#5337 SAT-457

Hepatoprotective Biomarkers, Amphiregulin and Soluble Fas, Increase During ELAD Treatment in Alcoholic Hepatitis Subjects

Jason Lapetoda[1], Lee K. Landeen[1], Ali Al-Khafaji[2], Amay Parikh[3], Andres Duarte-Rojo[4], Brian Borg[5], Geoff McCaughan[6], Lance Stein[7], Lewis Teperman[8], Mark Jacob[9], Michael Allison[10], Nikunj Shah[11], Ram Subramanian[12], Raza Malik[13], Robert Brown[14], Ross MacNicholas[15], Shahid Habib[16], Sumeet Asrani[17], Talal Adhami[18], and Patricia W. Bedard[1]

[1]Vital Therapies, Inc., San Diego, USA; [2]University of Pittsburgh Medical Center, Pittsburgh, USA; [3]Rutgers University, Newark, USA; [4]University of Arkansas for Medical Sciences, Little Rock, USA; [5]University of Mississippi Medical Center, Jackson, USA; [6]Royal Prince Alfred Hospital, Sydney, AU; [7]Piedmont Atlanta Hospital, Atlanta, USA; [8] New York University, NY, USA; [9]East Carolina University, Brody School of Medicine, Greenville, USA; [10]Cambridge University Hospital Addenbrookes, Cambridge, UK; [11]Rush University Medical Center, Chicago, USA; [12]Emory University, Atlanta, USA; [13]Beth Israel Deaconess Medical Center, Boston, USA; [14]Columbia University Medical Center, NY, USA; [15] Sir Charles Gairdner Hospital, Perth, AU; [16]University of Arizona Medical Center, Tucson, USA; [17]Baylor University Medical Center, Dallas, USA; [18]Cleveland Clinic Foundation, Cleveland, USA.

EASL THE INTERNATIONAL LIVER CONGRESS™ 2018 APRIL 11-15, PARIS, FRANCE

VITAL THERAPIES™ TARGETING LIVER DISEASE

INTRODUCTION

Hallmarks of alcoholic hepatitis (AH) are increased hepatocellular death, reduced liver function, and additional pro-inflammatory responses if dying cells are not removed. We previously reported that VTL C3A cells release hepatocyte mitogens (amphiregulin [AR], transforming growth factor alpha, and platelet-derived growth factor-BB) and produce a soluble form of Fas receptor (sFas) that can inhibit Fas receptor-mediated apoptosis in primary human hepatocyte cultures.[1,2] This study links the hepatoprotective effects of AR and sFas assessed by in vitro studies with biomarker analysis of severe AH (sAH) subjects' plasma from the VTI-208 Phase 3 trial conducted by Vital Therapies, Inc.[3,4]

For this study, plasma protein concentrations were measured in twenty-five VTI-208 subjects (n=14 ELAD-treated and n=11 untreated control subjects) at six time points: screen; study days 3, 5, 7 (i.e. during ELAD-treatment); and days 14 and 28 (follow up).

AIM

The aim of this study was to link the hepatoprotective effects of AR and sFas assessed by in vitro studies with biomarker analysis of severe AH subjects' plasma from the VTI-208 Phase 3 trial conducted by Vital Therapies, Inc.

MATERIALS & METHODS

Vital Therapies' (VTL) ELAD® System. The ELAD System is an investigational human cell-based liver treatment comprised of four metabolically active ELAD cartridges with ancillary device components and support circuitry intended to continuously treat subjects with sAH for up to 5 days.

Sample Collection. Venous whole blood was drawn into Li-heparin vacutainer tubes (BD, Franklin Lakes, NJ USA), centrifuged within 30 minutes, and the separated plasma was stored at -70°C. Samples were collected at screen (baseline), during the 5-day ELAD treatment, and at post-treatment follow up on days 14 and 28.

Immunoassays. Concentrations of AR and sFas were determined in previously frozen plasma samples using the ProteinSimple Ella custom SimplePlex assays (Bio-Techne, Minneapolis, MN USA).

Statistical Analysis. Immunoassay data and bilirubin (Bili) were longitudinally analyzed by ANOVA. Demographics and screening criteria were analyzed using Fisher's exact test. p ≤0.05 was deemed significant.

RESULTS

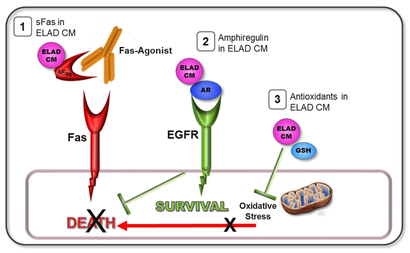


Fig 1. Mechanistic Model Developed Using in vitro Cell-based Assays. A primary human hepatocyte model of Fas-induced apoptosis suggests that factors measured in conditioned medium (CM) of ELAD's VTL C3A cells (sFas, AR, and as yet unidentified antioxidants) can reduce hepatocyte cell death.

RESULTS

Demographics of Evaluated Subjects

ELAD and Control subjects in this pre-defined subset were not significantly different at screen for demographics, diagnostic criteria for ACLF (associated with organ failure(s) and higher mortality rate[5]), concentration of AR, concentration of sFas, or MELD (Table 1).

Table 1. ELAD and Control Subjects in this Pre-defined Subset were not Significantly Different for Demographics at Screen. There were no statistically significant differences in age, ACLF criteria[5], concentrations of AR, concentration of sFas, or MELD at screen (see Methods section for specific statistical analyses used).

Group (ELAD/Control)	Subjects (No./Group)	Age (Avg±SEM)	Gender (M/F)	MELD (Avg±SEM)	No ACLF (1 Organ Failure) (No./Group)	ACLF Grade 2 (No./Group)	AR (ng/mL)	sFas (ng/mL)
ELAD	14	39 ± 1.3	4/10	25 ± 0.6	13	1	0.90	39.8
Control	11	39 ± 1.9	7/4	26 ± 0.5	10	1	0.95	37.1
P value	N/A	0.889	0.116	0.677	1.00	1.00	0.53	0.65

Selection Criteria for Subset of VTI-208 Evaluated

A subset of the subjects enrolled in the VTI-208 trial were selected based on the current criteria for the ongoing VTL-308 trial (Table 2). Subjects were younger, had lower INR, creatinine (CR), higher Bili and lower MELD than the population as a whole.

Table 2. Selection Criteria for the Subset of VTI-208 Plasma Samples Evaluated. The subset of subjects selected for analyses conformed to the subset selected for the current VTL-308 trial.

	Age (Years)	INR	CR (mg/dL)	BILI (mg/dL)	MELD
Available	≥18	≤3.5	NA	≥8	18-35
Selected Subset	**<50**	**≤2.5**	**<1.3**	**≥16**	**<30**

Increase in Hepatoprotective Factors

VTI-208 subjects' plasma (14 ELAD, 11 Control) meeting VTL-308 Phase 3 trial criteria (see Table 2, "Selected Subset") were immunoassayed for AR and sFas at screen and study days 3, 5, 7, 14, and 28.

AR levels in ELAD subjects were significantly increased vs. screen levels during study days 3, 5, and 7 (p=0.0003, D3; p=0.043, D5; p=0.027, D7). sFas levels in ELAD subjects were significantly increased vs. screen levels during study day 3 (p=0.0012). Study days 3 through 7 are during ELAD treatment, suggestive of receiving a dose of these factors from the VTL C3A cells (Figure 2).

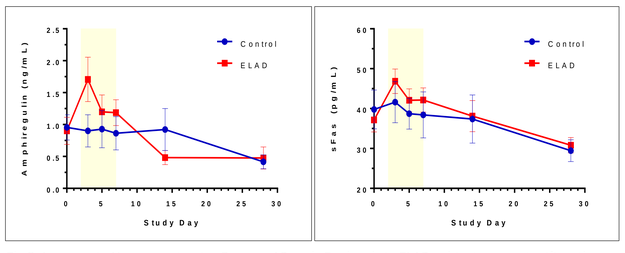
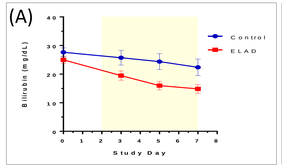


Fig 2. Increase in Hepatoprotective Factors. AR and sFas levels in ELAD subjects were significantly increased vs. screen levels during study D3, D5, and D7 (p=0.0003, D3; p=0.043, D5; p=0.027, D7), and D3 (p=0.0012), respectively (i.e. during ELAD treatment) .

Changes in Bilirubin and 90-day Survival in this Subset of Subjects

Bili levels were significantly decreased in ELAD-treated subjects at days 3, 5, and 7 compared to both screen and control subjects (p=0.033, D3; p=0.005, D5; p=0.011, D7, ELAD vs. Control; and p<0.0001 ELAD D3, 5, and 7 vs. Screen). Although the difference in survival by 91 days is not statistically different in this small sample set, there were fewer deaths in the ELAD group (Fig 3).

(A)



(B)

Group (ELAD/Control)	Deaths (No./Group)	Total (No./Group)	% Mortatlity (by 91 days)
ELAD	1	14	7%
Control	3	11	27%
P value	N/A	N/A	0.288

Fig 3. Decreased Bili and Increased 90-day Survival. (A) Bili levels decreased significantly more in ELAD-treated subjects than in Controls during the first seven days of treatment (i.e. days 3, 5, and 7) (p=0.033, D3; p=0.005, D5; p=0.011, D7, ELAD vs. Control; and p<0.0001 ELAD D3, 5, and 7 vs. Screen). (B) The difference in survival by 91 days is not statistically different with this small sample set, but there were fewer deaths in the ELAD group than in the Control group.

Model of Hepatoprotection During ELAD Treatment

AR and sFas, produced by VTL C3A cells and delivered to AH subjects during ELAD treatment, are hepatoprotective and promote restoration of liver function, as suggested by decreased Bili (Fig 4).

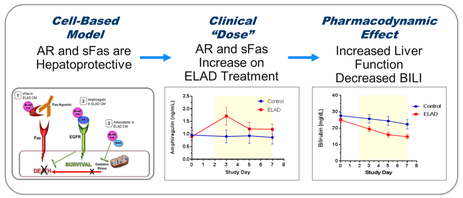


Fig 4. Hepatoprotective Factors Delivered by ELAD Promote Recovery of Liver Function. Cell-based models suggest that AR-mediated epidermal growth factor receptor (EGFR) signaling and blocked Fas activation by sFas reduce hepatocyte cell death; AR and sFas increase during ELAD treatment; and, concurrently, Bili is observed to decrease more in ELAD-treated than Control subjects.

DISCUSSION

VTL's Phase 3 randomized, open-label, multi-center, controlled trial (VTI-208) evaluating the ELAD System in subjects with sAH failed to meet primary and secondary endpoints of overall survival through at least 91 days. However, pre-specified subset analyses in subjects with screening MELD <28, and median age 46.9 years suggested efficacy trends and served as the design basis of a future study (VTL-308) focusing on subjects with MELD <30, age <50 years, CR <1.3 mg/dL, INR ≤2.5 and Bili ≥16 mg/dL.[3,4]

ELAD's VTL C3A cells secrete anti-apoptotic and regenerative factors. AR is the most necessary EGFR-ligand for liver regeneration after partial hepatectomy,[6] and sFas is widely known to block Fas-induced apoptosis. Cell-based in vitro models have shown that AR and sFas, both released by VTL C3A cells, reduce apoptosis in primary human hepatocytes.[1,2] These previous data support a working model of ELAD treatment contributing to survival and regeneration of hepatocytes by reducing apoptotic cell death associated with AH.

DISCUSSION (cont.)

To date, the majority of the data supporting this model was produced in vitro. However, this study measured changes in protein concentrations in the plasma of twenty-five subjects from the VTI-208 trial, meeting the VTL-308 trial inclusion criteria. Both AR and sFas increased significantly in subject plasma, relative to screen, during ELAD treatment. The same factors either did not increase (i.e. AR), or did not increase significantly (i.e. sFas) in Control subjects during the same time period.

These results are consistent with the hypothesis that the cell-based model is predictive of measurable clinical outcomes and that the benefit provided to the pre-specified subject subset may be due in part to hepatoprotective factors delivered during ELAD treatment.

Importantly, early changes in Bili levels (ECBL) are proposed as a surrogate endpoint predictive of survival in AH.[7,8,9] Although, ECBL predicts survival in both ELAD and Control subjects, significantly more ELAD-treated subjects in the pre-specified VTI-208 subset had a 20% ECBL and survived to 91-days. [7,8]

Oxidative stress is another hepatoptrotective mechanism noted in our cell-based models.[10,11] Identification and quantification of circulating biomarkers of oxidative stress in support of results of our in vitro work is the subject of current investigation. Additional work demonstrating anti-inflammatory mechanisms have already been made, but not yet published.

CONCLUSIONS

Subjects on ELAD experienced increases in AR and sFas during treatment, with corresponding early changes in Bili levels, which others have previously shown to be prognostic of survival.

These data suggest hepatoprotection occurs during ELAD treatment, perhaps by inhibiting apoptosis through EGFR activation, competition for Fas ligand, or reducing oxidative stress, as was previously demonstrated using in vitro primary human hepatocyte models.[1,2,10,11]

These clinical biomarker data and decreases in Bili, as a measure of liver function, suggest that the observed improvement in 91-d survival in these pre-defined ELAD subjects is likely due to a multi-therapeutic approach via cell-based treatment.

ACKNOWLEDGEMENTS

Thank you to Lisa Li, for her assistance in clinical data organization.

REFERENCES

1. Bedard PW, Lapetoda J, Michalopoulos GK, Landeen LK, et. al. Hepatology, 2015; 62(S1):1071A.
2. Lapetoda J, Landeen LK, Michalopoulos GK, Bedard PW. 17th International Symposium on Albumin Dialysis (ISAD), Sept 16 - 18, 2016. Rostock-Warnemünde, Germany.
3. Thompson J, Jones N, Al-Khafaji A, Malik S, Reich D, Munoz S, et al. Liver Transpl. 2018;24(3):380-393.
4. Thompson, J, MD, Reich, D, MD, Stange, J, MD, et al. Hepatology, 2015; 62(S6):1379A.
5. Moreau R, Jalan R, et al. Gastroentrology. 2013;144:1426–1437
6. Michalopoulos GK, Khan Z. Gastroenterology. 2005 Feb;128(2):503-6.
7. Hassanein T. et al. 26th Conference of APASL; Feb 15-19, 2017 Shanghai, China.
8. Hassanein T. et al. GTC Biomarker Summit, Mar 20-22, 2017. San Diego, CA USA.
9. Morris JM, Forrest EH. Eur J Gastroenterol Hepatol. 2005;17(7):759-62.
10. Dammanahalli JK, et al. Hepatology. 2017; 66(S1): 676A.
11. Dammanahalli JK, et al. EASL-AASLD joint meeting Sept 30 –October 1, 2017 in London, UK.

CONTACT INFORMATION

Patricia W Bedard, Vital Therapies, Inc., 15010 Ave. of Science, Suite 200, San Diego, CA 92128. pbedard@vitaltherapies.com