termis AMERICAS ANNUAL CONFERENCE and EXHIBITION
DECEMBER 3-6, 2017 CHARLOTTE, NC
Tissue Engineering & Regenerative Medicine
The Path Forward for Regenerative Medicine:
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Poster 588

Investigations into Potential Mechanisms of Action of a Cell-based Treatment for Severe Alcoholic Hepatitis

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VITAL THERAPIES® TARGETING LIVER DISEASE

INTRODUCTION

Only liver transplant extends long-term survival for severe alcoholic hepatitis (sAH), a disease characterized by decompensated hepatic function from chronic steatosis, inflammation, oxidative stress, and cholestasis. The ELAD® System (**Fig 1**) is an investigational (phase 3) extracorporeal human allogeneic cellular liver treatment comprised of four metabolically active cartridges containing VTL C3A cells (**Fig 2**), with a bedside delivery system and cell support circuitry, being evaluated as a treatment for sAH.

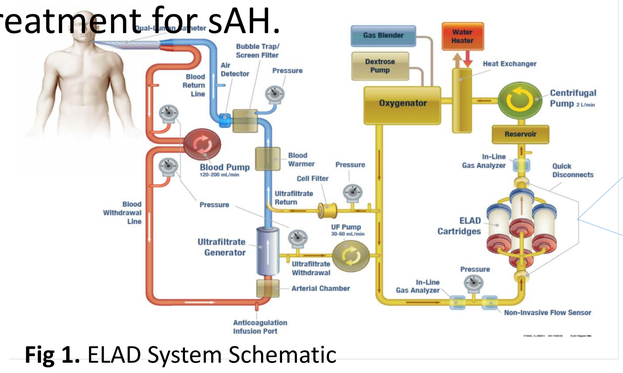

Fig 1. ELAD System Schematic

Fig 2. ELAD C3A Cell Cartridge (cross-section)

MATERIALS & METHODS

Numerous studies involving proteomics, metabolomics, gene expression, and in vitro signaling were completed, elucidating several hypothesized mechanisms of action in which C3A cell-secreted factors could provide benefit to this target population. Details can be found in the References.

RESULTS

The C3A cell secretome contains numerous proteins associated with immune modulation, regeneration, oxidative stress, angiogenesis, transport function (e.g. albumin, transferrin and lipoproteins), hemostasis, and homeostasis (**Fig 3**). C3A cells increase expression of anti-inflammatory IL-1 receptor antagonist (IL-1Ra) in response to pro-inflammatory cytokines, and IL-1Ra is significantly higher in ELAD-treated vs. control subject plasma (**Fig 4**). Amphiregulin activates the EGF receptor, soluble Fas blocks Fas-mediated apoptosis, and glutathione helps reduce reactive oxygen species (ROS), all having the potential to promote survival in hepatocytes (**Fig 5, 6**). VEGF and other unidentified factors may protect endothelial cells by blocking LPS-induced apoptosis and H_2O_2- or ethanol-induced ROS (**Fig 6, 7**). Our research shows that C3A cells produce energy-rich molecules (e.g. creatine phosphate), consume fatty acids through beta-oxidation (**Fig 8**), and detoxify through a dynamic cytochrome P450 enzyme system (**Fig 9**). Subjects on the ELAD System have reduced levels of bilirubin, decreased levels of primary bile acids and increased levels of secondary conjugated bile acids (**Fig 10**).

CONTACT INFORMATION

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RESULTS (cont.)

- 148 proteins identified to date via immunoassay; 62 proteins via LC/MS
  - 39 proteins secreted above 1 mg/day/cartridge
  - 15 proteins secreted 10x above normal serum levels

Inflammation:
Alpha-1-Antitrypsin
Complement C3
Ferritin
Gelsolin
Haptoglobin
Intercellular Adhesion Molecule 1
Interleukin-1 Receptor Antagonist
Interleukin-8
Tumor Necrosis Factor Alpha

Angiogenesis:
Angiopoietin-2
Placental Growth Factor
Vascular Endothelial Growth Factor
Vascular Endothelial Growth Factor-C

Regeneration:
Amphiregulin
Growth/differentiation factor 15
Heat-Shock protein 70
Heparin-Binding EGF-like Growth Factor
Hepatocyte Growth Factor
Platelet-Derived Growth Factor-BB
Tissue Inhibitor of Metalloproteinases 1
Tissue Inhibitor of Metalloproteinases 2
Tissue Inhibitor of Metalloproteinases 3
Transforming Growth Factor Alpha

Hematopoiesis:
Erythropoietin

Transport:
Albumin
Alpha-Fetoprotein
Apolipoprotein A-I
Apolipoprotein A-II
Apolipoprotein A-IV
Apolipoprotein B
Apolipoprotein C-I
Apolipoprotein C-II
Apolipoprotein C-III
Apolipoprotein E
Apolipoprotein H
Fatty Acid-Binding Protein, liver
Serotransferrin

Oxidative Stress:
Peroxiredoxin-4
Fatty Acid-Binding Protein, liver

**Proteins may have multiple roles depending on concentration and targeted cell type*

Fig 3. VTL C3A Cell-secreted Proteins

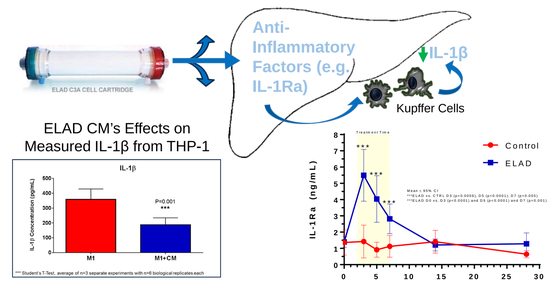

Fig 4. Current Model of VTL C3A Cell-secreted Factor Dampening of Inflammation

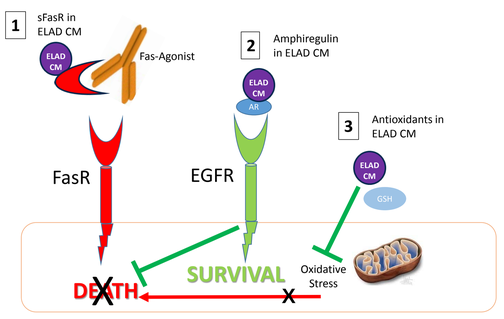

Fig 5. Current Model of VTL C3A Cell-secreted Factor Inhibition of Hepatocyte Apoptosis

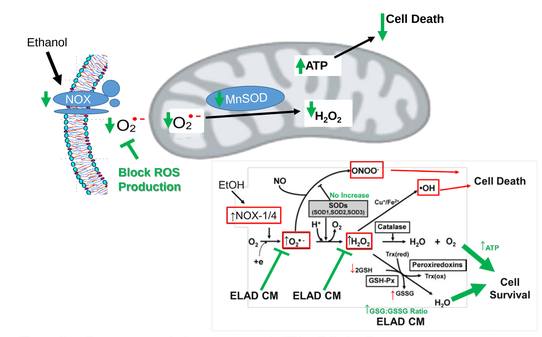

Fig 6. Current Model of VTL C3A Cell-secreted Factor Inhibition of Oxidative Stress

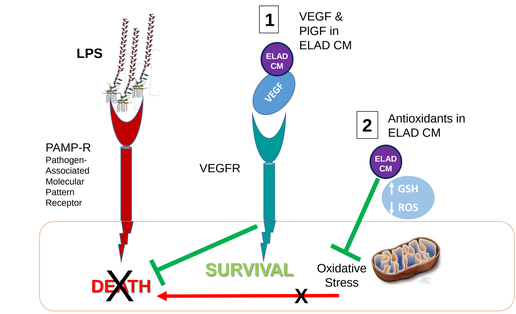

Fig 7. Current Model of VTL C3A Cell-secreted Factor Inhibition of Endothelial Cell Apoptosis

Biochemical Name	UF OUT 24h / UF IN 24h	UF OUT 48h / UF IN 48h	UF OUT 72h / UF IN 72h	UF OUT 96h / UF IN 96h	UF OUT 120h / UF IN 120h
glycerol 3-phosphate	12.27	9.92	15.64	9.97	8.1
creatine phosphate	18.01	7.73	6.58	7.95	6.56

glycerol —ATP→ glycerol-3 phosphate
creatine —ATP→ creatine phosphate

Biochemical Name	ELAD Screen / CONTROL Screen	ELAD Day 3 / CONTROL Day 3	ELAD Day 5 / CONTROL Day 5	ELAD Day 7 / CONTROL Day 7
phenylalanine	0.92	0.76	0.68	0.74
tyrosine	0.62	0.56	0.53	0.59
o-cresol sulfate	4.39	12.26	13.02	5.49
3-hydroxyisobutyrate	1.46	1.32	1.43	1.21
N1-methyladenosine	1.14	1.41	1.39	1.34

Fig 8. ELAD C3A Cell Cartridge Metabolomics

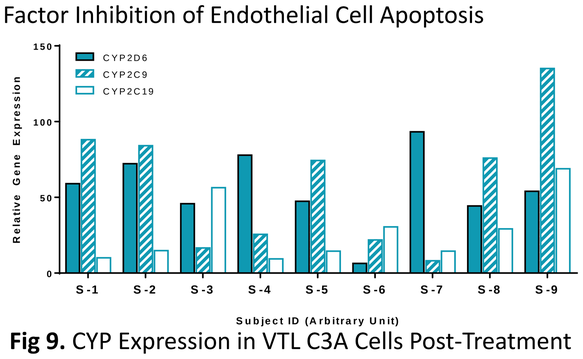

Fig 9. CYP Expression in VTL C3A Cells Post-Treatment

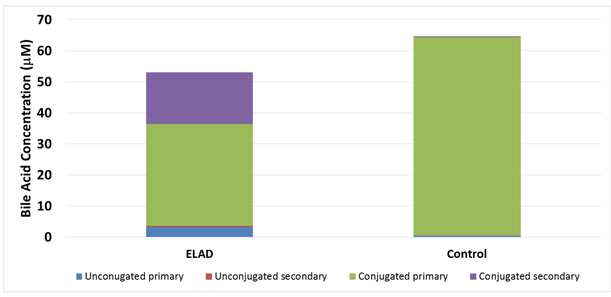

Fig 10. VTL C3A Cell Alteration in Bile Acid Profiles (Day 28)

CONCLUSIONS

Cell-based treatments offer advantages over monotherapies due to their ability to target multiple cell types through multiple mechanisms and dynamically respond to their environment.

ACKNOWLEDGMENTS

Metabolomics profiling was performed by Metabolon, Inc. Proteomics was performed by Rules Based Medicine, Rostock University/ProMed Tours, and Birmingham University.

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