

PREVENT

Rectal Microbicide Program

Kenneth E. Palmer, Ph.D.

Manufacturing of GRFT in *Nicotiana benthamiana*



Scaleable manufacture of HIV-1 entry inhibitor griffithsin and validation of its safety and efficacy as a topical microbicide component

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Towards a First-in Humans Clinical Trial of GRFT Rectal Microbicide

1. Formulation Development

- Lisa Rohan & team (University of Pittsburgh)
- Charlene Dezzutti (University of Pittsburgh)
- Josh Fuqua (University of Louisville)

2. Pharmacokinetics and Pharmacodynamics Assay Development

- Nobu Matoba & team (University of Louisville)
- David Garber (CDC)

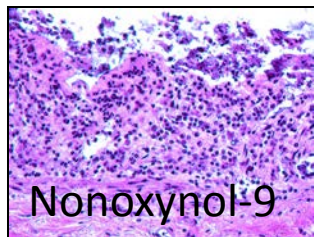
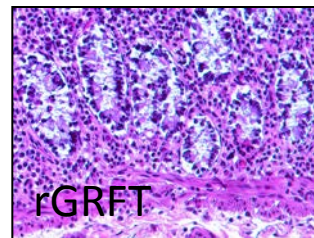
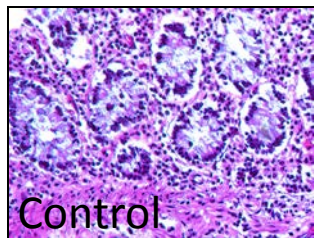
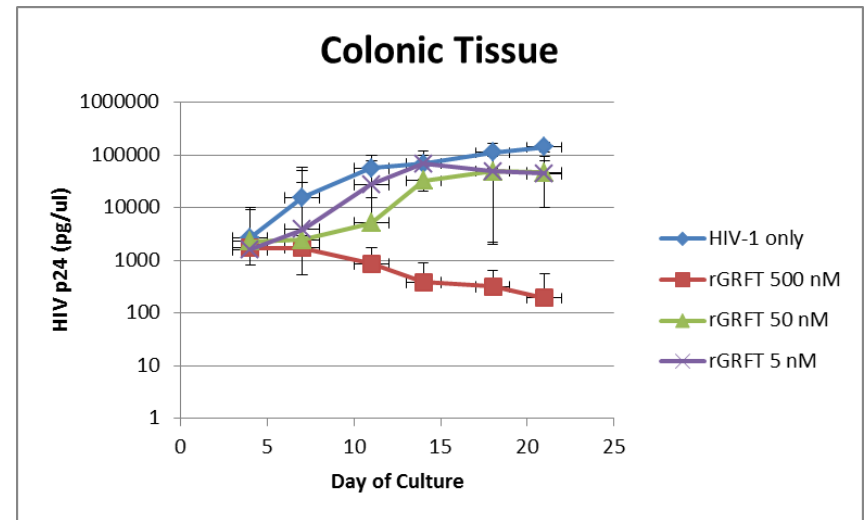
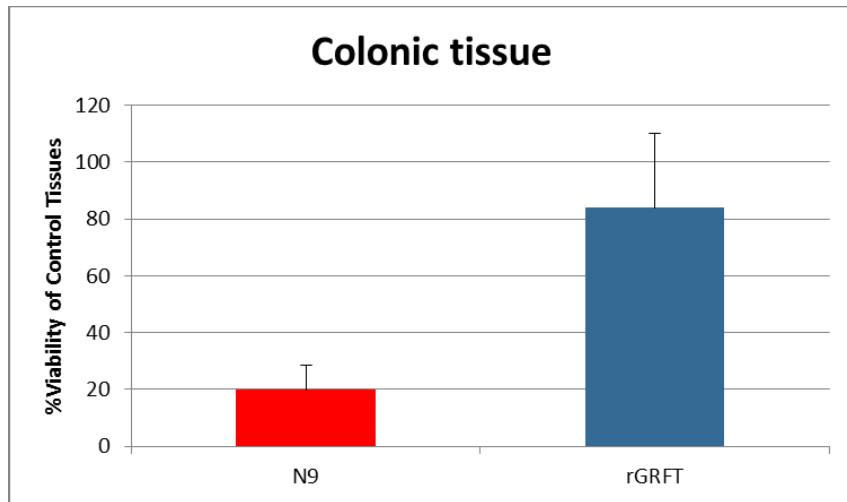
3. Clinical Trial Design

- Ross Cranston (University of Pittsburgh)
- Ian McGowan (University of Pittsburgh)

Formulation Development

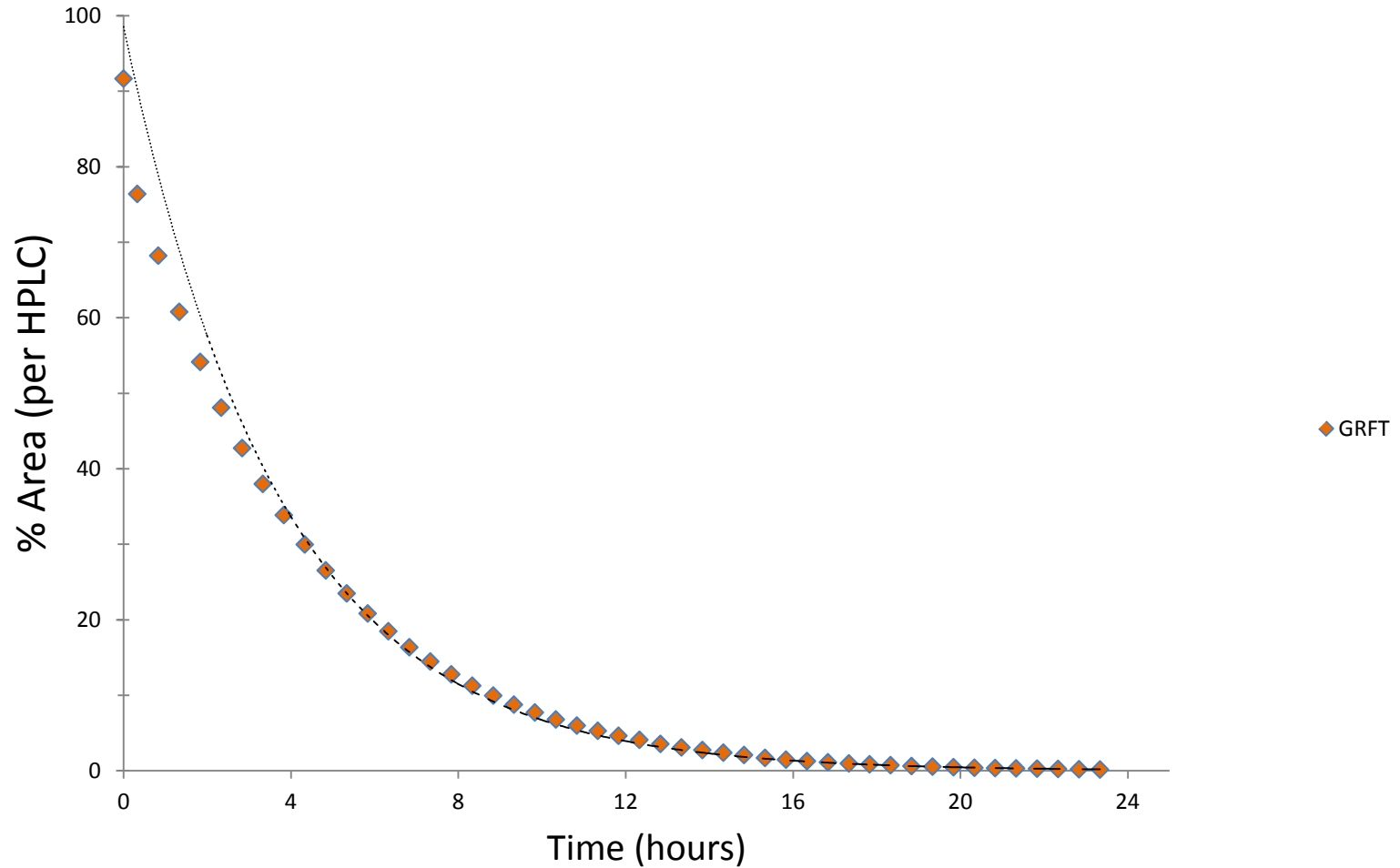
- Focus on rectal gel for pericoital use
- Initial formulation was 0.1% GRFT carbopol gel

Safety and Efficacy of Prototype GRFT Rectal Gel in Colon Tissue Explants

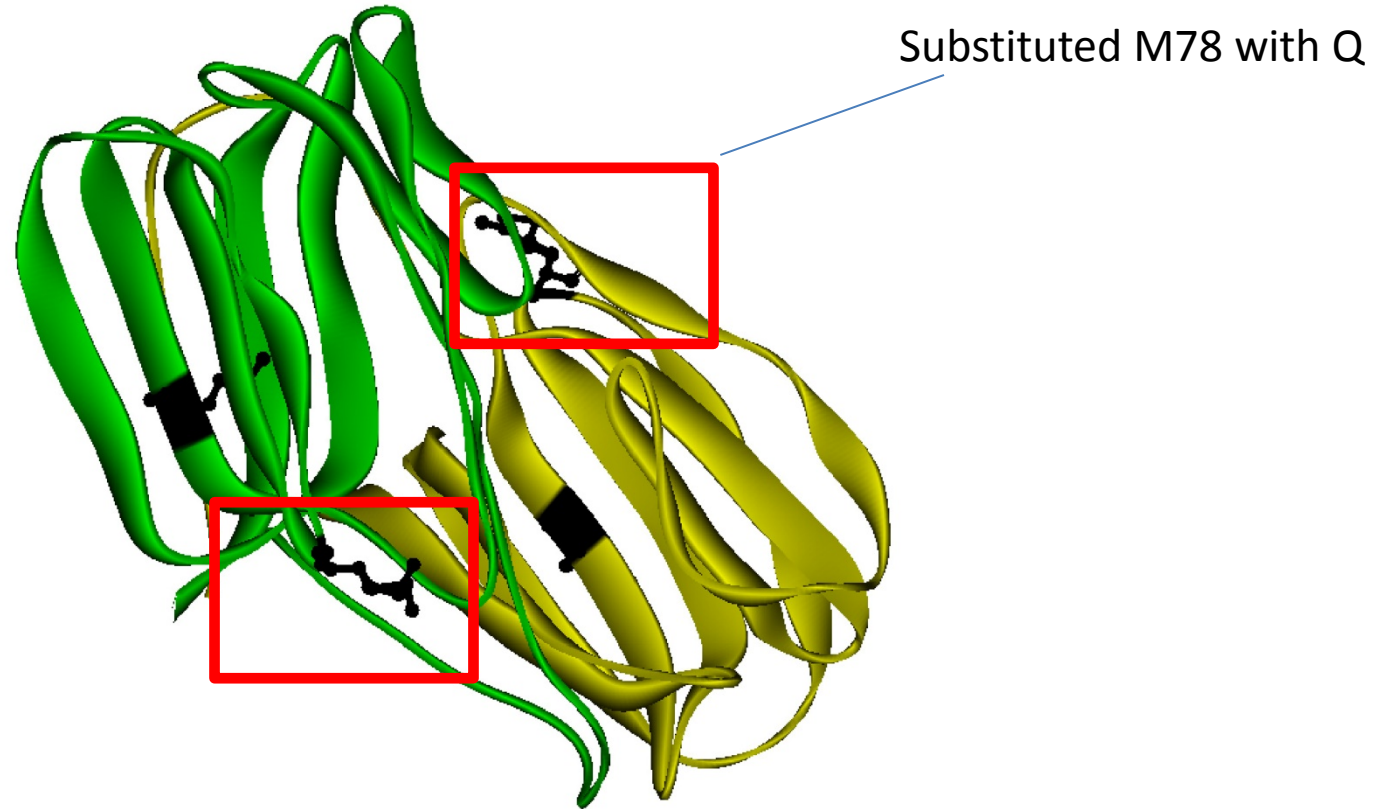


GRFT Preformulation Stability Concern: GRFT is susceptible to Oxidation

Griffithsin Oxidation in .02% Hydrogen Peroxide



Engineering GRFT for Oxidation Resistance

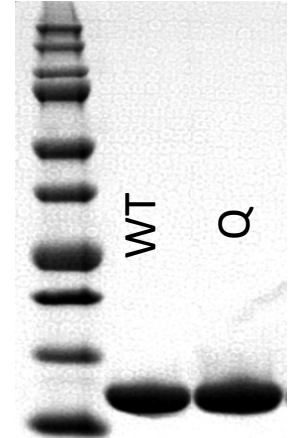


M78 is exposed with great potential for oxidation

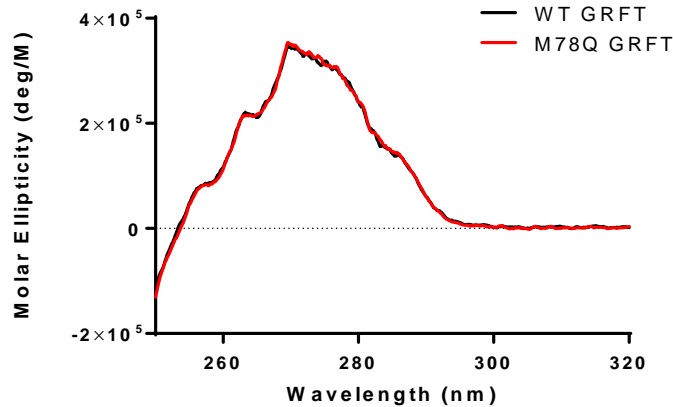
Q-GRFT Structure

Circular Dichroism

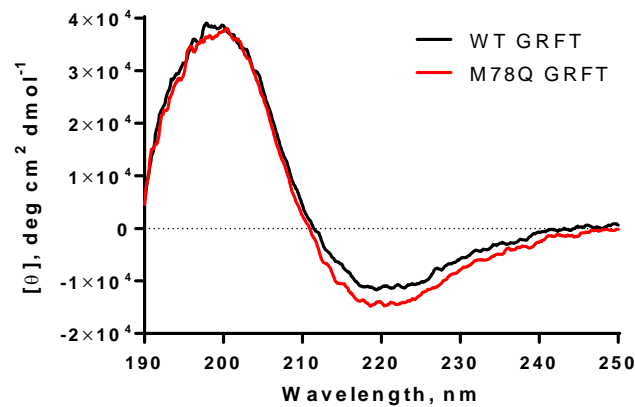
SDS-PAGE



Tertiary Structure



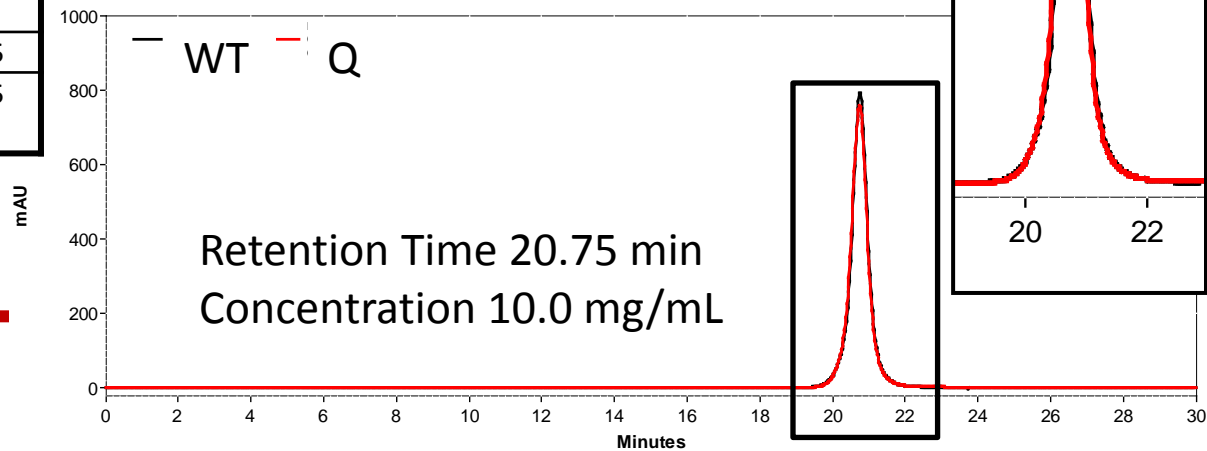
Secondary Structure



Differential Scanning Fluorimetry with Thermal Shift Assay

	Q GRFT	GRFT
Melting Temperature (°C)	77.1 ± 0.5	77.4 ± 0.5
Melting Temperature with Mannose (°C)	81.6 ± 0.6	82.0 ± 0.5

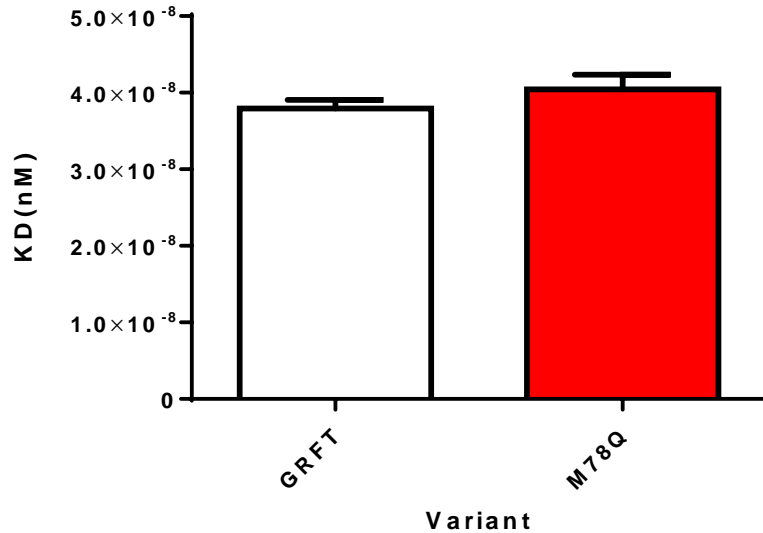
SEC-HPLC



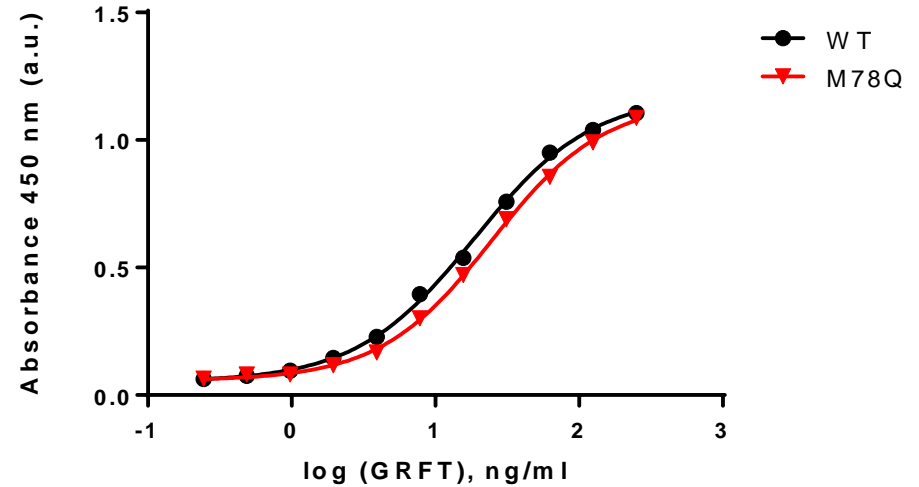
Fuqua, Hamorsky, Matoba, Palmer, unpublished

Activity of Q-GRFT

Surface Plasmon Resonance

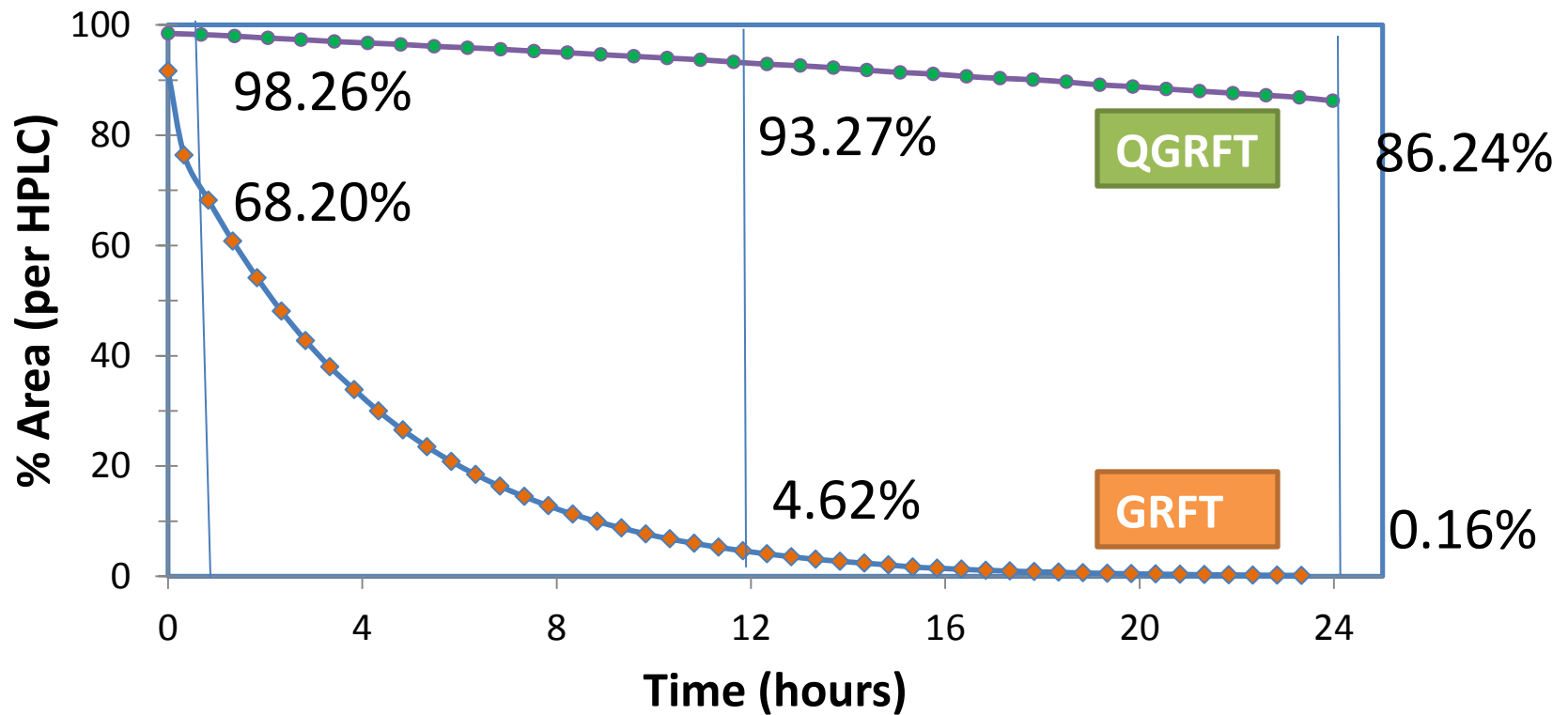


gp120 ELISA



		Q GRFT	GRFT
Antiviral Activity HIV Pseudovirus Q769.h5 (ng/mL)	IC 50	19.88 ± 9.75	14.74 ± 1.40
	IC 90	880.8 ± 424.6	1031 ± 4.59
	IC 99	55304 ± 15194	106973 ± 12067
Affinity – K _D (nM)		40.4 ± 3.3	37.9 ± 2.0
Potency – EC ₅₀ (ng/mL)		20.47 ± 2.1	12.12 ± 3.3

Comparison of GRFT and QGRFT Oxidation (with 0.02% H₂O₂)



Lisa Rohan and team, unpublished data

Q-GRFT Manufacturing for Formulation and Preclinical Toxicology

- Plants Harvested: 23Jun2015
- Total harvested material: 225.5kg
- Plant number harvested: 5983
- 87.2 grams purified Q GRFT
 - 20,000 4 ml/0.1% doses
- 366.3 mg of purified Q GRFT per kg of plant material



QGRFT Gel Formulation



Characteristic of the Gel	
pH	7.1
Viscosity at 25°C, <i>cp</i>	639.146 (RSD=2.437)
Osmolality, <i>mOs/kg</i>	356.67 (RSD=0.657)

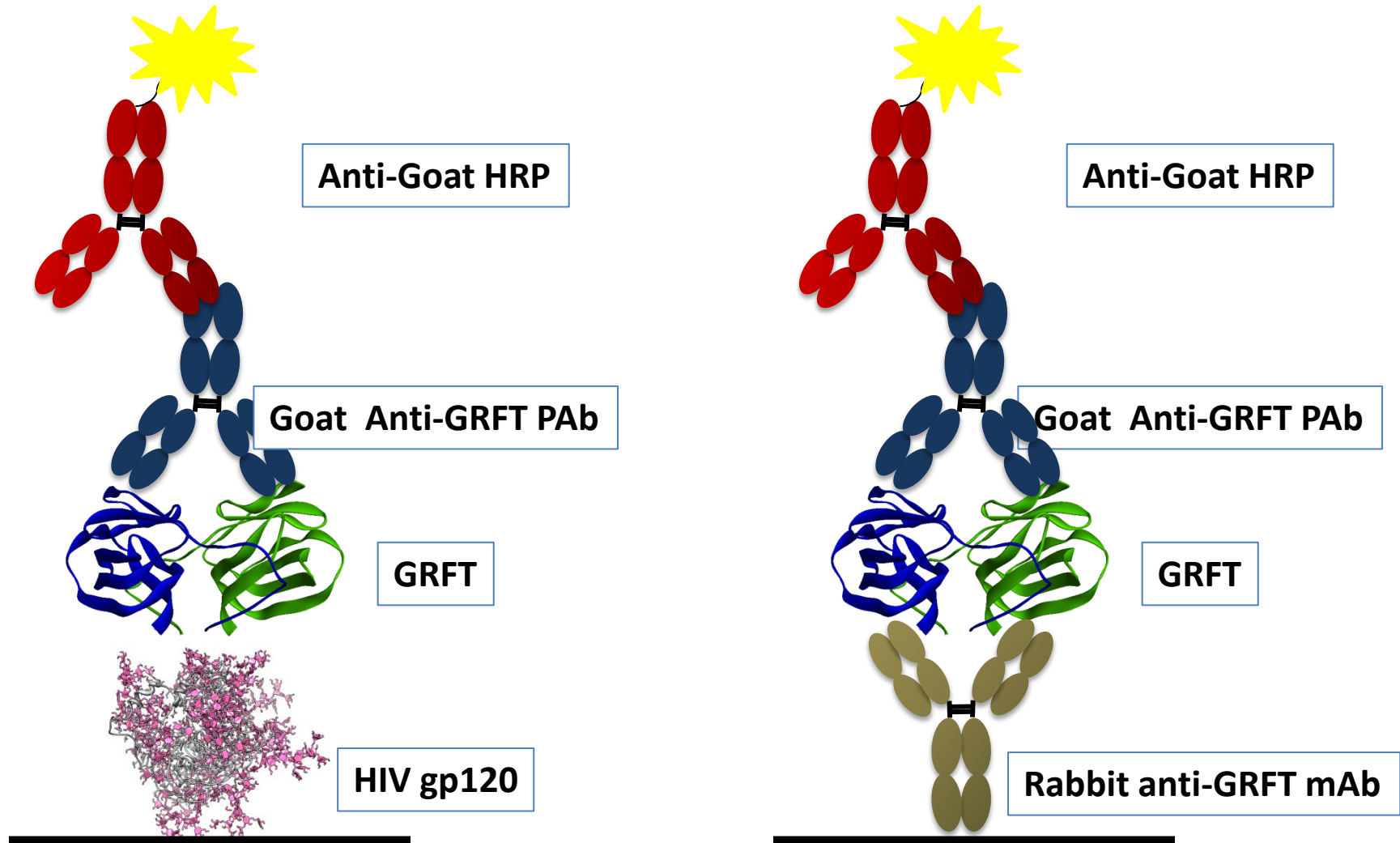
- Formulations available at concentrations of **0.1%**, **0.3%**, **1%** and **3%**

Pharmacokinetics and Pharmacodynamics

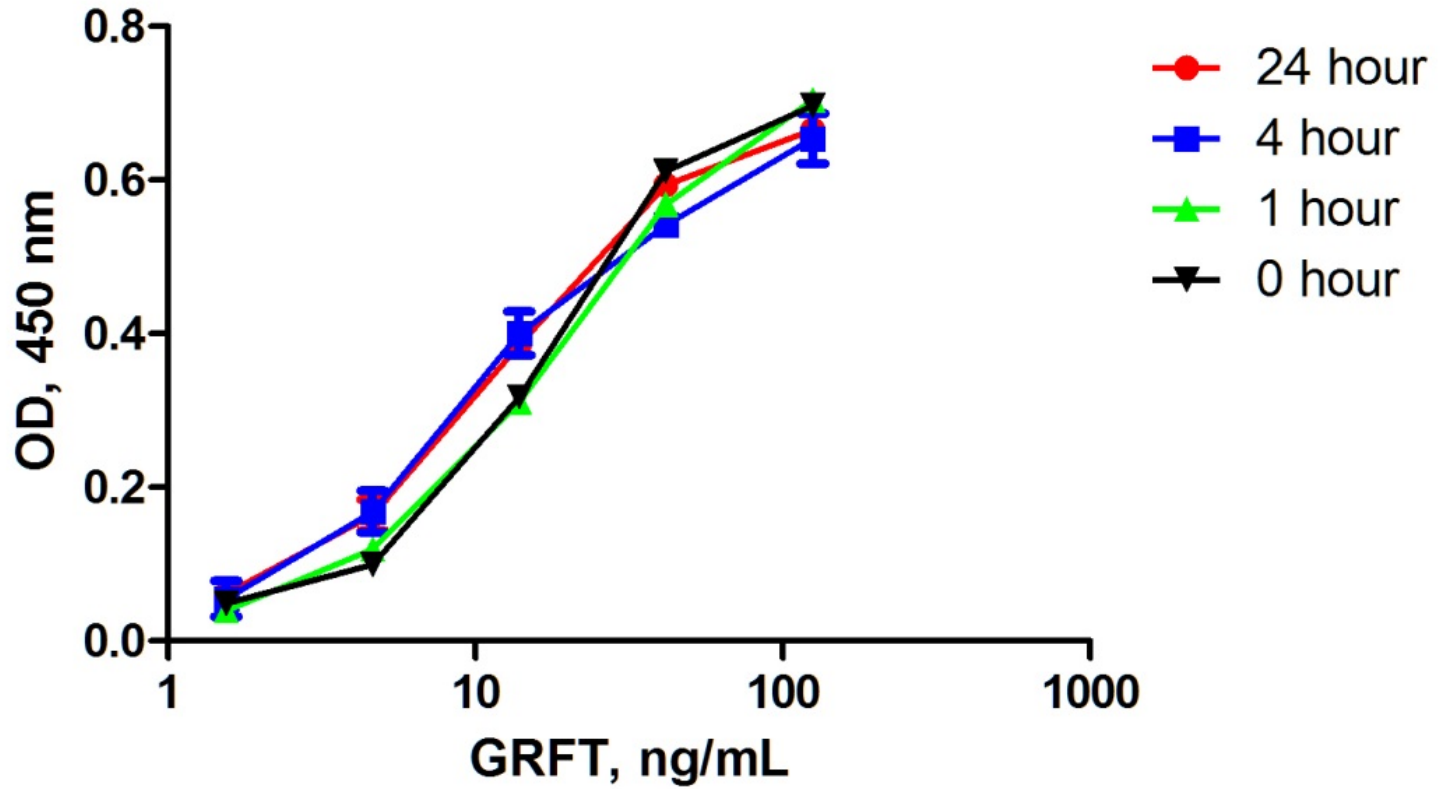
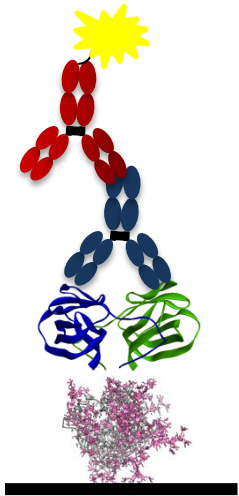
Assay Development

- Immunoassay methods (ELISA) for *active* GRFT in rectal fluids
- Detection of GRFT in blood plasma
- Detection of anti-drug antibodies

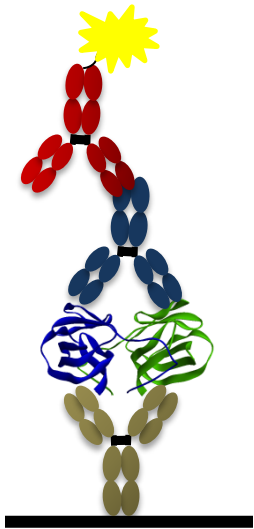
Immunoassay Design for Detection of GRFT in Biological Fluids



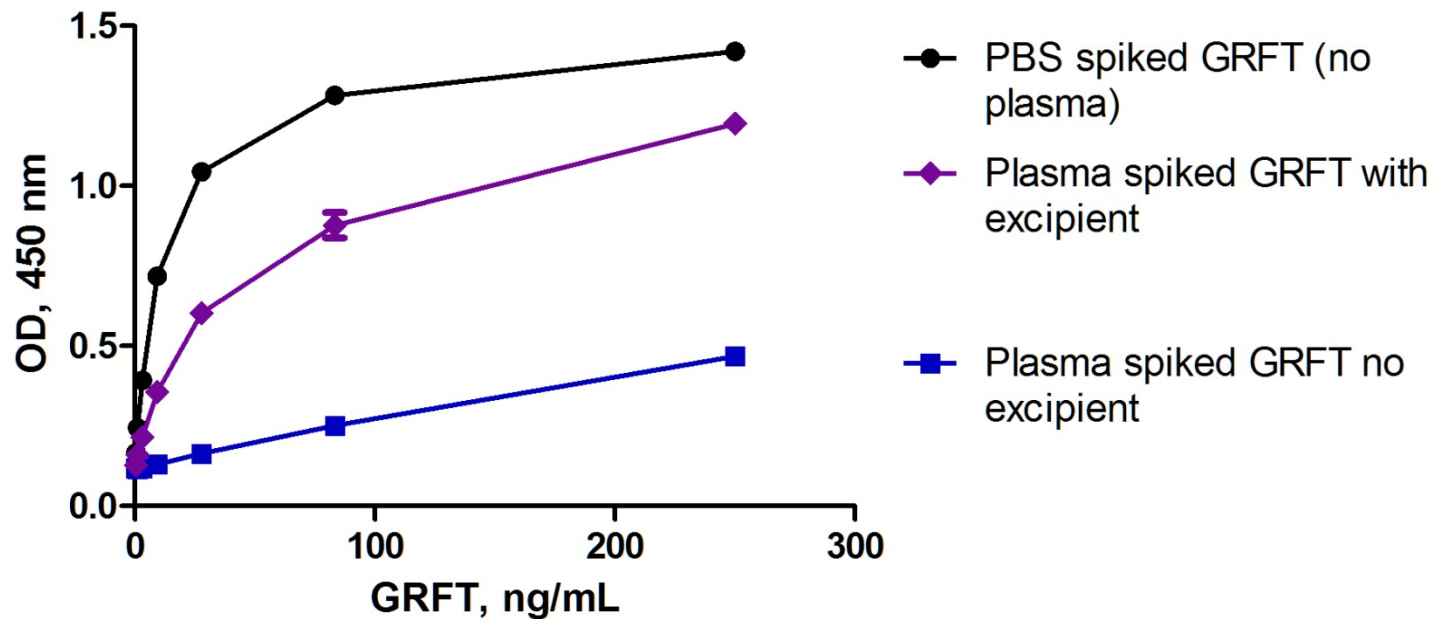
GRFT stability in macaque rectal fluids



Optimizing GRFT detection in rabbit plasma in support of GLP toxicology program



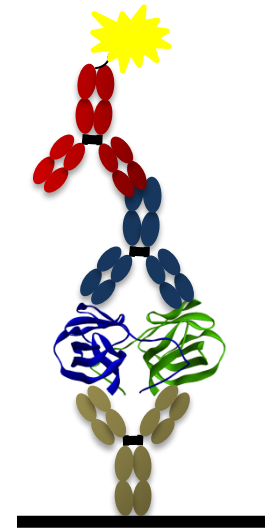
Detection of GRFT in Rabbit Plasma



LLOQ = 0.470 ng/mL

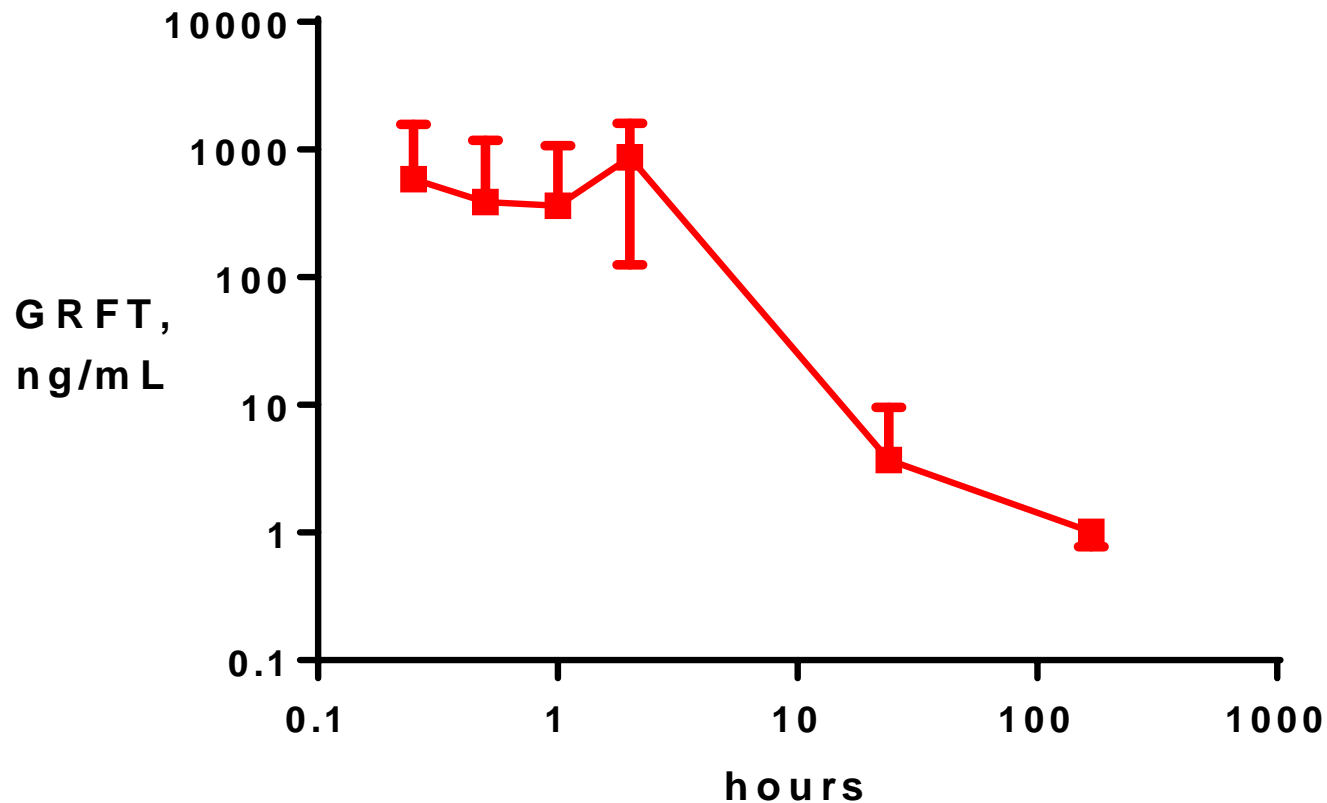
Pilot Study in Rhesus macaques: Carbopol 0.1% GRFT gel

- 6 Female Rhesus macaques treated
- Rectal fluids collected:
 - Pre-treatment
 - Post-treatment
 - 15 minutes
 - 30 minutes
 - 2 hours
 - 24 hours
 - 7 days
- Assayed by mAb-capture ELISA
- Assayed by HIV pseudovirus infection assay

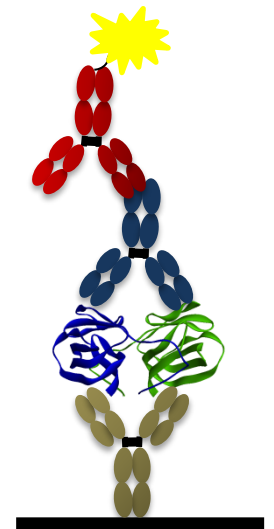


PK of GRFT in Macaque Rectal Fluids

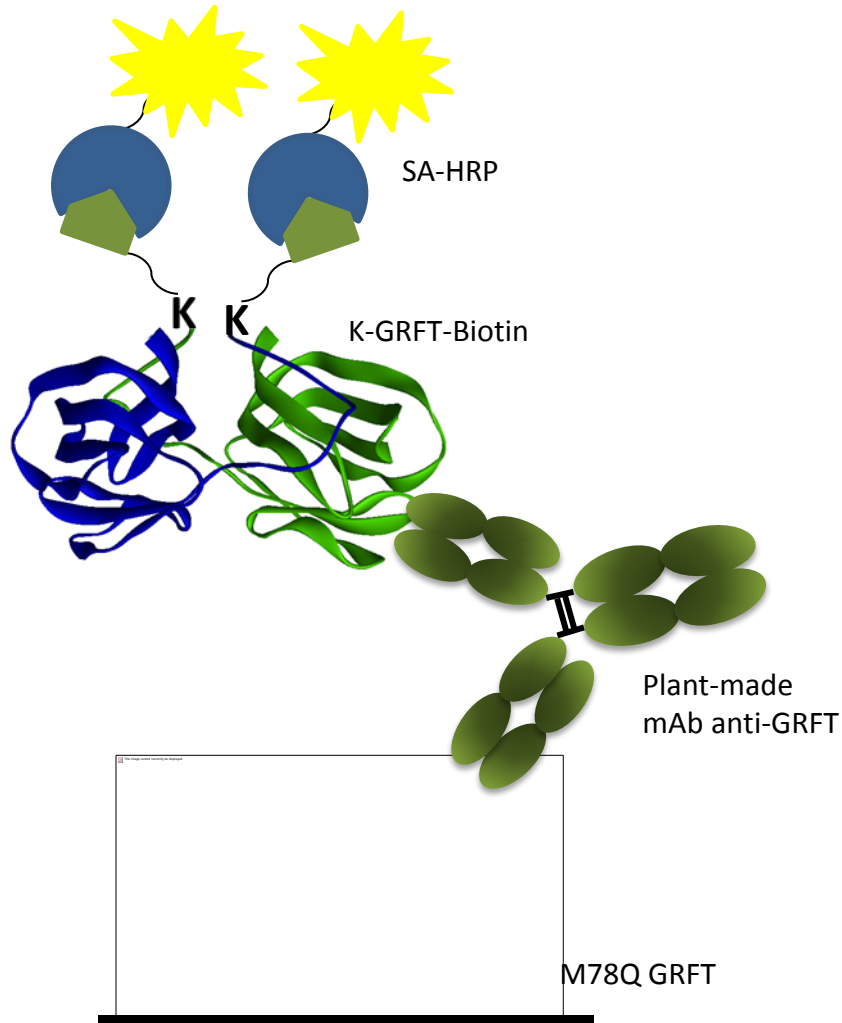
0.1% GRFT Carbopol Gel



GRFT Carb



Anti-drug (GRFT) antibody detection



LLOQ	1.96 ng/ml	0.196 ng
LLOD	0.64 ng/ml	0.064 ng

A Randomized, Double-Blind Phase 1 Safety and
Pharmacokinetic Study of Griffithsin Gel
Administered Rectally to HIV-1 Seronegative
Adults

Ross D. Cranston MB ChB MD FRCP

Ian McGowan MD DPhil FRCP

University of Pittsburgh

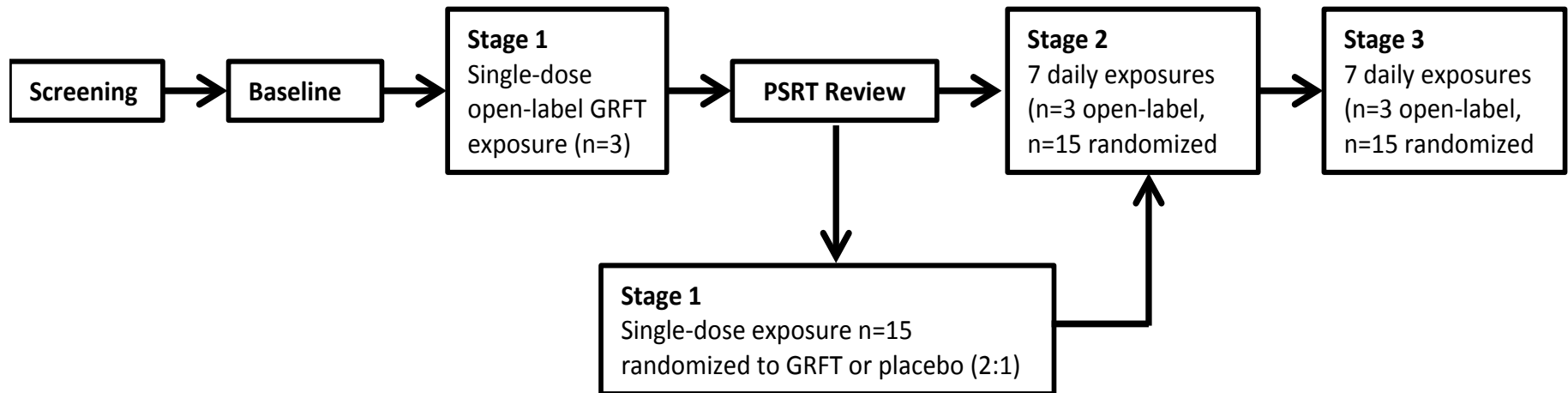
Protocol Summary

- Sample Size: Approximately 18 evaluable male participants
- Study Population: HIV-uninfected men between the ages of 18 – 45 years
- Study Sites: Magee-Womens Research Institute (MWRI) at University of Pittsburgh
- Study Design: Randomized, double-blind, single-site trial
- Study Duration: Participant accrual will take approximately 6 months. Participants will be on study for approximately 8 to 12 weeks. The total duration of the study will be approximately 9 months
- Study Products: Q-Griffithsin gel

Study Arms

Arm	N	Method of Assignment	Study Product	Exposures (1-2 week recovery period between stages)
1	3	Open-label	Q-GRFT	<ul style="list-style-type: none"> • Stage 1- Single dose • Stage 2- 7 daily exposures • Stage 3- 7 daily exposures
2	10	Randomized	Q-GRFT	<ul style="list-style-type: none"> • Stage 1- Single dose • Stage 2- 7 daily exposures • Stage 3- 7 daily exposures
3	5	Randomized	Matched placebo	<ul style="list-style-type: none"> • Stage 1- Single dose • Stage 2- 7 daily exposures • Stage 3- 7 daily exposures

Study Schema



ACKNOWLEDGEMENTS

- PREVENT Team
 - Lisa Rohan, Ph.D. University of Pittsburgh
 - **Lindsay Ferguson-Kramzer; Lin Wang**
 - Charlene Dezzutti, Ph.D. University of Pittsburgh
 - David Garber, Ph.D.
 - Janet McNicholl, M.D. CDC, Atlanta
 - Nobuyuki Matoba, Ph.D. University of Louisville
 - **Amanda Lasnik; Adam Husk; Krystal Hamorsky; Joshua Fuqua**
 - Ross Cranston M.D. University of Pittsburgh
 - Ian McGowan M.D., D.Phil.
 - **Jarret Engstrom, Rhonda Brand, Ph.D.**
- Funding from NIH/NIAID U19 AI 113182