel™s in ectocervical and rectal tissues—

The three gels (IQB3000, IQB3001, and IQB3002) were initially assessed for their capacity to protect mucosal tissue from HIV-1. The use of such *ex vivo* tissues to evaluate topical microbicides has been well established (Dezzutti *et al.*, 2014; Rohan *et al.*, 2010). A modest dilution of each gel was made to allow for even coating and applied to the mucosal surface. All of the gels protected ectocervical (Figure 6A) and colonic (Figure 6B) explants. Treated explants demonstrated a $\sim 2 \log_{10}$ decrease in HIV-1 replication compared to the untreated control explants. The antiviral activity of the gels was attributed to the active drug, IQP-0528, as all drugs retained ectocervical (Figure 7A) and colonic (Figure 7B) tissue viability and tissue architecture as defined by the MTT assay and hematoxylin and eosin staining, respectively. In contrast, the nonoxynol-9 (N9) treated tissues showed reduced mitochondrial activity with loss of tissue epithelium and cellularity.

3.1.9. Six (6) month stability of the DuoGel™ formulations—Stability was evaluated for the gel formulations IQB3000, IQB3001 and IQB3002 stored under accelerated conditions 40°C/75%RH for 6 months. All three formulations showed no significant changes in gel appearance, API stability, viscosity, or pH with all values falling within the defined TPP. IQP-0528 could be completely recovered ($\sim 100\%$ LC detection) from each gel. At 6 months, all three formulations maintained formulation stability at accelerated environmental conditions as defined by the TPP.

3.2. Additional Evaluations of DuoGel™ IQB3002, the lead formulation

The activity of IQP-0528 against HIV viral infection was comparable in the initial *in vitro* and *ex vivo* evaluations across all three DuoGel™ formulation candidates. Similarly, stability in all the formulation candidates was comparable. However, in the rheological and spreading evaluations, IQB3001 demonstrated the highest extent of spreading which was concluded to be “too leaky” for effective drug delivery. In drug delivery evaluations, IQB3000 released significantly less drug than the other formulations, and was therefore excluded. Given that all short-term stability and bioactivity results were equal, the physicochemical evaluations thus identified DuoGel™ formulation IQB3002 as the preferred formulation for further efficacy evaluation (Figure 1).

3.2.1. *In vitro* efficacy of IQB3002 in human PBMCs—IQB3002 was evaluated for anti-HIV activity in human PBMCs against a representative CCR5-tropic strain of each HIV-1 subtype (A through G) and O (CXCR4 tropism). The EC₅₀ value of IQB3002 ranged from 0.62 nM to 3.8 nM against each of the viral subtypes. The EC₅₀ of IQB3002 against subtype O was 161 nM, which is 75- to 391-fold higher relative to the other virus strains. This would be expected since Clade O is essentially a NNRTI-resistant virus harboring mutations which reduce the antiviral activity of IQP-0528 (Figure 8).

3.2.2. *In vitro* efficacy of IQB3002 in the presence of simulants representative of the vaginal environment—Due to the complex nature of the vaginal and/or rectal micro-environments, it is important to assess the anti-HIV activity of a candidate gel in the presence of vaginal and seminal simulants which are representative of the fluids that would be encountered during the sexual transmission event. IQB3002 was evaluated for activity

against the CCR-5-tropic HIV-1_{BaL} in TZM-bl-FcRI cells in the presence of simulated seminal fluid and simulated vaginal fluid relative to its activity in tissue culture medium alone. The antiviral activity following a 48 hour exposure was found to be similar in the presence of the additives relative to the activity in medium with EC₅₀ values of 1.75 nM, 3.67 nM and 2.73 nM for medium alone, simulated seminal fluid and simulated vaginal fluid, respectively (Figure 9). Maraviroc was evaluated in parallel as an assay positive control drug and it exhibited the expected level of activity in the assays with EC₅₀ values ranging from 5.0 to 17.6 nM (data not shown). Toxicity was evaluated in parallel and none was observed up to the highest concentration tested: 2.937 μ M. The placebo gel was evaluated in parallel in tissue culture medium in this assay system and was found to be inactive at a dilution of 1:1000. We did not further evaluate the placebo gel in vaginal or seminal simulants or at a lower dilution.

3.2.3. *In vitro* cytotoxicity of IQB3002—IQB3002 was evaluated for cytotoxicity to cells representative of those that would be found in the vaginal and rectal environments, including the established cell lines Ca Ski, HEC1A, ME180, END1, ECT1, VK2, and Caco-2, as well as fresh primary human PBMCs. Following a 24-hour exposure with the established cells, IQB3002 was found to be non-toxic up to the highest concentration evaluated: 2.937 μ M. IQB3002 was also found to be non-toxic to the normal vaginal flora *Lactobacillus* and to MatTek epivaginal tissue at these same concentrations. IQB3002 was found to be slightly toxic to human PBMCs following a 6 day exposure at a concentration of 1.36 mM (data not shown). The placebo gel was evaluated in parallel in ME180, Ca Ski, HEC1A cells and human PBMCs. The TC₅₀ in the established cell lines was less than a 1:5.5 dilution (highest concentration evaluated) and a 1:25 dilution in human PBMCs. The placebo was non-toxic to three species of *Lactobacillus* at a dilution of 1:11.

3.2.4. *Ex vivo* evaluation of IQB3002 formulation in ectocervical and rectal tissues—To better define the anti-HIV-1 activity of the lead gel, IQB3002, ten-fold dilutions were made starting at an initial 30-fold dilution of the DuoGel™ containing IQP-0528. The 30-fold dilution resulted in a final IQP-0528 concentration of approximately 1 mM. Ectocervical and colonic tissues were protected from HIV-1 infection down to 10 μ M levels of the original gel, which represents a 3000-fold dilution (Figures 10A and 10B). These results were confirmed by immunohistochemistry of the ectocervical explants at study end demonstrating the absence of HIV-1 p24 expressing cells at 10 μ M. Further diluting the gel to 1 μ M resulted in loss of IQP-0528 potency as HIV-1 replication was consistent with the untreated explant controls (HIV-1 only).

3.2.5. Twelve (12) month stability of IQB3002—Based upon the rheological data and *in-vitro* release testing, IQB3002 was chosen for continued stability for an additional 6 months (12 months total). At 12 months, analytical quantitation of IQP-0528 showed a 100% HPLC recovery of the API. The gel pH remained stable including the *in-vitro* release, flux, and permeability of IQP-0528 also remaining stable. Only the point viscosity and osmolality of IQB3002 exhibited any measurable change, falling from 89.79 ± 5.97 Pa·s to 75.55 ± 5.01 Pa·s and increasing from 260 ± 36 mOsm/kg to 340 ± 10 mOsm/kg for viscosity and osmolality, respectively. However, these values still fall within the defined

acceptable TPP. Based on these results, DuoGel™ formulation IQB3002 was determined to be stable under storage conditions.

4. DISCUSSION

In this study, a potentially safe and efficacious microbicide gel containing the dual-acting NNRTI IQP-0528 was created for use in the vagina and rectum. Formulation and evaluation were guided by experience at ImQuest BioSciences in developing IQP-0528-containing vaginal microbicide gels (Ham *et al.*, 2012; Mahalingam *et al.*, 2011). Until recently, the paradigm for microbicide development emphasized vaginal gels, resulting in a number of clinical trials (Abdool Karim *et al.*, 2010; Marrazzo *et al.*, 2015; McGowan *et al.*, 2011). A 1% tenofovir microbicide gel was formulated and evaluated in several studies (Abdool Karim *et al.*, 2010; Dezzutti *et al.*, 2012b; Rohan *et al.*, 2010). This was hyperosmotic (3111 mOsm/kg) and induced rectal mucosal epithelial sloughing in *ex vivo* evaluations (Rohan *et al.*, 2010). Human rectal application of this gel showed several adverse events that were consistent with gastrointestinal symptoms after use of a hyperosmolar product (Anton *et al.*, 2012). Subsequently, this product was reformulated to reduce its osmolality (846 mOsm/kg) (Dezzutti *et al.*, 2012b), although it remained hyperosmotic. The reduced osmolality product was found to be acceptable when used rectally (McGowan *et al.*, 2013). Rectal mucosal tissue appears to be affected more by hyperosmolality than vaginal tissue, although the latter may be compromised, at least to some extent, as well. In the end, a paradigm shift is needed toward creating microbicide gel products that are closer to being iso-osmotic with appropriate physiologic pH values compatible with their target compartments.

Although the vagina has minimal buffering capacity (Owen and Katz, 2005), it is exposed to 2 – 5 mL of semen with a typical pH of approximately 7.4 and good buffering capacity (Owen and Katz, 1999). As a result, the post-coital vagina remains basic for hours, largely re-acidifying after about 10 hours (Masters and Johnson, 1966). Although elevated human vaginal pH is associated with bacterial vaginosis (BV), this clearly is not induced following normal sexual activity. Thus, we posited that transient vaginal application of the DuoGel™ formulations would not likely induce a prolonged elevated pH that promotes BV. Ambient rectal fluid has low buffering capacity. However, in contrast to the vagina, the rectum actively responds to inserted materials that are not iso-osmolar or of neutral pH with secretions that act to restore ambient isoosmolality and neutral pH (Billich and Levitan, 1969; Bottger *et al.*, 1989).

Given that adherence plays a critical role in clinical trial outcomes (Marrazzo *et al.*, 2015; Rees *et al.*, 2015; Van Damme *et al.*, 2012) and to simplify usage for women who engage in vaginal and anal sex in the same sexual encounter, a single product DuoGel™ – that is physiologically safe for vaginal and rectal use – is of interest. The DuoGel™ formulation TPP of IQB3000, IQB3001, and IQB3002 was guided primarily by rectal compatibility (Figure 1). The pH 6 value of the DuoGel™s was defined as an acceptable characteristic between ambient values in the vagina and rectum. The DuoGel™s did possess a weak buffering capacity when mixed with solutions at vaginal pH 4.7. IQB3000 displayed the highest buffering capacity and IQB3002 displayed the lowest buffering capacity in vaginal fluid simulant (pH 4.7).

The three DuoGel™ formulations exhibited similar non-toxic behavior in cell-based *in vitro* assays. Despite the week buffering capacity of the DuoGel™s, none exhibited toxicity when exposed to either ectocervical or colonic tissues. These results compare favorably against existing microbicide gel formulations with all the DuoGel™ formulations exhibiting similar EC₅₀ values (4.11 – 4.79 nM), demonstrating similar efficacy of previously developed IQP-0528 gel formulations (Mahalingam *et al.*, 2011). Similar efficacy in biologically relevant ectocervical and colonic tissue explant assays was measured within the DuoGel™s as well. In terms of toxicity and efficacy, all three evaluated DuoGel™ formulations were equivalent. Finally, under accelerated stability conditions, all three DuoGel™ formulations maintained their physicochemical characteristics within acceptable ranges of the TPP for 6 months. IQP-0528 was fully recoverable from all formulations without degradation.

Osmolality has been identified as one of the key characteristics of a successful, rectally safe microbicide gel formulation (Dezzutti *et al.*, 2014; Mahalingam *et al.*, 2011; McGowan, 2011; Tien *et al.*, 2005). With decreased gel osmolality, increased rectal safety has been observed (Begay *et al.*, 2011; McGowan and Dezzutti, 2014; Rebe *et al.*, 2014; Wang *et al.*, 2011). Therefore, the TPP for the DuoGel™ was defined to be near physiological osmolality (290 mmol/kg). The osmolalities of IQB3000, IQB3001, and IQB3002 – 271 mOsm/kg, 256 mOsm/kg, and 260 mOsm/kg, respectively – all fall below previously formulated microbicide gels: TFV vaginal gel (3111 mOsm/kg) (Rohan *et al.*, 2010), a reduced glycerin TFV gel (846 mOsm/kg) (Dezzutti *et al.*, 2012b), IQP-0528 vaginal gel (~850 mOsm/kg) (Mahalingam *et al.*, 2011), a combination IQP-0528/TFV vaginal gel (882 mOsm/kg) (Ham *et al.*, 2012), and a TFV rectal gel (479 mOsm/kg) (Dezzutti *et al.*, 2014). The DuoGel™ formulations described here are near iso-osmolar and did not induce the epithelial damage observed for hyperosmolar commercial lubrications currently being used rectally (Dezzutti *et al.*, 2014).

From the onset of formulation development of the DuoGel™, a range of gel viscosities was chosen, from relatively “low” (IQB3001) to “high” (IQB3000), to vary the rate and extent of gel spreading (Gao *et al.*, 2015; Kieweg and Katz, 2006). As per the TPP, the gels were manufactured to have a point viscosity (@ 1 s⁻¹) of less than 120 Pa·s (Ham *et al.*, 2012; Mahalingam *et al.*, 2011; Rohan *et al.*, 2010). All gels exhibited non-Newtonian shear thinning rheological behavior, and one (IQB3000) had a yield stress. Computed spreading by the HEC placebo gel used in many microbicide trials was our basis for contrasting computed spreading and leakage behavior. IQB3001 exhibited 20% greater spreading over 2 hours than did the HEC placebo gel, suggesting it would be prone to vaginal leakage (Table 2). The lower gastrointestinal (GI) tract is a significantly larger chamber than the vagina, and so a less viscous fluid would provide better coverage over the mucosal layers. However, such “enema-like” products would tend to leak if applied in the vagina. Therefore, to accommodate both vaginal and rectal administration and coverage, the DuoGel™ was defined to provide minimal leakage in the vagina but still spread enough to have protective coverage in the rectum.

The permeability of IQP-0528 into the MatTek vaginal tissue was performed to provide a drug delivery comparison between the DuoGel™ formulations in an established *in vitro* model. After four hours application, IQB3001 and IQB3002 delivered similar drug

concentrations ($> 100 \mu\text{M}$ of IQP-0528 per cm^3 of tissue) which were approximately 65% greater than that for IQB3000. While there is no direct correlation between this tissue model and *in vivo* behavior and the permeability of the MatTek tissue is higher than that of human vaginal tissue (up to 40–50 times greater), the data suggest that the measured IQP-0528 tissue concentrations for the DuoGel™s after 4 hours are potentially several orders of magnitude greater than the EC_{50} of IQP-0528 and at least 10- to 100-fold greater than the calculated EC_{95} . The DuoGel™ formulations are designed to be effective in both the vagina and rectum; however, they represent an evolution of a vaginal specific formulation. Therefore, *in vitro* permeability of the DuoGel™ formulations was evaluated under vaginal conditions to both compare the formulations and to ensure that vaginal drug delivery was not detrimentally affected by formulation changes made to accommodate rectal administration. When compared to previously developed IQP-0528 vaginal gels, IQB3001 and IQB3002 compare favorably; however, IQB3000 resulted in reduced drug permeability flux (Ham *et al.*, 2012; Mahalingam *et al.*, 2011). While the primary physicochemical difference between the three DuoGel™ formulations is the gel viscosity, it is suggested that the major factor in the reduced flux is the increased viscosity. Under similar dissolution conditions, the increased viscosity of IQB3000 yields a slower dilution rate than both IQB3001 and IQB3002 which reduces the flux of IQP-0528 from the formulation into the tissue. However, it is suggested that higher viscosity formulations do not have a significant influence on overall drug transport once in the tissue as similar concentrations of IQP-0528 was measured in the basal medium for all three formulations (a result of both drug compound permeability and tissue model leakage).

Overall, multiple TPP characteristics (pH, osmolality, safety, efficacy, and stability) were equivalent across the three DuoGel™ formulations; the drug permeation evaluation eliminated IQB3000 and the spreading analysis eliminated IQB3001. DuoGel™ formulation IQB3002 was thus indicated for further evaluations.

The efficacy and toxicity of the IQB3002 was further analyzed in more detailed *in vitro* cell-based and explant tissue assays. At much higher concentrations than those that are evaluated in the efficacy assays, IQB3002 displayed no tested cellular toxicity following a 24 hour exposure in all representative cervical, vaginal, and colorectal cell lines, as well as the normal vaginal flora *Lactobacillus*. IQB3002 was observed to be minimally toxic in human PBMCs following a 6-day exposure, but only at an IQP-0528 concentration of 1.36 mM, a concentration that is well above that in the tissue. IQB3002 resulted in nanomolar EC_{50} concentrations against representative CCR5-tropic strains of HIV-1 from Clade A through G and a CXCR-4-tropic subtype O strain. These values are comparable to EC_{50} values for IQP-0528. The comparable EC_{50} values indicate that the DuoGel™ formulation does not negatively affect the *in vitro* activity of IQP-0528 to prevent HIV-1 infection. When evaluated in TZM-bl-FcRI cells against HIV-1_{BaL} in the presence of vaginal and seminal fluid simulants, the activity of IQB3002 was maintained relative to what was observed in assay media. These data indicate that IQB3002 will have activity against all virus subtypes and will maintain this activity in the vaginal and rectal vaults when exposed to other materials found there such as vaginal and seminal fluids. Dilutions of IQB3002 protected ectocervical and colonic tissues from HIV-1 infection down to $10 \mu\text{M}$ levels of the IQB3002

gel, which represents a 3000-fold dilution. Confirmed by immunohistochemistry, IQB3002 demonstrated that formulating to accommodate both rectal and vaginal administration does not compromise its safety profile in either chamber.

After identifying IQB3002 as the candidate DuoGel™ formulation, the accelerated environmental stability evaluations were continued for an additional 6 months (12 months total). At 12 months, the DuoGel™ formulation continued to show no changes in physical appearance, no API or excipient degradation, and no changes in IVRT. Only the point viscosity and osmolality exhibited any measurable changes which fell 15.8% and increased 30%, respectively. However, these changes in values are expected in gel formulations and fall within the defined acceptable TPP. The manufactured DuoGel™ IQB3002 demonstrated evidence of formulation stability under environmental conditions where the microbicide would be used.

5. CONCLUSION

Our microbicide gel formulation has achieved our goal of both rectal safety and product adherence, simplifying vaginal/rectal prevention into a single product. A single gel (DuoGel™ IQB3002) was identified from our testing. From established research on commercial products, on vaginal-only formulations, and on modified formulations, characteristics that would produce a safe microbicide gel for vaginal and rectal use were defined. The IQB3002 DuoGel™ has appropriate rheological characteristics that produce acceptable vaginal spreading. It is a slightly-buffering near-isotonic gel that minimally alters vaginal pH and is non-harmful to mucosal tissue. The DuoGel™ maintains its anti-HIV efficacy while being safe to ectocervical and rectal tissue. Creation of DuoGel™ IQB3002 demonstrates that developing a formulation for dual chamber use does not necessitate a compromise for either chamber. Dual chamber formulations show promise for future microbicide product development.

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Abbreviations

API	Active Pharmaceutical Ingredient
GRAS	Generally Regarded As Safe
HIV-1	Human Immunodeficiency Virus
HPLC	High Pressure Liquid Chromatography
MSM	Men who have Sex with Men
NNRTI	Nonnucleoside Reverse Transcriptase Inhibitor
PBMC	Peripheral Mononuclear Blood Cell
RAI	Receptive Anal Intercourse

STI	Sexually Transmitted Infection
TPP	Target Product Profile

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Figure 1. Formulation development flow chart of the single-agent DuoGel™

Flow diagram detailing each major decision point in the development of the DuoGel™ formulation. Beginning from a large screening panel of various microbicide formulated evolved from vaginal microbicide gels, the listed TPP resulted in three potential formulations (IQB3000, IQB3001, and IQB3002). These formulations were then iteratively evaluated under the stated “Formulation Evaluations”. IQB3002 was chosen as the lead formulation and finally evaluated under the stated “Detailed Formulation Evaluations”

Figure 2. Viscosity of the DuoGel™ formulations under increasing shear rate

The three DuoGel™ formulations were evaluated for their viscosity under a shear rate ramp from 0.05 Pa to 250 Pa and then from 1 s⁻¹ to 250 s⁻¹. Here the viscosity of IQB3000 (blue/red diamonds), IQB3001 (violet/orange squares), and IQB3002 (green/pink triangles) from 1 E-02 s⁻¹ to 250 s⁻¹ is shown. Each gel formulation underwent a stress sweep from 0.05 Pa to 250 Pa (blue, violet, and green) and then underwent a shear rate sweep from 1 s⁻¹ to 250 s⁻¹ (red, orange, and pink).

Figure 3. Permeation of IQP-0528 from DuoGel™ into vaginal tissue

In a vertical Franz cell system, 0.900 mL of the DuoGel™ formulation was applied to the apical side of the vaginal tissue model. At each time point, the tissue was removed, washed, and homogenized. The extracted IQP-0528 was determined via HPLC methods. IQB3000 (diamond); IQB3001 (square); IQB3002 (triangle). The concentration of IQP-0528 is given as the amount of drug per volume of tissue ((Abdool Karim *et al.*, 2010)). $n = 3 \pm \text{S.D.}$

Figure 4. IQP-0528 permeation into the basal tissue medium

In a vertical Franz cell system, 0.900 mL of the DuoGel™ formulation was applied to the apical side of the vaginal tissue model. At each time point, the basal tissue medium was collected. The IQP-0528 in basal medium was determined via HPLC methods. IQB3000 (diamond); IQB3001 (square); IQB3002 (triangle). $n = 3 \pm \text{S.D.}$

Figure 5. Anti-HIV activity of IQP-0528-containing DuoGel™s in human PBMCs

Gels IQB3000, IQB3001, and IQB3002 were evaluated for anti-HIV activity in human PBMCs against the CCR5-tropic subtype B strain HIV-1_{US/92/727}. The EC₅₀ values for each gel were similar, ranging from 4.11 nM to 4.79 nM. Each value is the result of one experiment with each of nine concentrations being evaluated in triplicate. AZT was evaluated in parallel as an assay control compound and had an EC₅₀ of 6.4 nM, which is in the expected range of activity (data not shown). Toxicity was evaluated in parallel and all of the gels were non-toxic up to the high test concentration of 1.47 μM.

Figure 6. Efficacy of the DuoGel™ in ectocervical and colonic tissue

The three DuoGel™s: IQB3000 (open triangle), IQB3001 (closed square), and IQB3002 (open diamond) were assessed for their capacity to protect mucosal tissue from HIV-1. A modest dilution of each gel was made to allow for even coating and applied to the mucosal surface. All of the gels protected ectocervical (Figure 6A) and colonic (Figure 6B) explants. Treated explants demonstrated a $\sim 2 \log_{10}$ decrease in HIV-1 replication compared to the untreated control explants. Data are the median $\pm$ 95% confidence interval of a minimum of three independent tissue donors.

Figure 7. The impact of DuoGel™ on ectocervical and colonic tissue viability

The three DuoGel™s: IQB3000, IQB3001, and IQB3002 were assessed for their toxicity and safety when applied to ectocervical (A) and colonic (B) tissue. All drugs retained tissue viability and tissue architecture as defined by the MTT assay and hematoxylin and eosin staining, respectively. In contrast, the nonoxynol-9 treated tissues showed reduced mitochondrial activity with loss of tissue epithelium and cellularity.

Figure 8. Evaluation of IQB3002 activity against representative clinical strains of HIV-1 in human PBMCs

IQB3002 was evaluated for activity against a representative CCR5-tropic strain of HIV-1 from each Clade A through G and a CXCR-4-tropic subtype O strain. The EC₅₀ value of IQB3002 ranged from 0.41 nM to 2.17 nM against each of the strains A–G, yielding a mean EC₅₀ value of 1.63 nM. The EC₅₀ of IQB-3002 against subtype O was 160.1 nM. Each reported value is the mean of two independent experiments with each of nine concentrations of IQB3002 evaluated in triplicate within each experiment. AZT was evaluated in parallel as a positive control compound and had EC₅₀ values ranging from 1.49 nM to 7.13 nM, which is within the expected range for AZT. Toxicity was evaluated in parallel and no toxicity was observed up to the high test concentration evaluated (1.47 μM).

Figure 9. Evaluation of IQB3002 activity in the presence of vaginal and seminal fluid simulants IQB3002 was evaluated for activity against CCR5-tropic HIV-1_{BaL} in TZM-bl-FcRI cells in the presence of tissue culture medium alone, simulated seminal fluid and simulated vaginal fluid to define the effects of the simulants on antiviral activity. The antiviral activity following a 48-hour exposure was found to be similar in the presence of the additives relative to the activity in medium with EC₅₀ values of 1.75 nM, 3.67 nM and 2.73 nM for media, simulated seminal fluid and simulated vaginal fluid, respectively. Each value is an average of two independent experiments with each of six concentrations being evaluated in triplicate within each experiment. No toxicity was observed up to the highest concentration evaluated (2.94 nM).

Figure 10. Efficacy of the DuoGel™ IQB3002 under dilution in ectocervical and colonic tissue
 Ten-fold dilutions of IQB3002 were made starting at after an initial 30-fold dilution. The 30-fold dilution resulted in a final drug concentration of approximately 1 mM. Ectocervical (A) and colonic (B) tissues were protected from HIV-1 infection down to 10 μ M levels of the original gel, which represents a 3000-fold dilution. Further diluting the gel to 1 μ M resulted in loss of IQP-0528 potency as HIV-1 replication was consistent with the untreated tissue controls (HIV-1 only). Data are the median $\pm$ 95% confidence interval of a minimum of three independent tissue donors.

Table 1

Single-Agent DuoGel Formulation

Ingredient (w/w, %)	IQB3000	IQB3001	IQB3002
IQP-0528	1	1	1
25 mM Phosphate Buffer pH 6.0	93.75	93.75	93.75
Glycerin	2.50	2.50	2.50
Methylparaben	0.20	0.20	0.20
Propylparaben	0.05	0.05	0.05
Hydroxyethyl cellulose	2.25	2.50	2.10
Carbomer 974P	0.25	\	0.25
Gel pH	5.94	5.76	6.08
Osmolality (mOsm/kg)	271	256	260
Viscosity @ 1 s ⁻¹ (Pa·s)	105.01 ± 2.48	43.77 ± 1.56	89.79 ± 5.97

(n = 3, mean ± SD)

Table 2

Rheological data and computed values of rheometric parameters and coated vaginal surface area for the three test gels.

Gel	m_0 (Pa·s)	m (Pa·s ₀)	n	τ_0 (Pa)	A (cm ²)	R
IQB3000	687	107	0.25	2.00	90	0.90
IQB3001	157	81	0.32	0.00	120	1.20
IQB3002	1197	81	0.28	0.00	84	0.84

A - Coated area at 120 min of spreading, $V = 4$ mL, m_0 , m and n - flow parameters, τ_0 - yield stress, R - Ratio of coated area of test gel to value computed for HEC placebo gel