Pharmacokinetic and pharmacodynamic evaluation of NNRTI IQP-0528 DuoGel™ in macaques

Lara E. Pereira[1], Pedro M. M. Mesquita[2], Anthony Ham[3], Tyana Singletary[4], Janet McNicholl[5], Karen W. Buckheit[3], Robert W. Buckheit, Jr.[3], James Smith[5]

[1]LifeSource Biomedical LLC, Moffett Field, California; [2]Albert Einstein College of Medicine, Bronx, New York; [3]ImQuest BioSciences, Frederick, Maryland; [4]Anyar Inc., Fort Walton Beach, Florida; [5]Centers for Disease Control and Prevention, Division of HIV/AIDS Prevention, Atlanta, Georgia.

ABSTRACT

Background: Unprotected receptive anal intercourse (RAI) is also practiced by women, providing strong rationale for the development of an HIV microbicide gel that is suitable for both rectal and vaginal applications. This would remove the need for separate products and dosages for women who engage in RAI and vaginal intercourse, thereby helping to increase user adherence and potentially cutting costs associated with separate products. A DuoGel™ product containing 1% wt/vol IQP-0528, which has non-nucleoside reverse transcriptase and entry inhibitory activities, was developed and first evaluated vaginally in pigtailed (PM) and rhesus (RM) macaques.

Methods: DuoGel™ formulation was optimized for dual compartment application. A 1.5 ml volume of 1% IQP-0528 DuoGel™ was applied vaginally to 6 SHIV+ female RM and 6 naïve female PM. Blood, vaginal pH measurements, cervicovaginal lavage, and vaginal and rectal spears were collected before, and up to 24 hours after gel application. Vaginal and rectal biopsies were also collected from PM at 24 hours. The RM were euthanized at 4 hours, and vaginal, cervical, rectal, and regional lymph node tissues were harvested for drug analysis. Anti-viral activity was tested *ex vivo* by co-culturing RM vaginal tissues with activated human peripheral blood mononuclear cells (PBMC), and measuring p24 levels up to 10 days after challenge with HIV-1Ba-L. Drug levels in plasma, vaginal fluids, and tissues were determined using LC-MS.

Results: In RM, median levels of IQP-0528 were between 10^4-10^5 ng/g in vaginal and cervical tissue, and 10^5-10^7 ng/ml in vaginal fluids for 4 hours after gel application. Bi-directional dosing was shown by median IQP-0528 levels being between 10^3-10^4 ng/g in rectal tissues. Similar levels were observed in PM vaginal fluids for 4 hours post gel application, but IQP-0528 was not detected in the rectum. Vaginal pH remained within normal range for both species (6.5-9). Vaginal tissues obtained from RM at necropsy protected co-cultured PBMC from HIV-1 infection *ex vivo*, with a viral inhibition range of 90-100%. Anti-viral activity was observed in vaginal tissues that were proximal, medial, and distal relative to the cervix. Viral inhibition was not detected in baseline tissue samples.

Conclusion: These data suggest that 1.5 ml of DuoGel™ delivers IQP-0528 throughout the macaque vaginal compartment at levels that are highly inhibitory to HIV-1 infection. Rectal PK studies are underway.

STUDY DESIGN AND METHODS

N=6 female SHIV+ rhesus macaques (terminal PK)

1.5 ml DuoGel™ applied vaginally in each macaque

Primary Endpoint: Drug levels in plasma, and vaginal/rectal fluids and tissues
Secondary Endpoint: Anti-viral activity in vaginal tissues

Sample	Pre-Necropsy N=6	Terminal PKs N=6, post gel application			
	No gel application	0 min	1 hour	2 hour	4 hr
Blood	---	X	X	X	X
Vaginal spears	---	X	X	---	---
Vaginal multiswab	---	---	---	X	X
Rectal spears	---	X	X	X	X
Vaginal Biopsies	X	---	---	---	---
CVL	---	X	---	---	X
Vaginal pH	---	X	X	X	X
Microflora	---	X	---	---	X
Necropsy	---	---	---	---	X

TISSUE COLLECTIONS
- Vaginal tissue: Fresh *and* frozen tissue
- Cervix - frozen
- Rectal tissue - frozen
- Lymph nodes: inguinal and iliac - frozen

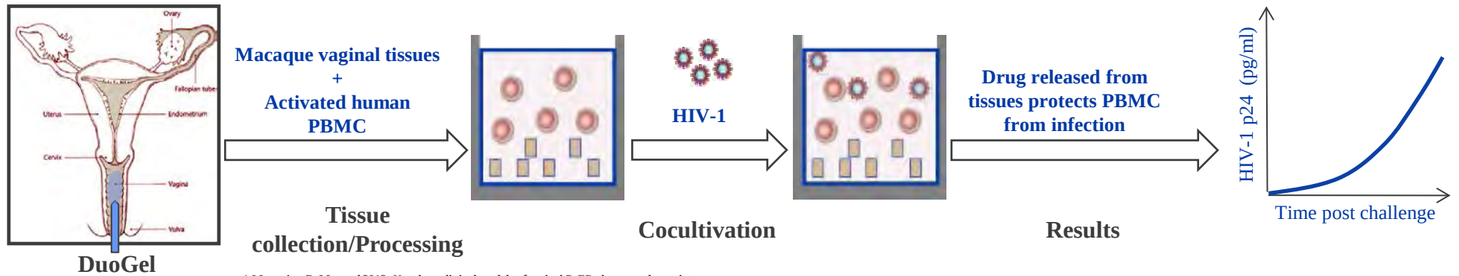
N=6 female naive pigtailed macaques (standard PK)

1.5 ml DuoGel™ applied vaginally in each macaque

Primary Endpoint: Drug levels in plasma, and vaginal/rectal fluids and tissues

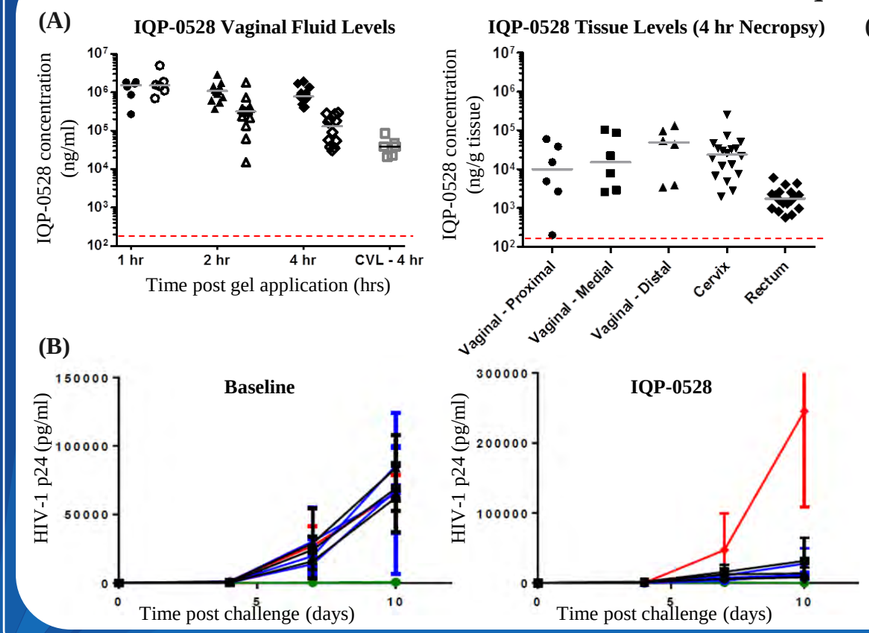
Sample	0 min*	1 hr	4 hr	24 hr
Blood[1]	X	X	X	X
Vaginal multiswab[2]	---	---	X	X
Vaginal spears[2a]	X	X	---	---
Rectal spears[3]	X	X	X	X
CVL[4]	X	---	---	X
Pinch biopsies[5] (vaginal and rectal)	---	---	---	X
Vaginal pH	X	X	X	X
Microflora[6]	X	---	---	X

Anti-viral assay for rhesus macaque samples



* Mesquita, P. M., *et.al* 2013. *Novel preclinical models of topical PrEP pharmacodynamics provide rationale for combination of drugs with complementary properties.* Retrovirology 10:113.

RESULTS – Rhesus macaques



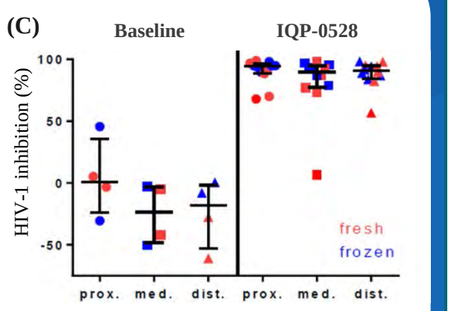


Figure 1. (A) Drug levels in proximal (closed symbols) and distal (open symbols) vaginal fluids (left panel), and in tissues at necropsy (right panel). The red dotted line indicates the *in vitro* IC90 (146 ng/ml). Drug was not detected in rectal fluids. (B) Representative data for 1 macaque showing p24 levels before (left panel) and 4 hours after (right panel) gel application. Activated human PBMC were incubated without (red lines) or with (black lines) fresh or previously frozen (blue lines) macaque vaginal tissue. (C) Summary of HIV-1 inhibition by untreated and gel-treated (4 hrs) vaginal tissues.

RESULTS – Pigtailed macaques

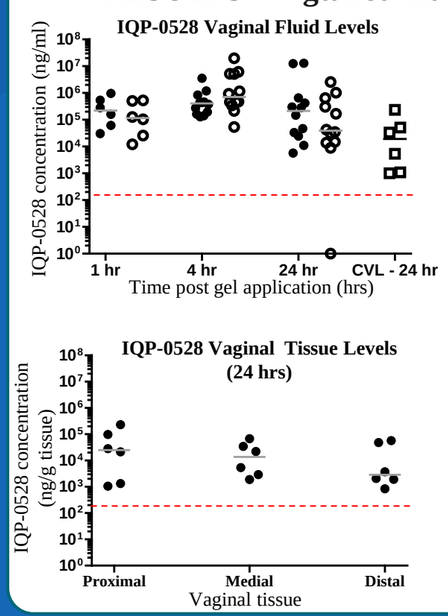


Figure 2. Top panel: Drug levels in proximal (closed symbols) and distal (open symbols) vaginal fluids up to 24 hrs after DuoGel vaginal application. Bottom panel: Drug levels in vaginal tissue biopsies obtained 24 hrs after DuoGel vaginal application. The red dotted line indicates the *in vitro* IC90 of IQP-0528 (146 ng/ml). Drug was not detected in rectal fluid and tissues.

SUMMARY AND CONCLUSIONS

- Median levels were between 10^3-10^5 ng/ml in CVL and in tissues (ng/g), and 10^4-10^7 ng/ml in vaginal fluid derived from spears.
- Levels are 2 to 4 logs above the *in vitro* IC90.
- These levels were observed at proximal, medial, and distal sites, suggesting that a sufficient degree of gel spread can be achieved in the vaginal lumen with a 1.5 ml gel volume.
  - Broader implications: Less is more - Do volumes less than the standard 4 ml used in clinical trials have the potential to be just as effective? Leakage/messy application avoided? Impact on adherence to gel use?
- Nearly 100% HIV-1 inhibition *in vitro* with DuoGel™-treated vaginal tissues from rhesus macaques.
  - Similar results were achieved with fresh and frozen tissues – Beneficial for clinical applications, when logistical constraints limit the immediate use of fresh biopsies in downstream assays.
- Bi-directional dosing observed in rhesus macaques: IQP-0528 levels in rectal tissues 1 to 2 logs above IC90. However, bi-directional dosing was not apparent in pigtailed macaques.

Supported by NIH Grant 5U19AI101961-03
Correspondence: ajo9@cdc.gov

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

E-mail: cdcinfo@cdc.gov | Web: www.cdc.gov
The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.