Hepatocellular death due to mitochondrial dysfunction is a key mechanism in alcoholic hepatitis (AH) and a leading cause of liver-related deaths. Both chronic and binge drinking can activate stress-mediated intracellular signaling pathways through induction of reactive oxygen species (ROS) and cause mitochondrial dysfunction. The ELAD System is an investigational human hepatic cell liver treatment comprised of four metabolically active caride cells containing VTL C3A with ancillary delivery device components and cell support circuitry, to treat severe AH.

Previous metabololme profiling studies of ELAD-treated clinical subject plasma ultrafiltered suggested that the cellular component of ELAD treatment could provide protective effects. In vitro studies further demonstrated a protective effect on primary human hepatocytes (PHH), at least in part by increasing the reduced glutathione ratio in these cells. We hypothesized that these anti-oxidant effects were produced through regulation of mitochondrial oxidative stress.

The aim of this study was to evaluate whether VTL C3A cell-secreted factors from the ELAD System could dampen ethanol-induced oxidative stress and improve mitochondrial function using a PHH model.

### MATERIALS & METHODS

ELAD Conditioned Media (ELAD CM) was prepared by aseptically infusing VTL C3A cell caride with Williams’ E media (WEM) containing insulin, transferrin, selenium, and glutamine, but without dexamethasone, and incubating caride at 37 °C for 4 h. ELAD CM was drained from the caride, pooled, aliquoted, and held at -80 °C, then filtered prior to use.

PHH Oxidative Stress Model: Cryopreserved PHH were plated in collagen-coated plates in WEM and treated with WEM or ELAD CM diluted 1:1 in WEM, 5 h prior to exposure to 100 mM ethanol (EtOH).

Total ROS in Intact PHH and Isolated Mitochondria was measured using 2,7'-dichlorofluorescin diacetate (DCF-DA) (Sigma).

MnSOD Activity (MnSOD) assay was performed using NADPH oxidase inhibitors and NOX1, NOX4.

Mitochondrial-specific Superoxide Anion (O2−) (i.e. a specific type of ROS) was measured using MitoSOX Red (ThermoFisher).

Adenosine-5′-triphosphate (ATP) was measured using CellTiter-Glo luminescence cell viability assay (Promega).

Mitochondria were isolated using Metrizamid (Sigma).

### RESULTS (cont.)

The next ROS species in the mitochondrial ROS pathway (Figure 4A) is H2O2. Consistent with the blunting of ETOH induction of O2− and MnSOD by ELAD CM treatment, H2O2 production was also lower in ELAD CM-treated mitochondria exposed to ETOH. ETOH treatment induced a significant increase in H2O2 in PHH-isolated mitochondria in WEM + EtOH medium (p=0.0001). Production of H2O2 in mitochondria incubated in ELAD CM + ETOH, although significantly increased relative to WEM (p=0.04), was increased less (14% over ELAD CM) than WEM + EtOH (Figure 4B).

### DISCUSSION

The mitochondrial ROS pathways are catalytic enzymes in oxidative stress and mitochondrial dysfunction in alcoholic liver disease (ALD). The current study provides evidence of a potential increase of ELAD CM on hepatocyte viability by attenuating ETOH-mediated ROS generation (specifically superoxide anion (O2−) and hydrogen peroxide (H2O2)) and protecting cellular ATP reserves. It also provides evidence for a potential mechanism of action of the ELAD System in the treatment of severe AH subclinical disease, where the multiple factors involved in the process of alcohol-induced liver injury, a crucial role is played by oxidative stress and decreased cellular antioxidant pool, including glutathione. It is becoming increasingly apparent that the hepatic mitochondrial compartment is an important target of alcohol toxicity. Studies have linked alcohol-mediated effects to mitochondrial dysfunction, apoptosis, increase in ROS production, loss of cellular ATP, and eventual mitochondrial DNA damage. Homologues of NADPH oxidase isoforms NOX1 and NOX4 are major sources of ROS, and these isoforms are widely expressed in the liver, mainly by hepatocytes, hepatic stellate cells, and endothelial cells.