



Ing B (ingenol-3-hexanoate)* is a potential PKC activator for the *Shock and Kill* strategy in HIV eradication

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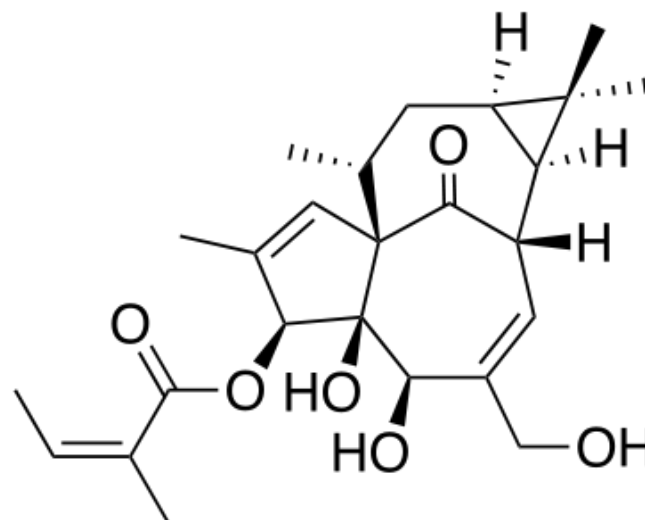


* Patent pending



Ingenol mebutate or Ingenol 3-angelate

Isolated from *Euphorbia peplus*
(milkweed)



Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine



Immunostimulatory cancer chemotherapy using local ingenol-3-angelate
and synergy with immunotherapies

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INGENOL



Euphorbia tirucalli

AVELOZ



Ingenol derivatives

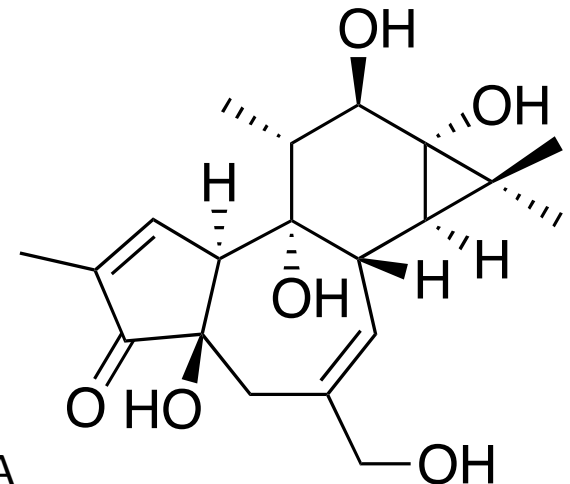
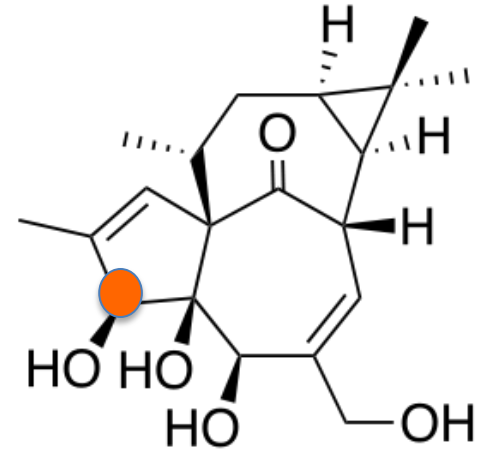
- The sap of *E. tirucali* presented a mix of “ingenols” with various unstable radicals at C3.
- **Solution:** modify the molecule, keeping the core intact.
- **Results:**
 - Ing A, B, and C
 - Also named Kyoll A, B, and C (KyoLab is the name of the company).



Dr. Luiz Pianowski

Ingenuol A, B, and C

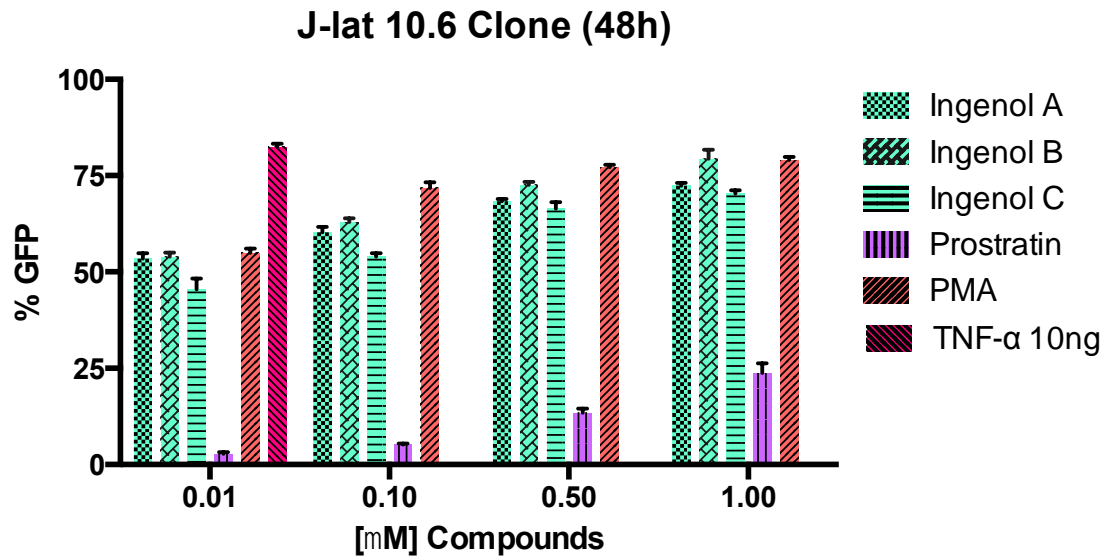
- Ing A:
 - 3-trans-cinnamate
- Ingenuol B:
 - 3-caproyl or hexanoate
- Ing C:
 - 3-dodecanoate



PMA

Ingenol

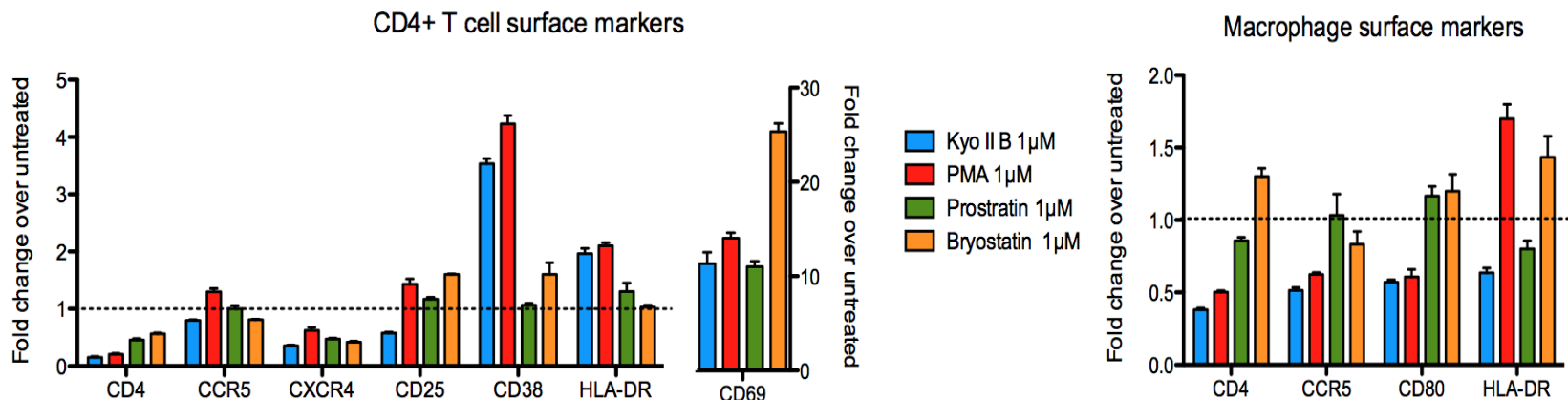
- Diterpene / Phorbol Ester (PMA, prostratin)
 - PKC activator – Mimic diacylglycerol
- Efficiently activates the HIV-LTR in reporter cells



Dr. Celina Abreu

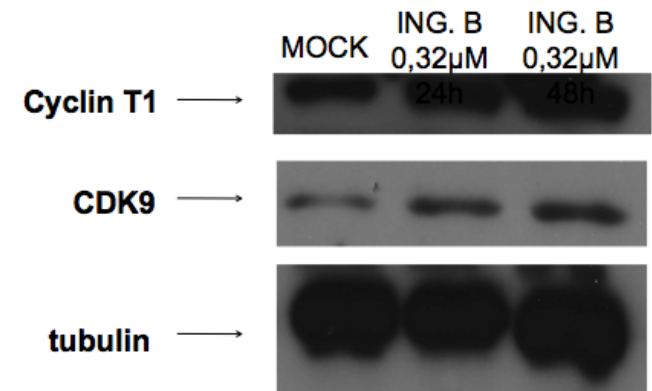
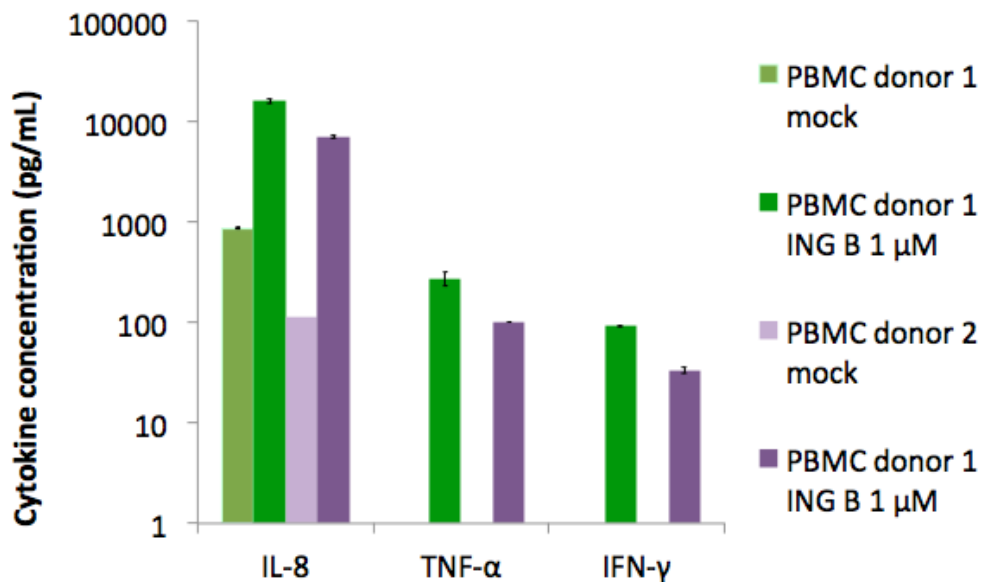
Ing B

- Decreases HIV and SIV replication in MT-4 / CEMx174
 - Downregulates **CD4, CCR5, CXCR4**
- In CD4+ T cells: Upregulates **CD38** & **CD69**. Slight upregulation of **HLA-DR**
- In Mø: Does not upregulate **CD80** or **HLA-DR**.



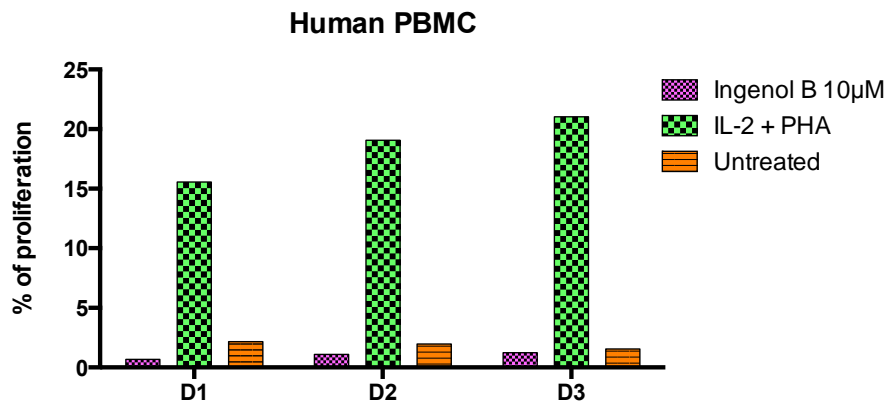
Ing B

- Upregulates *in vitro* expression of cytokines and PTEFb components.



Ing B

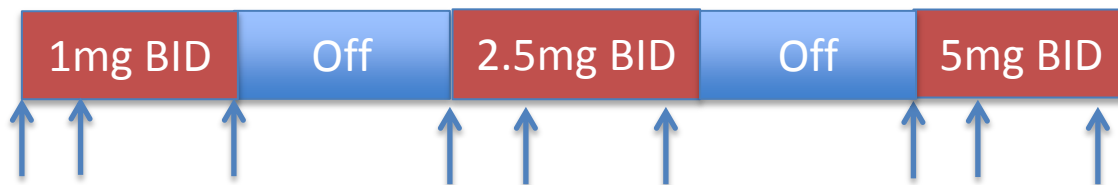
- Does not promote cell proliferation.



- Very well tolerated (orally)
 - Mice, rats, dogs
- Easy and cheap extraction

Rhesus macaque trial 1 – Proof of concept

Two SIVmac251-infected rhesus macaques (NOT SUPPRESSED)
Escalating dosage ORAL



↑ Blood collection at day 0, 3, and 7

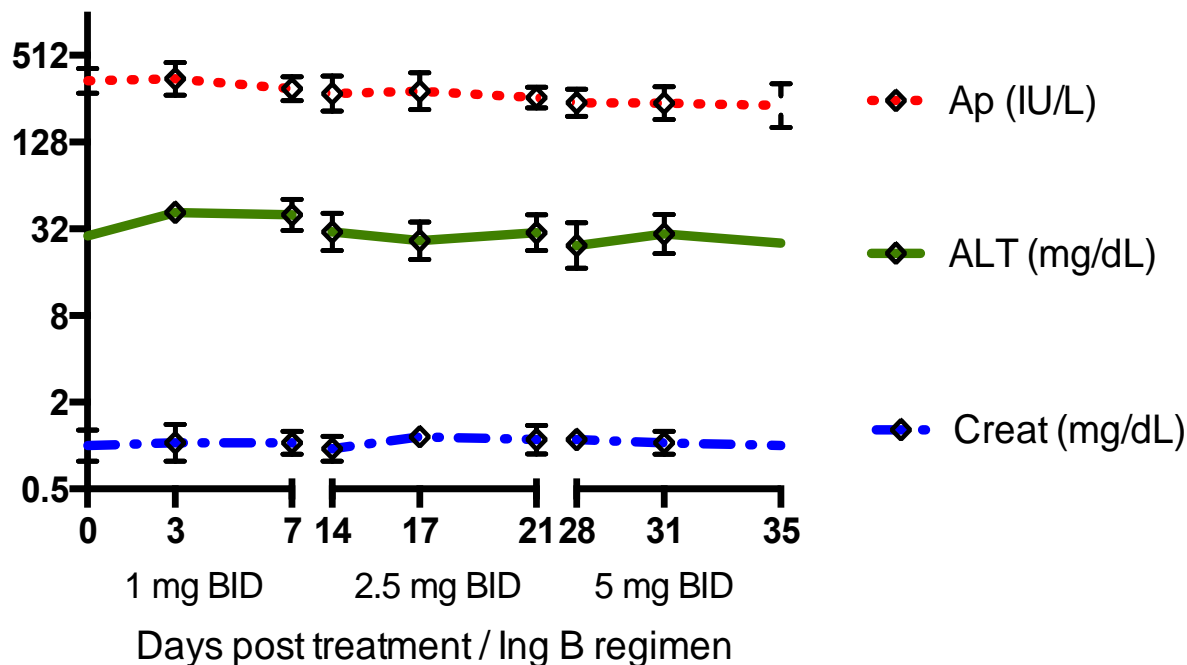
Each block: 7 days

CBC, Chemistry,
FACS, Viral load

Ingenol – Blood chemistry panel

Similar to our SIV-infected animals
* Low Ca, BUN, high globulin

Chemistry Panel

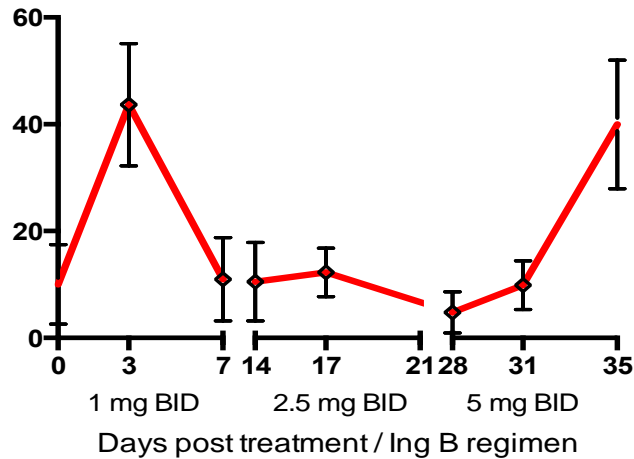


Within normal levels

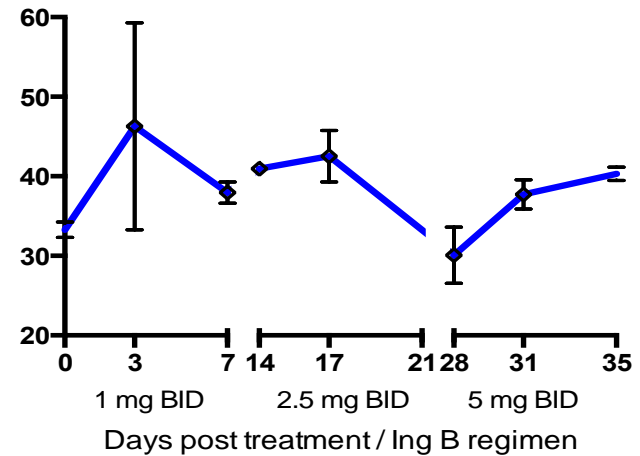
- * Protein
- * Bilirubin
- * (low) AST

Ingenol – CD69 (activation)

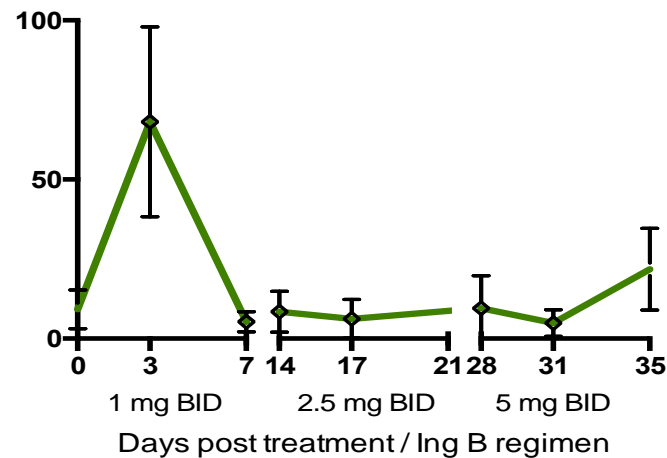
% CD4+ T cells positive for CD69



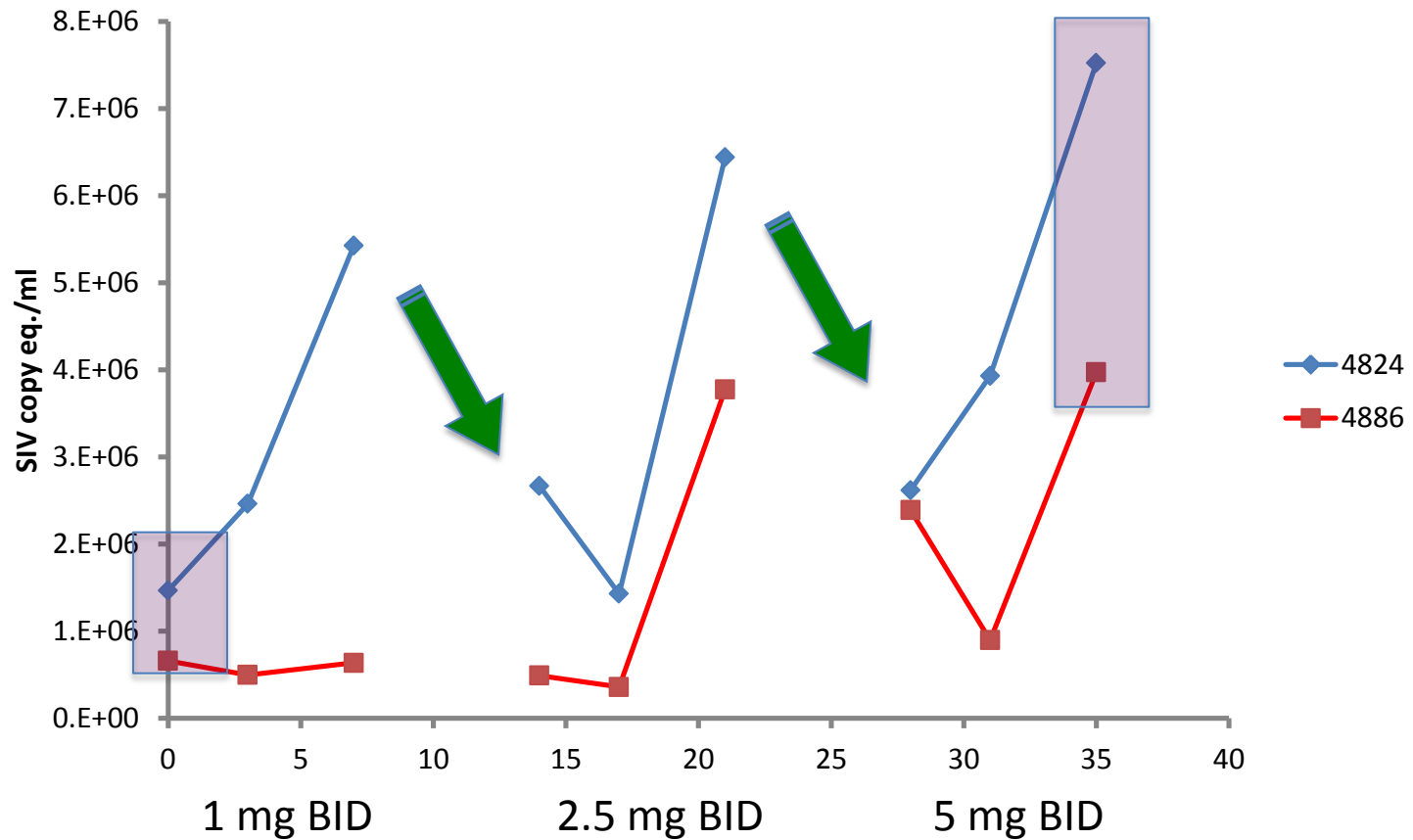
% CD8+ T cells positive for CD69



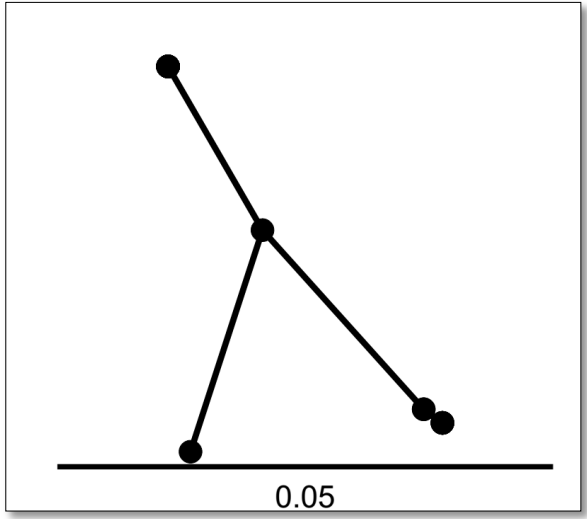
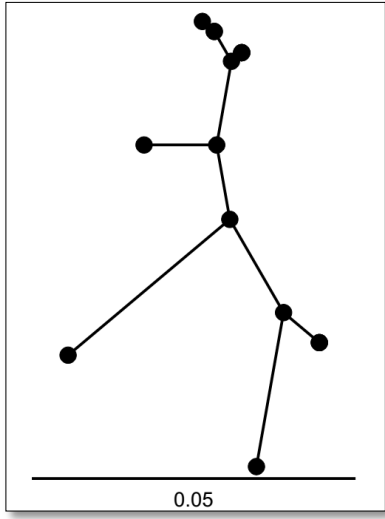
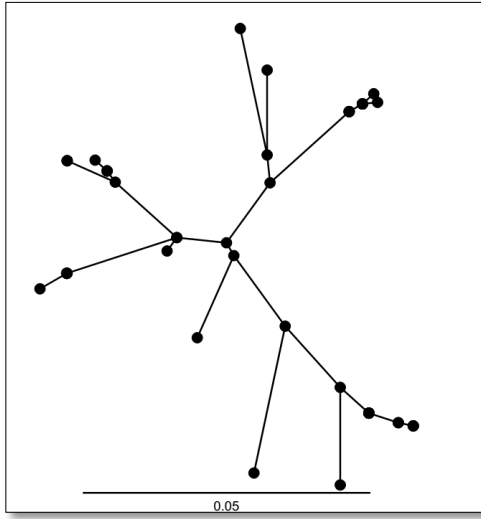
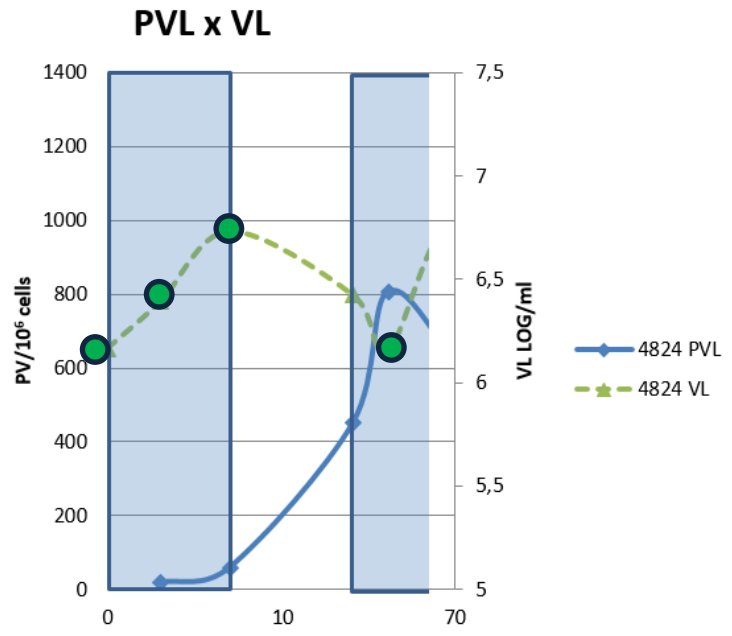
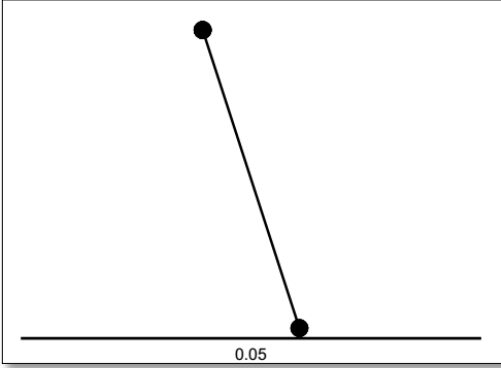
Monocytes positive for CD69



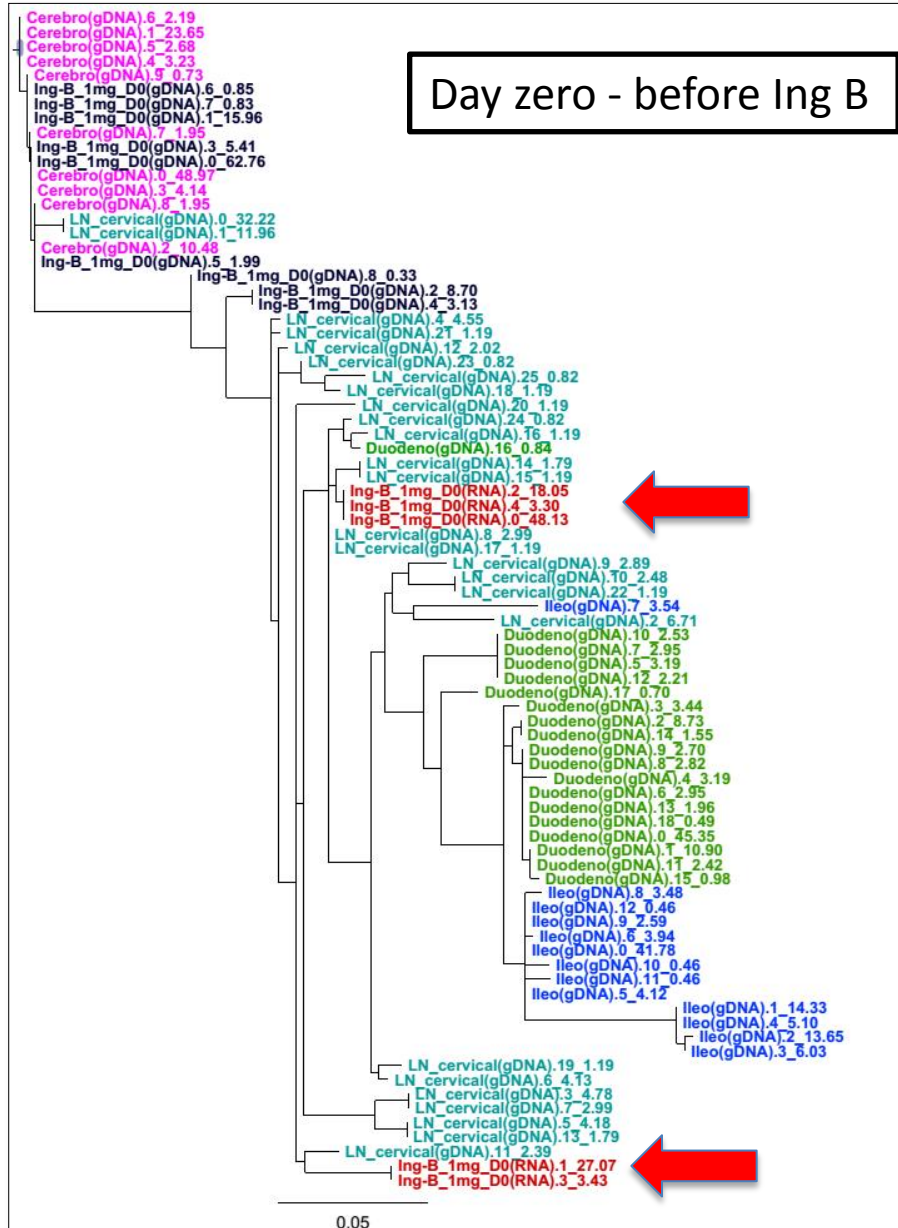
Ingenol increases viral load



Ingenol increases virus diversity in plasma



Brain DNA



Tissue DNA post necropsy

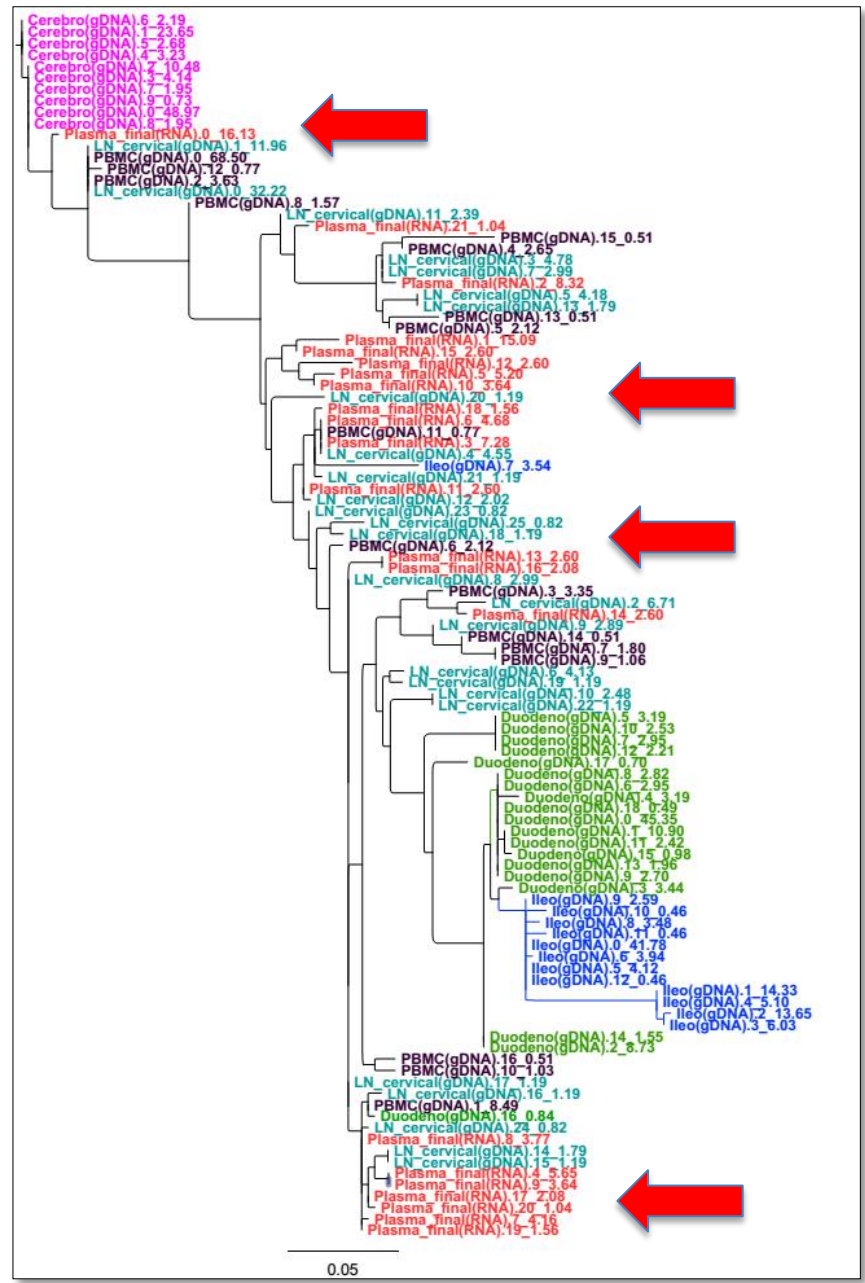
Plasma RNA

Gabriel Gonsalves

Post treatment Ing B



Brain DNA



Tissue DNA post necropsy

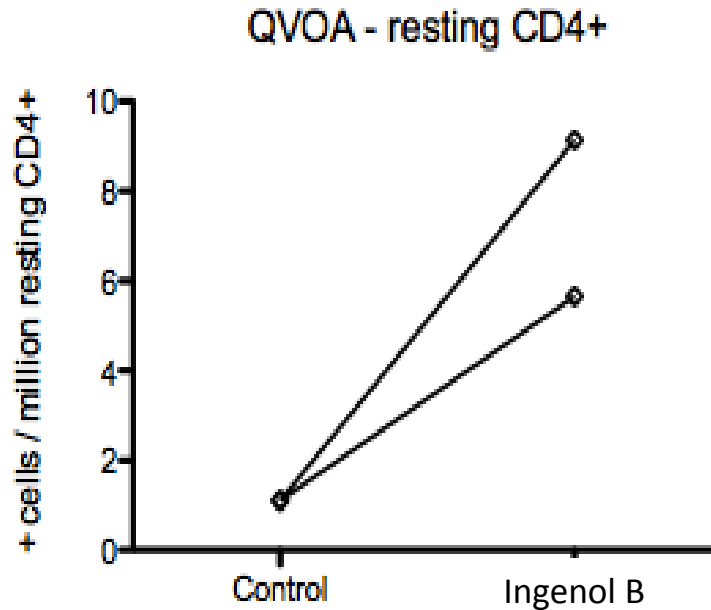
Plasma RNA

Gabriel Gonsalves

Studies in cART treated macaques - 1

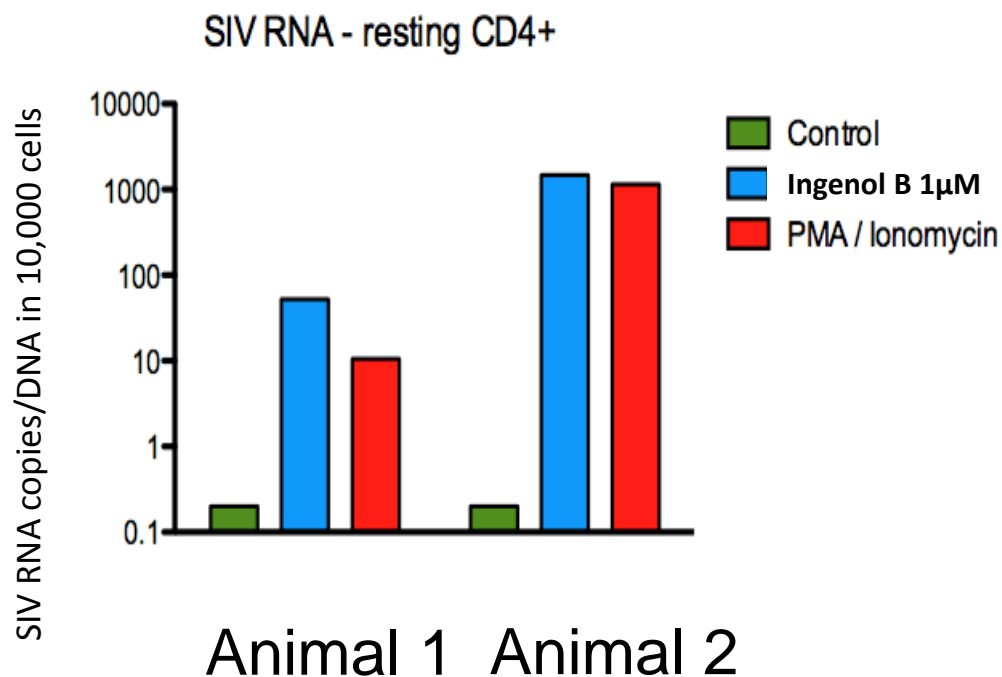
- **Group 1 – Hopkins Model**
 - * **3 Pigtailed macaques / SIVDeltaB670 + SIV17E-Fr**
 - * TNV / PMPA - 30 mg/kg/day
 - * DNV - 480 mg/kg BID
 - * RIV – 24 mg/kg BID
 - * L-870812 - 10 mg/kg BID
 - * **EX VIVO EXPERIMENTS**

Studies in cART treated macaques - 1



Resting CD4⁺ T cells were isolated from PBMCs, split in two sets, and serially diluted in duplicates. One set was kept as control while the other was treated with Ingenol B 1 μ M for 10 days

Studies in cART treated macaques - 1

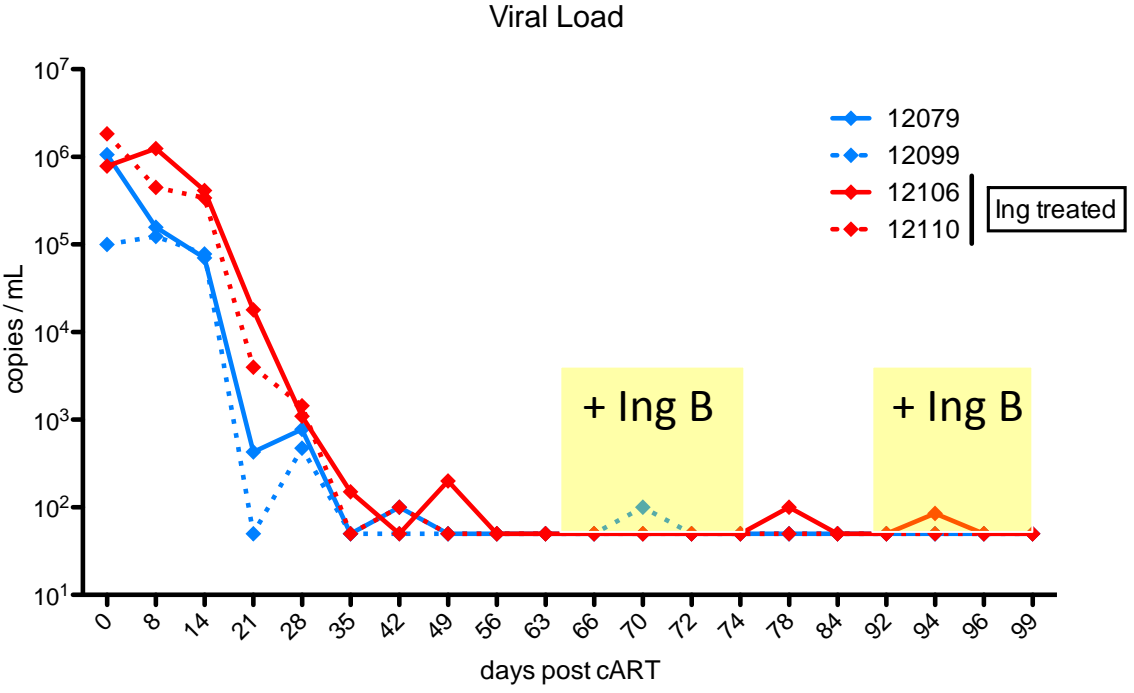


Resting CD4+ T cells were isolated from **spleen biopsies** and seeded on 24 well plates in AZT containing media. Cells were kept untreated or treated with Ingenol 1 µM or PMA 10 ng/mL + ionomycin 1 µM for 18 hours. After treatment, cells were collected and viral DNA and RNA were quantitated by qPCR. SIV RNA copy eq. was normalized by the levels of SIV DNA measured in 10,000 cells.

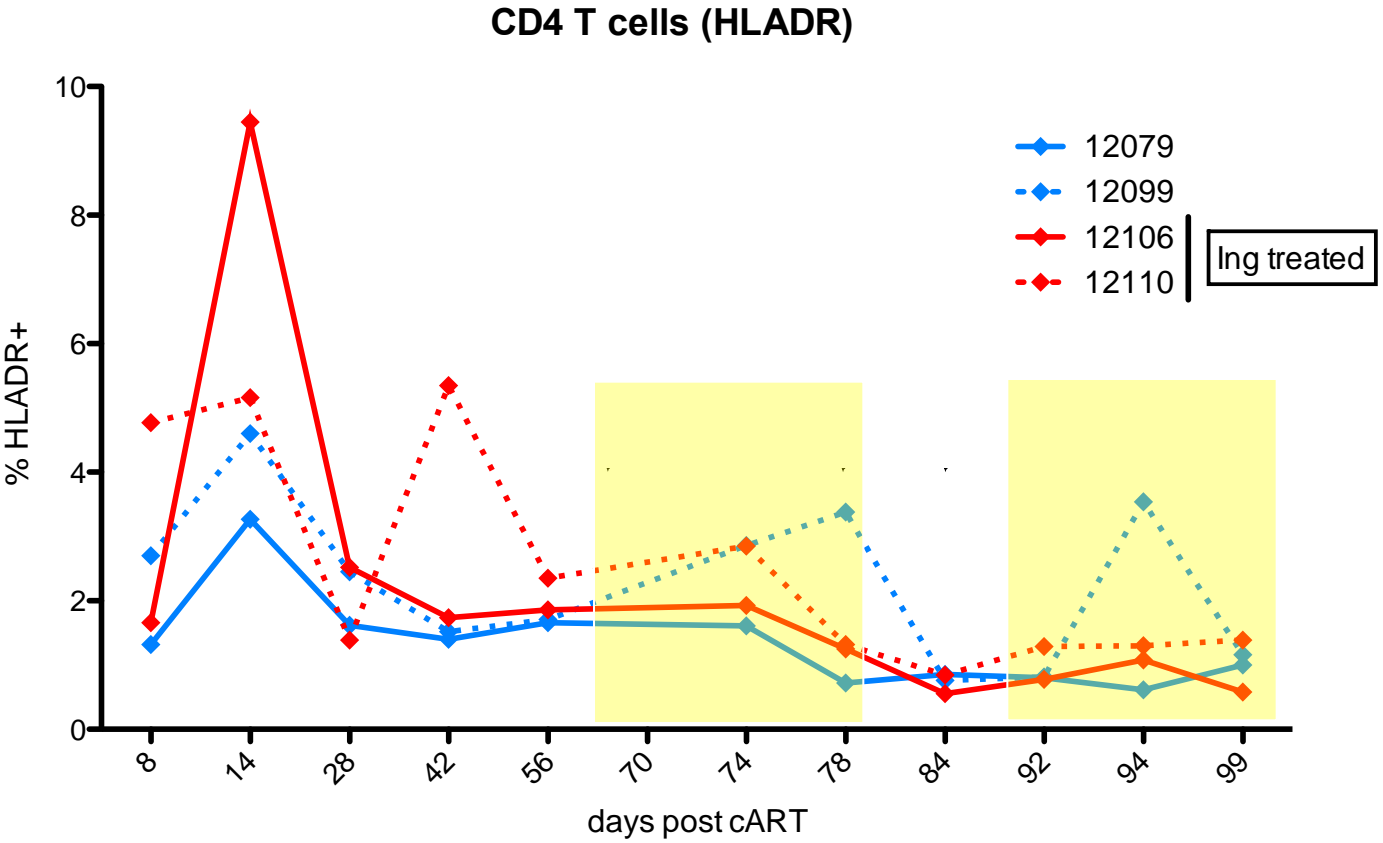
Studies in cART treated macaques - 2

- **Group 2A – Bioqual – 4 rhesus macaques / SIVmac251**
 - TNV / PMPA – 20 mg/kg/day
 - FTC – 50 mg/kg/day
 - RAL – 50 mg/kg BID
- **2 animals received ingenol 0.4 mg/kg/day**
 - **2 weeks / 1 week wash off / 2 weeks**
- **2 control animals**

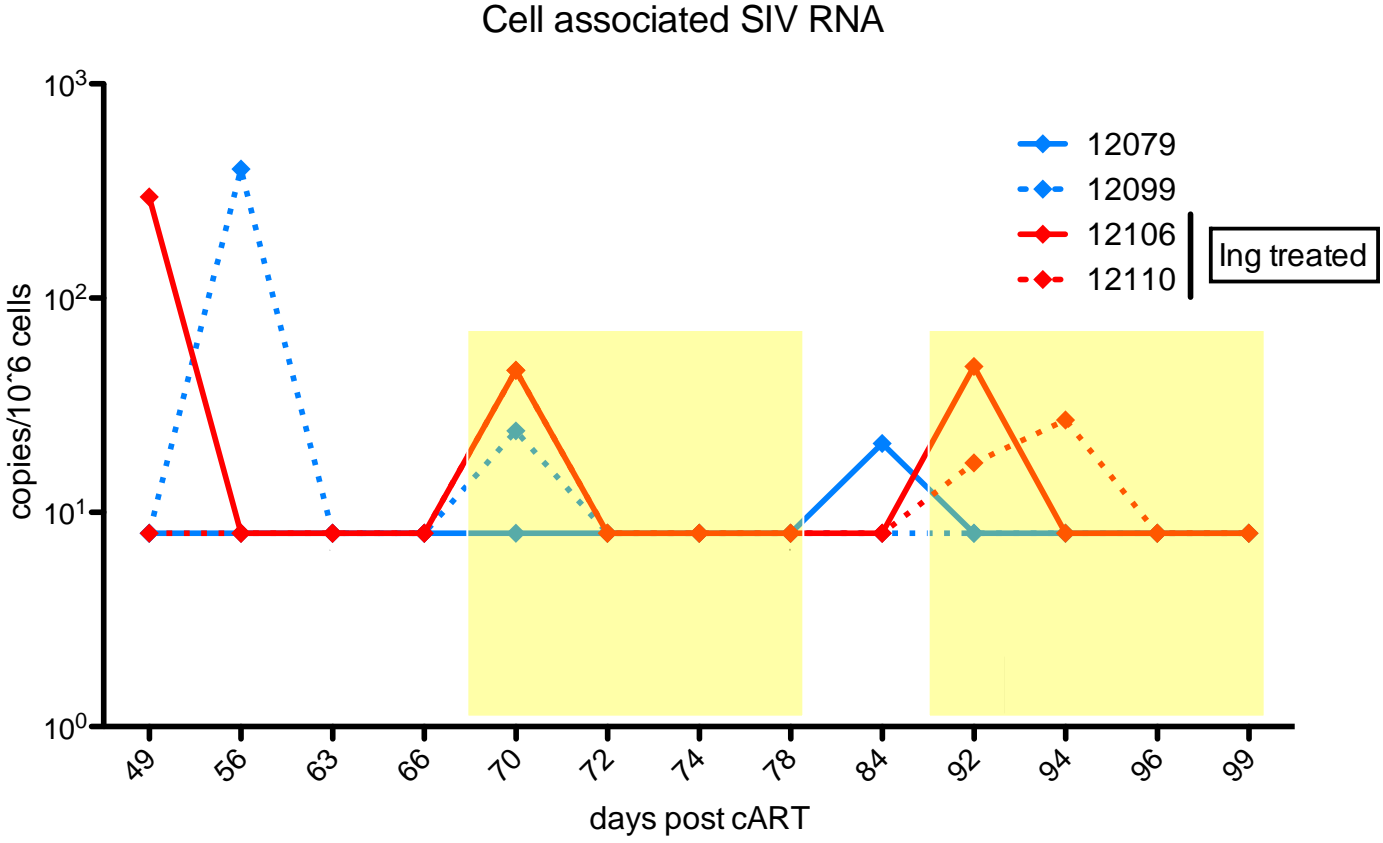
Studies in cART treated macaques - 2



Studies in cART treated macaques - 2



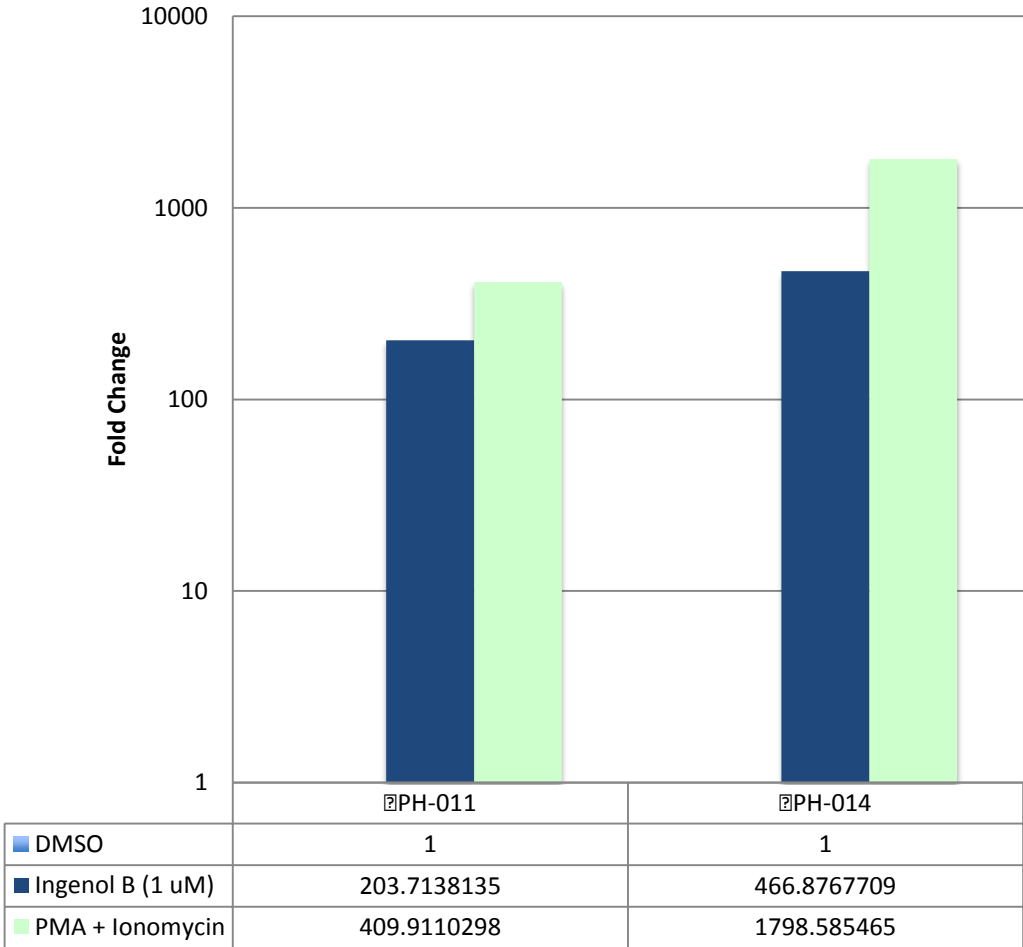
Studies in cART treated macaques - 2



Cell-associated HIV RNA – Siliciano’s Lab

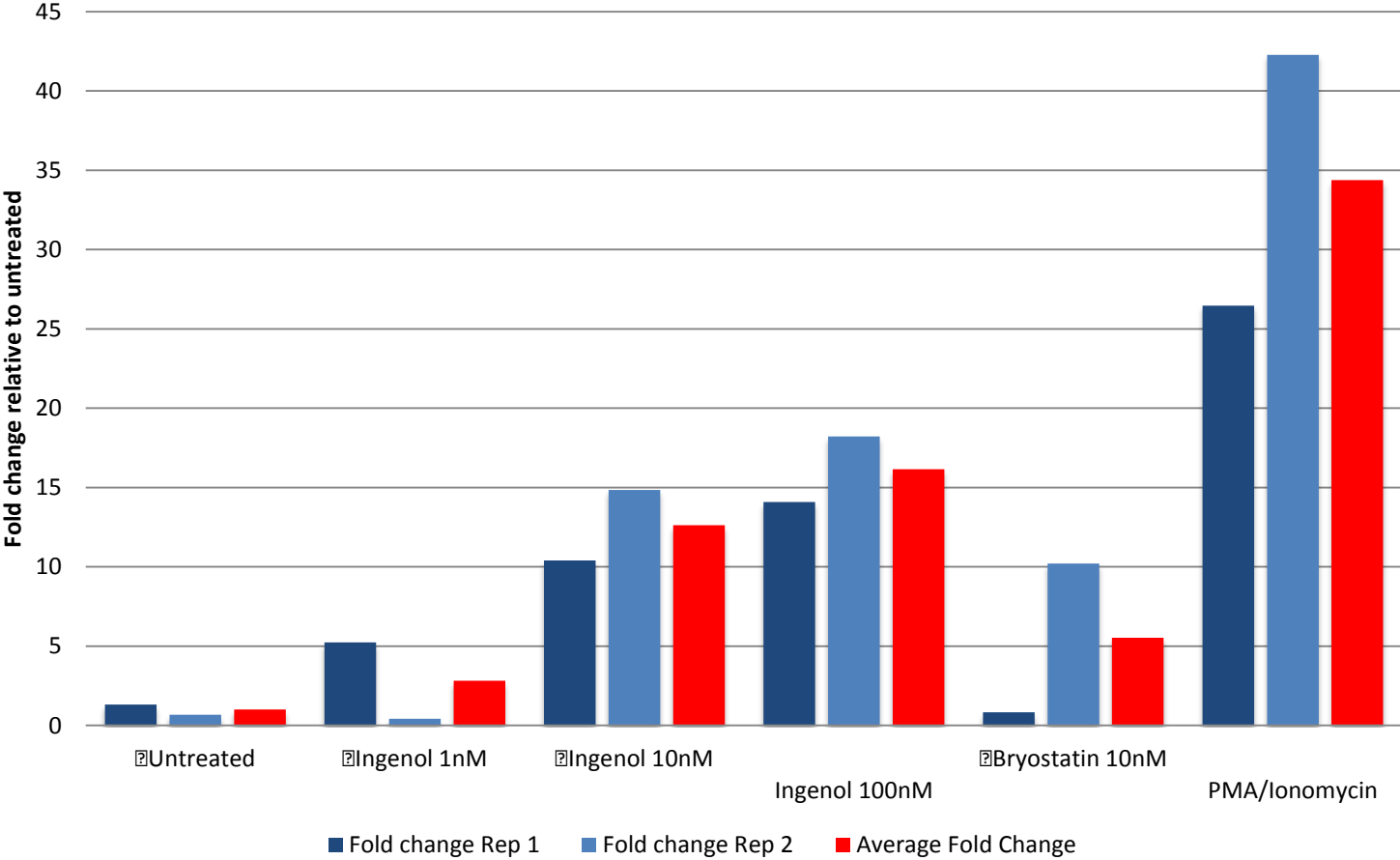
Patient 1

Meanwhile
At Hopkins...



Cell-associated HIV RNA – Siliciano's Lab

Patient 2

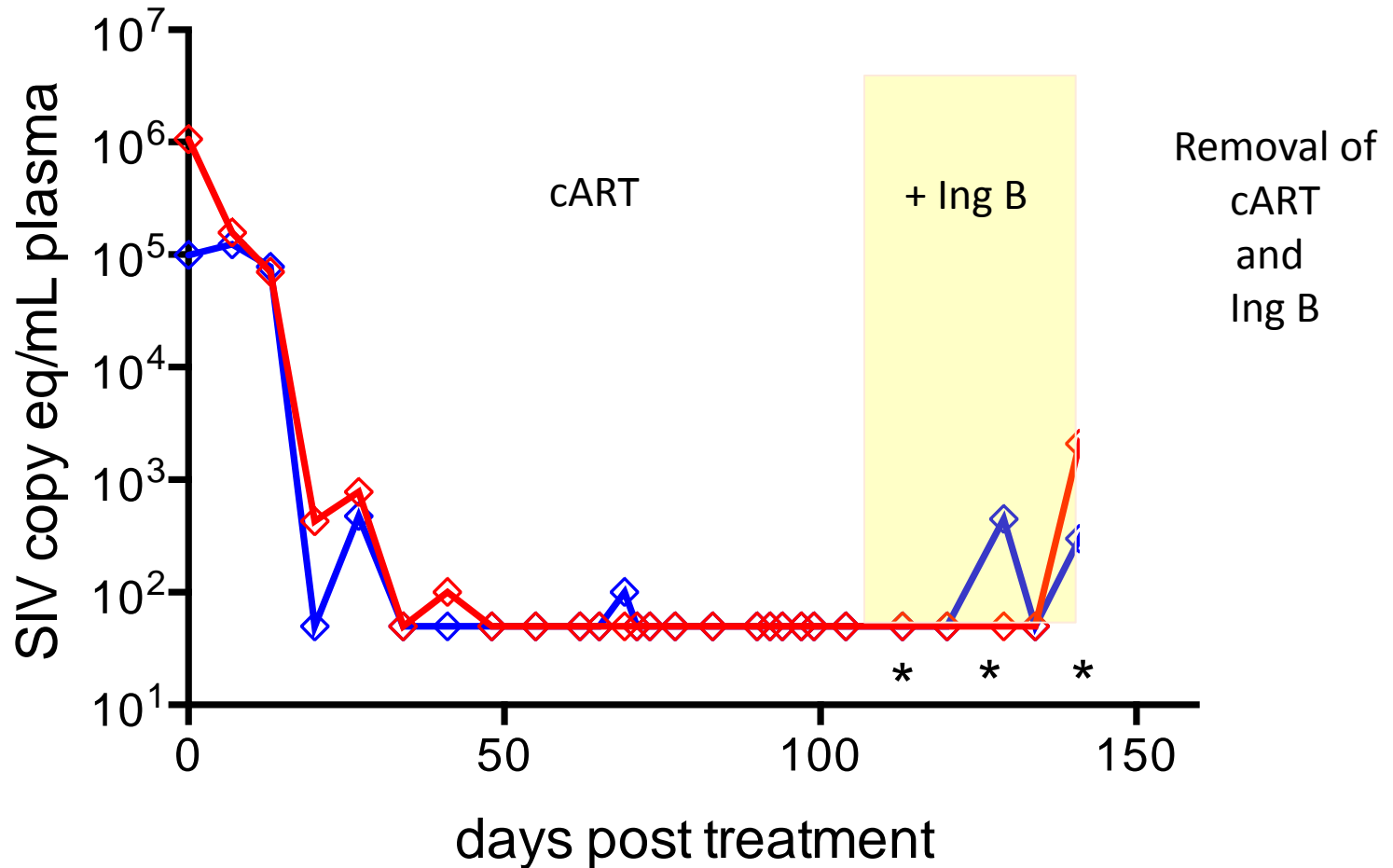


Studies in cART treated macaques - 3

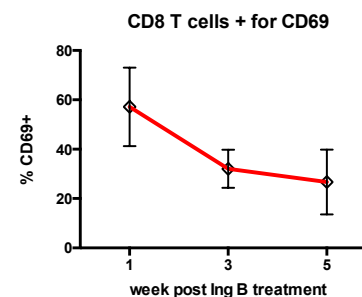
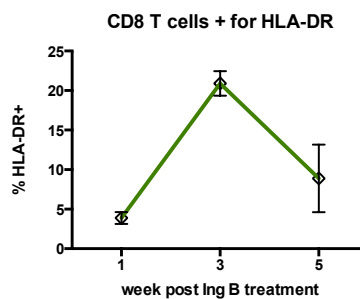
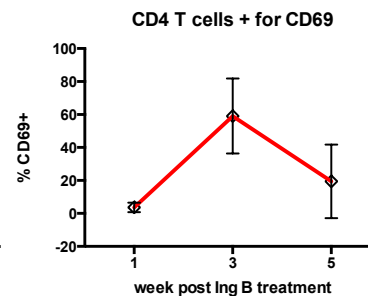
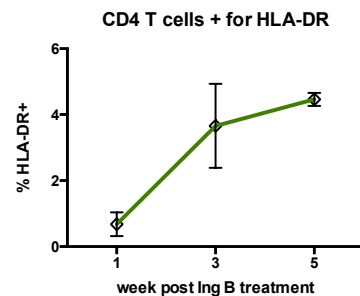
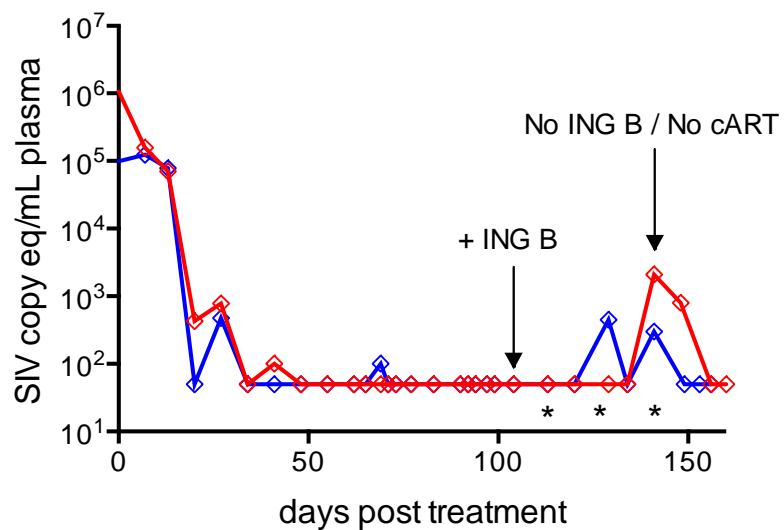
- **Group 2B – Bioqual – 2 rhesus macaques / SIVmac251**
 - TNV / PMPA – 20 mg/kg/day
 - FTC – 50 mg/kg/day
 - RAL – 50 mg/kg BID
- **2 animals received ingenol 0.4 mg/kg/day for 30 days**

Tests in SIV-infected cART-treated macaques

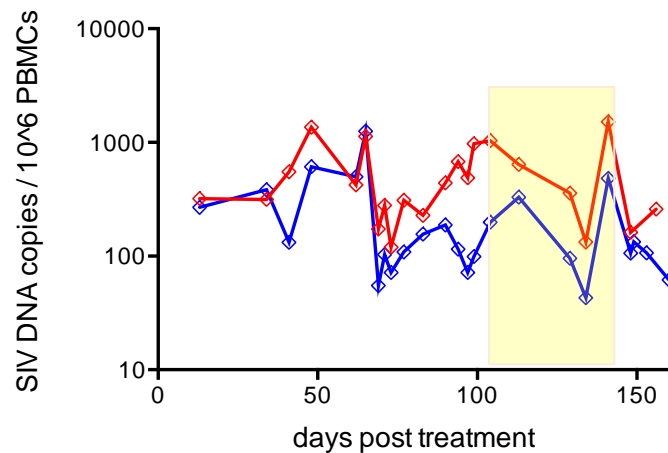
cART-suppressed macaques



cART-suppressed macaques



SIV DNA per 10⁶ cells



PK Studies

* In rats: 10 µg/kg i.v. – in macaque: 5 µg/kg i.v.

* In rats: 1 mg/kg p.o. – in macaque: 0.5 mg/kg p.o. (2.5 mg total)

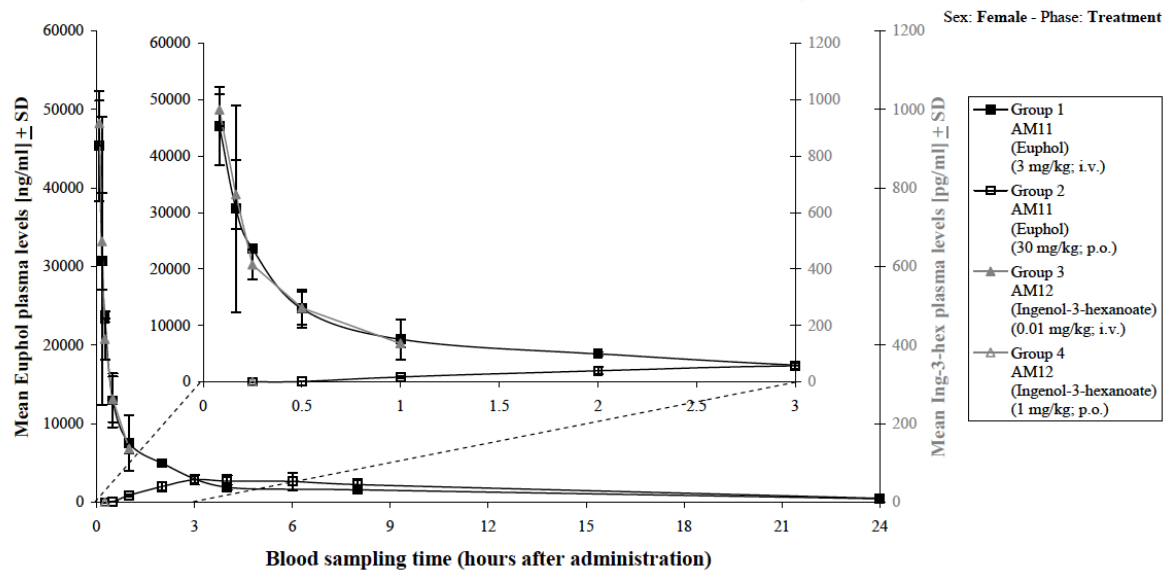
Study no.: 723.241.3671

APPENDIX IV/A

Euphol plasma levels [ng/ml] / Ingenol-3-hexanoate plasma levels [pg/ml]
(mean figure)



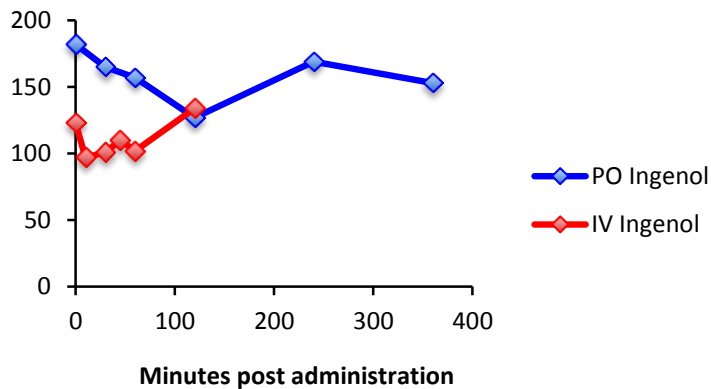
Start: 100,000 pg/mL



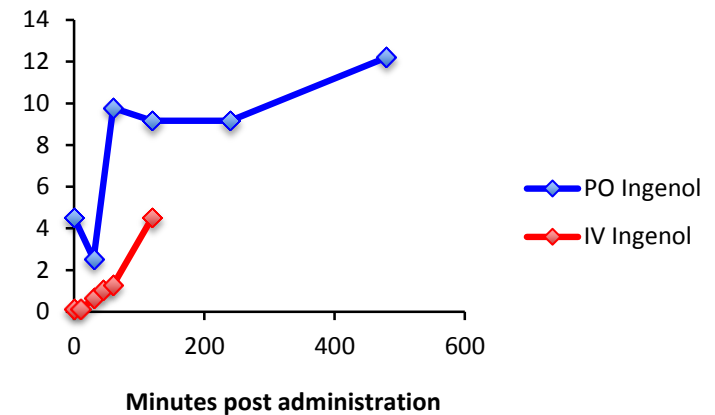
Ingenol in uninfected macaques

Cytokines (ELISA)

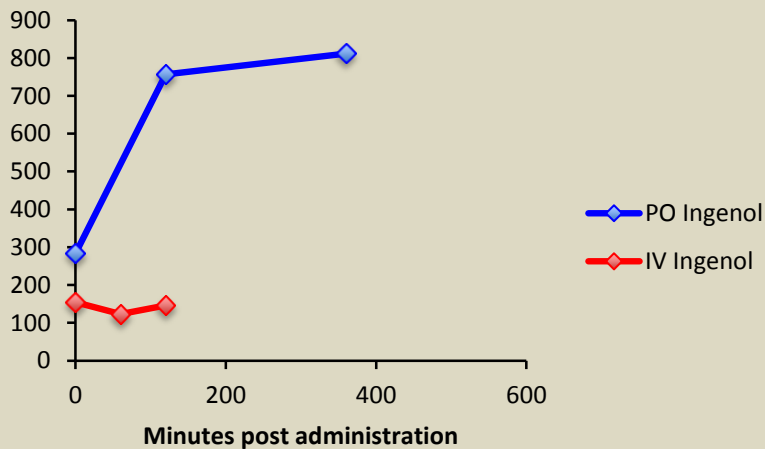
MCP1 levels in Plasma (pg/mL)



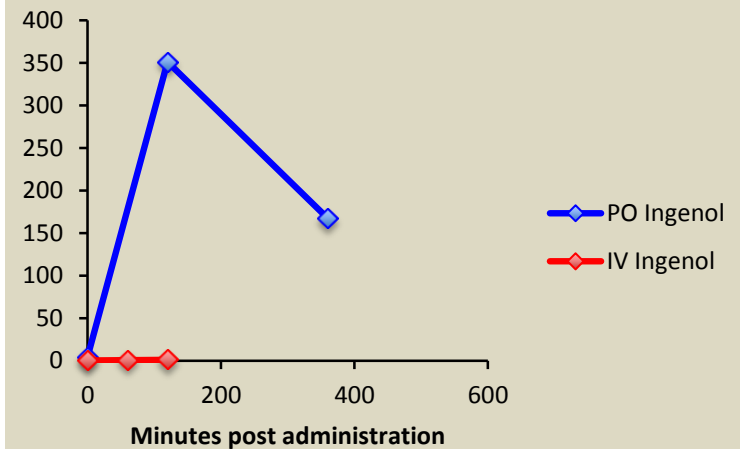
IL6 levels in plasma (pg/mL)



MCP1 in CSF (pg/mL)



IL6 in CSF (pg/mL)



Conclusions and Perspectives

- Ing B is a potent PKC activator and it is well tolerated when given to mammals orally.
- It is a potential drug for the reactivation of latent reservoirs
 - Evaluate bioavailability and pharmacokinetics
 - Detection in tissues (Dr. Angela Kashuba)
 - Radiolabeled compound (Aurigon – Germany)

As a proof of concept: PKC activators are not Satan

- * Molecule modifications
- * Tools to understand pharmacophysiology



Acknowledgments

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Ceereena Ubaida Mohien

Kelly Meulendyke

Claudia Avalos

Julia Russell

Julia Drewes

Jeanne Sisk

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