NaNots: A Novel Nanomedicine for Treating COVID-19 Sepsis

By Lou Hawthorne, CEO, and John Dodgson, PhD, CTO, NaNotics, LLC

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1.0 Document Purpose

To describe the ultra rapid development of a novel nanomedical toolkit for significantly reducing sepsis-associated mortality of COVID-19. This toolkit will comprise a set of injectable nanoparticles called "NaNots," engineered to deplete the specific molecules that drive sepsis – deeply, rapidly and safely – without reducing immune competence in COVID-19 patients. The NaNot platform has already been prototyped and tested in mice against certain targets.

2.0 About COVID-19

SARS-CoV-2, the novel coronavirus that causes COVID-19, is now spreading at an exponential rate across the planet. New cases are doubling every 3-4 days in the U.S. – which now has more cases than any other country – and every 2 weeks globally (see the Johns Hopkins Coronavirus [John Hopkins] global case tracker, for latest casualty data). The infection rate will decrease eventually through shelter-in-place and herd immunity from recovered patients, but it's likely that hundreds of millions or more will be infected by year end, with millions dead or dying.

COVID-19 mortality varies by patient age and other factors, with estimates of overall mortality ranging from 1.4% [University of Hong Kong] to 3.4% [WHO], making it 14-34 times the 0.1% mortality of seasonal flu [CDC]. There is much uncertainly re: both growth rate and mortality, but one fact is undeniable: COVID-19 is a true pandemic, with catastrophic potential.

Multiple teams around the world are developing accelerated plans for new diagnostics for tracking / isolating infected patients, thus slowing disease spread. However, it's clearly too late now to stop the spread of COVID-19 by quarantine alone, even with the best diagnostics. Therefore, the main hope for stopping COVID-19 rests on vaccine development; however, these efforts are at least a year away from yielding relevant quantities of an effective vaccine. And as we learn each year with seasonal flu, no vaccine is perfect; for instance, a recent CDC study [CDC flu vaccine] determined that the 2018-2019 flu vaccine was only 29% effective.

Another way of responding to COVID-19 (and future pandemics) is to develop tools for reducing mortality and debilitating sequelae stemming from viral infection. If this can be done, the most seriously affected COVID-19 patients would instead experience what *most* COVID-19 patients experience: a relatively mild flu. COVID-19 mortality mainly stems from sepsis or ARDS (Adult Respiratory Distress Syndrome, a lung-centered sepsis complication)[Liu 2020][Zhou 2020][Zumla 2020]. Even patients who do not die of COVID-19 can suffer permanent debilitating sepsis sequelae including lung fibrosis or other organ damage, amputated limbs, disfigurement and cognitive deficits among other serious maladies [Shankar-Hari 2016].

Elevated cytokine levels typical of sepsis/shock are found in seriously ill COVID-19 patients [Diao 2020]. These same cytokines are elevated in ARDS occurring with sepsis [Yang 2017][Meduri 1995][Zumla 2020] and ARDS driven by coronaviruses [Channappannavar 2017].

3.0 About Sepsis

Sepsis – from the Greek word for "rot" – is commonly triggered by infections – viral, bacterial, fungal or parasitic. Sepsis is not an infection *per se* but rather an escalating immunologic hyper-

response to the above triggers. The etiology, complications and potential treatment of sepsis are largely independent of the trigger. There is currently no effective treatment for sepsis, which strikes 49 million people per year worldwide and kills 11 million – more than all types of cancer combined. Sepsis is the #1 cause of death in hospital, despite the fact that the stages and molecular drivers of sepsis are well understood. 100+ clinical trials treating sepsis with the existing pharmacopeia and device repertoire have failed.

3.1 Sepsis Etiology

The innate immune system normally responds to infection by deploying NK cells, macrophages and neutrophils to the site of infection. They release a cytokine (signal molecule) called TNF-α [Chaudhry 2013], which can directly kill transformed human cells (whether transformed by viral infections like COVID-19 – or by cancer). TNF-α is sometimes referred to as a "master cytokine" because it can trigger release of other cytotoxic cytokines such as IL-1β, IL-6 and others [Calandra 1991] [Chaudhry 2013].

Just a few molecules of TNF-α can kill virtually any non-immune cell in the body, so the immune systems of healthy individuals normally deliver TNF-α in an intelligent, cell-mediated manner directly to bad cells. When a molecule of TNF-α binds a (type 1) TNF receptor on an infected cell, it triggers an evolved self-destruct program called "apoptosis" (from the Greek for "falling off," like leaves from a tree) – a cellular "implosion" designed to prevent the release of replicated viruses (the opposite of what the virus is trying to do, which is burst a cell to spread viral copies).

TNF-α is also a "chemokine" – a molecule that attracts other cells. The ability of TNF-α to induce two discrete effects – killing of transformed human cells and recruitment of other immune cells – is a form of "pleiotropy" (from the Greek meaning "many turns" or effects). The pleiotropy of TNF-α evolved to enhance immune response to infection; however, it also lays the groundwork for sepsis. Under certain circumstances – including COVID-19 infection – a positive feedback loop can arise whereby TNF-α molecules released to destroy transformed cells induce recruitment of more and more immune cells which in turn release more and more TNF-α and other cytotoxic cytokines – a process known as a "cytokine storm" [Qiu 2011][Channappannavar 2017].

Eventually, this "storm" generates so many cytotoxic molecules in patient blood that major organ damage – and death – becomes inevitable. Patients can die quickly – a few hours or days. Rapid infusion of IV antibiotics against opportunistic bacterial infections that can arise in COVID-19 patients can improve survival rates, but once a cytokine storm is underway, eradicating pathogens – even if successful – may not save the patient. The debilitation and mortality of sepsis stems directly from the cytokine storm and consequent organ damage, and only indirectly from the original pathogenic trigger.

3.2 Sepsis Therapeutics to Date

Despite over 100 clinical trials in sepsis, there are no current FDA-approved therapies that improve sepsis survival [Frydrych 2017], except very slightly in narrow cases. There is a growing consensus among sepsis researchers that single agent therapeutics are "doomed to fail" in sepsis [Delano 2016]. A 2014 meta-analysis of 17 studies documented the limited outcomes possible with conventional drugs: 28-day all-cause mortality was reduced from 36.8% death rate (1478/ 4017) among patients in the placebo group to

34.9% death rate (1731/4954) among patients receiving TNF-α inhibiting drugs – aka "TNFi's" [Lv 2014]. Antibody drugs against TNF-α and IL-6 have been tested in sepsis and showed little benefit in terms of overall survival [Dinarello 2002][Qiu 2011].

TNFi's and related drugs all reduce immune competence and thus clearance of pathogens of all types; short-term benefits were generally offset by pathogen rebound leading to longer-term mortality. A further reason for the failure of TNFi's in sepsis is that the organ damage/failure and mortality characteristic of severe sepsis/shock is driven by other cytokines – especially IL-1β and IL-6 – in addition to TNF-α. All three targets must be mitigated to reduce COVID-19 mortality throughout the inflammatory phase of sepsis.

Understanding specifically why conventional cytokine-inhibiting drugs fail against sepsis is useful for understanding the design parameters of a next-generation strategy like NaNots. TNF inhibiting drugs – e.g. Enbrel, Humira and Remicade – are designed to bind and neutralize excess circulating TNF-α, primarily to treat chronic autoimmune diseases like rheumatoid arthritis. However, there's a membrane form of TNF-alpha (mTNF-α) on the surface of immune cells in addition to the pathogenic soluble form of TNF-α (sTNF-α) that drives sepsis. mTNF-α is essential for maintenance of immune competence – including fighting off the original pathogen (e.g. COVID-19) that triggered sepsis in the first place. TNFi's also bind mTNF-α, causing the reduction of immune competence which is a well-documented side effect of TNFi's; all of these drugs carry warnings of increased risk of infection and, ironically, sepsis as well [Ali 2013].

Another problem in treating sepsis with present generation TNFi's and antibody drugs against other inflammatory cytokines is that they have a very long "tail" (persistence) in the bloodstream. Adalimumab (Humira) has a half-life of 10-20 days [FDA Humira], the Infliximab (Remicade) half-life is 7.5-9.5 days [FDA Remicade] and the Etanercept (Enbrel) half-life is ~four days [Zhou 2005] [FDA Enbrel]. These drugs persist in high concentrations not just in circulation but also within tissue at sites of infection, interfering with delivery of TNF-α by immune effector cells and slowing or blocking disease clearance. This factor has led to the failure of TNFi's to prolong life in sepsis [Aderka 1998]. The median half-life of the IL-6 inhibitor tocilizumab (Actemra) is 13 days [FDA Actemra], which is also likely to lead to immunosuppression in later stage sepsis.

3.3 Sepsis Therapeutics: Future Design Parameters

A next generation toolkit for treating for sepsis is certainly feasible. It must rapidly deplete inflammatory targets from circulation, yet not deplete them at all from tissue, thereby breaking the systemic cytokine storm without blocking anti-pathogen immune activity. NaNots meet both of these requirements.

Anti-sepsis NaNots would ideally address both key phases of sepsis – the initial inflammatory phase in which organ damage/failure occurs, and subsequent "immune paralysis" that can occur in response to acute inflammation, leading to pathogen bloom. However, just addressing the inflammatory phase of sepsis could save many COVID-19 patients who will otherwise die. This approach is the focus of the rest of this proposal.

The first cytokine that spikes in the early inflammatory stage of sepsis – meaning the first few hours – is TNF-α. If COVID-19 patients exhibiting this initial TNF-α spike can be identified and TNF-α can be scavenged from their blood before it reaches pathogenic

levels, the secretion of additional inflammatory cytokines – IL-1β, and IL-6 especially – can be prevented or at least reduced, along with the damage they cause. For later stage patients, all three of these inflammatory cytokines may need to be depleted to prevent further tissue damage and to prevent the patient from slipping into Immune Paralysis. The targets must be depleted deeply and rapidly from blood, without blocking delivery of these molecules by immune cells in tissue – essential for clearance of infected cells.

4.0 About NaNots

NaNot nano-scavengers are a new, patented, injectable, fully biocompatible tool for molecular depletion of *soluble* targets in blood without impact on *membrane* forms of the same target. NaNots are 120 nm nanoparticles that contain capture agents for specific molecular targets beneath a porous shield, which makes the NaNots specific to soluble target forms. NaNots are capable of very rapid and deep target scavenging. NaNots are also engineered to remain in circulation and *not* to extravasate into tissue, thus avoiding neutralization of key cytokines in pathogenic tissue microenvironments, where clearance of infection occurs. NaNots themselves are cleared from circulation by macrophages within ~30 minutes post-injection.

4.1 NaNot Safety

Current and projected NaNot safety far exceeds that of most approved drugs. NaNots are made from materials known to be safe, as demonstrated in other nanomedicines that have cleared phase-1 safety trials at much higher doses than projected for NaNots. Also, NaNot specificity for soluble vs membrane targets eliminates the main cause of drug side effects: unintended cell membrane interactions. No toxicity has been observed to date in mouse, rat or cell viability studies. MTD studies will begin shortly.

4.2 NaNot Performance

NaNotics has already engineered a NaNot against mouse sTNF-α and confirmed *in vitro* that it can deplete recombinant sTNF-α from mouse serum by >90% in <5 minutes. We followed up with *in vivo* studies using lethal bolus doses of mouse sTNF-α one minute before and one minute after injection of anti-sTNF-α NaNots, both of which significantly reduced 24-hour mortality versus controls. We have also conducted multiple tests that confirmed efficacy of NaNot shielding against membrane forms of a soluble target, and are planning tests to confirm specifically that NaNots against sTNF-α do not reduce immune competence.

4.3 Target Measurement

Administration of NaNots in COVID-19 patients with sepsis should ideally be based on measurement of all three inflammatory targets with a companion diagnostic – initially using standard ELISA kits, and later a low-cost lateral flow immunoassay (like a pregnancy strip) – followed by injection of appropriate scavengers at the needed dose. Several days of iterative measurement and target depletion may be required to guide COVID-19 patients through acute sepsis, after which full recovery is possible.

In acute emergency situations in which such testing is not feasible, a combination of NaNots against all three targets could be safely injected in the initial cytokine storm stage of COVID-19. Although this is a less efficient use of NaNots – a resource issue, especially initially – it would provide immediate and potentially life-saving counteraction to each of

the three critically damaging cytokines, even if the precise elevation of each target (and hence the stage of sepsis) is not known. Such use of NaNots will not compromise later immune competence and recovery from infection – versus use of a mixture of antibody drugs, which would lead to long-term immunosuppression.

4.4 Development Plan

NaNotics has a long-term plan to develop six NaNots for treating sepsis, cancer and other diseases. Given the global emergency posed by COVID-19 sepsis, our team is prioritizing ultra-rapid development of three anti-inflammatory NaNots for treating sepsis. Assuming adequate funding and maximum regulatory acceleration, we can develop all three NaNots – including safety studies – in just 6 months. Cost would be $30-40m USD, yielding enough NaNots for testing in 30 human COVID-19 patients with sepsis.

Significant additional capital will be required to scale NaNot production thereafter to treat sepsis in the rapidly expanding global population of patients with COVID-19 – and future emergent viruses. This could be achieved in 4-6 months following human trials, assuming sufficient resources and regulatory support.

We can't stop COVID-19, but NaNots can help us to weather the (cytokine) storm.

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