Potential role of cytochrome P450s in mediating the effects of alcohol on HIV pathogenesis

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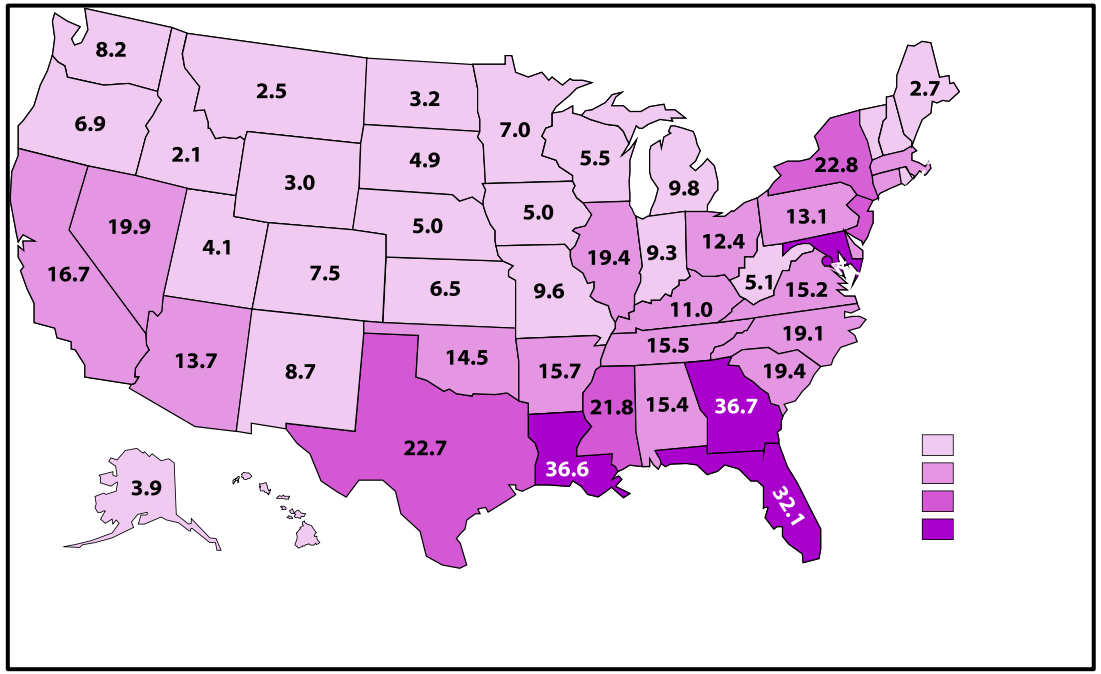
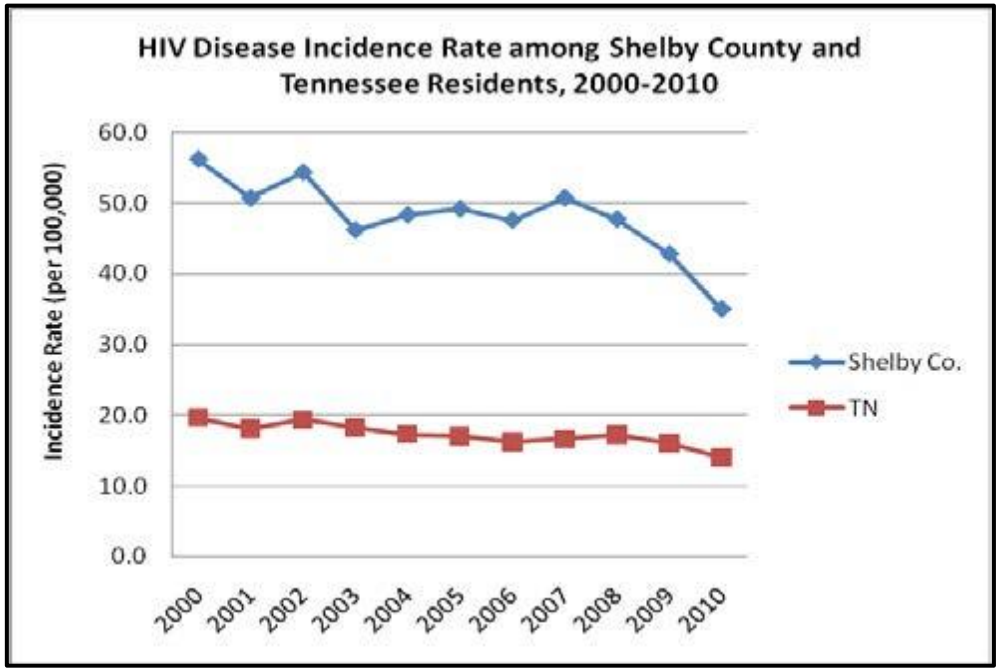
RATES OF HIV DIAGNOSES PER 100,000 POPULATION





- ~21,400 Tennesseans with HIV/AIDS.
- 10th in the country in the rate of new HIV infections.
- 5th in the country in the rate of HIV/AIDS deaths.
- Memphis among the top 10 cities for HIV infection rates.

Shelby county report, 2013

Alcohol use amongst HIV-infected population

- Prevalence of mild-to-moderate alcoholic (7-14 drinks/week for Men and 4-7 drinks/week for Women): 3-fold (50-60%) higher in HIV-infected population

- **Alcohol**
  - ↑ HIV-1 infection
  - ↑ AIDS and neuroAIDS
  - ↑ Neuronal damage and neuropsychological impairments
  - ↑ Mortality risk of AIDS and other diseases

Effects of alcohol on HIV-1 infection

↑ Oxidative stress

↓ Response to antiretroviral therapy (ART)

↑ Viral replication

↓ Immune function

↑ CNS barrier permeability

CYTOCHROME P450 (CYP)?

Alcoholism: Clinical and Experimental Research
Vol. 35, No. 1
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Effect of Alcohol on Drug Efflux Protein and Drug Metabolic Enzymes in U937 Macrophages

Mengyao Jin, Priyanka Arya, Kalpeshkumar Patel, Bhupendra Singh, Peter S. Silverstein, Hari K. Bhat, Anil Kumar, and Santosh Kumar

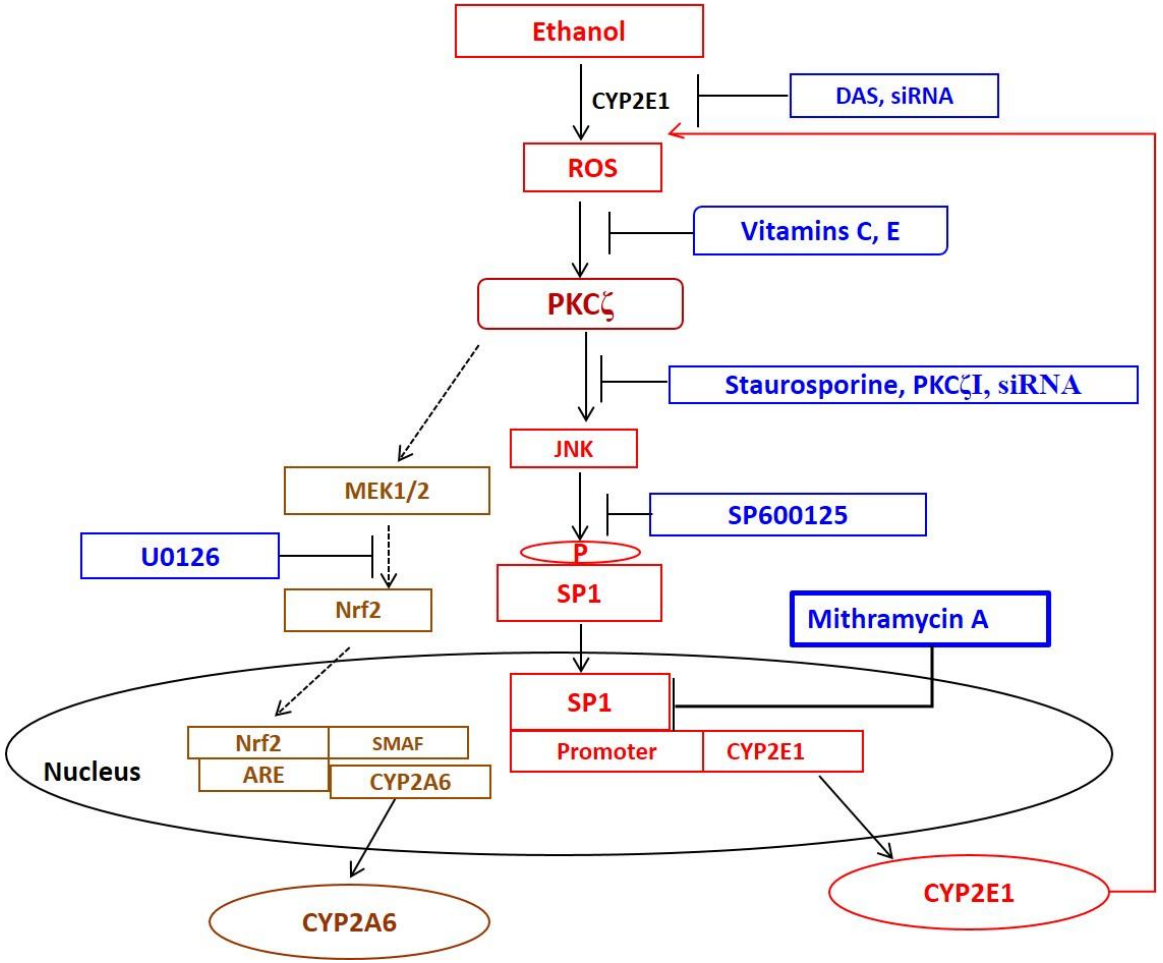
- Alcohol induces CYP2A6, CYP2E1, CYP3A4.
- Alcohol produces reactive oxygen species (ROS).
- Alcohol induces anti-oxidants: SOD1, catalase.

Citation: Cell Death and Disease (2013) 4, e554; doi:10.1038/cddis.2013.78

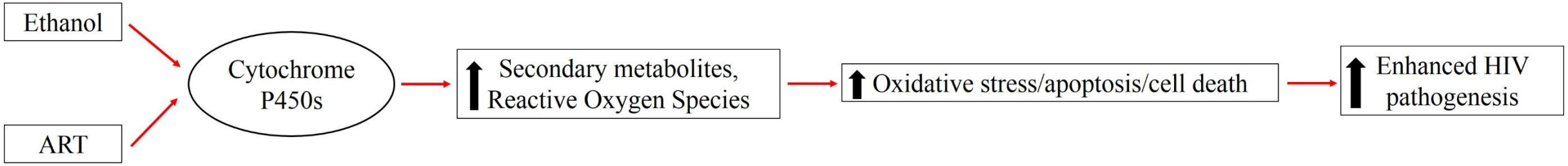
www.nature.com/cddis

Regulation of cytochrome P450 2e1 expression by ethanol: role of oxidative stress-mediated pkc/jnk/sp1 pathway

M Jin[1], A Ande[1], A Kumar[1] and S Kumar*[,1]



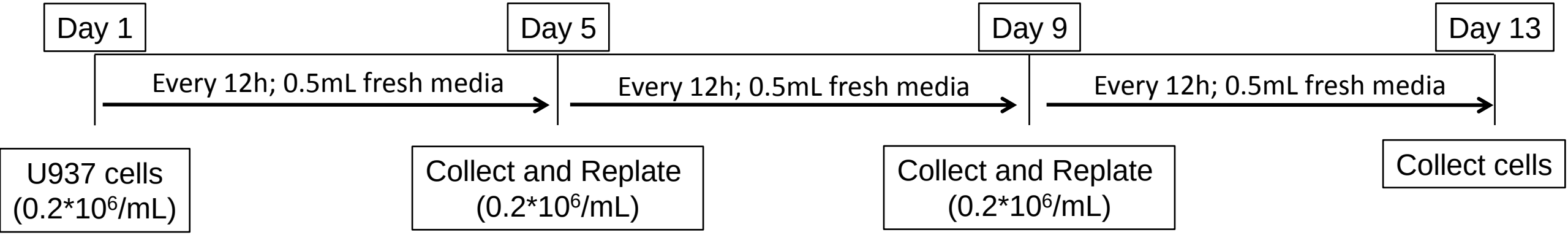
Hypothesis



Model:

- ***In vitro*:** U937 monocytic cells
- ***In vitro*:** Primary HIV-infected macrophages
- ***In vitro*:** ART metabolism: Effects of ethanol
- ***Ex vivo*:** Human monocytes/macrophages

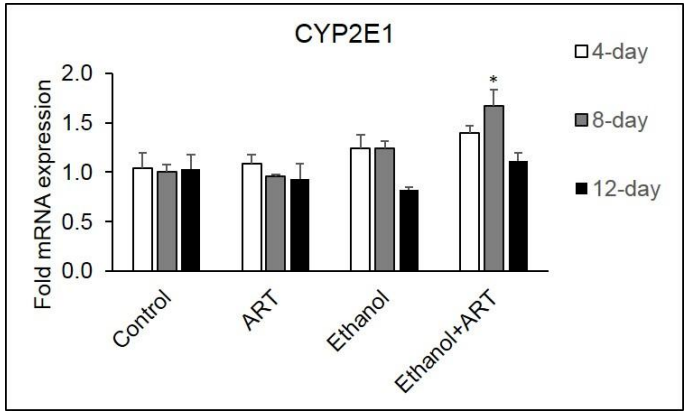
Experimental Design



Ethanol: 50 mM

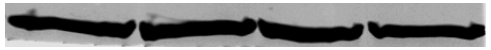
ART: Darunavir (Dar) 4µM and Ritonavir (1µM)

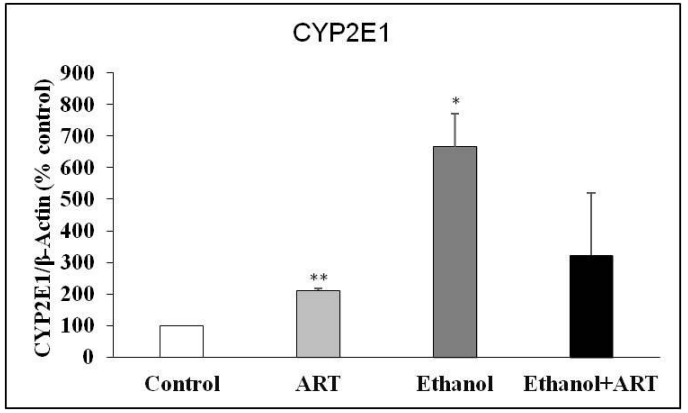
Results: Expression of ethanol metabolizing enzyme



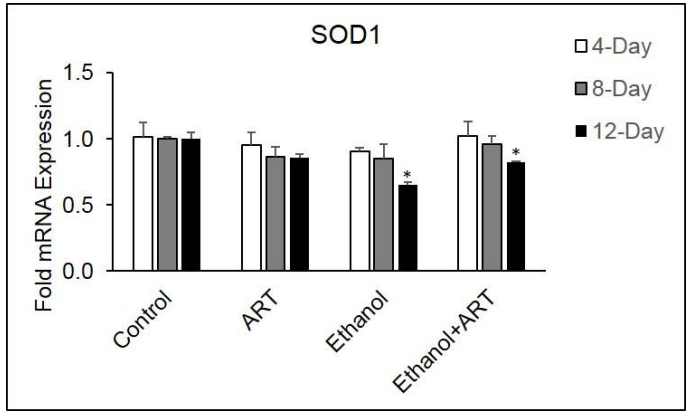
CYP2E1

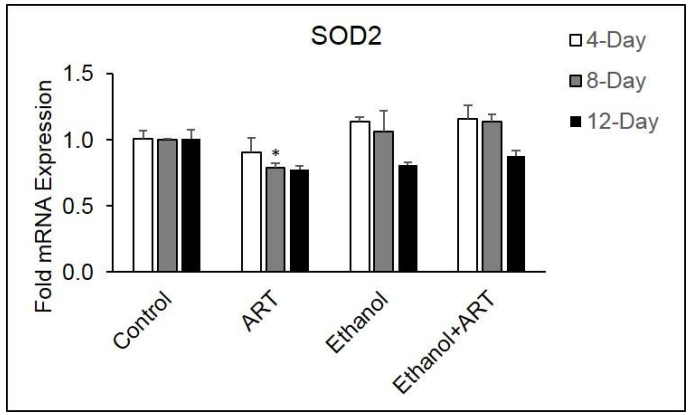
β-ACTIN





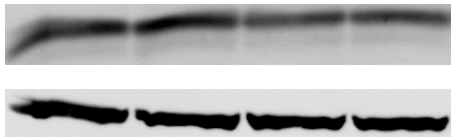
Expression of major AOEs





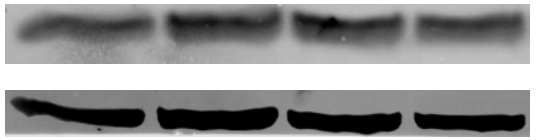
SOD1

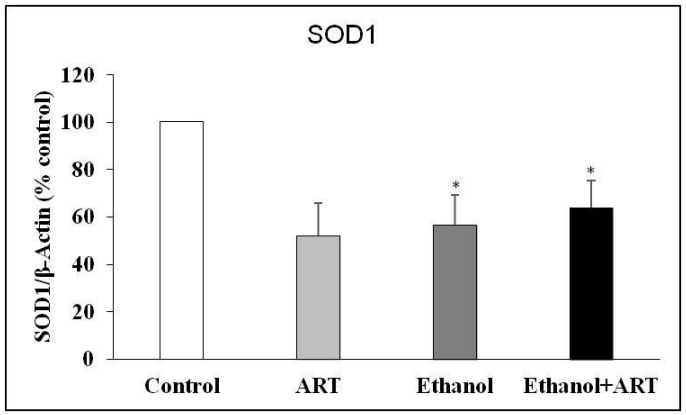
β-ACTIN

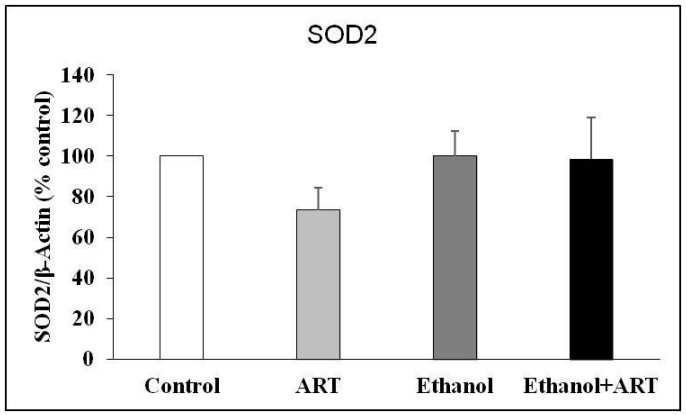


SOD2

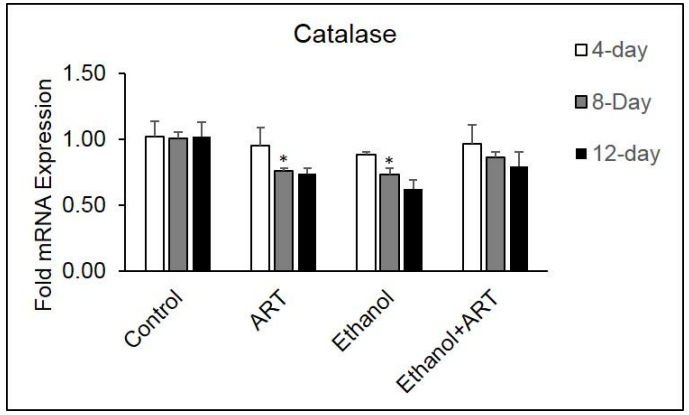
β-ACTIN

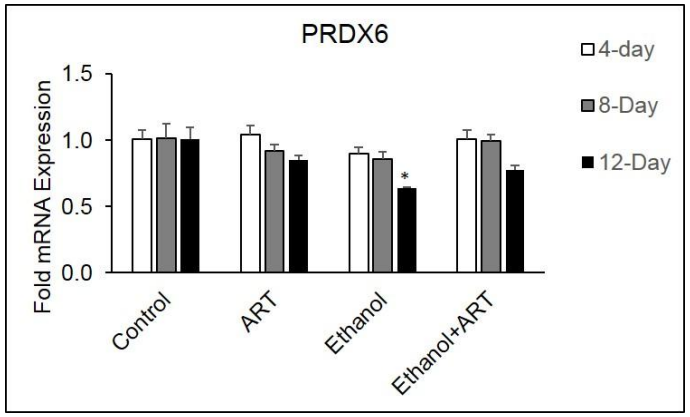


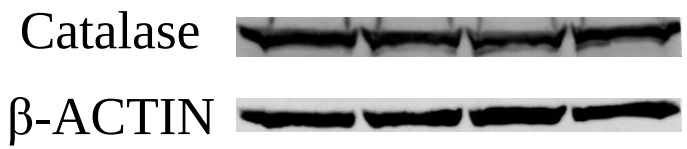


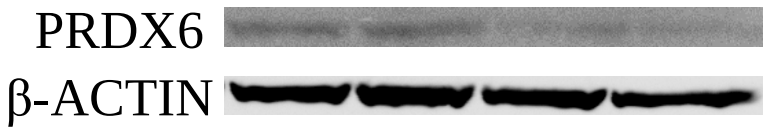


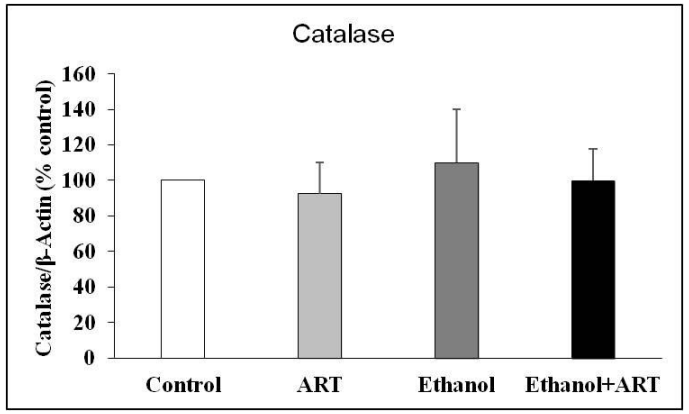
Expression of major AOEs

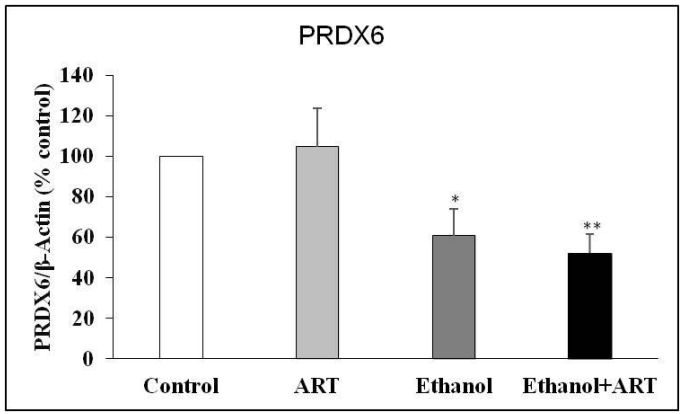




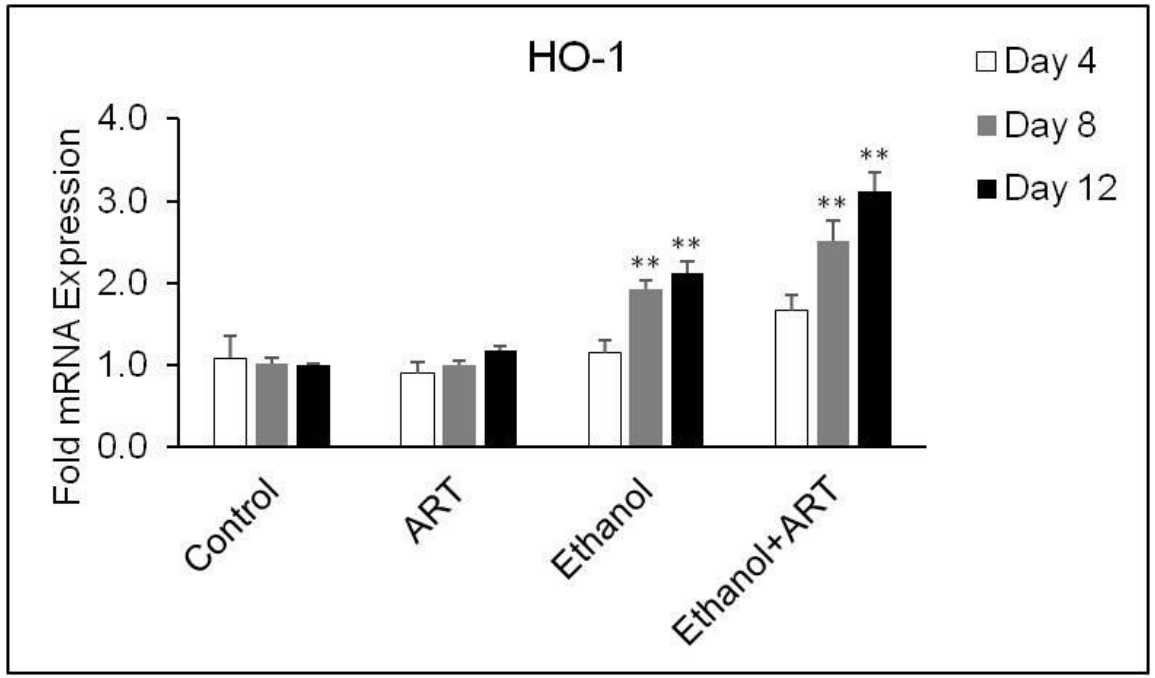






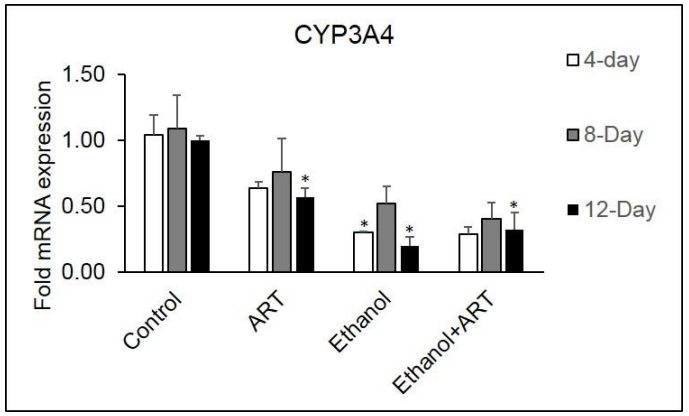


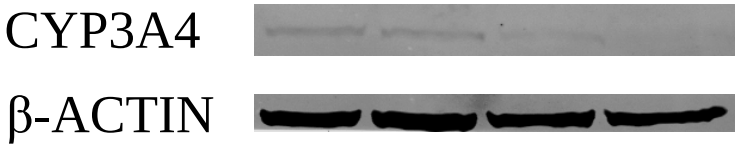
Transcription of HO-1

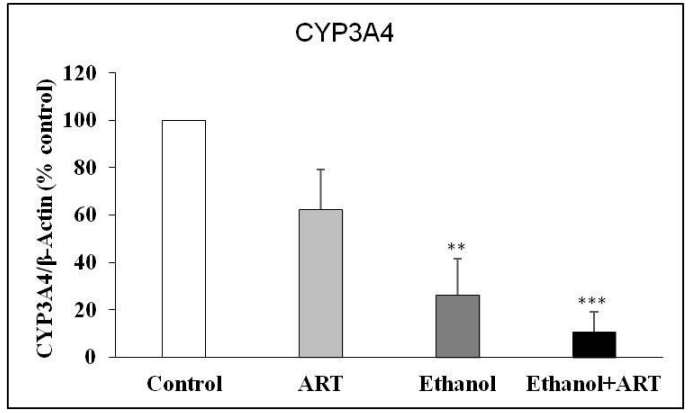


- Inducible heme degrading enzyme in cells
- Stress response protein induced under oxidative stress
- Reported to play a critical role in cellular protection

Expression of major ART metabolizing enzyme

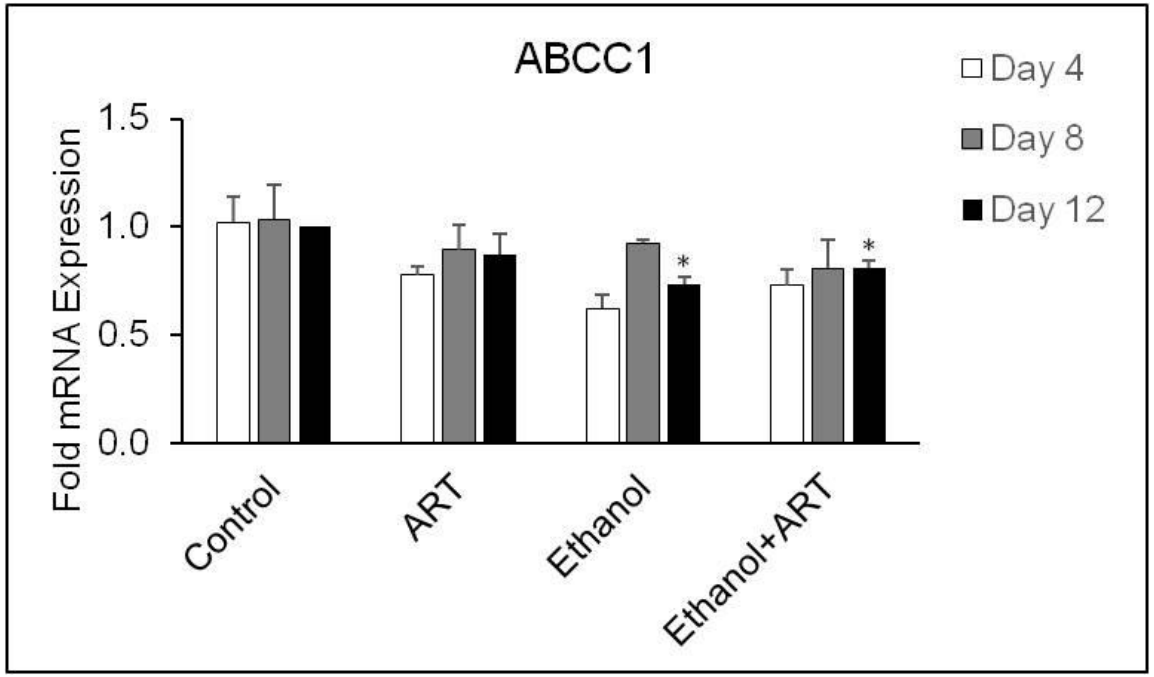






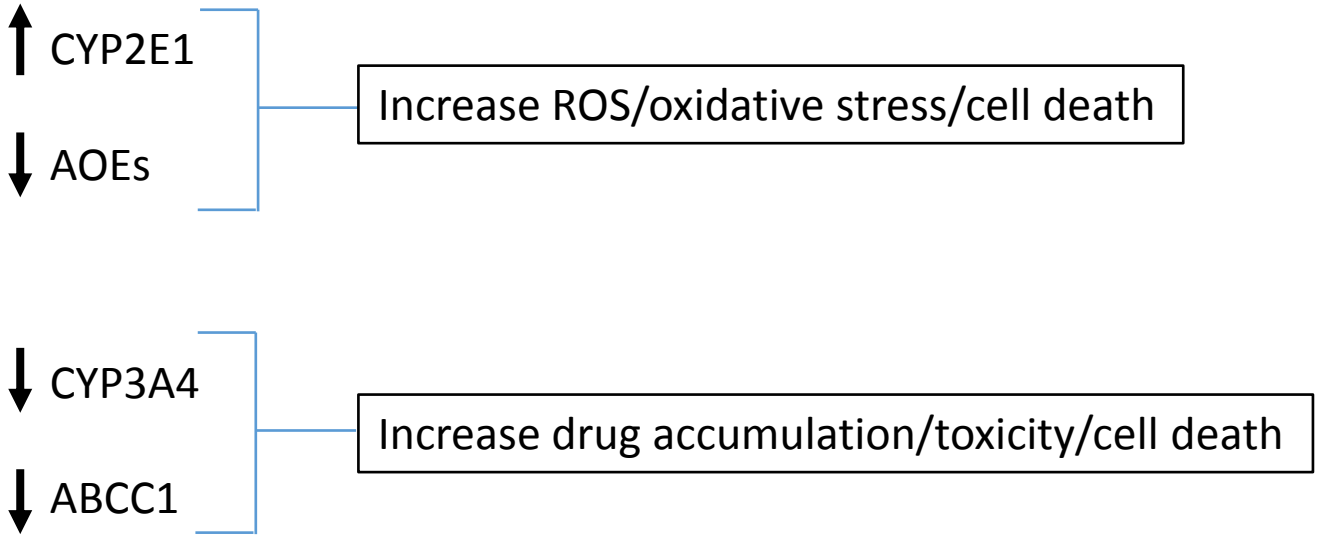
- Major ART metabolizing enzyme in U937 cells.

Transcription of major drug efflux transporter



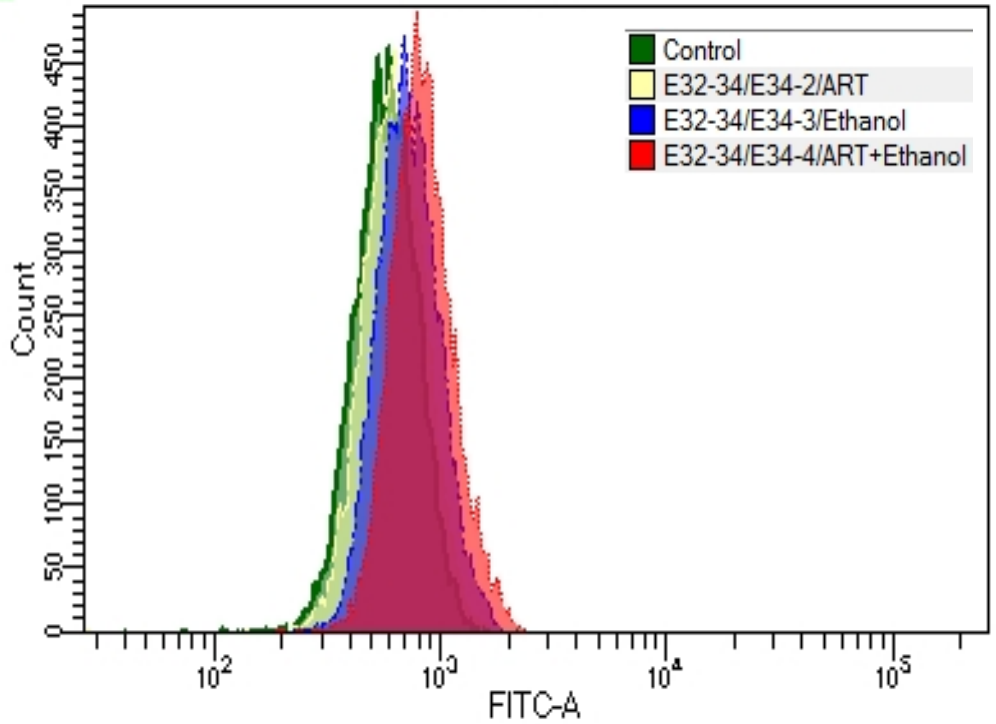
- Major drug efflux transporter expressed in U937 cells.

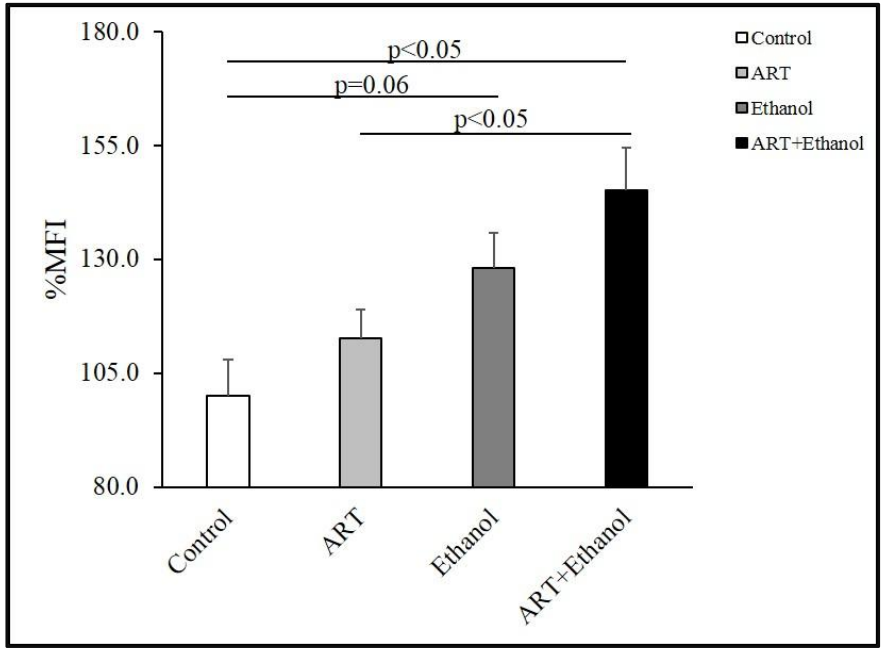
Results so far suggest---



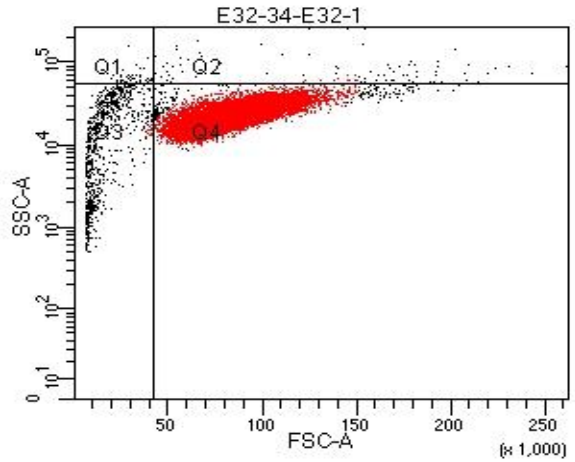
ROS/Cell death?

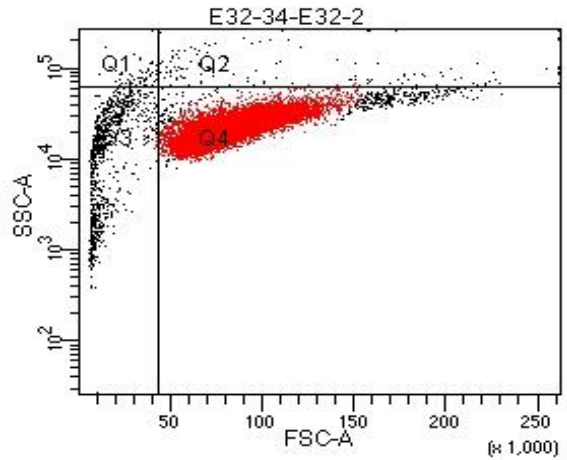
ROS measurement

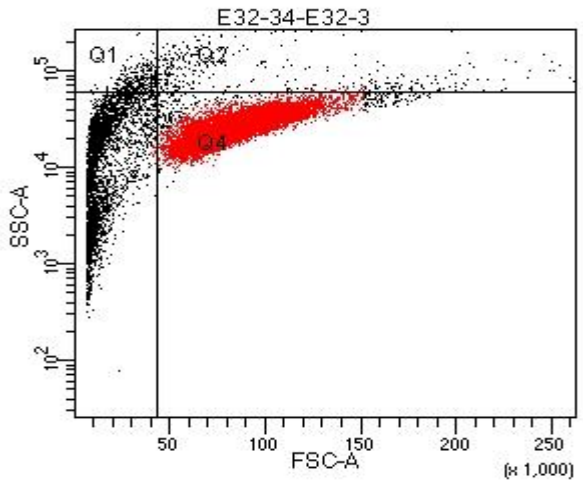


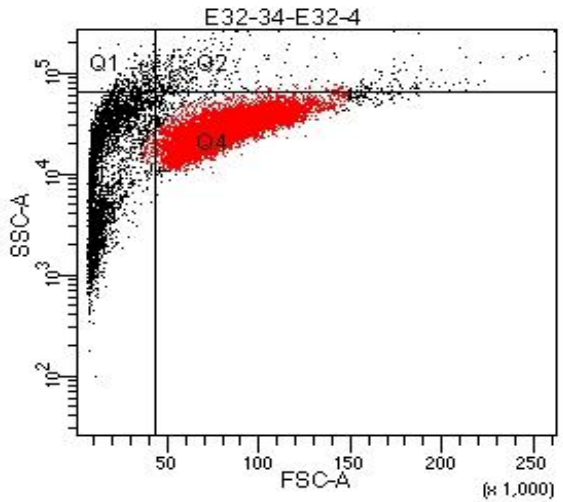


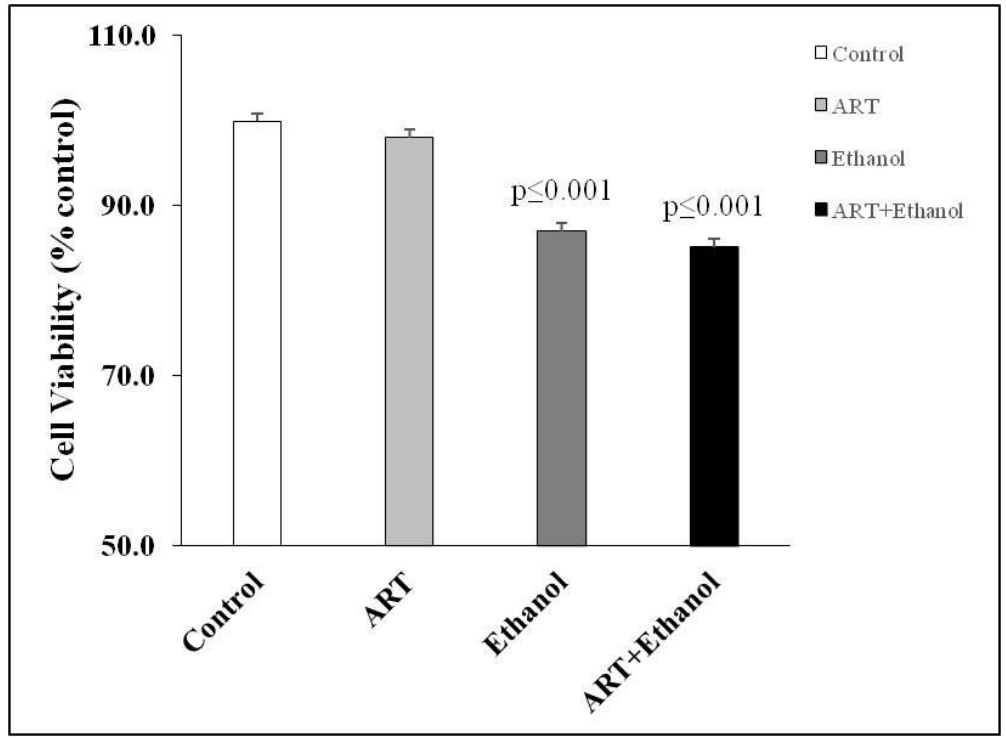
Cell viability (FACS)



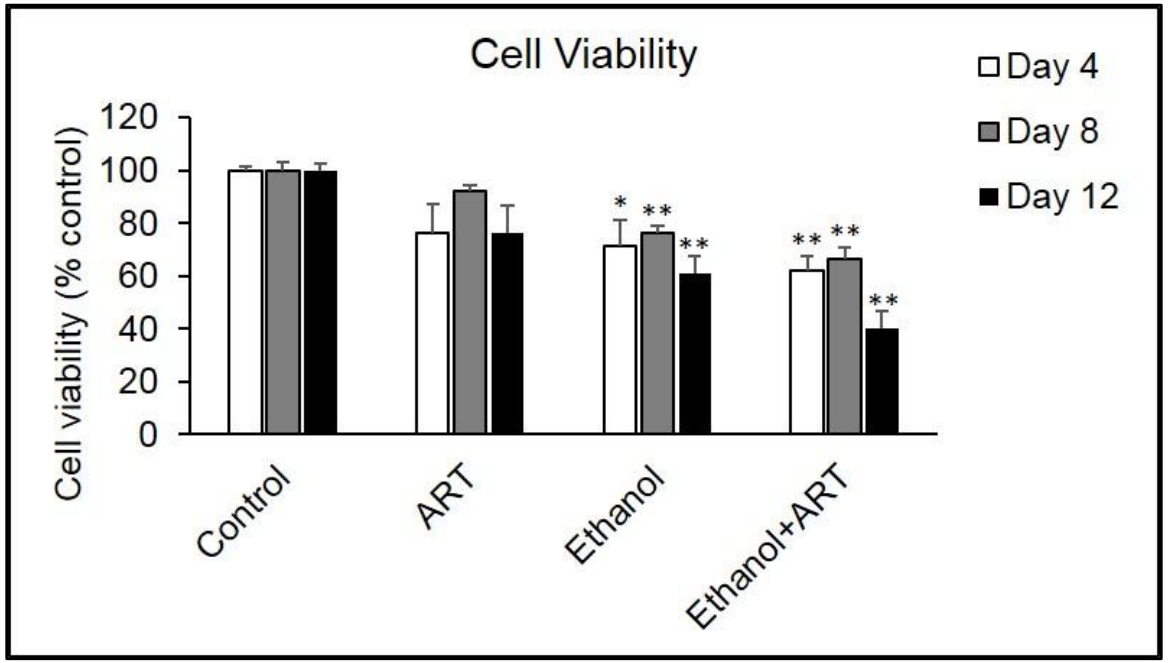




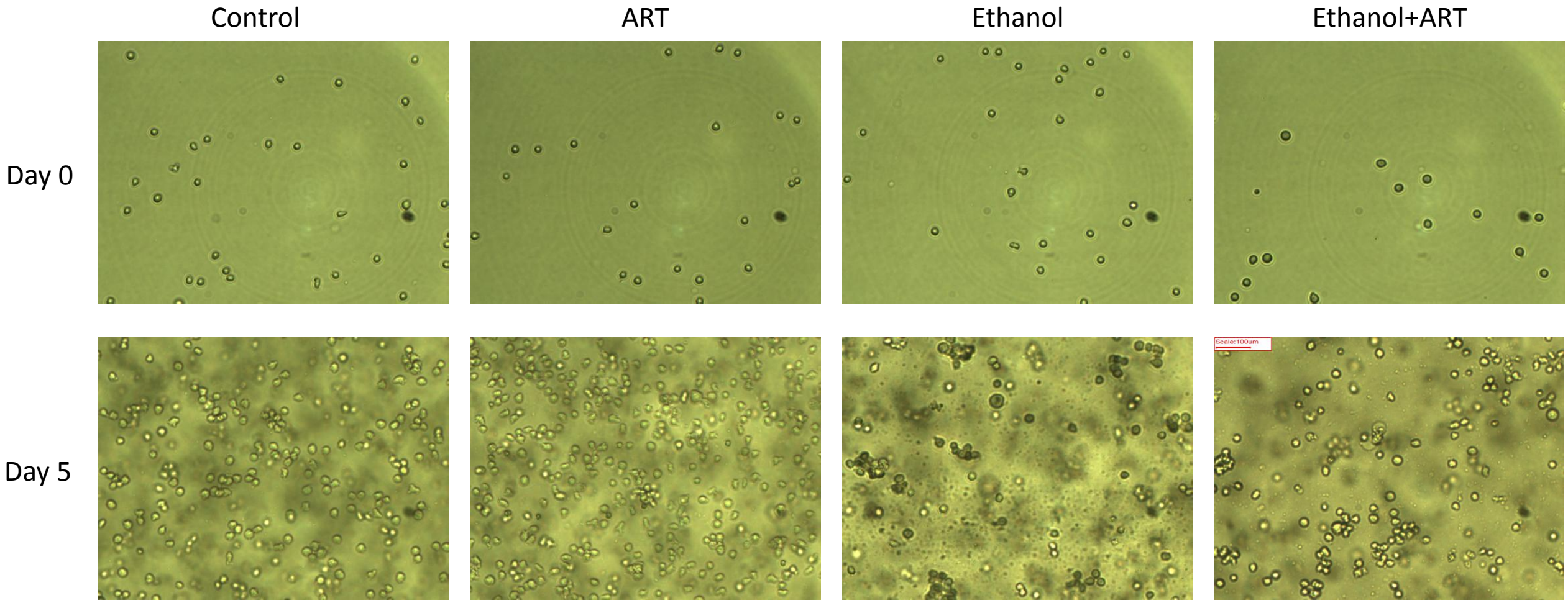




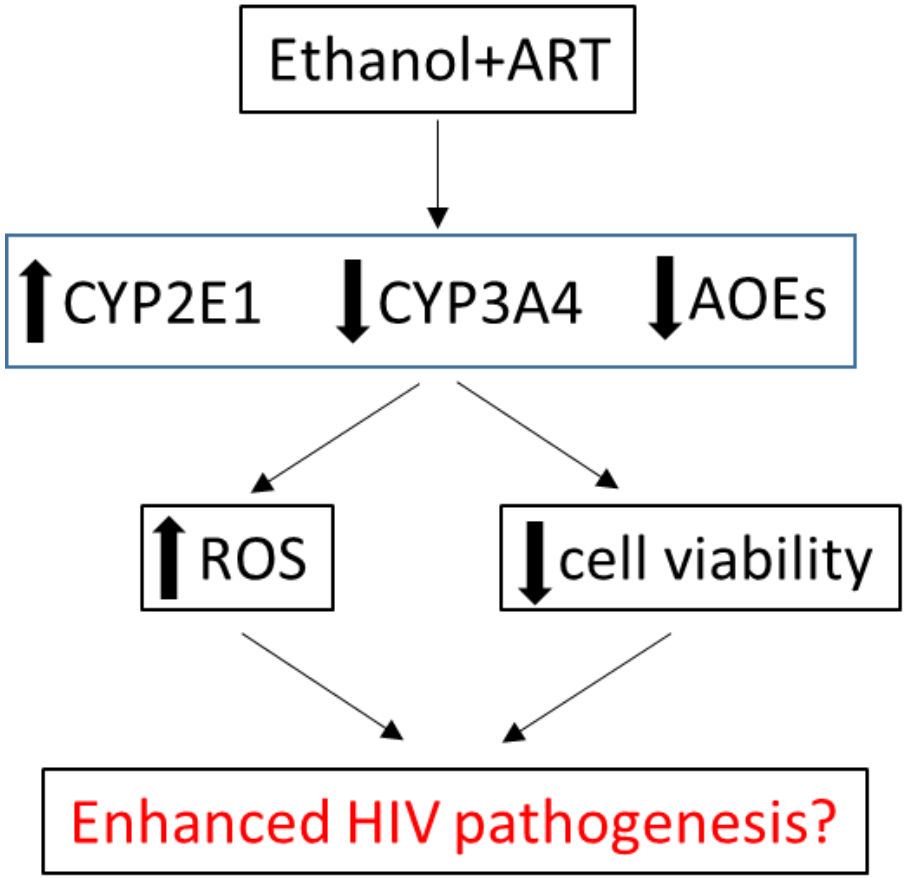
Cell viability (XTT)



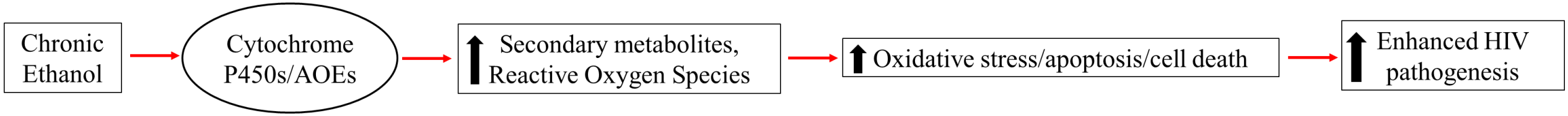
U937 microscopic image (10x)



Rationalized effects of chronic ethanol+ART on HIV pathogenesis



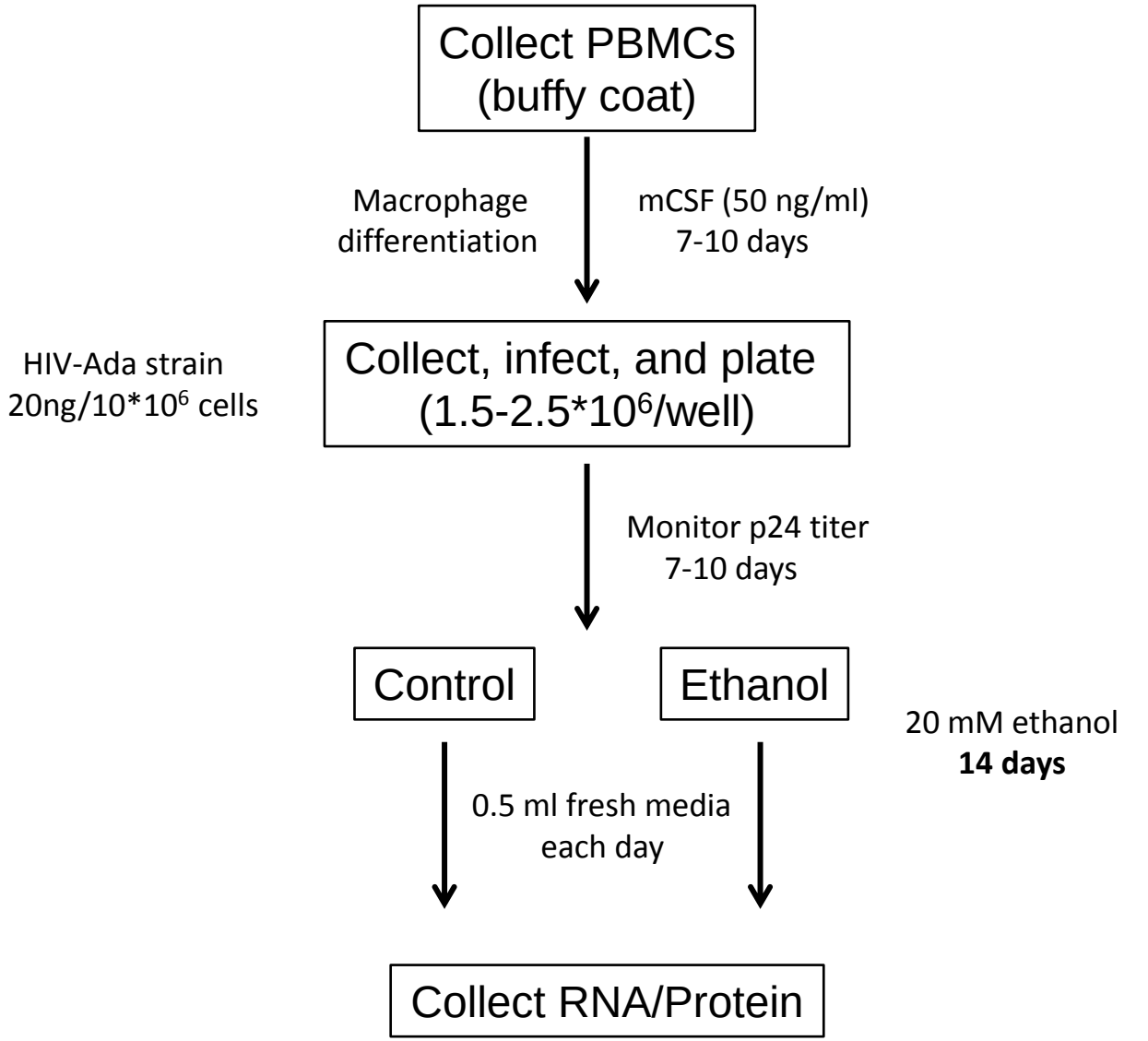
Hypothesis



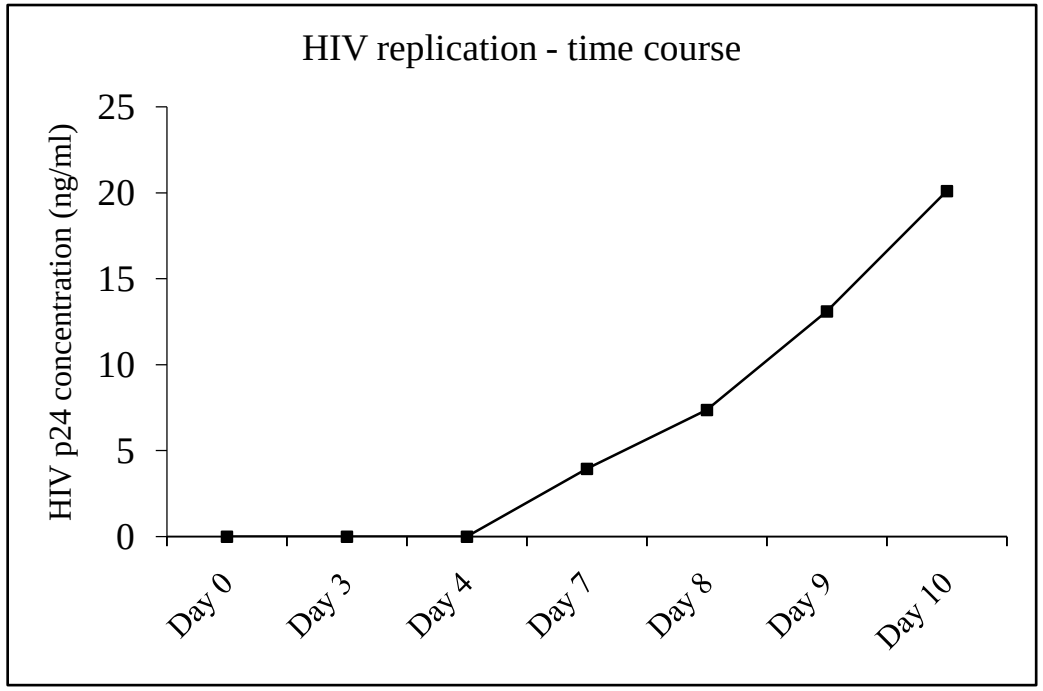
Model:

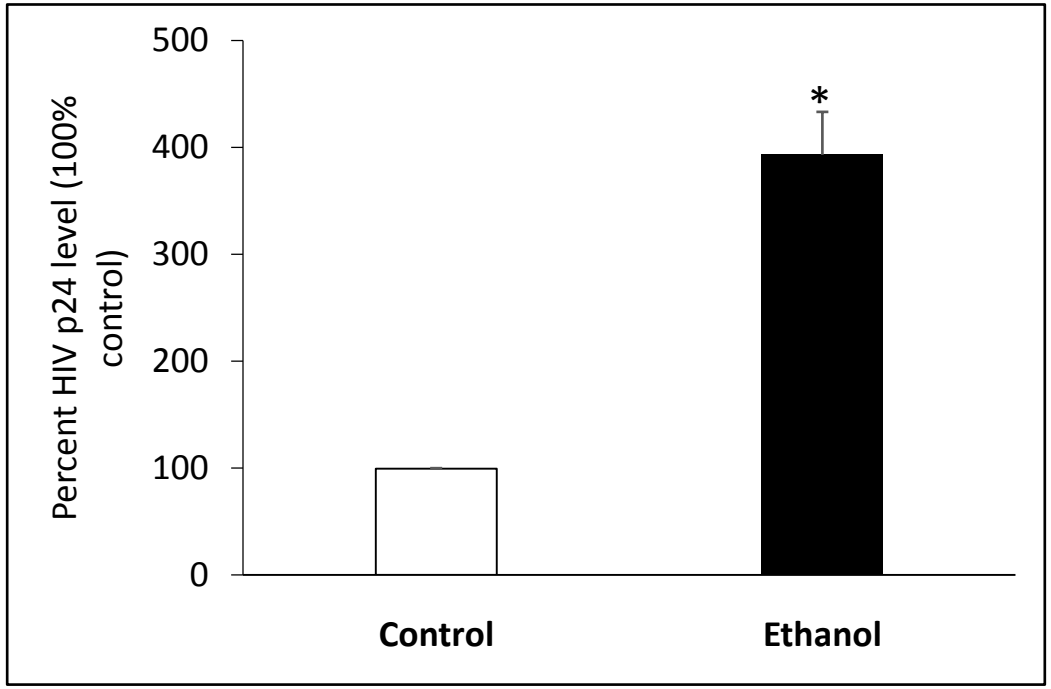
- ***In vitro*:** U937 monocytic cells
- ***In vitro*:** Primary HIV-infected macrophages
- ***In vitro*:** ART metabolism: Effects of ethanol
- ***Ex vivo*:** Human monocytes/macrophages

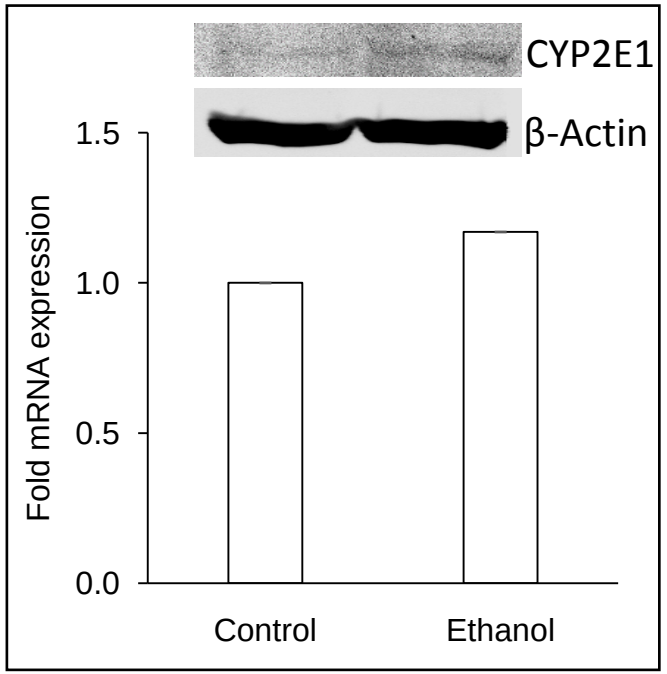
Experimental design

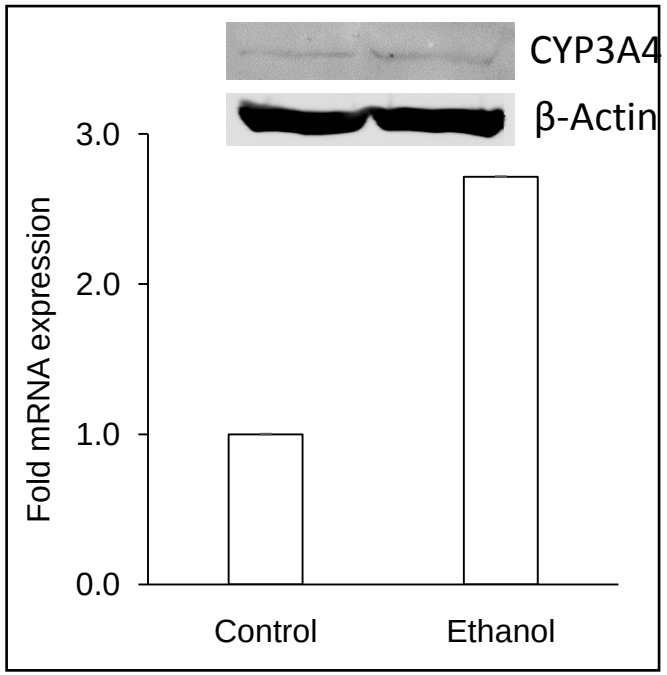


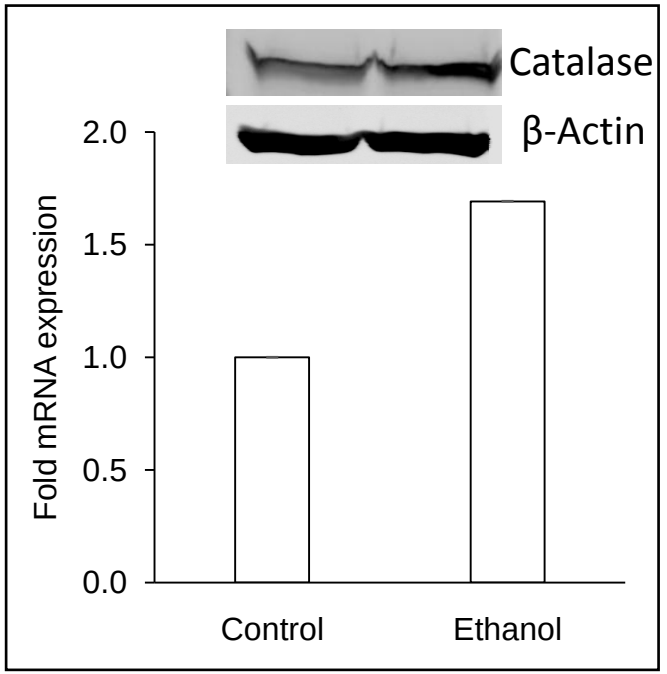
Effect of chronic ethanol on p24 production

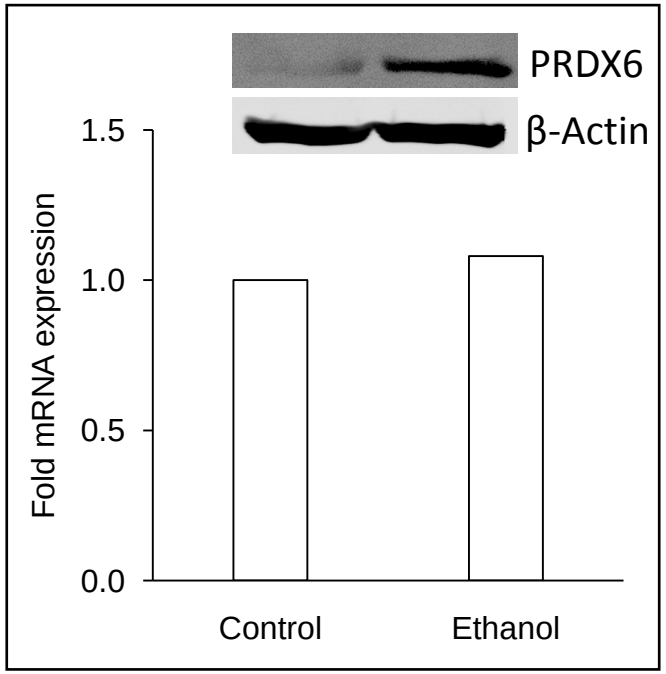


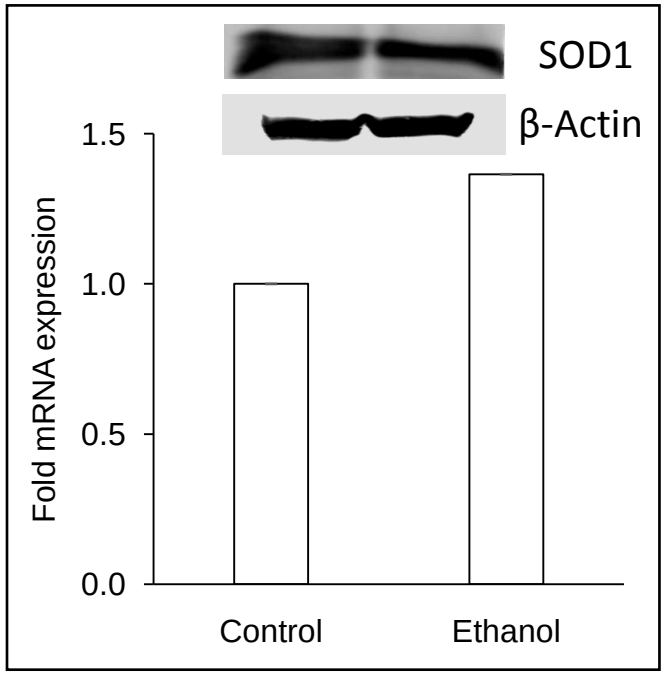


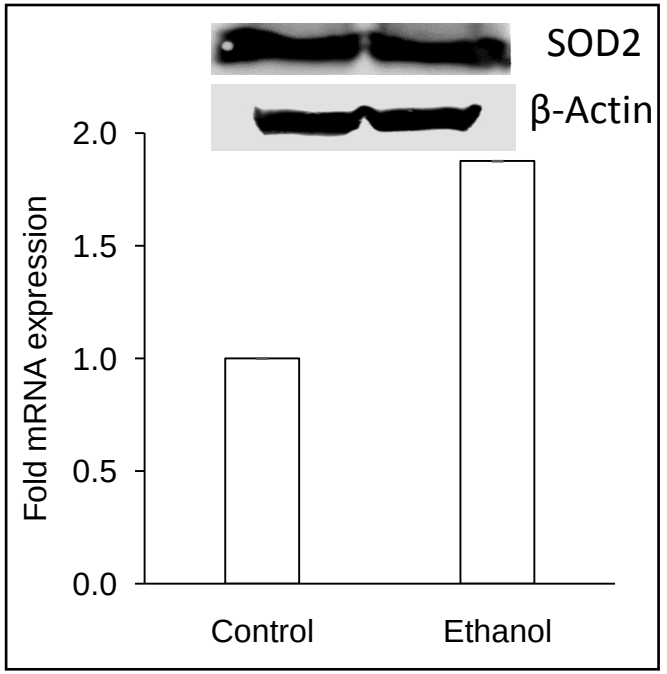

CYP2E1
β-Actin
Fold mRNA expression
1.5
1.0
0.5
0.0
Control
Ethanol


CYP3A4
β-Actin
Fold mRNA expression
3.0
2.0
1.0
0.0
Control
Ethanol

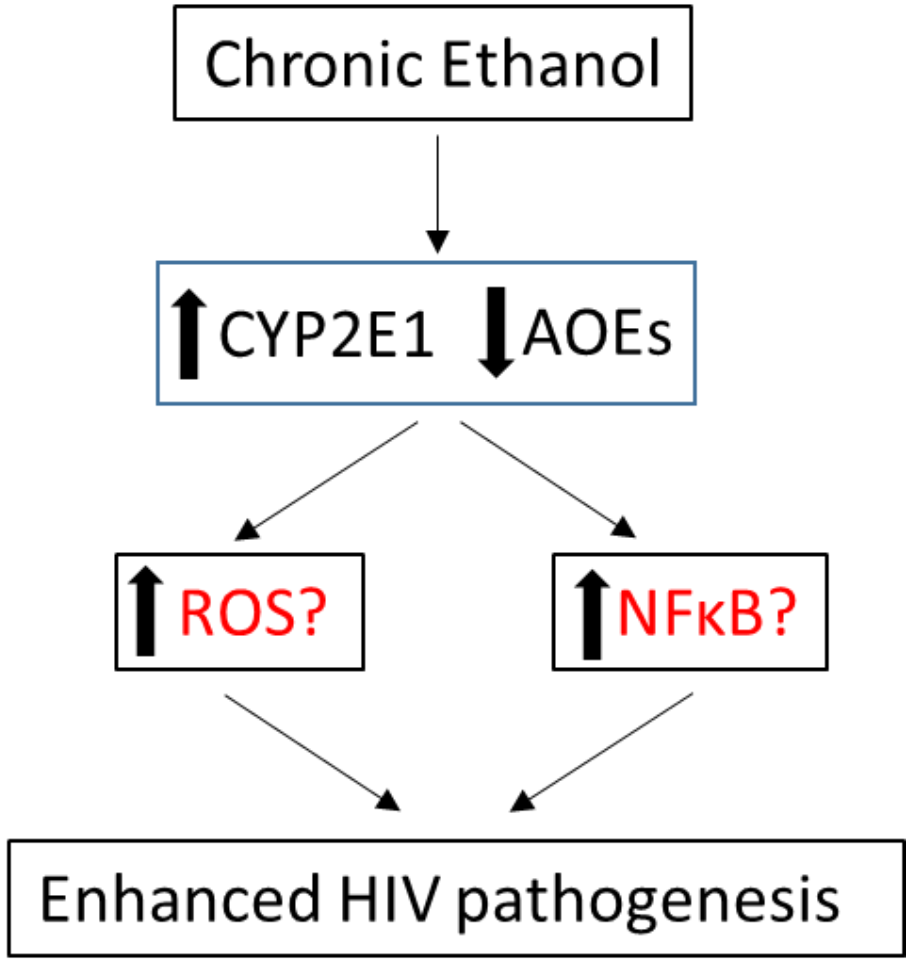

Catalase
β-Actin
Fold mRNA expression
2.0
1.5
1.0
0.5
0.0
Control
Ethanol


PRDX6
β-Actin
Fold mRNA expression
1.5
1.0
0.5
0.0
Control
Ethanol

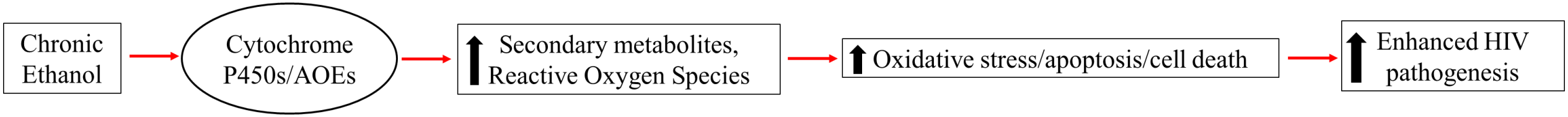

SOD1
β-Actin
Fold mRNA expression
1.5
1.0
0.5
0.0
Control
Ethanol


SOD2
β-Actin
Fold mRNA expression
2.0
1.5
1.0
0.5
0.0
Control
Ethanol

Possible cellular pathways mediating the effects of chronic alcohol



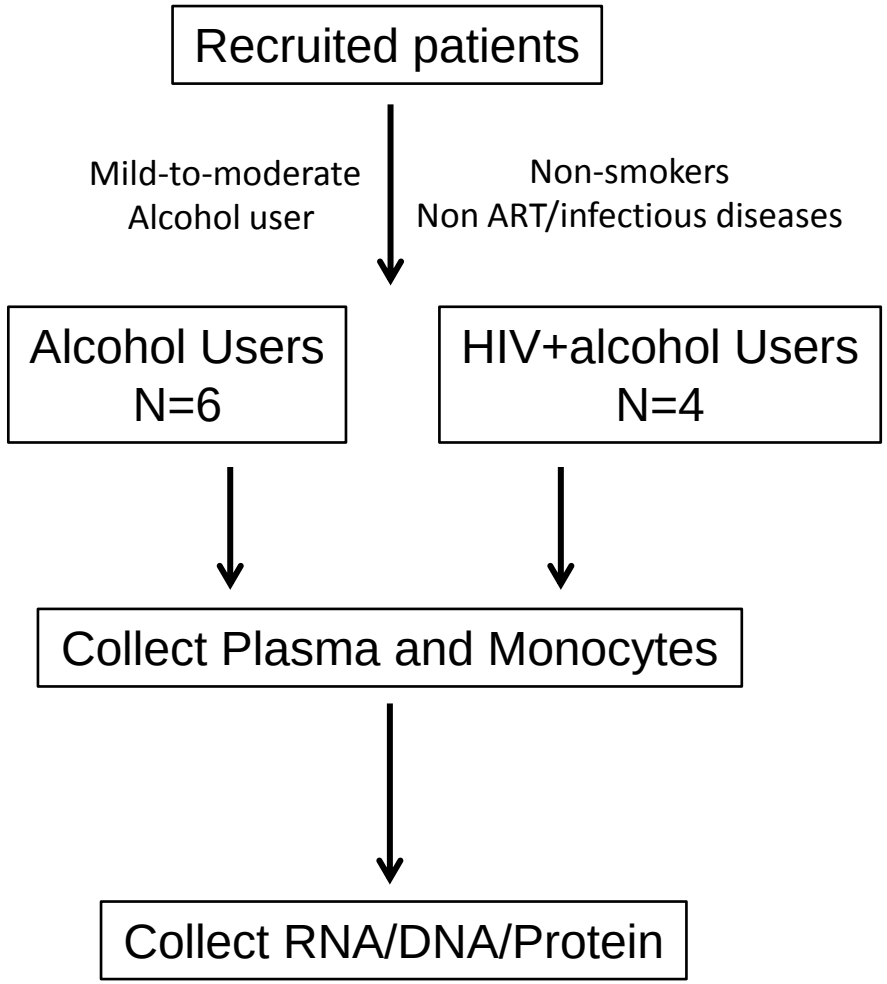
Hypothesis



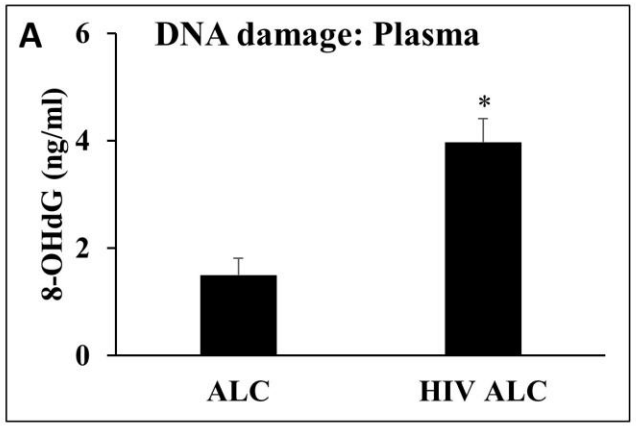
Model:

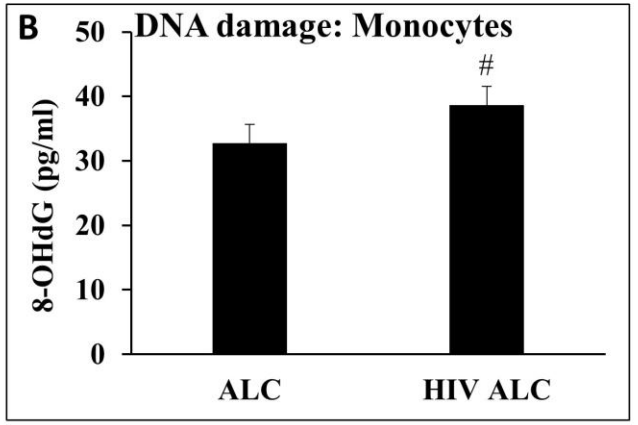
- ***In vitro*:** U937 monocytic cells
- ***In vitro*:** Primary HIV-infected macrophages
- ***In vitro*:** ART metabolism: Effects of ethanol (on-going)
- ***Ex vivo*:** Human monocytes/macrophages

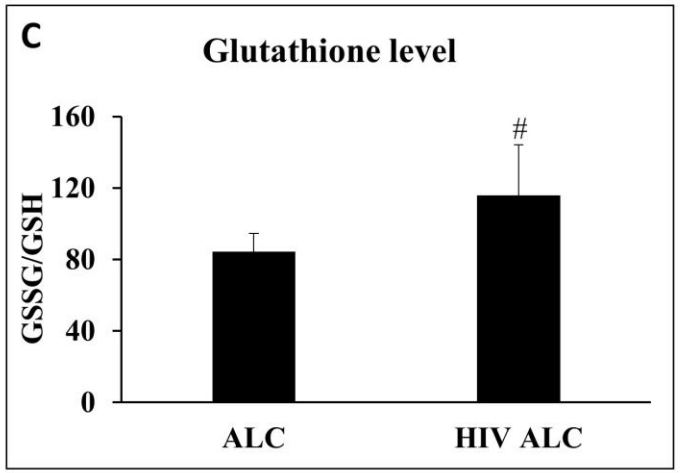
Experimental design



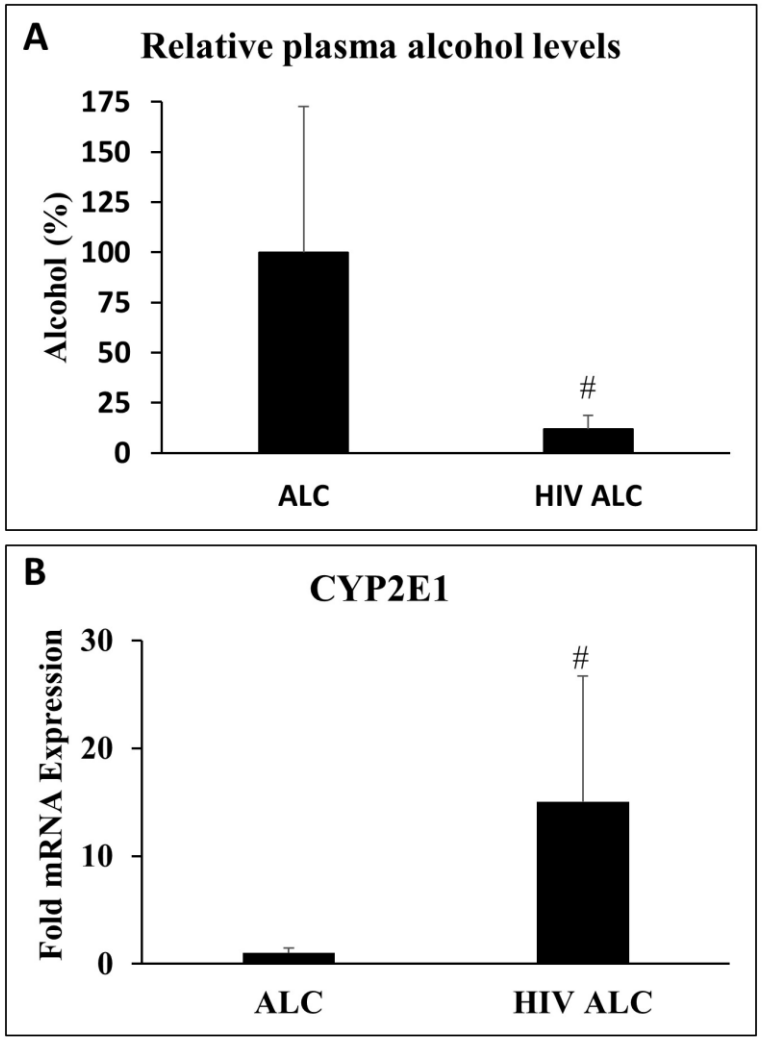
Oxidative stress parameters



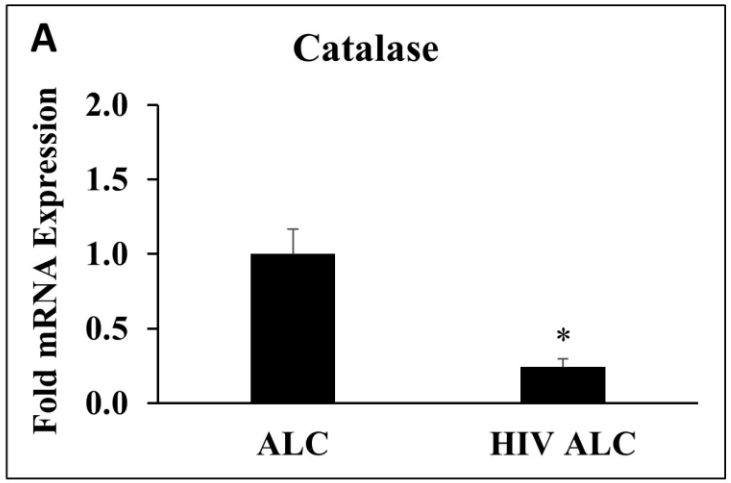


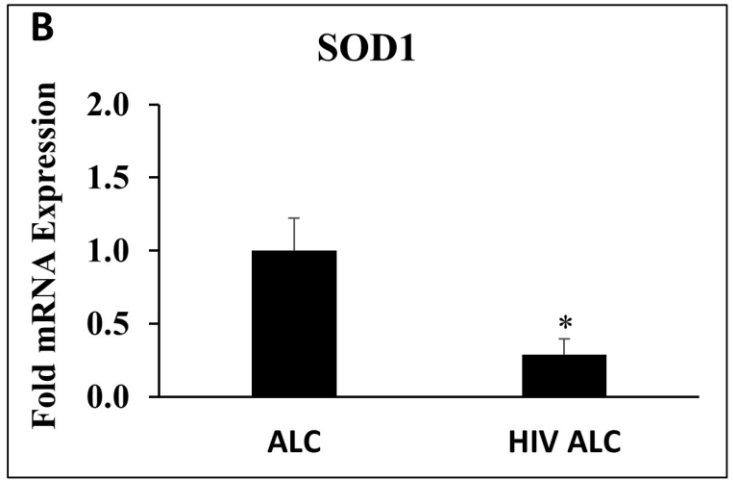


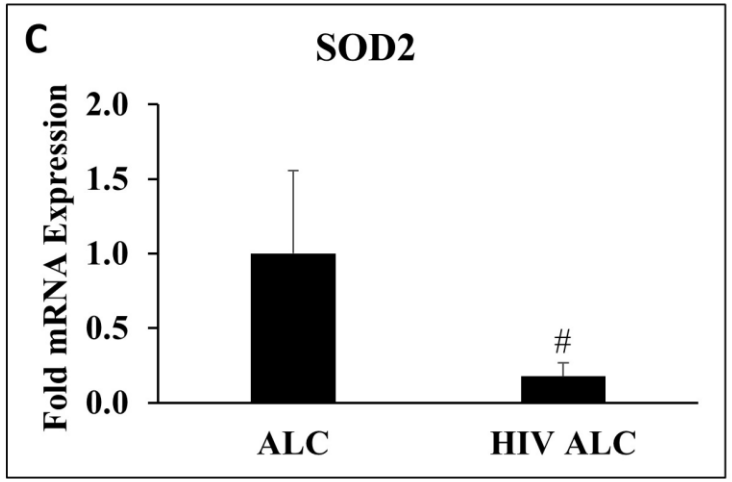
Relative alcohol metabolism

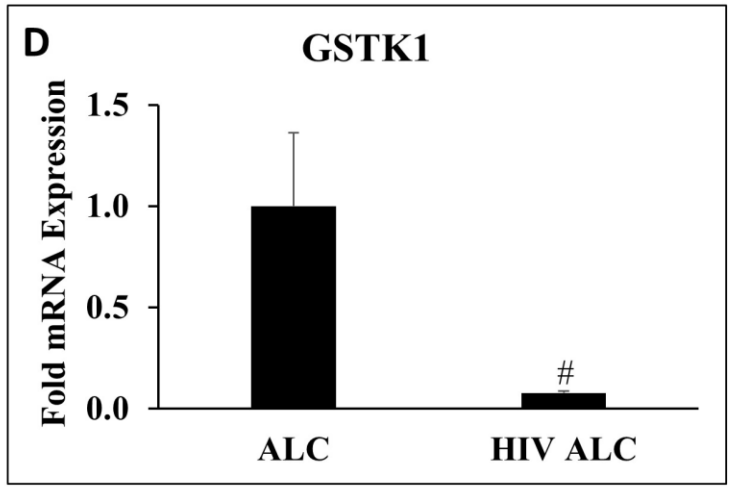


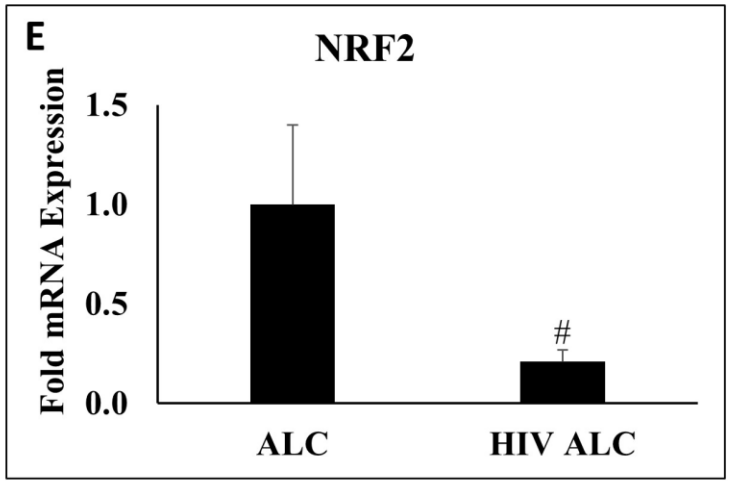
Antioxidant enzymes - expression in monocytes



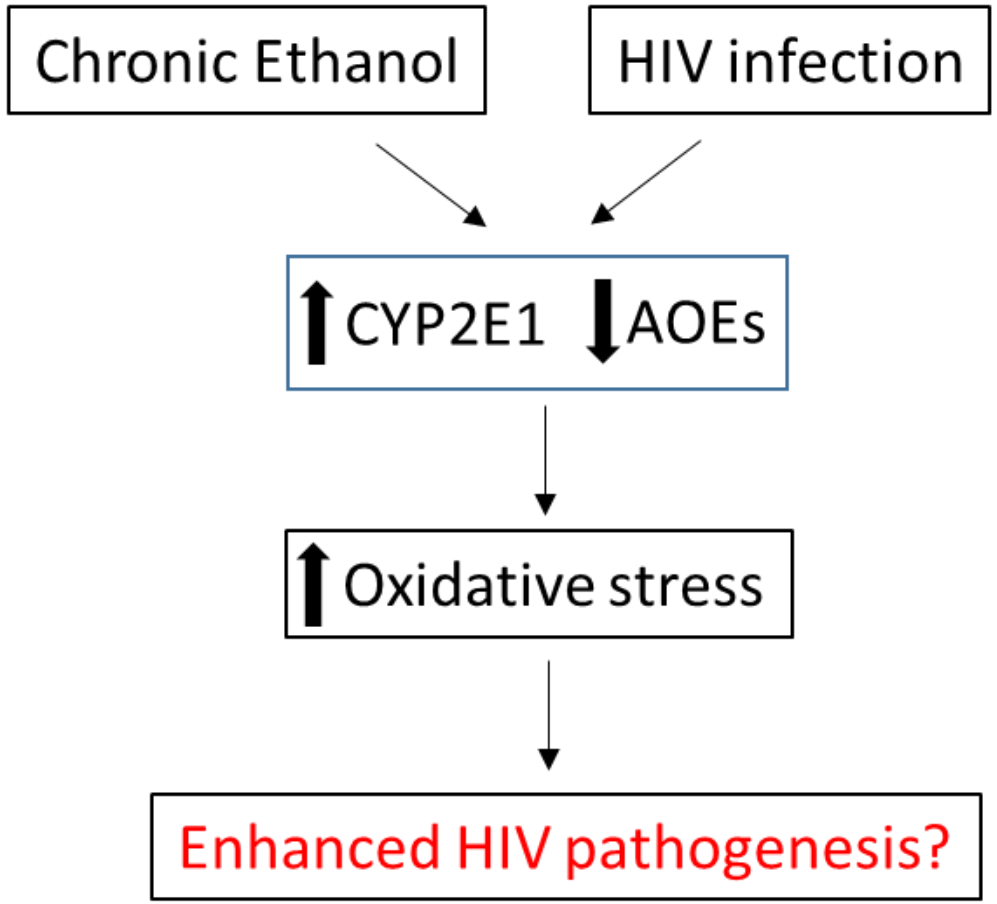








Observed interactions between alcohol intake and HIV infection

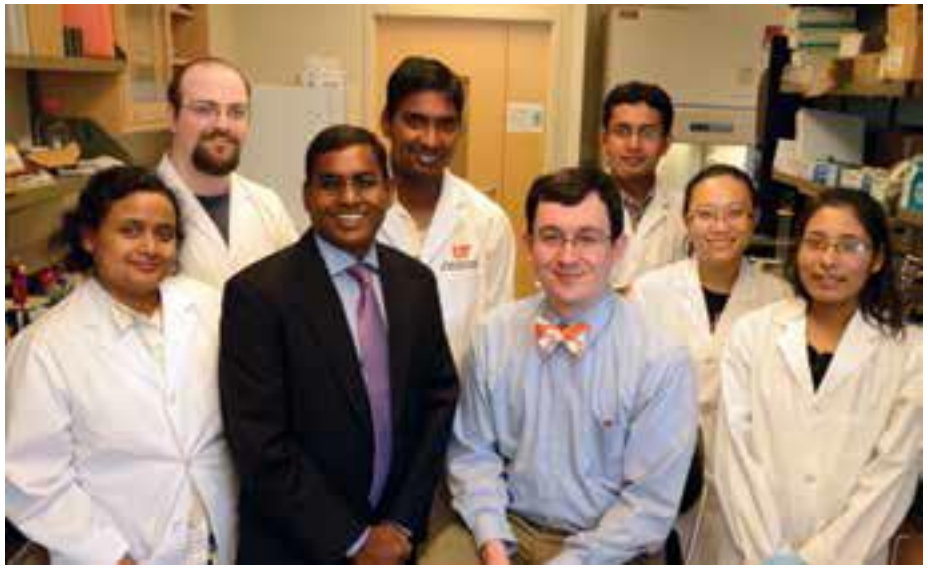


Conclusions

- In vitro studies
  - Chronic ethanol and/or ART treatment significantly alter the expression of CYPs and AOEs.
  - These changes were associated with enhanced production of reactive oxygen species and decreased cell viability.
  - HIV replication in primary MDM is enhanced following chronic ethanol treatment.
  - Preliminary data suggests associated changes in expression of CYPs and AOEs.
- Ex vivo study
  - Alcohol use amongst HIV infected patients was associated with enhanced oxidative stress compared to non-infected alcohol users.
- Overall, chronic ethanol mediated changes in CYPs and AOEs are rationalized for the enhancement in HIV replication.

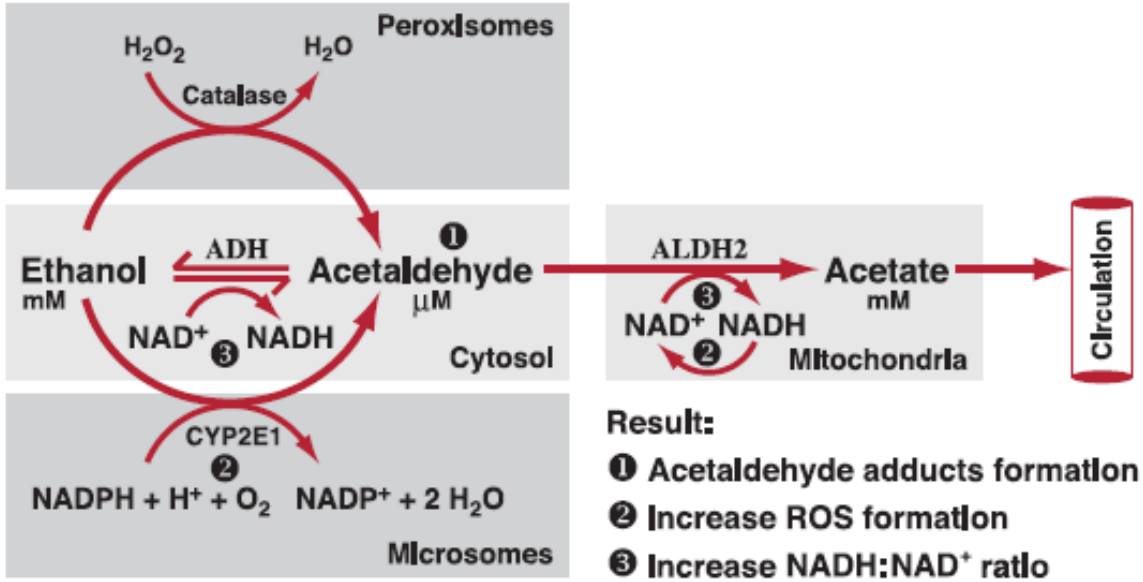
Acknowledgement

- Dr. Santosh Kumar (PI)
- Kumar research group
- College of pharmacy, UTHSC
- NIH - AA022063-01A1 and DA031616



Thank you for your attention!!!

Ethanol metabolism



- CYP3A4 and CYP1A2 (minor role)
- CYP2E1 (Induction by ethanol): Brain, Hearth, Lungs, Macrophages
- ADH/ALDH: primarily in Liver
- The Km of CYP2E1 for alcohol is 10 mM ,10-fold higher than the Km of ADH for ethanol

Samir Zakhari, Overview: How Is Alcohol Metabolized by the Body? NIAAA publication

Ethanol 50mM

Alcoholism: Clinical and Experimental Research

Vol. 30, No. 10
October 2006

Chronic Binge Ethanol Consumption Accelerates Progression of Simian Immunodeficiency Virus Disease

Gregory J. Bagby, Ping Zhang, Jeanette E. Purcell, Peter J. Didier, and Steve Nelson

Methods: “via an indwelling intragastric catheter to achieve an alcohol concentration of 50 to 60 mM for 4 consecutive days per week for the duration of the study”

Published in final edited form as:
Hepatology. 2012 August ; 56(2): 594–604. doi:10.1002/hep.25702.

HIV Protease Inhibitors Modulate Ca^{2+} Homeostasis and Potentiate Alcoholic Stress and Injury in Mice and Primary Mouse and Human Hepatocytes

Eddy Kao, **Masao Shinohara**, **Min Feng**, **Mo Yin Lau**, and **Cheng Ji***
Department of Medicine, Keck School of Medicine of USC, University of Southern California, Los Angeles, CA, USA

In vitro studies with primary human hepatocytes

- Alcohol (35-85 mM)

Naltrexone inhibits alcohol-mediated enhancement of HIV infection of T lymphocytes

Xu Wang,* Steven D. Douglas,* Jin-Song Peng,† David S. Metzger,‡ Charles P. O'Brien,‡ Ting Zhang,* and Wen-Zhe Ho*,[1]

Peripheral Blood Lymphocytes

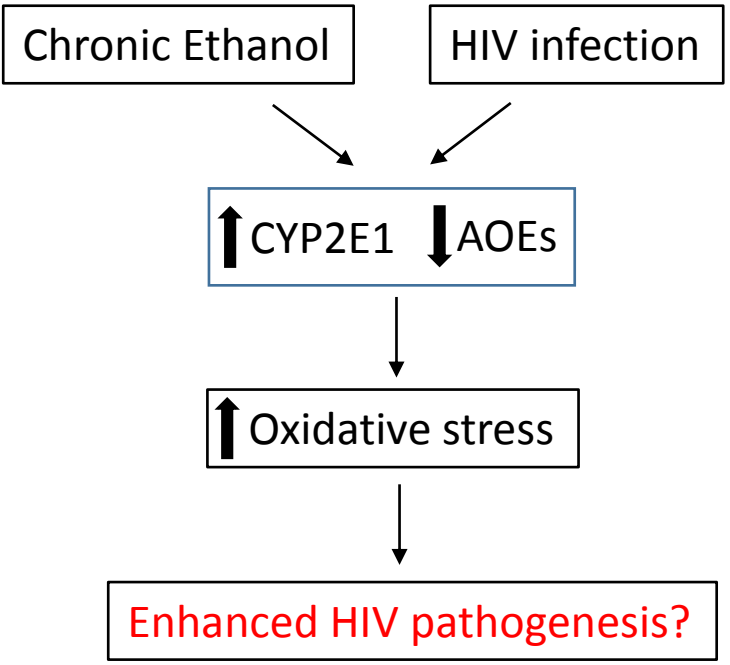
- Alcohol treatment: up to 40mM
- No effect on cell viability with 80 mM ethanol

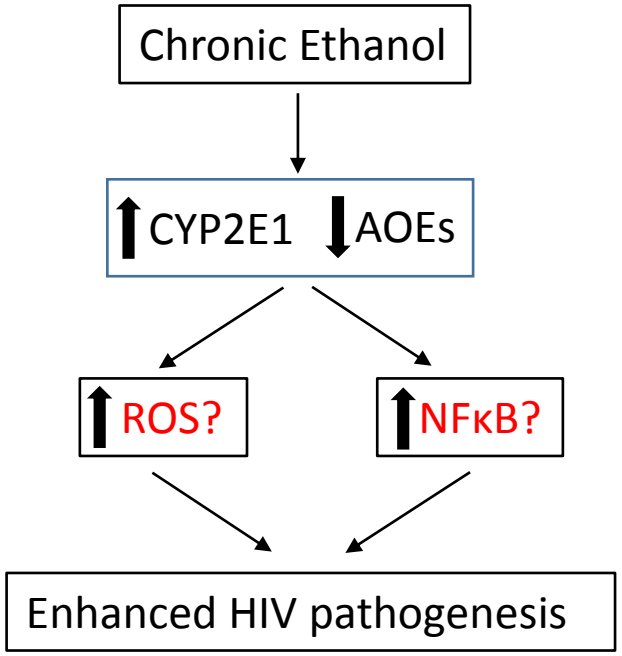
ART concentration

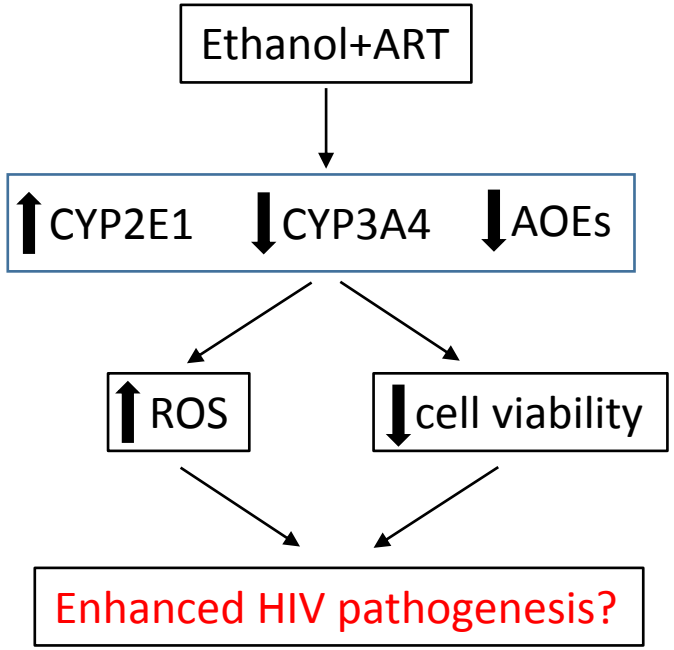
- Darunavir median plasma concentration (with ritonavir): 7.2µM
  - Yilmaz et al., AIDS Res Hum Retroviruses. 2009 Apr; 25(4): 457–461
- Combination ratio: PI+ritonavir: 4:1 (with lopinavir as Kaletra®)

ART affects ethanol metabolism

- Ethanol concentration in untreated HIV patients: 28mM (Dosed 1g/kg ethanol)
    - In patients on ART: 25 mM
    - DOI: 10.1097/QAI.0b013e318256625f, 2012.


Chronic Ethanol
HIV infection
CYP2E1
AOEs
Oxidative stress
Enhanced HIV pathogenesis?


Chronic Ethanol
CYP2E1
AOEs
ROS?
NFκB?
Enhanced HIV pathogenesis


Ethanol+ART
CYP2E1
CYP3A4
AOEs
ROS
cell viability
Enhanced HIV pathogenesis?