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Neo-Coley v2: A Unified Framework for Combinatorial PAMP Immunotherapy with Sustained Fever-Range Thermal Stress in Cancer

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Abstract

William Coley's bacterial cancer immunotherapy, practiced between 1891 and 1936 in more than one thousand patients with inoperable cancer, reported durable response rates that, in the historical record consolidated by McCarthy (2006), compare favourably with outcomes from modern checkpoint inhibitor monotherapy in similar

refractory disease — with the methodological caveats appropriate to a nineteenth-century case series. Modern attempts to replicate Coley's results have produced confirmed target engagement but rare objective regressions. Building on the published work of Hobohm and colleagues at THM University of Applied Sciences, Giessen, and on the clinical safety data published by Reuter, Oettmeier, and Hobohm (2018), this hypothesis paper articulates a convergent thesis: durable response may require 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, and preservation of host immune function. Five modern knowledge extensions are integrated: personalized neoantigen mRNA vaccines, computational modeling of PAMP combination synergy, biomarker-based patient selection, scheduling design informed by published clinical experience, and a clearer molecular account of fever's role in immune orchestration. The framework is implementable through three options: whole bacterial preparations, live attenuated vaccine pathogens, and defined synthetic PAMP cocktails. The synthetic option, developed in Section 5, addresses the manufacturing constraints that have limited every modern bacterial trial: each proposed component (MPLA, CpG ODNs, imidazoquinolines, STING agonists, Pam3CSK4, Poly ICLC) is either FDA-approved, in active clinical trials, or has documented human safety data, and the cocktail dose-titrated against fever produces the thermal stress component through the body's own pyrogenic pathway. A novel proposition (Component F) explores multi-recall mRNA-LNP tumor antigen redirection, leveraging pre-existing recall immunity to tetanus toxoid and SARS-CoV-2 spike epitopes. Sixteen empirical predictions and a single decisive falsifier are articulated. This paper presents a hypothesis to be tested, modified, or refuted by the field. It does not present new experimental data, and the framework is not a claim of demonstrated efficacy.

Keywords: cancer immunotherapy; Coley toxins; bacterial immunotherapy; pathogen-associated molecular patterns; PAMP; Toll-like receptor; STING agonist; fever-range thermal stress; whole-body hyperthermia; neoantigen vaccine; mRNA-LNP; tumor immunology; combinatorial immunotherapy; innate immunity; heat shock protein; hypothesis paper

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Disclaimer

This document presents a theoretical synthesis of public-domain scientific literature and articulates a testable hypothesis. It is intended as input to investigator-initiated scientific discussion. It is not medical advice and does not constitute a treatment recommendation. The framework has not been tested in a clinical trial. All clinical investigation of bacterial, synthetic-PAMP, or hyperthermia-based immunotherapy must be conducted under appropriate regulatory and institutional oversight.

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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). These figures must be interpreted with the methodological caveats appropriate to a nineteenth-century case series — including non-standardized diagnostic criteria of the era, the absence of contemporaneous control groups, selection bias inherent in clinical practice records, and outcome reporting standards that do not map cleanly onto modern RECIST or survival-curve methodology. The historical numbers cannot be directly compared with modern trial outcomes, and the framework presented here does not depend on the precise magnitude of Coley's reported response rates. It depends only on the

more modest empirical observation that durable responses occurred at a rate sufficient to motivate four decades of clinical practice and to merit modern mechanistic investigation.

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 underwent 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 introduction of cytotoxic chemotherapy in the 1940s and 1950s, with its directly demonstrable cytotoxic effect against rapidly dividing cells and its compatibility with the increasingly randomized-controlled-trial-oriented evidence framework of post-war oncology, displaced Coley's approach over the next decade. By 1965 Coley's practice was effectively extinct in academic oncology in the United States, and his preparation was withdrawn from the approved drug list by the US Food and Drug Administration in 1962 on grounds of insufficient modern evidence rather than demonstrated harm.

A question follows that the modern field has not adequately addressed. The response rates reported in Coley's case series, despite the methodological caveats noted above, appear to compare favourably with those produced by checkpoint inhibition in comparable refractory disease — the historical record consolidated by McCarthy (2006) reports five-year survival above 50% in inoperable sarcoma cases, while modern checkpoint inhibitor monotherapy produces 15–30% durable response rates across solid tumors. The two figures are not directly comparable, given differences in diagnostic criteria, patient selection, outcome standards, and the methodological rigor of the underlying data. Yet the gap is large enough, and consistent enough across multiple tumor types in the historical record, to merit serious examination of the question: if the underlying phenomenon — induced durable immune-mediated tumor regression — is operating in both eras, what features of the historical practice might explain the apparent difference in outcomes? 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 at least part of the gap in efficacy is the consequence of that loss.

This paper develops the second hypothesis, in synthesis with the published work of Hobohm and colleagues at THM University Giessen and their clinical collaborators (Hobohm 2001, 2009; Hobohm, Stanford, and Grange 2008; Orange, Reuter, and Hobohm 2016; Reuter, Oettmeier, and Hobohm 2018), which has 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 the present paper adds five integrations made possible by developments in cancer immunology since 2018: personalized neoantigen vaccines, computational modeling of PAMP combination synergy, biomarker-based patient selection, scheduling design informed by published clinical experience with sustained fever protocols, 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); the implementation options for delivering the required PAMP activation are surveyed in depth (Section 5); the framework is translated into a specific protocol design suitable as the basis of an investigator-initiated trial (Section 6); and Section 7 specifies the predictions and falsifiers by which the framework can be tested. The acknowledged limitations of the framework are stated explicitly in Section 8.

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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. Together, they reveal that all four shared a particular operational pattern: they administered antipyretics whenever the patient's body temperature reached or exceeded 38.5°C, treating fever as a side effect to be controlled rather than the pharmacodynamic endpoint of the treatment.

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 generated three findings 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, the protocol used acetaminophen administration whenever fever reached 38.5°C, a constraint imposed by ethical committee review that may have prevented the trial from sustaining the higher fever range (39–40°C) historically associated with Coley response.

The published interpretation of the Karbach data emphasized endotoxin tolerance and neutralizing antibody development as limiting factors. Subsequent clinical experience with sustained fever-induction protocols over multi-month durations (Reuter, Oettmeier, and Hobohm 2018) does not support this interpretation as a general phenomenon: the same dose continues to induce comparable fever responses over multi-month protocols in the clinical experience of the THM Giessen and Klinik im Leben groups. Tolerance kinetics may be relevant to the specific compounds used in the Karbach preparation, but they are not a universal limitation of sustained PAMP-based therapy. This is the first of several corrections incorporated in this revised version of the framework.

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. 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) and 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.

The SYNB1891 trial protocol mandated antipyretic administration whenever body temperature reached 38.5°C, treating cytokine release syndrome including fever as a managed toxicity rather than a pharmacodynamic endpoint.

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 and 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.

The MBVax program ended in 2017 not because of clinical failure but because of the manufacturing challenges of producing a consistent GMP-quality whole-bacterial preparation at scale. This is an important observation: even when the protocol pattern was approximately correct, the manufacturing constraints of whole-bacterial therapy proved limiting. Section 5 addresses this directly, and the synthetic implementation option developed in Sections 5.3–5.6 specifically addresses the manufacturing problem that has constrained the bacterial approach.

2.4 OK-432 (Picibanil) in Japan

OK-432 is a heat- and penicillin-treated preparation of *Streptococcus pyogenes* Su strain, in continuous clinical use in Japan since approval in 1975 for the treatment of cancer and lymphangiomas. Cumulative clinical experience involves over 100,000 patients, primarily in adjuvant settings for gastric, lung, and head-and-neck cancers (Sakamoto et al. 2002).

The published meta-analyses of randomized trials demonstrate a modest but consistent survival benefit when OK-432 is added to standard chemotherapy in gastric and lung cancer, with hazard ratios for overall survival generally in the 0.80–0.90 range.

The effect is small relative to that produced by modern checkpoint inhibitors in responsive populations, but the difference in trial design is informative. OK-432 has typically been administered to titrate against mild fever and constitutional symptoms, with no consistent attempt to drive fever into the 39–40°C range associated with the historical Coley protocols. As with the other modern programs, antipyretic intervention has been routine.

2.5 The Convergent Operational Pattern

The four modern bodies of evidence above share a striking operational pattern. In each, fever was treated as a side effect to be controlled rather than as the pharmacodynamic endpoint of the therapy. In each, the molecular signal Coley engaged most strongly — the multi-receptor combinatorial detection of bacterial molecular signatures — was either approximated (Karbach, MBVax, OK-432) or replaced with a single-pathway agonist (SYNB1891). And in each, durable objective response was rare. The next section articulates the framework that emerges from this convergent pattern.

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

The work of Hobohm and his collaborators over two decades has articulated a specific framework for understanding why modern Coley-derived trials have produced confirmed target engagement without the durable responses Coley reported. The framework identifies four operational conditions necessary for durable response. This section presents that framework. Sections 4 and 5 extend it.

3.1 Combinatorial PAMP Activation

Pathogen-associated molecular patterns are conserved molecular signatures of microbial origin that are detected by pattern recognition receptors on innate immune cells. The major PRR families relevant to bacterial detection include the Toll-like receptors (TLR2, TLR4, TLR5, TLR9), the cytosolic NOD-like receptors (NOD1, NOD2), the cytosolic STING pathway sensing cyclic dinucleotides, and the inflammasome-associated NLRP receptors.

A genuine bacterial infection presents all of these signatures simultaneously, in spatial and temporal correlation. The immune system has evolved to respond most strongly when this combinatorial pattern is detected. Synergy between receptors operates at multiple levels: shared and complementary signaling cascades, transcription factor co-activation, cytokine cross-regulation, and the integrated determination of dendritic cell maturation phenotype. A combinatorial PAMP signal does not produce the linear sum of single-receptor outputs; it produces qualitative responses that single-receptor activation cannot.

Modern PAMP-based immunotherapy programs have predominantly used single-pathway agonists: STING agonists (cyclic dinucleotides, ADU-S100, SYNB1891), TLR9 agonists (vidutolimod, lefitolimod), TLR7/8 agonists (imiquimod, motolimod).

When tested as monotherapy, these have produced response rates substantially below those reported for Coley's multi-PAMP preparations.

The framework's first claim is that this gap is not coincidental. Single-pathway PAMP agonism, however potent at its specific receptor, does not produce the integrated response that combinatorial multi-receptor activation produces. Durable response requires combinatorial activation.

3.2 Sustained Fever-Range Thermal Stress as the Dosing Endpoint

The framework's claim is that fever in the 39–40 °C range is not merely a side effect of cytokine release that must be tolerated in order to achieve the response, but rather an active and partially independent component of the immune response, engaging mechanisms that intratumoral cytokine release in the absence of systemic fever does not engage. The molecular basis of this claim is developed in Section 4.5; here the operational implication is stated.

Coley's clinical practice titrated dose against fever in the 39–40°C range, sustained for hours, and continued the treatment over months. Modern programs have engineered fever out: by mandating antipyretics at 38.5°C, by selecting routes of administration (intratumoral) that produce limited systemic response, by selecting agents (STING agonists with limited fever-induction profiles) that do not robustly produce sustained febrile response.

The framework's second claim is that this systematic suppression of the febrile response is one of the principal reasons modern programs have not reproduced Coley's results. Durable response requires sustained fever-range thermal stress as the dosing endpoint, with antipyretics used only as a ceiling intervention above the target range.

3.3 Multi-Month Treatment Duration

Coley administered his preparation for periods measured in months to years, not weeks. The MBVax compassionate-use cases included durable responders treated for twelve to twenty-four months. The Reuter, Oettmeier, and Hobohm (2018) safety dataset documents 523 fever inductions in 131 patients, with treatment durations typically extending over multiple months. The historical pattern is one of sustained engagement, not acute intervention.

Modern programs have generally used shorter durations: SYNB1891's intratumoral injection regimen extended over weeks, not months. The Karbach 2012 trial's induction phase was eight weeks. Checkpoint inhibitor monotherapy is typically given indefinitely until progression but at a fixed interval and dose that does not require titration to a pharmacodynamic endpoint.

The framework's third claim is that durable response requires sustained engagement of the immune response over months, not the acute pulse of a brief induction. The mechanism is at least three-fold: cumulative refinement of T-cell repertoire toward tumor-relevant specificities; sustained pressure against tumor immune escape mechanisms; and adequate time for the cellular reorganization (HEV remodeling, infiltrating

lymphocyte populations, memory T-cell formation) that durable response requires.

3.4 Preservation of Host Immune Function

The patient who responds to bacterial immunotherapy is the patient whose immune system can respond. Heavy cytotoxic chemotherapy depletes the rapidly dividing immune cell populations on which the response depends: lymphocytes, dendritic cells, neutrophil precursors. Long-term corticosteroid therapy suppresses cell-mediated immunity broadly. Both interventions are routine in modern oncology, often preceding any consideration of immunotherapy.

The framework's fourth claim is that durable response requires preserved immune function. This implies operational constraints on patient selection (preference for less heavily pretreated patients, exclusion of significant immunosuppression) and on combination therapy design (avoidance of lymphodepleting chemotherapy in the active treatment phase; restricted corticosteroid use).

3.5 The Convergent Pattern

The four conditions are not independent and cannot be substituted. They are jointly necessary. A trial that engages multiple PAMPs but suppresses fever fails. A trial that drives fever but uses single-pathway agonism fails. A trial that satisfies all three operational conditions in a patient whose immune system has been depleted fails. The historical Coley protocol satisfied all four; modern programs have satisfied none in combination. This is the framework's central operational thesis.

The next section adds five extensions from cancer immunology developments since 2018.

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4. What Modern Knowledge Adds

Five developments in cancer immunology since 2018 enrich the convergent framework articulated in Section 3 without altering its core claims. These additions sharpen patient selection, improve mechanistic specificity, suggest measurable correlates of response, and provide a substantially clearer molecular account of fever's role. Section 4.4 has been reformulated to reflect the published clinical experience of multi-month sustained fever-induction protocols (Reuter, Oettmeier, and Hobohm 2018), which documents continued fever responses over extended dosing without observable tolerance.

4.1 Personalized Neoantigen mRNA Vaccines

The KEYNOTE-942 trial (mRNA-4157/V940 plus pembrolizumab in resected high-risk melanoma) demonstrated, in 2023, that personalized neoantigen vaccines administered in combination with checkpoint inhibition produced a 44% reduction in recurrence at three-year follow-up compared with pembrolizumab alone (Khattak

et al. 2023; Weber et al. 2024 update). The vaccine is constructed from tumor-specific mutations identified by whole-exome sequencing of the resected tumor; the constituent peptides are restricted to neoantigens predicted to be presented by the patient's HLA class I and class II alleles.

For the Neo-Coley v2 framework, the personalized neoantigen vaccine offers a specificity layer that Coley's bacterial preparation could not provide. The bacterial preparation engaged broad immune activation; the vaccine directs that activation toward tumor-specific antigens. The combination is biologically rational: the bacterial PAMP cocktail produces the dendritic cell maturation and inflammatory milieu in which neoantigen-loaded vaccine peptides are presented with maximum immunogenicity. The two modalities engage different layers of the immune response (innate combinatorial activation versus adaptive antigen specificity) and are complementary by design.

4.2 Computational Modeling of PAMP Combination Synergy

Network-based modeling of PRR signaling has matured substantially in the past five years. Combinatorial PAMP signaling integrates inputs through shared transcription factors (NFκB, IRF3, IRF7), through cross-regulation of cytokine outputs (TLR9 amplification of TLR4 signaling, STING modulation of TLR4 trafficking), and through divergent or convergent dendritic cell maturation phenotypes. Computational models of these interactions have identified specific receptor combinations expected to produce maximal synergy, minimal redundancy, and acceptable cytokine release profiles.

A specific empirically validated example: Combinatorial TLR7/8 plus TLR9 dual agonism produces tumor regression in mouse models where neither single agonist alone is effective against large established tumors (Zhao et al. 2014). Other documented synergies and antagonisms include CpG-A TLR9 plus MPLA TLR4 (synergistic for IFN-γ induction), STING plus TLR7/8 (synergistic for DC maturation in some contexts), and STING pre-activation followed by TLR9 (antagonistic; STING desensitizes subsequent TLR9 responses) (Bhatnagar et al. 2022; Gehrcken et al. 2025).

For the Neo-Coley v2 framework, this provides a tool for rational protocol design that Coley did not have. Where Coley empirically combined two species of bacteria, modern protocol design can specify combinations of synthetic agonists predicted to produce specific cytokine and DC maturation phenotypes. This is taken up in Section 5.

4.3 Biomarker-Based Patient Selection

The SYNB1891 trial dataset, when reanalyzed with attention to baseline tumor immune profiles, demonstrates that patients with detectable CD11c+ dendritic cell populations in pretreatment biopsies showed both molecular activation and the longest stable disease responses, while "immune desert" tumors showed neither at any dose level (Luke et al. 2023, supplementary analyses). This pattern is consistent with the broader literature on tumor immune classifications: T-cell-inflamed tumors respond to immune therapies; immune desert tumors do not.

For the Neo-Coley v2 framework, the implication is that response to combinatorial PAMP activation requires an existing antigen-presenting cell scaffold. Patient selection must include assessment of baseline tumor immune profile (CD11c+ density, T-cell infiltration patterns, MHC expression).

4.4 Scheduling Considerations

The original draft of this paper proposed a pulse-rest dosing schedule (induction phase, rest interval, repeated pulses with strain rotation) on the basis of an interpretation of Karbach 2012 patient #11, where a three-month rest period appeared to reset cytokine and fever responses. The published clinical experience of sustained fever-induction protocols using approved PAMP-containing drugs, documented by Reuter, Oettmeier, and Hobohm (2018) across 523 fever inductions in 131 patients, indicates that exhaustion of fever induction is not observed in clinical practice at multi-month durations: the same dose continues to produce comparable fever responses over months. This published empirical observation supersedes the speculative interpretation from a single patient.

The revised framework therefore drops the pulse-rest dosing as a tolerance-mitigation strategy. Continuous dosing over multi-month durations remains the protocol baseline, consistent with Coley's historical practice and with the Reuter, Oettmeier, and Hobohm clinical experience. Scheduling decisions may still incorporate pragmatic considerations (allowing T-cell expansion phases to track adaptive immune timing, accommodating patient quality of life, managing logistic constraints of multi-month protocols), but these are matters of clinical judgment rather than necessary mitigation of a pharmacological limitation.

A related consideration concerns anti-PAMP antibody development. The available clinical and preclinical literature does not support neutralizing antibodies against PAMPs as a clinically meaningful obstacle to sustained dosing for most PAMP categories, with the possible exceptions of mistletoe lectin and flagellin. The framework therefore does not treat antibody-driven neutralization as a primary scheduling consideration.

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 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 conceptually significant. The framework's claim is that fever in the 39–40 °C range is not merely a side effect that must be tolerated for cytokine release to be achieved, but rather an active and partially independent contributor to immune orchestration. The mechanisms engaged by fever — HEV trafficking enhancement, HSP-mediated cross-presentation, NK cytolysis, DC maturation, neutrophil recruitment — appear not to be produced by intratumoral cytokine release alone, and are not engaged by modern bacterial immunotherapy approaches designed to avoid systemic thermal response. This offers a coherent mechanistic explanation, at the hypothesis level, for why approaches such as SYNB1891 may produce confirmed target engagement (the intratumoral cytokine component) without durable response (the systemic orchestration is absent): they may reproduce the local inflammatory component while losing the systemic orchestration that fever provides. It is also consistent with the observation that historical Coley protocols and the documented modern cases corresponding to them emphasize titrating to sustained 39–40 °C fever specifically. These claims are testable by the predictions specified in Section 7.

4.6 Cytokine-Induced Fever vs Whole-Body Hyperthermia

The mechanisms above operate at the level of systemic thermal stress, regardless of how that thermal stress is achieved. This is an important point for the implementation options developed in Section 5: the framework's claim about thermal stress is about the *temperature*, not about *how* the temperature is delivered.

In Coley's original protocol, fever was generated entirely by the cytokine response to the injected bacterial preparation. Coley had no external heating equipment; the bacterial dose was titrated upward over successive injections until each patient reliably reached the 39–40°C target range. This is also the operational pattern at Klinik im Leben and across the Hobohm clinical network in 2025: bacterial PAMP medications are administered, and the resulting cytokine release produces the fever directly.

A synthetic multi-PAMP cocktail produces the same pyrogenic response through the same biological pathway. The cytokines induced — IL-6, TNF-α, IL-1β — act on the hypothalamic preoptic area to raise the body's temperature set point, exactly as bacterial PAMPs do. Dose titration against fever as the endpoint, the method Coley used and Karbach 2012 validated in a modern GMP context, applies directly to the synthetic cocktail.

External whole-body hyperthermia (WBH) at 39–40°C is a clinically established alternative or supplement that has substantial literature support. Two principal temperature ranges are used: extreme WBH (41–42°C) for direct cytotoxic effects on tumor cells, and fever-range WBH (39–40°C) for immune activation. The fever-range protocol is the one relevant to the Neo-Coley v2 framework. Patients are placed in a controlled heating environment — radiant heat chamber, water-perfusion suit, or pressurized infrared cabin — and core body temperature is raised to the target range and maintained for one to several hours.

A 2025 systematic review documents the temperature-dependent biological effects of induced hyperthermia. At 39°C, the dominant mechanism is immune activation: enhanced leukocyte adhesion to endothelium, increased immune cell trafficking into the tumor microenvironment, HSP induction and release, and enhanced antigen presentation. The review notes that hyperthermia in this range "mimics fever" and that the cytokine profile induced is similar to that of a natural febrile response (Lukácsi, Munkácsy, and Győrffy 2024).

For protocol implementation, WBH is most useful as a supplement in three specific scenarios: patients whose cytokine response to dose-titrated PAMP administration does not reach the target fever range even at safety-limited doses; early-phase trial designs where directly-measured, externally-controlled temperature provides reproducibility advantages over patient-by-patient cytokine variability; and patients in whom cytokine-induced fever is contraindicated or limited (older patients, patients with conditions where cytokine release syndrome carries elevated risk, or patients on medications that blunt the pyrogenic response).

In standard clinical settings without WBH equipment, the cocktail-only protocol is the framework's recommendation. The cost difference is substantial: WBH-augmented sessions in dedicated facilities cost approximately $1,500–5,000 per session; cocktail-only sessions in standard clinics cost approximately $50–250 per session. Across a

full 12-month protocol with 30–50 sessions, the per-patient cost difference is in the tens to low hundreds of thousands of dollars.

Thermal monitoring is essential regardless of mechanism. Continuous core temperature monitoring during each session is essential whether the thermal component is delivered by cytokine-induced fever, WBH, or both. Several technologies are accessible at low cost: ingestible temperature pills (e-Celsius, CorTemp; approximately $50–75 per pill, gold-standard core temperature, wireless transmission for 24–36 hours), non-invasive heat-flux sensors (CORE by greenteg; approximately $300, validated against esophageal in research settings), and tympanic infrared thermometers for periodic checks. Any of these supports a protocol where temperature is checked every few minutes and operator intervenes if predefined thresholds are crossed: antipyretic (paracetamol) if core temperature approaches 40.0–40.5°C, active cooling (cold packs, room air) if it exceeds 41°C.

The framework's thermal stress mechanism operates in the 39–40°C range. Below 38.5°C, the predicted immune amplification is insufficient; above 41°C, safety concerns dominate. The dosing window is narrow enough to require monitoring but wide enough to be achievable with established titration methods.

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5. Implementation Options: Bacterial, Synthetic, and Hybrid Approaches

The convergent thesis of Section 3 and the modern knowledge extensions of Section 4 specify what the immune system needs to receive for durable response: combinatorial PAMP activation, sustained fever, multi-month duration, preserved host immune function. The framework is silent on how the activation signal is delivered. Three implementation options are reviewed in this section, with the synthetic option developed in detail as it addresses the manufacturing problem that has constrained every modern bacterial trial.

5.1 Whole Bacterial Preparations

The historical and most thoroughly studied implementation uses heat-inactivated whole bacterial preparations. The two principal options are:

Mixed bacterial vaccines (MBV-class). Coley's original preparation combined heat-inactivated *Streptococcus pyogenes* and *Serratia marcescens*. The Karbach 2012 trial used a GMP-quality contemporary MBV preparation. MBVax Bioscience's Febrivax-C represented an attempt to manufacture a consistent contemporary equivalent. The MBV approach engages multiple PAMPs (TLR2 from gram-positive cell wall components, TLR4 from LPS, TLR9 from unmethylated bacterial DNA, NOD1/NOD2 from peptidoglycan) within a single preparation that physically presents the bacterial signature pattern to the immune system. The principal limitation is manufacturing complexity: producing consistent GMP-quality whole-bacterial preparations at scale is technically demanding, and this limitation contributed materially to the end of the MBVax program in 2017.

OK-432 (Picibanil). Approved in Japan in 1975 and used continuously since, OK-432 is a heat- and penicillin-treated preparation of *Streptococcus pyogenes* Su strain. It is the most clinically experienced whole-bacterial PAMP source available globally, with cumulative experience in over 100,000 patients. As a single-species preparation, it engages a narrower PAMP profile than MBV but with established manufacturing and regulatory infrastructure.

5.2 Live Attenuated Vaccine Pathogens

A category worth including alongside the bacterial and synthetic options is the use of live attenuated vaccine pathogens as PAMP sources. The principal advantages are that these are already approved drugs with established manufacturing, that they replicate transiently and therefore provide sustained signal delivery from a single administration, and that they engage broader PAMP profiles than purified components.

BCG (Bacillus Calmette-Guérin). The most clinically established live attenuated bacterial immunotherapy. Intravesical BCG is standard of care for non-muscle-invasive bladder cancer, with multi-decade safety experience and well-characterized efficacy. BCG engages TLR2, TLR4, NOD2, and STING (through bacterial cyclic dinucleotides), providing a broader PAMP profile than any single synthetic agonist. Systemic and subcutaneous BCG protocols have been investigated for various cancers with intermittent positive signals.

Other approved vaccine pathogens. Live attenuated measles vaccine (Edmonston strain), live attenuated polio vaccine (Sabin strain), modified vaccinia virus (Ankara strain), and yellow fever vaccine (17D strain) have all been investigated as oncolytic or immunomodulatory agents in cancer immunotherapy. Each engages distinctive PAMP combinations (viral RNA via TLR3 and TLR7/8 for measles and polio; combinations including TLR2 and TLR4 for vaccinia). Talimogene laherparepvec (T-VEC), a modified herpes simplex virus, is approved for melanoma with established clinical efficacy.

The live attenuated vaccine pathogen approach offers a distinct advantage that purified synthetic agonists do not: temporal kinetics of antigen-and-PAMP presentation that mimic actual infection. A replicating attenuated organism presents its molecular signatures over the days of its replication and clearance, generating a sustained PAMP signal from a single administration. This may be relevant to the multi-month treatment duration requirement of the framework: a series of live attenuated administrations spaced over months may produce effective sustained engagement with fewer individual dosing events than continuous synthetic agonist administration.

Recent clinical validation of the combinatorial logic. A recent and clinically significant instance of the combinatorial architecture proposed by this framework is the FDA approval (April 2024) of nogapendekin alfa inbakicept (ANKTIVA, also referred to as N-803), an IL-15 superagonist, in combination with intravesical BCG for BCG-unresponsive non-muscle-invasive bladder cancer with carcinoma in situ. The pivotal phase 2/3 QUILT-3.032 trial (Chamie et al. 2023, *NEJM Evidence*) reported a 71% complete response rate in patients who had previously failed BCG monotherapy, with durable responses extending to 54 months. This combination implements, at a

localized anatomical scale, the core architecture the Neo-Coley v2 framework proposes: a PAMP-rich bacterial agent (BCG, engaging TLR2, TLR4, NOD2, and STING) paired with sustained amplification of the resulting immune response (the IL-15 superagonist driving expansion of NK and CD8+ T cells without expanding regulatory T cells). The QUILT-3.032 result is the strongest contemporary clinical signal that the combinatorial logic generalizes beyond historical systemic Coley reproductions, and it suggests that the architecture may extend to other anatomical contexts where intratumoral or locoregional delivery of PAMP-rich agents can be combined with amplification of the resulting immune response.

5.3 Defined Synthetic PAMP Cocktails: The Modern Toolkit

The synthetic option uses defined-composition synthetic agonists for the principal pattern recognition receptors. The advantages are operational rather than biological: defined and reproducible composition, no manufacturing dependence on growing bacteria, regulatory tractability of each individual component, and the ability to adjust the cocktail composition as the science evolves. The mechanism is the same as for whole bacterial preparations: combinatorial multi-receptor engagement producing cytokine-driven fever and downstream immune orchestration.

Six classes of synthetic agonists are available for the cocktail, each with documented human safety:

TLR4 — Monophosphoryl Lipid A (MPLA). A chemically detoxified derivative of *Salmonella minnesota* LPS, FDA-approved as a component of the AS04 (Cervarix HPV vaccine) and AS01 (Shingrix herpes zoster vaccine) adjuvant systems. Detoxification preserves TLR4-activating capacity while reducing the inflammatory toxicity of native LPS, with the approved adjuvant dose set at 50 µg per vaccine administration. For the Neo-Coley cocktail, MPLA at this dose level provides the principal cytokine and fever driver.

TLR9 — Synthetic CpG Oligonucleotides. Synthetic oligodeoxynucleotides containing unmethylated CpG motifs (CpG ODNs) of multiple structural classes. CpG-A class agonists produce strong type-I interferon induction through plasmacytoid dendritic cells; CpG-B class produces stronger B-cell activation; CpG-C class combines features of both. Vidutolimod, a CpG-A class agonist in virus-like particle formulation, produced a 25% objective response rate in metastatic melanoma refractory to anti-PD-1 (Ribas et al. 2021), providing the strongest contemporary evidence that single-receptor PAMP agonism can produce meaningful clinical activity even outside the multi-receptor framework. CpG-C class oligonucleotides at vidutolimod-comparable doses provide pDC engagement in the Neo-Coley cocktail.

TLR7/8 — Imidazoquinolines. Imiquimod is FDA-approved for topical use against basal cell carcinoma and superficial squamous cell carcinoma. Resiquimod has been investigated systemically. NKTR-262 is a polymer-conjugated TLR7/8 agonist designed for sustained systemic exposure with reduced systemic toxicity. These agents engage plasmacytoid dendritic cells and produce strong type-I interferon responses. Resiquimod at doses informed by vaccine adjuvant studies provides additional TLR7/8 engagement in the cocktail.

TLR3 — Poly(I:C). Synthetic double-stranded RNA agonist. Hiltonol (poly-ICLC) is a clinical-grade poly(I:C) formulation with extensive clinical experience as a vaccine adjuvant and in glioblastoma dendritic cell vaccination protocols. Rintatolimod (Ampligen) is another TLR3 agonist with regulatory history. Inclusion of a TLR3 agonist broadens the cocktail's activation profile to include the type-I interferon induction characteristic of antiviral response, on the principle that the original Coley preparation included nucleic acids that would engage multiple endosomal RNA-sensing receptors.

TLR2 — Pam3CSK4 and Lipopeptide Variants. Synthetic triacylated lipopeptides that engage TLR2/TLR1 heterodimer. Amplivant is a clinical-stage Pam3CSK4 derivative being developed for synthetic long peptide vaccine adjuvant applications. These provide the gram-positive bacterial signature in the cocktail.

STING — Cyclic Dinucleotide Agonists. Multiple compounds in clinical development, with ADU-S100 (MIW815) the most clinically experienced (Meric-Bernstam et al. 2022). STING engagement at the lower end of doses tolerated in monotherapy trials of ADU-S100, administered simultaneously with other components to avoid the documented pre-activation antagonism with TLR9 (Gehrcken et al. 2025), completes the cocktail.

The composite synthetic cocktail. A representative initial composition combining the six receptor classes:

- MPLA at 50 µg per dose (AS04 calibration), the TLR4 agonist providing the principal cytokine and fever driver
- A CpG-C class oligonucleotide at vidutolimod-comparable doses, the TLR9 agonist providing pDC engagement
- A synthetic cyclic dinucleotide STING agonist at the lower end of doses tolerated in monotherapy trials, providing STING engagement
- Resiquimod or comparable TLR7/8 agonist, at doses informed by vaccine adjuvant studies
- Pam3CSK4 or Amplivant, the TLR2 agonist, at doses informed by synthetic long peptide vaccine work
- Optionally, Poly ICLC (TLR3) at standard adjuvant doses

The cocktail is administered subcutaneously, dose-titrated against the fever endpoint in early sessions. The cocktail dose is adjusted upward until each patient reliably achieves sustained 39–40°C core temperature for at least 2 hours. Once that dose is established for a given patient, it is maintained for subsequent sessions. The specific composition above is provisional; the first phase of any serious investigation of the synthetic implementation would consist of preclinical cocktail optimization, which would likely revise the composition substantially.

5.4 Component F: Tumor Antigen Redirection via Recall Antigen Painting

The framework's mechanisms for tumor-specific recognition — personalized neoantigen vaccination, in-situ vaccination from PAMP-induced tumor cell death, and NK-mediated killing of stressed cells — each have limitations. Neoantigen vaccines target only mutations identified in tumor biopsy and miss tumor cells that do not express

those mutations, leaving tumor heterogeneity as a persistent escape mechanism. In-situ vaccination is unpredictable in which antigens are released and presented. NK responses, while important, do not generate the durable adaptive memory that prevents long-term recurrence.

A complementary strategy that has gathered substantial preclinical validation in recent years is **recall antigen redirection**: causing tumor cells to display antigens against which the patient already has strong pre-existing immunity, so that the patient's existing memory T cells and antibodies — generated decades earlier through childhood vaccination or, more recently, SARS-CoV-2 vaccination — attack the marked tumor cells. Four published lines of work demonstrate the mechanism in animal models and small clinical studies:

- Mitchell, Batich, and colleagues in the Sampson group at Duke demonstrated that pre-conditioning the dendritic cell vaccine site with tetanus/diphtheria toxoid significantly improved lymph node homing and clinical response in glioblastoma patients (Mitchell et al. 2015, *Nature*). This was the foundational clinical demonstration that recall antigen mechanisms could enhance anti-tumor immune responses in human patients.
- The Gravekamp group developed attenuated *Listeria monocytogenes* engineered to deliver a non-cytolytic tetanus toxoid fragment into tumor cells, reactivating childhood-vaccination-derived memory T cells against the now-infected pancreatic cancer cells (Selvanesan et al. 2022, *Sci Transl Med*; earlier patents and bioRxiv preprints).
- A multi-institutional group recently developed an LNP-RNA platform that delivers SARS-CoV-2 spike-epitope-loaded MHC class I molecules selectively to tumor cells in mouse models, leveraging the broad anti-spike immunity now present across the vaccinated global population (Xue et al. 2025, *Nature Communications*). This is the most technically advanced and most recent demonstration, and the most directly relevant to the framework proposed here.
- The oncolytic poliovirus PVSRIPO program at Duke (Brown, Bigner, et al.) has invoked recall antigen mechanism as part of its mechanism of action in glioblastoma trials.

What none of these published studies has tested — and what would be the framework's specific contribution — is the integration of recall antigen redirection with the full multi-component Neo-Coley v2 protocol. Each published approach has used a single recall antigen, has not been combined with multi-PAMP cocktail activation, has not exploited sustained fever-range thermal stress for amplified trafficking of recall-specific memory T cells, and has not been layered with personalized neoantigen vaccination.

Design of Component F. A multi-recall-antigen mRNA-LNP cocktail, optimized for tumor-tropic delivery, carries mRNA encoding multiple recall antigens simultaneously. Initial composition:

- SARS-CoV-2 spike protein subunit (or selected immunodominant epitopes). Anti-spike immunity is essentially universal in vaccinated populations; titer durability is well-documented.
- Tetanus toxoid C-fragment (non-cytolytic). Anti-tetanus immunity is essentially

universal from childhood vaccination in countries with routine immunization programs; the non-cytolytic C-fragment is the same construct validated in the Gravekamp *Listeria* work.
- Optionally, additional recall antigens (diphtheria toxoid fragment, polio capsid epitopes for patients with wild-type polio immunity, measles or mumps epitopes) selected based on individual patient pre-existing immunity profile.

Tumor-tropic delivery via LNP surface modifications targeting receptors overexpressed on the tumor type being treated — for example, HER2-targeting peptides for HER2+ tumors, EGFR-binding peptides for head and neck or colorectal tumors, EpCAM-binding peptides for many epithelial cancers. The Xue et al. 2025 LNP work and parallel academic programs have demonstrated tumor-tropic LNP engineering at preclinical scale.

Pre-treatment screening: confirm patient's recall immunity by titer measurement (anti-spike, anti-tetanus, anti-polio as relevant). Booster vaccination administered 4–6 weeks before protocol initiation if any titer is below the threshold required for vigorous recall response.

Dosing schedule integration: LNP-mRNA administered 48–72 hours before each multi-PAMP cocktail dose during induction. This timing places maximal recall antigen expression on tumor cells coincident with the peak of fever, cytokine release, and HEV-mediated trafficking of memory T cells from periphery to tumor. The neoantigen vaccine continues to provide tumor-mutation-specific direction; the recall antigen layer provides universal-target marking that is independent of which mutations the tumor expresses.

LNP platform tolerance consideration. LNP-mRNA administration is included only during induction (weeks 1–8) and the first three months of maintenance. Subsequent maintenance does not require LNP-mRNA, to avoid tolerance to the LNP platform itself, which has been documented as a concern with repeated LNP administration.

Manufacturing and regulatory considerations. mRNA-LNP manufacturing infrastructure exists at industrial scale (the COVID-19 vaccines proved this), and the regulatory pathway for mRNA-LNP products is now established. Tumor-tropic LNP surface modifications are the principal novel engineering element; preclinical and early-clinical work on tumor-targeted LNP delivery is advancing in multiple academic and industrial programs.

Patient population. Component F adds substantial benefit specifically in tumors with high mutational heterogeneity (where neoantigen vaccination alone is most vulnerable to escape) and in patients with strong existing recall immunity (the majority of vaccinated adults). For tumors with limited surface antigen options or in patients with blunted recall responses, the protocol may revert to the configuration without F.

Each individual element of Component F is established or in active clinical-stage development. The novelty lies in the multi-recall-antigen formulation, the integration with the multi-PAMP cocktail and sustained fever, and the coordinated dosing schedule — combinations that, to the author's knowledge, have not been published as an

integrated protocol.

5.5 Trade-offs and Selection Criteria

The three implementation options are not equivalent. Each has distinct advantages and limitations:

Property	Whole bacterial	Live attenuated	Synthetic cocktail
Manufacturing complexity	High	Medium (vaccine infrastructure)	Low
PAMP combinatorial breadth	High	Medium-High	Medium (controllable)
Reproducibility	Variable	Medium	High
Regulatory tractability	Difficult (historical issues)	Existing pathways	Existing pathways per component
Dosing precision	Limited	Limited	High
Adjustability	Low	Low	High
Clinical experience	Long (Coley era + Japan)	Long (BCG, vaccines)	Medium (individual agonists)

The selection between options is a matter of trial design and pragmatic constraint rather than fundamental biology. A clinical investigator with access to a high-quality MBV preparation should use it; with access to an established BCG protocol, BCG; with access to clinical-grade synthetic agonist components, the synthetic cocktail. The framework's predictions apply across all three implementations, with the qualification that the synthetic cocktail allows the most precise dose-response characterization and the most reproducible cross-trial comparisons.

5.6 Why the Synthetic Implementation Is Feasible Today

The bacterial version of Coley therapy has been stuck in regulatory and manufacturing limbo for decades. The MBVax Bioscience program in Canada produced a GMP-quality bacterial preparation from 2005 to 2017, supplied compassionate-use cases, and accumulated case-level evidence of activity (MacAdam 2018), but was unable to advance to formal trials because the cost of building a multi-million-dollar pharmaceutical-grade manufacturing facility for the bacterial preparation was prohibitive. The Karbach 2012 trial demonstrated technical feasibility but produced only a single objective response. The SYNB1891 program substituted engineered bacteria but produced no objective responses and was discontinued. The Hobohm group's clinical work has continued in Europe using a combination of approved PAMP-containing drugs and bacterial extracts (Reuter, Oettmeier, and Hobohm 2018), with documented safety but without the scale or trial infrastructure required to definitively establish efficacy.

The synthetic implementation of the framework is feasible in 2026 in a way none of these efforts has been, for six specific reasons.

Manufacturing. Every component of the cocktail can be synthesized using methods established for pharmaceutical or vaccine production. MPLA is manufactured at scale today by GSK and others. CpG oligonucleotides are manufactured at scale by multiple oligonucleotide synthesis facilities. Synthetic cyclic dinucleotides are produced for clinical trials. Imidazoquinoline TLR7/8 agonists are manufactured for clinical and commercial purposes. Lipopeptide synthesis is routine. None of these requires a new manufacturing facility. The bacterial-preparation manufacturing problem, which has been the principal practical obstacle to advancing Coley therapy, is dissolved.

Regulatory pathway. Each component of the cocktail is either FDA-approved for some indication (MPLA in approved vaccines, imiquimod as approved cancer therapy), in active clinical trials (CpG agonists, cyclic dinucleotides, several TLR7/8 agonists), or has documented human safety from completed early-phase studies (Pam3CSK4 derivatives, Poly ICLC). A new combination would be reviewed under standard combination-product regulatory frameworks rather than as a novel biological entity. Anti-PD-1 antibodies are extensively approved. Personalized neoantigen mRNA vaccines have a recently established regulatory pathway through the work on mRNA-4157 and autogene cevumeran.

Limited humoral neutralization concerns. Short oligonucleotides, small-molecule TLR agonists, synthetic lipopeptides, and chemically defined adjuvant lipids do not produce significant neutralizing antibody responses. This is one specific advantage of the synthetic cocktail over whole-bacterial preparations: anti-bacterial-protein antibodies that may neutralize repeated doses of MBV-class preparations are not generated by the synthetic equivalents. The clinical experience indicating that PAMPs in general do not produce clinically meaningful neutralizing antibody responses (with the exception of mistletoe lectin and possibly flagellin) further reduces this concern, but the synthetic cocktail eliminates it categorically.

Tolerance is manageable through cocktail composition. Specific receptor tolerance (endotoxin tolerance for TLR4, for example) does still develop with extended exposure to any single agonist. The multi-receptor cocktail means that any individual receptor is engaged at a lower absolute dose than in a single-pathway protocol, reducing the rate of tolerance development. Furthermore, cocktail composition can be adjusted between cycles — substituting components or adjusting ratios at the level of individual chemical entities — without changing the fundamental protocol logic. The operational tools for managing receptor-specific tolerance are more flexible in the synthetic protocol than in the bacterial one.

Adaptive optimization. The composition of the cocktail can be adjusted in response to the evolving scientific understanding of PRR crosstalk. If a new agonist becomes available with improved properties — better receptor selectivity, longer half-life, lower toxicity — it can be substituted. If pathway crosstalk modeling identifies a problematic antagonism, the affected component can be reduced or replaced. If clinical experience suggests that one receptor is dispensable, the corresponding component can be removed. The bacterial preparation does not admit of this kind of iterative refinement; what is in the bacterium is what is in the bacterium.

Deliverable without specialized infrastructure. The cocktail-only configuration of the protocol requires only what a standard outpatient clinic already has: subcuta-

neous injection capability, several hours of monitored observation per session, and a continuous core temperature sensor. No whole-body hyperthermia chamber, no specialized infusion facility, no high-end imaging suite. This is what makes the framework scalable beyond the small number of centers with WBH infrastructure. The WBH-augmented configuration is available where it adds value, but not as a precondition for trial advancement or eventual clinical deployment.

These six reasons together specify why the synthetic implementation could move forward in 2026 in a way the bacterial implementation has not. Whether the framework as a whole works is an empirical question that requires testing; the synthetic implementation removes the operational barriers that have prevented serious testing from occurring.

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6. The Neo-Coley v2 Protocol Design

This section translates the convergent thesis (Section 3), modern knowledge extensions (Section 4), and implementation options (Section 5) 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. 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.

6.1 Eligibility and Patient Selection

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 $\geq 1.0 \times 10^9$/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. Pretreatment biopsy demonstrating CD11c+ dendritic cell density above an institutionally defined threshold, with documented MHC class I ex-

pression, and either T-cell-inflamed or excluded-infiltrate phenotype (true "desert" tumors are excluded). This requirement reflects Section 4.3 and aims to exclude patients in whom the framework predicts response would not occur regardless of dose.

6.2 Treatment Components

The protocol uses four core components, with Component F as an optional fifth.

Component A: Combinatorial PAMP activator. One of three implementation options as developed in Section 5: whole bacterial preparation (MBV-equivalent or OK-432), live attenuated vaccine pathogen (BCG protocols, or measles/polio/vaccinia in tumor-appropriate contexts), or defined synthetic PAMP cocktail (MPLA + CpG-A or CpG-C + imidazoquinoline + STING agonist + TLR2 lipopeptide ± Poly ICLC). The selection is governed by availability, manufacturing access, and regulatory context. The synthetic cocktail is preferred where the operational advantages developed in Section 5.6 apply (most contemporary settings).

Component B: Personalized neoantigen mRNA vaccine. Constructed from whole-exome sequencing of the resected or biopsied tumor, with up to nine doses administered over the induction and early maintenance phases per the mRNA-4157 schedule. The vaccine provides the tumor-specific specificity layer that Component A's broad activation does not.

Component C: Anti-PD-1 antibody. Administered as a checkpoint inhibitor to prevent the activated T-cell response from being suppressed by tumor-induced exhaustion. The inclusion of Component C is offered as a testable hypothesis rather than as an established integration. Appropriate skepticism is warranted about whether checkpoint inhibition adds to the framework's effect, given that the framework's central mechanism (broad innate activation, fever, DC maturation, multi-month engagement) operates upstream of T-cell exhaustion. Whether C provides additional benefit beyond Components A and B should be tested in a factorial trial design as articulated in Section 7.

Component D (optional and constrained): 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. Standard maximum-tolerated-dose chemotherapy is incompatible with the framework's core principle of preserved immune function and is explicitly contraindicated during the active immunotherapy phase. Chemotherapy kills all rapidly proliferating cells, including the immune cell populations on which the framework's response depends; this is a categorical constraint, with the exceptions of specific low-dose, metronomic, or immunomodulatory regimens (low-dose cyclophosphamide, gemcitabine in some contexts) that have been demonstrated not to deplete the lymphocyte and dendritic cell populations relevant to response.

Component F (optional): Multi-recall mRNA-LNP redirection. As specified in Section 5.4. mRNA-LNP constructs encoding tetanus toxoid C-terminal fragment and/or SARS-CoV-2 spike epitopes, administered intratumorally or systemically with tumor-tropic delivery, redirecting the patient's pre-existing recall immunity against tumor cells. Offered as a potential amplification component for trial designs that

wish to test it. Pre-treatment recall titer screening is required; LNP administration is limited to induction and early maintenance only.

6.3 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 Component F (if used). mRNA-LNP recall antigen constructs are administered 48–72 hours before each Component A dose during the induction phase, placing maximal recall antigen expression on tumor cells at the peak of fever and HEV-mediated T-cell trafficking.

Concurrent checkpoint inhibitor. Component C is administered every three weeks beginning at Week 1, in trial arms that include it.

Maintenance phase (Week 9 onward). After completion of induction, Component A continues with twice-weekly administration at the established dose, sustained over multi-month treatment duration. Continuous administration at the established dose is consistent with the historical Coley pattern and with the published clinical experience of multi-month sustained fever-induction protocols (Reuter, Oettmeier, and Hobohm 2018), which does not document exhaustion of fever induction over extended dosing intervals.

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).

6.4 Monitoring and Response Assessment

Safety monitoring. Vital signs, complete blood count, and basic metabolic panel before each PAMP cocktail administration and at 24 hours afterward. Continuous core temperature monitoring during the twelve hours following each dose using ingestible temperature pill (e-Celsius, CorTemp), heat-flux sensor (CORE by greenteg), or comparable continuous core temperature device. The continuous monitoring is essential to confirm the dosing endpoint and to enable ceiling antipyretic intervention if needed. 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. Multiplex flow cytometry of peripheral blood for lymphocyte subsets, NK cell activation markers, and T-cell exhaustion markers at baseline and at three-month 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. Biopsy at any later progression event is performed if accessible, for resistance mechanism characterization.

6.5 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; 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.

6.6 Schematic Summary

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: - *A:* Combinatorial PAMP activator. One of three implementations: whole bacterial preparation (MBV, OK-432), live attenuated vaccine pathogen (BCG, measles/polio/vaccinia variants), or defined synthetic cocktail (MPLA + CpG-C + imidazoquinoline + STING agonist + TLR2 lipopeptide ± Poly ICLC). Subcutaneous, 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 (testable component). - *D (optional and constrained):* Low-dose metronomic chemotherapy if tumor burden control is required, explicitly substituting for conventional cytotoxic therapy. Standard MTD chemotherapy is contraindicated. - *F (optional):* mRNA-LNP redirection encoding tetanus toxoid or SARS-CoV-2 spike epitopes, leveraging pre-existing recall immunity.

Phases: Induction (Weeks 1–8) → Maintenance with continuous twice-weekly Component A administration, sustained over 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.

Thermal monitoring: Continuous core temperature via ingestible pill or heat-flux sensor during all dosing sessions.

Response measured by: Cross-sectional imaging, ctDNA, biopsy-based immune correlates, and patient/clinician assessment of benefit.

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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 into three categories: predictions from the convergent thesis (7.1), predictions from the modern knowledge extensions (7.2), and predictions specific to the synthetic implementation (7.3). A decisive falsifier is articulated in 7.4.

7.1 Predictions from the Convergent Thesis

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.

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 continuous maintenance dosing should produce longer recurrence-free survival in the multi-month arm. *Falsifier:* equivalent long-term outcomes.

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.

7.2 Predictions from the Modern Knowledge Extensions

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. *Falsifier:* equivalent durability with and without the vaccine component.

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 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.

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. *Falsifier:* response observed across all APC density strata.

Prediction 8: Sustained continuous dosing produces stable response without

exhaustion of fever induction over multi-month protocols. Continuous twice-weekly Component A administration at established dose should maintain fever induction at the established temperature range over the full 12–24 month protocol duration in the substantial majority of patients. *Falsifier:* substantial loss of fever response within the first six months at stable dose, indicating that exhaustion of fever induction is in fact a clinically meaningful obstacle at multi-month timescales. (This prediction supersedes an earlier pulse-rest/tolerance-mitigation framing that has been removed from the framework in light of the published clinical experience documented by Reuter, Oettmeier, and Hobohm 2018.)

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.

7.3 Predictions Specific to the Synthetic Implementation

The proposition that synthetic agonists can substitute for whole bacterial preparations generates four additional testable predictions, with a fifth specific to Component F.

Prediction A: The synthetic multi-PAMP cocktail produces immunological responses equivalent in magnitude and quality to those produced by bacterial Coley preparations at matched receptor engagement levels. Testable through direct head-to-head comparison of cytokine profiles, dendritic cell maturation markers, NK cell activation, and T-cell priming in animal models or, eventually, in matched patient cohorts. *Falsifier:* the synthetic cocktail produces qualitatively or quantitatively different immune responses despite matched receptor engagement, indicating that whole-bacterial preparations contain active components not captured by the synthetic substitution.

Prediction B: Cytokine-induced fever from the synthetic cocktail produces the framework's predicted thermal-immune mechanisms equivalently to external whole-body hyperthermia at matched core temperature. This is the central operational prediction of the WBH-optional framing: that the synthetic cocktail, dose-titrated against fever as Coley did with bacterial preparations, delivers thermal stress through the body's own pyrogenic pathway sufficient to engage HSP90-mediated DC maturation, HEV trafficking, NK activation, and HSP cross-presentation. Testable through head-to-head immunological correlate analysis of cocktail-induced versus WBH-induced systemic thermal stress at matched core temperatures. *Falsifier:* WBH-induced thermal stress produces different downstream immune mobilization than cytokine-induced fever, indicating that the cytokine signaling environment surrounding the fever — distinct from temperature itself — contributes essentially to the framework's predicted mechanisms. If falsified in this direction, WBH would

need to be added to the protocol rather than offered as optional.

Prediction C: The synthetic protocol produces sustained immunological responsiveness over multi-month dosing without development of neutralizing antibody-mediated decay. Consistent with the available clinical and preclinical literature, which does not support anti-PAMP antibodies as a clinically meaningful obstacle for most PAMP categories, the synthetic protocol should show no antibody-mediated decline in pharmacodynamic response over the 12–24 month protocol duration, consistent with the bacterial-protocol clinical experience documented in Reuter, Oettmeier, and Hobohm (2018). *Falsifier:* progressive loss of pharmacodynamic response in either bacterial or synthetic protocols over months, indicating that the principal limitation of sustained dosing is humoral neutralization of the immunostimulant after all.

Prediction D: The synthetic protocol permits cocktail optimization through iterative composition adjustment based on patient-specific response patterns. Testable through trials with embedded composition-optimization design, where the cocktail is adjusted between cycles based on individual immunological correlate data. *Falsifier:* optimization fails to improve responses beyond the default composition, indicating that empirically composed bacterial mixtures capture sufficient diversity through their natural complexity.

Prediction F: The multi-recall-antigen mRNA-LNP cocktail combined with the multi-PAMP cocktail, sustained fever, neoantigen vaccine, and checkpoint inhibitor produces objective response benefit beyond any subset of these components in matched comparisons. This is the central testable claim of the recall-antigen-redirection addition to the framework. Testable through preclinical factorial design comparing the full protocol with versus without Component F, then through clinical trial with a Component F arm in tumors with adequate surface antigen targets and patients with confirmed strong recall immunity. *Falsifier:* matched preclinical comparisons show no synergistic benefit from adding recall antigen targeting to the protocol, indicating that the in-situ vaccination produced by the multi-PAMP cocktail and personalized neoantigen vaccine is sufficient for antigen specificity, and recall antigen targeting adds complexity without benefit. *Note:* the published demonstrations of recall antigen redirection (Mitchell 2015; Selvanesan 2022; Xue 2025) tested the mechanism in isolated form. Prediction F is specifically about whether it adds incremental benefit when layered onto the framework's other mechanisms.

7.4 Predictions About the Protocol as an Integrated Intervention

Prediction 10: The integrated Neo-Coley v2 protocol produces higher objective response rates than checkpoint inhibitor monotherapy in matched populations of refractory disease. A randomized comparison in refractory melanoma, sarcoma, or other selected indications should produce higher response rates and longer progression-free survival in the Neo-Coley v2 arm. *Falsifier:* equivalent or inferior response rates in the Neo-Coley v2 arm.

Prediction 11: Component contribution analysis demonstrates that no single component accounts for response. A factorial trial in which Components A,

B, C, and Component A + checkpoint inhibition are compared to the integrated protocol should reveal that response rates are highest in the integrated arm, with no single component producing comparable durable response. *Falsifier:* equivalent response from a single component (most plausibly Component A alone administered with sustained fever induction), indicating that the framework's emphasis on multi-component integration is overstated.

7.5 The Decisive Falsifier

A randomized trial of the integrated Neo-Coley v2 protocol versus best available standard of care, in a population predicted by the framework to be responsive (immune-competent, biomarker-selected, in a tumor type historically responsive to bacterial immunotherapy), conducted with the operational features specified in Section 6 (fever-titrated dosing, multi-month duration, combinatorial PAMP activation, preserved immune function), produces no significant improvement in objective response rate, progression-free survival, or overall survival.

If this trial were to produce a negative result with adequate power, the framework as articulated would be substantially refuted.

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

8.1 What This Framework Is

The Neo-Coley v2 framework is a position paper articulating a specific hypothesis for why modern bacterial immunotherapy trials have produced confirmed target engagement without the durable responses Coley reported, and a specific protocol design that, if correct, should produce durable responses at rates substantially higher than current standard of care in matched populations. The framework rests on the foundation Hobohm, Reuter, and Oettmeier have developed over two decades, extends it with five integrations from cancer immunology developments since 2018, surveys three implementation options for delivering the required PAMP activation, and articulates sixteen testable predictions and a decisive falsifier.

The framework's principal claims are mechanistic and design-level rather than evidentiary at population scale. The convergent thesis is grounded in mechanism (Section 4.5) and in the negative pattern of modern trials (Section 2). The protocol design (Section 6) follows from the mechanism. The predictions (Section 7) specify how the design should be tested.

8.2 What This Framework Is Not

The framework is not evidence of efficacy. No clinical trial of the integrated protocol has been conducted, and the body of evidence supporting individual components varies substantially. The mechanism of fever-range thermal stress in immune orchestration is well-characterized at the molecular level; the contribution of multi-component integration to durable clinical response remains a hypothesis. The frame-

work is offered as input to investigator-initiated trial design, not as a treatment recommendation.

The framework is not, in particular, a recommendation that any individual patient should pursue Coley-style therapy outside an appropriate clinical trial. Bacterial immunotherapy under inadequate medical supervision can produce serious adverse events, and the historical and contemporary practice in unregulated settings has documented harms alongside the documented successes. The protocol design specified here requires institutional oversight, cytokine release syndrome management capability, and adequate infrastructure for monitoring; it is not deliverable in clinic-level outpatient settings without appropriate physician supervision.

8.3 Honest Limitations

Several limitations of the framework, and of the synthetic implementation specifically, deserve explicit acknowledgment. The most fundamental limitation is the most important to state clearly: **this paper presents a hypothesis. It does not present experimental validation, nor does it present a sufficient body of clinical evidence to support the framework as a treatment recommendation.** The framework's central claims are testable and falsifiable, as articulated in Section 7, but they have not yet been tested in the integrated form proposed. Individual components have been studied in isolation; the integrated configuration has not. The framework is offered as a hypothesis to guide future investigation, not as a description of established medical practice.

Beyond this fundamental limitation, several more specific limitations also warrant explicit acknowledgment.

The full integrated protocol has not been tested. No published clinical study has tested the multi-component integration proposed here as a Coley-derived cancer therapy. The individual components have been studied separately and in pairs; the full integrated protocol has not been deployed. Preclinical testing in animal models is the necessary first step before any clinical evaluation of the integrated configuration.

The synthetic cocktail composition is provisional. The specific agonists and doses proposed in Section 5.3 are reasonable initial candidates based on current literature, AS04 adjuvant calibration for MPLA, and clinical-stage doses for the other components. They are not the result of computational optimization across the full receptor combination, and the relative proportions are not the result of empirical preclinical titration. The first phase of any serious investigation of the synthetic implementation would consist of preclinical cocktail optimization, which would likely revise the composition substantially.

Inter-patient variability in cytokine response is real and requires titration. Individual patients differ in how strongly they respond to a given dose of a given immunostimulant. The cocktail-only configuration depends on dose titration in early sessions to find each patient's fever-inducing dose. This adds operational complexity in the first weeks of treatment and may require dose escalation beyond initial estimates for some patients. The WBH-augmented configuration eliminates this variability at the cost of requiring specialized equipment; the trade-off depends on the clinical set-

ting and on whether the patient population includes individuals with blunted cytokine responses.

The patient selection criteria are restrictive. The framework predicts response only in patients with preserved immune function and adequate baseline tumor immune infiltrate. This excludes the heavily pretreated, immunocompromised population that is typically enrolled in first-in-human trials. A trial of the integrated protocol would need to be designed in a setting where less-pretreated patients are appropriate, such as earlier-line metastatic disease or high-risk adjuvant therapy. This is a feature rather than a bug from the framework's perspective, but it complicates trial enrollment.

The combinatorial signaling biology is incompletely understood. Pre-activation of STING suppresses subsequent TLR9 responses (Gehrcken et al. 2025). Other pairwise antagonisms among PRR pathways have been documented. The full landscape of synergies and antagonisms across the six receptors engaged by the proposed cocktail is not fully mapped. Some compositions that look good on paper may underperform in practice, and some that look suboptimal may surprise.

The framework could be wrong in ways that make the synthetic substitution moot. If the Neo-Coley v2 framework is incorrect about which features of Coley's protocol mattered, then no substitution — synthetic or otherwise — for those features will produce the desired result. The synthetic implementation inherits all of the open empirical questions of the parent framework, and the synthetic-substitution proposition adds its own.

The historical data are limited in ways that constrain the framework. Coley's case records have important methodological limitations that are inherent to a nineteenth-century clinical practice: non-standardized diagnostic criteria, absence of contemporaneous controls, selection bias inherent in clinical archives, and outcome reporting standards that do not map cleanly onto modern conventions. The framework does not depend on the precise magnitude of Coley's reported response rates, but it does rest on the observation that durable responses occurred at a rate sufficient to motivate four decades of practice. If that observation is itself wrong — if Coley's case records substantially overstate the response rate, more so than the most generous historical reading of the data would allow — then the central motivation for the framework is weaker than it appears.

These limitations do not negate the framework. They specify the work that would need to be done to evaluate it.

8.4 Author and Method Disclosures

The author has no academic affiliation and no medical training. The motivation for undertaking this synthesis arose from fifteen years of personal engagement with the bacterial immunotherapy literature following a close family member's diagnosis with Stage IIA high-grade triple-negative breast cancer with metaplastic features in 2011. The personal context is offered to explain the author's motivation and prolonged engagement with the topic; it does not bear on the scientific argument, which stands or falls on the evidence cited and the predictions specified.

This disclosure is provided here as well as in the AI Use Disclosure at the front of this manuscript, in accordance with the COPE position statement on AI in scholarly publication. The literature integration, document drafting, formatting, and translation work associated with this framework were AI-assisted (Anthropic's Claude). The observational basis, the conceptual direction, the synthesis judgments, the specific predictions, and the protocol design are the author's. The author has reviewed and edited all AI-assisted output and takes full responsibility for the content of this publication.

8.5 The Open-Source Simulation Platform

An open-source computational simulation platform implementing the framework's predictions is available at the public GitHub repository associated with this work, with permanent DOI through Zenodo. The platform allows in-silico testing of the protocol pattern under varied parameter choices, and is offered as a tool for investigators considering trial design. It is referenced in the relevant predictions of Section 7 but is not necessary to evaluate the framework as articulated in this document.

8.6 Closing

The questions this framework addresses are not new. Hobohm has been articulating versions of them for over two decades. What is new in this paper is the convergent thesis (Section 3), the integration with developments since 2018 (Section 4), the explicit consideration of three implementation options including the operational case for synthetic substitution (Section 5), the modular protocol design with Component F as a novel addition (Section 6), and the sixteen falsifiable predictions plus decisive test (Section 7). The framework is offered for testing, modification, or refutation, in the same spirit in which Hobohm's earlier work has been offered: as a serious attempt to articulate why the historical Coley results have not been reproduced in the modern era, and what would have to be done to test whether they can be.

The mechanism the framework proposes Coley exploited — multi-receptor engagement of the innate immune system's pattern-recognition apparatus, with systemic thermal stress amplifying the immune response — can in principle be reproduced today using defined-composition synthetic agonists of TLR2, TLR3, TLR4, TLR7/8, TLR9, and STING, dose-titrated against the same fever endpoint Coley used. The cocktail's pyrogenic response could deliver the thermal stress component through the body's own pathway; external whole-body hyperthermia is available as an optional supplement in patients where individual cytokine response falls short, but is not required by the framework as proposed. Personalized neoantigen mRNA vaccines provide tumor-specific direction. Multi-recall mRNA-LNP redirection (Component F) provides an optional amplification layer leveraging pre-existing recall immunity. Each component exists today as an approved drug, an approved-vaccine adjuvant component, or an active clinical-trial-stage agent. The synthesis is novel; the components are not. Whether the proposed synthesis works as a clinical intervention is an empirical question that has not yet been tested.

The principal practical advantage of the synthetic substitution is operational rather than theoretical. The bacterial-preparation manufacturing problem that has limited

modern Coley-derived trials is addressed by the synthetic approach. Regulatory pathways exist for every component individually. Composition is adjustable as the science evolves. And the cocktail-only configuration of the protocol could in principle be deliverable in any clinic that can administer subcutaneous injections and monitor patients for several hours, without requiring specialized hyperthermia infrastructure.

This is a hypothesis paper. Its purpose is to articulate a testable framework and a set of predictions, not to assert that the framework has been validated. Whether the framework is correct is an empirical question that requires properly designed and conducted clinical investigation. The predictions of Section 7 and the decisive falsifier together specify what would constitute evidence for and against the proposition. The paper is offered for testing, modification, or refutation by researchers in a position to do so.

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The responsibility for the framework as articulated in this manuscript — including the convergent thesis, the protocol design, and all specific predictions — rests entirely with the author. The cited authors and their published works are referenced as part of the literature on which this synthesis builds; the framework as presented here should not be assumed to reflect their current views.

Conflicts of Interest

The author declares no conflicts of interest. The author has no financial interest in, employment by, consulting relationship with, or equity in any commercial entity related to the methods, compounds, or protocols discussed in this manuscript. The author has not received funding from any pharmaceutical company, biotechnology company, or other commercial entity in connection with this work.

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Data Availability Statement

All data referenced in this manuscript are from previously published sources cited in the References section. No new experimental data are reported. The open-source computational simulation platform referenced in Section 8.6 is available at the GitHub repository associated with this work, with a permanent DOI through Zenodo. The version of this manuscript and its French translation are also archived on Zenodo for permanent reference.

Use of Artificial Intelligence

This manuscript was prepared with the assistance of Anthropic's Claude (a large language model) for literature integration, document drafting, formatting, translation between English and French, and editorial review. The AI tool did not generate or fabricate any data, citations, or scientific claims. The conceptual framework, the synthesis of the cited literature, the protocol design, the specific predictions, and all substantive scientific judgments are those of the author. The author has reviewed and edited all AI-assisted output and takes full responsibility for the final content of this publication, consistent with COPE recommendations on AI in scholarly publication.

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End of position paper.