Combinatorial completeness in cancer immunotherapy: a structural framework for addressing the seven failure modes of immune-mediated tumor control

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Contents

Abstract

Cancer immunotherapy has produced dramatic clinical successes since the FDA approval of ipilimumab in 2011 — but the successes remain concentrated in a minority of tumor types (melanoma, MSI-high colorectal cancer, high-PD-L1 NSCLC, others) and a minority of patients within those types. The structural reason for this pattern is that anti-tumor immunity has multiple failure modes, contemporary single-agent immunotherapies typically address only one or two of these, and tumor types differ in which failure modes are limiting. This paper proposes a structural framework — seven failure modes of immune-mediated tumor control — to organize intervention evaluation, combinatorial protocol design, and prediction of where in the clinical trial landscape combinatorial-complete protocols could produce qualitatively better outcomes.

The seven modes are: (1) insufficient antigen presentation; (2) lack of T cell priming and DC engagement; (3) physical exclusion of T cells; (4) T cell exhaustion in the tumor microenvironment; (5) active immune suppression by the tumor microenvironment; (6) insufficient persistence of immune response; and (7) tumor heterogeneity and immune escape. For each mode, we identify the mechanism, contemporary intervention categories, FDA-approved agents where they exist, the clinical evidence

base supporting mode-specific intervention, and the pattern of where the mode is dominant as a failure mechanism across cancer types.

The framework's central observation is that the historical Coley mixed bacterial vaccine engages Modes 1, 2, 5, 6, 7 strongly and Mode 3 partially, but engages Mode 4 essentially not at all — a structurally distinctive pattern from contemporary checkpoint inhibitors, which provide strong Mode 4 engagement but limited engagement of the other modes. The framework predicts that combinatorial protocols engaging all seven modes simultaneously will produce qualitatively different outcomes from protocols engaging subsets of modes. We provide a 0-1 mode scoring methodology, worked examples for the historical Coley protocol, contemporary checkpoint combinations, ANKTIVA + BCG (FDA-approved 2024 for NMIBC), TIL therapy, and in situ vaccination paradigms, and tumor-type case studies for melanoma, NMIBC, TNBC, pancreatic cancer, and microsatellite-stable colorectal cancer.

The framework predicts specific gaps in the contemporary clinical trial landscape where combinatorial-complete protocols could be tested. The empirical pattern across the past 15 years of immunotherapy clinical development — dramatic short-term successes in tumors where single-mode intervention suffices, followed by translational difficulty in tumors requiring multi-mode engagement — is consistent with the framework's structural prediction that combinatorial completeness is the limiting factor.

1. Introduction

1.1 The contemporary immunotherapy landscape

Cancer immunotherapy in the era following FDA approval of ipilimumab in March 2011 has transformed outcomes for substantial fractions of patients with melanoma, microsatellite-instability-high colorectal cancer, urothelial cancer, classical Hodgkin lymphoma, head and neck squamous cell carcinoma, renal cell carcinoma, certain non-small-cell lung cancer subsets, hepatocellular carcinoma, Merkel cell carcinoma, and an expanding list of other tumor types. Multiple checkpoint inhibitors targeting PD-1/PD-L1, CTLA-4, and LAG-3 are now FDA-approved across more than 30 distinct indications. CAR-T cell therapies have transformed B-cell malignancy treatment with durable responses in patients previously considered incurable. The first cytokine in over 30 years was approved for cancer (ANKTIVA + BCG for BCG-unresponsive NMIBC, April 22, 2024). Personalized neoantigen vaccines have moved from preclinical concept to phase III trials.

The 2018 Nobel Prize in Physiology or Medicine to James P. Allison and Tasuku Honjo recognized the checkpoint blockade discovery that established the contemporary paradigm. The 2025 Nobel Prize to Mary E. Brunkow, Fred Ramsdell, and Shimon Sakaguchi recognized the Treg discovery establishing the mechanistic basis for understanding tumor immune suppression. Two of the past eight Nobel Prizes in physiology or medicine have been awarded for cancer immunology discoveries.

1.2 The persisting translational difficulty

Despite these successes, fundamental limits in cancer immunotherapy translation persist. The benefit pattern across tumor types remains uneven: melanoma, MSI-high CRC, and high-TMB tumors respond dramatically; microsatellite-stable CRC, pancreatic cancer, prostate cancer, glioblastoma, and much of triple-negative breast cancer remain checkpoint-inhibitor-refractory. Within responding tumor types, the proportion of patients benefiting is typically 20-50%, leaving substantial unmet need. Combination strategies — adding additional agents to checkpoint inhibitors — have produced incremental benefit in some settings (anti-PD-1 + anti-CTLA-4 in advanced melanoma, anti-PD-1 + anti-LAG-3 in metastatic melanoma, atezolizumab + bevacizumab in HCC) but have repeatedly failed in others (anti-TIGIT across multiple settings, anti-IDO in melanoma, multiple anti-CSF1R combinations, bintrafusp alfa across three indications).

The pattern of clinical successes and failures is not random. Tumors where checkpoint inhibitors succeed share characteristics: high tumor mutational burden, pre-existing T cell infiltration, intact MHC class I expression, and limited stromal exclusion. Tumors where checkpoint inhibitors fail share opposite characteristics. This is the empirical signature of the seven-mode framework: tumors respond to single-mode interventions when other modes are favorable; tumors fail to respond when other modes are unfavorable; combination interventions succeed when they address the operative failure modes and fail when they do not.

1.3 The historical Coley framework and its contemporary relevance

William B. Coley, a New York surgeon, observed in 1891 that patients with concurrent erysipelas infection sometimes experienced tumor regression. He developed a mixed bacterial vaccine (heat-killed Streptococcus pyogenes plus Serratia marcescens, “Coley toxins”) administered repeatedly over weeks to months, with documented sustained remissions in subsets of patients with various sarcomas and carcinomas. The historical clinical record is uncontrolled by contemporary standards, but the documented responses are difficult to attribute entirely to selection bias or coincidence.

Coley’s framework — sustained dosing of bacterial PAMP-rich preparations to induce systemic inflammation and febrile response — was eclipsed in mid-20th century oncology by chemotherapy and radiation. Recent decades have seen renewed interest as the molecular biology underlying Coley’s empirical observations has become understood: bacterial PAMPs activate dendritic cells through TLR signaling; sustained inflammatory cytokine signaling supports memory T cell formation; immunogenic cell death from inflammation releases tumor antigens; fever-range thermal stress upregulates MHC and promotes T cell trafficking.

This paper does not propose a return to the historical Coley protocol. The argument is structural: the Coley framework engaged six of the seven failure modes through its bacterial PAMP and sustained inflammation mechanisms, but engaged Mode 4 (T cell exhaustion) essentially not at all because the molecular biology of T cell exhaustion was unknown in Coley’s era. Contemporary checkpoint inhibitors provide the Mode 4 engagement Coley’s framework lacks. The integration of these — modern multi-

mode protocols that combine Coley-equivalent broad engagement with contemporary targeted Mode 4 blockade — is the structural prediction the framework recommends.

To make the lineage explicit, each element of Coley's empirical approach has a defined modern molecular descendant, and these descendants are precisely the components the framework's combinatorial protocols assemble:

- **Coley's bacterial toxins** (crude PAMP preparations injected at or near the tumor) are the direct ancestor of today's **intratumoral TLR agonists** — purified, dose-controlled danger signals such as poly-ICLC (TLR3) and the STING agonists — which trigger the same innate-immune alarm and dendritic-cell activation (Modes 2, 3, 5) without the variability and toxicity of live or crude bacterial preparations.
- **Coley's repeated dosing over weeks to months**, which sustained the immune response crudely, is the ancestor of today's **IL-15 superagonists** (such as N-803/ANKTIVA) and other defined persistence-supporting cytokine therapeutics that engage Mode 6 in a controlled fashion.
- **Coley's characteristic high fevers**, long suspected to contribute to his successes, correspond to **fever-range and whole-body hyperthermia**, which upregulates MHC expression, promotes T cell trafficking, and modifies the tumor microenvironment.
- **The nonspecific inflammatory antigen release** that Coley's approach achieved is now matched, far more precisely, by **mRNA and peptide neoantigen vaccines** that target defined tumor antigens (Modes 1, 7).

In this sense, the contemporary combinatorial protocols the framework recommends — and the trial designs developed in the companion paper (Paper 4 of this series) — can be read as the Coley approach made molecularly precise, dose-controlled, and completed by the one mode (checkpoint blockade) that Coley's era could not access. Throughout this paper, references to the "modern Coley" lineage denote specifically these defined descendants of Coley's intratumoral, sustained, innate-immune-activating strategy.

1.4 Relationship to previous papers in this series

This is the third paper in a series examining the structural relationship between historical immunotherapy frameworks and contemporary cancer immunology. The first paper proposed the seven-mode framework conceptually and showed how the Coley protocol's mechanism map across the seven modes (Monteiro EPD, Zenodo 2024, with current download count and engagement evidence). The second paper applied the framework to BCG-unresponsive NMIBC and predicted the structural pattern of the ANKTIVA + BCG combination prior to its FDA approval (April 22, 2024).

This third paper provides the comprehensive seven-mode framework with full literature integration, the scoring methodology, and tumor-type-specific case studies. The aim is to make the framework operational for trial design and intervention evaluation across cancer types.

1.5 Scope and limitations

This paper synthesizes published clinical and mechanistic evidence rather than presenting original experimental data. The framework is structural rather than quantitatively predictive — it identifies which modes a protocol engages and predicts qualitative patterns of clinical benefit, not specific magnitudes of effect. The author is an independent researcher without institutional affiliation; the paper has been developed with extensive AI-assisted literature review, and all specific citations have been verified against primary sources.

The framework is informed by the author's personal involvement in cancer immunotherapy as a family caregiver — specifically, the use of CHIPSA Tijuana-administered Coley vaccine plus tumor-antigen vaccine plus weekly fever-range whole-body hyperthermia plus assay-guided low-dose platinum chemotherapy in a 2011 case of Stage IIA (pT2 pN0) high-grade BRCA1-mutated triple-negative invasive ductal carcinoma with foci of metaplastic transformation, with sustained 15-year recurrence-free survival. The author acknowledges this personal context openly. The framework's structural arguments do not depend on the specific case for their validity, and the literature evidence reviewed in this paper would support the framework's conclusions independent of the personal context. However, the personal context provided motivation for the systematic literature review the paper presents.

The framework's predictions about specific combinatorial protocols have not been directly tested in head-to-head clinical trials. The paper identifies specific trials that would test the framework and acknowledges that until such trials are conducted, the framework's predictions remain structural rather than empirically validated at the protocol level. The component evidence base for each individual mode-engagement intervention is established; the integrated combinatorial prediction is the framework's contribution.

2. Mode One: Insufficient antigen presentation

The problem

For CD8+ cytotoxic T cells to recognize and kill cancer cells, the cancer cells must present tumor-derived peptides on the surface major histocompatibility complex class I (MHC-I, encoded by the human leukocyte antigen class I, HLA-I, gene complex). When MHC-I expression is reduced or lost, even strongly immunogenic tumors become functionally invisible to T cell-mediated immunity. This is among the most frequent and clinically consequential mechanisms of immune escape across human cancers.

The extent of the problem has been progressively clarified by genomic and immunohistochemical studies over the past two decades. The Garrido laboratory's systematic analyses across multiple tumor types established that HLA-I alterations are common in epithelial malignancies. In breast cancer specifically, Garrido MA, Rodriguez, Zinchenko, and colleagues (with Aptsiauri as senior author) found HLA-I alterations in 79 of 98 tumors examined (81 percent), including total HLA-I loss in 53 cases (54

percent) and partial loss in 16 (14 percent) — leaving only 19 percent of breast cancers with normal HLA-I expression. The same cohort showed loss of heterozygosity at chromosome 6 or 15 (the HLA-I and beta-2-microglobulin regions respectively) in 36 of 92 evaluable cases (39 percent), with eight tumors showing chromosomal LOH despite immunohistochemically positive HLA-I staining — indicating that genetic and expression-level defects can be partially uncoupled (Garrido MA et al., *Immunogenetics* 2018;70(10):647-659, doi:https://doi.org/10.1007/s00251-018-1074-2). Similar high frequencies of HLA-I alteration have been reported in laryngeal carcinoma, colorectal carcinoma, bladder carcinoma, and other tumor types.

A more recent line of work has focused on HLA loss of heterozygosity (HLA-LOH) as a specific, targetable mechanism. McGranahan and colleagues, using the LOHHLA computational tool on the TRACERx cohort of 327 tumor exomes from early-stage non-small-cell lung cancer (NSCLC) patients, found HLA-LOH in 40 percent of these tumors, with associations to elevated subclonal neoantigen burden, APOBEC-mediated mutagenesis, and PD-L1 positivity — consistent with selection of HLA-LOH variants under immune pressure (McGranahan et al., *Cell* 2017). Montesion and colleagues, analyzing a substantially larger pan-cancer dataset of 83,644 patient samples from the Foundation Medicine commercial sequencing database, found HLA-I LOH in 17 percent of tumors overall, with a non-linear relationship to tumor mutational burden: HLA-LOH was frequent at intermediate mutational loads but decreased above 30 mutations per megabase, consistent with the interpretation that highly mutated tumors require more complete antigen presentation defects to escape immunity (Montesion et al., *Cancer Discovery* 2021). Han and colleagues (ASCO Breakthrough 2024 conference abstract) reported preliminary findings that HLA-I LOH is more frequent in brain metastases from lung cancer than in matched primary tumors, with reduced CD8+ T cell infiltration and poorer immunotherapy response — though this remains conference-abstract material pending full peer-reviewed publication.

The implication is that any cancer immunotherapy strategy that depends on T cell recognition of tumor antigens must account for the high baseline frequency of antigen presentation defects. In the substantial fraction of tumors with reduced MHC-I expression, even maximally active T cells will not find their targets.

The mechanisms

The mechanisms of HLA-I downregulation are heterogeneous and have therapeutic implications. They divide into two broad categories.

Genetic (irreversible) defects. These include HLA-LOH at chromosome 6p21.3, loss of beta-2-microglobulin (B2M) at chromosome 15, mutations in HLA genes themselves, and mutations in antigen processing machinery components (TAP1, TAP2, tapasin, ERAP1, ERAP2). When the defect is genetic, treatment with inflammatory cytokines or epigenetic modifiers cannot restore HLA-I expression — the gene is missing or mutated. Garrido and colleagues have characterized these as “irreversible” or “hard” lesions (Garrido et al., *Cancer Immunology, Immunotherapy* 2002).

Regulatory (reversible) defects. These include transcriptional silencing of HLA-I and antigen processing machinery genes through epigenetic mechanisms, loss of expression of interferon regulatory factors (IRF1, IRF2) without underlying genetic

deletion, and defects in JAK/STAT signaling that prevent inducible HLA-I upregulation but leave the basic gene structure intact. When the defect is regulatory, treatment with interferons, HDAC inhibitors, or DNMT inhibitors can restore expression. These are “reversible” or “soft” lesions.

The clinical and therapeutic distinction matters: a regulatory defect is potentially correctable; a genetic defect is not. In practice, most tumors show a mix of mechanisms across clonal subpopulations, with the proportion of irreversible to reversible defects shifting under immune pressure during tumor evolution.

The contemporary interventions

Five intervention categories are clinically relevant for addressing Mode One, with substantially different evidence bases and translational maturity.

Interferon-gamma

Interferon-gamma (IFN-gamma) is the single most potent upregulator of MHC-I expression in human cells. The mechanism is direct: IFN-gamma binding to its receptor activates JAK1/JAK2 and downstream STAT1 phosphorylation, which transcriptionally upregulates HLA-A, HLA-B, HLA-C, B2M, TAP1, TAP2, and the immunoproteasome components. The intervention is, in mechanistic terms, exactly targeted to Mode One.

The clinical history of IFN-gamma in oncology has been mixed. Multiple historical trials of IFN-gamma as monotherapy showed limited efficacy, and the drug has FDA approval for chronic granulomatous disease and severe malignant osteopetrosis but not for cancer treatment. The reasons for monotherapy failure are now better understood: IFN-gamma upregulates not only MHC-I but also PD-L1, generating a compensatory immune brake that limits the efficacy of T cell activation. This finding suggested that IFN-gamma combined with PD-1/PD-L1 blockade could produce the benefit that IFN-gamma alone could not.

The phase 0 trial by Zhang and colleagues (*Cancer Immunology Research* 2019) tested this hypothesis directly. Eight patients with synovial sarcoma or myxoid/round cell liposarcoma — two cold-tumor archetypes — received weekly subcutaneous IFN-gamma (100 mcg/m²) with pre- and post-treatment biopsies. The trial demonstrated significant increases in tumor-surface MHC-I expression and significant T cell infiltration after IFN-gamma treatment. Gene expression analysis showed increased antigen presentation machinery expression and less exhausted phenotypes in tumor-infiltrating T cells. PD-L1 was upregulated as predicted, supporting the rationale for combination with checkpoint inhibition. The trial led to the ongoing CITN multicenter study (NCT03063632) testing IFN-gamma with pembrolizumab in sarcomas and other cold tumors.

The translational implication is direct: IFN-gamma plus checkpoint blockade is a contemporary, FDA-component combination that addresses Mode One together with Mode Four (T cell exhaustion). For Mode One specifically, IFN-gamma is the most mechanistically targeted intervention available.

HDAC inhibitors

Histone deacetylase inhibitors (HDACi) restore MHC-I expression in tumors where the underlying defect is epigenetic silencing rather than genetic loss. Multiple HDACi are FDA-approved (vorinostat, romidepsin, belinostat, panobinostat) for hematologic malignancies, with broader clinical use limited by toxicity and modest single-agent efficacy in solid tumors.

The MHC-I-restoring effect of HDAC inhibition has been demonstrated across multiple cancer cell line studies in melanoma, glioma, breast cancer, and other tumor types. The newer-generation class I selective HDAC inhibitors (mocetinostat, zabadinostat, entinostat) have been studied specifically for their immunomodulatory effects in combination with checkpoint inhibitors. The CCTG IND.226 trial and several industry-sponsored combination trials have explored HDACi-checkpoint inhibitor combinations with mixed but generally encouraging results.

The translational positioning of HDAC inhibitors for Mode One is complicated. Toxicity at doses required for tumor cell HDAC inhibition is substantial. Some preclinical work suggests that low-dose intermittent HDACi schedules may achieve MHC-I restoration with reduced toxicity. The optimal HDACi for immune restoration may differ from the optimal HDACi for cytotoxic effect — a fact that has not been adequately tested in formal trials.

DNA methyltransferase inhibitors

DNA methyltransferase inhibitors (DNMTi) include 5-azacitidine and decitabine, both FDA-approved for myelodysplastic syndrome. Like HDAC inhibitors, they restore expression of epigenetically silenced genes including HLA-I components, and they have been studied in combination with checkpoint inhibitors. Low-dose decitabine in combination with checkpoint inhibitors has shown encouraging signals in early trials in lung cancer and hematologic malignancies.

The mechanistic rationale is strong; the clinical translation has been slower than for HDAC inhibitors. The optimal dosing for immune modulation versus cytotoxic effect remains under investigation.

Fever-range thermal stress

Fever-range whole-body hyperthermia (39-40°C) upregulates MHC-I and MHC-II expression in tumor cells, increases heat shock protein expression which itself serves as a danger signal, improves T cell trafficking into tumors (the HSP90-α4 integrin signaling pathway characterized by the Repasky and Evans laboratories), and creates a generally immune-permissive tumor microenvironment.

This intervention is one of the two modes the historical Coley protocol engages directly. The Karbach 2012 phase I trial of MBV combined with NY-ESO-1 vaccination capped peak temperatures at 39.5°C through protocol-mandated dose reduction — the regulatory dose-attenuation pattern the Coley framework paper analyzes in detail. The dose attenuation likely reduced the magnitude of MHC-I upregulation achieved, contributing to the difficulty of replicating historical Coley response rates.

In the context of the broader combinatorial framework, fever-range thermal stress is a non-pharmacological intervention that engages Mode One simultaneously with Mode Two (dendritic cell maturation through HSP danger signals) and partially Mode Three (improved T cell trafficking). Its inclusion in any combinatorial-complete protocol is mechanistically justified.

Immunogenic chemotherapy

Certain cytotoxic chemotherapies induce immunogenic cell death (ICD) characterized by surface calreticulin exposure, ATP and HMGB1 release, and increased antigen presentation. Anthracyclines (doxorubicin, epirubicin), oxaliplatin, and some platinum agents have ICD-inducing properties at appropriate doses. Recent work has emphasized that the immunogenic effects depend on dose intensity and scheduling, and that conventional maximum-tolerated-dose regimens may actually impair the ICD effect through generalized lymphocyte suppression.

The implication is that low-dose, metronomic, or immunogenically-optimized chemotherapy schedules may engage Mode One more effectively than standard MTD scheduling — a finding directly relevant to the chemosensitivity-guided low-dose approach used in the Monteiro case described in the framework paper.

What current trials are not testing

Across the contemporary clinical trial landscape, individual interventions addressing Mode One are being tested almost exclusively in combination with checkpoint inhibitors (addressing Mode Four). This is rational as far as it goes — the synergy between Mode One restoration and Mode Four blockade is mechanistically sound and clinically demonstrated.

However, no current trial known to us combines Mode One interventions with simultaneous engagement of Modes Two through Six. The IFN-gamma plus pembrolizumab trial addresses Modes One and Four but not the others. The HDAC inhibitor combinations address Mode One and One Mode Four but typically do not engage Mode Five (active immune suppression by the TME) or Mode Six (insufficient persistence).

This is precisely the combinatorial completeness gap the framework analysis identifies. Mode One restoration is necessary but not sufficient. Tumors with HLA-LOH at the genetic level will not respond to any Mode One intervention; in these patients, alternative strategies (NK cell-based therapy, antibody-based recognition independent of HLA-I, oncolytic viruses) become necessary. Tumors with reversible HLA-I downregulation respond to Mode One interventions but require simultaneous engagement of other modes for durable benefit.

Coley framework engagement of Mode One

The integrated Coley protocol engages Mode One through two distinct mechanisms.

The bacterial preparation component triggers systemic IFN-gamma production through pattern recognition receptor activation on innate immune cells. This converges on the same molecular endpoint achieved by recombinant IFN-gamma

administration, produced endogenously and at the locations where bacterial PAMPs are sensed. Whether the intensity and duration of IFN-gamma signaling in a sustained Coley protocol matches, exceeds, or falls short of what is achieved with subcutaneous recombinant IFN-gamma dosing is not directly characterized in the available literature and warrants empirical comparison.

The fever-range thermal stress component directly upregulates MHC-I expression in tumor cells through heat shock response activation, with mechanistic overlap with the IFN-gamma pathway. The Karbach 2012 dose attenuation specifically reduced the magnitude of this effect.

In the framework paper's scoring methodology, the historical Coley protocol scores high on Mode One engagement. This is one of the framework's strongest mechanistic anchors for the argument that historical response rates reflected integrated activation of multiple modes simultaneously.

Gaps and open questions

Several questions emerge from this analysis that are relevant to the larger combinatorial framework.

First, no clinical trial has directly compared the magnitude of MHC-I upregulation achieved by IFN-gamma monotherapy, HDAC inhibitor monotherapy, fever-range thermal stress monotherapy, and combinations of these. The mechanistic literature suggests that IFN-gamma is the strongest single intervention, but the question of whether combinations produce additive or synergistic effects on MHC-I expression is not formally answered.

Second, the question of how to identify patients with reversible versus irreversible HLA-I defects in real time, before initiating treatment, is not yet solved. Standard tumor sequencing increasingly detects HLA-LOH (the Foundation Medicine and Tempus platforms now report it), but the distinction between genetic and regulatory defects in a given patient requires more nuanced assessment than current clinical practice provides.

Third, the temporal relationship between Mode One restoration and subsequent immune attack is poorly characterized. How long does MHC-I expression remain elevated after IFN-gamma treatment ends? How does the timing of checkpoint blockade administration relative to Mode One intervention affect outcomes? These pharmacodynamic questions are central to combinatorial protocol design and remain underexplored.

Fourth, the question of whether Mode One interventions can be effectively delivered intratumorally — bypassing systemic toxicity while concentrating effect at the tumor site — has been raised but not adequately tested. The localized delivery paradigm successful in non-muscle-invasive bladder cancer (BCG, ANKTIVA) suggests this approach may have broader applicability than current systemic-immunotherapy paradigms.

Implications for the combinatorial framework

Mode One is among the more tractable failure modes from a translational standpoint. Multiple interventions exist, with diverse mechanisms and dosing options. The Coley framework already engages this mode strongly through bacterial PAMP-induced IFN-gamma and fever-range thermal stress. Adding pharmacological interventions (IFN-gamma, HDACi, immunogenic-optimized chemotherapy) extends the Mode One engagement of any combinatorial protocol.

The framework's central argument predicts that combinatorial protocols engaging Mode One alongside Modes Two through Six will produce response patterns qualitatively different from those of single-agent or limited-combination approaches. The clinical signals from the ANKTIVA + BCG combination in bladder cancer, where Mode One restoration through bacterial PAMP exposure occurs alongside engagement of Modes Two and Six, support this prediction at localized scale. Whether the same combinatorial logic can be successfully translated to systemic-disease contexts remains the central open question of the broader analysis.

The next section addresses Mode Two: lack of T cell priming.

3. Mode Two: Lack of T cell priming and dendritic cell engagement

The problem

Effective adaptive immunity against cancer requires more than the presence of tumor antigens on MHC-I; it requires that those antigens be acquired, processed, and presented to naïve T cells in lymphoid tissue by professional antigen-presenting cells, primarily dendritic cells (DCs). When DCs are absent, immature, dysfunctional, or actively tolerogenic, even tumors with intact antigen presentation machinery fail to elicit productive T cell responses. Mode One ensures that tumor cells can be recognized by primed effector T cells; Mode Two governs whether T cells get primed in the first place.

DC dysfunction in cancer is now understood as a major and pervasive contributor to immune escape. Chen and colleagues, in a comprehensive 2024 review in *Cancer Communications*, characterize the multiple convergent mechanisms by which the tumor microenvironment impairs DC function: cytokine-driven differentiation arrest, hypoxia-induced functional dysregulation, exosome-mediated tolerization, metabolite-driven dysfunction (lactate, kynurenine, prostaglandin E2), and co-inhibitory ligand expression on DCs themselves (Chen et al., *Cancer Communications* 2024). The result is that even when tumor antigens are released into the microenvironment — through spontaneous cell death, irradiation, or cytotoxic chemotherapy — the DCs needed to capture, mature with, and cross-present those antigens to CD8+ T cells are insufficient in number, immature in phenotype, or actively tolerogenic.

The clinical consequence is that purely "T cell-focused" immunotherapy interventions — including most checkpoint inhibitors — depend on a functional priming step that is

often absent. This explains, in part, why checkpoint inhibitors work dramatically in some tumors (where DC function and prior T cell priming are intact) and fail in others (where they are not). The "hot" versus "cold" tumor distinction is largely a Mode Two phenomenon.

The mechanisms

DC dysfunction in cancer operates through several interrelated mechanisms.

Numerical insufficiency. Many tumors actively suppress DC differentiation and recruitment. Tumor-derived factors including VEGF, IL-10, and TGF-beta inhibit the differentiation of monocytes and bone marrow progenitors into functional DCs. The result is a tumor microenvironment with reduced numbers of conventional dendritic cells (cDCs), particularly the cross-presenting cDC1 subset that is essential for CD8+ T cell priming against tumor antigens. Sufficient cDC1 numbers in tumors are strongly correlated with response to checkpoint blockade.

Maturation defects. Even when DCs are present, they may remain in an immature state characterized by low surface expression of MHC-II, low expression of costimulatory molecules (CD80, CD86, CD40), and limited capacity to migrate to draining lymph nodes for T cell priming. Immature DCs presenting tumor antigens may actively tolerize T cells rather than activate them.

Active tolerization. Some DCs in the tumor microenvironment acquire actively suppressive phenotypes, expressing co-inhibitory ligands (PD-L1, PD-L2), producing immunosuppressive cytokines (IL-10, TGF-beta), and metabolizing tryptophan to immunosuppressive kynurenines via indoleamine 2,3-dioxygenase (IDO). Plasmacytoid DCs (pDCs) in particular can adopt tolerogenic phenotypes that promote regulatory T cell expansion rather than effector T cell priming (Chen et al., *Cancer Communications* 2024).

Cross-presentation failure. The cDC1 subset specializes in cross-presenting exogenous antigens on MHC-I to CD8+ T cells. In tumors deficient in cDC1, or where cDC1 function is impaired, this cross-presentation step fails — leaving even highly immunogenic tumor antigens unable to prime cytotoxic T cell responses.

The contemporary interventions

Four intervention categories address Mode Two with substantively different mechanisms and clinical profiles.

FLT3 ligand: DC expansion

Fms-like tyrosine kinase 3 ligand (FLT3L) is the master cytokine regulator of DC development. FLT3L binding to its receptor on hematopoietic progenitors drives proliferation, differentiation, and mobilization of all major DC subsets (cDC1, cDC2, pDC) into peripheral blood and tissues. Administration of recombinant FLT3L therefore provides a means to expand the DC pool available for tumor antigen capture and presentation.

The clinical development of recombinant FLT3L has been complicated but is producing useful evidence. Anandasabapathy and colleagues' phase I trial in healthy volunteers established that CDX-301 (a current recombinant FLT3L formulation developed by Celldex Therapeutics) safely expands all major DC subsets, with cDC1 and cDC2 levels increasing significantly and peaking approximately two weeks after the start of dosing (Anandasabapathy et al., *Bone Marrow Transplantation* 2015).

The most informative cancer-context trial of FLT3L to date is the phase II trial of CDX-301 with anti-DEC205-NY-ESO-1 vaccine and poly-ICLC in high-risk melanoma patients (NCT02129075), reported by Bhardwaj and colleagues in *Nature Cancer* 2020 (Bhardwaj N, Friedlander PA, Pavlick AC, et al. *Nature Cancer* 2020;1(12):1204-1217). FLT3L pre-treatment significantly increased peripheral cDC1, cDC2, and pDC populations, and the FLT3L-containing arm showed significant increases in NY-ESO-1-specific humoral and T cell responses compared to vaccine alone. The trial established that FLT3L meaningfully amplifies the immunogenicity of a defined-antigen vaccine through DC expansion.

Historical FLT3L monotherapy trials in ovarian, breast, and non-Hodgkin lymphoma did not show definitive anti-tumor activity, which is now interpreted as a consequence of mobilizing immature DCs in the absence of appropriate maturation signals. This finding clarifies a structural insight relevant to the broader combinatorial framework: DC expansion alone is insufficient; expansion must be combined with antigen exposure and maturation signals.

TLR agonists: DC maturation

Toll-like receptors (TLRs) are the prototypical pattern recognition receptors of innate immunity. TLR engagement on DCs drives maturation: upregulation of MHC and co-stimulatory molecules, production of pro-inflammatory cytokines including IL-12, migration to draining lymph nodes, and acquisition of cross-presentation capacity. Different TLRs respond to different pathogen-associated patterns: TLR3 to double-stranded RNA, TLR4 to lipopolysaccharide, TLR7/8 to single-stranded RNA, TLR9 to unmethylated CpG DNA. Each provides a distinct route to DC activation.

Several TLR agonists are FDA-approved for cancer or cancer-related indications. Imiquimod (Aldara), a TLR7 agonist, is FDA-approved for topical treatment of actinic keratoses, superficial basal cell carcinoma, and external genital warts, and is widely used off-label for superficial cutaneous lymphoma and superficial melanoma. Imiquimod acts directly on plasmacytoid DCs to drive IFN-alpha production and broader immune activation. Resiquimod (R-848), a more potent TLR7/8 agonist, has received EU orphan designation for cutaneous T cell lymphoma and is in active investigational development for systemic cancer immunotherapy applications.

Poly-ICLC (Hiltonol), a stabilized synthetic double-stranded RNA TLR3 agonist, is not formally FDA-approved as a stand-alone drug but is widely used in investigator-initiated trials of cancer vaccines. Its role as a TLR3 agonist that activates cross-presenting cDC1 is mechanistically specific and clinically validated in the in situ vaccination work described below.

The CpG oligodeoxynucleotides (TLR9 agonists) have a longer history in cancer vac-

cine adjuvant work, with multiple clinical trials in lymphoma, melanoma, and other indications producing variable but real anti-tumor activity.

STING agonists: cytosolic DNA sensing

The stimulator of interferon genes (STING) pathway is an intracellular sensor of cytosolic DNA that drives type I interferon production and DC activation through a mechanism distinct from TLR signaling. STING activation in tumor-infiltrating DCs is now understood as a critical bridge between tumor-derived DNA damage and adaptive immunity.

The clinical development of STING agonists has been technically challenging but is producing meaningful early data. Harrington and colleagues reported the phase I/II results of ulevostinag (formerly MK-1454, a synthetic cyclic dinucleotide STING agonist) administered intratumorally alone or with intravenous pembrolizumab in advanced solid tumors and lymphomas (Harrington KJ, Champiat S, Brody JD, et al., *Clinical Cancer Research* 2025;31(16):3400-3411). In the phase I study (NCT03010176, N=156), intratumoral ulevostinag was tolerable with dose-dependent pharmacokinetics and pharmacodynamics, though circulating inflammatory biomarker levels did not show a clear dose-effect relationship beyond the 540 µg dose. The randomized phase II study in previously untreated metastatic or recurrent head and neck squamous cell carcinoma (NCT04220866) showed evidence of greater antitumor activity for intratumoral ulevostinag plus intravenous pembrolizumab than for intravenous pembrolizumab monotherapy, though the sample size was small.

A novel approach to STING engagement is SYNB1891 (Synlogic), a live *Escherichia coli* probiotic engineered to produce STING-agonist cyclic dinucleotides in vivo when administered intratumorally. An ongoing phase I trial in advanced solid tumors has reported early data with two patients in initial cohorts showing stable disease, including refractory cases, and a manageable toxicity profile including cytokine release syndrome at higher doses. This intersection of bacterial-vehicle delivery with STING engagement represents a mechanistic convergence between the modern STING agonist approach and the historical Coley logic — engineered bacteria as a delivery system for endogenous immune activation.

A case-level demonstration of intratumoral STING agonist activity in PD-(L)1-refractory disease was reported in detailed biomarker analysis of a patient with Merkel cell carcinoma treated with ADU-S100 plus anti-PD-1 spartalizumab, who experienced durable regression of both injected and non-injected lesions (*Journal for ImmunoTherapy of Cancer* 2024;12(10):e009803). Single-cell sequencing showed cancer cells decreasing from 70% to 49% of the tumor microenvironment while T cells doubled from 18% to 36%, with documented Mode Two effects including increased DC infiltration and maturation.

Cancer vaccines: targeted antigen delivery

Cancer vaccines represent the most direct intervention approach for Mode Two: deliver tumor antigens to DCs in a context that promotes maturation and cross-presentation. The field is heterogeneous, including peptide vaccines, dendritic

cell vaccines (autologous DCs loaded with antigens ex vivo and reinfused), mRNA vaccines, viral vector vaccines, and antibody-targeted vaccines (such as the anti-DEC-205-NY-ESO-1 fusion construct used in the Bhardwaj 2020 trial).

A comprehensive review of the contemporary cancer vaccine landscape is beyond the scope of this section. The key structural insight relevant to the combinatorial framework is that vaccines alone — like FLT3L alone — generally fail to produce durable anti-tumor responses, but vaccines combined with DC expansion (FLT3L), maturation signals (TLR or STING agonists), and antigen release (radiotherapy or immunogenic chemotherapy) can produce qualitatively different outcomes. The integrated multi-component approach is the mechanistic vindication of the Coley logic at the level of contemporary molecular immunology.

The in situ vaccination paradigm: convergent contemporary expression of Coley logic

The most direct contemporary expression of integrated Mode Two engagement is the in situ vaccination (ISV) paradigm developed at Mount Sinai. Hammerich and colleagues, in *Nature Medicine* 2019, reported a three-step ISV protocol for indolent non-Hodgkin lymphoma (iNHL): (1) intratumoral FLT3L (CDX-301) to recruit and expand intratumoral DCs; (2) low-dose local radiotherapy to induce immunogenic tumor cell death and release tumor antigens; (3) intratumoral poly-ICLC to activate the antigen-loaded DCs through TLR3 (Hammerich et al., *Nature Medicine* 2019).

The trial (NCT01976585) enrolled patients with advanced iNHL and demonstrated systemic clinical tumor regressions — including regression of non-irradiated tumor sites (the abscopal effect) — in a population otherwise unresponsive to checkpoint blockade. Mechanistically, ISV recruited and matured intratumoral cross-presenting DCs, primed anti-tumor CD8+ T cells, and produced documented systemic immune responses. Critically, the trial also demonstrated that patients who did not initially respond to ISV developed PD1+ CD8+ T cells, and murine experiments showed that ISV converts PD1-refractory tumors into PD1-responsive ones. This led to a follow-up trial of ISV combined with pembrolizumab.

The ISV protocol is a contemporary recapitulation of the Coley logic at the molecular level: a multi-step intratumoral intervention that (i) recruits and amplifies professional antigen-presenting cells (FLT3L; analogous to the inflammatory recruitment Coley toxin induces), (ii) releases tumor antigens for capture (radiotherapy; analogous to thermal stress-induced antigen release), and (iii) activates antigen-loaded APCs through pattern recognition receptor engagement (poly-ICLC; analogous to the PAMP signaling of bacterial preparations).

The framework's central argument receives strong mechanistic support from this work: combinatorial-complete engagement of antigen release, APC recruitment, and APC maturation produces clinical responses that single-component interventions do not. The historical Coley protocol achieved similar combinatorial completeness through different molecular routes; the contemporary ISV achieves it through defined molecular components.

What current trials are not testing

The combinatorial framework's argument generalizes from the lymphoma ISV result to other cancer types where the principles should also apply. Across the contemporary solid tumor immunotherapy trial landscape, however, the most common pattern remains single Mode Two interventions (a TLR agonist, a STING agonist, FLT3L, or a vaccine) combined with anti-PD-1/PD-L1 — addressing Mode Two and Mode Four but rarely engaging the full ISV-style trinity of DC expansion, antigen release, and DC maturation simultaneously.

The structural-completeness gap is identifiable: most trials do not implement intratumoral FLT3L, low-dose radiotherapy, and intratumoral TLR or STING agonists together. Each component exists; each has been clinically validated; the combination has been demonstrated to produce systemic regressions in iNHL; yet the integrated protocol has not been tested across solid tumor types.

This is precisely the kind of combinatorial-completeness gap the framework analysis is designed to identify. The intervention components for full Mode Two engagement are available. The combinatorial trials are not being designed.

Coley framework engagement of Mode Two

The bacterial preparation component of the historical Coley protocol engages Mode Two through multiple convergent mechanisms.

Coley toxin (a heat-killed *Streptococcus pyogenes* and *Serratia marcescens* preparation, or modern MBV equivalents) delivers a broad spectrum of PAMPs including TLR2 ligands (lipoteichoic acid from streptococcal cell walls), TLR4 ligands (lipopolysaccharide from Serratia), TLR5 ligands (flagellin), and TLR9 ligands (bacterial CpG DNA). The simultaneous engagement of multiple TLR pathways drives broad DC maturation that no single synthetic TLR agonist replicates.

The fever-range thermal stress component induces release of heat shock proteins (HSPs) which serve as endogenous danger signals through pattern recognition receptor engagement, contributing to DC maturation independently of bacterial PAMPs.

The integrated systemic activation that Coley protocols produce — pyrogenic cytokine release, broad innate immune activation, sustained inflammation — also induces the kind of generalized DC expansion that recombinant FLT3L delivers in a more targeted molecular form.

The framework's analysis treats the historical Coley protocol as scoring high on Mode Two engagement because of this multi-pathway DC maturation effect. The contemporary in situ vaccination protocols achieve similar Mode Two engagement through defined components.

The structural argument that emerges: the molecular routes differ, but the combinatorial completeness principle is the same. A protocol that engages Mode Two through multiple convergent mechanisms — APC expansion plus antigen release plus pattern recognition signaling — produces qualitatively different outcomes than a protocol

that engages only one component of this triad. The Hammerich 2019 ISV trial provides contemporary clinical-grade evidence for this structural prediction.

Gaps and open questions

Several questions emerge that are relevant to the larger combinatorial framework.

First, the question of how the historical Coley protocol's bacterial PAMP delivery compares mechanistically to the defined contemporary triad (FLT3L + radiotherapy + poly-ICLC) has not been directly tested. Both engage Mode Two through convergent mechanisms; whether one produces stronger or more durable DC priming than the other is empirically unanswered.

Second, the optimal timing of Mode Two interventions relative to Mode One restoration and Mode Four blockade is unclear. The Hammerich 2019 work suggests that DC priming establishes the substrate that subsequent checkpoint blockade can act on; this temporal sequencing has therapeutic implications that current single-time-point trial designs may underrepresent.

Third, the extension of the ISV paradigm from iNHL to solid tumors remains poorly tested. Lymphoma offers a particularly favorable substrate (intratumoral injection is easy, lymphoma cells themselves can directly prime T cells under certain conditions, the cancer is inherently within the lymphoid system). Translating ISV principles to anatomically harder-to-access solid tumors will require delivery innovation.

Fourth, the comparative effectiveness of different TLR agonists (TLR3 via poly-ICLC, TLR7 via imiquimod, TLR9 via CpG) and STING agonists in different tumor types has not been systematically established. The structural framework treats them as functionally interchangeable Mode Two interventions; the empirical reality may show meaningful differentiation by tumor type.

Implications for the combinatorial framework

Mode Two is, like Mode One, addressable with multiple contemporary interventions, several of which already have FDA approval (imiquimod) or substantial clinical validation (CDX-301, poly-ICLC, multiple STING agonists in trials). The Hammerich 2019 ISV result is the strongest direct mechanistic and clinical demonstration that combinatorial Mode Two engagement produces qualitatively different outcomes than single-agent approaches — and that combinatorial Mode Two engagement can convert PD-1-refractory disease to PD-1-responsive disease.

The framework's combinatorial completeness argument predicts that protocols engaging Mode Two through the full triad (DC expansion + antigen release + maturation signaling) alongside engagement of Modes One, Four, and beyond will produce response patterns qualitatively different from current single-component approaches. The lymphoma ISV trial demonstrates this at one anatomical scale and tumor type. The framework's broader claim is that the same principle generalizes.

The next section addresses Mode Three: physical exclusion of T cells from the tumor microenvironment.

4. Mode Three: Physical exclusion of T cells from the tumor microenvironment

The problem

Effective T cell-mediated tumor destruction requires that primed effector T cells physically reach tumor cells. When stromal barriers, abnormal vasculature, dense extracellular matrix, or hypoxic regions prevent T cell entry into the tumor parenchyma, even well-primed T cells in the circulation cannot mediate cytotoxicity. This mechanism produces the immunologically "cold" tumor phenotype that has been the central obstacle to extending checkpoint inhibitor success to solid tumors with limited baseline T cell infiltration.

The clinical magnitude of this problem is substantial. Pancreatic ductal adenocarcinoma is the archetypal cold tumor, with response rates to single-agent checkpoint blockade in the low single digits. Cold-tumor patterns are also dominant in microsatellite-stable colorectal cancer, prostate cancer, much of breast cancer including most triple-negative breast cancer at presentation, and significant subsets of lung and other epithelial cancers. The "hot" tumors where checkpoint inhibitors succeed (melanoma, MSI-high colorectal cancer, lung cancer with high TMB) are characterized in part by pre-existing T cell infiltration that overcomes Mode Three.

Mode Three is distinct from Mode One and Mode Two in that it does not concern antigen recognition or T cell priming; it concerns the geometric and biochemical accessibility of the tumor to immune effectors. A protocol can perfectly address Modes One and Two yet fail clinically because the primed effector T cells cannot physically reach their targets.

The mechanisms

T cell exclusion operates through several interrelated mechanisms in the tumor microenvironment.

Cancer-associated fibroblasts (CAFs). CAFs are the dominant stromal cell population in many solid tumors, producing the dense extracellular matrix that physically constrains T cell movement. A subset of CAFs expressing fibroblast activation protein-alpha (FAP) is particularly relevant: FAP+ CAFs are reported across the majority of epithelial cancers including breast, colorectal, pancreatic, and lung adenocarcinomas, with FAP expression correlating with both poor prognosis and poor response to immunotherapy. CAF-derived TGF-beta, CXCL12, and other immunomodulatory factors compound the physical barrier with biochemical T cell exclusion.

Kieffer and colleagues, in single-cell analysis of breast cancer published in *Cancer Discovery* 2020, identified specific CAF clusters associated with immunotherapy resistance, providing molecular detail on the heterogeneity of the CAF population and the specific subsets driving immune exclusion (Kieffer Y, Hocine HR, Gentric G, et al. *Cancer Discovery* 2020;10(9):1330-1351, doi:https://doi.org/10.1158/2159-8290.CD-19-1384).

Extracellular matrix architecture. Beyond CAF biology, the physical architecture

of the tumor matrix itself excludes T cells. Real-time imaging of T cell dynamics in human non-small-cell lung cancer has demonstrated that dense collagen fibers oriented parallel to the tumor-stroma interface form a barrier around tumor masses, limiting T cell contact with tumor cells (Salmon and colleagues, multiple publications). The geometry of matrix deposition, not just its density, governs T cell penetration.

Abnormal tumor vasculature. Tumor blood vessels are structurally and functionally abnormal — tortuous, leaky, lacking the normal pericyte coverage and structured layered organization of healthy vessels. Abnormal vasculature impairs T cell extravasation from the bloodstream into the tumor parenchyma, with VEGF-driven endothelial cells expressing immune-modulating ligands including FasL and PD-L1 that actively suppress T cell entry (Motz et al., *Nature Medicine* 2014).

Hypoxia. Poorly vascularized tumor regions become hypoxic, and hypoxia drives a coordinated immunosuppressive program: HIF-1alpha stabilization upregulates immunosuppressive cytokines and adenosine production, downregulates MHC-I expression on tumor cells (linking Mode Three to Mode One), and impairs T cell function in cells that do reach the hypoxic region.

TGF-beta signaling. Across multiple cancer types, TGF-beta-activated myofibroblastic CAFs are increasingly recognized as central orchestrators of T cell exclusion. TGF-beta itself directly suppresses T cell function, drives CAF activation and matrix deposition, and promotes regulatory T cell differentiation. Multiple lines of evidence implicate the TGF-beta axis as a unifying mechanism across Mode Three biology.

The Coley framework does not directly engage Mode Three through its classical mechanisms. This represents a structural limitation that combinatorial extension can address.

The contemporary interventions

Five intervention categories address Mode Three through distinct mechanisms.

Anti-angiogenic therapy combined with checkpoint blockade

The most clinically validated approach to Mode Three is anti-VEGF therapy combined with checkpoint blockade. The biological rationale is that VEGF blockade normalizes the abnormal tumor vasculature, reduces VEGF-mediated immunosuppression, promotes T cell trafficking into the tumor, and enhances dendritic cell maturation — engaging Mode Three together with secondary effects on Modes Two and Five.

The landmark trial is IMbrave150 (Finn RS, Qin S, Ikeda M, et al., *New England Journal of Medicine* 2020;382:1894-1905, doi:https://doi.org/10.1056/NEJMoa1915745, NCT03434379). This phase III trial randomized patients with unresectable hepatocellular carcinoma 2:1 to atezolizumab (anti-PD-L1) plus bevacizumab (anti-VEGF) versus sorafenib, the prior standard of care. The combination produced significantly improved overall survival and progression-free survival, with the updated analysis showing a median OS 5.8 months longer than sorafenib and an objective response rate of approximately 30 percent including 8 percent complete responses. This established atezolizumab plus bevacizumab as the first-line standard of care for advanced

HCC and represents the strongest direct clinical validation of Mode Three engagement combined with Mode Four blockade.

The principle has been extended to other tumor types with mixed results. Combinations of VEGF receptor tyrosine kinase inhibitors (lenvatinib, cabozantinib) with checkpoint inhibitors have produced encouraging results in renal cell carcinoma and other cancers. The COSMIC-312 trial (cabozantinib plus atezolizumab in HCC) and LEAP-002 (lenvatinib plus pembrolizumab in HCC) extended this combinatorial approach with varied outcomes.

The mechanistic insight from this work generalizes: vascular normalization through VEGF blockade improves T cell infiltration into tumors that were previously T cell-excluded. The success of this approach validates Mode Three as a clinically tractable target.

Oncolytic viruses

Oncolytic viruses bypass Mode Three by being delivered directly into tumors (typically intratumorally), thereby achieving local immune activation regardless of pre-existing T cell exclusion. The viruses preferentially infect and lyse tumor cells, release tumor antigens, induce immunogenic cell death, and produce a strong local inflammatory response that converts the cold tumor microenvironment into a hot one — making the tumor susceptible to subsequent checkpoint blockade.

The most clinically advanced oncolytic virus is talimogene laherparepvec (T-VEC, IMLYGIC, Amgen), a genetically modified herpes simplex virus-1 expressing GM-CSF. T-VEC received FDA approval on October 27, 2015 for intralesional treatment of unresectable cutaneous, subcutaneous, and nodal melanoma lesions in patients with recurrence after initial surgery, based on the phase III OPTiM trial.

The combination of T-VEC with checkpoint blockade has been studied extensively. The phase Ib portion of the MASTERKEY-265 trial (NCT02263508) testing T-VEC plus pembrolizumab in advanced melanoma showed an objective response rate of 62 percent with a 33 percent complete response rate in the early cohort. However, the full randomized phase III portion of MASTERKEY-265 was stopped early for clinical futility — the combination did not significantly improve outcomes over pembrolizumab monotherapy in the broader study population. The phase Ib trial of T-VEC plus ipilimumab in 18 patients with unresectable melanoma reported an objective response rate of 56 percent including 33 percent complete responses.

These results illustrate a structural insight relevant to the combinatorial framework: oncolytic virus combinations show strong early-phase signals that have not always translated to phase III benefit. The interpretation includes the possibility that highly responsive patient populations selected by early-phase trials may already have sufficient T cell infiltration that the addition of Mode Three intervention does not change outcomes; the combinatorial logic may apply most where Mode Three is most operative.

Other oncolytic virus platforms in clinical development include Pexa-Vec (vaccinia), ONCOS-102 (adenovirus), Maraba (rhabdovirus), and engineered Newcastle disease

virus (NDV). Each has demonstrated some clinical signal in combination with checkpoint blockade, with the field still working through which platform-tumor pairings produce durable benefit.

FAP-targeted therapy

Direct targeting of FAP+ cancer-associated fibroblasts represents an emerging approach to Mode Three. Multiple preclinical studies have shown that depletion of FAP+ CAFs through CAR T-cell therapy, antibody-drug conjugates, or small-molecule inhibitors can disrupt the desmoplastic stroma and restore T cell infiltration in mouse models of pancreatic and other solid cancers (preclinical data, multiple sources including biorxiv preprints 2023). Clinical translation is in progress but no FAP-targeted therapy has yet achieved regulatory approval for cancer treatment. The technical challenge has been that FAP is expressed on some normal tissues (wound healing fibroblasts, certain fetal tissues) and depletion strategies need to avoid off-target toxicity.

TGF-beta inhibitors

TGF-beta inhibitors and TGF-beta-trap molecules represent another approach to disrupting CAF-mediated T cell exclusion. Bintrafusp alfa (a bifunctional fusion protein combining anti-PD-L1 with a TGF-beta receptor II trap) was developed by Merck KGaA and EMD Serono and tested in multiple phase II and III trials. The clinical results were disappointing — multiple phase III programs were discontinued in 2021 after failing to demonstrate benefit over comparators in lung and biliary tract cancers — but the mechanistic rationale for combining TGF-beta blockade with checkpoint inhibition remains active research. Selective TGF-beta inhibitors and combinations with newer agents continue to be explored.

NOX4 inhibitors (targeting the NADPH oxidase enzyme involved in CAF activation) and LRRC15-targeted approaches (a CAF surface marker increasingly recognized as a precision target) are in earlier development with preclinical evidence of restoring T cell infiltration (Ford et al., *Cancer Research* 2020; Hanley et al., *Journal of the National Cancer Institute* 2018).

Intratumoral delivery (delivery-based bypassing of exclusion)

The fifth approach to Mode Three is not pharmacological but logistical: deliver the immune-activating intervention directly into the tumor, bypassing the systemic barriers that exclude immune effectors. This is the principle behind the in situ vaccination work discussed in Mode Two (intratumoral FLT3L, intratumoral poly-ICLC), the intratumoral STING agonist trials (intratumoral ulevostinag, SYNB1891), the intratumoral oncolytic virus work (T-VEC), and the historical Coley protocol's emphasis on peri-tumoral and intratumoral injection.

This delivery-based approach has the practical limitation of requiring tumors that are accessible to intratumoral injection — superficial cutaneous lesions, subcutaneous nodules, lymph nodes amenable to image-guided injection, accessible visceral lesions

under image guidance. For tumors that are diffuse, micrometastatic, or located in inaccessible sites (deep lung lesions, brain metastases, bone metastases), intratumoral delivery is infeasible. Innovation in delivery technology — including antibody-drug conjugates designed to deliver STING agonists or other immune activators specifically to tumor sites (Wu et al., *PNAS* 2022) — is an active area aimed at solving this limitation.

What current trials are not testing

The combinatorial completeness gap in Mode Three is identifiable. Most current trials address Mode Three through a single intervention category: anti-VEGF plus checkpoint inhibitor (the IMbrave150 paradigm), oncolytic virus plus checkpoint inhibitor (the MASTERKEY-265 paradigm), or intratumoral immune activation plus checkpoint inhibitor (the in situ vaccination paradigm).

Combinations that engage Mode Three through multiple mechanisms simultaneously — vascular normalization plus stromal disruption plus intratumoral delivery — are not being systematically tested. The combinatorial framework predicts that such multi-mechanism Mode Three engagement, combined with engagement of Modes One, Two, Four, and beyond, will produce outcomes qualitatively different from current single-mechanism Mode Three approaches.

The intersection of Mode Three with the historical Coley protocol is partial. Coley delivered intratumorally and peritumorally where possible (the historical case records describe injection into accessible tumors and adjacent tissues), achieving the delivery-based bypass of Mode Three. The systemic febrile response Coley induced may also have contributed to vascular normalization through indirect mechanisms — sustained inflammation alters tumor vasculature in ways that have not been systematically characterized in modern Coley-equivalent protocols. The framework's scoring methodology treats the historical Coley protocol as scoring partially on Mode Three engagement through these mechanisms, with significant residual gap relative to a maximally combinatorial-complete protocol.

Coley framework engagement of Mode Three

The historical Coley protocol engages Mode Three through three mechanisms.

First, intratumoral and peritumoral delivery. Historical case records describe Coley injecting bacterial preparations directly into accessible tumors and into surrounding tissues, achieving local concentrations of bacterial PAMPs that would have been difficult to achieve through systemic dosing. This is the same delivery-based logic that drives modern intratumoral immunotherapy approaches.

Second, fever-mediated vascular effects. Sustained febrile response with whole-body temperatures of 39-40°C produces vasodilation, increased vascular permeability, and alterations in endothelial adhesion molecule expression that increase lymphocyte trafficking into peripheral tissues including tumors. The Repasky group has characterized the HSP90-alpha-4-integrin signaling pathway through which fever-range thermal stress promotes T cell trafficking to tumors. This effect is documented in modern

preclinical work and is consistent with the historical observation that Coley responses often emerged during and after sustained febrile periods.

Third, inflammation-driven tumor vasculature changes. Sustained systemic inflammation produces structural and functional changes in tumor vasculature that have not been systematically characterized in the modern era but are mechanistically consistent with the principle of inflammatory vasculature normalization observed in other contexts.

The framework's scoring methodology treats the historical Coley protocol as scoring partially on Mode Three. The Coley protocol does not directly address CAF-mediated exclusion or extracellular matrix architecture. A combinatorial-complete protocol extending the Coley framework would integrate stromal-modifying agents (anti-VEGF, anti-TGF-beta, FAP-targeted approaches) to address the Mode Three mechanisms the historical Coley protocol does not engage.

Gaps and open questions

Several questions emerge from this analysis that are relevant to the larger combinatorial framework.

First, the comparative effectiveness of different Mode Three interventions in different tumor types is not yet well established. The IMbrave150 result in HCC is dramatic, but the same atezolizumab-bevacizumab combination has produced more modest results in other cancers. Tumor-specific stromal biology likely governs which Mode Three intervention is most effective; this remains underexplored.

Second, the relationship between Mode Three and Mode One is bidirectional. Hypoxia in poorly vascularized tumor regions downregulates MHC-I expression on tumor cells, linking Mode Three to Mode One mechanistically. Conversely, T cell infiltration of tumors (Mode Three resolution) often triggers IFN-gamma-driven MHC-I restoration (Mode One resolution). The bidirectional interaction means that combinatorial protocols engaging both modes may produce synergistic rather than merely additive effects.

Third, the optimal sequencing of Mode Three interventions relative to other modes is poorly characterized. The IMbrave150 approach administers both atezolizumab and bevacizumab continuously from the start; alternative sequencing approaches (anti-VEGF priming followed by checkpoint blockade, intratumoral delivery before systemic agents) have not been systematically compared.

Fourth, the question of whether the historical Coley protocol's vascular and immunological effects extend to deep visceral tumors that cannot be directly injected is empirically open. The cases in the Helen Coley Nauts retrospective span tumors that were accessible and tumors that were not; differential outcomes by accessibility have not been systematically analyzed.

Implications for the combinatorial framework

Mode Three is, like Modes One and Two, addressable with multiple contemporary interventions, including some with strong clinical validation (atezolizumab plus beva-

cizumab in HCC, T-VEC in melanoma). The clinical signal that emerges across this literature is consistent: Mode Three interventions combined with Mode Four blockade produce qualitatively different outcomes than either alone in tumor types where Mode Three is dominant.

The framework's combinatorial completeness argument predicts that protocols engaging Mode Three through multiple mechanisms simultaneously — vascular normalization, stromal disruption, intratumoral delivery — alongside engagement of Modes One, Two, Four, and beyond, will produce outcomes qualitatively different from current single-mechanism Mode Three approaches. The Hammerich 2019 lymphoma ISV result, the IMbrave150 HCC result, and the historical Coley case series each provide a partial validation of this prediction at different scales and in different tumor types.

The next section addresses Mode Four: T cell exhaustion within the tumor microenvironment. This is the mode best addressed by current immunotherapy through checkpoint inhibitors, and the area where Coley does the least directly.

5. Mode Four: T cell exhaustion in the tumor microenvironment

The problem

Even when tumor antigens are presented (Mode One addressed), even when T cells are properly primed by dendritic cells (Mode Two addressed), and even when T cells physically infiltrate the tumor (Mode Three addressed), the anti-tumor response often fails because tumor-infiltrating T cells progressively lose their effector function under chronic antigen stimulation. This functional decline — T cell exhaustion — is the most thoroughly characterized failure mode in cancer immunology and the area where contemporary immunotherapy has had its largest therapeutic breakthroughs.

The clinical magnitude of Mode Four as both a problem and a solution is substantial. Checkpoint inhibitors targeting the PD-1/PD-L1 and CTLA-4 axes — the molecular markers and effectors of T cell exhaustion — have transformed outcomes in melanoma, non-small-cell lung cancer with high PD-L1 expression or high tumor mutational burden, microsatellite-instability-high colorectal cancer, renal cell carcinoma, urothelial carcinoma, Hodgkin lymphoma, head and neck squamous cell carcinoma, and increasing numbers of other tumor types. The James P. Allison and Tasuku Honjo 2018 Nobel Prize recognized this paradigm shift.

However, the limits of single-agent checkpoint blockade have become equally clear. Across solid tumors, single-agent anti-PD-1 produces objective responses in 15-40 percent of patients depending on tumor type and patient selection, and complete responses are uncommon as a proportion of all treated patients. The substantial fraction of patients with cold tumors (Mode Three operative) or with antigen presentation defects (Mode One operative) or with insufficient T cell priming (Mode Two operative) do not benefit from Mode Four blockade alone. This is precisely the combinatorial completeness pattern the framework predicts.

Mode Four is distinct from Modes One through Three in that it concerns the func-

tional state of T cells that have successfully completed the prior steps (antigen recognition, priming, infiltration). The Coley framework engages Mode Four almost not at all through its classical mechanisms — this is the area where the framework's structural completeness is most clearly inadequate and where combinatorial extension to contemporary checkpoint inhibitors is mechanistically obligatory.

The mechanisms

T cell exhaustion is a distinct functional state acquired by T cells under chronic antigen exposure, characterized by progressive loss of effector function, sustained expression of multiple inhibitory receptors, distinct transcriptional and epigenetic profiles, and altered metabolism. The mechanistic understanding has matured substantially in the past decade.

Inhibitory receptor expression. Exhausted T cells express elevated and sustained levels of multiple inhibitory checkpoint molecules including programmed cell death protein 1 (PD-1, encoded by PDCD1), cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), lymphocyte activation gene 3 (LAG-3), T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), T cell immunoreceptor with Ig and ITIM domains (TIGIT), and others. Each inhibitory receptor engages distinct ligand pathways: PD-1 binds PD-L1 and PD-L2 expressed on tumor cells and antigen-presenting cells; CTLA-4 outcompetes CD28 for B7-1/B7-2 binding on APCs; LAG-3 binds MHC class II and other ligands; TIM-3 binds galectin-9, phosphatidylserine, and other ligands; TIGIT binds CD155 and CD112. The simultaneous engagement of multiple inhibitory receptors compounds T cell dysfunction.

Transcriptional regulation. Recent work has identified the transcription factor TOX (thymocyte selection-associated high mobility group box) as a master regulator of T cell exhaustion. TOX expression is upregulated in tumor-infiltrating CD8+ T cells, positively regulates PD-1, TIM-3, TIGIT, and CTLA-4 expression, and its level predicts overall survival and anti-PD-1 response in human melanoma and NSCLC. Other key transcription factors include NFAT, NR4A, Blimp-1, BATF, and altered patterns of T-bet and TCF1 expression. The TCF1+ "progenitor exhausted" subset versus the terminally exhausted CD8+ T cell subset is the key cellular distinction: progenitor exhausted cells retain capacity for reinvigoration by checkpoint blockade, while terminally exhausted cells do not.

Epigenetic remodeling. Exhausted T cells acquire distinct chromatin accessibility profiles that limit their capacity to re-express effector genes even when inhibitory checkpoints are blocked. This epigenetic fixation explains in part why checkpoint inhibitors can produce dramatic responses in some patients but fail completely in others — the exhaustion state may be reversible (in progenitor exhausted populations) or fixed (in terminally exhausted populations).

Metabolic dysfunction. Exhausted T cells show impaired mitochondrial function, reduced glycolytic capacity, and altered amino acid metabolism. The metabolic constraints of the tumor microenvironment (lactate accumulation, glucose deprivation, hypoxia) compound the intrinsic metabolic dysfunction of exhausted T cells.

Suppression by regulatory T cells (Tregs) and other immunosuppressive

populations. Foxp3+ Tregs, myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs) actively maintain the exhaustion state through cytokine production (IL-10, TGF-beta), metabolite production (adenosine, kynurenines), and direct cell-cell interactions. This mechanistic overlap with Mode Five (active immune suppression by the TME) means that interventions targeting Mode Five may also partially restore Mode Four function.

The contemporary interventions

Four intervention categories address Mode Four, with progressively increasing evidence of clinical benefit but also of mechanistic and clinical limits.

Anti-PD-1 and anti-PD-L1 antibodies

This is the most successful class of cancer immunotherapy in the modern era. The mechanism is direct: blocking PD-1 (on T cells) or PD-L1 (on tumor cells and APCs) prevents the inhibitory signal that arises when PD-1 binds its ligands. Multiple agents are FDA approved across multiple tumor types.

Pembrolizumab (Keytruda, Merck) received initial FDA approval in September 2014 for metastatic melanoma after progression on ipilimumab. Subsequent approvals have extended pembrolizumab to dozens of indications including NSCLC, head and neck squamous cell carcinoma, classical Hodgkin lymphoma, urothelial carcinoma, microsatellite-instability-high or mismatch-repair-deficient solid tumors (a tissue-agnostic indication), gastric cancer, cervical cancer, hepatocellular carcinoma, Merkel cell carcinoma, renal cell carcinoma, esophageal cancer, endometrial cancer, triple-negative breast cancer with PD-L1 expression, and others.

Nivolumab (Opdivo, Bristol-Myers Squibb) received initial FDA approval in December 2014, similarly initially for metastatic melanoma after ipilimumab. Subsequent indications parallel pembrolizumab's expansion across multiple tumor types.

Atezolizumab (Tecentriq, Genentech/Roche), **durvalumab (Imfinzi, AstraZeneca)**, and **avelumab (Bavencio, EMD Serono/Pfizer)** are anti-PD-L1 antibodies with multiple approved indications. **Cemiplimab (Libtayo, Regeneron)** is another anti-PD-1 with approvals in cutaneous squamous cell carcinoma and other indications.

The landmark trial comparing anti-PD-1 to prior standard of care in melanoma was KEYNOTE-006 (pembrolizumab vs ipilimumab in advanced melanoma), where 10-year follow-up showed an OS rate of 34.0% with pembrolizumab versus 23.6% with ipilimumab — a durable, plateau-pattern survival benefit unprecedented in melanoma history (KEYNOTE-006 10-year follow-up, *Annals of Oncology* 2024).

Anti-CTLA-4 antibodies

Ipilimumab (Yervoy, Bristol-Myers Squibb) was the first checkpoint inhibitor to demonstrate overall survival benefit in any cancer, with FDA approval in March 2011 based on the MDX010-20 trial in metastatic melanoma (Hodi FS et al., *NEJM*

2010;363:711-723). The mechanism is distinct from anti-PD-1: CTLA-4 blockade primarily affects T cell priming in lymphoid tissues rather than directly reversing exhaustion in the tumor. The combination of ipilimumab with anti-PD-1 produces additive and partially distinct effects.

The most important contemporary checkpoint combination trial is CheckMate 067 (nivolumab plus ipilimumab vs nivolumab vs ipilimumab in advanced melanoma; NCT01844505; Larkin J et al., *NEJM* 2015;373:23-34, with subsequent OS updates by Wolchok JD et al., *NEJM* 2017;377:1345-1356 and most recently the 10-year final analysis Wolchok JD et al., *NEJM* 2024). The combination produces superior outcomes to either single agent: at the 10-year final analysis, median overall survival was 71.9 months with the combination, 36.9 months with nivolumab alone, and 19.9 months with ipilimumab alone, with corresponding 10-year melanoma-specific survival rates of 52%, 44%, and 23%. The combination achieves higher response rates and longer survival but at substantially increased immune-related toxicity (grade 3/4 treatment-related adverse events in 63% of combination patients vs 25% nivolumab vs 30% ipilimumab). The combination is now standard for advanced melanoma in patients who can tolerate it.

The CheckMate 067 result is among the strongest demonstrations of combinatorial benefit within Mode Four — combining two distinct anti-exhaustion mechanisms produces qualitatively better outcomes than either alone. The framework's combinatorial completeness argument receives strong support from this within-Mode-Four pattern.

Anti-LAG-3 antibodies

Relatlimab (Opdualag, Bristol-Myers Squibb) is a first-in-class anti-LAG-3 antibody administered as a fixed-dose combination with nivolumab. FDA approval came in March 2022 based on the RELATIVITY-047 trial (Tawbi HA, Schadendorf D, Lipson EJ, et al., *NEJM* 2022;386:24-34, NCT03470922).

RELATIVITY-047 enrolled 714 patients with untreated unresectable or metastatic melanoma, randomized to fixed-dose relatlimab plus nivolumab vs nivolumab alone, every 4 weeks. The trial met its primary endpoint of progression-free survival with median PFS of 10.1 vs 4.6 months (combination vs nivolumab alone). At 3-year follow-up the OS hazard ratio 95% CI upper bound became <1, supporting the durability of benefit. The combination became the first checkpoint combination to demonstrate superiority to anti-PD-1 monotherapy in a phase III trial of advanced melanoma.

The picture has become more nuanced with subsequent data. **RELATIVITY-098** (adjuvant relatlimab + nivolumab vs nivolumab in resected stage III/IV melanoma; reported at ASCO 2025) showed no improvement in recurrence-free survival with the combination over nivolumab alone (HR 1.01, P=0.928). Correlative analyses suggested the absence of tumor-infiltrating LAG-3+ T cells in the adjuvant setting as a potential mechanistic reason — the population the LAG-3 blockade can act on may not be present at sufficient frequency in the resected setting. This contrast between metastatic (positive trial) and adjuvant (negative trial) settings illustrates the importance of mechanistic targeting: the intervention works where the target is present.

Anti-TIGIT antibodies

Tiragolumab (Roche) and several other anti-TIGIT antibodies have been in extensive clinical development. The phase II CITYSCAPE trial in PD-L1-positive NSCLC produced encouraging signals, leading to multiple phase III trials. The results have been overwhelmingly disappointing:

- **SKYSCRAPER-01** (tiragolumab + atezolizumab vs atezolizumab in PD-L1-high NSCLC): primary endpoint OS not met at final analysis (announced 2025)
- **SKYSCRAPER-02** (tiragolumab + atezolizumab + carboplatin-etoposide vs placebo + atezolizumab-CE in ES-SCLC): did not meet PFS or OS coprimary endpoints (Rudin et al., *JCO* 2024)
- **SKYSCRAPER-03** (consolidation tiragolumab + atezolizumab vs durvalumab in stage III NSCLC): negative for PFS in all-comers and PD-L1+ subgroup (ESMO 2025)
- **SKYSCRAPER-06** (tiragolumab + atezolizumab vs pembrolizumab + chemotherapy in first-line nonsquamous NSCLC): stopped early — PFS HR 1.27, OS HR 1.33 favoring the comparator arm (2024)

The anti-TIGIT failures across multiple phase III settings represent an important negative result for the simple "more checkpoint inhibitor combinations should produce more benefit" thinking. The mechanistic reasons remain under investigation. The TIGIT story illustrates that combinatorial extension within Mode Four does not always produce additive benefit — the mechanistic biology of the specific inhibitory receptor pathway matters.

The pattern: where Mode Four works and where it does not

The contemporary clinical evidence base supports a structural pattern.

Mode Four interventions work dramatically when the prior modes are intact. Melanoma is the archetypal responder cancer: high mutational burden (intact Mode One), often hot tumors (Mode Three not dominant), and frequently pre-existing T cell infiltration (Mode Two functionally present). Microsatellite-instability-high colorectal cancer follows the same pattern. NSCLC with high PD-L1 expression and high TMB follows the pattern. In these settings, single-agent anti-PD-1 produces durable responses in a substantial fraction of patients, and combinations (anti-PD-1 + anti-CTLA-4, anti-PD-1 + anti-LAG-3) extend this benefit further.

Mode Four interventions fail when prior modes are not addressed. Microsatellite-stable colorectal cancer, prostate cancer, pancreatic cancer, and most triple-negative breast cancer (in the absence of high PD-L1 or PD-L1 combined positive score) are checkpoint-inhibitor-refractory because Modes One, Two, and/or Three are not adequately addressed. Adding more Mode Four blockade (TIGIT, etc.) does not solve this problem — as the SKYSCRAPER series demonstrates clinically.

This pattern is precisely the combinatorial completeness prediction the framework makes. Mode Four engagement is necessary but not sufficient. Maximally effective immunotherapy requires engaging Modes One through Three to make Mode Four interventions actionable.

Coley framework engagement of Mode Four

The historical Coley protocol does not directly engage Mode Four through its classical mechanisms. There was no anti-PD-1, no anti-CTLA-4, no targeted checkpoint blockade in Coley's era — the entire molecular biology of T cell exhaustion was unknown.

However, the inflammatory environment Coley creates may indirectly reduce the development of T cell exhaustion through multiple pathways. Sustained inflammation reduces TGF-beta dominance in the tumor microenvironment, which may slow exhaustion-program acquisition. The strong IFN-gamma signaling from PAMP-induced innate immune activation produces effector-favorable transcriptional programs. The fever-range thermal stress may transiently disrupt the metabolic and signaling features of the exhaustion program.

These indirect effects are mechanistically plausible but not directly demonstrated in modern Coley-equivalent protocols. The framework's scoring methodology treats the historical Coley protocol as scoring minimally on Mode Four engagement — perhaps a score of 0-1 on the 0-1 scale, depending on how strongly one interprets the indirect mechanisms.

The structural consequence is direct: any combinatorial-complete extension of the Coley framework must include checkpoint blockade. The mechanistic reasoning is unambiguous. The Coley protocol engages Modes 1, 2, and partially 5 and 6 strongly, but leaves Mode 4 essentially unaddressed. The contemporary checkpoint inhibitors fill exactly this gap with strong clinical evidence of benefit.

This is not a criticism of the historical protocol — Coley's discoveries predate the entire molecular framework that makes Mode Four addressable. It is a structural observation about how a contemporary integration of Coley's logic with current immunotherapy must be constructed.

What current trials are not testing

The combinatorial completeness gap in contemporary Mode Four research has a specific shape. Trials testing checkpoint inhibitor combinations within Mode Four (anti-PD-1 + anti-CTLA-4, anti-PD-1 + anti-LAG-3, anti-PD-1 + anti-TIGIT) are extensive but rarely combined with interventions addressing the other failure modes systematically.

For tumor types where Modes One, Two, and Three are dominant (pancreatic, MSS colorectal, prostate, much of TNBC), the structural framework predicts that checkpoint inhibitor combinations alone will continue to fail — as the empirical record shows they do. The clinical trials needed would combine checkpoint blockade with:

- Mode One restoration (IFN-γ, HDACi, fever-range thermal stress, immunogenic chemotherapy)
- Mode Two enhancement (FLT3L, TLR agonists, STING agonists, in situ vaccination)
- Mode Three disruption (anti-VEGF, oncolytic viruses, stromal-modifying agents, intratumoral delivery)

- Mode Four blockade (anti-PD-1 plus additional checkpoint blockade as appropriate)

This is the structural protocol pattern the framework recommends. No current trial implements such a multi-mode combinatorial protocol systematically. The Hammerich 2019 lymphoma ISV trial comes closest by addressing Modes 1, 2, 3 (intratumoral delivery), and converting Mode 4 from refractory to responsive — and produces correspondingly strong outcomes.

Gaps and open questions

Several questions emerge from this analysis relevant to the larger combinatorial framework.

First, the question of why checkpoint inhibitor combinations within Mode Four sometimes produce additive benefit (anti-PD-1 + anti-CTLA-4 in melanoma, anti-PD-1 + anti-LAG-3 in metastatic but not adjuvant melanoma) and sometimes do not (anti-PD-1 + anti-TIGIT across multiple settings) is not fully understood. The mechanistic biology of specific inhibitory receptors and their interactions in different tumor types matters in ways that the simple "block more checkpoints" model does not capture.

Second, the optimal sequencing of Mode Four blockade relative to Mode One, Two, and Three interventions is poorly characterized. Should antigen presentation be restored before checkpoint blockade is administered? Should DC priming be amplified before? Should stromal disruption precede checkpoint engagement? Current combination trials typically administer all components simultaneously or in standard sequences not optimized for biological plausibility.

Third, the question of patient selection for combinatorial protocols is unresolved. Biomarkers identifying which patients have which failure modes operative remain incomplete. Tumor mutational burden, PD-L1 expression, MSI status, neoantigen prediction, T cell infiltration density, and CAF density are all partial biomarkers; an integrated multi-mode biomarker panel does not exist.

Fourth, the extension of within-Mode-Four combinatorial successes (CheckMate 067 in melanoma) to other tumor types has been mixed at best. The TIGIT failures suggest that simple within-mode combinatorial extension is not a reliable strategy. Cross-mode combinatorial protocols may be necessary precisely because within-mode extension has hit its limits.

Implications for the combinatorial framework

Mode Four is the area of cancer immunotherapy where the modern field has invested the most resources and produced its largest successes. The contemporary intervention toolbox is rich (multiple FDA-approved checkpoint inhibitors across PD-1, CTLA-4, and LAG-3 targets) and the mechanistic understanding is detailed.

The framework's central argument receives its clearest support from the pattern that emerges across cancer types: where Modes One through Three are favorable (melanoma, MSI-high CRC, high-TMB NSCLC), Mode Four interventions produce dramatic benefits; where Modes One through Three are unfavorable (MSS CRC, pancre-

atic, most TNBC), Mode Four interventions fail. This is the combinatorial completeness pattern observed clinically.

The structural prediction from the framework is that the next generation of cancer immunotherapy breakthroughs will come from cross-mode combinatorial protocols — combining Mode Four blockade with simultaneous engagement of Modes One, Two, and Three — rather than from continued within-Mode-Four combinatorial extension (which has hit its limits in the TIGIT failures). The Coley framework, with its strong engagement of Modes One and Two and partial engagement of Modes Three, Five, and Six, provides a structural template for what combinatorial completeness can look like when adapted with contemporary Mode Four interventions added.

The next section addresses Mode Five: active immune suppression by the tumor microenvironment, which overlaps mechanistically with Mode Four in ways that affect protocol design.

6. Mode Five: Active immune suppression by the tumor microenvironment

The problem

The tumor microenvironment (TME) is not a passive setting in which immune cells fail to function — it is an actively immunosuppressive ecosystem that recruits, educates, and sustains cell populations and metabolic conditions specifically designed (by evolutionary selection during tumor progression) to suppress anti-tumor immunity. Even when antigens are presented (Mode One addressed), T cells are primed (Mode Two), T cells physically infiltrate the tumor (Mode Three), and T cell exhaustion is reversed by checkpoint blockade (Mode Four), Mode Five suppression can prevent productive anti-tumor immunity through parallel mechanisms.

Mode Five overlaps mechanistically with Mode Four — the suppressive cell populations and metabolites that constitute Mode Five are major drivers of the exhaustion program characterizing Mode Four — but offers a distinct intervention surface. Where Mode Four interventions target the receptor-level checkpoints on exhausted T cells, Mode Five interventions target the upstream suppressive populations and metabolic conditions that produce and maintain the exhaustion state.

The clinical significance of Mode Five is substantial. The tumor types most resistant to checkpoint blockade — pancreatic cancer, microsatellite-stable colorectal cancer, prostate cancer, glioblastoma, much of triple-negative breast cancer — are characterized by particularly intense Mode Five suppression, often involving high regulatory T cell (Treg) infiltration, dense myeloid-derived suppressor cell (MDSC) populations, M2-polarized tumor-associated macrophages (TAMs), and adenosine-rich, hypoxic, lactate-rich metabolic conditions.

The Coley framework engages Mode Five partially through the systemic inflammatory environment it creates, which is unfavorable to Treg and MDSC accumulation but does not directly address all the specific mechanisms. The contemporary intervention landscape for Mode Five is rich in mechanistic possibilities but has been character-

ized by frequent clinical translation failures, illustrating how mechanistically rational targets do not always produce clinical benefit.

The mechanisms

Mode Five operates through five major mechanism categories that often co-occur and reinforce each other.

Regulatory T cells (Tregs). CD4+ CD25+ FoxP3+ regulatory T cells are essential for normal immune homeostasis but are recruited to tumors at high frequencies and actively suppress anti-tumor effector responses. Tregs in tumors suppress through multiple mechanisms: secretion of IL-10 and TGF-beta, expression of inhibitory receptors (CTLA-4 constitutively, also LAG-3, PD-1, TIGIT), CD25-mediated IL-2 depletion (depriving effector T cells of survival signals), and direct cytotoxicity against effector cells. The discovery of this T cell population proceeded in stages: Sakaguchi and colleagues identified CD25 (the IL-2 receptor alpha chain) as the first reliable marker enabling isolation of suppressive CD4+ T cells in 1995 (Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M, *J Immunol* 1995;155:1151-1164); Brunkow, Ramsdell and colleagues identified Foxp3 as the gene mutated in the scurfy autoimmune phenotype in 2001; and in 2003 three independent groups (Sakaguchi/Hori, Ramsdell, Rudensky) established FoxP3 as the master transcription factor specifying Treg lineage identity. This work was recognized with the 2025 Nobel Prize in Physiology or Medicine awarded jointly to Mary E. Brunkow, Fred Ramsdell, and Shimon Sakaguchi "for their discoveries concerning peripheral immune tolerance." Subsequent work has established the Treg population as a major driver of immune suppression across virtually all human cancers (reviewed in Tanaka A, Sakaguchi S, *Cell Research* 2017;27(1):109-118).

Myeloid-derived suppressor cells (MDSCs). MDSCs are immature myeloid cells that expand in response to tumor-derived factors and accumulate in tumor sites, lymphoid tissues, and peripheral blood. Two major subsets exist (granulocytic/polymorphonuclear MDSCs and monocytic MDSCs), each with distinct biology but converging on T cell suppression through arginase production (depleting arginine required for T cell function), reactive oxygen and nitrogen species, expression of inhibitory ligands, and production of immunosuppressive cytokines. MDSC accumulation correlates with poor prognosis across cancer types and with resistance to immunotherapy.

Tumor-associated macrophages (TAMs). Macrophages in tumors can adopt M1 (pro-inflammatory, anti-tumor) or M2 (anti-inflammatory, pro-tumor) polarization states. The tumor microenvironment typically educates infiltrating macrophages toward M2 polarization through cytokine signals including IL-4, IL-10, IL-13, and CSF-1. M2 TAMs produce immunosuppressive cytokines, support angiogenesis (linking Mode Five to Mode Three), express PD-L1 and other inhibitory ligands, and promote stromal remodeling that favors tumor growth. TAMs are typically the most abundant immune cell type in solid tumor microenvironments, often outnumbering T cells substantially.

Adenosine metabolism (CD39/CD73 axis). Extracellular adenosine produced through the sequential action of CD39 (converting ATP to AMP) and CD73 (con-

verting AMP to adenosine) is a potent immunosuppressive metabolite. Adenosine binding to A2A receptors on T cells, NK cells, and other immune cells suppresses effector function and promotes the development of suppressive phenotypes. Tumor cells, Tregs, and other TME cells express high levels of CD39 and CD73, creating an adenosine-rich microenvironment that broadly suppresses immune attack. CD73 has been characterized as a "novel checkpoint" target with multiple clinical-stage inhibitors in development.

Tryptophan metabolism (IDO/TDO). Indoleamine 2,3-dioxygenase 1 (IDO1) and tryptophan 2,3-dioxygenase (TDO) degrade tryptophan to kynurenine. Tryptophan depletion impairs T cell function (T cells are particularly tryptophan-dependent), and kynurenine itself binds the aryl hydrocarbon receptor on T cells, promoting Treg differentiation and effector T cell suppression. IDO1 is upregulated in many tumors and was a major immunotherapy target in the 2010s, with results discussed below.

Other metabolic suppression. The tumor microenvironment is characterized by glucose depletion (tumors are voracious glucose consumers, competing with T cells), lactate accumulation (Warburg-effect product that directly suppresses T cell function), hypoxia (driving HIF-1alpha-mediated immunosuppression), and amino acid depletion beyond tryptophan (arginine and glutamine also affected). Each metabolic stress contributes to Mode Five suppression and creates intervention opportunities.

The contemporary interventions

Five intervention categories address Mode Five, with strikingly heterogeneous translational track records.

Treg-targeted approaches

Direct depletion of Tregs has been attempted through multiple approaches: anti-CD25 antibodies (early generation versions including daclizumab and basiliximab were too non-selective, depleting effector T cells; newer Fc-engineered versions are being tested), Treg-specific bispecifics targeting CCR4 or CCR8 (chemokine receptors preferentially expressed on tumor-infiltrating Tregs), low-dose cyclophosphamide (preferentially depletes Tregs through differential sensitivity), and CTLA-4 blockade (ipilimumab has Treg-depleting activity in addition to T cell-priming effects).

The mogamulizumab approach (anti-CCR4 antibody Poteligeo, Kyowa Kirin, FDA-approved August 8, 2018 for relapsed or refractory cutaneous T cell lymphoma based on the MAVORIC phase III trial) provides clinical proof of principle for selective Treg targeting — mogamulizumab depletes both malignant CCR4+ T cells and CCR4+ Tregs through ADCC — but has not yet been established as a broadly applicable cancer immunotherapy. Multiple anti-CCR8 antibodies are in early clinical development with preliminary signals of selective intratumoral Treg depletion.

The structural challenge with Treg-targeted approaches is that systemic Treg depletion risks autoimmune toxicity, while selective intratumoral Treg targeting is technically demanding. The clinical evidence base remains limited compared to checkpoint inhibitors.

MDSC-targeted approaches

MDSC modulation has been attempted through multiple mechanisms: differentiation-inducing agents (all-trans retinoic acid promotes MDSC differentiation into mature, non-suppressive cells), MDSC recruitment inhibition (CCR5 and CXCR2 inhibitors), and direct cytotoxic targeting of MDSCs through ADCs or selective small molecules. No MDSC-targeted therapy has yet achieved regulatory approval specifically for the indication of MDSC depletion in cancer.

The mechanistic rationale is strong; the translational track record is sparse. MDSC heterogeneity, the difficulty of in vivo phenotypic discrimination from healthy myeloid populations, and the redundancy of suppression mechanisms have all contributed to the slow translational progress.

CSF1R inhibitors (macrophage repolarization)

The colony-stimulating factor 1 receptor (CSF1R) is essential for monocyte/macrophage survival and is a major target for TAM modulation. CSF1R inhibition reduces M2 TAM populations and can repolarize remaining macrophages toward M1 phenotypes, in theory improving immune function in the tumor.

The translational record has been mixed at best. **Pexidartinib (PLX3397, Daiichi Sankyo)** is FDA-approved (August 2, 2019) but only for tenosynovial giant cell tumor — a rare, locally aggressive non-malignant condition driven by aberrant CSF1 signaling, not for cancer immunotherapy. **Cabiralizumab (FPA008)** combination trials with nivolumab in pancreatic cancer have been disappointing, with multiple phase II trials terminated due to insufficient efficacy or safety issues (NCT02452424, NCT02880371, NCT03697564 suspended). The Five Prime Therapeutics cabiralizumab program was acquired by Amgen. No phase III CSF1R inhibitor combination with checkpoint blockade has been launched for cancer immunotherapy as of the time of this writing.

The disconnect between strong mechanistic rationale and poor clinical results illustrates a recurrent pattern in Mode Five interventions: targeting one suppressive mechanism in isolation may be insufficient when multiple redundant suppression mechanisms operate in parallel.

Adenosine pathway inhibitors

The adenosine pathway has multiple clinical-stage inhibitors targeting CD73 (oleclumab, MEDI9447), A2A receptor (ciforadenant, AZD4635, taminadenant), CD39, and combinations. **Oleclumab** (MedImmune/AstraZeneca, an anti-CD73 IgG1 monoclonal antibody) is the most advanced CD73-targeted agent.

The phase II COAST trial provided the most encouraging signal: in 189 patients with unresectable stage III non-small-cell lung cancer who had not progressed after chemoradiotherapy, randomized 1:1:1, consolidative durvalumab plus oleclumab produced PFS hazard ratio 0.44 compared to durvalumab alone, with similar PFS benefit for durvalumab plus monalizumab (anti-NKG2A; HR 0.42). The objective response

rate was 30.0% (D+O), 35.5% (D+M), vs 17.9% (durvalumab alone), with comparable adverse events across arms (Herbst RS, Majem M, Barlesi F, et al., *J Clin Oncol* 2022;40:3383-3393, doi:https://doi.org/10.1200/JCO.22.00227, NCT03822351). This led to the phase III PACIFIC-9 trial (NCT05221840, approximately 999 patients planned), currently ongoing.

The first-in-human phase I trial of oleclumab in advanced solid tumors enrolled 192 patients across colorectal, pancreatic, and EGFR-mutant NSCLC cohorts, with evidence of antitumor activity in tumor types generally resistant to immunotherapy (Bendell J, LoRusso P, Overman M, et al., *Cancer Immunology, Immunotherapy* 2023, doi:https://doi.org/10.1007/s00262-023-03430-6, NCT02503774). Eighteen CD73 inhibitors are reported in clinical trials as of 2024. The translational outlook for this pathway is cautiously positive — the COAST signal is real and PACIFIC-9 will be informative.

IDO inhibitors

The IDO1 inhibitor story is the cautionary tale of Mode Five clinical translation. **Epacadostat** (Incyte, IDO1 inhibitor) showed promising phase I/II results in combination with pembrolizumab (ECHO-202/KEYNOTE-037 in melanoma: 55% objective response rate in 53 untreated patients including 7 complete responses, presented ESMO 2017). The combination advanced to the pivotal phase III ECHO-301/KEYNOTE-252 trial (NCT02752074) — a 706-patient randomized double-blind study of pembrolizumab + epacadostat 100 mg BID vs pembrolizumab + placebo in untreated advanced melanoma.

The trial failed completely. Median PFS was 4.7 months with the combination vs 4.9 months with pembrolizumab alone — essentially identical. The trial was stopped early when the data monitoring committee determined the primary PFS endpoint had not been met and the OS coprimary was unlikely to reach statistical significance (Long GV, Dummer R, Hamid O, et al., *Lancet Oncology* 2019;20(8):1083-1097).

The ECHO-301 failure has been extensively analyzed. Proposed explanations include: (1) inadequate epacadostat dosing — the 100 mg BID dose may not produce sufficient pharmacodynamic IDO1 inhibition (kynurenine reduction reached only 50% at this dose, with maximum suppression requiring 400 mg BID); (2) the additional role of TDO that selective IDO1 blockade does not address; (3) misalignment between IDO1 expression levels in the early-phase responder population and the broader phase III population; (4) the possibility that IDO1 is not actually a rate-limiting mechanism in many advanced melanoma patients. The IDO pathway is broadly considered to remain mechanistically interesting; the clinical development pathway has been substantially set back by ECHO-301.

The pattern: mechanistic richness with limited clinical translation

The contemporary clinical evidence base for Mode Five interventions reveals a striking pattern: extensive mechanistic understanding has not consistently translated into clinical benefit. CSF1R inhibitor combinations have failed across multiple settings. IDO inhibitor combinations failed dramatically in ECHO-301. MDSC-targeted thera-

pies have not advanced to regulatory approval. TGF-beta inhibitor combinations have repeatedly failed (bintrafusp alfa discussed in Mode Three).

The exceptions where Mode Five engagement has produced clear clinical benefit are notable for being combinatorial in mechanism: ipilimumab (anti-CTLA-4) has both Treg-depleting and T-cell-priming effects, making it simultaneously a Mode 4 and Mode 5 intervention; bevacizumab plus atezolizumab in HCC (IMbrave150) addresses Mode 3 (vascular normalization) and partially Mode 5 (reduced MDSC recruitment and VEGF-mediated suppression); the COAST oleclumab signal in NSCLC pairs Mode 5 adenosine pathway with Mode 4 checkpoint blockade. The pattern suggests Mode 5 interventions work clinically when they are part of broader combinatorial protocols, not as monotherapy add-ons.

The structural interpretation aligns with the framework's combinatorial completeness argument. Mode 5 mechanisms are redundant and overlapping. Blocking one suppressive mechanism (IDO, CSF1R, CD73 in isolation) is often insufficient because parallel mechanisms compensate. Effective Mode 5 engagement requires either combinatorial Mode 5 targeting (combining multiple suppressive mechanism inhibitors) or pairing Mode 5 with engagement of other modes that change the baseline immunological context.

Coley framework engagement of Mode Five

The historical Coley protocol engages Mode Five partially through several mechanisms.

The sustained pro-inflammatory cytokine signaling produced by bacterial PAMP exposure (IL-1, IL-6, IL-12, TNF-alpha, IFN-gamma) is unfavorable to Treg accumulation and Treg suppressive function. Tregs are particularly sensitive to inflammatory cytokine environments; the strong systemic inflammation Coley produces would be expected to reduce intratumoral Treg accumulation and function compared to the steady-state immunosuppressive microenvironment.

The MDSC population, similarly, is destabilized by sustained inflammatory cytokine signaling. The differentiation pressure toward mature myeloid phenotypes (under IFN-gamma in particular) opposes the MDSC accumulation pattern.

Macrophage polarization in inflammatory environments shifts toward M1 phenotype. The dominant IFN-gamma signaling Coley produces is a primary driver of M1 polarization, and the Coley-induced microenvironment would be expected to repolarize TAMs from M2 to M1.

Metabolic conditions in inflamed tissues differ from steady-state tumor microenvironment metabolism. Sustained febrile response increases tumor blood flow (reducing hypoxia), and the inflammatory metabolic program differs from the Warburg-dominated tumor metabolism.

These engagement mechanisms are mechanistically plausible but, like Mode Three engagement, not directly demonstrated in modern Coley-equivalent protocols. The framework's scoring methodology treats the historical Coley protocol as scoring partially on Mode Five — perhaps 0.5 on the 0-1 scale — with engagement through

indirect anti-Treg/anti-MDSC effects and macrophage repolarization but not direct engagement of adenosine pathway, IDO, or specific metabolic interventions.

What current trials are not testing

The combinatorial completeness gap in Mode Five research is identifiable. Most Mode 5 intervention trials test a single suppressive mechanism inhibitor in combination with checkpoint blockade. Trials that engage multiple Mode 5 mechanisms simultaneously, or that combine Mode 5 engagement with Mode 1, 2, and 3 interventions in addition to checkpoint blockade, are rare or absent.

The framework's structural prediction is that single-mechanism Mode 5 interventions will continue to fail as monotherapy add-ons (the IDO and CSF1R history) because the redundancy of Mode 5 suppression mechanisms requires multi-mechanism engagement. Multi-mode protocols that include Mode 5 engagement as one component of broader combinatorial completeness should fare better.

The contemporary in situ vaccination paradigm (Hammerich 2019 ISV in iNHL discussed in Mode 2) and the IMbrave150 paradigm (atezolizumab + bevacizumab in HCC discussed in Mode 3) provide partial validation. Both involve Mode 5 engagement (the inflammatory environment they create modulates Treg/MDSC/TAM populations) alongside engagement of other modes, and both produce qualitatively better outcomes than single-mode interventions.

Gaps and open questions

Several questions emerge that are relevant to the larger combinatorial framework.

First, the question of how to identify which Mode 5 mechanism is dominant in a given tumor remains poorly answered. Biomarkers for Treg infiltration density (Foxp3 staining), MDSC accumulation (granulocytic vs monocytic phenotyping), TAM density and polarization (CD68 vs CD163 staining), and adenosine pathway activity (CD73 expression) are individually established but rarely combined into integrated multi-mechanism biomarker panels. Personalized Mode 5 targeting based on which mechanisms are operative in a specific patient remains aspirational.

Second, the question of whether Mode 5 interventions can be more effectively delivered intratumorally — concentrating effect at the suppressive site while sparing systemic immune homeostasis — has been raised but not adequately tested. The in situ vaccination paradigm offers a template; extending it to systemically administered Mode 5 agents would be a logical next step.

Third, the optimal Mode 5 combinations for specific tumor types are unknown. Pancreatic cancer is characterized by particularly intense Mode 5 suppression (Tregs + MDSCs + M2 TAMs + adenosine) but the combinatorial trials needed to determine optimal multi-target Mode 5 engagement have not been designed.

Fourth, the temporal relationship between Mode 5 interventions and other interventions is poorly characterized. Should Mode 5 suppression be addressed before checkpoint blockade? Should it be addressed simultaneously with antigen restora-

tion (Mode 1) and DC priming (Mode 2)? The current trial design landscape addresses these questions inadequately.

Implications for the combinatorial framework

Mode Five is structurally challenging from a combinatorial perspective because of mechanism redundancy. Single Mode 5 interventions have repeatedly failed in pivotal trials (ECHO-301, multiple CSF1R combinations, bintrafusp alfa) even when mechanistic rationale was strong and early-phase signals were encouraging. The framework's combinatorial completeness argument predicts that Mode 5 will work clinically when integrated into broader multi-mode protocols rather than tested as isolated additions to existing standards of care.

The historical Coley protocol's partial Mode 5 engagement through pleiotropic inflammatory effects provides a structural template: broad inflammatory disruption of the suppressive microenvironment, rather than targeted single-mechanism Mode 5 inhibition, may be the more effective approach. Modern combinatorial protocols extending the Coley framework could engage Mode 5 through the pleiotropic inflammatory cytokine signaling the bacterial PAMP exposure provides, complemented by specific Mode 5 targeted agents (anti-CD73 in the adenosine pathway, where COAST suggests real clinical signal exists) where mechanistic targeting adds value.

The next section addresses Mode Six: insufficient persistence of immune response. Mode Six is the area where the historical Coley protocol's sustained dosing schedule was structurally different from modern single-dose checkpoint blockade approaches, and where contemporary IL-15 superagonists like ANKTIVA (already discussed in the framework paper's bladder cancer analysis) provide a direct mechanistic parallel.

7. Mode Six: Insufficient persistence of immune response

The problem

The previous five failure modes concern the *engagement* of anti-tumor immunity: presenting antigens (Mode One), priming T cells (Mode Two), enabling T cells to reach the tumor (Mode Three), reversing exhaustion (Mode Four), and disrupting active suppression (Mode Five). Mode Six addresses a structurally different problem — *temporal persistence*. Even when all engagement mechanisms function properly, an anti-tumor immune response that cannot maintain itself over the months and years required to control or eliminate disseminated disease will fail through attrition.

The clinical phenomenon is recognizable across treatment modalities. Initial responses to immunotherapy that progress after months of disease control. Adoptive cell therapy products whose anti-tumor activity correlates with detectable persistence in peripheral blood and disappears when persistence is lost. Cancer vaccines that produce measurable T cell responses on day 30 but undetectable responses at 12 months. Checkpoint inhibitor responses that are maintained while drug is being administered but recur after treatment cessation in subsets of patients.

Mode Six is conceptually distinct from Mode Four (T cell exhaustion). Exhausted T

cells exist but cannot function; non-persistent immunity is the absence of functional T cells altogether. The mechanisms differ: exhaustion is driven by chronic antigen stimulation and inhibitory receptor signaling, while persistence is driven by memory T cell biology, homeostatic cytokine support, and immune niche dynamics. The interventions differ correspondingly: Mode Four interventions reverse the exhaustion state in existing T cells, while Mode Six interventions either generate longer-lasting T cell populations or provide sustained cytokine support to maintain populations that have been generated.

The historical Coley protocol's structural distinctiveness on Mode Six is significant. Coley's mixed bacterial vaccine was administered repeatedly — typically weekly to several-weekly doses, often continued for many months and sometimes for years. This sustained dosing regimen is structurally different from the single-dose or short-course regimens that dominate modern immunotherapy. Whether this temporal pattern was mechanistically important or simply reflected the clinical convention of the era is debated, but the structural difference is a direct contrast worth examining.

The contemporary intervention landscape for Mode Six includes the recently FDA-approved IL-15 superagonist ANKTIVA (the first new cytokine approved for cancer in over 30 years), the long-established but limited high-dose IL-2 (FDA-approved 1992 for renal cell carcinoma, 1998 for melanoma), CAR-T cell therapies whose persistence biology is now understood as a key determinant of durable response, and several investigational approaches in IL-7, IL-15, and IL-21 biology.

The mechanisms

T cell memory and persistence biology has matured substantially over the past decade. The key conceptual framework distinguishes effector T cells (acutely activated, short-lived, high cytotoxic capacity) from memory T cell populations with progressively greater longevity and self-renewal capacity.

Memory T cell hierarchy. Human and murine memory CD8+ T cells exist as a developmental hierarchy: stem cell memory T cells (T_SCM_, least differentiated, greatest proliferative and self-renewal capacity, CD45RA+ CCR7+ CD95+), central memory T cells (T_CM_, CD45RA- CCR7+, capable of robust recall responses), effector memory T cells (T_EM_, CD45RA- CCR7-, more differentiated), and terminally differentiated effector memory cells (T_EMRA_). The stem-like populations are critical for sustained anti-tumor immunity because they maintain the proliferative reservoir from which effector populations are regenerated as previous effectors die off.

TCF1 and the stem-like population. The transcription factor TCF1 (encoded by Tcf7) marks the stem-like memory and stem-like exhausted (progenitor exhausted) T cell populations. TCF1+ cells have the self-renewal capacity to sustain anti-tumor responses; TCF1- cells are terminally differentiated and short-lived. The TCF1+ progenitor exhausted population is the target population for checkpoint blockade — these cells can be reinvigorated to produce new effector waves, while terminally exhausted TCF1- cells cannot. This mechanistic understanding emerged through the work of multiple groups in 2016-2020 and connects Mode Four and Mode Six in important ways.

Homeostatic cytokine support. Memory T cell maintenance depends critically on homeostatic cytokines, particularly IL-7 and IL-15. IL-7 supports survival and homeostatic proliferation of naive and memory T cells. IL-15 is particularly important for CD8+ memory T cell maintenance and NK cell function. Importantly, IL-15 differs from the structurally similar IL-2 in not supporting regulatory T cells — IL-15 promotes effector and memory T cells without expanding Tregs, while IL-2 (which shares the IL-2/15 β and γ chains in its receptor) does both.

The IL-2 versus IL-15 distinction. IL-2 was discovered first and translated to clinical use earlier. High-dose IL-2 (aldesleukin, Proleukin) was the first effective immunotherapy for solid tumors, FDA-approved for renal cell carcinoma in 1992 and metastatic melanoma in 1998 based on the durable complete responses observed in 6-8% of patients despite low overall response rates (~15-16%). The mechanism of IL-2's benefit was understood only after subsequent biology emerged: IL-2 stimulates T cell expansion broadly but also expands Tregs (which constitutively express high-affinity IL-2 receptors) and induces activation-induced cell death in effector populations. IL-15 supports the desired populations (effector and memory CD8+ T cells, NK cells) without the Treg expansion and AICD problems of IL-2.

CAR-T persistence biology. Adoptive cell therapies have made the importance of persistence quantitatively clear. Tisagenlecleucel (Kymriah, the first FDA-approved CAR-T, August 2017 for pediatric/young adult relapsed/refractory B-cell ALL) data from the ELIANA trial (Maude SL, Laetsch TW, Buechner J, et al., *NEJM* 2018;378(5):439-448, doi:https://doi.org/10.1056/NEJMoa1709866, NCT02435849) demonstrated that the durability of clinical response correlates directly with persistence of tisagenlecleucel in peripheral blood and with persistent B-cell aplasia (the on-target effect indicating active CAR-T cells continue to recognize and eliminate CD19+ cells). Five-year follow-up of ELIANA showed that 44% of patients who achieved initial remission remained relapse-free at 5 years — the patients in whom CAR-T persistence was sustained.

Memory niche dynamics. Memory T cells reside in specific anatomical niches (bone marrow, lymph nodes, tissue-resident memory cells in non-lymphoid sites) where they receive trophic signals supporting their maintenance. Disruption of these niches (through chemotherapy, radiation, or aging) compromises memory maintenance. Conversely, niche-supportive interventions could extend memory persistence.

The contemporary interventions

Four intervention categories address Mode Six, with maturity ranging from FDA-approved (IL-2, ANKTIVA, CAR-T) to early clinical investigation.

IL-2 and engineered IL-2 variants

High-dose IL-2 (aldesleukin, Proleukin) was the foundation of solid-tumor cytokine immunotherapy. The Rosenberg group at NCI established the dosing regimen (600,000-720,000 IU/kg every 8 hours, up to 14 doses per cycle), and subsequent series across multiple centers documented durable complete responses in a small but reproducible

fraction of patients. The toxicity profile is severe — capillary leak syndrome with hypotension, multi-organ effects requiring ICU-level care — which has restricted IL-2 use to specialized centers.

The contemporary landscape includes multiple engineered IL-2 variants designed to retain therapeutic effects while reducing toxicity and Treg expansion. **Bempegaldesleukin (NKTR-214)**, a PEGylated IL-2 with biased CD122 signaling, was tested in pivotal trials in combination with nivolumab in melanoma (PIVOT IO-001) and other tumor types. The phase III PIVOT IO-001 trial failed its primary endpoints in 2022. Subsequent IL-2 variants in development include Treg-sparing IL-2 muteins, CD25-biased IL-2 forms (engaging IL-2 high-affinity receptor on Tregs preferentially for autoimmune indications), and CD122-biased forms (engaging effector and memory T cells preferentially for cancer).

The translational record for engineered IL-2 variants in oncology has been disappointing to date despite extensive investment. The mechanistic understanding suggests that simply enhancing IL-2 signaling — even with engineered selectivity — may not produce the temporal persistence benefit Mode Six requires, perhaps because IL-2 acts more on activation than on memory maintenance.

IL-15 and IL-15 superagonists

IL-15 was discovered in 1994 by Grabstein and colleagues, characterized by its similarity to IL-2 in proliferative effects on T cells and NK cells but with mechanistically distinct biology favoring memory T cell maintenance. Native IL-15 has poor pharmacokinetics (short half-life, requires presentation in complex with IL-15Rα for efficient signaling), limiting its direct therapeutic utility.

Nogapendekin alfa inbakicept-pmln (ANKTIVA, N-803, ImmunityBio) is an IL-15 superagonist comprising an IL-15 mutant (N72D) bound to a soluble IL-15Rα-Fc fusion. The structure substantially improves pharmacokinetics and bioactivity over native IL-15. ANKTIVA in combination with intravesical BCG received FDA approval on April 22, 2024 for BCG-unresponsive non-muscle invasive bladder cancer with carcinoma in situ — the first new cytokine FDA-approved for cancer in over 30 years.

The approval was based on the QUILT-3.032 trial (Chamie K, Chang SS, Kramolowsky E, et al., *NEJM Evidence* 2023;2(1):EVIDoa2200167, NCT03022825). Cohort A enrolled patients with BCG-unresponsive carcinoma in situ with or without papillary tumors (84 patients in the efficacy cohort); the complete response rate was 71% and the median duration of response was 26.6 months. Cohort B enrolled patients with BCG-unresponsive high-grade papillary disease (77 patients); the complete response rate was 62%. Critically, the dosing regimen includes a sustained component: weekly induction for 6 weeks, then maintenance dosing weekly for 3 weeks at months 4, 7, 10, 13, and 19, for a total of 15 maintenance doses spanning ~19 months. This sustained dosing pattern is structurally important and represents a return to the temporal model that historical Coley dosing exemplified.

ANKTIVA's clinical pattern — strong activity in combination with a PAMP-rich agent (BCG, a live attenuated Mycobacterium bovis), sustained dosing, durable responses extending beyond cessation — provides a contemporary structural template for Mode

Six engagement. The framework's prediction that sustained dosing with cytokine support produces qualitatively different outcomes from single-dose regimens receives direct empirical support from this clinical experience.

CAR-T cell therapy persistence enhancements

The CAR-T cell therapy field has converged on persistence as a key determinant of durable response. Multiple strategies are being pursued to enhance persistence:

- **Costimulatory domain choice:** 4-1BB-based costimulation (tisagenlecleucel, lisocabtagene maraleucel) produces more persistent CAR-T populations than CD28-based costimulation (axicabtagene ciloleucel, brexucabtagene autoleucel). The 4-1BB versus CD28 distinction maps onto memory versus effector bias in the resulting CAR-T population.
- **Membrane-bound IL-15 fusion (mbIL15):** CAR-T cells engineered to express membrane-bound IL-15 show enhanced persistence and T_SCM_ phenotype maintenance in preclinical and early clinical studies (Hurton LV, Singh H, Najjar AM, et al., *PNAS* 2016;113(48):E7788-E7797, doi:https://doi.org/10.1073/pnas.1610544113).
- **Manufacturing optimization:** Short CD3/CD28 costimulation plus IL-21 supplementation during ex vivo expansion enriches for T_SCM_ populations in the final product.
- **Lymphodepletion:** Adequate pre-infusion lymphodepleting chemotherapy (fludarabine-cyclophosphamide regimens) creates the homeostatic cytokine sink that supports CAR-T expansion and persistence.

The persistence framework has progressively reshaped CAR-T development, with newer products generally outperforming earlier ones on persistence-related metrics.

Cancer vaccine boost regimens and prime-boost approaches

Cancer vaccines (peptide, mRNA, viral vector, DC-based) have historically produced measurable immune responses with limited clinical durability. The vaccine field has progressively converged on prime-boost regimens (using different vector platforms for priming and boosting to avoid anti-vector immunity), repeated dosing schedules, and adjuvant optimization to enhance persistence.

The ongoing personalized neoantigen vaccine trials, including the **autogene cevumeran** (BNT122/RO7198457; renamed intismeran autogene) program in pancreatic and other cancers (Rojas LA, Sethna Z, Soares KC, et al., *Nature* 2023;618(7963):144-150, doi:https://doi.org/10.1038/s41586-023-06063-y, NCT04161755; extended T-cell durability data in Guasp P, Sethna Z, Reiche C, et al., *Nature* 2025;639:1042-1051), illustrate the contemporary paradigm. The pancreatic cancer phase I (16 patients) reported persistence of vaccine-induced T cell responses for at least 2 years in patients who remained disease-free, with 8 of 16 patients developing detectable vaccine-induced T cell responses and hazard ratio for recurrence of 0.14 favoring responders at 3-year follow-up, providing proof of

principle that vaccine-induced memory can be durable when biological conditions support persistence.

Prime-boost mRNA-LNP vaccine regimens for melanoma (mRNA-4157/V940 in combination with pembrolizumab, KEYNOTE-942/mRNA-4157-P201) extended adjuvant therapy duration to ~9 cycles over multiple months, with reported recurrence-free survival benefit in the phase IIb readout (HR 0.561, p=0.053 in initial readout). The pattern of sustained dosing producing better outcomes than shorter regimens is consistent with the Mode Six framework prediction.

The pattern: persistence as the limiting factor

The contemporary clinical evidence base supports a structural pattern. Where persistence is achieved, durable responses follow; where persistence fails, even initially robust responses eventually fail.

The CAR-T data illustrate this most directly: patients with sustained CAR-T persistence and persistent B-cell aplasia maintain remission; patients who lose CAR-T persistence relapse. The IL-2 data show the same pattern: the small fraction of patients who achieve complete responses to high-dose IL-2 frequently maintain those responses for years, while the larger fraction of partial responders or non-responders progress. The ANKTIVA + BCG data show the contribution of sustained dosing to durable response.

The framework's combinatorial completeness argument has a temporal dimension: Mode Six engagement is necessary for sustained immune control regardless of how well Modes One through Five are addressed. A protocol that engages Modes 1-5 effectively but addresses Mode Six only through single-dose intervention will produce a wave of initial response followed by relapse as the response attenuates. A protocol that addresses Mode 6 through sustained dosing or cytokine support will produce durability of the response engagement Modes 1-5 establish.

This temporal dimension is implicit in the historical Coley protocol's sustained dosing pattern. The repeated injections over weeks and months were not simply because Coley lacked the means to deliver a "one-shot" treatment — they reflected his clinical observation that responses required sustained intervention to mature and durable benefits required prolonged treatment. Modern molecular biology has explained the mechanism (homeostatic cytokine support, memory niche dynamics, sustained antigen exposure for memory formation), but the empirical observation predates the mechanism by a century.

Coley framework engagement of Mode Six

The historical Coley protocol engages Mode Six structurally through three mechanisms.

First, sustained dosing. The Coley vaccine was administered repeatedly over weeks to months. The persistence of PAMP exposure, inflammatory cytokine signaling, and antigen release sustained the conditions favoring memory T cell formation and maintenance.

Second, sustained inflammatory cytokine signaling. The repeated febrile responses and the inflammatory cytokine cascades (IL-12, IL-15 produced by activated DCs and macrophages, IFN-gamma from NK cells and Th1 T cells) provided sustained trophic support for the memory T cell populations being generated. The contemporary recognition that IL-15 in particular is critical for CD8+ memory T cell maintenance provides mechanistic interpretation of what the Coley protocol was inadvertently providing.

Third, sustained antigen exposure. The repeated bacterial PAMP exposure and the sustained inflammatory response that produced ongoing tumor antigen release maintained the antigen presentation conditions favorable to memory formation. This stands in contrast to single-dose tumor vaccines where antigen exposure is brief and memory formation conditions are correspondingly limited.

The framework's scoring methodology treats the historical Coley protocol as scoring strongly on Mode Six — perhaps 0.75-1.0 on the 0-1 scale — through these sustained-dosing and sustained-cytokine-signaling effects. This is one of the modes where the Coley framework's structural completeness is highest, comparable to its engagement of Modes 1 and 2.

The structural prediction is direct: contemporary protocols that abandon the sustained dosing pattern in favor of single-dose or short-course regimens will lose the Mode 6 benefit even if they preserve engagement of other modes. Conversely, integration of IL-15 superagonists or other persistence-supporting interventions can substitute for the sustained dosing pattern Coley used directly.

What current trials are not testing

The combinatorial completeness gap on Mode Six has a temporal shape. Most contemporary immunotherapy combinations adopt short-course or single-dose protocols (CAR-T as one-time infusion, checkpoint inhibitors as fixed-duration regimens, vaccines as 4-6 dose series). Few trials sustain dosing for the months-to-years range that historical Coley dosing and ANKTIVA's maintenance schedule suggest.

The framework's prediction is that protocols with stronger Mode 6 engagement (either through sustained dosing or through IL-15 superagonist combination) will produce more durable responses than equivalent protocols with shorter dosing schedules. The ANKTIVA + BCG result in NMIBC is one direct demonstration of this principle in a tumor setting where the BCG component provides Mode 1 + Mode 2 engagement (PAMP-driven antigen presentation and DC priming) and the ANKTIVA component provides Mode 6 engagement (sustained IL-15 cytokine support).

Trials that would extend this principle to other tumor types — IL-15 superagonist plus a Mode 1/2 engaging agent (PAMP-rich bacterial preparation, oncolytic virus, intratumoral therapy) plus checkpoint blockade (Mode 4) — are limited. The ImmunityBio program is testing ANKTIVA in multiple other indications, but the specific cross-mode combinatorial protocols the framework recommends are sparse in the trial landscape.

Gaps and open questions

Several questions emerge that affect the larger framework.

First, the question of how long sustained dosing must continue is unanswered. ANKTIVA's regimen extends maintenance dosing through month 19; the historical Coley regimen often continued for many months and sometimes for years. CAR-T cell persistence in successful cases extends for years from a single infusion (different mechanism — the cells self-renew). The optimal duration of sustained cytokine or immunomodulator dosing for maintenance of anti-tumor memory is empirically uncharacterized.

Second, the trade-off between sustained dosing and accumulated toxicity is poorly characterized. High-dose IL-2 illustrates the extreme — the same property that produces durable complete responses in a small fraction also produces severe toxicity in the majority. ANKTIVA's tolerability profile is reported as substantially better than high-dose IL-2 (grade 3/4 adverse events 0-3% in QUILT-3.032), suggesting engineered cytokine variants may produce favorable benefit-risk profiles for sustained dosing.

Third, the optimal cytokine for Mode 6 support is unresolved. IL-15 has theoretical advantages over IL-2 (Treg-sparing, memory-favoring) but the clinical comparative evidence base is limited. IL-7 supports memory differently and has been less developed in cancer immunotherapy. IL-21 has roles in T cell stem-like differentiation that have been exploited in CAR-T manufacturing but less in systemic therapy.

Fourth, the question of whether Mode 6 can be addressed through sustained dosing of the upstream-mode-engaging agent (Coley approach — repeated PAMP exposure providing both Mode 1/2 engagement and indirect Mode 6 support through sustained inflammatory cytokine signaling) versus through separate cytokine-providing agents (IL-15 superagonist approach — direct Mode 6 support added to a separate upstream-mode-engaging agent) has not been compared directly. The clinical evidence suggests both approaches can work; the combinatorial protocol implications differ depending on the answer.

Implications for the combinatorial framework

Mode Six is the temporal dimension of combinatorial completeness. A protocol that engages all five other modes well but fails Mode Six will produce initial response followed by attrition. A protocol that addresses Mode Six well but neglects upstream modes will provide cytokine support for an immune response that has nothing to act on.

The framework's structural argument receives clear support from contemporary clinical evidence: - CAR-T persistence determines durability of response - ANKTIVA's combination with BCG produces durable NMIBC responses through sustained Mode 6 engagement - High-dose IL-2's durable complete responses correlate with the sustained activity its dosing regimen enables - Vaccine programs with prime-boost and sustained dosing patterns outperform single-shot regimens

The historical Coley protocol's strong Mode 6 engagement through sustained dosing provides a structural template that contemporary protocols can either replicate (through repeated dosing of an upstream-mode-engaging agent) or substitute (through addition of an IL-15 superagonist or equivalent cytokine support to a

single-dose upstream-mode-engaging agent).

The framework's protocol design implication is that any combinatorial-complete contemporary protocol must address Mode 6 explicitly. The empirical track record of immunotherapy approaches that ignored persistence — many cancer vaccines, some checkpoint inhibitor regimens stopped early, single-dose adoptive cell products without persistence-enhancement strategies — is consistent with the framework's prediction that Mode 6 neglect produces transient responses regardless of upstream engagement.

The next section addresses Mode Seven: tumor heterogeneity and immune escape. Mode Seven is the spatial and clonal dimension of immune evasion — even when persistent immune responses are mounted against tumor antigens, sub-clonal variation within tumors and selection pressure during treatment can produce antigen-loss variants that escape established immunity.

8. Mode Seven: Tumor heterogeneity and immune escape

The problem

The previous six failure modes concern *engagement* of anti-tumor immunity in time and mechanism: antigen presentation, T cell priming, infiltration, exhaustion reversal, suppression of suppression, and temporal persistence. Mode Seven addresses a structurally different dimension — the *spatial and clonal heterogeneity* of the tumor itself. Cancer is not a uniform population of identical cells but a clonally evolving ecosystem in which subclonal variation, selective pressure, and Darwinian evolution operate continuously. An immune response that effectively eliminates the dominant clones may leave subclonal variants that escape recognition and become the next dominant population, producing relapse despite initially robust response.

The clinical phenomenon is well-recognized across treatment modalities. CD19 antigen loss after CD19-targeted CAR-T therapy in B-cell ALL — across multiple cohorts, CD19-negative or CD19-low relapses account for a range of approximately 30% to 65% of all relapses (variation reflecting differences between clinical trial populations and real-world cohorts). Neoantigen loss through copy-number deletion of mutation-containing chromosomal regions during checkpoint inhibitor treatment in NSCLC. HLA class I loss of heterozygosity emerging or expanding during immunotherapy in multiple tumor types. The MART-1/MAGE-A3/gp100 antigen loss patterns observed historically in melanoma vaccine and TIL therapy studies. Lineage switch in B-cell ALL (B-cell to myeloid) under CD19-targeted pressure.

Mode Seven is conceptually distinct from Mode One (insufficient antigen presentation) in an important way. Mode One concerns baseline antigen presentation dysfunction — tumors that arrive at clinical attention with HLA defects, MHC class I downregulation, antigen processing defects already present. Mode Seven concerns *acquired* antigen loss under immune selection pressure — tumors that started with intact antigen presentation but evolved escape variants in response to immune attack. The mechanisms can overlap (HLA LOH appears in both contexts) but the temporal dynamics and intervention strategies differ.

The Coley framework's engagement of Mode Seven through its mechanisms — broad polyclonal antigen exposure, bystander killing via fever-induced damage, danger signals released during immunogenic cell death — represents a structurally different solution from targeted single-antigen approaches like CD19 CAR-T or single-neoantigen vaccines. Mode Seven is one of the modes where the framework's structural prediction about Coley protocol benefit aligns directly with the modern understanding of cancer evolution.

The contemporary intervention landscape for Mode Seven includes multi-antigen CAR-T constructs (bispecific, tandem, sequential), polyepitope and clonal-neoantigen-targeted vaccines, immunogenic cell death-inducing therapies that release diverse antigens, oncolytic viruses, and combinatorial approaches designed to provoke epitope spreading.

The mechanisms

Cancer evolution operates through Darwinian selection on heritable variation in clonal populations, with immune pressure as one of the dominant selective forces. The mechanistic understanding has matured substantially through next-generation sequencing of multi-region tumor samples and longitudinal sampling.

Clonal versus subclonal mutations. Tumor mutations exist along a continuum from clonal (present in essentially all tumor cells, arising early in tumor evolution before extensive clonal expansion) to subclonal (present in only a fraction of tumor cells, arising later during clonal evolution). Clonal mutations represent the "trunk" of the tumor's evolutionary tree; subclonal mutations represent the "branches." McGranahan, Furness, Rosenthal, and colleagues demonstrated that tumors with predominantly clonal neoantigens (low subclonal heterogeneity) had stronger anti-tumor T cell responses and better response to checkpoint blockade than tumors with predominantly subclonal heterogeneity (McGranahan N, Furness AJS, Rosenthal R, et al., *Science* 2016;351(6280):1463-1469). This established the principle that an immune response is qualitatively limited by the clonal architecture of its target — even effective immunity against subclonal antigens cannot eliminate cells lacking those antigens.

Immunoediting and tumor evolution. Tumors are not passive targets but active participants in coevolution with the immune system. The Rosenthal-McGranahan-Swanton TRACERx analysis of 88 early-stage untreated NSCLCs (258 multi-region samples; Rosenthal R, Cadieux EL, Salgado R, et al., *Nature* 2019;567(7749):479-485) demonstrated that immune infiltration levels actively shape tumor evolution: heavily infiltrated tumor regions show ongoing immunoediting (loss of neoantigens, HLA LOH, promoter hypermethylation of neoantigen-containing genes); sparsely infiltrated tumors show evidence of historical immunoediting (waning of neoantigen editing, copy-number loss of previously clonal neoantigens). Approximately 40% of tumors had consistently low immune infiltration; 28% had consistently high infiltration; 28% had intra-tumoral variation in infiltration that correlated with mutation heterogeneity.

HLA loss as evolutionary response. Loss of heterozygosity at the HLA class I locus, allele-specific HLA loss, and outright deletion of HLA class I genes appear as

recurrent evolutionary responses to immune pressure. McGranahan and colleagues demonstrated HLA LOH in 40% of early-stage NSCLC tumors, usually as a subclonal event, and showed that HLA LOH permits branched evolution associated with expansion of mutations predicted to bind the lost HLA allele (McGranahan N, Rosenthal R, Hiley CT, et al., *Cell* 2017;171(6):1259-1271). This is the molecular signature of immune selection: the tumor evolves to lose specifically the antigen presentation machinery that was effectively presenting its mutations.

Loss of clonal neoantigens under treatment pressure. Anagnostou and colleagues demonstrated that clonal neoantigens can be lost during disease progression after checkpoint blockade in NSCLC: in 4 NSCLC patients with matched pretreatment and resistant samples, 7-18 putative mutation-associated neoantigens were lost in resistant clones, through either subclone elimination or deletion of chromosomal regions containing truncal alterations (Anagnostou V, Smith KN, Forde PM, et al., *Cancer Discovery* 2017;7(3):264-276). Functional T-cell responses to the eliminated neoantigens were demonstrated, confirming that the loss was immunologically meaningful rather than incidental. This work established that what appears as "checkpoint inhibitor resistance" can be the predictable outcome of evolutionary selection acting on the heterogeneous tumor population.

Lineage switch. In B-cell ALL treated with CD19-targeted therapy (CAR-T cells or blinatumomab bispecific T cell engager), some tumors escape immune pressure by undergoing lineage switch — converting from B-lymphoid to myeloid phenotype, with loss of CD19 expression as part of the transcriptional reprogramming. This is the most dramatic form of antigen escape, involving complete reprogramming of cellular identity, and it illustrates the extent to which tumor heterogeneity includes plasticity in differentiation state, not just genetic variation.

Multiple mechanisms of CD19 loss. Even within the single-antigen escape pattern in B-ALL, multiple mechanisms operate: truncated CD19 mutations producing nonfunctional protein, disruption of CD19 trafficking to the cell surface, CD19 mRNA misplicing producing isoforms lacking the CAR-recognized epitope, and lineage switch as above. CD19 expression can sometimes recover after relief of immune pressure, indicating that some "loss" represents reversible downregulation rather than genetic deletion.

Bystander killing and antigen spreading as counter-mechanisms. Anti-tumor immune responses can spread from initial antigen specificities to additional antigens through several mechanisms: T cell killing of tumor cells releases tumor-derived antigens that can be cross-presented by dendritic cells to prime new T cell specificities; inflammatory cell death from cytotoxic activity releases damage-associated molecular patterns (DAMPs) that activate broader immune responses; bystander killing of antigen-negative cells by activated T cells in proximity to antigen-positive targets can occur through cytokine release or other non-specific mechanisms. Epitope spreading is the clinical phenomenon by which an initially narrow immune response broadens over time, and it provides a partial counter to Mode 7 escape.

The contemporary interventions

Five intervention categories address Mode Seven with varying clinical maturity.

Multi-antigen targeting in CAR-T therapy

The CAR-T cell therapy field's confrontation with CD19 antigen escape after initial successes has driven extensive development of multi-antigen approaches.

Bispecific and tandem CAR constructs. Single CAR-T cell products expressing receptors recognizing multiple antigens. Tandem CD19/CD20 CARs use a single chimeric receptor with binding domains for both targets in series. Bispecific CD19/CD22 CARs use either single chimeric receptors or separate CARs on the same cell. Multiple constructs are in clinical development.

Sequential CAR-T therapy. Patients who relapse after CD19 CAR-T with CD19-negative disease can be treated with CD22-targeted CAR-T or blinatumomab-type bispecific T cell engagers (BiTE) as salvage. The Fry group and others established CD22-targeted CAR-T as effective in CD19-relapsed B-ALL, though CD22 downregulation can subsequently produce CD22-negative relapse.

Cotransduction approaches. The CARPALL study (NCT02443831, reported in *Blood* 2024;143(2):118) used cotransduction of separate CD19 and CD22 CAR vectors into the same T cell product. In 12 patients with advanced B-ALL, 10/12 achieved MRD-negative CR at 2 months. With median follow-up of 8.7 months, no antigen-negative relapses had occurred — though 5 patients did experience MRD emergence or relapse with CD19- and CD22-expressing disease (loss of CAR-T cell persistence, which is a Mode 6 problem rather than Mode 7 antigen escape).

The CAR-T multi-antigen approach addresses Mode 7 directly at the single-antigen-escape level. However, the approach has theoretical limits: sufficient subclonal heterogeneity could escape any finite combination of targeted antigens, and Mode 7 escape mechanisms include cellular identity changes (lineage switch) that go beyond simple antigen modulation.

Multi-neoantigen and clonal-neoantigen vaccines

The personalized neoantigen vaccine field has explicitly addressed Mode 7 in its design philosophy.

Polyepitope mRNA vaccines. The autogene cevumeran and mRNA-4157 programs (discussed in Mode 6) target up to 20 neoantigens per patient simultaneously. The rationale is to require simultaneous escape of multiple neoantigens for tumor escape — increasingly difficult as the number of targeted neoantigens increases.

Clonal-neoantigen-focused approaches. The Swanton group's bioinformatics pipeline identifies clonal (truncal) neoantigens from multi-region sequencing, allowing vaccine design or TIL enrichment focused on the mutations most likely present in all tumor cells. Two first-in-human phase I/II trials in advanced melanoma and NSCLC have been launched using this approach.

TIL therapy with clonal neoantigen enrichment. Adoptive transfer of tumor-infiltrating lymphocytes selected for recognition of clonal neoantigens — addressing both the persistence (Mode 6) and the heterogeneity (Mode 7) dimensions simultaneously.

Oncolytic viruses and immunogenic cell death

Oncolytic viruses provide antigen-agnostic tumor killing — they replicate selectively in tumor cells and kill them through viral cytopathic effect, releasing the full diversity of tumor antigens (clonal and subclonal, known and unknown) along with viral PAMPs and cellular DAMPs. The mechanism inherently produces broad antigen exposure and is not limited by the patient's specific neoantigen profile.

T-VEC (talimogene laherparepvec, FDA-approved October 27, 2015 for advanced melanoma) provides clinical proof of principle, though the MASTERKEY-265 phase III trial of T-VEC + pembrolizumab in advanced melanoma failed to meet its primary endpoint (discussed in Mode 3).

Several other oncolytic platforms are in clinical development. The mechanistic argument for oncolytic virotherapy as a Mode 7 intervention is strong; the clinical translation has been mixed.

Immunogenic chemotherapy and radiation

Some chemotherapy agents (anthracyclines, oxaliplatin) and radiation produce immunogenic cell death — calreticulin exposure, HMGB1 release, ATP release, type I IFN signaling — that releases broad tumor antigen profiles in inflammatory contexts favoring cross-presentation. This addresses Mode 7 indirectly by exposing the immune system to the full antigenic complexity of the tumor including subclonal variation.

Radiation-induced abscopal effects in patients receiving immunotherapy plus radiation illustrate the mechanism: local radiation produces immunogenic cell death and antigen release that primes systemic responses against distant tumor sites containing the same and related antigens.

Combinatorial epitope spreading promotion

The combination strategy of single-antigen targeted therapy plus broad-immune-engagement therapy attempts to leverage the initial precise targeting while promoting epitope spreading to address heterogeneity. The Hammerich 2019 in situ vaccination paradigm in iNHL (discussed in Mode 2) provides one example: intratumoral Flt3L + radiotherapy + poly-ICLC produced abscopal regressions in PD-1-refractory lymphoma, and importantly converted PD-1-refractory disease to PD-1-responsive disease — consistent with epitope spreading promoting the recognition of additional tumor antigens.

ANKTIVA + BCG in NMIBC (discussed in Modes 5 and 6) provides another example: BCG creates broad inflammatory antigen release while ANKTIVA provides sustained cytokine support for the resulting polyclonal T cell response.

The pattern: heterogeneity is structural and requires structural solutions

The contemporary clinical evidence base supports a structural pattern. Single-antigen targeting approaches consistently encounter Mode 7 escape mechanisms — even when they achieve initial deep responses. The CD19 CAR-T trajectory is the clearest illustration: dramatic initial responses (>80% remission in pediatric B-ALL), followed by relapse from antigen-loss disease in a substantial fraction of patients, driving the multi-antigen development response. Single-neoantigen targeting (early peptide vaccines) was similarly limited; the field has moved toward polyepitope and clonal-focused approaches in response.

Broad-engagement approaches — oncolytic viruses, immunogenic cell death-inducing chemotherapy, in situ vaccination paradigms, bacterial PAMPs — engage the full antigenic complexity of the tumor and resist Mode 7 escape better in principle. Their clinical translation has been mixed in practice, suggesting that broad antigen exposure alone is insufficient without effective engagement of the other failure modes.

The framework's combinatorial completeness argument has its clearest spatial-clonal expression in Mode 7. A protocol that engages all six other modes against a single antigen (or single antigen family) is vulnerable to Mode 7 escape regardless of how thoroughly the other modes are addressed. A protocol that engages multiple modes against broad antigenic targets — through either deliberate multi-antigen targeting or through broad-engagement mechanisms — produces more robust responses.

Coley framework engagement of Mode Seven

The historical Coley protocol engages Mode Seven strongly through several mechanisms.

First, broad antigen exposure. The bacterial PAMP-driven inflammation produces immunogenic cell death in tumor tissue, releasing the full diversity of tumor antigens. This is not selective for clonal versus subclonal antigens — the inflammatory damage exposes whatever antigens are present in the cells being killed.

Second, fever-induced tumor cell stress and damage. The repeated febrile responses produced by the Coley vaccine create heat-shock protein-mediated immunogenic cell death in tumor cells, with chaperone-bound antigens released into the inflammatory environment for cross-presentation. The mechanism is antigen-agnostic — heat-shock-induced cell death does not depend on which specific antigens the cell expresses.

Third, danger signal release. The Coley-induced inflammation produces DAMPs (HMGB1, ATP, type I IFN, etc.) in addition to the PAMPs from the bacterial preparation itself. The combined danger signal environment promotes broad cross-presentation of the released antigens by activated dendritic cells, favoring epitope spreading.

Fourth, polyclonal T cell activation. The bacterial PAMPs activate T cells through multiple TLR-driven and bystander pathways, producing polyclonal activation that does not depend on pre-existing specific recognition of any particular tumor antigen.

This complements the antigen-specific responses by providing inflammatory effector function.

The framework's scoring methodology treats the historical Coley protocol as scoring strongly on Mode Seven — perhaps 0.75-1.0 on the 0-1 scale — through these broad-antigen-exposure and broad-T-cell-activation mechanisms. The structural argument is that contemporary single-antigen-targeted approaches are vulnerable to Mode 7 escape in ways the Coley framework structurally is not.

This is one of the modes where the Coley framework's structural completeness is highest and where the contrast with modern targeted approaches is sharpest. A contemporary protocol that combines bacterial PAMPs (or PAMP-equivalents like TLR agonists, BCG, attenuated bacterial preparations) with checkpoint blockade, IL-15 superagonist, and other contemporary interventions inherits the Coley protocol's Mode 7 robustness while gaining the contemporary modes' targeted engagement of Modes 1-6.

What current trials are not testing

The combinatorial completeness gap on Mode 7 has a specific shape. Most contemporary immunotherapy trials test either single-antigen-targeted approaches (CAR-T against one antigen, vaccines against one or few neoantigens) or broad-engagement approaches without explicit Mode 7 emphasis. Trials that explicitly combine targeted approaches with broad-engagement approaches to address Mode 7 systematically are limited.

The framework's structural prediction is that: - Single-antigen targeted approaches will continue to encounter Mode 7 escape (CD19 CAR-T pattern, single-neoantigen vaccine pattern) - Broad-engagement approaches without targeted assistance will produce intermediate efficacy (oncolytic virus monotherapy pattern) - Combinatorial approaches that pair targeted engagement with broad-engagement mechanisms will produce more durable responses

The ANKTIVA + BCG result in NMIBC partially demonstrates this principle — BCG provides broad antigen exposure (Mode 7 + Modes 1, 2, 5) while ANKTIVA provides sustained T cell support (Mode 6). The lymphoma in situ vaccination paradigm (Hammerich 2019) similarly combines targeted intratumoral DC activation with broad immune engagement.

The specific cross-mode combinatorial protocols that would systematically test the framework's prediction — for example, an oncolytic virus or PAMP-rich preparation paired with checkpoint blockade and IL-15 superagonist support in TNBC, pancreatic cancer, or MSS colorectal cancer — are limited in the current trial landscape. The Coley-equivalent biological reasoning suggests these combinations should produce qualitatively different outcomes from the same individual components tested separately.

Gaps and open questions

Several questions emerge that affect the larger framework.

First, the relative contribution of clonal versus subclonal targeting to durable response is not fully quantified. The McGranahan 2016 *Science* paper established that clonal neoantigen-rich tumors respond better to checkpoint blockade. But the optimal targeting strategy — whether to focus vaccines and adoptive cells on clonal neoantigens, broaden to include subclonal, or pursue broad antigen-agnostic strategies — depends on the specific tumor context and remains incompletely characterized.

Second, the epitope spreading phenomenon is poorly quantified across treatment modalities. How often does it occur? In what tumor contexts? Which interventions promote it most reliably? The mechanistic understanding is qualitative; the quantitative parameters for protocol design remain to be established.

Third, the question of whether Mode 7 can be addressed prophylactically (before relapse) versus only therapeutically (after antigen-escape relapse) is unresolved. Bispecific or tandem CAR-T from the outset is a prophylactic Mode 7 approach; sequential CAR-T after relapse is therapeutic. The relative effectiveness of these strategies likely depends on the rate of antigen-escape variant generation in each tumor type.

Fourth, the integration of cancer evolution biology with combinatorial immunotherapy design is at an early stage. The Swanton-McGranahan group's TRACERx-derived insights have informed individual trial designs but have not been systematically applied to multi-mode combinatorial protocol development.

Implications for the combinatorial framework

Mode Seven completes the framework's seven-mode structure and provides the strongest argument for the framework's combinatorial completeness logic. The Mode 7 escape pattern — tumors that evolve to escape any narrowly-targeted immune attack — means that targeted immunotherapy without broad-engagement support faces a structural limit that no amount of targeted optimization can overcome.

The Coley framework's strong Mode 7 engagement through broad antigen exposure, fever-induced immunogenic cell death, and danger signal release is one of its most structurally distinctive features. The contemporary single-antigen targeted approaches that have produced the most dramatic short-term immunotherapy successes (CAR-T in B-ALL, neoantigen vaccines in select cancers) are paradoxically the most vulnerable to Mode 7 escape — their precise targeting is also their evolutionary Achilles heel.

The framework's protocol design implication is that any combinatorial-complete contemporary protocol must address Mode 7 either through deliberate multi-antigen targeting or through broad-engagement mechanisms. The Coley framework's bacterial PAMP-rich preparation provides one direct mechanism. Contemporary alternatives — oncolytic viruses, immunogenic chemotherapy, TLR agonists, bacterial preparations like BCG — provide several others. A protocol that engages all the other modes intensively but addresses Mode 7 only through narrow targeting will produce dramatic initial responses followed by Mode 7-mediated relapse.

Mode Seven and the framework as a whole

With Mode Seven complete, the seven-mode framework provides a structural account of all the major failure modes of immune-mediated tumor control:

1. **Insufficient antigen presentation** — engaged by IFN-γ, HDAC inhibitors, fever-range thermal stress, immunogenic chemotherapy
2. **Lack of T cell priming and DC engagement** — engaged by Flt3L, CDX-301, TLR agonists, STING agonists, in situ vaccination
3. **Physical exclusion of T cells** — engaged by anti-VEGF, oncolytic viruses, stromal modulation, intratumoral delivery
4. **T cell exhaustion** — engaged by anti-PD-1, anti-CTLA-4, anti-LAG-3 checkpoint blockade
5. **Active immune suppression by TME** — engaged partially by checkpoint blockade Treg depletion, partially by CD73/A2A inhibitors where signal exists
6. **Insufficient persistence of immune response** — engaged by IL-15 superagonists (ANKTIVA), sustained dosing patterns, CAR-T persistence engineering
7. **Tumor heterogeneity and immune escape** — engaged by multi-antigen targeting, oncolytic viruses, immunogenic cell death, broad-antigen-exposure mechanisms

The framework's combinatorial completeness prediction is that protocols engaging all seven modes simultaneously will produce qualitatively different outcomes from protocols engaging subsets of modes. The historical Coley protocol — engaging Modes 1, 2, 5, 6, 7 strongly and Mode 3 partially, but Mode 4 essentially not at all — was structurally incomplete by exactly the dimension that contemporary checkpoint inhibitors now fill. The contemporary single-mode interventions (checkpoint inhibitors alone, CAR-T alone, single-target vaccines alone) are each structurally incomplete by other dimensions the Coley framework filled.

The framework's protocol design recommendation is direct: combinatorial-complete immunotherapy requires engagement of all seven modes. The Coley framework provides a structural template for Modes 1, 2, 5, 6, 7 engagement; contemporary checkpoint inhibitors provide Mode 4; contemporary anti-VEGF, stromal-modifying agents, and intratumoral delivery provide Mode 3. The integration of these is the contemporary form of what the framework predicts will produce the next generation of immunotherapy advances.

The next section will address the scoring methodology that operationalizes this framework for specific cancer types and protocol designs.

9. Scoring framework and cancer-type case studies

Operationalizing the seven-mode framework

The seven-mode framework derives its practical utility from a scoring methodology that operationalizes mode engagement for specific interventions, intervention combinations, and tumor types. The scoring is intentionally simple: each intervention or protocol is assigned a score from 0 to 1 on each of the seven modes, where 0 rep-

resents no engagement of that mode, 1 represents strong direct engagement, and intermediate values represent partial or indirect engagement.

The simplicity is deliberate. More elaborate scoring schemes — for example, continuous magnitude scoring on each mode with weighting by tumor type — would offer apparent quantitative precision but would require parameter choices that the available clinical evidence does not adequately constrain. The 0-1 scoring captures the key structural information (which modes a protocol engages and how strongly) without overcommitting to quantitative precision that the evidence cannot support.

The scoring methodology has three components:

Mode engagement scoring (0-1 per mode). Each intervention is assigned a score reflecting the strength of its engagement of that failure mode. Score 1.0 reflects direct, mechanistically robust engagement supported by clinical evidence in at least one indication. Score 0.5-0.9 reflects partial or indirect engagement, or engagement supported by preclinical or early clinical evidence. Score 0.1-0.4 reflects minor or speculative engagement. Score 0 reflects no meaningful engagement.

Coverage assessment. The sum or pattern of mode scores across an intervention or protocol indicates its combinatorial coverage. A protocol scoring 1.0 on Mode 4 only (single-agent anti-PD-1) has narrow coverage. A protocol scoring 0.8-1.0 across multiple modes (Coley protocol on Modes 1, 2, 5, 6, 7; modern combination protocols across additional modes) has broader coverage. The framework's central prediction is that broader coverage produces better outcomes, particularly in tumors where multiple modes are operative as failure mechanisms.

Tumor-type weighting. Different cancer types have different dominant failure modes. Melanoma is often dominated by Mode 4 (exhaustion of pre-existing T cell responses); pancreatic cancer by Modes 1, 3, and 5; microsatellite-stable colorectal cancer by Modes 1, 2, and 5. The framework's predictions for specific tumor types depend on which modes are operative as failure mechanisms in that tumor type, modulated by which modes the proposed intervention engages.

Scoring worked examples

Historical Coley protocol (mixed bacterial vaccine, sustained dosing)

Mode	Score	Basis
1: Antigen presentation	0.8	Strong IFN-γ induction via PAMP signaling; fever-mediated MHC upregulation; immunogenic cell death releases antigens

Mode	Score	Basis
2: T cell priming	0.9	Broad-spectrum PAMP-driven DC maturation; the historical paradigm closest to contemporary in situ vaccination
3: Physical exclusion	0.4	Partial engagement via intratumoral inflammation and fever-induced vasodilation; no specific anti-VEGF or stromal-modifying mechanism
4: T cell exhaustion	0.1	Minimal direct engagement; possible indirect effects through sustained inflammation reducing exhaustion-program acquisition
5: Active immune suppression	0.6	Inflammatory environment unfavorable to Tregs and MDSCs; macrophage repolarization toward M1 phenotype; no direct adenosine or IDO targeting
6: Persistence	0.9	Sustained dosing pattern over weeks to months; sustained inflammatory cytokine signaling including IL-15-relevant pathways; sustained antigen exposure
7: Tumor heterogeneity	0.9	Broad antigen exposure through immunogenic cell death; fever-induced damage is antigen-agnostic; danger signals promote epitope spreading; polyclonal T cell activation

Total mode coverage: strong on five modes (1, 2, 6, 7 strongly; 5 moderately), partial on Mode 3, essentially absent on Mode 4. This is the structural signature of the Coley framework: broad coverage with one clear gap that contemporary checkpoint blockade fills.

Modern combination checkpoint blockade (nivolumab + ipilimumab)

Mode	Score	Basis
1: Antigen presentation	0.1	No direct engagement
2: T cell priming	0.3	Indirect via CTLA-4 blockade enhancing priming in lymphoid tissues
3: Physical exclusion	0.2	Minimal direct engagement; some indirect effects through immune-mediated vascular changes
4: T cell exhaustion	1.0	Direct, mechanistically robust engagement; the paradigmatic Mode 4 intervention with strong clinical evidence
5: Active immune suppression	0.5	Partial Treg depletion via ipilimumab; no direct MDSC, adenosine, IDO, or metabolic engagement
6: Persistence	0.4	Fixed-duration dosing schedule; no specific persistence-supporting mechanism; durable responses depend on intrinsic memory formation
7: Tumor heterogeneity	0.3	No direct broad-antigen mechanism; relies on pre-existing T cell repertoire diversity

Total mode coverage: very strong on Mode 4, partial on Modes 2 and 5, minimal on others. This is the structural signature of contemporary checkpoint blockade: narrow but powerful single-mode engagement that depends on the other modes being adequately addressed by the patient's own biology (high TMB, hot tumor, intact antigen presentation) for clinical benefit.

ANKTIVA + BCG (FDA-approved combination, NMIBC)

Mode	Score	Basis
1: Antigen presentation	0.7	BCG PAMP-driven IFN-γ; immunogenic cell death from BCG-induced inflammation

Mode	Score	Basis
2: T cell priming	0.8	BCG-driven DC maturation through TLR signaling; sustained PAMP exposure
3: Physical exclusion	0.5	Intravesical delivery achieves direct intratumoral inflammation; vasodilation from inflammatory cascade
4: T cell exhaustion	0.2	No direct checkpoint blockade; some indirect effects from sustained inflammatory environment
5: Active immune suppression	0.5	Inflammatory disruption of suppressive cell populations; no direct targeted Mode 5 mechanism
6: Persistence	1.0	IL-15 superagonist provides direct memory T cell support; sustained dosing schedule through month 19
7: Tumor heterogeneity	0.8	BCG-induced broad antigen exposure; immunogenic cell death releases full tumor antigen complexity

Total mode coverage: strong on Modes 2, 6, 7; moderate on Modes 1, 3, 5; weak on Mode 4. The combination is structurally Coley-like in its broad coverage with the IL-15 superagonist providing explicit Mode 6 engagement.

TIL (tumor-infiltrating lymphocyte) therapy (lifileucel, FDA-approved for advanced melanoma 2024)

Mode	Score	Basis
1: Antigen presentation	0.4	Indirect — relies on intact MHC class I presentation in patient's tumor; selects for tumors where presentation occurs
2: T cell priming	0.6	Bypasses in vivo priming through ex vivo expansion of pre-primed TILs

Mode	Score	Basis
3: Physical exclusion	0.3	Adoptive transfer delivers cells systemically; relies on intact homing to tumor
4: T cell exhaustion	0.5	Ex vivo expansion partially reverses exhaustion; IL-2 support helps maintain effector function
5: Active immune suppression	0.3	Lymphodepleting chemotherapy provides temporary suppression of Tregs and MDSCs
6: Persistence	0.6	IL-2 support post-infusion; lymphodepletion creates cytokine sink supporting expansion; persistence variable
7: Tumor heterogeneity	0.7	Polyclonal TIL population recognizes diverse antigens; selected for reactivity against autologous tumor including heterogeneous targets

Total mode coverage: moderate across multiple modes, with no single mode at maximum strength. The pattern reflects TIL therapy's complex multi-mode mechanism but also explains its variable clinical results — depending heavily on the quality of the autologous TIL preparation.

In situ vaccination paradigm (Hammerich 2019 — Flt3L + radiotherapy + poly-ICLC in iNHL)

Mode	Score	Basis
1: Antigen presentation	0.8	Radiotherapy-induced immunogenic cell death; IFN-γ induction from poly-ICLC
2: T cell priming	1.0	Direct, mechanistically robust DC engagement via Flt3L expansion plus poly-ICLC TLR3 activation; paradigmatic Mode 2 intervention

Mode	Score	Basis
3: Physical exclusion	0.7	Intratumoral delivery directly addresses spatial barrier; radiotherapy provides immunogenic vascular changes
4: T cell exhaustion	0.4	No direct checkpoint blockade in original protocol; later combination work showed conversion to PD-1 responsive state
5: Active immune suppression	0.4	Inflammatory environment disrupts local Treg/MDSC accumulation
6: Persistence	0.5	Sustained intratumoral inflammatory environment; not specifically targeted but mechanistically present
7: Tumor heterogeneity	0.8	Immunogenic cell death from radiation releases broad antigen profile; epitope spreading documented

Total mode coverage: very strong across the antigen-engagement modes (1, 2, 3, 7), with epitope spreading and conversion of PD-1-refractory disease to PD-1-responsive demonstrated clinically. This intervention pattern is the contemporary expression of Coley framework logic — multi-mode broad engagement rather than single-mode targeting.

Proposed combinatorial-complete protocol (hypothetical)

A protocol combining intratumoral bacterial PAMP preparation, IL-15 superagonist (ANKTIVA-equivalent), anti-VEGF or stromal-modifying agent, checkpoint blockade (anti-PD-1 + anti-CTLA-4), and immunogenic chemotherapy would score:

Mode	Score	Basis
1: Antigen presentation	0.9	Bacterial PAMP IFN-γ + immunogenic chemotherapy
2: T cell priming	0.9	Bacterial PAMP TLR signaling + DC engagement
3: Physical exclusion	0.8	Anti-VEGF or stromal-modifying agent + intratumoral delivery

Mode	Score	Basis
4: T cell exhaustion	1.0	Anti-PD-1 + anti-CTLA-4 checkpoint combination
5: Active immune suppression	0.7	Ipilimumab Treg depletion + inflammatory disruption
6: Persistence	1.0	IL-15 superagonist + sustained PAMP dosing
7: Tumor heterogeneity	0.9	Broad antigen exposure from PAMP-induced damage + immunogenic chemotherapy

This is the structural signature of combinatorial-complete coverage: 0.7-1.0 on all seven modes. The framework's prediction is that protocols approximating this coverage pattern will produce qualitatively different outcomes from protocols with narrower coverage.

The biological feasibility of such a protocol is established by component approvals — every component is FDA-approved or in advanced clinical development for at least one indication. The translational challenge is not novel agent development but combinatorial protocol design and clinical testing.

Cancer-type case studies

The framework's predictions are tumor-type specific because different cancers have different dominant failure modes. Five cancer types provide useful worked examples.

Melanoma

Dominant modes: Mode 4 (often the limiting factor in pre-existing T cell responses); Mode 7 (subclonal heterogeneity in late-stage disease). Modes 1, 2, 3 are often adequately addressed by the tumor's high mutational burden, intrinsic immunogenicity, and frequently hot tumor immune microenvironment.

Framework prediction: Mode 4 interventions (single-agent anti-PD-1, combination checkpoint blockade) produce dramatic benefits in this tumor type because the other modes are adequately addressed by intrinsic biology. This matches the empirical record: melanoma has been the leading responder cancer for checkpoint immunotherapy since the original Hodi 2010 trial.

Limits in melanoma: Mode 7 (subclonal heterogeneity in late-stage disease) and Mode 6 (persistence in patients without TCF1+ progenitor populations) account for many of the patients who do not respond to or relapse after checkpoint blockade. The framework predicts that adding Mode 6 and Mode 7 engagement to checkpoint blockade (IL-15 superagonist, intratumoral therapies, neoantigen vaccines) should extend benefit to additional patients. The KEYNOTE-942 mRNA-4157 + pembrolizumab phase IIb result (HR 0.561 for RFS) supports this prediction in the adjuvant setting.

Non-muscle invasive bladder cancer (NMIBC)

Dominant modes: Modes 1, 2 (urothelial cancers often have intermediate immunogenicity and good MHC class I expression but require activation); Mode 6 (the recurrent nature of NMIBC requires sustained anti-tumor immunity). BCG has been standard of care since the 1970s and addresses Modes 1, 2, 5, 7 partially through its bacterial PAMP mechanism.

Framework prediction: Adding Mode 6 engagement (IL-15 superagonist) to BCG should produce qualitatively better outcomes than BCG alone, particularly in BCG-unresponsive disease where the limitation is presumably insufficient persistence and/or insufficient Mode 4 engagement (chronic antigen stimulation may produce exhaustion in the responder T cell populations).

The ANKTIVA + BCG result in BCG-unresponsive NMIBC (FDA approval April 22, 2024; CR rate 71% in CIS cohort; median DOR 26.6 months) directly validates this prediction. The combination provides combinatorial completeness across Modes 1, 2, 5, 6, 7 — essentially the Coley framework engagement pattern with explicit IL-15 superagonist Mode 6 enhancement.

The framework's further prediction is that adding checkpoint blockade (Mode 4 engagement) to ANKTIVA + BCG should extend benefit to additional patients. This combination is in clinical development.

Triple-negative breast cancer (TNBC)

Dominant modes: Varies substantially. PD-L1-high TNBC: Mode 4-dominant similar to melanoma. PD-L1-negative TNBC: Modes 1, 2, 3, 5 all potentially operative. BRCA1/2-mutant TNBC: higher tumor mutational burden makes Mode 1 less limiting; platinum sensitivity reflects intrinsic genomic instability that creates more antigens but also more clonal heterogeneity (Mode 7).

Framework prediction: Single-agent checkpoint blockade should produce variable outcomes in TNBC depending on PD-L1 status and TMB. This matches the empirical record: pembrolizumab has FDA approval in PD-L1-positive metastatic TNBC and as neoadjuvant therapy in early TNBC, but response rates remain substantially lower than in melanoma.

For BRCA1/2-mutant TNBC specifically, the framework predicts platinum-based chemotherapy provides Modes 1, 7 engagement through immunogenic cell death and broad antigen exposure. The TNT trial (Tutt et al., *Nature Medicine* 2018) demonstrated platinum benefit in BRCA1/2-mutated TNBC. The framework predicts combining platinum chemotherapy with checkpoint blockade should extend benefit further — and this is the structural logic behind KEYNOTE-522 (pembrolizumab + chemotherapy in early TNBC; pCR rate improvement and EFS benefit established).

The framework's further prediction is that protocols engaging Modes 2, 5, 6 alongside platinum + checkpoint should produce further benefit. Specific options include intratumoral PAMP preparations, IL-15 superagonists, and stromal modulation. No current trial implements this multi-mode combination systematically.

Pancreatic ductal adenocarcinoma (PDAC)

Dominant modes: Mode 1 (low antigen presentation in many PDAC); Mode 3 (intense desmoplastic stroma excluding T cells); Mode 5 (intensely suppressive microenvironment with Tregs, MDSCs, M2 TAMs); Mode 7 (substantial subclonal heterogeneity in late-stage disease). PDAC is the paradigmatic immunotherapy-refractory solid tumor.

Framework prediction: Single-agent checkpoint blockade should fail in PDAC because Modes 1, 3, 5 are not adequately addressed by intrinsic biology. This matches the empirical record: pembrolizumab and nivolumab monotherapy have essentially no activity in unselected PDAC.

The framework's positive prediction is that protocols engaging Modes 1, 2, 3, 5, 6, 7 simultaneously with checkpoint blockade (Mode 4) should produce benefit where single-mode interventions fail. The autogene cevumeran result (Rojas et al., *Nature* 2023) provides preliminary support: an mRNA neoantigen vaccine (Modes 1, 2, 7 engagement) plus atezolizumab (Mode 4) plus mFOLFIRINOX (Modes 1, 7 via immunogenic chemotherapy) produced responses correlating with vaccine-induced T cell responses, with HR 0.14 for recurrence in responders vs non-responders at 3-year follow-up.

The framework's structural recommendation for PDAC is to add Mode 3 engagement (anti-VEGF or stromal-modifying agents) and Mode 6 engagement (IL-15 superagonist or sustained PAMP dosing) to the autogene cevumeran + atezolizumab + chemotherapy backbone. No current trial implements this full combinatorial pattern.

Microsatellite-stable colorectal cancer (MSS CRC)

Dominant modes: Mode 1 (often low TMB; antigen presentation defects common); Mode 3 (cold tumors); Mode 5 (suppressive microenvironment).

Framework prediction: Single-agent checkpoint blockade should fail in MSS CRC because Modes 1 and 3 are not adequately addressed. This matches the empirical record: anti-PD-1 monotherapy has minimal activity in MSS CRC; only the MSI-high subset (where Mode 1 is well-addressed by high mutational burden) responds to checkpoint blockade.

The framework's positive prediction is that interventions engaging Modes 1, 2, 3 alongside checkpoint blockade should produce benefit in MSS CRC. Specific options include immunogenic chemotherapy (Mode 1 via antigen release; Mode 7 via heterogeneity coverage), anti-VEGF (Mode 3 via vascular normalization), TLR agonists or intratumoral PAMP preparations (Mode 2), and checkpoint combinations.

No current trial in MSS CRC implements this multi-mode combinatorial protocol systematically. The framework predicts this is a major translational opportunity.

Limitations of the scoring framework

The scoring methodology has acknowledged limitations.

First, the 0-1 mode scoring is qualitative rather than quantitative. Assigning a score of 0.8 versus 0.7 on a specific mode is a judgment based on integrated evidence

rather than a measured quantity. Different reviewers might assign different specific scores while agreeing on the overall pattern. The framework's structural conclusions are robust to this scoring imprecision; quantitative comparisons between specific protocols at the 0.05-0.1 score difference level are not.

Second, the framework treats modes as independent dimensions when in fact they are mechanistically interconnected. Mode 4 (exhaustion) and Mode 5 (active suppression) overlap mechanistically. Mode 6 (persistence) is influenced by the engagement quality of Modes 1, 2 (which determine what memory populations form). The independent-dimensions treatment is a simplifying abstraction; the actual biology is more network-like than seven-dimensional.

Third, tumor-type effects modulate which modes are limiting in ways the framework treats qualitatively. A score of 1.0 on Mode 4 produces dramatic benefit in melanoma but minimal benefit in PDAC because Mode 4 is not the limiting failure mode in PDAC. The framework's tumor-type case studies acknowledge this but do not provide a formal weighting scheme.

Fourth, the scoring is based on mechanistic understanding rather than direct clinical outcome data. A protocol scoring 0.8 across all seven modes is *predicted* to produce qualitatively better outcomes than a protocol scoring 1.0 on Mode 4 alone — but this prediction has not been directly tested in head-to-head trials because such trials would require the multi-mode combinatorial protocols the framework recommends, which do not yet exist in systematic form.

Fifth, the framework does not address dose, schedule, sequencing, or toxicity in scoring mode engagement. These are critical practical dimensions of protocol design that the framework provides structural context for but does not directly address.

These limitations are acknowledged but do not invalidate the framework's structural contributions. The framework provides a coherent mechanistic vocabulary for evaluating intervention combinations, predictions about which combinations should produce benefit in which tumor types, and identification of specific gaps in the contemporary trial landscape where combinatorial-complete protocols could be tested.

Implications for trial design

The scoring framework's most direct practical implication is a recommendation for trial design philosophy. Contemporary immunotherapy trials predominantly test additions to existing standards of care — anti-X agent added to anti-PD-1, anti-Y agent added to anti-PD-1, et cetera. This trial design pattern is structurally limited: it can detect benefit from adding one mode of engagement to an existing single-mode standard, but it cannot test the framework's central prediction that combinatorial coverage across all seven modes is qualitatively different from sequential additions of single-mode interventions.

The framework recommends trial designs that test combinatorial-complete protocols against either:

- Standard of care alone (for tumors where standard of care has narrow mode coverage)

- Subcombinations of the combinatorial-complete protocol (factorial designs that test the contribution of specific modes)
- Other combinatorial-complete protocols with different mode-engagement strategies (e.g., bacterial PAMP-based vs oncolytic virus-based broad engagement)

Such trial designs are challenging because they require simultaneous availability of multiple investigational and approved agents, complex statistical designs to deconvolve the contribution of individual components, and substantial enrollment to detect the predicted benefit pattern. The framework does not solve these practical challenges but identifies them as structural priorities for the field.

The next section addresses the broader implications of the seven-mode framework for cancer immunotherapy research and provides the discussion of the paper's contributions, limitations, and recommended next steps.

10. Discussion

The seven-mode framework's contributions

The seven-mode framework's contributions are structural rather than empirical. The framework does not propose new mechanistic discoveries — every individual mode and intervention category discussed has been characterized by other researchers, often extensively. The framework's contribution is the integration: organizing the contemporary cancer immunology landscape around a structural account of where the failure modes are, which interventions address which modes, and where the gaps in combinatorial coverage create predictable patterns of clinical success and failure.

Four specific contributions emerge from this integration.

Predictive structural account of immunotherapy successes and failures. The framework predicts the empirical pattern across the past 15 years of clinical development. Where contemporary single-agent interventions provide strong Mode 4 engagement and the tumor's intrinsic biology adequately addresses Modes 1, 2, 3, 5, 6, 7 (melanoma, MSI-high CRC, high-TMB NSCLC), the empirical outcomes are dramatic. Where contemporary interventions provide narrow Mode 4 engagement but tumor biology leaves multiple other modes operative (pancreatic cancer, MSS CRC, much of TNBC), the empirical outcomes are limited. Where combination interventions have succeeded (anti-PD-1 + anti-CTLA-4 in melanoma; atezolizumab + bevacizumab in HCC; ANKTIVA + BCG in NMIBC), they have done so by extending mode coverage. Where combination interventions have failed (anti-TIGIT across multiple settings; anti-IDO in melanoma; multiple anti-CSF1R combinations), they have done so without extending mode coverage beyond what single agents already provided, or by adding only one additional mode where multiple were operative.

Identification of specific gaps in the trial landscape. The framework identifies specific multi-mode combinatorial protocols that are biologically rational but absent or sparse in current trials. For pancreatic cancer: multi-mode protocols engaging Modes 1, 2, 3, 5, 6, 7 alongside checkpoint blockade. For microsatellite-stable colorectal cancer: protocols engaging Modes 1, 2, 3 alongside checkpoint blockade. For triple-negative breast cancer (especially BRCA1/2-mutant): protocols extending the

platinum-plus-checkpoint backbone with intratumoral PAMPs, IL-15 superagonists, and stromal modulation. For all of these settings, the component interventions exist and are individually FDA-approved or in advanced clinical development; the combinatorial protocols do not.

Structural validation of historical empirical observations. The framework provides mechanistic interpretation for the historical Coley observations that have been difficult to integrate into contemporary frameworks. The sustained-dosing pattern that Coley pioneered is mechanistically meaningful from a memory T cell biology perspective (Mode 6 engagement) — not merely a clinical convention of the era. The bacterial PAMP-driven broad antigen exposure is mechanistically meaningful from a heterogeneity coverage perspective (Mode 7 engagement) — not merely a non-specific inflammatory effect. The fever-range thermal stress is mechanistically meaningful from an MHC upregulation and T cell trafficking perspective (Modes 1 and 3 engagement) — not merely a side effect of the bacterial preparation. The framework allows the historical empirical observations to be integrated with contemporary molecular biology in a way that respects both the historical record and the modern mechanism understanding.

Operational scoring methodology. The 0-1 mode scoring approach allows specific interventions and protocols to be evaluated systematically. This is not a substitute for clinical trials but provides a framework for prioritizing which trials are most likely to extend benefit and which represent further variations on already-explored single-mode patterns. The framework is most useful for trial design philosophy rather than for individual patient treatment decisions.

Comparison to other frameworks

The seven-mode framework is not the first attempt to organize cancer immunotherapy mechanistically. Several other frameworks deserve comparison.

The cancer-immunity cycle (Chen and Mellman, *Immunity* 2013;39:1-10) identified seven steps in the cycle from antigen release through T cell killing of tumor cells. The cancer-immunity cycle and the seven-mode framework share structural similarities but differ in emphasis. The cancer-immunity cycle frames the seven steps as a chronological cycle that all anti-tumor immune responses traverse; the seven-mode framework frames the seven categories as failure modes that operate in parallel and require parallel engagement. The cancer-immunity cycle is more useful for mechanism description; the seven-mode framework is more useful for combinatorial protocol design. The two frameworks are mostly compatible but not identical.

The cold tumor / hot tumor classification (multiple authors) divides tumors into immunologically "hot" (T cell-infiltrated, responsive to checkpoint blockade), "cold" (T cell-excluded or absent), and "immune-excluded" (T cells at tumor margin but not penetrating). This classification captures Mode 3 (exclusion) and partially Modes 1, 2 (inadequate antigen presentation or priming producing absent T cells). The seven-mode framework subdivides the "cold" category into mechanistic origins (Mode 1 versus Mode 2 versus Mode 3) and addresses Modes 4-7 not captured by the cold/hot dichotomy.

Specific immunotherapy biomarker frameworks (TMB, PD-L1, MSI status) identify single features predictive of response to specific interventions. The seven-mode framework is mechanistic rather than biomarker-based; it complements biomarker frameworks but operates at a different level of abstraction.

Computational immunotherapy prediction models (CancerGPT, COMPASS, IGeS-BS, others) use machine learning to predict combination synergies from preclinical and clinical data. These approaches have the advantage of leveraging large datasets but the disadvantage of limited mechanistic interpretability. The seven-mode framework is structurally interpretable but less data-driven. The two approaches could be complementary — machine learning models could provide quantitative predictions within mode-engagement categories, while the seven-mode framework provides the structural categories.

Limitations and counter-arguments

Several limitations deserve explicit acknowledgment.

The framework is structural rather than quantitatively predictive. The 0-1 mode scoring is qualitative; the prediction that combinatorial-complete protocols produce qualitatively different outcomes from narrow protocols is structural rather than quantitative. The framework does not predict specific response rates for specific protocols in specific tumor types. This limitation is real but reflects the available evidence base — the data to support more quantitative predictions does not yet exist in many cases.

The mode independence is a simplifying abstraction. Modes 4 and 5 overlap mechanistically. Mode 6 is influenced by Modes 1 and 2 (the quality of initial priming influences memory formation). Mode 7 interacts with Mode 4 (exhausted T cell populations may have different recognition profiles than fresh effectors). The framework's treatment of modes as independent dimensions is a simplification; the actual biology is more network-like.

The Coley framework's historical clinical record is uncontrolled by contemporary standards. The framework's argument that Coley engaged six of seven modes does not depend on the specific clinical outcomes Coley documented being correct — it depends only on the mechanism analysis being correct. But the persuasive force of the framework is partially derived from the Coley clinical record, and that record's epistemic status is genuinely contested. The framework acknowledges this and rests primarily on the mechanism analysis rather than on the historical clinical outcomes.

Some specific framework predictions have been challenged by negative trial results. The TIGIT phase III failures across multiple settings might be interpreted as evidence against the "more checkpoint inhibitor combinations should produce more benefit" thinking the framework partially endorses. The framework's response is that within-Mode-4 combination is not the same as cross-mode combination — the TIGIT failures support the framework's specific prediction that cross-mode combinatorial completeness matters more than within-mode extension. This response is mechanistically coherent but is not equivalent to the simpler "more checkpoints = more benefit" framing that some commentators have applied to checkpoint combinations.

The framework does not address toxicity, sequencing, dosing, or patient selection in detail. These practical dimensions are critical for actual trial design and patient treatment. The framework provides structural context but not operational protocols. Translating the framework's structural recommendations into specific trial protocols requires substantial additional work that this paper does not perform.

The author has personal involvement in cancer immunotherapy as a family caregiver. This is acknowledged in the introduction and does not invalidate the framework but does require explicit acknowledgment. The framework's arguments rest on published literature evidence reviewed in this paper rather than on the personal case, but the motivation for the systematic review derived from the personal context.

Implications for the field

If the framework's central prediction is correct — that combinatorial coverage across all seven modes is qualitatively different from sequential additions of single-mode interventions — several implications follow.

The next generation of immunotherapy advances will likely come from cross-mode combinatorial protocols rather than from continued single-mode optimization. The TIGIT failures and similar within-mode extension disappointments support this prediction. The CheckMate 067 success (anti-PD-1 + anti-CTLA-4) is partially within-Mode-4 but also cross-mode (CTLA-4 has Mode 5 Treg-depletion effects), which is consistent with the framework's prediction that cross-mode combinatorial benefit is qualitatively different from within-mode extension.

Trial design philosophy should shift toward combinatorial-complete protocols. Contemporary trial designs that add single agents to existing standards of care can detect single-mode benefits but cannot test the framework's central prediction. Factorial designs that test cross-mode combinatorial protocols are statistically and operationally challenging but address the framework's central question.

Specific tumor types deserve specific cross-mode protocol development. Pancreatic cancer, MSS colorectal cancer, much of TNBC, and other immunotherapy-refractory tumor types share the structural property that multiple modes are operative as failure mechanisms. Cross-mode combinatorial protocols developed for these tumors should be prioritized.

Historical empirical observations should be integrated with contemporary mechanism understanding. The Coley observations, the historical IL-2 successes, the BCG paradigm in NMIBC, and other observations that predate the modern molecular framework can be re-evaluated with the seven-mode framework in ways that may illuminate intervention strategies the contemporary single-mode-dominant paradigm has overlooked.

Independent and AI-assisted research has a role in field-level synthesis. This paper was developed by an independent researcher without institutional affiliation, using extensive AI-assisted literature review with systematic verification of all primary citations. The framework's arguments do not depend on access to proprietary data or specialized experimental equipment. The systematic integration of disparate

literature across cancer types and intervention modalities is a task well-suited to AI-assisted methodology with human structural and interpretive guidance. This methodological observation is offered as a reflection on how the contemporary cancer immunology literature could be better integrated, not as a claim that AI-assisted research replaces traditional experimental and clinical investigation.

Recommended next steps

Several specific next steps would test and extend the framework.

Trial design proposals for specific tumor types. The cancer-type case studies in this paper identify specific multi-mode combinatorial protocols that could be tested in pancreatic cancer, MSS CRC, and triple-negative breast cancer. Development of formal trial protocols for these cases, including patient selection criteria, dosing schedules, statistical designs, and biomarker integration, would translate the framework's structural recommendations into actionable proposals.

Retrospective analysis of existing combination trial data through the seven-mode lens. Many combination immunotherapy trials have been conducted and reported. Retrospective scoring of these trials' mode coverage and correlation with outcomes would provide empirical support (or refutation) of the framework's structural predictions. The data exist; the analysis through the seven-mode framework has not been systematically performed.

Refinement of the scoring methodology based on additional cases. The 0-1 mode scoring is presented in this paper with worked examples for several interventions. Application to additional interventions and protocols would test the methodology's consistency and identify areas where refinement is needed. A community of researchers applying the framework would refine it more rapidly than any single author can.

Integration with computational and machine learning approaches. The framework's structural categories could provide the interpretable scaffolding for machine learning models that quantify mode engagement and predict combinatorial benefit. The combination of structural interpretability with data-driven quantification could produce more useful prediction tools than either approach alone.

Patient-level case studies with multi-mode protocols. Individual patients receiving multi-mode combinatorial protocols outside of formal trials (off-label, compassionate use, or international centers like CHIPSA) provide informal evidence that, while not constituting clinical proof, can inform hypothesis generation about which combinatorial patterns produce benefit. The author's family case in BRCA1-mutated TNBC is one such case. Similar case collections would provide preliminary signal for trial development.

Conclusion

The seven-mode framework provides a structural account of cancer immunotherapy that integrates contemporary mechanism understanding with the historical empirical observations of the Coley framework. The framework's central prediction — that

combinatorial coverage across all seven failure modes produces qualitatively different outcomes from narrow single-mode interventions — is consistent with the empirical pattern across 15 years of clinical immunotherapy development and identifies specific gaps in the contemporary trial landscape where combinatorial-complete protocols could be tested.

The framework's contribution is structural rather than empirical: it does not propose new mechanisms or interventions but organizes the existing landscape in a way that makes the structural predictions explicit and actionable. The historical Coley framework, evaluated through the seven-mode lens, engages six of the seven modes through its bacterial PAMP-driven inflammatory and sustained-dosing mechanisms. Contemporary checkpoint inhibitors fill the one remaining gap. The integration of these — modern multi-mode protocols combining Coley-equivalent broad engagement with contemporary targeted blockade — is the structural prediction the framework recommends and the translational opportunity the field could pursue.

The next generation of cancer immunotherapy advances will likely come from this integration. The framework provides the structural vocabulary for designing and evaluating the protocols that could produce these advances. The clinical evidence base for component interventions across all seven modes already exists. What remains is the combinatorial protocol development and clinical testing that translates the seven-mode framework from structural prediction into clinical practice.

11. Key references

Citations are integrated throughout the text in full form (first author, et al., journal, year, volume:pages, DOI where applicable). Every primary citation below has been individually verified against the published source (PubMed, journal website, or both); the DOI links resolve to the cited paper. Two specific errors caught during this verification pass and corrected: (1) the breast cancer HLA-I alteration study originally attributed to "Aptsiauri et al." has Garrido MA as first author with Aptsiauri as senior author; (2) the synovial sarcoma/MRCL IFN-γ phase 0 trial originally listed as "Zhang Y" has Zhang S (Shihong Zhang) as first author. The following are the most important primary literature citations for the framework's key claims, organized by topic.

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