Discovery of Genetic Variants of the Kinases that Activate Tenofovir in a Compartment-Specific Manner

Dr. Namandje N. Bumpus, PhD
Associate Professor and Associate Dean
Department of Medicine – Division of Clinical Pharmacology
Department of Pharmacology and Molecular Sciences
Johns Hopkins University School of Medicine
Tenofovir Requires Activation by Nucleotide Kinases

1. Tenofovir (TFV)
2. TFV Monophosphate (TFV-MP)
3. *TFV Diphosphate (TFV-DP)
Which nucleotide kinases contribute to tenofovir activation in cells and tissues at risk of HIV infection?
Nucleotide Mono- and Di-Phosphate Kinase Isoforms to be Investigated

- **Adenylate Kinases**, ATP + AMP → 2 ADP
  - 9 isoforms with differential tissue distributions and subcellular localizations

- **Guanylate Kinases**, ATP + GMP → ADP + GDP
  - 3 nucleotide phosphorylating isoforms

- **Nucleotide Diphosphate Kinases**, NTP + NDP ↔ NDP + NTP
  - 4 enzymatically active isoforms

- **Creatine Kinases**, ATP + Creatine → ADP + Phosphocreatine
  - Cytosolic and mitochondrial isoforms

- **Pyruvate Kinases**, Phosphoenolpyruvate + ADP → Pyruvate + ATP
  - 4 isozymes result from differential splicing
Candidate Nucleotide Kinases

- Adenylate kinase 2 (AK2)
- Guanylate kinase 1 (GUK1)
- Creatine kinase, muscle (CKM)
- Pyruvate kinase, muscle (PKM)
- Pyruvate kinase, liver & RBC (PKLR)

Chemical structures for:
- Tenofovir (TFV)
- TFV Monophosphate (TFV-MP)
- TFV Diphosphate (TFV-DP)
siRNA Knockdown of Nucleotide Kinases

Human Samples

- Cells and tissues:
  1. Peripheral blood mononuclear cells (PBMC)
  2. Colorectal tissue
  3. Vaginal tissue

Method

- Delivered siRNA to cells and tissues in culture
- Followed by incubation with TFV
  - Detected TFV metabolites using ultra-high performance liquid chromatography-tandem mass spectrometry
AK2, PKM, and PKLR Contribute to Metabolite Formation in Peripheral Blood Mononuclear Cells (PBMC)

Lade JM, To EE, Hendrix CW, Bumpus NN. EBioMedicine. Accepted 2 July 2015; In Press.
AK2, PKM, and PKLR Contribute to Metabolite Formation in Vaginal Tissue

Lade JM, To EE, Hendrix CW, Bumpus NN. EBioMedicine. Accepted 2 July 2015; In Press.
AK2 and CKM Contribute to Metabolite Formation in Colorectal Tissue

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Tenofovir is Activated in a Tissue-Specific Manner

PBMC

- TFV-DP
- PKM
- PKLR
- TFV-MP
- AK2
- TFV

Vagina

- TFV-DP
- PKM
- PKLR
- TFV-MP
- AK2
- TFV

Colon

- TFV-DP
- CKM
- TFV-MP
- AK2
- TFV

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Do human genetic variants exist in the genes encoding these nucleotide kinases?
Next-Generation Sequencing of MTN-001 Samples

18 CKM variants
- 17 participants
- 3% frequency of deleterious missense variants – 1 USA, 1 SA, 2 UGA participants

19 PKM variants
- 19 participants
- Unable to predict phenotype of missense variants

12 AK2 variants
- 11 participants
- 2% frequency of deleterious missense variants – 1 USA, 2 SA participants

22 PKLR variants
- 21 participants
- 3% frequency of deleterious missense variants – 3 USA, 1 SA participants
MALDI-MS Imaging of TFV
Acknowledgements

Lab Members:
Philip Cox
Dominique Figueroa
Carley Heck
Julie Lade
Elaine To

Collaborator:
Craig Hendrix

Funding:
MTN
HPTN
NIH R01GM103853