The Sisyphean Odyssey of the ELAD: Why We Think VTL is a Short

Chris Lau, Louis Gaye, and Martin Shkreli

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In this article we will prove that the Vital Therapies ("The Company" or "Vital") "VTI-208" clinical trial of the extracorporeal liver assist device ("ELAD" or "the device") for liver failure will fail to demonstrate a meaningful difference in 91-day mortality between ELAD and control (standard of care only). The ELAD is the Company's only asset. Given Vital has approximately $3 per share in cash and no other assets, the results of the upcoming trial are critical to Vital's share price.

The process of **proving** that the future outcome of an uncertain event will occur is a difficult one. We must begin by defining "uncertain", which is no simple task. A recent paper suggests that there are two types of uncertainty: epistemological and ontological uncertainty (Tannert, 2007). Epistemological uncertainty, as its name implies, is uncertainty that can be solved by accruing knowledge and making correct inferences. Ontological uncertainty is related to systems which are inherently unpredictable due to their systematic chaos. We view the upcoming data release of the VTI-208 trial results with epistemological uncertainty and will explain our framework for determining the trial's likely conclusion.

"When one admits that nothing is certain one must, I think, also add that some things are more nearly certain than others" (Russell, 1947). There is a common belief that it is, in fact, impossible to predict a future event with 100% certainty. Philosophers like Kant and Hume argued whether 100% certainty even exists. But we are concerned with the real and tangible world of mathematics, not the metaphysical.

Logic affords certainty if all deductive inferences are mathematically correct. In mathematics, proofs rely on axioms to establish, build, and extend truths into new findings. The idea that two parallel lines never intersect is an axiom. We take it for granted and for good reason. You can draw two parallel lines over and over again and keep extending them, but they will never cross because of the governing laws of mathematics. By relying on axioms we can infer and deduce bigger concepts, such as practical well-known theorems like the Pythagorean Theorem and other esoteric theorems such as those encountered in topology and number theory.

In medicine, we have some core axioms that are important. Some are so simple that they are laughable, but we often take them for granted and prefer fear of uncertainty. Armed with axiomatic ideas like, "All else being equal, drugs need to be lipophilic or have a certain logP to enter the brain," or "A drug that undergoes complete metabolism where its metabolites are inert cannot have its intended function despite its nature as an active drug," give us a powerful weapon against uncertainty. Thankfully, pharmaceuticals are not mathematics—although we prefer 100% certainty, we do not need it in our conclusions. Here is how we approach a reasonably large subset of decisions.

We rely on the concept of "conjunctive low probabilities" to gain comfort on many predictions. For example, the likelihood that outcome A, requiring conditions B, C and D to be

positive, is in itself positive, is distorted by the conjunctive probability. If condition B's probability of reasonable likelihood is 10%, and C and D are 50%, the likelihood outcome A will occur is 2.5%. We have found this method of analysis useful in the past in predicting complex compounded events. It appears obvious at first, but it is akin to making a three-point shot. Perhaps you have a 20% chance of doing so. If I told you that you had to make the shot while blindfolded and using only your left hand, the probability that you could accomplish such a feat would drop to such tiny levels, we would argue for practicality's sake that you have proven it will not happen (or at least that you'd bet on it).

Designing a therapy is a lot like making a hard basketball shot blindfolded. There is a good reason why most drug ideas never make it to fruition. Even when you have designed what you think is a rational drug, you are confronted with obstacles which may limit your likelihood of success. This is why when designing and discovering drugs, we focus on the most rational targets with the most extreme endpoints and favor a diversified portfolio of risk to reduce reliance on a necessarily difficult-to-predict process.

Vital Therapies is developing the ELAD for liver failure. There are a number of reasons to be skeptical of this technology, but when we short a stock, we don't want to just be skeptical. We want to—**we need to**—be right. This is why unlike many of our short-selling peers, we short one to three stocks per year in large size. We bet big when inevitably a company has advanced a therapy so absurd that it simply cannot work. This is one of those times. Our recent shorts using this approach have included: Celladon (2015), Sunesis (2014), Prana (2014), Oncothyreon (2012), Mannkind (2011), Genvec (2010), Novelos (2010), AtheroGenics (2007), and Regeneron (2003). As you can see, these opportunities don't come around every day.

Building a complete and proven case for a short sale is a time-consuming endeavor. We have been analyzing Vital's situation for months and have reached our conclusion that the ELAD will fail in the VTI-208 study and demonstrate no discernable or meaningful difference between ELAD-treated patients and control patients. The theorem is based on two vital pillars which support the claim.

> **<u>Condition A</u>: The probability that the purported benefit seen in the subgroup analysis of the preceding trial, VTI-206, that is, that the results of the acute alcoholic hepatitis subgroup represented a meaningful and reproducible survival benefit over control despite the negative data seen in the non-alcoholic subgroup and that this finding cannot be ascribed to chance alone: 20%.** Post-hoc subgroup observations are unreliable conclusions and hypothesis-generating, at best (Cui L, 2001) (Naggara, 2011) (Wittes, 2009). This is especially the case when there is no plausible explanation as to why one subgroup outperformed the other. One must be tremendously skeptical of drawing any conclusions. Instead of assigning the rationale for the subgroup performance to the obligate nature of one subgroup necessarily outperforming another, it is erroneously deemed to be a repeatable phenomenon. Even if taken for granted, the results

of the subgroup analysis in the VTI-206 trial weren't good. The overall intent-to-treat (ITT) results from the VTI-206 study proved that the device had no overall therapeutic benefit. Therefore, it is a necessary condition for VTI-208's success that the Company's subgroup analysis was appropriate and reasonable. We are certain that this was not the case.

We will also analyze the Company's predecessor's (Hepatix Inc.) study, which failed, and the Company's VTIC-301 study in China, which at first glance appeared successful but is found to be deeply flawed after critical analysis. For conservatism's sake, we will assume there is an 80% probability we are right on condition A—I believe you will feel we are 100% right and there is no "reasonable doubt" after reading our logic. In summary, the clinical performance of the ELAD has proven to be therapeutically useless and any assertions to the contrary are born from a conflicted perspective, rely completely on hope, and represent erroneous logic which ignores fact and therefore must be discarded.

Condition B: The probability that Vital's ELAD device was designed correctly and is a reasonable surrogate for a human liver and is not, as we feel, a marginalized and non-functional device: 20%. We feel at least 80% confident that ELAD is a non-functional and therapeutically inert device. We will explain our proposition by carefully dissecting the anatomy of the device and juxtaposing an understanding of the fundamental human biology and physiology of the liver required for emulating this organ. We will reveal flaws in not only the basic mechanics of the ELAD, but also the biological platform of the cell type used. The ELAD simply cannot work as currently designed, which obviates the need for studying the clinical data generated to date. Condition A only serves to underscore the validity of Condition B without being interdependent.

It is important to understand that both conditions are critical and requisite criteria for success—they are co-dependent. In other words, both conditions need to be "true" for the device to "work" and demonstrate a clinical benefit.

Condition A is simple—The Company has advanced the theory that because the alcoholic subgroup outperformed the non-alcoholic subgroup in VTI-206, the new VTI-208 study may succeed since it is enrolling only alcoholic patients. It is logical to expect that for VTI-208 to have any chance of success, the Company's theory that the alcoholic subgroup experienced a meaningful and repeatable benefit in survival must be true, or any hypothetical treatment effect would have already been seen in the VTI-206 study, but was not. In short, the Company is relying on the idea that alcoholic patients respond to the ELAD while admitting that non-alcoholic patients do not.

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Condition B is critical. We can agree that an intrinsically flawed device will not restore or support liver function in dying patients. To illustrate an extreme example, you would quickly agree that a device that only filtered one mL of blood per day would stand no chance of rescuing a human with liver failure given humans have 5L of blood that the liver processes continuously. You would also agree that an ELAD-like device with neuronal cells would be unlikely to support a dying acute liver failure patient. While these extremes are not the case for the ELAD, we believe that a functioning and rationally-designed device is a necessary condition for success in VTI-208. We will prove that the Company's ELAD is too flawed to be seriously considered as a functional device.

Because we are at least 80% confident of these co-dependent conditions, the probability that the VTI-208 study will meet its primary endpoint is less than 4%. The markets are offering a 50% probability of success, which we can infer from the value of puts and calls. This sounds good, but it is imperative we build evidence for each pillar systematically and completely. Weak syllogisms can be devastating if not investigated thoroughly. If the probability of one of these conditions is actually 100%, the whole theorem is broken and the trade is not worth the risk.

Condition A: ELAD has proven to not work in each clinical trial it has been tested in and the Company's decision to focus on the alcoholic subgroup is without merit

In this section, we will focus on the remarkable finding that despite being trialed since 1990 and having been owned by three successive companies, the ill-fated ELAD has only generated negative data. Perhaps it is only remarkable that the concept continues to find funding. The continuous stream of negative results with the ELAD was only interrupted recently by the Company's attempt to re-characterize the failed VTI-206 study as a potential success if one ignores half of the trial participants. As we will demonstrate, this is a very difficult proposition to accept in light of not only the VTI-206 study, but the other ELAD failed data. You will agree after reviewing the evidence that the ELAD unfortunately does not have a therapeutic benefit.

Condition A, Point 1: VTI-206 data is worse than commonly understood

The VTI-206 study failed. The ELAD device showed clear lack of efficacy (53% survival for ELAD vs. 50% for control) when the entire PP population (non-subgroup) was analyzed (Teperman, 2013). As seen in Figure 1, the VTI-206 trial demonstrated a lack of therapeutic benefit in the per-protocol (PP), modified intent-to-treat (mITT), and ITT populations. Amazingly, the company never discloses this, instead choosing to focus only on the subgroup analysis that demonstrated a benefit. We were forced to calculate the results ourselves and find this conduct by the Company to be highly misleading and shameful.

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PP, n=45	ELAD	Control
90 day OS	**53%**	**50%**
mITT, n=51	ELAD	Control
90 day OS	**46%**	**48%**
ITT, n=62	ELAD	Control
90 day OS	**38%**	**39%**

Figure 1. 90-day overall survival by study population

The company owes it to patients, investigators, investors, and themselves to at least mention the lack of overall efficacy before moving to a subgroup analysis. It is not a surprise that the Company's DSMB reported that the overall population did not have the possibility of meeting the primary endpoint (Vital Therapies, Inc., 2014). It is also not surprising that despite the study concluding in 2011, the study results have not yet been published. This is a large red flag as eminent journals often refuse to publish data which draws conclusions that are plainly invalid.

The failure in the overall population should give any short-seller of Vital Therapies much comfort that a repeated study of VTI-206 will fail. The near-overlap of survival data is stunning for a device that purportedly serves as an artificial liver. However, we must give the Company's hypothesis the benefit of the doubt and analyze it carefully. Vital is positing that the benefit in survival seen in the "alcoholic" subgroup will reoccur in VTI-208. What difference did they see?

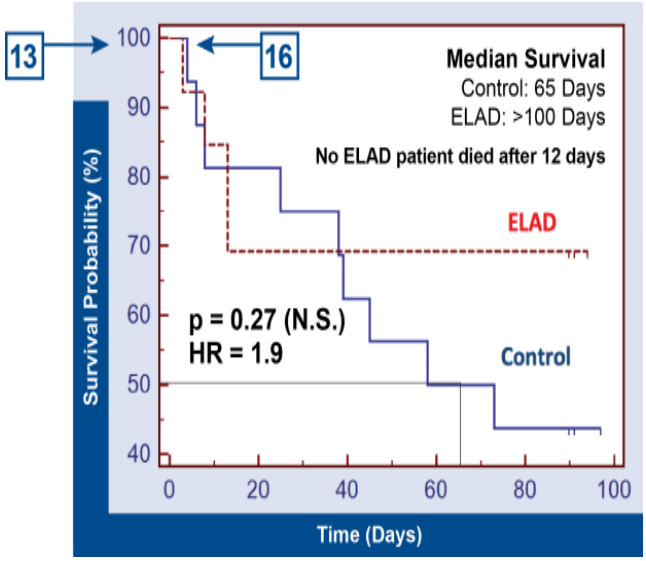

Figure 2. Subgroup analysis of VTI-206. (Teperman, 2014)

As you can see in Figure 2, the subgroup analysis (again, ignoring the non-alcoholic patients) seems to demonstrate a survival benefit as the ELAD group has a longer "tail" of survival than control starting at day 40 after initially having been similar to control. The hazard

ratio of 1.9 indicates that the patients in the control arm were twice as likely to die compared to the ELAD-treated arm, but the p-value is 0.27, which is still very far from statistical significance. It is not clear if Vital did the appropriate multiplicity (some may say duplicity) adjustment required by statistics in post-hoc subgroup analyses. Some authors even claim that a post-hoc analysis should never use a p-value because of its inherent invalidity (Cui L, 2001).

There are two other major issues with this data that aren't obvious at first sight. Firstly, the device has an overlapping efficacy during the first 40 days (Figure 2), despite the fact that the average use of the device was only for a few days (Teperman, 2013). As we will see later, the ELAD seems to be unusable after a few days due to its design flaws. The reason this finding is of interest is that a putative artificial liver should begin separation during its use, and not after. It is hard, or even absurd, to believe that using this artificial organ does not benefit the survival of its user during actual use and only has a delayed effect 40 days after its initial use. Other "artificial organs" like dialysis and the heart-lung machine certainly don't work this way. The alleged sustained benefit from an initial brief use of ELAD does not seem to be plausibly causal of the delayed benefit more than a month later. A recent author was similarly concerned that a limited duration of therapy may be insufficient in liver failure (Banares R. , 2014).

The second and more important reason the alcoholic subgroup is even worse than it looks (and this is a very high hurdle) is the removal of two patients from the alcoholic subgroup dataset (Teperman, 2014). It is reckless and absurd to disqualify two patients from an n=15 *subgroup*, resulting in an n=13 *sub-subgroup*. The Company claims that because the patients only received less than 72 hours of ELAD therapy (note that the average duration of therapy in the trial is not substantially more than this) they were not "per-protocol". This is akin to removing cancer patients from an overall survival analysis because they progressed or could not tolerate therapy—it is not allowed by modern medicine and statistics and is preposterous. It is certainly ridiculous considering the cutoff for disqualifying these two patients is 72 hours whilst the average patient received 93 hours of treatment—why is 3 days of ELAD so much worse than 4? Should one assume that patients receiving ELAD for only 3 days do not benefit but those who are lucky enough to receive it for 4 will have a response? Finally, we are told that the two patients in question did die, so their exclusion dramatically benefited this already very small sample size. By excluding these two patients, the company is touting a 69% 90-day survival for ELAD vs. a 44% 90-day survival for control. The 30-day survival appears to favor the control and it is not clear which endpoint was pre-specified. In an acute life-threatening setting like acute liver failure, the 30-day endpoint would seem more appropriate. Anyway, despite the post-hoc and subgroup nature of the analysis, a 25% absolute reduction is seen. The benefit shrinks when one (properly) includes the two extra patients, resulting in a 60% ELAD vs. 44% control survival—only a 16% absolute difference, again on a *subgroup* analysis.

All of this sounds quite bad, but it gets so much worse. In the non-alcoholic subgroup, 90-day survival in the ELAD-treated arm was 17% compared to ***60%*** in the control arm. (Figure 3).

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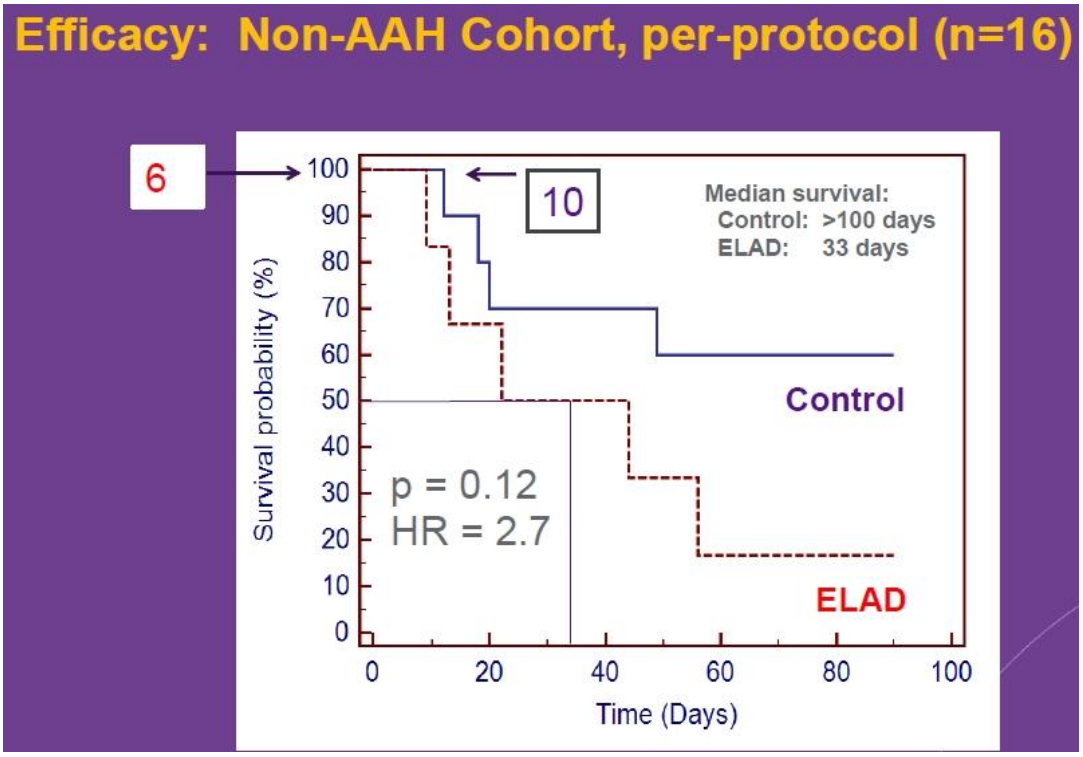

Figure 3. Non-alcoholic subgroup of VTI-206, control in blue, ELAD in red. (Teperman, 2013)

Vital never explains why the ELAD-treated non-alcoholic subgroup did so much *worse* than the ELAD-treated alcoholic subgroup. They merely brush the findings off and state that the ELAD was "not effective" in this subgroup, instead of telling the truth, which is that the data were markedly worse than in control (no device, standard of care only). It would seem to us that an artificial organ that is applied to a patient suffering from said organ's failure would at least not show harm, but this data suggests otherwise. It is very difficult to take Vital's alcoholic subgroup "benefit" theory at face value, when a similar leap of faith hypothesizing that ELAD causes harm would require the same benefit of the doubt and discredit any argument claiming that the ELAD is "better than nothing."

We calculated the Kaplan-Meier curve of the entire VTI-206 PP population (Figure 4). Amazingly, to our knowledge, the Company has never disclosed this piece of data for VTI-206. As you can see, the data show no survival benefit between ELAD and control. In fact, for a majority of the first 74 days, the control curve is actually above the ELAD curve.

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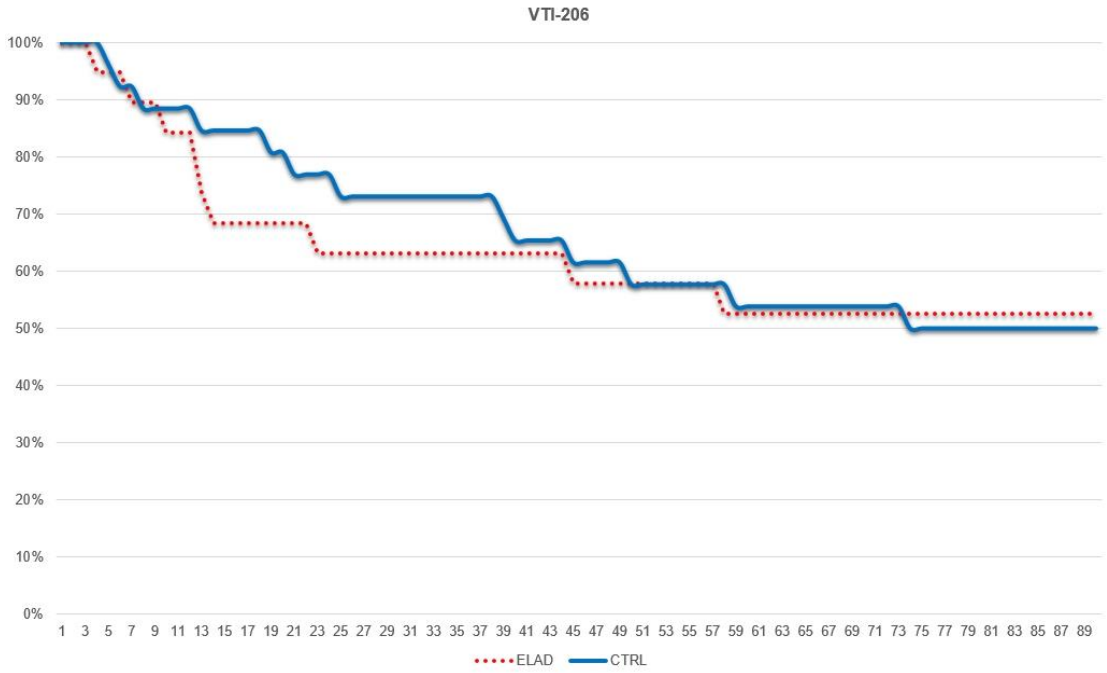

Figure 4. Kaplan-Meier Curve of VTI-206 PP population (drawn by authors, collected primary data from various sources). Control in blue, ELAD in red.

Unfortunately for Vital, the story is still not over. While we are nearing the limits of imagination for re-characterizing a failed clinical trial, Vital breaks new ground by claiming the alcoholic subgroup was successful and the "non-alcoholic" subgroup was not, despite the fact that approximately half of the "non-alcoholic" subgroup were alcoholics. Figure 5 is one for the record books. We are perplexed as to why the Company calls this cohort "non-alcoholic."

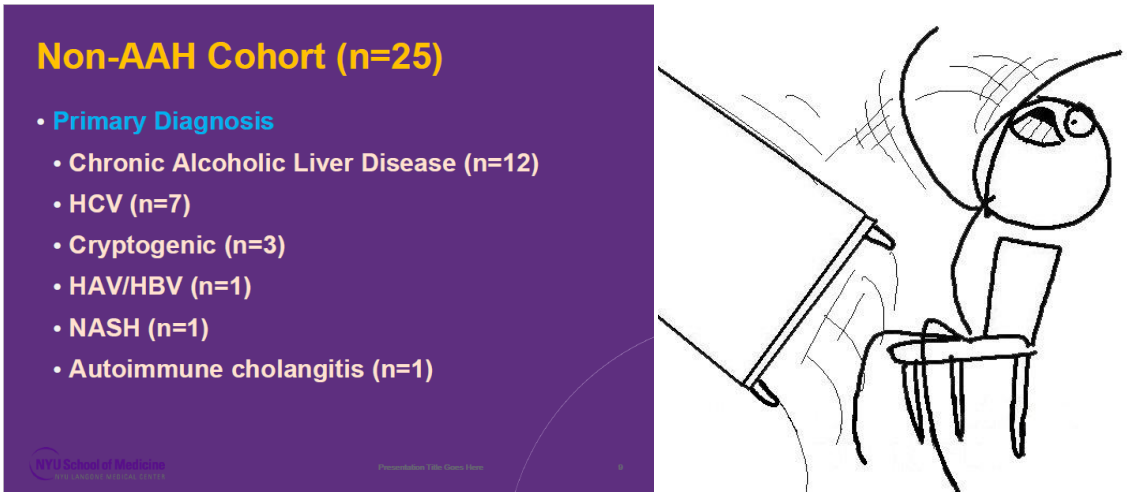

Figure 5. Non-Alcoholic Alcoholics. (Teperman, 2012)

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Condition A, Point 2: Why subgroup analyses generally do not work

The scientific community is overwhelmingly skeptical of the usefulness and predictive value of subgroup analyses (Cui L, 2001) (Naggara, 2011) (Wittes, 2009) (George, 2004) (Rothwell, 2005) (Sleight, 2000) (Wang R. , 2007). Science is skeptical of the reliability of subgroup analyses because conclusions derived from them have a strong likelihood of being false positives. Another major pitfall is the frequent lack of correction for what is known as "multiplicity" testing, which requires strict adjustments to p-values. The reader is encouraged to learn more about this somewhat abstract and esoteric concept.

Many humorous anecdotes have invalidated the practice of post-hoc observations and subgroup analyses, almost to the point that we are shocked that the Company's only therapy can be predicated on such an observation. For example, in a clinical trial called ISIS-2, investigators showed that aspirin had no effect on certain subgroups based on astrological birth sign (Collins, 1996), despite having a positive finding in the overall study population. History shows that subgroup analyses are not reliable unless the basis for its use is rational.

We often find that companies in trouble and lacking alternative assets will resign themselves to subgroup analyses as they have no other choice (giving up and declaring failure is not an option some are willing to tolerate). Examples include Pharmacyclic's Xcytrin, La Jolla's Riquent, Alexion's pexelizumab, and many other embarrassing situations. An unforgettable quote from FDA's Dr. Richard Pazdur encapsulates the flawed logic of subgroup analysis perfectly: "that's akin to shooting an arrow and having it land on a wall and then drawing a target around it" (Arnst, 2008).

The lack of a solid rationale for the selection of the subgroup analysis in VTI-206 (alcoholic vs. non-alcoholic) appears random. It feels like the Company would have chosen *any* subgroup that worked. Given the large number of baseline characteristics (MELD score, age, diagnosis, hepatic encephalopathy status) and two endpoints (30- and 90-day overall survival), it is almost certain that the company could find a successful subgroup. With the overall trial failing, it is difficult to put stock into the subgroup analysis being anything but a false positive.

Some subgroup analyses make sense. Benlysta was wisely resurrected after a failed Phase III trial (Human Genome Sciences, 2006) by careful selection criteria changes (Human Genome Sciences, 2009). It is important to know that the first Benlysta phase III trial came very close to meeting its primary endpoint in the *overall* ITT study population and tweaks to the study design resulted in a successful repeated study. Unfortunately for Vital, the selection of alcoholic vs. non-alcoholic patients appears random at best and deliberately misleading at worst.

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While there is no rationale why alcoholic patients would benefit from the ELAD and non-alcoholic patients would not, that outcome is not the one Vital has to explain. In the case of VTI-206, the alcoholic patients benefited, but unlike in most subgroup analysis situations, the non-alcoholic patients did not merely "not benefit." They were materially worse. This is the worst kind of subgroup, which is not simply teasing out which patients did best, but actively ignoring the patients who did worse. **In our view, this is not a "subgroup analysis" but a deliberate ranking of heterogeneous delta of a data set and misrepresenting the results as an important *a priori* finding**.

Using a random number generator, we simulated the VTI-206 study with ten subgroups, each of which represented patients with different baseline characteristics. Although a survival benefit was not seen in the overall ITT population and failed to reach statistical significance (p=0.44), our own post-hoc subgroup analysis found that 1 out of the 10 subgroups showed remarkable efficacy (p=0.02). Recall that not even Vital's subgroup analysis reached statistical significance.

There are a number of papers we cited earlier which demonstrate that as the number of subgroups analyzed increases, the probability that a subgroup reaches statistical significance *without* correction for multiplicity becomes almost certain. It is just as nonsensical to ascribe meaning to our random number generator as it is to assume the alcoholic subgroup experienced a positive treatment effect.

Condition A, Point 3: The biology of Alcoholic vs. Non-Alcoholic patients is inconsequential

It is not plausible that the observed subgroup difference in VTI-206 is due to a real treatment effect, not only because there is no actual observed treatment effect, but also because the subgroup is not different enough biologically to logically conclude a disparate treatment effect is possible.

Although, we cannot expect a functional and effective bio-artificial liver (BAL) to be a perfect substitute for a human liver, it should provide a survival benefit in *all* subgroups of acute liver failure. Consider how successful and supportive kidney dialysis machines have been in patients with different types of kidney failure.

The clinical picture in acute liver failure is the same regardless of etiology (Lee, 2012). The pathological mechanisms of alcoholic liver disease and non-alcoholic liver disease are similar and both result in cytokine and oxidative stress-mediated injury (Day, 2002) (Stewart, 2001) (Day, 2006). If the ELAD were to effectively treat acute liver failure, it would provide a survival benefit to both alcoholic and non-alcoholic patients. However, in VTI-206, the ELAD demonstrated a reverse performance in the non-AAH subgroup compared to the AAH subgroup (Figures 2 and 3). When combined, these results led to slightly worse performance for the ELAD-treated patients versus control. Vital Therapies has not given any explanation as to why

the alcoholic subgroup would perform better than the non-alcoholic subgroup on ELAD. This is not what the rare successful subgroup analysis looks like.

AAH patients are slightly more complex than non-AAH, as alcoholics are more likely to develop cirrhosis (O'Shea, 2010) (Louvet, 2015). Symptomatic patients with either mild or advanced presentations of alcoholic liver disease have a 40-50% likelihood of developing, or being diagnosed as already having, cirrhosis (O'Shea, 2010) (Louvet, 2015). Ignoring the other reasons why the subgroup analysis is invalid, it would make more sense that ELAD would have a treatment effect in non-AAH as there are fewer variables confounding outcomes.

Condition A, Point 4: Hepatix's, VitaGen's and VTI-201 trials all failed

Hepatix, the predecessor company to VitaGen, which is the predecessor company to Vital Therapies, ran a clinical trial with a similar device to the ELAD in 24 patients with acute liver failure, all of whom can be classified as having non-alcoholic acute liver failure (Ellis, 1996). ELAD-treated patients had a survival rate of 67% (8/12) while control patients had a survival of 58% (7/12) (Figure 6). This study, like VTI-206, failed to show a significant difference in survival versus control. It also failed to show a negative treatment effect in non-alcoholic acute liver failure patients, which questions the validity and consistency of the ELAD's performance in the VTI-206 trial.

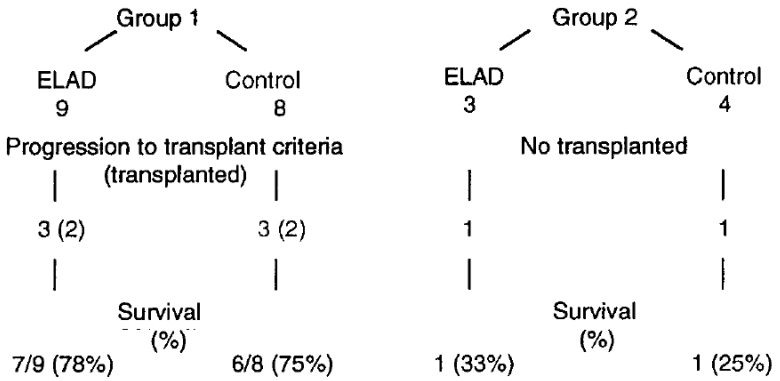

FIG. 1. Survival figures for ELAD-treated and control patients in Group 1 and Group 2.

Figure 6. Survival figures for ELAD-treated and control patients in Group 1 and Group 2. (Ellis, 1996)

VTI-201 was a clinical trial of only n=18 patients with n=14 enrolled in the ELAD group and only n=4 enrolled in control, making any comprehensive interpretation of results impossible (Hillebrand, 2010). The ELAD group had survival of 46% at 30 days, *less* than control which demonstrated 50% survival. The difference swings in ELAD's favor at 90 days, with 39% survival versus only 25% survival for control. Keep in mind there were 4 patients in the control group.

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VitaGen, the successor to Hepatix, conducted two trials from 1999 to 2003, PS-0698 and CR-202. In PS-0698, 24 patients with either late stage fulminant hepatic failure or primary graft non-function were randomized into two groups (7 of whom were controls), 19 listed for liver transplantation and 5 not listed. There was a positive trend for survival at day 30 in the ELAD-treated patients versus those on standard of care only, 83% vs. 43% (p=0.12) (Hakim, 2009). PS-0698's results informed the design of a subsequent phase 2 trial, CR-202.

We did not find any data on CR-202 except for what was provided in Vital Therapies' 2014 10-K. 19 patients with fulminant hepatic failure were enrolled with 13 randomized to ELAD and 6 randomized to standard of care (Vital Therapies, Inc., 2014). Although, there is no survival data, Vital concedes that "no intergroup differences in mortality were observed." They also concede that both PS-0698 and CR-202 failed to demonstrate a statistically significant improvement in outcomes and were not powered or designed to do so, but thanks to the convenience and wisdom of post-hoc meta-analysis, which combined PS-0698 and CR-202 into one trial since the endpoints and inclusion criteria were similar, 30-day survival of 75% in ELAD vs. 50% in standard of care was demonstrated (p=0.12).

Again, we can see that, for 15+ years, the ELAD has been unable to break free from statistically insignificant "positive trends" and "post-hoc meta-analyses," an unfortunate pattern that we believe will be repeated in VTI-208.

Condition A, Point 5: VTIC-301 was not successful despite its appearance

Clinical trials conducted in China have come under some scrutiny regarding their data integrity. Nonetheless, we will assume Vital's China-based study was not influenced improperly in any way. The VTIC-301 study enrolled 54 patients, with 35 in ELAD and 19 in control (Duan, 2007). The results appear remarkable with an 86% survival in ELAD and a 47% survival in control. However, the data have fatal flaws which limit their credibility. The protocol of the trial was changed during the trial, which was open-label (Vital Therapies, Inc., 2014). The second cohort of patients enrolled after the change in protocol did dramatically better (in favor of ELAD) than the first. In fact, the first cohort of patients did not meet statistical significance for survival at 14 and 84 days (p=0.074 and p=0.058).

The most interesting finding of this "successful" Chinese clinical trial is VTI-206's failure to reproduce similarly remarkable results. The patients in VTIC-301 were diagnosed with acute-on-chronic liver failure due to various causes (Duan, 2010). What is striking about the VTIC-301 patient population is that most had an etiology of viral hepatitis (Duan, 2007), or, as Vital would call, "non-alcoholic acute-on-chronic hepatitis," a whole subgroup that was essentially discarded in the VTI-206 trial. It is difficult to give credence to this "remarkable" treatment effect of 86% versus 47%, when the VTI-206 study showed a 17% survival in ELAD-treated versus 60% in control in the non-AAH subgroup. In fact, averaging the two studies would

result in a slight advantage for control. It is difficult to explain the diametrically opposite outcomes in this non-AAH population.

Condition A, Point 6: The ELAD's success is not realistic given the clinical experience of MARS, Prometheus, and HepatAssist

All other ELAD-type devices have failed in acute liver failure. Of these, three were involved in large clinical trials.

The HepatAssist 2000, a BAL based on porcine hepatocytes developed by Circe BioMedical, was tested in a phase 3 trial in 171 patients with acute liver failure (Demetriou, 2004). The primary endpoint of 30-day overall survival was not met. In the ITT population, survival for the HepatAssist-treated + standard of care (SOC) was 71% vs. 62% in the SOC only arm (p=0.26). A mITT analysis, which excluded a subgroup comprised of primary graft non-function patients (n=24), also failed to reach statistical significance with survival of 73% in treated + SOC vs. 59% in SOC only (p=0.12). Within the primary graft non-function subgroup, 30-day survival in the treated arm was worse than in the SOC arm, 58% vs. 75% (p=0.67). Although porcine hepatocytes and C3A cells are different, the failure of the HepatAssist 2000 calls into question the efficacy and utility of the ELAD and there are reasons (which we will go into detail later) to believe that porcine hepatocytes are actually superior to C3A cells.

The MARS albumin dialysis system, developed by Gambro, is an acellular liver assist device that removes toxins via adsorption (Stange, 1993). It was tested in a phase 3 trial (the RELIEF trial) in 179 patients with acute-on-chronic liver failure (Banares R. , 2013). The primary endpoint was 28-day transplant-free survival in the ITT and PP populations with 90-day transplant-free survival as a secondary endpoint. In the ITT population, 28-day survival in MARS-treated + SOC vs. SOC was 60.7% vs. 58.9%, respectively. The PP population (n=156) had similar survival rates (60.0% vs. 59.2%). The rates for 90-day survival were similarly unremarkable: 46.1% vs. 42.2 % in ITT (p=0.71) and 44.7% vs. 43.7% in PP (p=0.97). In other words, MARS therapy failed to show any survival benefit. Note that almost all the patients enrolled in this trial had alcohol-induced liver failure (Banares R. , 2013). Although the MARS albumin dialysis device failed to improve survival, it is FDA-approved in the treatment of drug overdose and hepatic encephalopathy (Gambro, 2011). The MARS system's inability to improve survival in patients with acute liver failure does not bode well for the future of the ELAD.

The PROMETHEUS albumin dialysis system, developed by Fresenius Medical Care, is based on the concept of fractionated plasma separation and adsorption (FPSA) (Kribben, 2012). It was tested in a phase 3 trial (the HELIOS trial) in 145 patients with acute-on-chronic liver failure. The primary endpoints were 28-day and 90-day survival. In the ITT population, 28-day survival was 66% in the FPSA-treated + SOC vs. 63% in SOC only (p=0.70). 90-day survival was 47% in FPSA-treated + SOC vs. 38% in SOC only (p=0.35). 58% of the FPSA-treated +

SOC patients and 68% of the SOC only patients had alcoholic liver disease. The authors concluded that the FPSA "does not increase the probability of survival." The failure of the HepatAssist 2000, MARS system, and PROMETHEUS system to improve survival in patients with liver failure only highlight the difficulty of effectively duplicating the native liver's functions.

Condition A, Point 7: The biochemical results of ELAD with regard to albumin and bilirubin are misleading

Albumin is synthesized exclusively by hepatocytes (Quinlan, 2005) (Boyer, 2012) (Orlando, 2014). In VTI-208 patients, depressed serum albumin levels are observed (Ashley, 2015), which confirms that albumin synthesis is impaired in these patients (Schreiber, 1975) (Tuma, 1984) (Mitzner, 2006) (Singh, 2012) (Higgins, 2013). Restoring albumin levels in patients with liver failure is critical for the removal of toxins, heavy metals, drugs, etc. (Nicholson, 2000) and is a strategy that many liver dialysis devices are predicated on. This suggests that the ELAD and the acute liver failure patient must achieve a collective rate of albumin synthesis that is higher than normal to alleviate hypoalbuminemia. We will explain how the C3A cells' capacity for albumin production does not accomplish this and is, therefore, therapeutically insignificant.

The normal level of human serum albumin is 3.5-5.0g/dL (Dasgupta, 2015). Given that humans have ~5L of blood, it follows that the total serum albumin is 175-250g, or an average of ~213g. VTI-208 patients had a mean serum albumin level of 2.7g/dL at baseline or a total serum albumin of 135g, which translates into an average deficit in total serum albumin of 78g (Ashley, 2015). If the ELAD is expected to help acute liver failure patients recover, then the ELAD needs to overcome this shortfall.

A healthy adult synthesizes 10-15g of albumin per day (Garcovich, 2009) (Boldt, 2010) (Nicholson, 2000) (Caraceni, 2013) (Boyer, 2012). In a presentation on the VTI-206 trial, a slide shows that a single ELAD cartridge produces albumin at a peak rate of ~300 mg/day, or 1.2g of albumin per day for four ELAD cartridges (Figure 7) (Teperman, 2012). Although the VTI-208 patients' albumin synthesis rate is less than normal and, therefore, less than 10g of albumin per day, we will be conservative and assume that they are producing albumin at the normal rate of 10g per day. Also consider that at least 4% of total serum albumin is degraded every day (Nicholson, 2000) (Dancygier, 2010) (Miller T. , 2006).

We modeled the serum albumin levels in the average VTI-208 patient with and without ELAD treatment for five days, which is the maximum treatment time in the trial. We found that the total serum albumin after Day 5 in the patient on ELAD experienced a net gain of ~4.2g compared to the patient not on ELAD (Figure 8). This net gain is only ~5.3% of the 78g albumin deficit and this is assuming the patient is synthesizing albumin at a normal rate. In other words,

even in a best case scenario, the ELAD's rate of albumin synthesis is drastically insufficient and will not significantly address the total serum albumin deficit in VTI-208 patients.

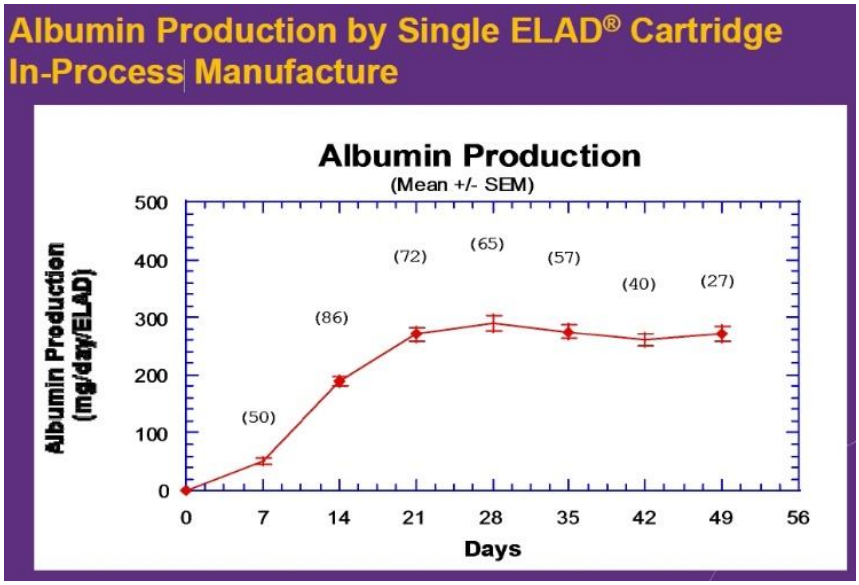

Figure 7. Albumin Production by a Single ELAD Cartridge (Teperman, 2012)

Albumin Profile of the Average VTI-208 pt assuming NORMAL albumin synthesis rate without ELAD

Control	Total serum ALB, g	New ALB/day, g	Daily ALB degradation, g	EOD total serum ALB, g
Day 1	135.0	10.0	-5.8	139.2
Day 2	139.2	10.0	-6.0	143.2
Day 3	143.2	10.0	-6.1	147.1
Day 4	147.1	10.0	-6.3	150.8
Day 5	150.8	10.0	-6.4	154.4
			Change in serum ALB from Days 1-5	15.2

Albumin Profile of the Average VTI-208 pt assuming NORMAL albumin synthesis rate with ELAD (+1.2g/day)

ELAD	Total serum ALB, g	New ALB/day, g	Daily ALB degradation, g	EOD total serum ALB, g
Day 1	135.0	11.2	-5.8	140.4
Day 2	140.4	11.2	-6.1	145.5
Day 3	145.5	11.2	-6.3	150.4
Day 4	150.4	11.2	-6.5	155.2
Day 5	155.2	11.2	-6.7	159.7
			Change in serum ALB from Days 1-5	19.4
			ELAD albumin benefit	**4.2**

Figure 8. Model of serum albumin in the average VTI-208 patient with and without ELAD treatment

Bilirubin is a product of heme catabolism, which starts with the breakdown of dead or damaged red blood cells (Boyer, 2012) (Zucker, 2004). Elevated bilirubin, or hyperbilirubinemia, is typically a sign of hepatocyte dysfunction (Nagasue, 1987) (Stickel, 2010)

(Garg, 2012). Hyperbilirubinemia occurs when the bilirubin concentration is greater than 2.0-2.5mg/dL (Hauser, 2011) (Mauss, 2014). While the ELAD has been shown to decrease serum bilirubin levels, we will provide some much needed context as to why such a decrease is insignificant.

Normal levels of total bilirubin in human serum are <1.2mg/dL (Kwak, 2012) (Arora, 2009) (Zucker, 2004) (Lum, 1988). Total bilirubin levels in the VTI-208 population at baseline are an average of 25mg/dL (Ashley, 2015), which would be described as hyperbilirubinemia. In VTI-206, the per-protocol AAH subgroup experienced the largest decline in serum total bilirubin at day 3, which was 5mg/dL (Figure 9). Since the VTI-208 population shares many of the same patient characteristics as the VTI-206 AAH subgroup, we believe the VTI-208 population will experience a similar decline in serum total bilirubin. In other words, the VTI-208 patients will still suffer from hyperbilirubinemia by end of ELAD treatment.

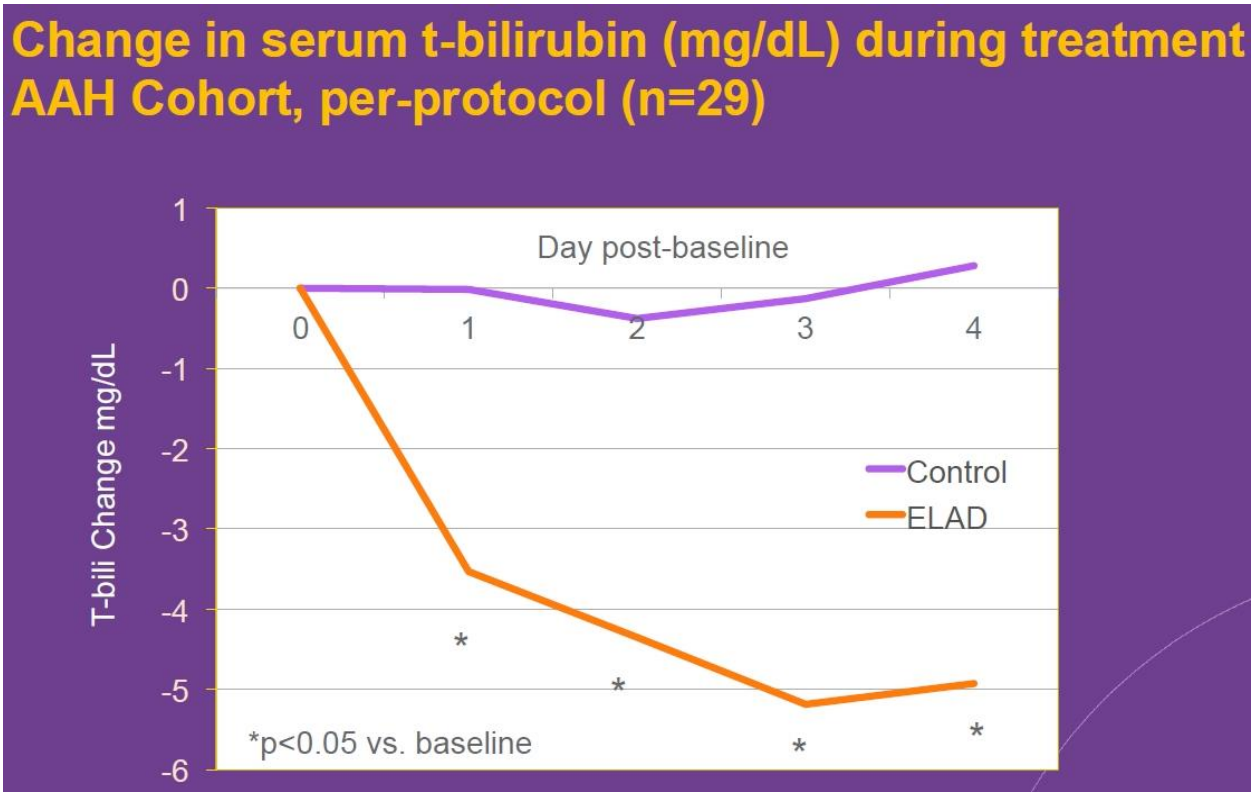

Figure 9. Change in serum t-bilirubin in the per-protocol AAH subgroup (Teperman, 2013)

It is alarming that there is no data at days 30, 60, or 90, which would show, unequivocally, that ELAD treatment is indeed helping patients with acute liver failure regain lost liver functions. Vital's inability or unwillingness to provide this additional, but crucial, data is incomprehensible and is suggestive that the data are not good.

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Condition A, Point 8: The reduction in bilirubin and ammonia is simply due to blood displacement

Once ELAD therapy is initiated, a patient experiences a temporary loss of blood that circulates in the ELAD. The human body compensates for this loss via erythropoiesis, new red blood cell production (Koury, 2004). We believe there is a strong likelihood that the drop in the biochemical parameters associated with clinical improvement is due to simple subtraction and hemodilution, not to the alleged efficacy of the ELAD. Several studies show that patients on kidney dialysis also experience hemodilution evidenced by lower serum liver enzymes (Liberato, 2012) (Sette, 2014).

In a 1996 Hepatix study, "blood ammonia levels elevated at the start of the study (median 98 umol/L, range 60-205) fell over the first 6 hours of haemoperfusion (mean fall, -3.52 umol/L) but were unchanged (106 umol/l, range 38-162) at 48 hours" (Ellis, 1996). We believe that the drop in ammonia levels in this 1996 study was simply due to blood displacement and resulted in hemodilution. We also believe this occurred in VTI-206 and will reoccur in VTI-208. This would explain the return to baseline in levels of bilirubin and MADDREY score (Figures 9, 10, 11).

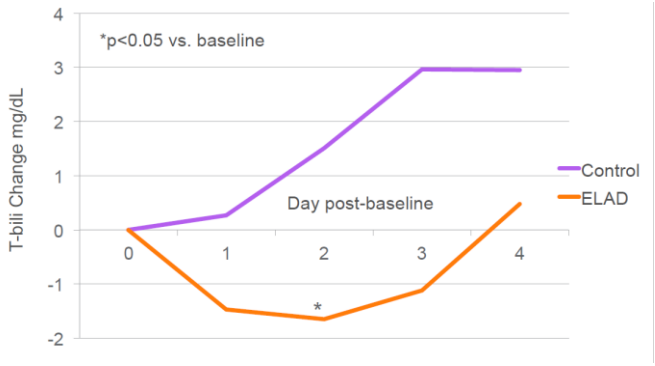

Figure 10. Change in t-bilirubin (mg/dL) during treatment Non-AAH cohort, per-protocol (n=16) (Teperman, 2013)

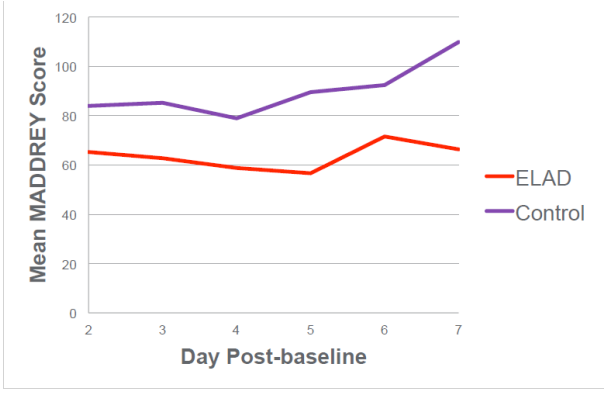

Figure 11. Mean MADDREY score post-baseline: AAH subjects (mITT) (Teperman, 2013)

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We believe that any clinical improvement attributed to the ELAD occurs only within the five days that the patient is on the therapy and is, therefore, inconsequential.

Condition B: Vital's ELAD is a non-functional device

Drs. Norman Sussman and James Kelly were the co-founders of Hepatix, the original company behind the ELAD. Dr. Sussman is now a board member of HepaHope, Inc. and an associate professor of surgery at the Baylor College of Medicine, while Dr. Kelly is the CEO of Cell Machines (Sussman & Kelly, 2014). Dr. Sussman and Dr. Kelly are not professional medical device engineers and their inability to design a coherent device will be made clear shortly.

It is critical to analyze the ELAD device as a distinct theoretical entity that is detached from the clinical trial results in VTI-206. In this section, we will examine the liver cells which make up the bulk of the device's intended metabolic action, the transportation of blood from the patient to the device and back, and an analysis of the hollow fiber bioreactor tubes where the cells are grown. You will come to learn that the ELAD is a non-functional device which does not detoxify blood from patients with liver failure.

Condition B, Point 1: Limited flow rate and small cartridge surface area eliminate the ability for ELAD to function properly

ELAD cartridges are hollow fiber devices (Sussman, 1992) (Gislason, 1994) (Sussman, 1994) (Sussman, 1994) (Ellis, 1996) (Nyberg, 2012) (Teperman, 2012). Hepatix used two cartridges, each containing 200g of C3A cells and 10,000 cellulose acetate fibers with a functional surface area of $2m^2$ (Gislason, 1994). Vital Therapies currently uses four cartridges, each containing 110g of C3A cells and 8,000 hollow fibers (Vital Therapies, 2009). In-vivo, liver sinusoids, hepatic vessels where portal and arterial blood combine and where separation of blood and plasma occurs, have a total surface area of $400m^2$ (Kuntz, 2006). In other words, the modern-day ELAD (which has four 110g cartridges) replicates ~1% of the surface area of a normal adult liver.

We believe the lack of a more intricate 3-dimensional architecture in the ELAD results in a severe compromise of surface area. The human liver is designed to maximize surface area and vasculature to allow for greater efficiency. Several papers have corroborated the opinion that new ELADs should try to resemble the liver's 3-dimensional environment, especially with consideration towards the highly vascularized nature of the organ (Banares R. , 2014) (Tilles, 2002). Another paper contains the results of an experiment that tests this hypothesis (Figure 12). It demonstrated that 3-dimensional bioreactors, which house hollow fibers anchored to a

scaffold, a feature that Vital's ELAD lacks, performed better than hollow fiber bioreactors in ureagenesis, albumin secretion, ammonia removal, and CYP450 activity (Zhang, 2014).

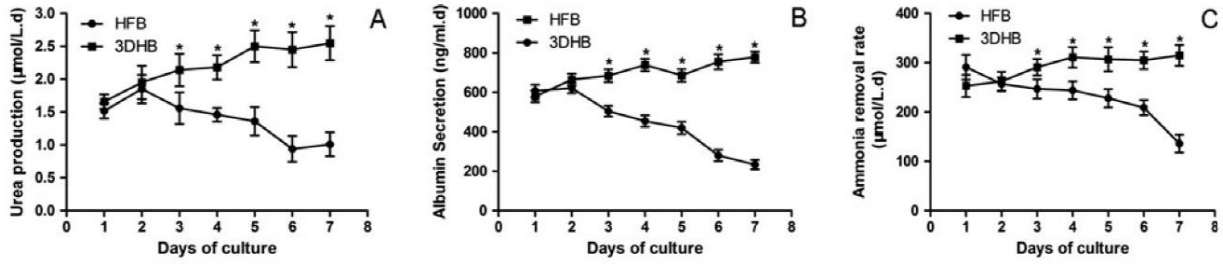

Figure 12. Urea production, albumin secretion, and ammonia removal of cultured hepatocytes in a 3-D hollow fiber bioreactor with a scaffold vs. a hollow fiber bioreactor.

In a recent review article, the co-founders of Hepatix and designers of the ELAD device, Drs. Sussman and Kelly agree that scaling up a liver assist device does not allow for access by "diffusion alone" (Sussman & Kelly, Artificial Liver., 2014).

Another important function of the liver that the ELAD designers did not take into consideration was bile excretion. Indeed, in a recent review, the co-inventors of the ELAD device acknowledge that a next-generation liver device should have a "drainage system for bile excretion" (Sussman & Kelly, 2014).

Normal hepatic blood inflow is approximately 1,500 ml/min (Bradley, 1952) (Iwata, 2004) (Williams, 2012) (Ciccone, 2016). According to other publications, including a review paper on the pharmacokinetics of BALs, higher extracorporeal perfusion rates of 600-1,000 ml/min and toxin clearance rates of 600-700 ml/min are needed in BALs, conditions met only by orthotopic liver transplantation (Iwata, 2004) (Ronco, 2009). Given that the extracorporeal perfusion rate of the ELAD is 120-200 ml/min and the plasma separation rate is 30-60 ml/min (Vital Therapies), it would appear that the rate at which it detoxifies blood has an upper limit defined by the rate of plasma separation, which seems insufficient when compared to normal hepatic plasma flow (700-950ml/min) (Suren, 1991) (Clemmesen, 1998). Iwata et al. conclude that "it is difficult to rationalise the usage of the BAL system to treat patients for simple removal of low-molecular-weight toxins" (Iwata, 2004). In a review article on BAL devices, one can see that they all fail to meet the aforementioned flow requirements or show an unambiguous positive treatment effect (Figure 13).

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TABLE 3. Characteristics of Eight Different BAL Systems

	ELAD	HepatAssist	TECA-HALSS	BLSS	RFB	LSS and MELS	AMC-BAL	HBAL
Center	Amphioxus Cell Technologies, Houston, TX	Cedars-Sinai Medical Center, Los Angeles, CA, and Circe Biomedical, Lexington, KY	Chinese PLA General Hospital, Beijing, China	Excorp Medical, Inc, Oakdale & University of Pittsburgh, PA	University of Ferrara and RanD, Cavezzo Italy	Charité, Humboldt University, Berlin, Germany	Academic Medical Center, University of Amsterdam, The Netherlands	Nanjing University, Nanjing, China
Cell type	Human, tumor derived	Porcine	Porcine	Porcine	Porcine	LSS → porcine MELS → human	Porcine	Porcine
Cell source	Cultured C3A	Cryopreserved	Freshly isolated	Freshly isolated	Freshly isolated	Freshly isolated	Freshly isolated	Freshly isolated
Cell amount	200–400 gram	$5–7 \times 10^9$	$10–20 \times 10^9$	70–120 g	200–230 g	Up to 600 g	10×10^9	10×10^9
Device function pretreatment	Not reported	Not reported	Not reported	Not reported	Not reported	LSS → not reported MELS → yes	Urea synthesis	Not reported
Device sterility pretreatment	Not reported	Not reported	Not noted	Microbial culture	Not noted	LSS → not reported MELS → yes	PCR and microbial culture	Not reported
Mass transfer	70 kD cutoff membrane	0.2 μm porous membrane	Membrane, details not reported	100 kD cutoff membrane	Polyester screen, cutoff 1 μm	LSS → 300 kD cutoff MELS → 400 kD cutoff	Direct hepatocyte–plasma contact	100 kD cutoff membrane
Barrier filter	1 μm	No	Not reported	No	0.4 μm	No	Leukocyte filter and 0.47 μm filter	No
Plasma/blood perfusion	Blood	Plasma	Plasma	Blood	Plasma	Plasma	Plasma	Plasma
Blood filtration rate	150–200 mL/min	90–100 mL/min	Not reported	100–250 mL/min	80 mL/min	150–300 mL/min	100 mL/min	100 mL/min
Plasma filtration rate	Not applicable	50 mL/min	Not reported	Not applicable	22 mL/min	31 mL/min	40–50 mL/min	Not reported
Bioreactor flow rate	15–200 mL/min	400 mL/min	Not reported	100–250 mL/min	1.0–1.5 mL/min/g hepatocytes	100–200 mL/min	150 mL/min	Not reported
Treatment time	Up to 168 hrs	6 hrs	Up to 5 hrs	12 hrs	Maximal 24 hrs	LSS → 7–46 hrs MELS → 7–74 hrs	Maximal 24 hrs	6 hrs
Oxygenation level	Prebioreactor	Prebioreactor	Not reported (prebioreactor)	Prebioreactor	Prebioreactor	Local, inside bioreactor	Local, inside bioreactor	Not reported
Oxygenation gas	Not reported	Not reported	Not reported	Mixture $O_2/CO_2/N_2$, → pH and O_2 guided	95% O_2/5% CO_2	Not reported	95% O_2/5% CO_2	Not reported
Anticoagulation	Heparin	Citrate	Heparin	Heparin	Heparin/citrate	Heparin	Heparin	Not reported
Extra detox devices	No	Charcoal column prebioreactor	Charcoal column prebioreactor	No	No	LSS → no MELS → albumin dialyzation	No	Hetrogeneous, (charcoal or bilirubin column)

BAL, bioartificial liver; ELAD, Extracorporeal Liver Assist Device; TECA-HALSs, TECA-Hybrid Artificial Liver Support System; BLSS, Bioartificial Liver Support System; RFB, Radial Flow Bioreactor; LSS, Liver Support System; MELS, Modular Extracorporeal Liver Support; AMC-BAL; AMC-Bioartificial Liver; HBAL, Hybrid Bioartificial Liver; PCR, polymerase chain reaction.

TABLE 4. Clinical Outcome of Eight Different BAL Systems

	ELAD	HepatAssist	TECA-HALSS	BLSS	RFB	LSS and MELS	AMC-BAL	HBAL
Type of trial	1. Safety evaluation 2. Controlled trial	1. Safety evaluation 2. Controlled trial	Safety evaluation	Safety evaluation	Safety evaluation	Safety evaluation	Safety evaluation	Safety evaluation
No. of patients	1. 11 2. 24	1. 10 2. 171	6	4	7	1. LSS (porcine) → 7 2. MELS (human) → 8	12	12
Indication of BAL treatment	1. ALF 10 PNF 1 AOC 0	1. ALF 7 PNF 1 AOC 2	ALF 3 PNF 0 AOC 3	ALF 2 PNF 0 AOC 2	ALF 4 PNF 3 AOC 0	1. LSS → not reported 2. MELS → ALF 2 PNF 2 AOC 4	ALF 12 PNF 0 AOC 0	ALF 12 PNF 0 AOC 0
1. safety study, 2. controlled trial	2. ALF 24* PNF 0 AOC 0	2. ALF 147 PNF 24 AOC 0						
Bridge to OLT and survival of OLT patients	1. Bridge to OLT 4 Survival no OLT 1 Died no OLT 6	1. Bridge to OLT 8 Survival no OLT 0 Died no OLT 2	Not reported, at least 2 patients survived without OLT	No OLT waiting list: 3 deaths 5–10 days post-BAL, 1 survival → auxillary transplant	Bridged to OLT 6 Survival no OLT 0 Died before OLT 1	1. Bridged to OLT 6 Survival no OLT 0 Died before OLT 0	Bridge to OLT 11 Survival no OLT 1	Bridged to OLT 0 Survival no OLT 9 Died post BALT 3
1. safety study, 2. controlled trial	2. Bridge to OLT 6† Survival no OLT 0 Died no OLT 7	2. Bridge to OLT n.r Survival no OLT n.r Died no OLT n.r				2. Bridged to OLT 6 Survival no OLT 1 Died before OLT 0 Died no OLT 1		
Complications during treatment	Hypotension in 1 patient; 2 patients → treatment discontinued due to bleeding and pyrexia	Occasionally hypotension	No	Transient hypotension	No, treatment in 2 patients was discontinued due to external reasons	LSS → not reported MELS → no	2 × short period of hypotension, easily corrected → treatment continued	No
Survival improvement	No	Only in subgroups improved survival was found (n = 171)	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable
Neurological improvement	Probably	Yes	Yes	No, possible due to sedation	Yes	LSS → not reported MELS → yes	Yes	Unclear
Ammonia elimination	−8% (increased)	18%‡	Not reported	33%	33%	1. No improvement 2. Not reported	44%	Unclear
Total bilirubin elimination	−20% (increased)	18%‡	Not reported	6%	11%	1 & 2 Not reported	31%	Unclear
Tested for PERV	Not applicable	Yes → negative	Not reported	Yes → negative	Yes → negative	LSS → Yes, negative MELS not applicable	Yes → negative	Not reported

Average ammonia and total bilirubin elimination percentage is represented as a decrease of concentration after bioartificial liver transplantation treatment compared to the concentration before treatment.

*Only orthotopic liver transplantation waiting list patients included.

†Two groups of patients (see text, ELAD).

‡Average of data published in 5 different papers.[67,70,71,73,74]

BAL, bioartificial liver; ELAD, Extracorporeal Liver Assist Device; TECA-HALSS, TECA-Hybrid Artificial Liver Support System; BLSS, Bioartificial Liver Support System; RFB, Radial Flow Bioreactor; LSS, Liver Support System; MELS, Modular Extracorporeal Liver Support; AMC-BAL, AMC-Bioartificial Liver; HBAL, Hybrid-Bioartificial Liver; ALF, acute liver failure; PNF, primary graft nonfunction; AOC, acute-on-chronic liver failure; OLT, orthotopic liver transplantation; BALT, bioartificial liver transplantation.

Figure 13. BAL system comparison (van de Kerkhove, 2004)

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The ELAD also has a fatal flaw with filter pressure build-up which results in clotting in the cartridge (Figure 14) (Gislason, 1994). This flaw may explain why we do not see bilirubin levels beyond day 5 and is the only explanation we have found for why ELAD treatment is so short compared to kidney dialysis.

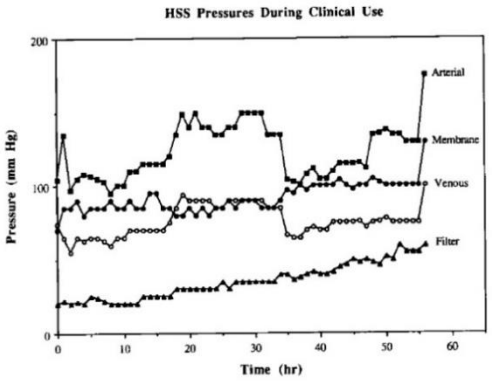

FIG. 3. An example of hepatic support system pressure readings obtained in clinical use. A sudden increase in arterial venous and membrane pressures at the end was due to clotting in the venous drip chamber.

Figure 14. HSS Pressures during clinical use. (Gislason, 1994)

The ELAD has a surface area of ~1% of a normal liver, with a cell mass which is 30% of a normal liver, and a flow rate which is 13% of a normal liver (assuming the upper limits of the ELAD's specifications). Using simple arithmetic, it would appear that the metabolism of the ELAD is equal to the product of these values, or less than 0.04%. This calculation is too aggressive, as the C3A cell line used in the ELAD is deficient relative to normal hepatocytes (Sinz, 2008) (Tostoes, 2010) and, furthermore, it is unclear if the cellular mass is sufficiently oxygenated, so assuming 30% of the cell mass is functioning is inappropriate.

Condition B, Point 2: Non-parenchymal cells are not optional and perform important liver functions that the ELAD ignores

The liver does not consist entirely of parenchymal cells, or hepatocytes. 60% of the cells in the liver are hepatocytes while the other 40% consists of non-parenchymal cells, namely sinusoidal endothelial cells, stellate (Ito) cells, and Kupffer cells (Godoy, 2013) (Boyer, 2012), which all perform critical liver functions that the ELAD plainly ignores.

Liver sinusoidal endothelial cells (LSECs) represent 19% of the total liver cell population (Godoy, 2013) (Boyer, 2012). They have large pores that allow nutrients, toxins, hormones, proteins, etc. to flow from plasma to the hepatocytes for processing. The ELAD attempts to mimic this function by using semi-permeable hollow fiber tubes to act as a barrier between the C3A cells and plasma ultrafiltrate. It would succeed in this regard were it not for the fact that sinusoidal endothelial cells do more than act as a leaky wall. LSECs perform receptor-mediated

removal of gut-derived endotoxin, the accumulation of which is associated with alcoholic hepatitis (Wheeler, 2003) (Purohit, 2008) (Rao, 2009) (Nolan, 2010) (Boyer, 2012) (Mauss, 2014). These cells also secrete cytokines involved in tissue regeneration and cell growth (DeLeve, 2013) and participate in immune responses to pathogens (Boyer, 2012).

Hepatic stellate cells (HSCs) represent 6% of the total liver cell population (Godoy, 2013) (Boyer, 2012). They are found between LSECs and hepatocytes and known mostly for synthesizing collagen, but like their non-parenchymal counterparts, they perform an array of functions that include regeneration, intermediary metabolism, and immunoregulation (Godoy 2013).

Kupffer cells represent 15% of the total liver cell population (Godoy, 2013) (Boyer, 2012). Macrophages exclusive to the liver, they reside in the sinusoidal endothelium and perform key functions in host defense (Godoy, 2013). They actually represent the largest macrophage population in mammals (Movita, 2012) (Kmiec, 2001). While Kupffer cells release pro-inflammatory cytokines like TNF-alpha, which participate in the inflammatory response, they, along with LSECs, prevent endotoxin and foreign substances from reaching the systemic circulation, regulate liver regeneration, and influence the metabolic and detoxification functions of hepatocytes (Godoy, 2013) (Boyer, 2012).

In liver injury, all non-parenchymal cells are activated (Taub, 2004), can regenerate, possess both pro-inflammatory and anti-inflammatory qualities, and participate in cell-to-cell communications with each other and with hepatocytes (Kang, 2012) (Boyer, 2012). The intricacies of the signaling and molecular pathways involved are beyond the scope of this paper, but the functions of LSECs, stellate cells, and Kupffer cells raise an important question. If non-parenchymal cells were optional, which the ELAD certainly suggests by omission, why would the human body use resources to restore them?

Given the intricacy of the hepatic ecosystem and the important roles that non-parenchymal cells play in metabolism, immunity, cell-to-cell communication, and detoxification in conjunction with hepatocytes, we believe that absence of their functions in any liver dialysis device is a major oversight and any strategy to circumvent the inclusion of their functions is extremely naïve.

Condition B, Point 3: During treatment, ELAD cells are likely anoxic and unlikely to be metabolically active

Not all hepatocytes are created equal. In fact, there are three types of hepatocytes, each of which have distinct metabolic functions and levels of oxygenation: periportal, centrilobular, and perivenous, that comprise the functional unit of the liver, called the acinus (Figure 15) (Katz, 1992). In the liver acinus, periportal hepatocytes, in Zone 1, are the closest to the portal vein and hepatic artery blood inflows, which means that they are very oxygen rich. Perivenous

hepatocytes, in Zone 3, are the furthest from the blood supply, which means that they are very oxygen poor. Centrilobular hepatocytes, in Zone 2, are a mix of Zone 1 and Zone 3 hepatocytes.

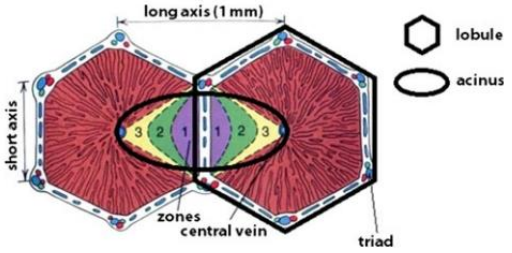

Fig. 2 Organization of the liver lobule and acinus. Based on the local blood composition, the acinus is roughly divided into three zones, *1* periportal, *2* transitional and *3* perivenous. The periportal zone is close to the portal triad vasculature and supplied by highly oxygenated blood (O_2 partial pressure 60–70 mmHg). The perivenous zone is proximal to the central vein and receives poorly oxygenated blood (O_2 partial pressure 25–35 mmHg). If no specific zonal mechanisms are active (such as pericentral metabolic activation of many hepatotoxic compounds, because many CYP enzymes are preferentially expressed in the center of the liver lobules), toxicity becomes visible at first in the periportal region, as this is the first zone to filter blood (Allen and Bhatia 2003). Adapted from Bacon et al. (2006)

Figure 15. The liver acinus (Godoy, 2013)

Periportal hepatocytes predominantly perform gluconeogenesis, oxidative energy metabolism, beta oxidation, cholesterol synthesis, and urea synthesis. Perivenous hepatocytes predominantly perform glycolysis, fatty acid synthesis, and glutamine synthesis (Dancygier, 2010). The functional heterogeneity of primary hepatocytes calls into question the identity of the C3A cell line. Vital has stated that the cell line is "extremely well differentiated" (Sussman, 1992) which we interpret as meaning it cannot differentiate further into the required hepatocyte subtypes that would actually allow for realistic liver replacement.

Two bioengineers have determined that the design of the ELAD is unlikely to correctly account for the high oxygen consumption of hepatocytes (Hay, 2000) (Smith, 1997). In an analysis of oxygen transfer in a model of a plasma-perfused hollow fiber bioreactor (much like the ELAD), the authors note that BAL designs need to consider the extremely high oxygen demands of primary hepatocytes given their wide range of oxygen-dependent metabolic functions (Hay, 2001). And yet, the results of the analysis show that "a substantial proportion of the hepatocytes contained in the model device would be subjected to hypoxic conditions," conditions that would impair hepatocyte metabolism.

In another analysis of oxygen transfer in hollow fiber bioreactors, the authors emphasize the efficiency of the acinar structure of the liver in oxygen distribution and the need to meet the high oxygen consumption rate of hepatocytes. HepG2 cells, the parental cell line of the current ELAD's C3A cells, were used in this model, the results of which show that oxygen "is completely depleted at distances equivalent to 1-2 cell layers from the membrane outer wall" (Figure 16) and that "while cells further from the membrane may well be viable, they are

unlikely to be metabolically active" (Smith, 1997). Smith et al. conclude that BAL designs require the oxygenation of hepatocytes within the bioreactor, a feature that the current ELAD lacks.

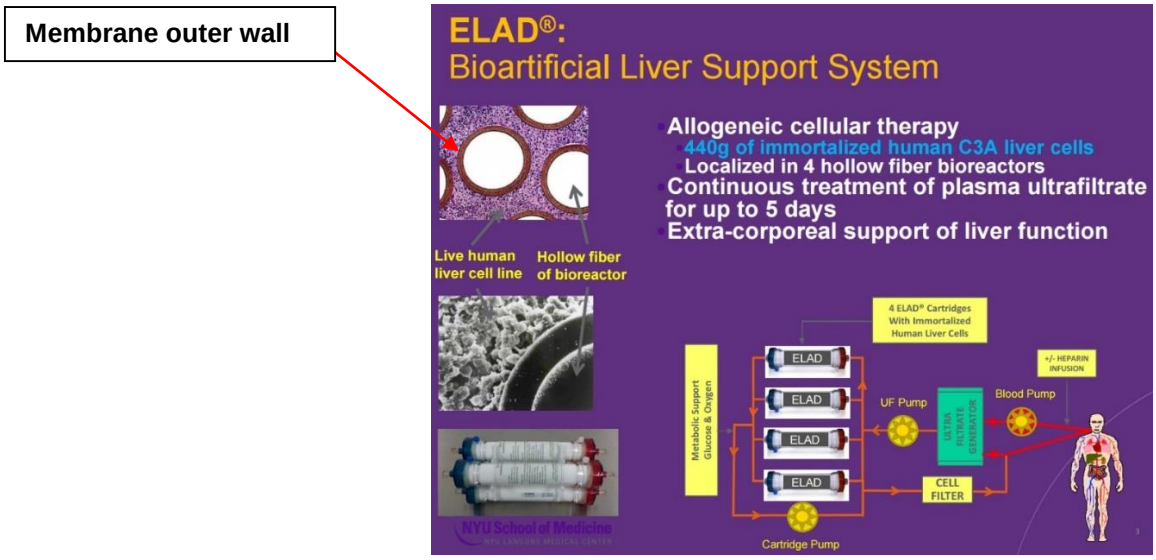

Figure 16. Cross-section of an ELAD cartridge. (Teperman, 2012)

Condition B, Point 4: Prior to ELAD treatment, C3A cells are likely exposed to hyperoxic or hypoxic conditions and may be undergoing apoptosis during culture

The C3A cell line is derived from the HepG2 cell line, which is derived from human hepatocytes. Like human hepatocytes, C3A cells have a high demand for oxygen (Gislason, 1994) (Ellis, 1996). In the setting of BALs, it is important that these cells receive adequate oxygenation in culture (Martin, 2005) (Nahmias, 2006) (Kidambi, 2009). However, oxygen is known to be poorly soluble in aqueous solution such as plasma (Sussman, 1994) (Webster, 1997) (Smith, 1997) (Hay, 2000). The co-founders of Hepatix, Norman Sussman and James Kelly, admit that "plasma does not have the carrying capacity of whole blood and will not satisfy the oxygen requirements of a significant mass of living cells" (Gislason, 1994).

In a more recent paper on oxygenation in BALs, "hepatic hollow fiber bioreactors (HFBs)…suffer from oxygen limited transport mainly due to the low solubility of oxygen in the cell culture medium, long diffusion path-lengths, and high demand for oxygen by the hepatocytes cultured in the extracapillary space" (Chen, 2010). In the same paper, Chen attempts to improve the oxygen transport in a hollow fiber bioreactor containing C3A cells by inducing bovine hemoglobin oxygen carriers in the culture medium. Note that the cells in this experiment and the C3A cells in the current ELAD were cultured in 10% bovine serum (Vital Therapies, Inc., 2015). Within 14 days of the experiment, the lactate production and glucose consumption of C3A cells cultured without bovine hemoglobin had nearly tripled whereas they had remained stable in the C3A cells that were cultured with bovine hemoglobin (Figure 17). This suggests an

increase in the number of C3A cells undergoing anaerobic respiration in the control HFB (no bovine hemoglobin) compared to the active group (bovine hemoglobin) (Madonna, 2013) (Woods, 1971). In other words, it is likely that at 14 days, many of the cells in the control HFB were hypoxic and did not produce enough ATP to maintain viability. The results of the experiment show that the active HFB had slightly more than twice the number of functioning cells compared to the control HFB.

Furthermore, Chen states "the cell culture medium in most cell culture systems need to be oxygenated to supraphysiological levels (>160 mm Hg) to deliver enough oxygen to cultured cells." However, "prolonged exposure to these conditions will induce the formation of reactive oxygen species, which will eventually kill cells" (Chen, 2010). This experiment tests the oxygen levels in hollow fiber bioreactors *in culture*, suggests the C3A cells are defective *before* ELAD therapy starts, and stresses the importance of sufficient oxygenation in HFBs. In short, we believe many of the C3A cells in Vital's ELAD are hypoxic and undergo apoptosis during culture.

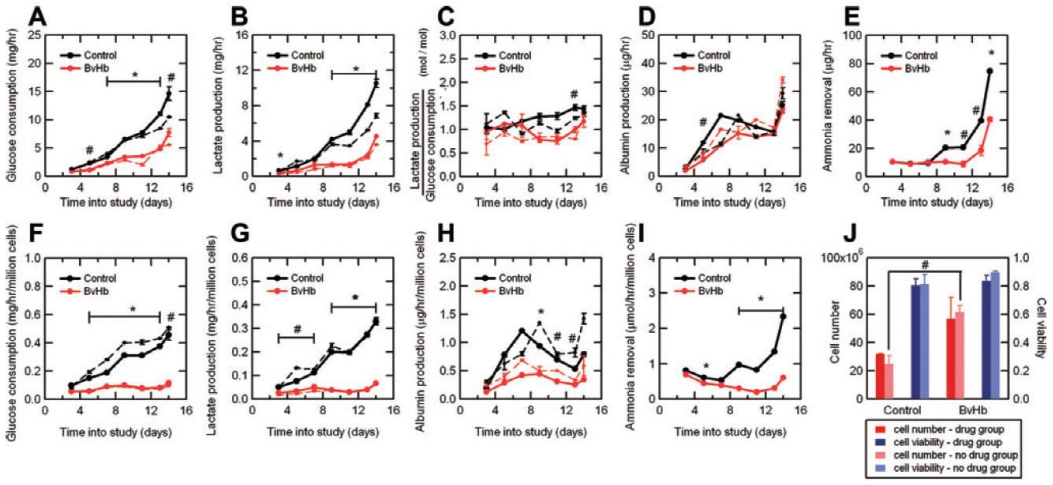

Figure 17. Metabolic, synthetic, and detoxification functions of hepatic HF bioreactors cultured at 10% O2. The cell culture medium was supplemented with BvHb at 15 g/L. The solid lines represent the drug group; dashed lines represent the group with no added drugs. (A) Global glucose consumption rate (mg/h). (B) Global lactate production rate (mg/h). (C) Molar ratio of lactate production to glucose consumption. (D) Global albumin synthesis rate (mg/h). (E) Global ammonia removal rate (mg/h). (F) Glucose consumption rate per cell (mg/h/million cells). (G) Lactate production rate per cell (mg/h/million cells). (H) Albumin synthesis rate per cell (mg/h/million cells). (I) Ammonia removal rate per cell (mg/h/million cells). (J) Final cell count and viability at the end of the cell culture period. All data are shown as the mean_standard deviation. n=2 for each group. *p<0.05; #p<0.10 throughout the study if not specified otherwise. BvHb, bovine hemoglobin. (Chen, 2010)

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Condition B, Point 5: The ELAD C3A cell line is a poor choice which limits or eliminates therapeutic efficacy

The cell source that is the gold standard for BALs are primary hepatocytes as they have been proven to perform all essential liver functions (Catapano, 1996) (Tostoes, 2010), but their use is limited by "inadequate supply, high cost, high variability, and low in vitro proliferation capacity" (Eva, 2014). And yet, the best-performing cell source is not enough to create a functioning, effective BAL. Primary hepatocytes in a BAL should also replicate the "complex in-vivo environment in an effort to enhance and stabilize long-term in-vitro function" (Tilles, 2002). Other methods to improve primary hepatocyte performance include adding "growth factors and hormones to the culture medium," "the use of hepatocyte spheroids," and "co-culturing hepatocytes with nonparenchymal cells" (Tilles, 2002). If the ideal liver assist device requires these conditions of *primary hepatocytes* for optimal performance, then the C3A cell line, a derivative of a hepatoblastoma (not a primary hepatocyte), is no exception.

The liver plays a major role in metabolism. The liver's CYP450 enzyme system metabolizes drugs and also converts prodrugs into their active metabolites (Guengrich, 2008) (Nelson, 1982). Therefore, it is important that the CYP activity of the cells used in a BAL perform on a level comparable to that of primary hepatocytes. HepG2 cells, from which C3A cells are derived, have been shown to have disturbingly low levels of CYP expression compared to those of primary hepatocytes (Rodriguez-Antona, 2002). Rodriguez-Antona et al. found that the most active CYP enzyme in the HepG2 cell line, CYP1A1, was only 20% as active as in human hepatocytes after 24 hours of culture. Furthermore, the activity of CYP2E1, one of the most important CYP enzymes in the metabolism of xenobiotics, was found to be more than 100-fold higher in human hepatocytes than HepG2 cells after 24 hours of culture. We believe the diminished CYP inhibitory activity of the HepG2 line further highlights another important liver function that C3A cells perform poorly.

In a comparison of the metabolic and synthetic functions of porcine hepatocytes and C3A cells, C3A cells were shown to have lower levels of P4501A1 activity, ammonia removal, and amino acid metabolism (Figure 18) (Wang L. , 1998).

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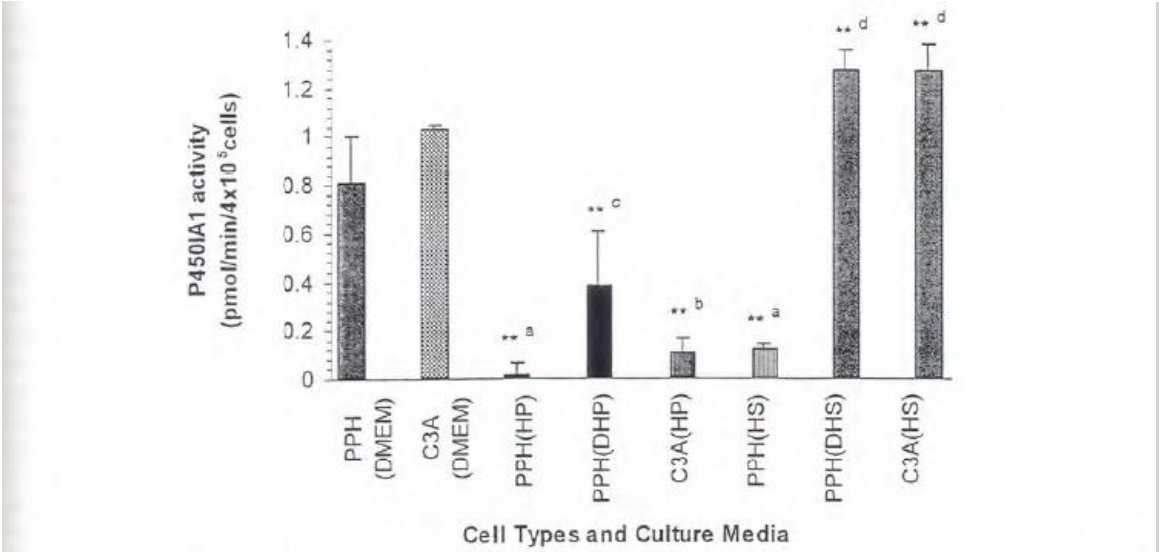

Fig. 4. Comparison of P450IA1 activity of PPH and C3A in different media (each group $n = 7$). The data presented are based on cell numbers (4×10^5 cells/mL). The abbreviations are same as for Fig. 3. $^{**}p < 0.01$; a = compared with all other groups excluding C3A(HP); b = C3A(HP) vs. C3A(DMEM) and C3A(HS); c = PPH(DHP) vs. PPH(DMEM) and PPH(DHS); d = compared with all other groups.

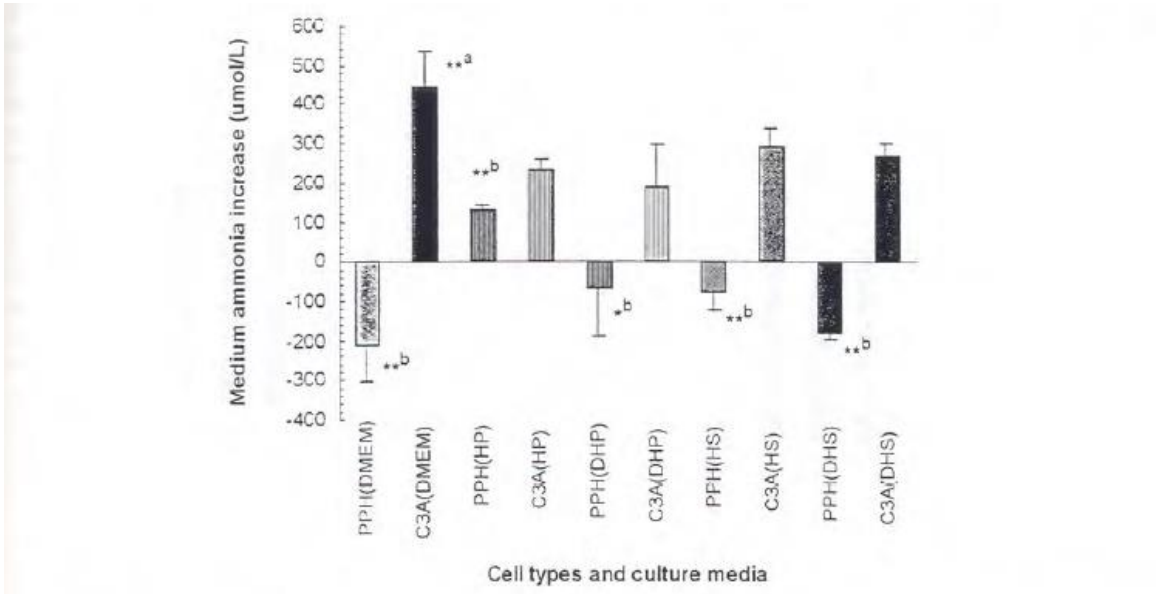

Fig. 5. Comparison of ammonia levels in different media for PPH and C3A (each group $n = 7$). The data presented are based on cell numbers (4×10^5 cells/mL). The abbreviations are same as for Fig. 3. $^{*}p < 0.05$; $^{**}p < 0.01$; a = compared with all other groups; b = compared with C3A in the same medium.

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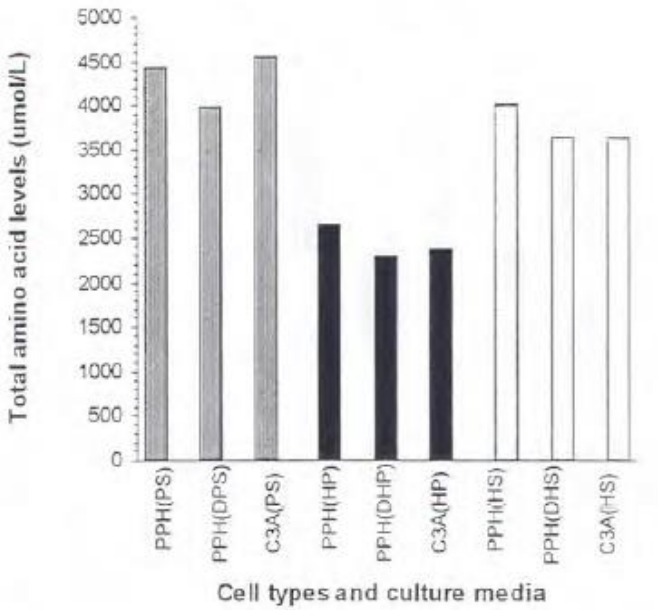

Fig. 7. The total amino acid levels recorded for PPH and C3A in different media (each group $n = 7$). The data presented are based on cell numbers (4×10^5 cells/mL). The abbreviations are same as for Fig. 3.

Figure 18. P4501A1 activity, ammonia removal, and amino acid metabolism in C3A cells

In view of these findings, the authors conclude that primary porcine hepatocytes "are to be preferred to C3A for clinical application of BALSS" (bioartificial liver support systems). These findings foreshadow the VTI-208 results considering the failure of the porcine-based HepatAssist.

A very damaging paper to the C3A hypothesis elegantly demonstrated this cell line's inability to detoxify ammonia using the urea cycle, one of the major causes of death in liver failure (Mavri-Damelin, 2008) (Nyberg, 2012) (McManus, 2014). Tellingly, Drs. Sussman and Kelly, the Hepatix co-founders, failed to provide a rebuttal, only indicating in a review paper that the data were "at odds" with their previously published animal data (Sussman & Kelly, Artificial Liver., 2014). The Mavri-Damelin experiments conclusively demonstrated the C3A cells' inability to detoxify ammonia through the urea cycle by using radiolabeled ammonium chloride, which primary hepatocytes detoxified but C3A cells did not (as measured by radiolabeled urea) (Figures 19, 20).

Normally, periportal hepatocytes convert ammonia into urea via the urea cycle. Perivenous cells convert ammonia into glutamine via glutamine synthetase. The glutamine synthetase route of removing ammonia is appropriate for *trace* amounts only, and is unlikely to be of use if it is relied on solely (Tilles, 2002). The finding that C3A cells do not detoxify

ammonia is alarming for multiple reasons. Firstly, ammonia is a major cause of death in liver failure and toxic levels must be reduced. Secondly, the lack of an enzymatic process to detoxify ammonia in C3A cells calls into question the cell lineage of C3A. Thirdly, why was ELAD designed with C3A cells, a subclone of HepG2 cells, which are known to not detoxify ammonia (Mavri-Damelin, 2007)? Are these purported hepatocytes zone 1, 2, or 3 cells, or none of the above, or one of those cells without these crucial enzymes? Is it possible crucial detoxification enzyme expression levels are lost in this cell line? Mavri-Damelin measured mRNA expression in C3A cells, primary human hepatocytes, and whole human liver. It appears that culture-like consistency, including that experienced in the ELAD device, may reduce key protein expression, including urea cycle proteins (Figure 21). These questions and the results of the Mavri-Damelin paper ask fundamental questions about the viability of the C3A cell line that are still unanswered.

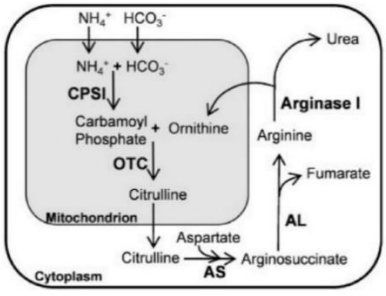

Figure 1. The urea cycle. CPSI, carbamoyl phosphate synthase I; OTC, ornithine transcarbamylase; AS, arginosuccinate synthetase; AL, arginosuccinate lyase; arginase I.

Figure 19. The urea cycle, CPSI, carbamoyl phosphate synthase I; OTC, ornithine transcarbamylase; AS, arginosuccinate synthetase; AL, arginosuccinate lyase; arginase I.

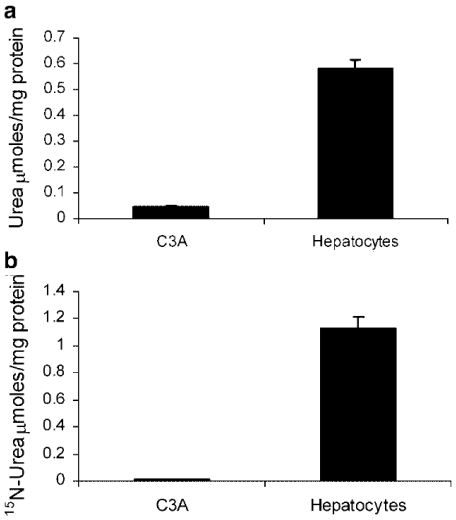

Figure 3. a: GC–MS shows that unlabelled urea can be detected in the conditioned medium of C3A cells (black bars; $n = 4$, mean ± SD, repeated twice). b: De novo synthesis of urea. ^{15}N-labelled urea, which would indicate incorporation of $^{15}NH_4Cl$ into the urea cycle, is not detected in conditioned medium of C3A cells in comparison to that produced by primary human hepatocytes (shaded bars), in the presence of 1 mM $^{15}NH_4Cl$.

Figure 20. Urea synthesis in C3A cells and primary hepatocytes.

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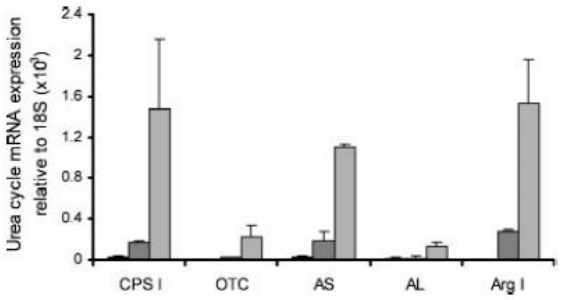

Figure 4. Urea cycle gene expression. Real-time RT-PCR results showing the urea cycle gene expression levels in C3A cells (black), primary human hepatocytes (dark grey) and whole human liver (grey). OTC and arginase I are completely absent, with very low expression of the other three urea cycle genes ($n=6$, mean $\pm$ SD, repeated 3 times). Values are relative to the expression of ribosomal RNA 18S.

Figure 21. Urea cycle gene expression. Real-time RT-PCR results showing the urea cycle gene expression levels in C3A cells (black), primary human hepatocytes (dark grey) and whole human liver (grey). OTC and arginase I are completely absent, with very low expression of the other three urea cycle genes (n=6, mean +- SD, repeated 3 times). Values are relative to the expression of ribosomal RNA 18S.

Condition B, Point 6: The feasibility of the ELAD in the context of clinical utility and inventors' actions

The lack of dramatic efficacy with ELAD raises questions about its clinical utility—if the ELAD were a true liver support system, patients would have 90-100% survival instead of similar survival to those on standard of care. The 1-year, 5-year, and 10-year survival rates in a retrospective study of 300 alcoholic liver disease patients who underwent orthotopic liver transplantation, the gold standard treatment in patients with liver failure, were 96%, 88%, and 76% respectively (Pfitzmann, 2007). If the ELAD led to liver regeneration, we would expect it to achieve comparable survival rates and be a viable option for *all* patients with acute liver failure.

Drs. Sussman and Kelly, the original creators of the ELAD, also seem to agree that ELADs are not realistic devices stating "…the underlying cirrhosis is not reversible" and "recovery…leaves the patient in the same frail state, still in need of a liver transplantation" (Sussman & Kelly, Artificial Liver., 2014). Furthermore, Dr. Sussman's affiliation with HepaHope, which has been working on a BAL device based on "liver slices" since 1999 (Sussman & Kelly, 2014), and Dr. Kelly's affiliation with Cell Machines, which has "patented liver cell factories" (Cell Machines, 2015) indicate their lack of confidence in the ELAD technology. If the ELAD was the game-changing liver dialysis system that Vital Therapies thinks it is, wouldn't Drs. Sussman and Kelly be working on the ELAD?

The current academic climate indicates that the interest in artificial livers persists but it seems that the field has moved on to other cell lines and technologies: HepaRG cells in the AMC

bioreactor (Nibourg, 2013), cryopreserved human hepatocytes in 3D spheroids (Xia, 2015), stem cell-derived hepatocyte-like cells in a 3D culture (Kim J. , 2015), mesenchymal stem cells in a novel 3D system (Li, 2015), porcine hepatocytes in a spheroid reservoir BAL (Glorioso, 2015), etc. A PubMed search of "bioartificial liver" produces 6,263 results, while a search of "bioartificial liver hepatoma" produces 399 (6.4%), "bioartifical liver HepG2" produces 112 (1.8%), and "bioartificial liver C3A" produces 37 (0.6%). It appears that human hepatoma cell lines are a niche category receiving little attention in the field of bioartificial livers. We believe Vital Therapies' ELAD is the culmination of 25 years of unsuccessful experiments in a technology that has stagnated in three successive companies, undergone no marked improvements, and consistently produced questionable clinical trial results.

A 2012 paper, citing the MARS, PROMETHEUS, HepatAssist, and ELAD systems, had this to say about the advances in artificial liver support systems: "more than 30 different cell-based support devices have been reported since 1987," "more than 14 systems have been reported in clinical trials," "more than 400 patients have been treated with bioartificial liver systems," and "none of these bioartificial liver systems have yet obtained FDA approval for the treatment of liver failure" (Nyberg, 2012).

We assigned a 20% probability to both Condition A, which is the likelihood that the survival benefit seen in the VTI-206 acute alcoholic subgroup (n=29) is legitimate and not due to chance, and Condition B, which is the likelihood that the ELAD was designed properly and fully functional. We are confident in these probabilities and have determined that there is a 96% probability that the VTI-208 trial will fail.

In conclusion, we believe that the ELAD suffers from many mechanistic flaws and an extraordinary inability to meet the metabolic needs of a normally functioning liver. Combined with the unconvincing clinical data, these significant deficiencies all but preclude the success of VTI-208.

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Appendix

Acute liver failure – Acute deterioration of liver function in patients in the absence of preexisting liver disease. (Kim T. , 2013) (Asrani, 2014) (Sarin, 2014) (Polson, 2005). The different precipitating events in acute liver failure include viral infections (such as Hepatitis A, B, and E), drugs (with Acetaminophen being the most common), and alcohol (Bernal, 2013) (Lee, 2012).

Acute-on-chronic liver failure (ACLF) – Occurs in patients with chronic liver disease, characterized by a precipitating event (known or unknown) often resulting in acute deterioration in liver function, multi-organ failure, and high short-term mortality. (Kim T. , 2013) (Asrani, 2014) (Sarin, 2014) (Jalan, 2012)

Acute alcoholic hepatitis (AAH) – Alcohol-induced liver disease characterized by hepatic inflammation and acute onset. May be observed in chronic alcoholics with or without noticeable liver impairment or in moderate drinkers after a short-term alcoholic binge. Clinical presentation includes fever, liver enlargement and tenderness, neutrophilic leukocytosis, hyperbilirubinemia, and coagulation impairment. (Ceccanti, 2006) (Pang, 2015)

Hepatic sinusoid – The hepatic sinusoid is a blood vessel which receives oxygen-rich blood from the hepatic artery and nutrient-rich blood from the portal vein. Hepatocytes are separated from the hepatic sinusoid by the space of Disse and receive nutrients and oxygen from the plasma that is filtered from the hepatic sinusoid into the space of Disse. (Boyer, 2012)

Endotoxin (lipopolysaccharide) – Endotoxins are present in intestinal bacteria and only minimally absorbed by the intestine in healthy patients. Alcohol increases the amount of endotoxin in the intestine and the permeability of the intestine to endotoxins. As a result, an excess amount of endotoxins are absorbed into the systemic circulation and into the liver sinusoids where they internalized TL4 and CD14 receptors on Kupffer cells, thereby causing an inflammatory response (Purohit, 2008).

Extracorporeal Liver Assist Device (ELAD ®) – "ELAD is an investigational extracorporeal, human cell-based liver support system designed with the proposed intent to supplement hepatic function in order to improve survival rates among subjects with liver failure." (Figure 23) (Vital Therapies, n.d.)

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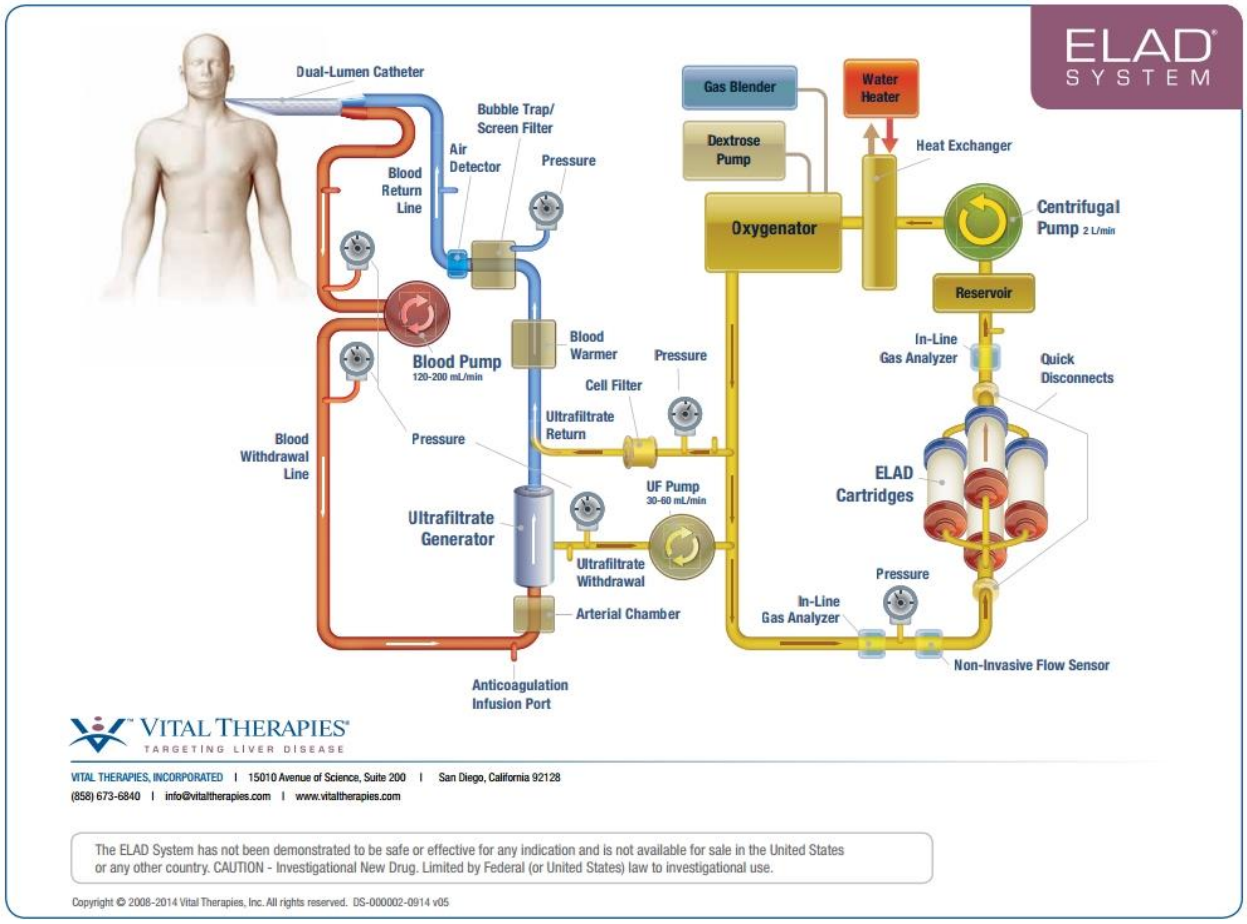

Figure 23. The Extracorporeal Liver Assist Device (ELAD). (Vital Therapies)

Liver basics (Kuntz, 2006):

Number of liver cells - 300 billion liver cells

Number of hepatocytes per g of liver - 171 million hepatocytes

Diameter of hepatocytes - 20-40um

Lifespan of hepatocytes - 150 days

Mitosis rate per 10,000-20,000 liver cells - 1

Liver acinus - The functional and microcirculatory unit of the liver (Dancygier, 2010). The liver acinus is a mass of liver parenchyma which surrounds the hepatic arterioles and portal venules (Katz, 1992).

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Periportal hepatocytes (Zone 1) - Functions include oxidative energy metabolism, glycogen synthesis from amino acids and lactate, gluconeogenesis, beta-oxidation, cholesterol synthesis, and urea synthesis (from NH_4, amino acids, nitrogen).

Perivenous hepatocytes (Zone 3) - Functions include glycogen synthesis from glucose, glycolysis, lipogenesis, ketogenesis, bile acid synthesis, and glutamine synthesis (from NH_4, glutamate, alpha-oxyglutarate, ornithine).

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