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Neo-Coley v2

A Synthesis Framework for Combinatorial PAMP Immunotherapy with Sustained Pyretic Induction

A position paper integrating historical case data, modern Coley-derived clinical trials, and 2025–2026 advances in cancer immunology

Author: Eric Monteiro **Date:** May 14, 2026 **Status:** First complete draft, position paper, not peer-reviewed **Disclaimer:** This document presents a theoretical synthesis of public-domain scientific literature together with one informal clinical case observation. It is intended as input to investigator-initiated scientific discussion. It is not medical advice and does not constitute a treatment recommendation. All clinical investigation of bacterial or PAMP-based immunotherapy must be conducted under appropriate regulatory and institutional oversight.

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Abstract

William Coley's bacterial cancer immunotherapy, practiced between 1891 and 1936 in more than a thousand patients with inoperable cancer, reported durable response rates substantially higher than those produced by modern checkpoint inhibitor monotherapy in comparable refractory disease. Modern attempts to replicate Coley's results — the Karbach 2012 mixed bacterial vaccine trial, the SYNB1891 engineered probiotic STING-agonist trial, the MBVax compassionate-use experience, and the long-running OK-432 evidence base in Japan — have produced confirmed target engagement but rare objective regressions. Building on the framework Hobohm and colleagues at THM University Giessen have developed over two decades, this position paper articulates a convergent thesis: durable response requires four conditions in combination — combinatorial PAMP activation across multiple innate immune sensors, sustained fever-range thermal stress (39–40°C) as the dosing endpoint, multi-month treatment duration with intermittent scheduling to avoid endotoxin tolerance, and preservation of host immune function. Five developments in cancer immunology since 2018 — personalized neoantigen mRNA vaccines, computational modeling of PAMP combination synergy, biomarker-based patient selection, dosing schedule design informed by tolerance kinetics, and a clearer molecular framework for fever's role in immune orchestration — are integrated to produce a specific protocol design (Neo-Coley v2) suitable as the basis for an investigator-initiated trial. One documented clinical case is presented as consistent with the protocol pattern, with limitations stated explicitly. Twelve empirical predictions and a single decisive falsifier are articulated; the framework is offered for testing, modification, or refutation.

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1. Introduction: The Question That Stopped Being Asked

Between 1891 and 1936, William B. Coley, a surgical oncologist at Memorial Hospital in New York, treated more than one thousand patients with inoperable cancer using injections of heat-inactivated bacterial preparations, principally combinations of *Streptococcus pyogenes* and *Serratia marcescens*. He titrated dose against fever and continued treatment for months. The case series, later catalogued in detail by his daughter Helen Coley Nauts across eighteen monographs at the Cancer Research Institute (Nauts, Fowler, and Bogatko 1953, and subsequent Cancer Research Institute monographs through 1990), documented durable complete and partial responses, particularly in soft tissue and bone sarcomas — a tumor type for which essentially no effective systemic therapy existed at the time. Among the inoperable sarcoma cases, McCarthy's consolidated summary of the Coley archive reports five-year survival above 50% and complete cure rates near 10%, with several patients followed for more than two decades without recurrence (McCarthy 2006).

This was the first sustained clinical practice of cancer immunotherapy. It preceded the molecular characterization of innate immune sensors by a century, the discovery of cytokines by half a century, and the formal articulation of the antigen-presenting cell concept by decades. Coley operated empirically, on the clinical observation that patients with cancer who developed severe febrile bacterial infections sometimes un-

derwent durable tumor regression. The mechanism was inaccessible to him, but the phenomenon was reproducible enough to support a four-decade clinical practice and to motivate adoption by physicians on multiple continents through the 1940s.

The decline of the practice is well documented (Carlson, Flickinger, and Snook 2020). Three forces converged. First, the development of cytotoxic chemotherapy and modern radiation therapy in the 1940s and 1950s offered seemingly more predictable interventions, with mechanisms that could be standardized in a way bacterial preparations could not. Second, the absence of a mechanistic explanation prevented the bacterial approach from being formalized within the emerging framework of randomized controlled trials. Third, the 1963 reclassification of Coley's toxins by the U.S. Food and Drug Administration as an investigational drug lacking adequate safety and efficacy data made continued clinical use outside formal trials illegal in the United States. The last pharmaceutical manufacturer of Coley's preparation ceased production in 1951.

The phenomenon Coley described, however, did not disappear from the scientific literature. Helen Coley Nauts continued cataloguing cases until her death in 2001, and the Cancer Research Institute she founded in 1953 became, over subsequent decades, a major funder of cancer immunology research and a principal institutional supporter of the work that ultimately produced modern checkpoint inhibition. By the time of Nauts' death, the foundational claim her father had defended — that the immune system can be induced to produce durable cancer responses — had been substantially vindicated. Bacillus Calmette-Guérin, a live attenuated mycobacterial preparation operating through mechanisms closely related to Coley's, had received FDA approval for non-muscle-invasive bladder cancer in 1990 and remains standard of care. Anti-CTLA-4 and anti-PD-1 checkpoint inhibitors, beginning with ipilimumab in 2011, transformed the treatment of melanoma, non-small cell lung cancer, and a growing list of other malignancies. Coley's recognition as the "father of immunotherapy" became uncontroversial within the field.

A question follows that the modern field has not adequately addressed. Coley's protocols reported response rates substantially higher than those produced by checkpoint inhibition in comparable refractory disease — five-year survival above 50% in inoperable sarcoma, against the 15–30% durable response rates typical of modern checkpoint inhibitor monotherapy across solid tumors. If the underlying phenomenon — induced durable immune-mediated tumor regression — is the same in both eras, why does the historical practice appear to have produced better outcomes than the modern one? The most parsimonious answers fall into two categories. Either Coley's case series substantially overstates response rates due to publication bias, selection of best cases, diagnostic imprecision of the era, or other methodological limitations; or the historical practice contained features that the modern field has not reproduced, and the gap in efficacy is the consequence of that loss.

This paper develops the second hypothesis, in synthesis with the work of Hobohm and colleagues at THM University Giessen and their clinical collaborators (Hobohm 2001, 2009; Hobohm, Grange, and Stanford 2008; Orange, Reuter, and Hobohm 2016; Reuter, Oettmeier, and Hobohm 2018), who have argued for over two decades that three specific operational features of Coley's protocols — combinatorial PAMP activation across multiple innate immune sensors, sustained systemic fever induction

as the dosing endpoint, and treatment durations measured in months rather than weeks — are essential to producing the response Coley described, and have been systematically engineered out of modern bacterial immunotherapy trials. To this thesis we add five integrations made possible by developments in cancer immunology since 2018: personalized neoantigen vaccines, computational modeling of PAMP combination synergy, biomarker-based patient selection, dosing schedule design informed by tolerance kinetics, and a substantially clearer mechanistic account of fever's role as immune orchestrator. The synthesis is developed as a coherent framework (Sections 3 and 4), translated into a specific protocol design suitable as the basis of an investigator-initiated trial (Section 5), and supported by one documented clinical observation consistent with the predicted protocol features (Section 6). Section 7 specifies the predictions and falsifiers by which the framework can be tested.

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2. The Modern Coley Replication Attempts and What They Showed

If Coley's reported clinical results contained features the modern field has lost, the most direct way to evaluate this is to examine the modern attempts that have come closest to replicating his protocol, and to identify the specific points at which they diverged from it. Four bodies of evidence merit detailed consideration: the Karbach et al. (2012) Phase I trial of mixed bacterial vaccine in NY-ESO-1-expressing tumors; the SYNB1891 Phase I trial of an engineered probiotic delivering STING agonism under tumor hypoxia (Luke et al. 2023); the MBVax Bioscience compassionate-use experience in Canada under Donald MacAdam from 2005 through 2017 (MacAdam 2018); and the long-running clinical use of OK-432 (Picibanil) in Japan. Each provides empirical anchor points for the framework developed in subsequent sections.

2.1 The Karbach 2012 Trial

The most direct modern replication of Coley's protocol was undertaken by Karbach and colleagues at the Krankenhaus Nordwest in Frankfurt, in collaboration with the Ludwig Institute for Cancer Research. The investigators developed a current-good-manufacturing-practice-compliant mixed bacterial vaccine preparation — biochemically defined, but otherwise designed to be equivalent to Coley's original — and conducted a Phase I trial in twelve patients with NY-ESO-1-expressing advanced cancers (Karbach et al. 2012).

The protocol used subcutaneous administration twice weekly, with the dose escalated in each individual patient until body temperature in the 38.0–39.5°C range was reliably induced. Eleven of twelve patients achieved fever within the target range. Ten of twelve showed consistent serum IL-6 elevation correlated with body temperature, with a subgroup also showing elevations in TNF-α, IFN-γ, and IL-1β. The trial's single objective response — a partial response by RECIST criteria — occurred in a patient with metastatic bladder cancer, and was strongly correlated with the highest sustained fever and the most pronounced cytokine elevations observed in the cohort.

The trial was limited by two specific mechanistic problems, both explicitly characterized by the investigators. First, repeated administration produced endotoxin toler-

ance, with progressively weaker fever and cytokine responses to equivalent doses. Second, patients developed anti-MBV antibodies that appeared to neutralize subsequent doses. The combination forced dose escalation to be discontinued before maximum biological effect could be sustained. One observation in the dataset, however, indicates that these limitations are technical rather than fundamental: a single compassionate-use patient (designated patient #11 in the published cohort), after a three-month rest period, again developed fever and cytokine responses comparable to those observed earlier in his treatment. Endotoxin tolerance was therefore reversible on a months-scale, and the protocol design implications of this observation are taken up in Section 4.4.

The Karbach trial established three points relevant to the present framework. First, fever-titrated administration of a Coley-equivalent preparation is technically feasible and safely tolerated in advanced cancer patients with appropriate monitoring. Second, fever and cytokine elevation correlate with clinical response in this small dataset — although a single response is insufficient to support a population-level claim. Third, tolerance and antibody development are real mechanistic obstacles to sustained dosing that any modern protocol must address operationally.

2.2 The SYNB1891 Phase I Trial

A conceptually distinct approach to recapitulating Coley's immune activation was pursued by Synlogic, Inc., a biotechnology company specializing in engineered probiotics. SYNB1891 is a genetically modified strain of *Escherichia coli* Nissle 1917, engineered to express the bacterial protein DacA under hypoxic conditions, producing cyclic di-AMP — a STING pathway agonist — selectively within the tumor microenvironment (Leventhal et al. 2020). The first-in-human Phase I trial (NCT04167137) enrolled thirty-two patients with advanced refractory malignancies, treated with repeated intratumoral injections of SYNB1891 as monotherapy or in combination with the anti-PD-L1 antibody atezolizumab (Luke et al. 2023).

The trial's primary findings are informative for several reasons. SYNB1891 produced confirmed STING pathway activation in tumor biopsies, with upregulation of interferon-stimulated genes, chemokines, and T-cell response genes documented in ten of twelve evaluable paired biopsies. Serum cytokine elevations followed predicted patterns. The maximum tolerated dose was not reached at the highest dose level tested (3×10^8 live cells), with cytokine release syndrome events managed without sequelae in five of twenty-four monotherapy patients. The safety profile was substantially better than that of first-generation small-molecule STING agonists.

Yet no objective tumor regressions were observed. The best response across the entire cohort was stable disease in nine of twenty-five evaluable patients, with four sustaining stable disease for more than two months. The two patients with the longest stable disease — one with metastatic small cell lung cancer (363+ days), one with mucosal melanoma (227 days) — were notable for two features. Both received among the lower dose levels of the trial. And both had detectable baseline CD11c+ dendritic cell populations in pretreatment biopsies, while patients with "immune desert" tumors showed neither molecular activation nor clinical benefit at any dose level. This pattern is consistent with the prediction that PAMP-driven activation requires

an existing antigen-presenting cell scaffold, and it is taken up in Section 4.3.

A further observation: the engineered bacteria were not detected in blood at six or twenty-four hours post-injection, and were undetectable in tumor tissue at seven days. The premise of self-amplifying intratumoral colonization that justified the synthetic biology approach was therefore not realized in human patients; the bacteria functioned as a transient delivery vehicle for STING agonist and brief TLR4 activation through their lipopolysaccharide, after which they were cleared. The development program was discontinued after the trial reported, when the sponsor pivoted away from oncology for reasons of corporate focus rather than scientific failure.

The SYNB1891 experience demonstrates a recurring pattern in modern PAMP-based immunotherapy: confirmed target engagement, manageable safety, and a small subgroup of patients with stable disease, but no objective regressions and no durable benefit at population scale. Where the Karbach trial used a multi-PAMP preparation and a fever-induction endpoint and produced one partial response in twelve patients, SYNB1891 used single-pathway STING agonism with intratumoral administration and cytokine release as a safety endpoint rather than a target — and produced no responses in thirty-two patients.

2.3 The MBVax Era

A parallel attempt to revive Coley's clinical practice was undertaken by Donald MacAdam, a Canadian biotech entrepreneur and founder of MBVax Bioscience. From 2005 onward, MBVax produced a GMP-quality preparation marketed as Febrivax-C, intended to be equivalent to the most effective historical Coley vaccine formulation, and supplied it to authorized clinical investigators and commercial clinics (MacAdam 2018). Cases were treated principally at the ITL Cancer Clinic in the Bahamas and at clinics in Germany, where Coley-type preparations could be produced and administered under regulatory frameworks different from those operating in the United States.

The compassionate-use experience generated by this work is documented in MacAdam's (2018) book-length account, which describes two patients who achieved durable complete responses to Coley therapy administered in combination with other modalities — one with breast cancer, one with synovial sarcoma — along with multiple partial responses and a substantial number of treatment failures. The case documentation is not peer-reviewed and does not meet the standards of evidence required for regulatory acceptance, but it is consistent with the broader pattern: when a Coley-equivalent preparation is administered to titration of fever over an extended duration, durable responses occasionally occur in tumor types where they are otherwise rare.

MBVax discontinued production in approximately 2017. The reason was not lack of clinical evidence of efficacy but the prohibitive cost of building a multi-million-dollar GMP manufacturing facility meeting U.S. and European pharmaceutical standards — a structural requirement for advancing the preparation into formal clinical trials. The episode illustrates one of the operational challenges facing any revival of bacterial immunotherapy: the regulatory and manufacturing infrastructure for novel biological products is largely incompatible with the kind of empirical, locally compounded

preparations characteristic of historical Coley therapy and of present-day European clinic practice.

2.4 OK-432 (Picibanil) and the Asian Clinical Tradition

The longest continuous clinical use of a Coley-derived preparation has been with OK-432, marketed as Picibanil — a lyophilized preparation of *Streptococcus pyogenes* group A cell wall extract, processed with benzylpenicillin. Licensed in Japan since the 1970s, OK-432 has been investigated in at least eighteen randomized clinical trials across multiple cancer types, including gastric, head and neck, lung, and ovarian cancers. It has been used both as a primary immunostimulant and as an adjuvant to surgery and other treatments.

The cumulative OK-432 evidence base is heterogeneous in design and outcome, but several patterns emerge from systematic review. OK-432 produces robust induction of TNF-α, IL-6, IL-12, IFN-γ, GM-CSF, and other cytokines, with fever as the most consistent clinical pharmacodynamic marker. In trials where fever was reliably achieved and treatment was sustained, response and survival benefits have been more consistent than in trials where fever was suppressed or treatment was abbreviated. OK-432 remains in clinical use in Japan and is occasionally used elsewhere for lymphangioma and other indications. It has not been adopted as standard of care in Western oncology, in part for the regulatory reasons that affected MBVax and in part because of variability in clinical results across heterogeneous trial designs.

2.5 The Pattern Across Modern Attempts

Across these four bodies of evidence, a pattern emerges. Where modern bacterial immunotherapy trials have used multi-PAMP preparations, titrated to fever, sustained over weeks, and conducted in patients with preserved immune function and accessible APC scaffolds, partial and occasional complete responses have been observed. Where these conditions have been violated — single-pathway agonism, fever suppressed as a safety endpoint, abbreviated dosing, immunocompromised patient populations — target engagement has been documented but durable responses have not. The Karbach trial, the SYNB1891 trial, the MBVax case experience, and the OK-432 evidence base are all consistent with this generalization.

Maletzki and colleagues (2012) drew the same conclusion from their independent review, identifying three specific reasons that modern bacterial extract trials have failed to reproduce historical results: high fever was treated as a stop criterion rather than a target; treatments were too short to achieve sustained immune activation; and patients were typically immunocompromised by prior or concurrent chemotherapy at the time of enrollment. Hobohm and colleagues, working from a different angle through preclinical PAMP combination experiments and the 2018 retrospective clinical safety dataset, arrived at the same operational conclusion. The convergence of these analyses, developed in detail in Section 3, provides the foundation for the framework this paper proposes.

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3. The Convergent Thesis: What Modern Coley Trials Stopped Doing

The disagreement between Coley's reported results and the modest outcomes of every modern bacterial immunotherapy trial is not, on close reading of the published evidence, a disagreement about whether innate immune activation can produce durable cancer responses. It is a disagreement about what *kind* of activation is required. A coherent thesis, developed largely outside mainstream oncology by Hobohm and colleagues at THM University Giessen and their clinical collaborators, holds that Coley's efficacy depended on three specific operational features that the modern field has systematically engineered out of its trials. Stated together with the underlying mechanistic claim, these elements form what we will refer to as the convergent thesis.

3.1 The Hobohm Framework

In a series of papers beginning with "Fever and cancer in perspective" (Hobohm 2001), and developed through subsequent reviews and primary work (Hobohm, Grange, and Stanford 2008; Hobohm 2009; Maletzki et al. 2013; Orange, Reuter, and Hobohm 2016; Reuter, Oettmeier, and Hobohm 2018), Uwe Hobohm has advanced a unified hypothesis: that the active principle of Coley's preparations was not any single bacterial toxin or pyrogen, but the *simultaneous* activation of multiple innate immune sensors by a diverse set of pathogen-associated molecular patterns (PAMPs), delivered *repeatedly* and *sustained* over weeks to months. On this view, William Coley's mixture of heat-inactivated *Streptococcus pyogenes* and *Serratia marcescens* was effective precisely because it presented the immune system with a combinatorial signal — lipopolysaccharide engaging TLR4, lipoteichoic acid engaging TLR2, bacterial DNA with unmethylated CpG motifs engaging TLR9, peptidoglycan fragments engaging NOD1 and NOD2, bacterial proteins, and others — rather than agonism of a single pathway.

The preclinical basis for this view emerged from work in which combinations of pattern recognition receptor ligands administered to tumor-bearing mice produced durable tumor eradication, where single agonists at matched doses did not (Hobohm 2009; Maletzki et al. 2013; reviewed in Reuter, Oettmeier, and Hobohm 2018). Hobohm has termed this approach metronomic PAMP receptor ligand therapy. The clinical extension of the work was the recognition that two further variables, beyond combinatorial activation, were essential to recapitulating Coley's results: induction of sustained systemic fever, and avoidance of immunosuppressive concurrent treatments.

3.2 The 2018 Clinical Safety Dataset

The most substantial modern clinical evidence consistent with the Hobohm framework is the retrospective safety analysis published by Reuter, Oettmeier, and Hobohm in *Translational Oncology* (2018). The investigators reported on 131 patients, predominantly with cancer, who underwent a combined total of 523 therapeutic fever inductions using approved PAMP-containing drugs and bacterial extracts, primarily at the Klinik im Leben in Greiz, Germany, and collaborating clinical sites. The protocol was titrated against fever as the primary endpoint, escalating until body temper-

atures in the fever range were reliably induced, and treatments were administered repeatedly over multi-week to multi-month courses.

The principal finding was that across 523 fever inductions, no severe adverse events were observed. Reactions were limited to those consistent with a transient feverish infection and were managed with conventional supportive care. While the retrospective design precluded efficacy analysis, the safety dataset is, to our knowledge, the largest contemporary documentation of repeated fever-range thermal stress induction in cancer patients. It establishes that the operational components of a classical Coley-style protocol — repeated dosing, fever-range thermal endpoint, multi-week duration — can be administered safely under current clinical monitoring conditions.

3.3 The Three Constraints Modern Trials Violated

A reading of the major modern trials of bacterial or PAMP-based immunotherapy alongside the Hobohm framework reveals a striking pattern. Where these trials produced target engagement without objective tumor regressions, they did so under conditions that systematically violated the operational principles the Hobohm group has identified. The same observation has been made independently by Maletzki and colleagues in their 2012 review of Coley's toxin reevaluation (Maletzki et al. 2012), who identified three specific failure modes in modern attempts.

Fever as stop criterion, not endpoint. In trials including SYNB1891 (Luke et al. 2023), ADU-S100 (Meric-Bernstam et al. 2022), and many TLR agonist studies, cytokine release syndrome and systemic febrile response have been treated as dose-limiting toxicities to be avoided through dose ceiling design. The Karbach et al. (2012) trial of mixed bacterial vaccine in NY-ESO-1-expressing tumors represents a partial exception: fever in the 38–39.5°C range was explicitly used as the dosing endpoint, with doses escalated in each patient until the target temperature was achieved. The single partial response observed in that trial — in a patient with metastatic bladder cancer — was associated with both the highest fever and the most pronounced cytokine elevations. Across the field as a whole, however, sustained febrile activation has been characterized as a safety signal to suppress rather than a therapeutic state to maintain.

Treatment duration too short. Modern PAMP-based trials have typically employed treatment courses of weeks at most, often only a single induction cycle of several doses. The Karbach trial's first phase consisted of escalating doses over a limited number of administrations before tolerance and antibody development limited further escalation. The SYNB1891 protocol involved three weekly intratumoral injections in cycle 1, with up to four cycles total. By contrast, Coley's original protocols frequently extended over months, and Nauts' documentation of his case series records patients receiving 100 or more injections over a year or longer (Nauts, Fowler, and Bogatko 1953). The 2018 Reuter et al. clinical experience similarly emphasizes multi-month protocols. Maletzki et al. (2012) explicitly note short treatment duration as a likely contributor to the failure of modern bacterial extract trials to reproduce historical results.

Patients immunocompromised by prior or concurrent chemotherapy. A consistent feature of modern bacterial immunotherapy trials, including SYNB1891 and

Karbach 2012, is the enrollment of heavily pretreated patients with advanced refractory disease, typically following multiple lines of cytotoxic chemotherapy. In the SYNB1891 trial, participants had received a median of three prior lines of systemic therapy, with a range of up to eight (Luke et al. 2023). The expected immunological consequence — lymphopenia, reduced dendritic cell and natural killer cell numbers, impaired cytokine responsiveness — is well documented across cytotoxic regimens. Maletzki et al. (2012) and the Hobohm group have argued that this represents a major confound in interpreting modern Coley-derived trial outcomes: the patients in whom the approach has been tested are those whose immune systems are least capable of mounting the response the approach is designed to elicit.

A separate observation from the SYNB1891 trial supports the broader thesis from a different direction. Among the small number of patients with prolonged stable disease in that study, both had detectable CD11c+ dendritic cell populations in pretreatment tumor biopsies, while patients with "immune desert" tumors showed neither molecular activation nor clinical benefit at any dose level (Luke et al. 2023). This pattern is consistent with the prediction that PAMP-driven activation requires an existing antigen-presenting cell scaffold on which to operate, and that the most heavily pretreated patients are the least likely to retain such a scaffold.

3.4 The Convergent Hypothesis Stated Precisely

Drawing the elements together: the framework as articulated by Hobohm and colleagues, supported by the convergent evidence from the historical Coley case series, the 2012 Karbach trial, the SYNB1891 experience, the 2018 Reuter clinical safety dataset, and the Maletzki et al. critical reviews, predicts that **durable antitumor response to bacterial immunotherapy or its PAMP-receptor-ligand analogues requires the following four conditions in combination**:

1. **Combinatorial PAMP activation**, engaging multiple innate immune sensors in parallel rather than agonism of a single pathway. Single-pathway approaches (STING-only, TLR9-only, TLR4-only) produce target engagement and measurable molecular changes but rarely produce durable regressions.

2. **Sustained systemic activation reaching fever-range thermal stress**, with fever induction in the 38.5–39.8°C range used as the dosing endpoint rather than as a dose-limiting toxicity. The thermal component is not incidental: it engages distinct biological mechanisms that are addressed in Section 4.5.

3. **Treatment duration measured in months rather than weeks**, with intermittent scheduling sufficient to avoid endotoxin tolerance and the development of neutralizing antibodies against the immunostimulant. The Karbach trial's observation that a 3-month rest period restored responsiveness in a previously tolerant patient is consistent with this requirement.

4. **Preservation of host immune function**, avoiding concurrent or recent lymphodepleting chemotherapy where possible. Where cytotoxic agents are required for tumor burden control, low-dose metronomic regimens — which do not produce significant lymphopenia, and which have independent immunostimulatory effects through immunogenic cell death — represent a more compatible

combination than standard maximum-tolerated-dose chemotherapy.

The hypothesis is testable. It generates specific empirical predictions, which are developed in Section 7. It is consistent with the historical Coley case series, the partial successes of the modern Coley-derived trials reviewed in Section 2, the failures of single-pathway PAMP approaches, the documented modern safety of fever-induction protocols, and the patient-level findings from the SYNB1891 biomarker analysis. The remainder of this paper examines what further integration of 2025–2026 immunological knowledge would add to this framework (Section 4), proposes a specific protocol design that incorporates both the convergent thesis and the modern additions (Section 5), presents a documented clinical observation consistent with the framework (Section 6), and articulates the predictions by which the framework can be falsified (Section 7).

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4. What 2025–2026 Knowledge Adds

The convergent thesis articulated in Section 3 is, in its essential elements, the framework Hobohm and colleagues have developed over more than two decades. It is consistent with the historical Coley case series, the partial successes of modern bacterial immunotherapy trials, and the documented modern clinical safety of fever induction. What it does not yet incorporate are five developments in cancer immunology that have either matured or emerged since the 2018 Reuter et al. publication, and which substantially strengthen and constrain the proposed framework. This section addresses each in turn. The five additions are: a personalized neoantigen specificity layer using mRNA platforms; predictive modeling of PAMP combination synergy; biomarker-based patient selection; intermittent dosing schedules informed by tolerance kinetics; and a substantially clearer molecular framework for the role of fever itself in immune orchestration.

4.1 Personalized Neoantigen Specificity as a Complementary Layer

The principal scientific criticism leveled at Coley's protocols, both historically and against modern bacterial extract approaches, is that they elicit broad, non-specific immune activation without tumor-directed targeting. The PAMP cocktail rationale defended in Section 3 produces innate awakening, dendritic cell maturation, and a permissive cytokine environment, but it does not by itself instruct the adaptive immune response to recognize tumor-specific antigens. In the original Coley protocols, the source of antigen-specificity was the tumor itself, presented through cross-presentation of tumor-derived material released during the inflammatory response — an effect today characterized as in-situ vaccination.

The mRNA neoantigen vaccine platforms that matured between 2022 and 2025 supply precisely this missing specificity layer. mRNA-4157 (V940), developed by Moderna in collaboration with Merck, encodes up to 34 patient-specific neoantigens identified by sequencing the patient's tumor and predicting immunogenic epitopes via bioinformatic algorithms (Weber et al. 2024). In the randomized Phase IIb KEYNOTE-942 trial, the addition of mRNA-4157 to pembrolizumab in patients with completely

resected high-risk stage IIIB–IV cutaneous melanoma reduced the hazard for recurrence or death by approximately 44% relative to pembrolizumab alone (hazard ratio 0.561; 95% confidence interval 0.309–1.017). The 2.5-year recurrence-free survival rate improved from 55.6% with pembrolizumab alone to 74.8% with the combination, with a similar magnitude of benefit observed for distant metastasis-free survival (Weber et al. 2024). A Phase III trial in 1089 patients with high-risk melanoma is ongoing (NCT05933577), with regulatory submissions anticipated in 2026.

The Memorial Sloan Kettering and BioNTech collaboration on autogene cevumeran in pancreatic ductal adenocarcinoma — a tumor type with substantially lower mutational burden than melanoma, and historically considered immunologically "cold" — demonstrated vaccine-induced neoantigen-specific T-cell responses persisting for years post-treatment in a substantial proportion of patients, with corresponding reductions in recurrence at extended follow-up (Rojas et al. 2023). The platform's demonstrated efficacy extending from a tumor with very high mutational burden to one with low burden suggests broad applicability across tumor types likely to be addressed by a Coley-style framework.

The relevance to the convergent thesis is direct and bidirectional. The Coley framework provides innate awakening and a permissive context for adaptive immune priming. The mRNA neoantigen platform provides specific direction for the awakened adaptive response. Each addresses a deficit of the other: classical Coley therapy lacks tumor-specific direction; classical neoantigen vaccines frequently fail in the absence of adequate dendritic cell activation and a non-suppressive tumor microenvironment, a deficit that PAMP cocktails and fever-induced systemic effects are designed to address. The hypothesis that the combination would be additive or synergistic is testable, and represents a clear design choice for the Neo-Coley v2 protocol developed in Section 5.

4.2 Computational Modeling of PAMP Combination Synergy

Section 3 articulated the empirical case for combinatorial over single-pathway PAMP activation. Preclinical work in the past two years has made this case substantially more precise. A 2024 preprint from the Gajewski laboratory examined the combination of the STING agonist DMXAA with the TLR4 agonist LPS, demonstrating that suboptimal doses of each agent administered together produced significantly improved control of B16 melanoma in mice compared to either agent alone, even when comparison was made at matched total cytokine output (Gajewski group, bioRxiv 2024). The mechanism identified was co-engagement of both the NFκB and IRF3 transcriptional pathways. The authors note that this combinatorial co-engagement is the natural pattern of innate signaling during pathogen exposure, and that single-pathway agonists fail to recapitulate it.

The same body of work has also identified important antagonisms. Pre-activation of the STING pathway suppresses subsequent TLR9-mediated interferon production from plasmacytoid dendritic cells (Gehrcken et al. 2025), an observation with direct implications for the temporal sequence of agonist administration: if certain agents must be given concurrently rather than sequentially to avoid mutual interference, the protocol design must accommodate this. The detail matters because it suggests that

not all PAMP combinations are simply additive, and that empirical mixtures such as the classical mixed bacterial vaccine may benefit from rational decomposition into their pathway-engaging components, followed by recombination according to known signaling interactions.

This is the domain in which AI-assisted analysis adds genuinely new capability. Single-cell transcriptomic data, signaling network models, and the increasingly detailed maps of pattern recognition receptor pathway crosstalk now permit predictive modeling of which agonist combinations will produce synergy versus antagonism, at which relative concentrations, and under which temporal sequences. The classical Coley preparation engaged perhaps a dozen distinct receptor pathways simultaneously through the natural complexity of two heat-killed bacterial species. A modern reconstruction can be more deliberate, identifying the subset of pathway combinations predicted to produce maximal synergy with minimal antagonism, and matching the activation profile to the immune context of the individual patient's tumor. This is the rationale for the cocktail composition logic proposed in Section 5.

4.3 Biomarker-Based Patient Selection

Section 3.3 noted the SYNB1891 trial finding that baseline CD11c+ dendritic cell density in pre-treatment tumor biopsies predicted response, while patients with "immune desert" tumors showed neither molecular activation nor clinical benefit at any dose level (Luke et al. 2023). This observation generalizes. The 2025 literature on cold versus hot tumors has converged on the recognition that the immunologic state of the tumor microenvironment at the time of treatment initiation is a stronger predictor of response to innate-immune-modulating therapy than any property of the agent itself.

Multiple lines of evidence support the operational claim that pre-treatment tumor profiling should function as a selection criterion rather than as a post-hoc explanation of variable response. Multi-omics analyses have identified intrinsic and extrinsic mechanisms of immune evasion that distinguish responders from non-responders to checkpoint inhibition, and similar patterns are emerging from analyses of innate immune agonist trials. Tumor mutational burden, loss of heterozygosity at HLA loci affecting neoantigen presentation, TGF-β-driven T-cell exclusion, and the density of suppressive cell populations such as regulatory T cells and tumor-associated macrophages each independently affect response probability.

For the Neo-Coley v2 framework, three biomarkers are of particular relevance and should inform enrollment criteria:

- *Baseline antigen-presenting cell density* in tumor tissue, measured by CD11c+ and CD11b+ immunofluorescence, as a direct proxy for the APC scaffold on which the therapy operates;
- *T-cell exclusion versus T-cell-inflamed signature* in the tumor microenvironment, distinguishing patients in whom APC-recruiting pretreatment would be needed from those in whom direct PAMP activation may suffice;
- *Tumor mutational burden and HLA loss-of-heterozygosity status*, predicting the quality of neoantigen presentation possible under the personalized vaccine component of the protocol.

The implication for protocol design is operationally important: the framework should not be tested in the unselected refractory-advanced patient population that has been standard for first-in-human bacterial immunotherapy trials. It should be tested in patients pre-stratified by these biomarkers to be capable of mounting the response the framework predicts. This is one of the principal reasons modern bacterial immunotherapy trials have produced disappointing results — the enrollment criteria selected against the population in which response was biologically possible.

4.4 Dosing Schedule Design Against Endotoxin Tolerance

The principal mechanistic obstacle to sustained PAMP-based therapy is the development of endotoxin tolerance — a transient refractory state in which repeat exposure to TLR4 agonists in particular produces progressively weaker cytokine and fever responses — and, in the case of complex bacterial preparations, the development of anti-bacterial antibodies that neutralize subsequent doses. The Karbach et al. (2012) trial documented both phenomena explicitly, with the authors attributing the limited efficacy of sustained MBV administration in their cohort substantially to these mechanisms.

A critical observation from the same trial points toward the solution. Patient #11 of the Karbach cohort, having developed tolerance during the initial dose-escalation phase, was placed on a three-month rest period before resuming MBV under a compassionate-use single-patient protocol at the highest dose level, with sixteen additional injections. The patient again developed fever and cytokine responses comparable to those observed earlier in treatment, indicating that endotoxin tolerance is reversible on a timescale of months rather than permanent. This finding has direct protocol-design implications: a sustained Coley-style regimen administered over many months must incorporate scheduled rest periods of sufficient duration to permit tolerance reset, and may further benefit from strain rotation — periodic substitution between preparations of distinct bacterial compositions — to avoid antibody-mediated neutralization accumulating against any single preparation.

The dosing schedule proposed in Section 5 reflects this insight: an induction phase of weekly administration to establish fever and cytokine responses, followed by maintenance cycles consisting of two-to-four-week pulses separated by rest intervals of comparable or greater duration, with strain or preparation rotation introduced after the second pulse cycle. This pattern is testable against the simpler continuous-dosing approach typical of historical Coley protocols, and against fixed-interval pulse-rest patterns without rotation.

4.5 Fever as Molecular Orchestrator

The most consequential addition to the convergent thesis is that the molecular case for fever's role in immune orchestration has become substantially clearer than was available to Hobohm in 2001 or even at the time of the 2018 Reuter et al. publication. The body of work emerging from the Repasky and Evans laboratories at Roswell Park, together with the broader heat shock protein immunology literature, now provides a mechanistic account of why fever-range thermal stress at 39–40°C is not merely a

marker of cytokine release but an active and partially independent component of the immune response.

Five distinct effects of thermal stress in the febrile range have been characterized:

First, **dendritic cell maturation is induced directly by fever-range temperature through HSP90 upregulation** (Basu and Srivastava 2003). Brief exposure to elevated temperature produces an immature-to-mature DC transition that is independent of, and complementary to, PAMP-driven maturation. This implies that PAMP and fever acting together produce more complete DC activation than either alone — a prediction the framework makes explicit.

Second, **T-cell trafficking across high endothelial venules is enhanced by fever-range thermal stress through IL-6 trans-signaling mechanisms** (Evans, Repasky, and Fisher 2015). Fever upregulates intravascular ICAM-1 density in HEV, promotes L-selectin adhesion through MEK1-ERK1/2 signaling in lymphocytes, and increases T-cell extravasation into lymph nodes and into tumors. The molecular basis includes HSP90 binding to α4 integrins, which activates the FAK-RhoA pathway driving adhesion-dependent migration; this effect has been characterized specifically at 40°C (Lin et al. 2019).

Third, **NK cell cytolytic activity is enhanced by thermal stress**, through induction of MHC class I polypeptide-related sequence A (MICA) expression on target cells and through NKG2D receptor clustering on the NK cell surface (Evans, Repasky, and Fisher 2015). This effect operates independently of T-cell priming and provides a parallel cytotoxic arm engaged by fever but not by intratumoral PAMP delivery in the absence of systemic thermal stress.

Fourth, **antigen cross-presentation to MHC class I is augmented by extracellular heat shock proteins released during thermal stress**, which bind and chaperone peptide antigens into the cross-presentation pathway of dendritic cells (Srivastava 2002). This is the molecular substrate of the in-situ vaccination effect classically attributed to Coley's therapy: tumor antigens released during the inflammatory response are bound by HSPs released from the same context, and are then presented as CD8+ T-cell targets with substantially higher efficiency than in the absence of thermal stress.

Fifth, **neutrophil recruitment and function are upregulated** by fever-range hyperthermia through G-CSF and CXCL8-dependent mechanisms, with increased respiratory burst capacity that contributes to both bacterial killing and tumor cell stress (Evans, Repasky, and Fisher 2015).

The collective implication is consequential. Fever in the 39–40°C range is not a side effect that must be tolerated for cytokine release to be achieved. Fever is an active and partially independent contributor to immune orchestration, engaging mechanisms — HEV trafficking enhancement, HSP-mediated cross-presentation, NK cytolysis, DC maturation, neutrophil recruitment — that are not produced by intratumoral cytokine release alone, and that are not engaged by modern bacterial immunotherapy approaches engineered to avoid systemic thermal response. This provides a coherent mechanistic explanation for why approaches such as SYNB1891 produce confirmed target engagement (the intratumoral cytokine component) without durable response

(the systemic orchestration is absent): they reproduce the local inflammatory component while losing the systemic orchestration that fever provides. It also explains why historical Coley protocols and the documented modern cases consistent with them emphasize titrating to sustained 39–40°C fever specifically.

This is the strongest mechanistic argument the framework offers against the prevailing direction of the modern field. The five additions described in this section, taken together with the convergent thesis of Section 3, define both the rationale and the design constraints for the Neo-Coley v2 protocol developed in Section 5.

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5. The Neo-Coley v2 Protocol Design

This section translates the convergent thesis of Section 3 and the five additions of Section 4 into a specific protocol design that could serve as the basis for an investigator-initiated trial under appropriate regulatory and institutional oversight. The protocol is specified at the level of design principles, component categories, dosing logic, and endpoints — not at the level of exact drug dosages, which require clinical input from investigators with direct experience of the candidate agents. The design is intentionally modular: components may be substituted or omitted based on availability, regulatory context, or specific tumor type, without compromising the integrity of the underlying framework.

5.1 Eligibility and Patient Selection

The framework predicts that response requires preserved immune function and an existing antigen-presenting cell scaffold. Eligibility criteria follow directly.

Tumor types of primary interest. Histologically confirmed solid tumors known to have demonstrated response to bacterial immunotherapy historically, or to innate immune modulation in modern trials: soft tissue sarcoma, osteosarcoma, melanoma, renal cell carcinoma, urothelial carcinoma, triple-negative breast cancer, head and neck squamous cell carcinoma. Other tumor types are not excluded but require additional rationale.

Disease setting. Two settings are of particular interest. The first is adjuvant or perioperative therapy in high-risk resected disease, where the framework would be tested against documented recurrence patterns and would benefit from a minimal residual disease context. The second is advanced metastatic disease after no more than one or two prior lines of therapy, where the immune system has not been substantially compromised by extended cytotoxic exposure.

Immune competence requirements. Absolute lymphocyte count ≥1.0 × 109/L, preferably ≥1.5. No lymphodepleting chemotherapy within 30 days. No corticosteroid use exceeding prednisone 10 mg/day equivalent within 14 days. Adequate hematologic, renal, hepatic, and cardiopulmonary function to safely tolerate fever induction to 39.8°C.

Tumor immune profile. Pre-treatment biopsy with multiplex immunofluorescence demonstrating baseline CD11c+ dendritic cell density above a threshold to be cali-

brated against the SYNB1891 reference data and clinical assay validation. A T-cell-inflamed or moderately inflamed signature is acceptable. Tumors classified as "true desert" by Galon-style immunoscore criteria are either excluded, or assigned to a separate stratum receiving APC-recruiting pretreatment.

Key exclusions. Active autoimmune disease requiring systemic immunosuppression. Pregnancy. Inability to safely tolerate fever to 39.8°C (severe cardiopulmonary compromise, uncontrolled seizure disorder, recent severe systemic infection). Concurrent uncontrolled infection of any kind.

5.2 Treatment Architecture and Components

The protocol consists of four components administered in a defined temporal architecture.

Component A: Combinatorial PAMP activator. A defined mixture engaging multiple pattern recognition receptors in parallel. Three candidate compositions are listed in descending order of regulatory accessibility and historical evidence base:

1. *MBV-equivalent preparation* (heat-inactivated *Streptococcus pyogenes* and *Serratia marcescens*), administered subcutaneously following the dose-titration approach validated by Karbach et al. (2012). This composition engages TLR2, TLR4, TLR9, NOD1/2 and other receptors in the natural combinatorial pattern of Coley's original preparation.
2. *OK-432 (Picibanil) in combination with a CpG-A class TLR9 agonist*, where MBV-equivalent preparations are unavailable. OK-432 is currently licensed in Japan and has been investigated in more than 18 randomized clinical trials. CpG-A class agents, including vidutolimod, have shown clinical activity in melanoma refractory to PD-1 blockade (Ribas et al. 2021).
3. *Rationally constructed multi-agonist cocktail* consisting of a detoxified LPS variant (TLR4), a CpG-A TLR9 agonist, mistletoe lectin (TLR-engaging Shiga-toxin-family lectin per Maletzki et al. 2013), and a STING agonist administered concurrently to avoid the pre-activation-mediated TLR9 antagonism described by Gehrcken et al. (2025). This option requires computational modeling of relative concentrations and timing, and represents the longest-term form of the protocol once supporting evidence accumulates.

Component B: Specificity layer. Personalized neoantigen mRNA vaccine designed from pre-treatment tumor exome sequencing, following the architecture demonstrated by mRNA-4157 (encoding up to 34 patient-specific neoantigens). Administered intramuscularly. The timing of vaccine doses relative to PAMP cocktail dosing is specified in Section 5.3.

Component C: Checkpoint inhibitor. Anti-PD-1 antibody (pembrolizumab or equivalent), administered intravenously at standard dosing every three weeks throughout the protocol. The role is to rescue the antigen-specific T-cell response from PD-L1-mediated exhaustion in the tumor microenvironment, after the inflammatory and adaptive priming components have established the immune context in which checkpoint blockade can act.

Component D (optional): Low-dose metronomic chemotherapy. In patients requiring tumor burden control, low-dose oral cyclophosphamide (typically 50 mg/day) or comparable agents documented to produce immunogenic cell death without lymphodepletion. This component explicitly substitutes for, rather than supplements, conventional maximum-tolerated-dose chemotherapy. Its inclusion is governed by the principle articulated in Section 3.4: preservation of host immune function is non-negotiable.

5.3 Dosing Endpoint and Schedule

The protocol uses **fever induction as the primary dosing endpoint** for Component A. This is the defining operational feature of the design, and the principal divergence from modern bacterial immunotherapy convention.

Induction phase (Weeks 1–8). Component A is administered subcutaneously twice weekly, with the dose escalated at each administration until body temperatures in the 39.0–39.8°C range are reliably induced. Paracetamol (1 g) is permitted only as a ceiling intervention if body temperature exceeds 39.8°C or remains sustained above 39.5°C for more than six hours. Antipyretics are otherwise withheld, in contrast with standard clinical practice but consistent with the framework's central thesis that the febrile response is the medicine, not a side effect. The induction phase continues until the patient achieves at least four consecutive sessions with fever reliably reaching the target range at a stable dose, or for a maximum of 8 weeks.

Concurrent vaccine administration. Component B (neoantigen vaccine) is initiated at Week 1 if vaccine manufacture has been completed, or as soon as available thereafter, with up to nine doses administered over the induction and early maintenance phases per the mRNA-4157 schedule. Vaccine and PAMP cocktail are administered on the same day where possible, with vaccine administration following the febrile peak by 24–48 hours, to align adaptive priming with the most immunogenic phase of the innate response.

Concurrent checkpoint inhibitor. Component C is administered every three weeks beginning at Week 1.

Maintenance phase (Week 9 onward). After completion of induction, the regimen transitions to a pulse-rest pattern: a three-week pulse cycle of twice-weekly Component A administration, followed by a three-week rest interval. This pattern continues for at least twelve months in metastatic disease, twenty-four months in high-risk adjuvant settings. After the second pulse cycle (approximately Week 18), **preparation rotation** is introduced: an alternative PAMP cocktail composition is substituted for one pulse cycle, then the original composition resumed. The rotation is designed to circumvent anti-bacterial antibody accumulation per the rationale developed in Section 4.4.

Total duration. Minimum twelve months of protocol, extended to twenty-four months for high-risk adjuvant settings. This duration is consistent with the historical Coley protocols, which extended over months to years, and with the documented modern multi-month fever induction experience (Reuter, Oettmeier, and Hobohm 2018).

5.4 Monitoring and Response Assessment

The protocol generates substantial monitoring requirements, both for safety and for biological correlate assessment.

Safety monitoring. Vital signs, complete blood count, and basic metabolic panel before each PAMP cocktail administration and at 24 hours afterward. Continuous body temperature monitoring during the twelve hours following each dose, with the option of outpatient remote monitoring after the first three weeks. Adverse event assessment per CTCAE.

Biological correlate monitoring. Serum cytokine panel (IL-6, TNF-α, IFN-γ, IL-1β) at baseline, six hours after dose, and twenty-four hours after dose for the first three Component A administrations, then weekly during induction. Anti-PAMP-preparation antibody titers at baseline, end of induction, and every three months thereafter, to track tolerance and neutralizing antibody development. Multiplex flow cytometry of peripheral blood for lymphocyte subsets, NK cell activation markers, and T-cell exhaustion markers at the same intervals.

Tumor response monitoring. Cross-sectional imaging (CT or MRI per tumor type) every eight weeks during the first year, every twelve weeks thereafter. Circulating tumor DNA (ctDNA) at baseline, end of induction, and every six weeks thereafter. ctDNA dynamics are increasingly recognized as a more sensitive early signal of treatment effect than imaging-based RECIST assessment, particularly in inflammatory contexts where pseudoprogression is biologically expected.

Tumor biopsy timepoints. Pre-treatment biopsy is mandatory, both for eligibility (CD11c+ density) and for baseline molecular profiling. End-of-induction biopsy (Week 8–9) is optional but strongly recommended for biological correlate analysis, particularly for documenting changes in immune cell infiltration, PD-L1 status, and T-cell receptor repertoire. Biopsy at any later progression event is performed if accessible, for resistance mechanism characterization.

5.5 Response Definitions and Stopping Rules

The framework's response logic differs from conventional clinical trial response assessment in one important respect: durable benefit may follow apparent early radiographic progression, particularly during the induction phase when inflammatory pseudoprogression is biologically expected. Stopping rules are designed to accommodate this.

Continuation criteria. The protocol is continued if any of the following apply: stable or improving disease by imaging; ctDNA stable or declining; clinical benefit by patient and physician assessment; biopsy-confirmed increase in tumor immune infiltration at the end-of-induction timepoint.

Discontinuation criteria. The protocol is discontinued for: unequivocal progression confirmed by both imaging and ctDNA at two consecutive eight-week assessments; cumulative grade 3 or higher toxicity not adequately managed by standard supportive care; persistent inability to achieve fever range despite dose escalation

and preparation rotation (indicating refractory tolerance not responsive to scheduled rest); or patient withdrawal.

Adverse event management. Grade 1–2 fever and chills are expected and constitute the protocol's intended pharmacodynamic effect. They are managed with hydration and limited paracetamol per the dosing rules above. Cytokine release syndrome grade 3 or higher is managed per standard institutional CRS protocols, with tocilizumab available as a rescue if needed. The Reuter, Oettmeier, and Hobohm (2018) safety dataset of 523 fever inductions in 131 patients provides the principal evidence base for the expected adverse event profile of the protocol.

5.6 Schematic Summary

The protocol can be summarized as follows.

> **Patient:** Selected by tumor type, disease setting (adjuvant high-risk or metastatic post-one-to-two-lines), preserved immune competence (lymphocyte count, no recent lymphodepleting therapy), and tumor immune profile (CD11c+ APC scaffold present; not "true desert").
>
> **Treatment components:** Four agents administered in concert. - *A:* Combinatorial PAMP activator (MBV-equivalent, or OK-432 plus CpG-A, or rationally constructed multi-agonist cocktail), administered subcutaneously twice weekly, dose-titrated to induce fever 39.0–39.8°C. - *B:* Personalized neoantigen mRNA vaccine, intramuscular, up to nine doses, timed to follow fever peaks. - *C:* Anti-PD-1 antibody, intravenous, every three weeks. - *D (optional):* Low-dose metronomic chemotherapy if tumor burden control is required, explicitly substituting for conventional cytotoxic therapy.
>
> **Phases:** Induction (Weeks 1–8) → Maintenance pulse-rest cycles with preparation rotation, continuing 12–24 months total.
>
> **Dosing endpoint:** Sustained fever in the 39.0–39.8°C range, with antipyretics used only as a ceiling above that range.
>
> **Response measured by:** Cross-sectional imaging, ctDNA, biopsy-based immune correlates, and patient/clinician assessment of benefit.

Each design choice traces directly to a specific element of the framework developed in Sections 3 and 4. Section 6 documents one clinical observation consistent with a subset of these protocol features. Section 7 articulates the predictions by which the protocol — and the framework underlying it — can be falsified or confirmed.

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6. Clinical Observation: A Documented Case Consistent with the Framework

The preceding sections have developed the convergent thesis (Section 3) and the 2025–2026 integrations (Section 4) that together form the Neo-Coley v2 framework, and have translated that framework into a specific protocol design (Section 5). The

framework is supported by historical case series (Section 1), modern small-trial evidence (Section 2), preclinical multi-PAMP work, and a mechanistic foundation in fever-range thermal stress immunology (Section 4.5). What it has not yet been tested against is a contemporaneously documented patient case in which the framework's predicted protocol pattern — fever-titrated, sustained, with preserved immune function — was administered.

This section documents one such case. The patient is the spouse of the document's author. The author has no medical training. The case is presented not as evidence of efficacy at population scale, which a single patient cannot provide, but as a documented example of the protocol pattern the framework predicts, in a tumor type and historical context where the outcome observed is uncommon by the standard of care of the era. The limitations of this case are stated explicitly in Section 6.5 and should be read alongside the documented features.

6.1 Clinical Context and Treatment Decision

In late 2010 or early 2011, the patient — a thirty-six-year-old woman, BRCA1-mutation carrier, with no prior history of malignancy — was diagnosed with triple-negative breast cancer following workup for a palpable left breast mass. The histopathologic diagnosis was confirmed by core needle biopsy and immunohistochemistry, demonstrating absence of estrogen receptor, progesterone receptor, and HER2 expression — the standard immunohistochemical definition of triple-negative disease. The lesion was subsequently characterized at surgical pathology (April 2011) as a 2.4 cm Stage IIA (pT2 pN0) invasive ductal carcinoma, Nottingham grade 3 (overall score 9/9), with a small component of metaplastic carcinoma showing squamous differentiation; three sentinel lymph nodes from the left axilla were negative for carcinoma on multi-level examination and keratin AE1/AE3 immunostaining.

The standard of care for triple-negative breast cancer in 2010–2011 consisted of surgical resection where appropriate, neoadjuvant or adjuvant cytotoxic chemotherapy according to anthracycline- and taxane-based regimens, and radiation therapy as indicated. Immunotherapy was not part of the standard of care for any breast cancer subtype at that time. The KEYNOTE-522 trial establishing pembrolizumab plus chemotherapy as standard neoadjuvant treatment for high-risk triple-negative breast cancer would not report until 2020. The five-year overall survival for triple-negative disease at the time of the patient's diagnosis was substantially worse than for hormone-receptor-positive or HER2-positive subtypes, and the metaplastic subtype the patient carried was — and remains — particularly difficult to treat: published 5-year overall survival for Stage II metaplastic TNBC ranges from approximately 50% to 70% across series, with documented resistance to standard anthracycline–taxane regimens.

The author, through independent research at the time of diagnosis, became aware of two bodies of work that informed the treatment decision: the historical record of bacterial immunotherapy associated with William Coley, and the contemporaneously emerging clinical use of in vitro chemosensitivity testing — a laboratory technique in which a panel of chemotherapeutic agents and combinations is tested against the patient's tumor cells ex vivo, with the goal of identifying drug combinations to which

the specific tumor is most sensitive. The sensitivity panel performed for the patient's tumor reportedly indicated that the standard anthracycline–taxane regimen would be of limited efficacy against her tumor cells in vitro, while an alternative combination demonstrated measurable activity.

After consultation with multiple oncologists, and the eventual identification of one willing to administer chemotherapy according to the sensitivity-panel-guided regimen rather than the published standard, the patient received the sensitivity-panel-guided regimen (cisplatin plus gemcitabine) at reduced dose. In parallel, the patient was admitted to CHIPSA Hospital in Tijuana, Mexico — a center with established practice in combination integrative protocols under COFEPRIS authority — for a three-week intensive immune-priming phase consisting of (a) Coley toxin administration (mixed bacterial vaccine of *Streptococcus pyogenes* and *Serratia marcescens* lineage, sourced via the institution from MBVax Bioscience, the Canadian manufacturer of modern Coley toxin preparations), (b) autologous tumor antigen vaccine prepared from the patient's biopsy specimen, and (c) weekly whole-body fever-range hyperthermia at 39–40°C, sustained for two to four hours per session. The patient was then discharged with a continuing supply of Coley toxins, and the author continued home administration of the Coley toxins under fever titration throughout the subsequent months — including during the surgical recovery period and during the parallel low-dose chemotherapy phase. The home administration was without direct medical supervision but proceeded from the dosing schedule and titration protocol the patient had received at CHIPSA.

6.2 Protocol Administered

The protocol features, reconstructed from the author's memory in the absence of contemporaneous written clinical records, were the following.

The Coley-type preparation was a mixed bacterial vaccine (*Streptococcus pyogenes* and *Serratia marcescens* lineage) sourced via CHIPSA Hospital from MBVax Bioscience, Canada — the principal modern manufacturer of Coley toxin preparations during the relevant period. The composition followed the historical Coley formulation, with batch-to-batch variation characteristic of biologic preparations.

Administration during the initial three-week intensive phase at CHIPSA was subcutaneous, performed by clinical staff, with weekly whole-body fever-range hyperthermia (39–40°C, sustained two to four hours per session) administered in parallel. The dosing endpoint was fever induction. Doses were escalated progressively until body temperatures approaching but not exceeding 40°C were reliably induced, with associated rigors and chills consistent with the classical pyrogen response. During the subsequent home-continuation phase, the author administered the Coley toxins subcutaneously following the titration schedule established at CHIPSA. Paracetamol (1 g) was used as a ceiling intervention if body temperature exceeded the threshold the author had selected as the upper safety limit, and was otherwise withheld during the febrile episodes. The protocol was administered repeatedly over multiple months, with the precise total number of administrations no longer documented.

In parallel with the bacterial preparation administration, the patient received the sensitivity-panel-guided reduced-dose chemotherapy regimen (cisplatin plus gemc-

itabine) described in Section 6.1. Standard surgical intervention — bilateral mastectomy with sentinel lymph node biopsy (therapeutic left, prophylactic right reflecting BRCA1+ status) — was performed at South Miami Hospital on April 5, 2011, with the resulting surgical pathology confirming the Stage IIA (pT2 pN0) classification documented in Section 6.1.

The protocol pattern corresponds to several specific features of the framework developed in Sections 3–5: a combinatorial multi-PAMP preparation rather than single-pathway agonist; fever induction as dosing endpoint, sustained at or near the framework's predicted optimal range of 39–40°C; multi-week duration rather than single-cycle administration; and parallel reduced-dose rather than maximum-tolerated-dose chemotherapy. It does not correspond to all features of the Neo-Coley v2 protocol as designed — most notably, it predated the personalized neoantigen mRNA vaccine, anti-PD-1 checkpoint inhibitor, and biomarker-based patient selection components, none of which were available in 2010–2011 — and it was administered outside any institutional or regulatory framework.

6.3 Outcome and Follow-up

At the time of writing, approximately fifteen years after diagnosis, the patient is alive and without documented recurrence. Routine surveillance imaging and oncologic follow-up have continued throughout the interval. The absence of recurrence after this duration is uncommon in metaplastic triple-negative breast cancer, by the standards of both the diagnostic era and present clinical experience. Population-level recurrence-free survival data for triple-negative breast cancer in 2010–2011 indicated that most recurrences occurred within the first three years following primary treatment, with the great majority within five years; for the metaplastic subtype specifically, published 5-year overall survival ranged from approximately 50% to 70% across series, with recurrence patterns considered more aggressive than conventional TNBC. Durable disease-free survival beyond ten years without recurrence in patients with high-risk metaplastic disease was an outcome with which the contemporaneous standard of care was not consistently associated.

6.4 Secondary Observation: An Associated Case

The author is acquainted with a second patient whose clinical course was briefly observed in proximity to the index case. This patient, an eighteen-year-old woman at the time of diagnosis approximately ten or more years prior to the present writing, was diagnosed with stage IV cutaneous melanoma. She was evaluated at Memorial Sloan Kettering Cancer Center in New York, where her family was reportedly advised to prepare for end-of-life care given the absence of effective therapy for her disease at that time. The patient was subsequently treated overseas with a Coley-type bacterial immunotherapy protocol, the specific details of which are not available to the author. At the time of writing, the patient is alive and reportedly without evidence of disease.

This secondary observation is presented with two specific caveats. First, the details of the protocol administered are not available to the author, and the case therefore cannot be characterized as a documented administration of the protocol pattern proposed in this paper. Second, melanoma is unusual among cancers in that documented cases

of spontaneous regression — including durable complete regression of metastatic disease — exist independent of any therapy (Bramhall, Mahady, and Peach 2014). The relative immunogenicity of melanoma, evidenced by the higher rate of response to checkpoint inhibition in this tumor type than in most others, also implies that a single case of durable remission, even in stage IV disease, cannot be confidently attributed to any specific intervention without contemporaneous documentation of the therapy administered. The case is included as an associated observation contributing context, not as supporting evidence for the framework.

6.5 What This Observation Shows and Does Not Show

The index case is a single patient who received parallel cytotoxic therapy, was treated outside any institutional or research framework, and has no contemporaneous biological correlate documentation. It is incapable, by its nature, of establishing causation, or of supporting any conclusion about the population-level efficacy of the protocol pattern described.

What it does demonstrate is the following. The protocol pattern the framework predicts — combinatorial multi-PAMP preparation, fever-titrated dosing endpoint at or near the 39–40°C range, sustained multi-week administration, parallel reduced-dose rather than maximum-tolerated-dose chemotherapy — was administered to one patient in 2011, in the absence of any institutional support for such an approach, and was tolerated without observed serious adverse events. The patient — diagnosed with Stage IIA (pT2 pN0) high-grade BRCA1-mutated triple-negative invasive ductal carcinoma with foci of metaplastic transformation, a subtype with published 5-year overall survival of approximately 50–70% across series — has remained recurrence-free at fifteen-year follow-up. The outcome is consistent with the framework's predictions, but the case design does not isolate the contribution of the bacterial preparation from the contributions of the parallel chemotherapy, the surgical and radiation interventions, or the natural history of the patient's specific tumor.

The case is presented in this paper for one reason. The framework developed in the preceding sections rests on a substantial body of preclinical, mechanistic, and historical evidence, but has been tested in modern clinical settings only under operational conditions (single-pathway agonism, fever suppressed as toxicity, abbreviated duration, immunocompromised patients) that the framework predicts will not produce durable response. The contemporary clinical evidence base for the protocol pattern the framework does predict to produce durable response — combinatorial, fever-titrated, sustained, in immune-competent patients — is sparse to nonexistent in formally documented form. One documented case, with its substantial limitations acknowledged, contributes one data point in a setting where no such data point has previously been presented in the literature this synthesis draws on.

This case is presented neither as proof of concept nor as a recommendation for treatment. It is presented as the observation that motivated the author to undertake the synthesis represented by this paper, and as one piece of clinical context that future investigators of the Neo-Coley v2 protocol pattern may wish to consider.

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7. Predictions and Falsifiers

A framework that cannot be falsified is not a scientific framework, regardless of how much supporting evidence can be assembled for it. This section articulates the specific empirical predictions generated by the Neo-Coley v2 framework, and the experimental results that would constitute evidence against each prediction. The predictions are organized in four categories: predictions from the convergent thesis of Section 3; predictions from the 2025–2026 additions of Section 4; predictions about the Neo-Coley v2 protocol as an integrated intervention; and the set of results that would, taken together, constitute decisive evidence that the framework as a whole is wrong.

7.1 Predictions from the Convergent Thesis

The convergent thesis (Section 3.4) makes four operational claims, each generating a specific testable prediction.

Prediction 1: Multi-PAMP activation outperforms single-pathway agonism at matched cytokine output. A randomized comparison of a multi-PAMP cocktail (engaging at least TLR4, TLR9, and STING) versus single-pathway STING agonism, calibrated to produce equivalent peak serum IL-6, TNF-α, and IFN-γ levels, should produce higher objective response rates and longer progression-free survival in the multi-PAMP arm. *Falsifier:* equivalent response and survival in both arms at matched cytokine output, with any difference attributable to cytokine magnitude alone rather than to the qualitative pattern of receptor engagement.

Prediction 2: Sustained fever-range thermal stress contributes to response independently of peak cytokine levels. A randomized comparison of fever-permitted versus fever-suppressed administration of the same agonist at the same dose should produce higher objective response rates in the fever-permitted arm. *Falsifier:* equivalent response in both arms, indicating that the systemic thermal component does not contribute independently of intratumoral cytokine release.

Prediction 3: Treatment duration measured in months produces longer durable benefit than treatment measured in weeks. A randomized comparison of an 8-week induction-only protocol versus a 12-month protocol with maintenance pulse-rest cycles should produce longer recurrence-free survival in the multi-month arm, with all other features held equivalent. *Falsifier:* equivalent long-term outcomes, indicating that brief intense induction is sufficient and that maintenance dosing does not contribute to durability.

Prediction 4: Preserved immune function is required for response. A prospective cohort analysis of response stratified by baseline absolute lymphocyte count, prior cytotoxic exposure, and dendritic cell density should reveal substantially higher response rates in immune-competent patients than in heavily pretreated, lymphopenic, or APC-depleted patients. *Falsifier:* comparable response rates across immune-status strata, indicating that the framework's emphasis on preserved immune function is misplaced.

7.2 Predictions from the 2025–2026 Additions

The five integrations introduced in Section 4 each generate distinct predictions.

Prediction 5: The personalized neoantigen specificity layer increases durability of response over PAMP cocktail alone. A randomized comparison of PAMP cocktail with versus without concurrent personalized mRNA neoantigen vaccine should produce longer recurrence-free survival in the combination arm, with detectable neoantigen-specific T-cell responses correlating with the durability advantage. *Falsifier:* equivalent durability with and without the vaccine component, indicating that broad innate activation alone is sufficient for the observed long-term immune memory.

Prediction 6: PAMP combinations selected by predictive modeling of synergy outperform empirically combined cocktails at equivalent total dose. A randomized comparison of an empirically constructed PAMP mixture (such as MBV) versus a rationally combined cocktail with components and ratios specified by signaling synergy/antagonism modeling should produce higher response rates or lower toxicity at matched total cytokine output. *Falsifier:* equivalent results, indicating that empirical combinations capture sufficient synergy and that rational decomposition and recombination add nothing of clinical value.

Prediction 7: Baseline CD11c+ dendritic cell density predicts response independent of dose. A prospective biomarker analysis of response stratified by baseline APC scaffold should reveal a density threshold below which response rates are near zero regardless of dose, and above which response rates correlate with the magnitude of immune activation. *Falsifier:* response observed across all APC density strata, indicating that the framework's emphasis on the pre-existing immune scaffold is incorrect.

Prediction 8: Endotoxin tolerance is reversible by scheduled rest, and pulse-rest dosing produces sustained response where continuous dosing fails. A randomized comparison of continuous weekly dosing versus pulse-rest cycles with strain rotation should produce longer-maintained fever and cytokine responses, and longer-duration treatment with maintained response, in the pulse-rest arm. *Falsifier:* equivalent response durability across schedules, indicating that tolerance is either not the limiting factor in continuous dosing or is not mitigated by rest periods.

Prediction 9: Fever-range thermal stress engages immune mechanisms not engaged by intratumoral cytokine release alone. A prospective immunological correlate analysis should reveal that fever-permitted protocols produce, relative to fever-suppressed protocols at matched intratumoral cytokine output, greater increases in (a) tumor-infiltrating CD8+ T cells, (b) NK cell activation markers in peripheral blood, (c) circulating HSP-bound tumor antigens, and (d) high endothelial venule remodeling markers consistent with the IL-6 trans-signaling and HSP90-α4 integrin mechanisms described in Section 4.5. *Falsifier:* equivalent immunological profiles with and without permitted systemic fever, indicating that the additional mechanisms described do not operate at clinically meaningful magnitude.

7.3 Predictions About the Protocol as an Integrated Intervention

Beyond the component predictions above, the Neo-Coley v2 protocol generates several integrative predictions that can be tested only by deploying the protocol as a single intervention.

Prediction 10: The Neo-Coley v2 protocol produces objective response rates substantially higher than checkpoint inhibitor monotherapy in matched populations. In patient populations defined by the eligibility criteria of Section 5.1 (eligible tumor types, preserved immune competence, APC scaffold present), the protocol should produce objective response rates exceeding those of checkpoint inhibitor monotherapy in comparable populations. *Falsifier:* equivalent or inferior objective response rates, indicating that the integrated protocol fails to add benefit over current best standard.

Prediction 11: Apparent early radiographic progression (pseudoprogression) is followed by subsequent regression in a substantial subset of responders. ctDNA dynamics should diverge from imaging in this subset, with declining ctDNA preceding eventual imaging response. *Falsifier:* pseudoprogression is rare and indistinguishable from genuine progression by all available markers, eliminating the rationale for the protocol's modified stopping rules.

Prediction 12: The protocol produces durable immune memory detectable in peripheral blood for years after treatment completion. Long-term immunological follow-up should reveal sustained neoantigen-specific T-cell responses and altered T-cell receptor repertoire characteristic of antigen-experienced memory in responders. *Falsifier:* response without durable detectable immune memory, indicating that the response mechanism does not involve adaptive memory formation in the manner the framework predicts.

7.4 The Decisive Falsifier

Taken together, the predictions above admit of partial confirmation or partial refutation in many configurations. A single failed prediction does not falsify the framework as a whole; it falsifies the specific claim and prompts revision. The set of results that would constitute decisive evidence against the framework, however, is the following: a well-designed, adequately powered, biomarker-selected, randomized clinical trial of the full Neo-Coley v2 protocol against current standard of care, conducted in immune-competent patients with the predicted favorable tumor immune profile, that demonstrates neither objective response benefit nor durable disease-free survival benefit over the comparator.

Such a trial would be conclusive in a way no individual component prediction can be, because it would test the integrated framework under exactly the conditions the framework predicts to produce response. A negative result under those conditions would not be explainable by reference to the operational failures of previous modern bacterial immunotherapy trials, because those failures would have been corrected by design. The framework would, in that scenario, simply be wrong, and the modest response rates produced by single-pathway PAMP approaches in unselected refractory populations would represent the genuine ceiling of what bacterial immunotherapy

can achieve.

The framework's defenders, including the present author, should commit in advance to this falsifier. The discipline of stating in advance what would constitute decisive evidence against one's own framework is what distinguishes scientific work from advocacy. The framework developed in this paper is offered in that spirit.

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8. Discussion: What This Framework Is, and What It Is Not

This paper has developed a synthesis framework for combinatorial PAMP immunotherapy with sustained pyretic induction, drawn together a body of historical and modern evidence consistent with the framework, translated the framework into a specific protocol design, presented one documented clinical case consistent with the protocol pattern, and articulated the predictions and falsifiers by which the framework can be tested. This concluding section addresses three questions explicitly: what this paper is and is not, what limitations attach to its synthesis approach, and what next steps would be appropriate for researchers who find the framework worth pursuing.

8.1 What This Paper Is

This paper is a position paper. It articulates a research-program-level framework for revisiting Coley-derived bacterial immunotherapy under contemporary scientific conditions, building substantially on the published work of Hobohm and colleagues and integrating five subsequent developments in cancer immunology. It is intended as input to scientific discussion among researchers actively working on bacterial immunotherapy, fever therapy, cancer vaccines, innate immune modulation, and related areas, and as a structured proposal for the design of investigator-initiated clinical research.

The framework is offered as a coherent hypothesis with explicit predictions, not as a settled scientific claim. The protocol design in Section 5 is offered at the level of design principles and component categories, intended as the conceptual basis for an investigator-initiated trial — not as a clinical guideline ready for direct implementation. The documented clinical case in Section 6 is offered as one consistent observation, not as supporting evidence at population scale.

8.2 What This Paper Is Not

This paper is not peer-reviewed. It has not been subjected to formal review by the scientific community whose work it synthesizes. Errors of interpretation, omission, or emphasis remain possible at any point in the synthesis and would benefit from correction by researchers with direct expertise in each constituent area.

This paper is not a treatment recommendation. None of its content should be interpreted as advising the use of any of the agents described, the administration of any of the protocols described, or any modification of standard medical care. Patients with cancer, and the clinicians who care for them, should consult their institutional treatment recommendations and any clinical trials for which they are eligible.

This paper is not a comprehensive review of cancer immunotherapy or of bacterial immunotherapy more broadly. It is structured around a single specific hypothesis — that combinatorial PAMP activation with sustained fever induction in immune-competent patients is the protocol pattern modern bacterial immunotherapy has lost. Adjacent fields receive attention only where directly relevant to that hypothesis. Other approaches to cancer immunotherapy, including approaches that disagree with or supersede the Coley-derived framework, are not given the attention they would receive in a comprehensive review.

This paper is not based on original experimental data. The author has performed no wet-lab work, no animal studies, no clinical trials, and no preclinical research of any kind. All experimental claims are drawn from the published literature of other investigators, cited in the references.

8.3 Limitations of the Synthesis Approach

The synthesis presented in this paper was conducted from outside any academic, industrial, or clinical research institution. This has several specific implications.

The literature accessible to the synthesis is limited to public sources — peer-reviewed publications, preprints, registered trial records, regulatory filings, and similar publicly available material. Unpublished observations, lab-internal data, conference abstracts not indexed in major databases, and personal communications among researchers in the field are not accessible to the synthesis. Where the framework draws conclusions from published evidence that conflicts with unpublished evidence known within a specific research community, the synthesis would be expected to err in that area.

The synthesis was conducted with substantial assistance from AI-based research and writing tools, including for literature search, paper summarization, drafting, and structural organization. The author is responsible for all interpretive choices and for the framework's claims; the AI tools were used as research and writing aids, not as authors. Where the framework makes specific scientific claims, those claims have been checked against primary sources where possible, but the possibility of error in interpretation of specialist literature remains, particularly in subsections drawing on areas adjacent to the author's primary focus — heat shock protein immunology, mRNA vaccine immunology, computational PAMP signaling modeling. Expert review of those subsections in particular would strengthen the document.

The clinical case documented in Section 6 has substantial limitations stated explicitly within that section. The case is one data point, not a case series; it is from memory, not contemporaneous records; it is confounded by parallel chemotherapy; it lacks biological correlate documentation. These limitations should be read alongside the case features.

The framework's five 2025–2026 integrations (Section 4) are each grounded in real and active areas of research, but the combination of all five into a single integrated protocol design has not been previously tested or, to the author's knowledge, previously proposed in this specific combination. The synergy among them is hypothesized on the basis of the framework's underlying claims; the actual interactions among com-

ponents when deployed together require empirical testing before any confident claim about their combined effect can be made.

8.4 Acknowledgments

The framework developed in this paper rests substantially on the work of others, and the author wishes to acknowledge specific debts.

The convergent thesis of Section 3 is, in its essential features, the framework Uwe Hobohm and his clinical collaborators Uwe Reuter, Ralf Oettmeier, and Maurice Orange have developed over more than two decades of publication. The author's contribution is to integrate five subsequent developments in cancer immunology with their framework, propose a specific protocol design synthesizing the integrated framework, and present one supporting clinical observation. The intellectual core of the convergent thesis belongs to Hobohm and colleagues, and the present author is in their debt.

The historical case archive on which Sections 1 and 3 depend was assembled, organized, and preserved by Helen Coley Nauts at the Cancer Research Institute over the course of her professional life from 1953 until her death in 2001. Without her decades of careful documentation, the empirical foundation for any modern engagement with Coley's clinical observations would be substantially weaker than it is.

The modern Coley replication trial reviewed in Section 2.1 was conducted by Julia Karbach and colleagues at the Krankenhaus Nordwest in Frankfurt and the Ludwig Institute for Cancer Research. The fever-titration dosing approach they validated in a current GMP context is the most direct modern operational precedent for the protocol pattern this paper proposes.

The molecular framework of fever-range thermal stress immunology synthesized in Section 4.5 draws principally on the laboratories of Elizabeth Repasky and Sharon Evans at Roswell Park Comprehensive Cancer Center. The clinical case for fever as immune orchestrator rests substantially on their preclinical and translational work.

Donald MacAdam and the MBVax Bioscience compassionate-use experience preserved Coley therapy as a living clinical practice through years when it would otherwise have disappeared. His 2018 documentation is one of the few sources for case-level information on modern Coley protocol use outside formal trials.

The patient documented in Section 6, and the medical professionals who collaborated on her care, are not named for reasons of privacy. Her case is the proximate cause of the author's interest in the framework developed here, and the present synthesis would not exist without her.

8.5 Next Steps

For researchers who find the framework worth engaging, several specific next steps are suggested.

The most useful immediate step would be critical review of the synthesis by investigators actively working in the constituent areas — particularly the Hobohm group,

researchers working on engineered bacterial cancer therapy, researchers working on mRNA cancer vaccines, and researchers working on fever-range thermal stress immunology. Errors of interpretation in the synthesis would be most efficiently identified by researchers with direct expertise in each component literature.

Component predictions from Section 7 are individually testable through investigator-initiated trials that do not require the full Neo-Coley v2 protocol. Prediction 1 (multi-PAMP versus single-pathway at matched cytokine output) and Prediction 9 (immunological correlates of fever-permitted versus fever-suppressed protocols) in particular are testable within existing trial infrastructure, with patient populations and dosing schedules that resemble current bacterial immunotherapy trials.

Reanalysis of the Helen Coley Nauts historical case archive with modern statistical and computational methods would substantially strengthen or weaken the framework's empirical foundation. The archive contains case-level data on more than one thousand patients; the relationship between fever intensity, treatment duration, parallel therapies, and outcome has never, to the author's knowledge, been formally analyzed across the full archive using modern methods.

For industrial interest, the development of a current GMP-compliant multi-PAMP preparation — whether based on the historical Coley vaccine formulation, on a rationally constructed cocktail, or on an engineered hybrid — represents a high-value manufacturing target. The MBVax precedent demonstrates that the manufacturing barrier, not the scientific question, has been the principal obstacle to formal trial advancement of Coley-derived therapies for the past two decades.

For patients and families with documented clinical cases that fit the protocol pattern described in this paper, the Hobohm group at THM University Giessen and their clinical collaborators are an active research community that may be receptive to case documentation contributions. The author's intent in producing this synthesis is to support that work, not to compete with it.

The framework developed in this paper is offered with the explicit hope that it will be tested, modified, and either confirmed or refuted by researchers in a position to do so. If the framework is correct in its essential features, the most important consequence of this paper would be to accelerate the design of trials testing it. If the framework is wrong, the most important consequence would be to clarify why, and to redirect attention toward the approaches that will work.

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