Motivated by the Hydraulic Permeability of Tumesced Tissue We Will Carry Out a Clinical Trial for the Treatment of Chronic Wounds Via Direct Antibiotic Injection

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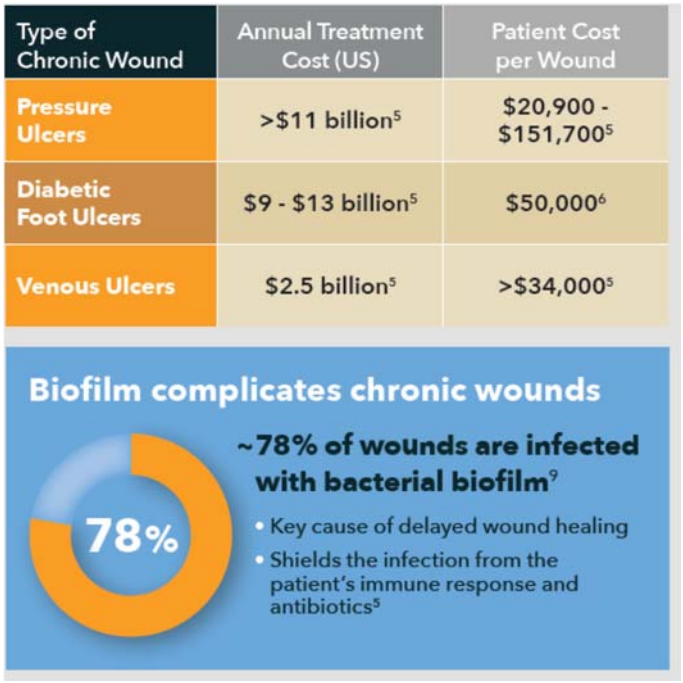
Submitted to the Veneklasen Foundation

Requested Donation is $100,000.

Background and Total Addressable Market: Chronic wounds are an immense medical problem. As the chart below shows over 25B is spent in the US alone on wound care [see also Sen et al 2009, *Human Skin Wounds: A Major and Snowballing Threat to Public Health and the Economy*, Wound Repair and Regneration, 17, 763]. Diabetic limb complications are more costly than each of the 5 most costly cancers. [Barshes, N. R. *et al.* 2013, *The System of Care for the Diabetic Foot: Objectives, Outcomes, and Opportunities Diabetic Foot & Ankle*, 4: 21847].



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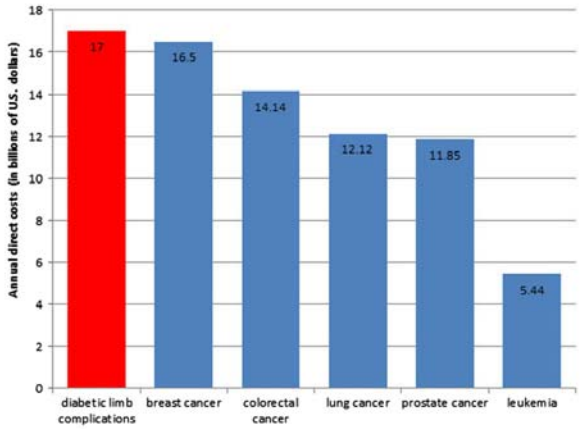

"The estimated annual direct costs of diabetic limb complications in comparison to the annual direct costs of the five most costly cancers in the United States."[Barshes et al, 2013]

Yet: despite: "**Billions in Global Wound Product Sales, Chronic Wounds Remain a Chronic Problem;"** Based on New Research from MedMarket Diligence**.**
Even with "the legion of products developed for wound care, from dressings to negative pressure wound therapy to bioengineered skin and growth factors, the obesity- and age-driven increase in chronic slow-healing and non-healing wounds plague healthcare systems globally."
https://www.pr.com/company-profile/press-releases/250307
The incorrigibility of chronic wounds could be due to the presence of highly persistent biofilms – colonies of bacteria that display emergent behavior and group resistance to antibiotics [Zhao et al 2013 *Biofilms and Inflammation in Chronic Wounds*, Adv. Wound Care 2, 389]. It was found that 60% of chronic wound specimens contain biofilms whereas only 6% of acute wounds do [James et al 2007, *Biofilms in Chronic Wounds*, Wound Repair Regen.16,37], indicating their prevalence in chronic wounds and scarcity in acute wounds. Furthermore these biofilms can be composed of antibiotic resistant pathogens such as MRSA. It is very important to note that antibiotic resistance does not mean antibiotic "proof ". MRSA for example exhibits a minimum inhibitory concentration [MIC] even for common antibiotics such as cefazolin [Nicolau and Silberg, 2017, *Cefazolin Potency Against Methicillinresistant Staphylococcus Aureus: a Microbiologic Assessment in Support of a Novel Drug Delivery System for Skin and Skin Structure Infections*,. Infect Drug Resist. 10, 227]. At a concentration of 1,024 mg/L, 1238 out of 1239 MRSA isolates were inhibited. To achieve such a concentration in body tissue via intravenous injection would be fatal. But to locally achieve this concentration by direct injection is straightforward and safe. The key challenge to such a treatment is to densely permeate (with antibiotic) the unhealthy colonized subcutaneous tissue which constitutes the chronic wound.

Previous Hypothesis for Permeation via Ultrasound Drug Dispersal: A concern is that a direct antibiotic injection with sufficient fluid to swell up the tissue would form pools and not densely permeate the tissue so as to reach all the pathogens. These untreated pockets of bacteria would then be a source of continuing disease. The Veneklasen Foundation funded research at UCLA whose goal was to test the hypothesis that externally applied ultrasound would break up the

pools and disperse the drug via cavitation. This hypothesis was tested "in-vivo" on pigs using CT , diffusion MRI, passive cavitation detection, and 3D imaging of the extremity.

To our surprise we found that therapeutic levels of ultrasound did not lead to cavitation of injected aerated saline. These intensity levels [4.5atm] are sufficient to cause cavitation in bulk. This insight led to our publication "*Interstitial Matrix Prevents Therapeutic Ultrasound from Causing Inertial Cavitation in Tumescent Subcutaneous Tissue.*"[Ultrasound Med Biol. 2018;44:177–86.]

Our measurements using CT employed an iodine contrast agent which has a molecular size similar to a small antibiotic such as cefazolin. The contrast agent was injected to form a welt which was imaged from different directions as shown in Figure 1a.

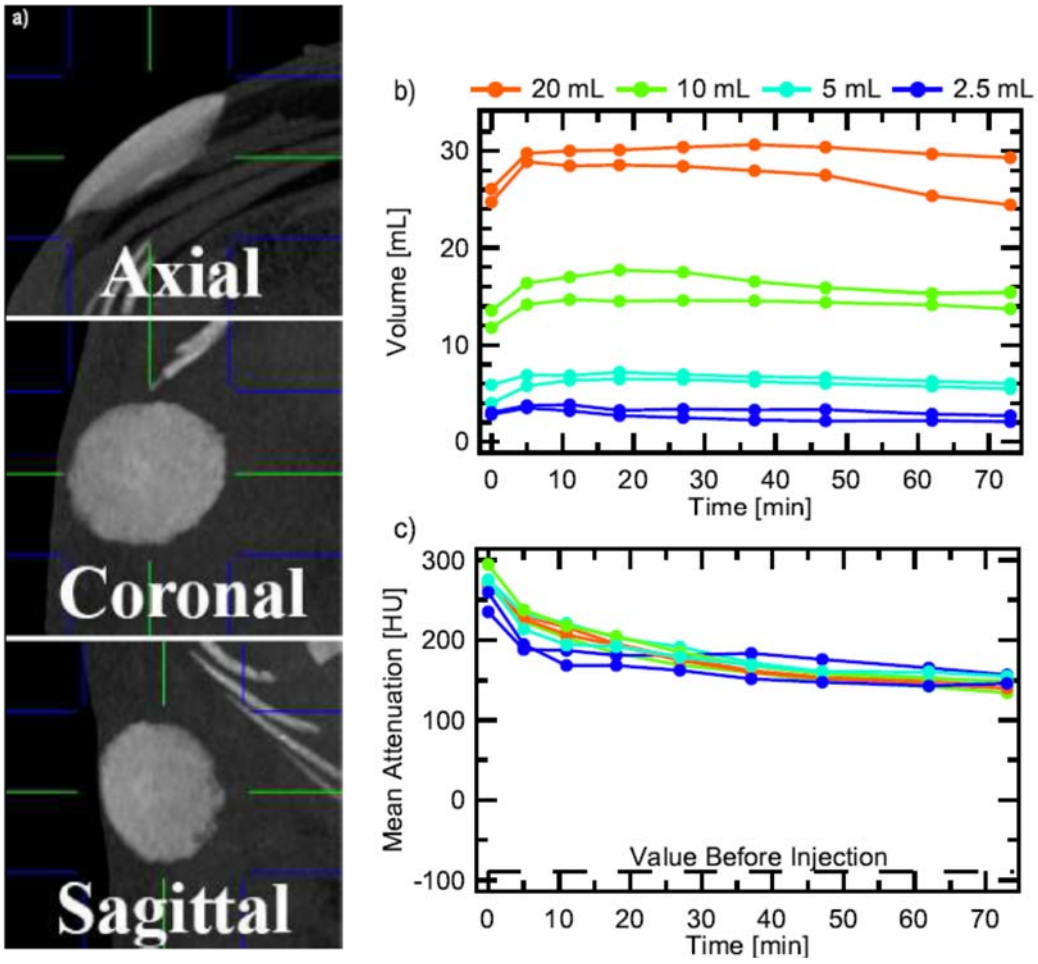

Figure 1: Computerized Tomography of an injection of contrast agent into the subcutaneous tissue of a live pig. The cheated iodine contrast agent has the same molecular weight as a small molecule antibiotic such as Cefazolin. From CT data one can determine the volume of the injected solution (b) as well as the mean attenuation (c). Note that the volume increases for the first 5 minutes. This is due to the permeation of the tissue by the contrast agent. The permeation is drvien by the pressure of the injection which swells the tissue. The lowering of the mean attenuation is consistent with the spreading out of the contrast agent. It is important to note that the contrast molecules are present near the injections site for over an hour.

From a sequence of these images we determine the permeation of the molecules in the subcutaneous tissue as a function of time. This led to another surprise: the injected fluid and molecules spread out on their own. There was no pooling or "fracking' of the subcutaneous tissue. External application of ultrasound did not enhance the spreading of the injected molecules. One can see that in figure 'b' the volume occupied by the contrast agent increases over time on its own. This is consistent with a decrease in mean attenuation in "c". These results were published by us "*Tumescent Injections in Subcutaneous Pig Tissue Disperse Fluids Volumetrically and Maintain Elevated Local Concentrations of Additives for Several Hours, Suggesting a Treatment for Drug Resistant Wounds*" [Phar Res 2020, 37, 51].

To interpret these findings we invoke Darcy's law of hydrodynamics for a porous medium. In the tumesced state, tissue permeability dramatically increases, enhancing fluid dispersal. Indeed, the hydraulic conductivity (Darcy permeability) of normal subcutaneous tissue is low, about 10^{-11} cm^4/dyne-sec [Swabb et al; 1974 *"Diffusion and Convection in Normal and Neoplastic Tissues."* Cancer Res. 34:2814], but can increase by over four orders of magnitude upon swelling [Guyton, et al 1966, "*Interstitial Fluid Pressure: III its Effect on Resistance to Tissue Fluid Mobility*." Cancer Res.;19:412]. This decrease in resistance to fluid flow accounts for the dispersal of a drug injected under pressure that is sufficient to swell up the tissue. As the pathogens reside in the subcutaneous tissue the direct injection by itself causes the antibiotic to reach them. According to Figure 'c' the contrast agent remains in the tissue for over an hour. This means that an antibiotic will have sufficient time to interfere with the pathogen. In other words the TMIC [Time above Minimum Inhibitory Concentration] is much greater than the reproduction time of the pathogens.
Although our hypothesis about ultrasound dispersing the drug didn't hold up it got replaced with a new insight. The same hydrodynamic theory which is the fundamental theory of high frequency sound also describes the response to pressure gradients. Here it is the very low frequency hydrodynamic motion that leads to tissue permeation.

The enhanced permeability allows for visualization of tumesced tissue with diffusion-weighted magnetic resonance imaging (DW-MRI). DW-MRI is a non-invasive method of measuring the apparent diffusion coefficient (ADC) - a measure of the molecular diffusion of water - within tissue [32]. These measurements are also consistent with the revised hypothesis and are report in our key paper: Koulakis, J.P. *et al.2020 mentioned above.* Based upon this insight we applied for had issued a US patent **July, 2022** US 11,389,396 B2- *"Tumescent Antibiotic Injections for Treatment of Chronic Skin and Soft Tissue Infections."*

Proposal for Funding of a Phase 2 Clinical Study Implementing Our Revised Hypothesis that a Direct Antibiotic Injection Can Cure Chronic Wounds: Based upon the revised hypothesis we applied to the FDA for an IND for a phase 2 clinical trial to treat people with chronic wounds. Although we are using a known antibiotic the dosage and method of delivery is sufficiently different that this activity is classify as a New Drug. This study comes under the auspices of the Division of Infectious Disease. If the study is successful we will be issued an NDA [New Drug Application], which we expect will carry the opportunity for "Regulatory Exclusivity" when marketed. Our application to the FDA was approved and assigned IND# 167527; "A Phase 2 Randomized Safety and Efficacy Study Comparing Subcutaneous Tumescent Antibiotic

Administration with Standard of Care to Standard of Care only for the Treatment of Chronic Wounds."[Seth Putterman is the Principal Investigator]. We have applied to UCLA for approval of the Institutional Review Board [IRB] and are told that with an IND this should be straightforward. The complete clinical protocol [as approved by the FDA] is being sent as a separate attachment. In short Dr Vardanian will initially treat 30 patients with chronic wounds- 15 by "Standard of Care" [SoC] and 15 with direct injection plus SoC. The key data will be wound size which will be monitored over time after treatment. Dr Vardanian will not charge for his time. The donated funds will be used for the procedure room , investigational pharmacy expenses, study coordinators, biostatistics and "redcap" team; IRB and FDA coordinating. We estimate the study cost per patient as being about $3.5k. The breakdown is below. A donation of 100k will get us to the point where we have an excellent perspective on safety and a very good sense of efficacy.

Budget $100,000.

UCLA Investigational Pharmacy	9k
Procedure Room and expendable costs [$800./patient]	24k
Study coordinator	22k
IRB and FDA interfacing	10k
REDCap data base	11k
BioStats Including one month at 20% Dr Koulakis	18k
UCLA Donation Administration Charge	6k

Donation of $100,000. will be made in 3 installments:

1st installment. $60,000 by Nov. 30, 2023.

2nd installment. $20,000 by Dec. 31, 2023.

3rd installment. $20,000 by March 31, 2024.

The Donation would be through UCLA. Their tax determination letter is here:
https://ucla.app.box.com/tax-pdf-irs-determ-letter

The same letter also states that we are exempt from filing the Form 990 because we are a state institution. UCLA doesn't have audited financial statements, but the whole UC system does, and we are covered as part of that. The most recent is here: http://finreports.universityofcalifornia.edu/index.php?file=a133/2021report.pdf

UCLA will need a donation letter from the Veneklasen Foundation stating what the donation is for and the check can be made out to UC Foundation and mailed to :

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