

A new era of infection control

Immunity and medicine, in unison
against infection

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Executive Summary

The time has come for the biopharma industry to develop a better way to control infections than the century-old approach of antibiotic chemicals. Antibiotics changed medicine with their almost miraculous ability to treat infection. But today, we know that they:

- i Often cause severe microbiome damage
- ii Often fail to control many troublesome infections
- iii Typically generate resistance along the way, limiting their effectiveness

The solution is not to develop another antibiotic: the world already has over 100 good ones. We need a new approach that can treat infection in the settings where the antibiotic approach often fails. The body's natural immune system already identifies invaders, attacking pathogens while sparing the beneficial bacteria in the microbiome. We propose a therapeutic strategy that disables bacterial defenses to the immune system, thus working *in unison* with the body's natural responses. We call such molecules "*bacterial defense disablers*".

We envision our “bacterial defense disablers” idea as a bold new approach to managing infection control, and an idea with the potential to disrupt a century of thinking about the problem. The concept takes advantage of millions of years of evolution to allow the immune system to target pathogens, as it does so well, and when it attacks those pathogens, a bacterial “defense disabler” blocks the ability of bacteria to repair damage from the attack. This is hugely advantageous compared to killing bacteria wholesale with antibiotic chemicals, not least because it:

- i Preserves the healthy microbiome (which has not been targeted by the immune system)
- ii Enables eradication of “hidden” pathogens, and
- iii Avoids the usual paths to antibiotic resistance

A sweeping innovation like this could revolutionize infection control across many fields, and by virtue of its broad application represent a multi-billion-dollar business.

In the material that follows, we provide more detail on

- The clinical problems with existing antibiotics and how related, narrow spectrum strategies are merely false hope
- The expensive economic consequences of antibiotic treatment failure
- Detrimental health consequences of damage to the healthy microbiome
- The transformative potential of the new bacterial defense disabler approach as demonstrated *in vitro* and *in vivo* using a molecule in development by our company, Unison Medicines
- Application of bacterial defense disablers to recalcitrant urinary tract infections, as an example of active infection control
- Application of bacterial defense disablers to immuno-oncology, as an example of passive infection control

We envision a world of better infection control, without antibiotics.

Introduction

Why is a better way needed to control infections than the centuries-old approach of antibiotic chemicals? Antibiotics fail to control many troublesome infections, may generate resistance along the way, and have destructive off-target effects on the desirable bacteria of the microbiome. Why should we accept that? Instead, we propose working *in unison* with the body's natural immune system to identify the invader pathogen and disable its defenses so that the immune system can clear it away. We call our approach "*bacterial defense disablers*".

Bacterial defense disablers dramatically empower the patient's own immune system to clear infection, yet induce little disturbance to the healthy microbiome. The key: bacterial defense disablers do not any kill bacteria outright. By not exposing the gut to a killing agent, the amount of resistance generated by bacterial defense disablers falls to a minimum—many orders of magnitude less than what antibiotics generate. The gut is preserved, which in turn eliminates tolerability problems and the harmful side effects caused by many antibiotics. Because the therapeutic mode of action differs from all previous medications, it works whether pathogens have previously developed resistance to antibiotics or not.

Beyond this, bacterial defense disablers can treat infectious syndromes where antibiotics have always struggled, even in the days before antibiotic resistance. The most difficult chronic infections often defeat antibiotic treatment by the infectious agents embedding themselves inside host cells, where antibiotics typically cannot reach, and where immune clearance is of limited efficacy. For example, antibiotics often cannot treat recurrent urinary tract infections (UTIs), which persist because bacteria shelter inside bladder cells, making themselves inaccessible to antibiotics.

Bacterial defense disablers will bring great benefit to all infection control stakeholders. Patients will benefit from new, non-antibiotic options, especially in the many settings where antibiotics do not work well today (resistant infections, chronic infections, non-life-threatening infections, intracellular infections, and infections during cancer care). Physicians gain a new, patient-friendly therapeutic option. The stewardship community can support an effective, non-antibiotic option that allows the most critical antibiotics to be reserved for the dire situations where they are most needed.

Importantly, bacterial defense disablers will also reward financial investors with a sizable market opportunity. Bacterial infection remains a health threat approximately the size of cancer (see Figure 1). Bacterial resistance makes the problem worse, even in developed countries, and treatment failure rates have risen to shockingly high levels and cost our health systems dearly. Modern and expensive medical procedures assume

functioning infection control: surgery, cancer care, transplantation, and auto-immune care. In many of these settings, it is understood that antibiotics are a poor way to meet patient need. This is particularly true in the emerging field of immuno-oncology. Attempts to develop more sophisticated antibiotics are unlikely to work in any of these settings, because the problems are too fragmented for antibacterials to solve. Bacterial defense disablers offer a new strategy that can solve these fragmented problems by harnessing the comprehensive power of the immune system. Bacterial defense disablers are a general solution with numerous clinical and market opportunities.

DEATHS EACH YEAR, WORLDWIDE

Same order of magnitude

Cancer



Bacterial infection



Figure 1. Bacterial infection carries 79% of the cancer death toll. Antibiotic resistance makes infections increasingly harder to treat.

Bacterial defense disablers are not a theoretical idea: Unison Medicines has developed a bacterial defense disabler that has demonstrated the ability to work with the immune system to defeat infection in reliable models. Our agents are administered orally and show no discernible effect on the healthy microbiome. They work against the most challenging, recalcitrant, and resistant Gram-negative bacteria designated as priority pathogens by the WHO and CDC.

We are excited by the observation that when other fields have developed new treatment paradigms, they have rewarded investors with healthy returns. To make the parallel explicit, innovators in rheumatoid arthritis, cardiovascular disease, and melanoma developed novel treatment paradigms that were not simply better versions of steroids, statins, and

chemotherapy. Instead, they found novel treatment strategies to address the underlying medical need, and in doing so, created blockbuster medications (see Figure 2). Defense disablers are the way to repeat this playbook for infection control, which has equally great—if not greater—unmet need.



Figure 2. Approved therapeutic strategies per indication, plotted at the year the first agent acting on that lever was approved.

Bacterial defense disablers will solve infection control problems in ways a new antibiotic never could. They will underwrite medicine in the 21st Century in the way that antibiotics did in the 20th Century.

Let us tell you how.

01

The end of the antibiotic era

THE LIMITATIONS OF THE ANTIBIOTIC TREATMENT PARADIGM

Modern medicine relies on the effectiveness of antibiotics. They are true wonder drugs, but—as a class—antibiotics are not a perfect answer to the management of pathogenic bacteria, for at least three major reasons.

- i Antibiotics are usually insufficiently selective, indiscriminately killing most bacteria they encounter, including beneficial ones. Killing beneficial bacteria causes harm. More selective, better targeted anti-pathogen therapies are needed.
- ii Antibiotics have great difficulty in dealing with pathogens where they hide. Antibiotics easily kill free-floating planktonic bacteria, but pathogens commonly employ evasion strategies such as secreting and coating themselves with protective biofilms, or invading host cells and subverting them to act as protective, nutrient-rich havens. Most antibiotics have difficulty penetrating and/or killing once inside these niches (see Figure 3).

1.27 M

deaths per year attributed directly to antibiotic-resistant infections

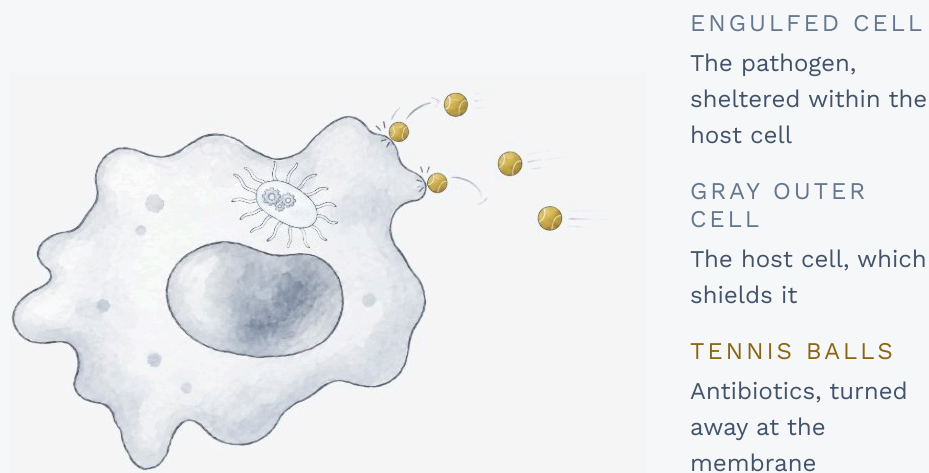


Figure 3. Some bacteria (engulfed cell) adapt to survive inside host cells (gray outer cell), which shield the bacteria from antibiotics (tennis balls). Most antibiotics either cannot enter host cells in any meaningful quantity (e.g., beta-lactams), or have dramatically reduced activity against intracellular bacteria (e.g., fluoroquinolones).

- iii Finally, bacteria quickly evolve resistance, and “wonder drugs” lose their effectiveness. Society constantly needs new antibiotics, in a never-ending arms race, but, for economic reasons, few new antibiotics are being developed. This phenomenon of antimicrobial resistance (AMR)¹ threatens to undo much of the medical progress of the 20th Century and cause literally trillions of dollars of economic harm.²

Collectively, these three reasons render antibiotics far less clinically successful than might be expected. Indeed, antibiotics fail with shocking frequency and deadly consequences. For serious infections, treatment failure rates have grown as high as 20% (sometimes much higher). When antibiotics fail, risks to patients and costs to healthcare systems rise dramatically. We estimate that antibiotic treatment failure costs the US healthcare system a conservative \$27B every year in excess costs.

~\$27 B

annual US excess cost of
antibiotic treatment failure

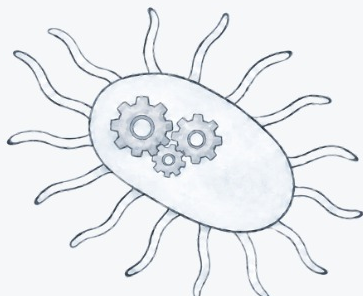
*DISABLING BACTERIAL DEFENSES INSTEAD OF OUTRIGHT KILLING WILL
TRANSFORM INFECTION CONTROL*

We propose a completely new approach to handling bacterial infection that we are convinced can be as transformative in the 21st Century as antibiotics were in the 20th: rather than kill bacteria outright with a chemical, we propose a therapeutic strategy of disabling bacterial defenses so that the immune system can handle the infection. Disabling bacterial defenses weakens bacteria against the immune system, which then clears the infection much more easily than it otherwise would. In principle, many bacterial enzymes are suitable targets for this strategy, particularly those involved in stress responses which are primarily triggered by immune assault. In this way, a bacterial defense disabler deprives bacteria of a critical response to the immune system (see Figure 4).

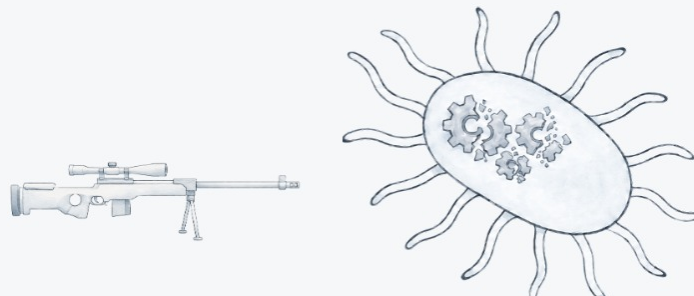
We see profound advantages implied by this “disable defenses” strategy, which can succeed in each of the three ways antibiotics fall short:

- i Achieve exquisite selectivity in targeting pathogens. This works by taking advantage of millions of years of co-evolution between the immune system and the bacteria our bodies host. The human immune system has a remarkable ability to distinguish between desirable bacteria, which it identifies as benign, and pathogens, against which it mounts a response. Weakening bacterial defenses matters hardly at all for the benign and healthy bacteria, but tremendously for the pathogens facing immune attack. The disable-defenses strategy works in unison with the immune system to achieve the desired selectivity which antibiotics lack.

1 · Bacteria have various internal cellular machinery



2 · The immune system specifically targets pathogens and damages their internal machinery



3 · Bacterial repair enzymes repair the damage and the pathogens live



4 · Defense disablers deactivate those repair tools. Pathogens cannot repair damage, so they die



Figure 4. Mechanism of action for defense disablers. 1. Bacteria have various internal cellular machinery. 2. The immune system identifies the pathogen and targets it with various methods of inducing damage. 3. Bacterial repair tools (enzymes) mend the damage caused by the immune system, allowing the pathogen to survive. 4. Bacterial defense disablers deactivate the repair tools (enzymes). The damage caused by the immune system remains unrestored, and the bacteria die.

- ii Target pathogens where they hide. Most antibiotics in the armamentarium have been co-opted from natural sources. They were then optimized by scientists to increase the spectrum of bacteria they could kill. They were not optimized, either by evolution or by chemists, to penetrate intracellular host niches or biofilms. A bacterial defense disabler could and should be designed with these goals in mind.
- iii Sidestep the problem of antibiotic resistance. A bacterial defense disabler would, by definition, target bacterial machinery never

previously targeted by a drug, rendering most current resistance mechanisms irrelevant. It could treat susceptible and resistant infections alike.

02

The birth of a new treatment paradigm

UNISON MEDICINES HAS INVENTED A PRACTICAL BACTERIAL DEFENSE DISABLER

We describe not merely a theoretical idea, but a real scientific achievement. At Unison Medicines, we have developed first-in-class, orally bioavailable small molecules which target an enzyme critical to bacterial defense. The concept works: we have demonstrated proof of principle in animal models of infection (see Figure 5). Moreover, our bacterial defense disablers achieve the ideal profile, succeeding in each of the three characteristics where antibiotics fail. A single molecular entity, designated as UNI-568911, generated the data below.

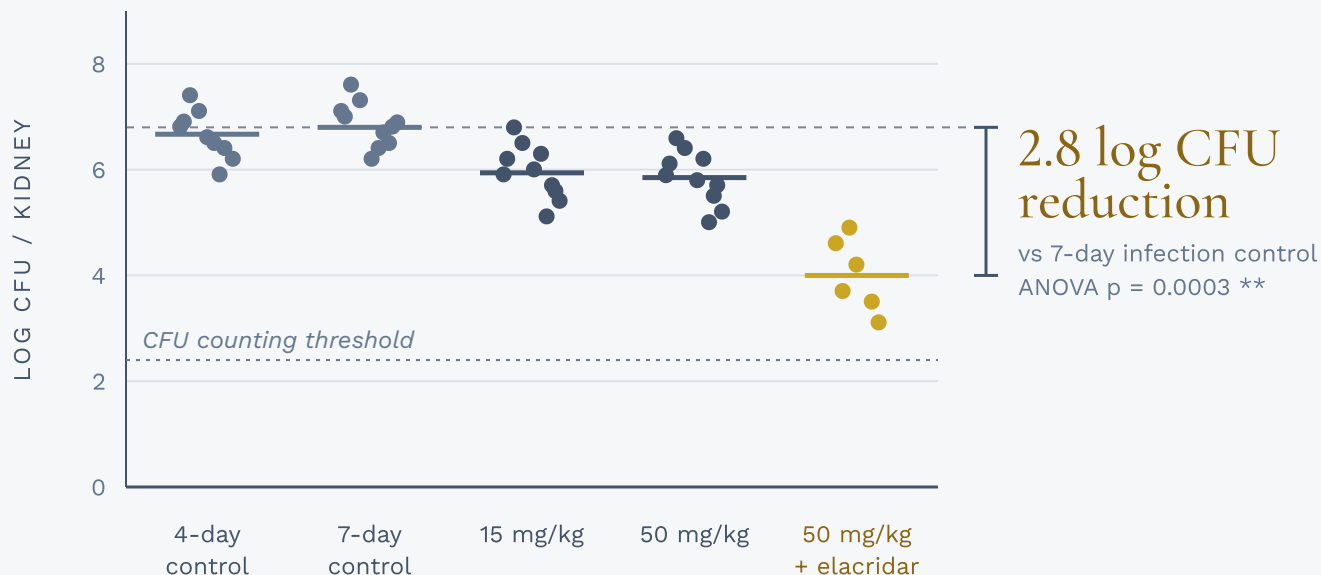


Figure 5. Monotonic reduction in bacterial Colony Forming Units (CFU) produced by oral administration of UNI-568911 in a chronic, 7-day model of mouse urinary tract infection (UTI). Administration of UNI-568911 on days 4–7 post-infection, dosed every 6 hours. The highest exposure group included combination with elacridar, which traps higher concentrations of UNI-568911 in macrophage immune cells. ** indicates statistical significance by ANOVA $p = 0.0003$. Dose response trend was statistically significant by the Jonckheere–Terpstra test for ordered exposure: $Z = -2.83$, one-sided $p = 0.002$. Calculated with $N=6$ in highest group.

- i UNI-568911 has no discernible impact on the healthy microbiome, demonstrating the selectivity achieved by working with the immune system against pathogens only (see Figure 6).
- ii UNI-568911 accumulates in the intracellular reservoirs where many of the most intractable pathogenic bacteria hide (see Figure 7). As described above, many of the most virulent pathogens invade and co-opt immune cells. Inside, they raid host nutrients and can shield themselves from antibiotics and the action of other immune system responses. UNI-568911 flips the advantage back to the immune system. Immune cells actively take up UNI-568911, where it then concentrates inside specialized killing vesicles (lysosomes)—the very location that pathogens themselves hide (see Figure 8). The drug goes to the exact site where it is needed to weaken bacterial defenses and

restore the immune cells from co-opted hiding places back into pathogen-killing machines.

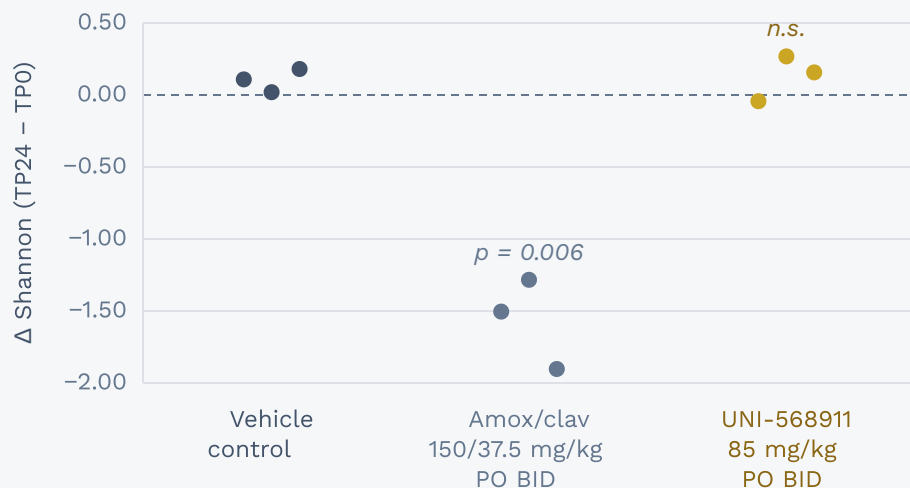


Figure 6. Mice receiving high-dose oral UNI-568911 showed no reduction in microbiome diversity (Shannon index), while amoxicillin/clavulanate caused significant disruption. Shannon diversity change calculated from stool samples collected before and after 24 hours of test article administration. 150/37.5 mg/kg PO BID for amoxicillin/clavulanate chosen as clinical dose often used in mouse studies. For each group, N=3.

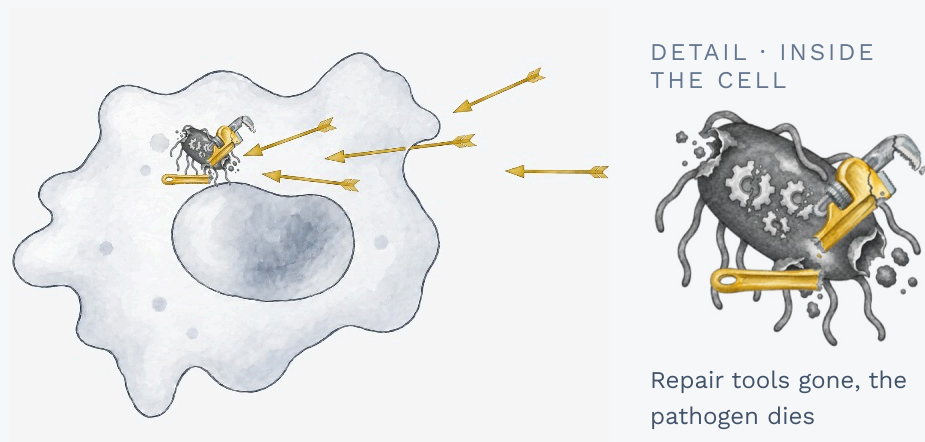


Figure 7. UNI-568911 (gold arrows) accumulates within the host immune cell, where bacteria attempt to hide. UNI-568911 weakens bacterial defenses to the host response. Unable to effectively repair damage, bacteria die (dark blue) and infection is cleared.

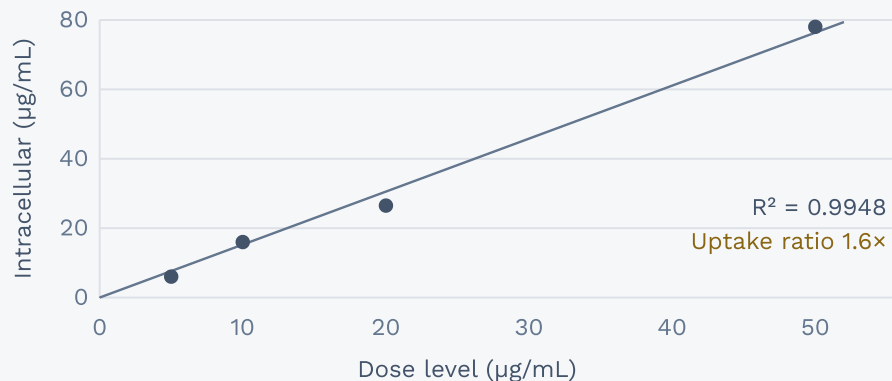


Figure 8. UNI-568911 uptake in THP-1 macrophages (4-hour incubation). UNI-568911 is actively concentrated within macrophages at a ratio of 1.6x across doses, showing excellent cell penetration. Furthermore, UNI-568911 shows lysosomotropism: a phenomenon whereby basic molecule enters the lysosome and becomes protonated, “trapping” the molecule in the lysosome. When we repeat the experiment with lysosome formation inhibited, drug concentration nearly disappears.

- iii UNI-568911 both makes the problem of antibiotic resistance by current mechanisms largely irrelevant, and minimizes the rate of resistance development towards itself. UNI-568911 uses a mechanism that bacteria have not encountered before. Thus, UNI-568911 works whether the pathogen has previously developed resistance, or not. The graph below demonstrates nearly identical performance of UNI-568911 against a susceptible and multi-drug-resistant strain of *E. coli* (see Figure 9). And, because it is so much more selective than 20th Century antibiotics (see point 1), it puts far less pressure on the bacteria in the healthy microbiome, which we anticipate will slow the development of resistance to UNI-568911 itself. Finally, because it is new to the world, there are no pre-existing resistance genes in nature, which is a key vulnerability of current antibiotics adapted from natural sources and another reason we expect slow resistance development towards UNI-568911. The graph below demonstrates nearly identical performance of UNI-568911 against a susceptible and multi-drug-resistant strain of *E. coli*, which uses a laboratory damaging agent as a surrogate for damage induced by the immune system (see Figure 9).

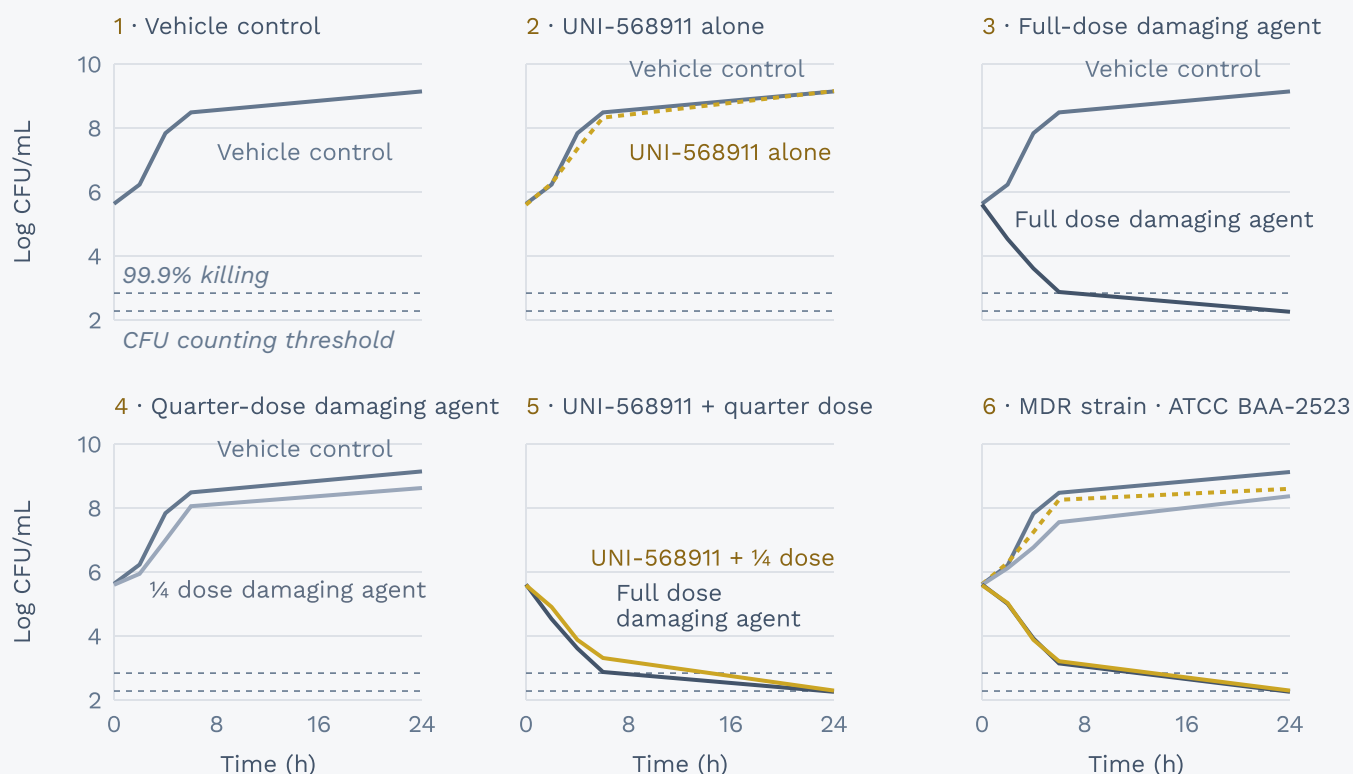


Figure 9. UNI-568911 bacterial killing curves against a non-resistant, laboratory quality control strain of *E. coli* (ATCC 25922) (steps 1-5) and a multi-drug resistant, clinical strain of *E. coli* (ATCC BAA-2523) (bottom right). Activity is essentially unchanged between the two, despite the resistance of the second strain. Explaining the assay: Step 1: Vehicle control shows pathogen, *E. coli*, growth (gray). Step 2: Treatment with UNI-568911 alone shows pathogen growth is undisturbed (dashed gold line). Explanation: *E. coli* is undamaged, so the presence of a defence disabler (UNI-568911) has no effect. Step 3: At full dose, a damaging agent kills all pathogen (dark blue). Hypothesis: to mimic the in vivo immune environment, use a low-dose damaging agent Step 4: At ¼ dose, a damaging agent has minimal effect (light blue) and is insufficient to clear pathogen. Step 5: Combine small amount of damaging agent with UNI-568911 (gold line). Conclusion: Defence disabler combined with damaging ability typically produced by immune responses is dramatically effective at killing *E. coli*.

BACTERIAL DEFENSE DISABLERS WILL SERVE AN ENORMOUS MARKET

Novel antibiotics developed over the last decade have arguably been the biopharmaceutical industry's worst-performing commercial products. As evidence for this, large developers have exited the field, and the specialist developers who have commercialized novel antibiotics have generally gone

bankrupt (e.g., Achaogen, Aradigm, Melinta, Nabriva, Iterum, and others). Frequently cited reasons for antibiotic commercial underperformance include the widespread use of low-cost generic alternatives to new agents, which limits both pricing power and patient numbers; and short courses of treatment compared to chronic conditions, which limits revenue further.^{3,4}

The disabling defenses approach treats needs unmet by antibiotics, and therefore not subject to competition with cheap, generic antibiotics. The advantages described above—selectivity for pathogens; sparing the microbiome; killing bacteria where they hide; sidestepping antibiotic resistance—allow for the treatment of devastating and costly diseases in the many cases when antibiotics routinely fail.

Urinary tract infections (UTIs) are extremely common with 11% of American,^{5,6} and a similar percentage of British women developing one every year.⁷ Although the majority of UTIs respond to current antibiotics, a meaningful minority of infections do not. Chronic urinary tract infection (UTI) is a debilitating, life altering condition currently with no good solution. The primary reason for this is that the pathogen can embed itself into bladder cells, where it can hide from antibiotics and the immune system.⁸⁻¹⁴ We estimate that UTI infections will become chronic and unresponsive in 3.3 million American and 660,000 British women. As an initial use case, targeting chronic UTIs, which represent a multi-billion dollar market, is a very promising strategy.

We understand and can solve chronic UTIs. The Unison molecule, UNI-568911, is an ideal treatment because of its oral bioavailability, its gentleness to the microbiome, its activity against *E. coli* and other Gram-negative pathogens commonly involved in UTI, and its ability to reach the reservoirs in bladder cells where the pathogen hides. We recently generated proof of principle with UNI-568911 in a mouse model of chronic UTI. We see a multi-billion-dollar market for such a drug.

But the scope of the market for our compounds is far larger:

- i The next, highly valuable market segment we will pursue is respiratory infection, which conventional antibiotics fail patients at an alarming

rate with severe consequences, including high mortality. These infections include streptococcal pharyngitis (strep throat), pertussis (whooping cough), pneumococcal pneumonia, tuberculosis (TB), epiglottitis, atypical pneumonia (walking pneumonia) and diphtheria. We calculate the size of the Community Acquired Pneumonia market alone to be somewhere in the range of \$9B.

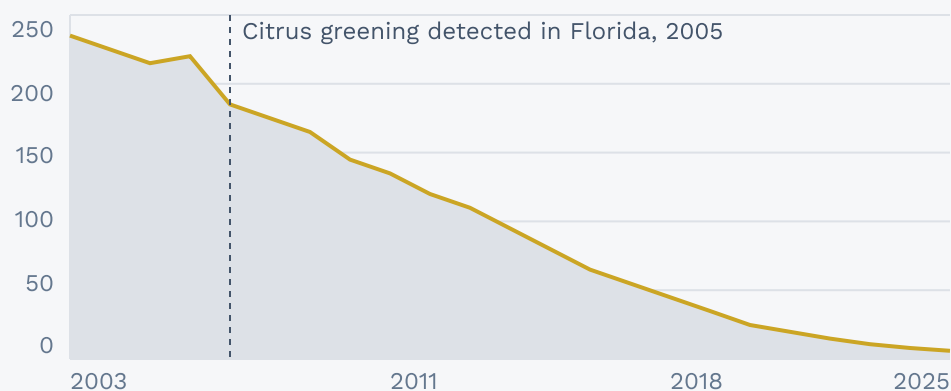
- ii Biothreat pathogens—bacteria that pose a national security risk—often hide in intracellular niches. All five priority bacterial biothreat pathogens are either intracellular: *Y. pestis* (plague),¹⁵ *F. tularensis* (a highly infectious bacterium that causes a severe zoonotic disease called tularemia),¹⁶ *B. mallei* (glanders, an often-fatal respiratory disease),¹⁷ and *B. pseudomallei* (melioidosis, a life-threatening infection)¹⁸ or have significant intracellular components as part of their infectious lifecycle, such as *B. anthracis* (anthrax)¹⁹. We think we can address several of these, too, with Unison’s technology.
- iii Our most successful immuno-oncology medicines require a healthy microbiome to function properly. Patients who receive antibiotics at the beginning of their PD-1 inhibitor therapies or CAR-T therapies experience dramatically worse outcomes, yet these patients are at high risk of infection.^{20,21} Bacterial defense disablers offer a way out of the paradox. They could be used prophylactically to prevent infection and reduce the need for patients to receive gut-damaging antibiotics. Value for this sort of treatment could easily surpass that of Neupogen/Neulasta, which sold over \$4B per year at peak and is now a mainstay of chemotherapy protocols. Mechanistically, Neupogen/Neulasta and related medicines are of little value to immuno-oncology patients. We see a future where bacterial defense disablers are a cornerstone of 21st Century cancer care.
- iv Livestock producers desperately need antibiotic alternatives. Regulators strongly prefer “antibiotic-free” meat products, and consumers will pay a premium. But intensive farming practices necessary to achieve unit cost efficiencies mean that, in practice, it has been hard for producers to avoid antibiotic use. Even so, antibiotics have only moderate efficacy. Defense disablers could be what

regulators, consumers, and producers all want: a non-antibiotic way of effectively dealing with infection. As it happens, many of the most valuable livestock health indications involve intracellular pathogens hiding from the immune system: mastitis in dairy cows,²² *Lawsonia* in hogs,²³ and *Piscirickettsiosis* in Chilean salmon (a \$700M annual problem).^{24,25}

96%

decline in Florida citrus
production since 2003

- Perhaps most surprisingly, bacterial diseases are devastating crop agriculture. New bacterial diseases afflicting crops emerge seemingly every year. Bacteria and their insect vectors thrive in warmer environments, and the rise of climate change appears to be driving an explosion in new diseases, many affecting premium crops.²⁶ Agrichemical companies have little to offer and most of these diseases are considered incurable. A particularly salient example, citrus greening disease, has infected nearly all of Florida's orange production capacity, which has declined 96% over the last 20 years (see Figure 10),²⁷ effectively ending the citrus industry in that once-productive state. Infection rates are now rising in other major global citrus producers, including California and Brazil. There is no cure.



MILLION BOXES OF CITRUS, FLORIDA · VERTICAL

Figure 10. Decline in Florida's citrus production since 2003, as reported by USDA. Citrus greening disease is almost solely responsible for the 96% decline.

But fortunately, plants *do* have immune systems²⁸ and bacterial defense disablers could work in crops to overcome disease. Bacterial defense disablers could be an ideal, environmentally friendly way to address the

multi-billion-dollar need for bacterial crop protection. Because they are not antibiotics, they would not have the same environmental damage potential as using antibiotics directly on our crops (which is the current “solution” for citrus greening). Here, too, this is not a theoretical idea: Unison has demonstrated that UNI-568911 works at least as well in clearing citrus greening bacteria, *Candidatus Liberibacter asiaticus* (CLAs), as the antibiotic currently in use (see Figure 11).²⁹

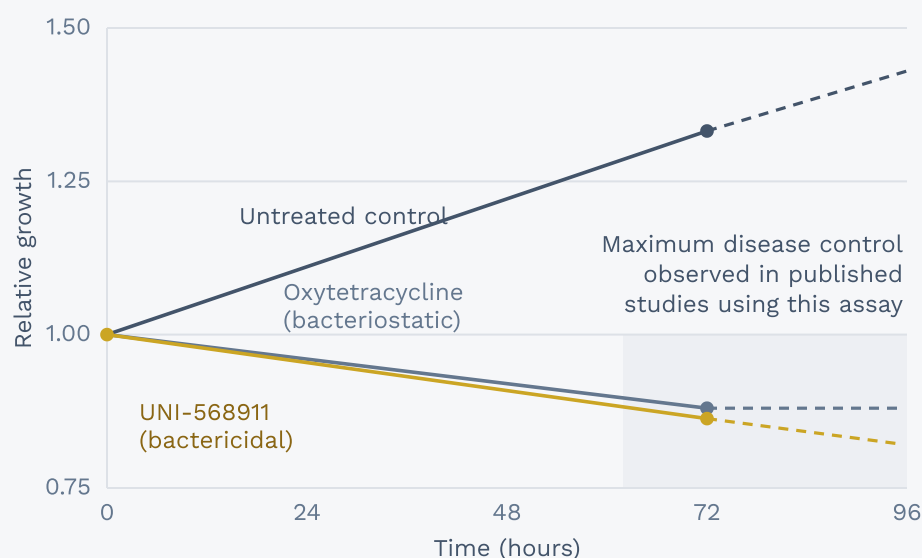


Figure 11. Unison’s lead asset, UNI-568911, achieves the highest bacterial clearance reported in the industry-leading assay. The assay uses miniaturized citrus roots infected with CLAs to evaluate treatment performance in a laboratory setting. Until this assay was developed, studying citrus greening treatments was extremely difficult, because CLAs does not otherwise grow in the lab. In addition to the environmental benefits over oxytetracycline (OTC), we anticipate that UNI-568911 will continue to reduce CLAs concentration over time, unlike OTC, which is bacteriostatic. Dashed segments are projections beyond the 72-hour measured timepoint.

The field of bacterial disease treatment is ripe for disruption. A better solution in any of these fields could command a multi-billion-dollar market. A platform technology that can be proven in one field and then expanded into others will produce a category-defining company.

Beyond the markets, bacterial defense disablers could make a major impact on global public health. Unison's technology could be deployed efficiently to low- and middle-income countries, because our small molecules can be manufactured and distributed at scale with a highly competitive cost structure.

Our ambition is to redefine infection treatment in the 21st Century, the way antibiotics did in the 20th.

03

The antimicrobial resistance paradox and the real crisis of treatment failure

At Unison, we think the way the world analyzes the antimicrobial resistance (AMR) problem is misguided. For many years now, highly credible professionals in the field have alerted us to the problem of “superbugs”—pathogens which carry resistance mechanisms to many or all of our current drugs. These superbugs are terrifying and real, and the specter of a world filled with them is even more petrifying. As we write today, however, superbugs present rather infrequently and are a narrow, highly specific use-case. Even aggregating them together, the number of patients infected by various superbugs each year cannot create a viable patient population for a commercial drug product.

In stark contrast, antibiotic treatment failure (whatever the cause) is common and costly. Treatment failure can be addressed, to a degree, by using stronger front-line antibiotics. In fact, the field responded this way over the past several decades. Third-generation cephalosporin antibiotics, once considered last-resort, now serve as the first-choice antibiotics for severe infections like pneumonia.³⁰ Doing so perpetuates the development of resistance in a never-ending arms race. It is time to end the arms race by taking a new approach.

THE DRAMA OF THE AMR PROBLEM

Dame Sally Davies, the former Chief Medical Officer for England, brought the rise of AMR to popular attention in her 2013 short book, *The Drugs Don't Work*, in which she placed the AMR problem on the same scale as climate change.³¹ Indeed, AMR has become a widespread clinical problem and can render even basic medical procedures life-threatening.³² If AMR continues to rise, we face the alarming prospect of the world reverting to a pre-antibiotic era, where routine surgeries bring risk of death and cancer patients succumb not to their malignancy, but to secondary infection.

An oft-cited report published in 2016, commissioned by the UK Prime Minister David Cameron and chaired by economist Lord Jim O'Neill, alerted the world that barring intervention, AMR would take 10M lives globally each year and cause a cumulative \$100 trillion in economic damage by 2050. If reality is anything close to this, the world will have paid dearly. The report asserted that AMR is one of the most pressing health issues of the 21st Century.²

More immediately, an extremely rigorous investigation by the AMR Collaborators, led by Christopher Murray at the University of Washington, reported in *The Lancet* that AMR already kills 1.27M people per year, and contributes to the death of a total of 4.95M.^{1,33}

A PARADOXICALLY SMALL PATIENT POPULATION

Once we look at specific resistance problems, an entirely different picture emerges. The Centers for Disease Control and Prevention (CDC) reports numbers of infections per year for its priority, antibiotic-resistant pathogens.³⁴ Carbapenem-resistant *Acinetobacter* infections cause 8,500

hospitalizations and 700 deaths annually.³⁴ Carbapenem-resistant Enterobacteriaceae infections cause 13,100 hospitalizations and 1,100 deaths annually.³⁴ Each of these pathogens causes a small subset of more general infection categories: for example, *Acinetobacter* is often implicated in ventilator-associated pneumonia or catheter-associated bloodstream infections. Compared to the tens of millions of antibiotic prescriptions written every year in the US, the number of patients with these very scary, drug-resistant infections is vanishingly small. This is partly because our current antibiotic armamentarium is very large, with somewhere between 80–100 distinct molecular antimicrobial agents commercially available in the US. The odds of finding a drug that can kill any given causative pathogen are high, even if the pathogen has resistance genes.

What we see in this mismatch between the very real AMR crisis headline numbers, and very real but very small priority pathogen patient populations, is that the world has narrowly defined the AMR crisis in terms of specific pathogen-drug resistance combinations: Carbapenem-resistant Enterobacteriaceae, Methicillin-resistant *S. aureus* (MRSA), etc. When framed this way, the target populations are relatively small and so there appears not to be an urgent need for new medicines. Indeed, pharmaceutical companies have essentially stopped investment in novel antibacterial drugs because the market demand for new agents is close to zero. This trend is well-documented and reported elsewhere.³⁵

THE SCALE OF ANTIBIOTIC TREATMENT FAILURE IS LARGE

Drug-pathogen resistance combinations focus on the *bacteria*, which is only part of the clinical story. Our view is that we should be defining the unmet need in terms of the many millions of at-risk *patients* and their *clinical outcomes*. The real unmet medical need is antibiotic treatment failure—whatever the cause—not antibiotic resistance per se. We initially presented this view recently in the *Journal of Antibiotics*, and elaborate here with more detail.⁴

When we survey the medical literature, we find that rates of treatment failure are high, often shockingly so. The costs are high, too, both for

patients and the healthcare system. Mortality rates are far higher in these treatment failure patients, as are the total costs of care for them. They require extended time in the hospital, the intensive care unit, or additional procedures to manage complications.

CAUSES OF TREATMENT FAILURE

As stated before, we posit the primary causes of treatment failure are:

- i AMR means many empiric drugs do not work well.
- ii Antibiotics have great difficulty in dealing with pathogens where they hide.
- iii Antibiotics are usually insufficiently selective, damaging the healthy microbiome, which is necessary for a healthy immune response.

To elaborate on these ideas and reference the underlying research:

The largest and most straightforward cause of antibiotic treatment failure is antibiotic resistance. Most intuitively, if the pathogen is resistant to the drug being administered, treatment will have no utility. By definition, a patient with an infection resistant to empiric therapy will be a treatment failure case, requiring an antibiotic switch. The global study published in *The Lancet* in 2022 brought this clearly to light: AMR kills 1.27M people per year, and contributes to the death of a total of 4.95M.¹ Other studies have correlated rates of antibiotic resistance to rates of sepsis.⁷³⁻⁷⁵ This observation is consistent with the idea that treatment failure patients are more likely to progress to sepsis, and antibiotic resistance is a cause of treatment failure.

Second, pathogens' defense mechanism of "hiding" drives treatment failures. The hiding, itself, takes at least three major forms:

INFECTIOUS SYNDROME	ANNUAL U.S. EPISODES	FAILURE RATE	EXCESS COST PER FAILURE	ANNUAL BURDEN	EVIDENCE BASIS
Diabetic foot infection	750,000 ³⁶⁻³⁹	20% ^{36,40,41}	\$25,000 ³⁸⁻⁴¹	\$3.75 B	Evidence-informed proxy
Community-acquired pneumonia, inpatient	1,500,000 ^{42,43}	15% ^{44,45}	\$25,000 ^{44,46}	\$5.63 B	Direct failure comparison; charges
Skin and soft tissue infection	18,000,000 ^{47,48}	15% ^{49,50}	\$2,500 ^{49,50}	\$6.75 B	Direct failure comparison
Complicated urinary tract infection	2,900,000 ⁵¹	25% ⁵²	\$5,000 ^{51,52}	\$3.63 B	Evidence-informed proxy
Community-acquired pneumonia, outpatient	4,500,000 ^{42,53}	22% ^{53,54}	\$2,800 ⁵³	\$2.77 B	Direct failure comparison
Ventilator-associated pneumonia	100,000 ⁵⁵	45% ⁵⁶⁻⁵⁸	\$25,000 ^{55,58}	\$1.13 B	Evidence-informed proxy
Complicated intra-abdominal infection	300,000 ^{59,60}	20% ^{59,60}	\$10,000 ⁵⁹⁻⁶¹	\$0.60 B	Direct failure comparison; charges
Hospital-acquired pneumonia, nonventilated	200,000 ^{62,63}	30% ^{56,57,62}	\$15,000 ^{62,63}	\$0.90 B	Evidence-informed proxy
Uncomplicated urinary tract infection	8,000,000 ⁶⁴	13% ⁶⁵⁻⁶⁷	\$1,000 ^{65,67}	\$1.04 B	Direct failure comparison
Primary bloodstream infection	300,000 ⁶⁸	20% ⁶⁹⁻⁷¹	\$17,500 ^{70,72}	\$1.05 B	Resistance and delay proxy
Total	—	—	—	\$27.25 B	Rounded headline \$27 B

Table 1. Summary of bacterial infectious syndromes with costs of treatment failure estimated at over \$0.5B per indication. Dollar estimates are approximate 2025 values. Direct comparison means that the source compared failure with success, although some studies reported charges rather than accounting costs. Proxy rows are explicit analytical estimates anchored to disease, resistance, readmission, delay, or length-of-stay evidence.

i *Biofilm formation*: Chronic infections, non-responsive to antibiotics, appear increasingly to be associated with biofilms.⁷⁶⁻⁷⁸ Biofilms are a self-protective matrix of extra-cellular DNA held together by lynchpin proteins.^{79,80} Bacteria live in the matrix, shielded from both host immune response and antibiotics. Biofilms have been associated with chronic infected wounds;^{81,82} the respiratory tract of cystic fibrosis patients;⁸³⁻⁸⁵ the sinuses of chronic rhinosinusitis patients;⁸⁶ deep tissue infections of bone⁸⁷ and heart valve;⁸⁸ and recurrent urinary tract infections.⁸⁹ In short, most chronic infections that do not respond to antibiotics appear to be associated with biofilms,⁹⁰ and bacteria within biofilms are up to 1000x more tolerant of antibiotics than when free-living.⁹¹

1000×

antibiotic tolerance of bacteria
inside a biofilm

ii *Intracellular niches*: Bacterial survival strategies can include invading host cells, which then become a shelter from most antibiotics (most of which penetrate poorly), and from adaptive immune responses from T cells and antibodies. For many pathogens, concealment within an intracellular niche is the dominant survival strategy. These diseases are marked by the need for extremely long courses of multiple antibiotics, precisely because antibiotics penetrate host cells poorly. As previously described, this is the case with antibiotic-refractory (“chronic”) UTI, and all of the biothreat pathogens. It is also famously the case for tuberculosis^{92,93} and nontuberculosis mycobacteria, both of which are caused by pathogens that invade respiratory immune cells, then require multi-drug treatments for six months or longer, and still result in high treatment failure rates.⁹⁴

Beyond these well-known examples, other intracellular infectious syndromes include: leprosy (Hansen’s disease);^{95,96} typhoid fever;^{97,98} invasive *Salmonella*;⁹⁹ Legionnaires’ disease;^{100,101} chlamydia;¹⁰²⁻¹⁰⁴ listeriosis;^{105,106} Whipple’s disease;¹⁰⁷ and more.

Perhaps more surprisingly, many other infectious syndromes driven by more common organisms have treatment failures driven by otherwise susceptible pathogens hiding in intracellular niches. This includes: *S. aureus*, which forms intracellular small-colony variants in chronic/recurrent osteomyelitis and relapsing MRSA bacteremia.^{108,109}

It can also include *S. pyogenes* in recurrent pharyngitis with penicillin treatment failure.^{110,111} Intracellular *E. coli* have even been strongly linked to Crohn's disease.^{112–114}

- iii *Persister populations*: A third way bacteria can hide is by a “persister” phenotype.^{115,116} These bacteria lack genetic determinants of resistance, yet are not killed by antibiotics. In these cases, the pathogens typically enter a dormant metabolic state under antibiotic stress. In this state, the antibiotics have little efficacy. Once antibiotic pressure subsides, the pathogens revert to metabolic activity, causing disease relapse. The persister phenotype is often found within bacteria hiding in intracellular niches and biofilms, so this mechanism is often complementary to other hiding strategies already described.

Third, because antibiotics are insufficiently selective, they kill many helpful bacteria in our microbiomes as collateral damage to the pathogen of concern. Increasingly, we understand that the immune system and microbiome work together as an integrated defense to infection. Antibiotics work at cross purposes by killing the pathogens, but also by damaging the very system they are meant to protect. We discuss these problems in detail in our next chapters on [the link between the immune system and microbiome](#) and [the specific case in immuno-oncology](#).

For completeness, we acknowledge that there are other causes of treatment failure. Examples include: immune-compromised hosts, lack of adequate source control with infected prosthetics or catheters,¹¹⁷ and good drugs that are under-dosed.¹¹⁸ We argue that these causes, too, would not be improved simply with “stronger”, next-generation antibiotic. Under-dosing is solved with better pharmacology. Source control is improved by careful protocols. Even immune-compromised hosts would benefit greatly from some sort of combination therapy: adding a mechanism that is distinct from antibiotics to help the host clear the infection sooner.

WHAT SHOULD WE DO?

Up to this point, the world has responded to treatment failure by engaging in an arms race with bacteria. As resistance rates have risen, guidelines have moved stronger drugs into empiric therapy. Consequently, bacteria

developed more resistance with the result that still stronger drugs are moved into empiric therapy, and more “last resort” medicines developed. The arms race response has run its course and is no longer a viable solution. As mentioned, very few new antibiotics have been developed over the past several decades, and most of those that have received approval have failed in commercialization. Conglomerates have exited the field, and smaller developers have gone bankrupt.

The treatment history for gonorrhea provides a textbook example of the antibiotic arms race. Treatment began with sulfonamides when antibiotics first became widely available; then soon escalated to penicillin; followed by tetracyclines, then fluoroquinolones. Resistance development to front-line treatment precipitated each escalation. Cephalosporins are now the treatment mainstay, with resistance to ceftriaxone, an often-used member of the class, already emerging.¹¹⁹ Most other infectious syndromes exhibit similar escalation patterns over time.

Many in the field would suggest that the solution to escalation lies in a precision medicine approach: narrow-spectrum antibiotics targeted at very specific pathogens. While this would help overcome resistance, most approaches do not address hiding bacteria. Moreover, we think this direction is a commercial dead-end limited by extremely small biopharmaceutical markets, far below the one billion dollar a year threshold often used to benchmark a candidate drug’s commercial attractiveness. Compared to other opportunities a drug financier could fund with the same development dollars, the return on narrow-spectrum antibiotics is simply not compelling. We discuss this at length in [our chapter on narrow-spectrum antibiotics](#).

The stage is set for Unison and other innovators to do something different. Infectious disease treatment failure is an enormous problem, with massive unmet need. It will not be solved by making more potent antibiotics. A therapeutic that addresses the root causes of failure will be a clinical and commercial success. Defense disablers unlock the opportunity.

METHODOLOGY FOR ASSESSING COST OF TREATMENT FAILURE

Our analysis provides a bottom-up model of the US burden of bacterial treatment failure based on values reported in the literature. For each major infectious syndrome we estimate four quantities from the published literature: (i) the annual US incidence of the syndrome; (ii) the frequency of antibiotic treatment failure; (iii) the excess direct medical cost of a failure episode over an uncomplicated, successful course; and (iv) the excess mortality associated with failure. The annual cost of treatment failure for each syndrome is the product of the first three:

$$\text{Annual cost} = \text{Incidence} \times \text{Failure rate} \times \text{Excess cost per failure}$$

The cost of failure is defined as the excess resources consumed when first-line therapy fails, driven principally by additional hospital length of stay, complications and additional morbidity, re-intervention or surgery, and readmission. It is a healthcare-system (direct medical cost) perspective; indirect costs such as lost productivity are excluded. We seek to report the incremental cost of treatment failure in one individual as compared to a similar individual with the same infection type who does not experience treatment failure.

Definition: An intuitive definition of antibiotic treatment failure is “any situation where bacteria survive antibiotic treatment and the clinical symptoms of the infection persist”.¹²⁰ A working technical definition is: “any of the following events within 30 days of initial antibiotic: antibiotic refill, antibiotic switch, emergency room visit, or hospitalization”.⁵³

Unfortunately, there is not a standardized definition used in the field, which contributes to a wide reported range of treatment failure rates. In accordance with our objective of determining an order of magnitude to the cost of treatment failure, we do our best to apply this definition to the papers we cite, even if the authors do not use the same definition themselves.

Cost normalization (2025 USD). Source figures span publication years from 2008 to 2023. Every cost was inflated to 2025 dollars using the US Bureau of

Labor Statistics Consumer Price Index for Medical Care (CPIMEDSL, 1982–84 = 100). The deflators applied were approximately $\times 1.42$ (2012), $\times 1.32$ (2015), $\times 1.24$ (2017), $\times 1.22$ (2018), $\times 1.18$ (2019), and $\times 1.05$ (2023). The 2025 index (~590) was cross-checked against the mid-2026 published value (~594).

Excess mortality. Excess deaths are the additional deaths associated with failure (failure-vs-success mortality difference applied to the failing population) and are associational, not strictly causal: patients who fail therapy are frequently sicker at baseline, so the estimates should be read as an upper-bound signal of the human cost, not an attributable-death count.

Scope decisions. Nine types of infections were included as the most economically significant, with CAP split into inpatient and outpatient, yielding a total of ten analyses. Sepsis is deliberately excluded because it is a downstream state arising from a primary syndrome (counting it would double-count CAP, intra-abdominal, urinary, and bloodstream sources). Otitis media is excluded because the cost of failure per episode is low. Gonorrhea, endocarditis, osteomyelitis (as a standalone), and brain abscess were considered and excluded: gonorrhea has very high incidence but low cost per failure; endocarditis and brain abscess are comparatively infrequent; and diabetic-foot osteomyelitis is captured within the diabetic foot infection row. To avoid double-counting bacteremias that are secondary to the pneumonias, UTIs, and intra-abdominal infections already modeled, the bloodstream-infection row counts primary bloodstream infection only (we estimate approximately half of the ~600,000 annual US episodes).

Limitations. This work is meant to:

- i Illustrate what the literature already reports in terms of treatment failure frequency and costs.
- ii Provide an aggregate estimate across major infectious syndromes.

Our methodology is therefore secondary research, limited by the different definitions, methods, and sources that the underlying studies used, and the difficulties inherent in combining diverse sources. Our results are estimates, not firm calculations.

04

One integrated system: the immune system and microbiome

We think it appropriate to consider the immune system and microbiome as one integrated system working together to ward off infection. While antibiotics are wonder drugs, they are often very damaging to the microbiome. By damaging the microbiome, they significantly impair a patient's ability to fight the infection on his/her own—the very infection that the antibiotics are meant to treat.

Antibiotics cause damage to the microbiome because they are usually insufficiently selective. By this, we mean that antibiotics inhibit or kill most bacteria that they interact with, whether that bacterium is pathogenic or helpful. We see at least three distinct mechanisms whereby indiscriminate antibiotic action is harmful for a patient's ability to fight infection, working at cross purposes with killing the pathogens:

- i Removing beneficial competitors.
- ii Preventing the adaptive immune response from “learning” the pathogen, a phenomenon called arrested immunity.
- iii Interrupting the gut-immune axis.

First, antibiotics deplete our natural bacterial flora. Commensal (non-pathogenic) bacteria serve many beneficial roles, helping in metabolism and competing for nutrients and adhesion sites. Antibiotics inhibit susceptible bacteria, but not resistant bacteria present in our microbiomes. After antibiotic treatment, the niches once occupied by commensals are now free for resistant and often pathogenic species to colonize.^{121–123}

Infection by *Clostridioides difficile* is the most striking example of this phenomenon. *C. difficile* is itself considered a CDC urgent threat pathogen, causing over 223,000 infections annually,³⁴ perhaps as many as 460,000.¹²⁴ Unlike other threat pathogens, *C. difficile* infection happens almost exclusively *after antibiotic use*, in particular, clindamycin, fluoroquinolones, and cephalosporins,¹²⁵ precisely because these antibiotics decolonize the gut of competitors. When appropriate competitors are restored to patients after antibiotics, they are protected from *C. difficile*.¹²² Similarly, women who take antibiotics have triple the risk of vaginal yeast infections, as bacteria which normally compete with fungi are removed by antibiotics.^{126,127}

In another manifestation of this problem, after commensal depletion by antibiotics, patients are more at risk for invasive bloodstream infections from resistant bacteria, previously present in the gut and greatly expanded after susceptible competitors were killed. In particular, vancomycin-resistant *Enterococcus* (VRE) can

223,000

US *C. difficile* infections a year,
almost all after antibiotic use

bloom in the gut after a course of antibiotics, prior to bloodstream invasion.¹²⁸

In the second mechanism, antibiotics interrupt the process of adaptive immunity in a phenomenon called arrested immunity. Antibiotic treatment prevents the adaptive immune system from fully learning how to defend against the pathogen, which means that the risk of reinfection is higher than when the immune system clears the infection on its own. Reinfection is much more likely after antibiotic treatment than when infection resolves naturally. Antibiotics short-circuit the antigen exposure that is necessary to train T cells and develop antigen specific antibodies. It appears that for successful antigen recognition, the adaptive immune system requires exposure to an antigen for a certain period of time, which is cut short when antibiotics kill pathogens early in the infection cycle.^{129–131}

Finally, antibiotics impair the immune response via the gut-immune axis. Damaging the healthy microbiome impairs immune function. While the specific mechanisms remain an area of intense, active investigation, it is clear from emerging laboratory, animal model, and clinical evidence that broad-spectrum antibiotics and the resulting gut dysbiosis disrupt proper immune response. The microbiome is particularly involved in instructing the adaptive immune response, including the differentiation and balance of effector and regulatory T cells and antibody responses.^{132,133} The largest body of evidence links the healthy microbiome to lung immunity. Gut-damaging antibiotics impair alveolar immune function and reduce the ability to fight infection.^{134–136} A clear, clinical example is that healthy adults given a 5-day course of broad-spectrum antibiotics develop gut dysbiosis and measurable blunting of antibody response to influenza vaccine.¹³⁷

Antibiotics are, to be sure, wonder drugs. The net benefits of antibiotics are most clear in response to acute, severe infection where the immune system has been overrun. However, in cases where infections are more likely to be chronic, or less likely to be immediately life-threatening, or where antibiotics are likely to fail, or where the goal is infection

prevention, killing bacteria indiscriminately with antibiotics—pathogens and healthy microbiome alike—is probably not the best strategy. As the scientific community has come to appreciate the harmony in which the immune system and microbiome work, we see bacterial defense disablers as a therapeutic strategy that contributes to the harmony in fighting infection, rather than disrupts it.

05

False hope: narrow-spectrum bacteria killers

Given our previous discussion on immune/microbiome integration and the trouble with antibiotic collateral damage to healthy bacteria, the obvious technical strategy to solve this problem would be a “precision-medicine approach”. The idea is to find a way to target and kill the pathogens only, sparing the healthy microbiome.

Indeed, it appears that much of the (preclinical) field has moved in this direction.¹³⁸ These strategies focus on generating “narrow-spectrum bacteria killers” to solve AMR and deal with the problem of insufficiently selective broad-spectrum antibiotics. The technologies to do this are genuinely innovative and exciting. They range from targeted small molecules (often employing AI in the process), to antibodies, to engineered bacteriophages (bacteria-specific viruses), to lysins (engineered molecules sourced from bacteriophages which kill bacteria directly), and more. Despite the diverse technological approaches, these techniques share the objective of killing a specific pathogen while sparing the rest.

~2 days

typical turnaround for diagnostics
deployed in healthcare settings

Narrow-spectrum bacteria killers have a certain appeal, because they are conceived of as “personalized medicine”. A patient could be tested, the specific pathogen identified, and the drug administered, exactly matched to the pathogen causing the infection. The idea has elegance.

Our view, though, is that as a strategy, this is a dead end, independent of the technology harnessed to kill the bacteria.

First, this strategy relies on a robust diagnostic infrastructure operating very quickly and at scale. We do not have that in any meaningful sense, and should not expect to in the foreseeable future.

Current diagnostics deployed in healthcare settings are slow and unreliable. Slow, meaning they usually take two days or more to deliver results.¹³⁹ Unreliable, meaning that they often do not return an actionable result (either a null, or a polymicrobial report).¹⁴⁰ Respiratory infections only identify a culprit pathogen about half the time. Hiding bacteria (biofilm, intracellular, persisters) may not be collected by the biopsy and/or identified in the diagnostic. Many new technologies in development could conceivably speed up results, fixing the “slow” part of the diagnostic problem if deployed at scale.¹³⁹ But these technologies, even if widely deployed, cannot address the unreliability of sample collection.

Partly as a result of the limitations in sampling and diagnostic technology, severe, resistant infection patient populations are small. As mentioned before, Carbapenem-resistant *Acinetobacter* infections cause 8,500

hospitalizations and 700 deaths annually.³⁴ Recruiting enough patients for a narrow-spectrum bacteria killer clinical trial presents a serious challenge: not only are the patient numbers small, but the nature of the acute infection means that they are hard to enroll in a trial in the small window of time when medication needs to start—often just a few hours.

Assuming a trial can be completed and drug approved, patient populations defined in this way result in extremely narrow commercial markets, which must still compete against generic, approved alternatives. Generic competition limits pricing power. As a drug product, narrow-spectrum bacteria killers suffer from low volume and low price. Most of these technologies have complex manufacturing processes and high cost of goods. As commercial products, most (or all) will struggle to break even, and are unlikely ever to recover development costs.

The problem gets worse. To cover all the resistant pathogens of concern, the world would require a *portfolio* of narrow-spectrum bacteria killers. We would probably need a minimum of five such drugs to cover the specific pathogens of highest clinical importance. More realistically, 10–20 would be required. Each with the same commercial dynamics.

Such a program seems difficult, at best. At worst, wasteful of scarce talent and capital.

At Unison, we seek to change the entire paradigm for how bacterial infections get treated, managed, and controlled. Narrow-spectrum bacteria killers are unlikely to be anything beyond that: narrowly applied. We focus our energy on bacterial defense disablers, which we believe can and will change the paradigm for infection control.

10–20

narrow-spectrum drugs a full portfolio would require

06

The unsolved problem of chronic urinary tract infections

UTIs afflict an estimated 400 million patients each year, predominantly women, making UTIs one of the most common bacterial infectious syndromes.^{141,142} Over one in ten women will contract a UTI every year in the United States and other Western countries.⁵⁻⁷ Of these, the majority respond to antibiotics, but a recurrence is frequent. A reported 27%–44% of women will contract a second UTI within one year of their first.^{143,144}

For some of these women, their symptoms never fully resolve, a condition referred to informally as “chronic UTI”. A RAND study found 2.70–6.53% of American women have symptoms aligning with chronic UTI.¹⁴⁵ This is corroborated by a 2024 population-wide study of Welsh health databases finding 1.7–6.0% of women could have chronic UTI, indicated by their use of long-term suppressive antibiotics prescribed for UTI management.¹⁴⁶ Using a conservative 2.70%, we estimate that 3.3 million US women (and 660,000 British women) suffer from chronic UTI.

Chronic UTI devastates lives. UTIs cause excruciating pain and many sufferers of chronic UTI are unwilling to be more than a few steps away from a bathroom during (frequent) symptom flares. UTI recurrence is closely linked to sexual intercourse^{147,148} and chronic UTI can put severe strain on intimate relationships. Work from Chronic UTI Australia details these symptoms and estimates that one in ten sufferers have seriously contemplated or attempted suicide.¹⁴⁹

There are no good therapeutic options for chronic UTI patients. Long-term (continuous) antibiotics do help some patients.¹¹ Many cannot tolerate such lengthy treatment courses, which come with toxicity and severe gut dysbiosis. Clinicians are reluctant to prescribe such treatments as they conflict with good stewardship principles and increase the likelihood of generating antibiotic resistance.

Mounting evidence indicates that antibiotics fail in chronic UTI because the culprit bacteria hide deep within bladder cells. Indeed, some of the most consistent treatment successes for chronic UTI patients have come from a surgical procedure called electrofulguration, where pockets of bladder cells containing bacterial lesions are vaporized and cauterized.^{14,150–153}

Two decades of research underpin the clinical evidence. The work began in mouse models, where researchers first identified that uropathogenic *E. coli* (UPEC) resist clearance by antibiotics and immune cells by invading the bladder epithelium.^{154,155} Intriguingly, antibiotics could sterilize the urine of these mice, but the bacteria would persist embedded in bladder tissue.^{10,156,157} Next, researchers identified that the intracellular bacteria

form biofilm-like clusters inside host cells called intracellular bacterial communities (IBCs). Inside IBCs, bacteria can replicate and then eventually efflux from the host cell and invade another, repeating the cycle.^{9,158–163} The host cells shield IBCs from antibiotics: antibiotic activity is restored in the lab when bladders are removed and homogenized to break down the cell walls.¹⁰ It is the lesions formed by IBCs that likely correspond to those removed by electrofulguration (see Figure 12).

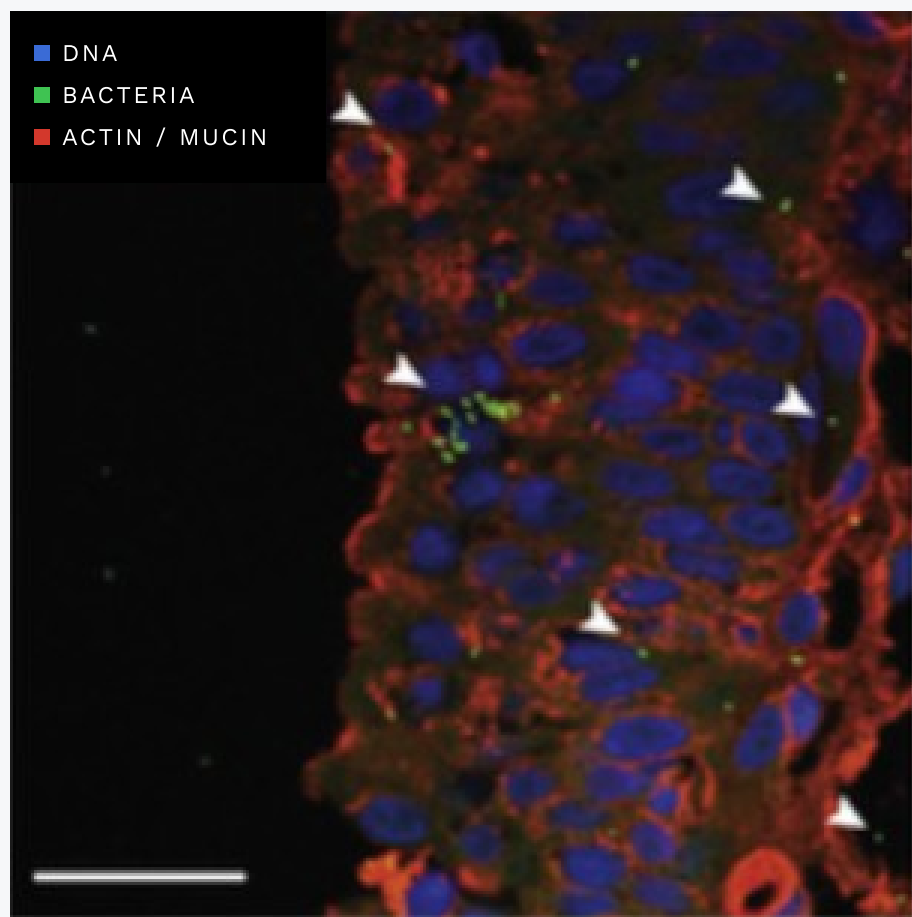


Figure 12. Images of a bladder biopsy taken from a patient undergoing bladder electrofulguration for late-stage treatment of antibiotic-refractory recurrent UTI. White arrows point to bacteria, which are showing in green from an imaging technique that labels them with a fluorescent marker. Bacteria have embedded in the bladder tissue and resisted years of chronic, antibiotic therapy. Work performed by De Nisco and colleagues.

A further, even smaller subset of solitary bacteria hide within lysosomes, termed quiescent intracellular reservoirs (QIRs).¹⁶⁴ Like IBCs, QIRs hide within host cells from antibiotics and the immune system and can drive cycles of reinfection. Shockingly, QIRs have adapted to survive within lysosomes, the very organelles designed to kill them.¹⁶⁴

Organoid models allow researchers to better understand infection dynamics as they are easier to work with than are animal models. Recent advances in organoid technology have allowed detailed mechanistic studies, which have confirmed the findings in earlier animal models.^{165–167} Robust models which include immune cells showed that neutrophils and antibiotics alike are unable to remove intracellular bacteria (IBCs and QIRs), all visualized under high power microscopy.^{168,169}

Finally, bladder tissue sampled from human, antibiotic-refractory UTI patients has revealed embedded bacteria.^{12,170,171} This finding held for patients with sterile urine, again supporting the idea that antibiotics are ill-suited to solve chronic urinary tract infections.

Chronic UTI is an archetypal syndrome of a disease refractory to antibiotics, not so much because of antibiotic resistance, but because bacteria hide where antibiotics cannot reach and where the immune system is less effective.

Patients need a new way to treat chronic UTI. Antibiotics do not work for them. Surgery with electrofulguration cannot possibly remove all hidden bacteria, not to mention how difficult the procedure is for patients.

Bacterial defense disablers, and in particular, Unison's lead asset UNI-568911, could be the exact right technology. UNI-568911 can successfully treat chronic UTI in mice, a major demonstration of proof of principle (see Figure 5 in Chapter 1, repeated as Figure 13 here). Working "in unison" with the immune system, UNI-568911 produces a dose-response in clearing bacteria out of the kidney and urine, and at the highest dose, reduces bacteria by 99.8%. No antibiotic is required, and because of that, there is no discernible impact on the healthy gut microbiome (see Figure 6 from the opening chapter).

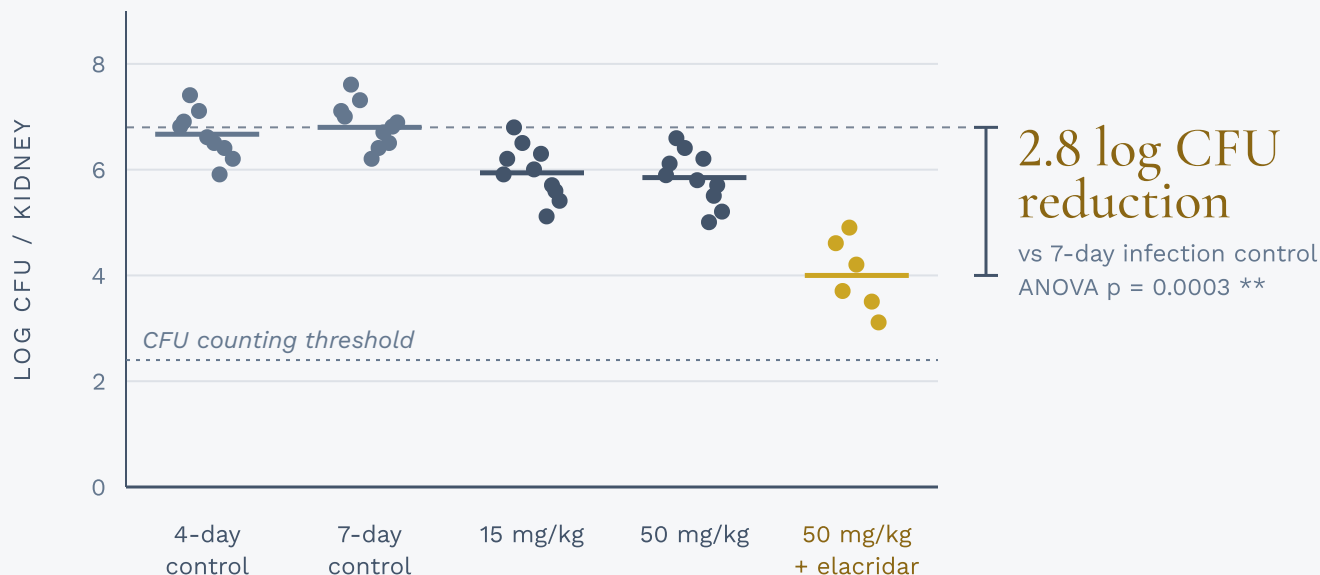


Figure 13. Monotonic reduction in bacterial Colony Forming Units (CFU) produced by oral administration of UNI-568911 in a chronic, 7-day model of mouse urinary tract infection (UTI). Administration of UNI-568911 on days 4–7 post-infection, dosed every 6 hours. The highest exposure group included combination with elacridar, which traps higher concentrations of UNI-568911 in macrophage immune cells. ** indicates statistical significance by ANOVA $p = 0.0003$. Dose response trend was statistically significant by the Jonckheere–Terpstra test for ordered exposure: $Z = -2.83$, one-sided $p = 0.002$. Calculated with $N=6$ in highest group. Repeated from Figure 5 for ease of viewing.

We further distinguish our results from antibiotics in terms of which bacteria our compound kills. As we described above, sterile urine accompanied by continuing infection is a common phenomenon in chronic UTI patients. Indeed, antibiotics clear more bacteria in the urine than does UNI-568911. But urine gets flushed regularly anyway. UNI-568911 concentrates inside immune cells, and further inside of lysosomes. We reason that because of this, it is aiding the immune system in killing the most recalcitrant, tissue-resident bacteria that antibiotics miss. These same bacteria provide the reservoirs for reinfection. Our future work centers around demonstrating this hypothesis and moving our first asset into clinical trials.

Chronic UTI is a compelling clinical problem in its own right. It is also the first proving ground for bacterial defense disablers. Chronic UTI is just the beginning of what bacterial defense disablers can do.

07

The unsolved problem of antibiotics in immuno-oncology

Not only do antibiotics impair the ability of patients' bodies to fight infection, they also impair the ability to fight cancerous malignancy.

The introduction of immuno-oncology in the past decade and a half revolutionized cancer care. The big idea is to harness the power of the immune system to kill cancer, instead of using cytotoxic chemotherapy agents. The most successful class of these agents, the PD-1 inhibitors (also known as checkpoint inhibitors), work by “releasing the brake” from immune cells. PD-1 inhibitors have brought many solid tumor cancer patients into complete remission, treating tumors where medicine previously had little to offer. In the process, they have become some of the best-selling drugs of all time.

In a surprise finding, patients who receive antibiotics at the beginning of their PD-1 course of treatment find that the PD-1 no longer works *against cancer*. The rationale: immuno-oncology drugs rely on a healthy immune system. Antibiotics damage the gut microbiome, and the tight linkage between microbiome and immune system means that a damaged gut microbiome leads to a dysfunctional immune system.

As the oncology world moves increasingly towards more sophisticated immunotherapies, and more patients receive these treatments, a non-antibiotic treatment for bacterial infection will become increasingly important. We do not want to find ourselves in a position where cancer patients receive highly effective, modern oncology therapies but succumb to infection treated with antibiotics developed in the middle of the 20th Century.

To be more concrete with the evidence, we start with the clinical observation that patients receiving antibiotics up to 2 months prior and as late as 1 month after starting their immuno-oncology regimen show remarkably different survival outcomes. For the sake of illustration, one of the more dramatic study results is shown in Figure 14, taken from the work of Pinato and colleagues.¹⁷² These findings have been consistently repeated across the solid tumor cancer types treated with PD-1 inhibitors. A meta-analysis that incorporated 41,663 patients found a consistent, pronounced immuno-oncology impairment in both overall survival and progression-free survival in non-small cell lung cancer, urothelial cancer, melanoma, renal cell carcinoma, hepatocellular carcinoma, and others.¹⁷³

Results like this underline a strong and consistent clinical observation of immuno-oncology impairment correlated with antibiotic therapy at the beginning or two months before checkpoint inhibitor therapy.

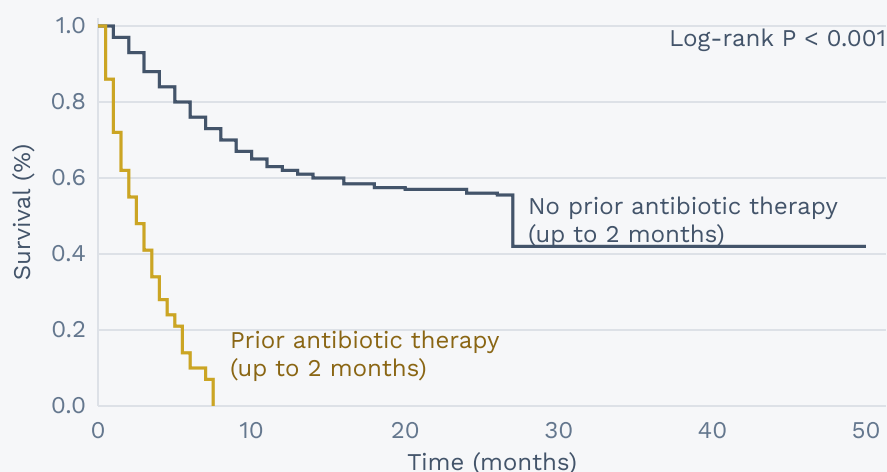


Figure 14. Kaplan-Meier curve of a prospective study reported in *JAMA Oncology* comparing survival between cancer patients who did (N=29) and did not (N=166) receive antibiotic therapy in the two-month window prior to initiating PD-1 therapy. Patients included those with non-small cell lung cancer (N=119), melanoma (N=38), and other tumor types (N=39). Overall survival for those who did not receive antibiotics was 26 months, vs 2 months for those who did receive antibiotics ($p < 0.001$). Work performed by Pinato and colleagues.

The correlation is striking, but an obvious possible confounding variable is that patients who receive antibiotics are often sicker, with more comorbidities, than those who do not. The difference in survival could simply be that the antibiotic subgroup of patients is more frail. To evaluate correlation versus causation in this setting, Genentech studied the results from their Phase 3 programs for atezolizumab, their checkpoint inhibitor. In these studies, they had compared efficacy of atezolizumab head-to-head with the chemotherapy drug docetaxel in non-small cell lung cancer patients. They reasoned that patients in both groups would have received antibiotics. If the antibiotic subgroup is simply a sicker group of patients, then these patients should succumb faster in the chemotherapy group, too. What they found was the opposite: a non-significant difference in the chemotherapy group, but a significant difference in the atezolizumab

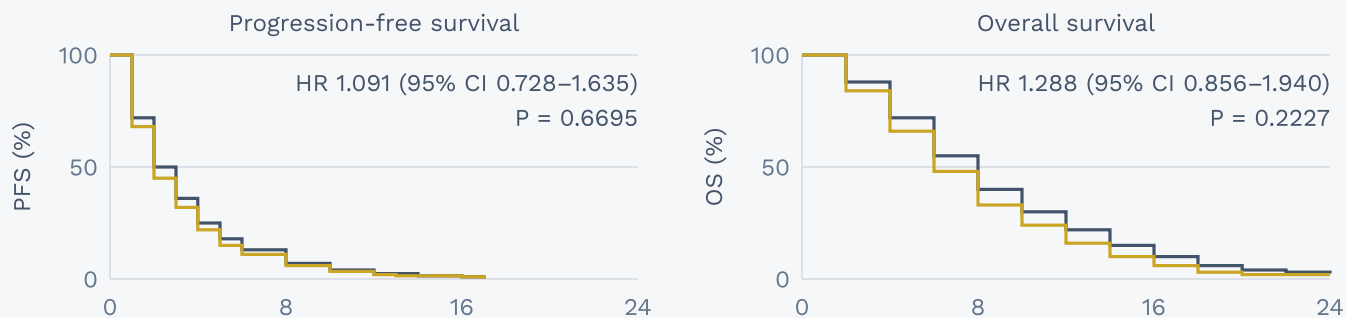
group (see Table 2).¹⁷⁴ This finding supports the hypothesis that antibiotics interfere mechanistically with checkpoint inhibitors. It has been replicated in other major studies,¹⁷⁵ including a prospectively evaluated study in gastric cancer patients (see Figure 15).¹⁷⁶

	MEDIAN OVERALL SURVIVAL (MONTHS)	
	Antibiotic exposure	No antibiotic exposure
Atezolizumab (Tecentriq)	8.54	14.06
Docetaxel	9.10	10.28

Table 2. Impact of prior antibiotic therapy on non-small cell lung cancer overall survival in checkpoint inhibitor patients (atezolizumab) versus chemotherapy patients (docetaxel). The difference in the chemotherapy group is non-significant. Work done by Chalabi and colleagues at Genentech.

Furthermore, laboratory studies in mice recapitulate the effect and build the case for a mechanistic understanding of the link between a healthy microbiome and a functioning immune system. In a study conducted at a leading PD-1 lab at Dana Farber, mice required a healthy microbiome for their checkpoint inhibitor therapy to control tumor growth (see Figure 16).¹⁷⁷

IRINOTECAN COHORT



PD-1 PROSPECTIVE COHORT

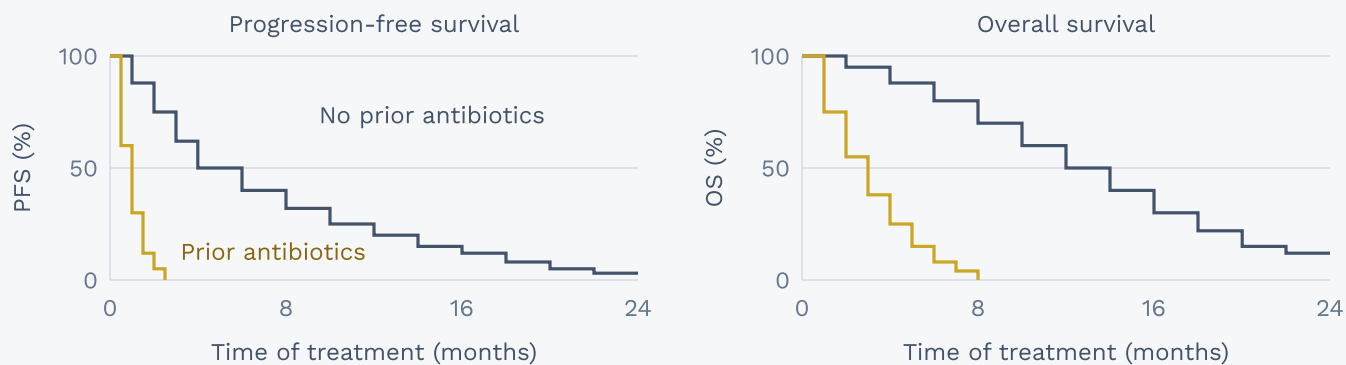


Figure 15. Gastric cancer patients receiving antibiotics versus no antibiotics. The top row shows progression free survival and overall survival for chemotherapy patients. Antibiotics have no discernible impact on outcomes. The bottom row shows a prospectively evaluated PD-1 cohort. Prior antibiotic therapy exerts a clear detrimental effect on PD-1 outcomes. Work performed by Kim and colleagues.

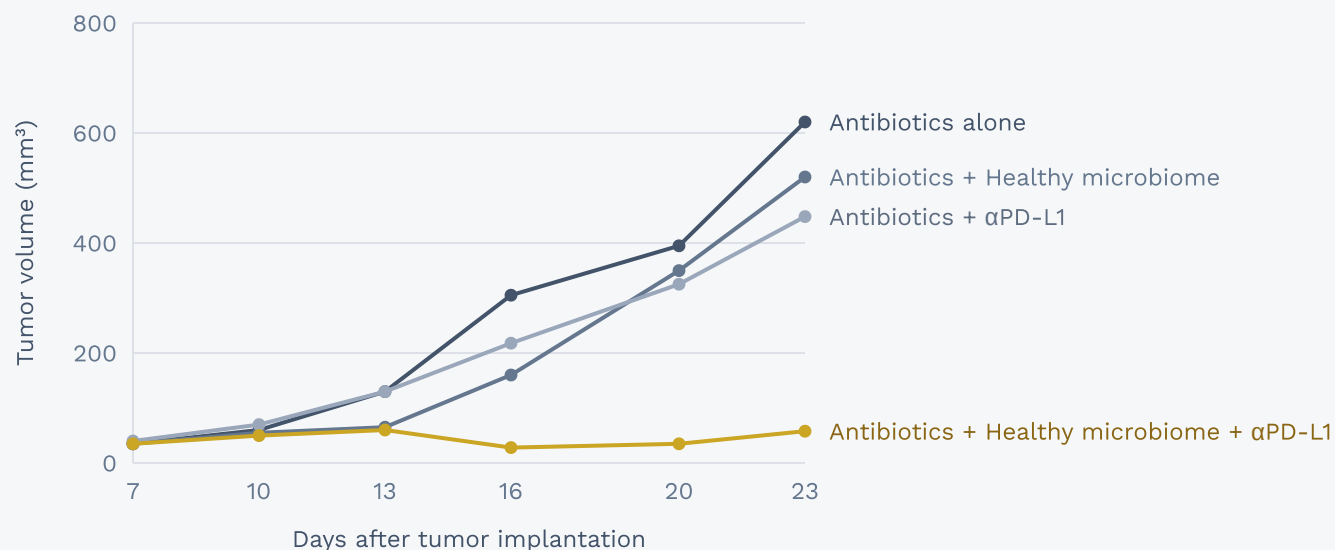


Figure 16. Study from Dana Farber using MC38 colon carcinoma tumor growth in mice treated with broad-spectrum antibiotics versus mice reconstituted with healthy human microbiota. Anti-PD-L1 therapy effectively controls tumor growth only in mice with intact microbiome. Reconstitution with healthy microbiota restores checkpoint inhibitor responsiveness. Work performed by Park and colleagues.

Tightening the causal chain, the microbiomes of PD-1 responders carry distinct signatures from non-responders, relatively depleted in some species and enriched in others (see Figure 17).^{20,21,178} The non-responders lack strains corresponding to those most susceptible to antibiotics, primarily anaerobes.²¹ Furthermore, not all antibiotics have a negative impact on PD-1 outcomes, but rather those with broader spectrum and those more likely to deplete anaerobes.²¹ These differences carry through to peripheral T cell populations, linking antibiotic-induced anaerobe depletion to reduced T cell function (see Figure 18).²¹ Mechanistically, this helps explain why antibiotics can abrogate immuno-oncology therapeutic agents reliant on functioning T cells. The detailed workings of the mechanism underlying the gut-immune linkage remains an active field of investigation as researchers seek to understand the molecular basis of the gut-immune relationship.

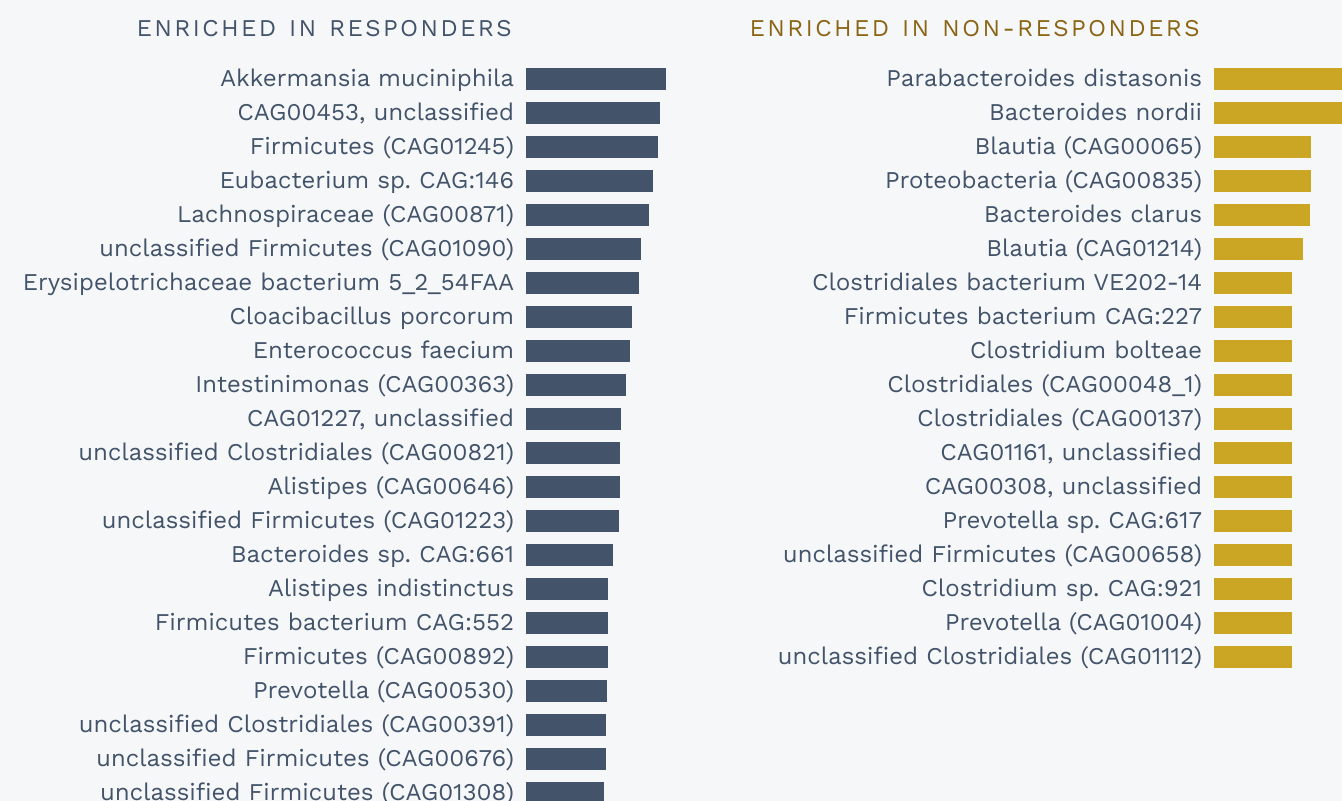
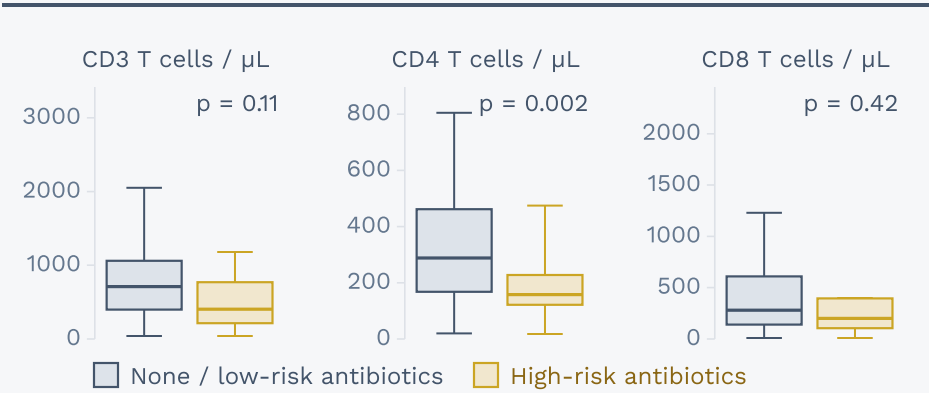


Figure 17. Microbiome analysis of PD-1 patients. The microbiomes of PD-1 responders are highly likely to be enriched in the species listed in navy, while the microbiomes of non-responders are highly likely to be enriched in the species shown in gold. For our purposes here, the individual strains are not as important as showing the distinct microbiome signatures correlated with PD-1 responders and non-responders. Antibiotics with anaerobic coverage tend to remove microbial species necessary for PD-1 response. Work performed by Routy and colleagues.

About 150,000 US patients every year receive checkpoint inhibitors for their cancer diagnoses.^{*179} Their PD-1 treatment typically costs \$150,000 per patient.¹⁸⁰ About 30% of these patients require antibiotic treatment, which measurably worsens their cancer.^{20,173} From a financial standpoint, treating these patients with generic antibiotics costs a few dollars and wastes tens or hundreds of thousands already spent on their cancer care.

* Merck does not report the number of patients treated by Keytruda. We estimated this number by dividing the reported revenue in their annual 10-K report by the estimated net price per patient of \$150,000, and then accounting for other PD-1 products in the marketplace.



Box: interquartile range and median. Whiskers: range of observations.
Individual values as reported in the source.

Figure 18. CAR-T, another form of immune-oncology, exhibits similar features with respect to antibiotics. Here, antibiotics significantly reduce circulating CD4 T cell populations and show a trend to reduction for CD3 and CD8 T cells. Work performed by Stein-Thoeringer and colleagues.

Solid tumor patients on PD-1 therapy have well-documented, elevated rates of community infection driven by their underlying disease and demographics. Non-small cell lung cancer patients are at disproportionate risk for respiratory infection. Post-menopausal women (the majority of female PD-1 recipients) face chronically elevated UTI risk.^{181–183} See Table 3 for common infections contracted by PD-1 patients.

INFECTION TYPE	SHARE OF INFECTIONS
Respiratory	50–60%
Urinary tract	15–25%
Skin & soft tissue	15–20%

Table 3. Share of infection diagnoses in PD-1 patients.

Infectious disease guidelines explicitly classify malignancy as a comorbidity that escalates empiric antibiotic selection.^{30,184,185} A community infection that would receive narrow-spectrum treatment in a healthy adult receives broader, more potent coverage in a cancer patient, by guideline.

We envision that bacterial defense disablers could be the modern infection control medicine that complements immuno-oncology. Like the PD-1 medicines, bacterial defense disablers rely on the exquisite power of the immune system to identify pathogens. Like the PD-1 inhibitors with cancer, bacterial defense disablers make the immune system more able to clear infection. Because bacterial defense disablers do not damage the microbiome, they would not interfere with the gut-immune axis necessary for PD-1 efficacy. Bacterial defense disablers could be given in response to an infection, or more likely, could be given in a preventive sense, in the way that colony stimulating factors are already given to chemotherapy patients to reduce infection risk. Colony stimulating factors are a cornerstone of many chemotherapy treatment protocols, allowing patients to be treated in outpatient settings because of their reduced infection risk. As such, they collectively provide more than \$4B in revenue each year. Immuno-oncology needs a new paradigm for infection control. The product that meets that need will be valuable for patients and shareholders alike.

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