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EPIGENETIC STATES ACROSS IMMUNE MEMORY, TRAINING AND EXHAUSTION: A CLAIM-CALIBRATED SYNTHESIS FROM MECHANISM TO THERAPEUTIC REPROGRAMMING

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ABSTRACT

Immune memory cannot be explained solely by antigen-receptor clonotypes. Immune cells can also retain regulatory configurations whose effects depend on lineage, tissue and the conditions under which recall occurs. In this mechanistic review, we evaluate the literature available through July 2026 on DNA methylation, histone modification, chromatin accessibility, three-dimensional genome organization, non-coding RNA, and immunometabolism in adaptive memory, trained and tolerized innate responses, exhaustion, and disease-associated inflammatory memory. The evidence is interpreted within a five level framework. The foundation is composed of descriptive maps. Prospective prediction and functional recall are distinct but complementary pathways to causal perturbation and, ultimately, clinical usefulness. The level provided is the strongest inference supported by one study. It is not a formal bias assessment, nor a numeric quality score. Single-cell, longitudinal and perturbational studies are increasingly suggesting that the fate of the immune system is determined not by a single epigenetic mark, but by the order of incoming signals, the tissue context and cell identity. Interventions under investigation include chromatin-modifying drugs, manipulation of trained immunity, combinations with checkpoint blockade, and locus-specific epigenome editing. But persistent accessibility is not enough to suggest memory, predictive association is not enough to infer causation, and a causal regulatory element is not necessarily a safe therapeutic target. Hence, clinical translation will rely on longitudinal validation, cell-resolved biomarkers, confirmed target engagement, controllable persistence, selective delivery, and careful monitoring of unintended immune effects.

Keywords: Epigenetic Immunity; Immune Memory; Trained Immunity; Chromatin Accessibility; Single-Cell Multi-Omics; CRISPR Epigenome Editing

1 INTRODUCTION

The term epigenetics was coined to describe how the cellular phenotype can be stably maintained without changing the DNA sequence. In current molecular biology, epigenetics is defined as regulatory information inscribed on DNA, histones, chromatin structure and RNA that modulates gene expression and can be transmitted between cell divisions

or preserved as a stable cellular state [1–4]. This concept is particularly powerful for immunology as immune cells have to be sufficiently plastic to respond to infection but also maintain lineage identity, self-tolerance and memory. Despite a broadly shared inherited genome, immune-cell types read distinct regulatory landscapes; antigen-receptor rearrangement adds lymphocyte-specific genomic diversity [5–7].

Early ideas about immunological memory focused on antigen-specific lymphocyte clones and their receptors. The explanation is still needed but not enough. A memory T cell is not just one that is present. The metabolic programs, transcription factor networks, enhancers and promoters that allow for rapid recall must also be maintained. Without rearranged antigen receptors, innate cells deliver the instructive message that diverse microbial or vaccine exposures can enhance subsequent responsiveness. This expanded the notion of immune memory from clonal specificity to epigenetically regulated functional adaptation, thus introducing the concept of trained immunity [8–11].

To be a useful mechanistic account of epigenetic immunity, it has to link the immune phenotype that molecular regulators induce to the disease context in which that phenotype becomes detrimental and to the experimental tools needed to distinguish association from causation. Thus, each claim is evaluated against the study design that underlies it. Descriptive profiles can provide information for prospective prediction or functional-recall experiments. Clinical utility and locus-specific perturbation are evidence of utility. More rigorous designs are needed. This is an important distinction, because a persistent mark does not necessarily mean memory, just as an epigenetic difference seen in disease does not make it a therapeutic target. This line of reasoning is illustrated in Figure 1, beginning with regulatory state and exposure, through immune phenotype to translational interpretation.

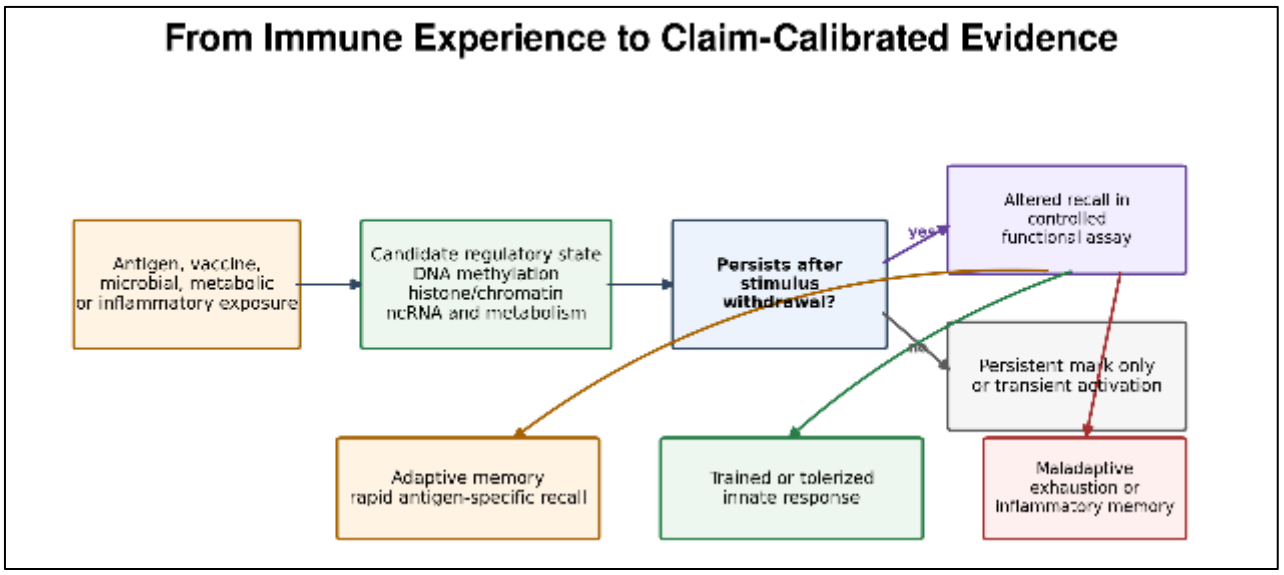


Figure 1: Conceptual model of epigenetic immune memory evaluation. Antigenic or environmental exposure may induce a persistent regulatory state, but its interpretation depends on prospective observation, a defined recall response, causal perturbation, and (in the case of translational claims) clinical validation.

2 LITERATURE SCOPE AND REVIEW STRATEGY

The review addresses three practical questions. What evidence is needed to determine a stable immune epigenetic state? When can we say that a state is predicting or producing a change in recall? What mechanisms are sufficiently robust to be considered as therapeutic targets? Topics of discussion include inflammatory memory in disease, exhaustion, trained and tolerated innate responses, and adaptive T- and B-cell memory. It also points to novel treatment platforms. Older studies were included when an important mechanism, technology or conceptual shift was identified. Highlighting newer reports that substantially altered the translational or mechanistic interpretation of the field [11–16].

In many cases the terminology of epigenetics is used more broadly than the underlying experiments warrant. To prevent overinterpretation, each claim was rated at the highest level acceptable to the claim design. Level 1: Descriptive evidence. Regulatory difference, Immune State. At Level 2, a state that is predicted to lead to a subsequent immune outcome is evaluated prospectively. For level 3, persistence beyond the acute response must functionally link to altered recall, cytokine production, killing or other immune output following a defined secondary challenge. Levels 2 and 3 are complementary but not necessarily consecutive stages, since prediction and functional recall yield different types of evidence. Level 4: Causal intervention. To change the phenotype, we need to perturb a regulator or locus. Level 5 is

defined as an intervention that has demonstrated immune target engagement, clinical meaningful benefit, and acceptable safety in the intended population. These levels relate to individual claims, rather than the overall quality of an article. Also, even strong causal evidence does not ensure that a target is therapeutically viable.

2.1 Literature search, evidence selection and reference verification

Searches of PubMed/MEDLINE, Web of Science Core Collection and Scopus covered each database from inception to 31 July 2026. A two-part concept search was translated into the syntax used by each platform. The core query was: ("immune memory" OR "immunologic memory" OR "trained immunity" OR "innate immune memory" OR "inflammatory memory" OR "T-cell memory" OR "B-cell memory" OR "T-cell exhaustion") AND (epigenetic* OR chromatin OR methylat* OR histone* OR "chromatin accessibility" OR "three-dimensional genome" OR "3D genome" OR "non-coding RNA" OR immunometaboli* OR "epigenome editing"). PubMed combined title/abstract searching with relevant controlled vocabulary. Scopus used TITLE-ABS-KEY, whereas Web of Science used Topic (TS). Google Scholar, reference lists and forward-citation searches served only to locate landmark papers or recent records not yet consistently indexed. Publication details and DOI links were checked against publisher pages, PubMed and Crossref.

We included peer-reviewed studies conducted in humans, animals or primary cells that studied an epigenetic characteristic of adaptive memory, trained or tolerant innate immunity, exhaustion or inflammatory memory. Studies were prioritised if they were primary studies, prospectively linked a measured state to a downstream immune outcome, resolved purified or single cell populations, perturbed a specific regulator or locus, reported on clinical target engagement and safety, used a defined secondary challenge, or examined the durability of a state after withdrawal of the initiating stimulus. Studies restricted to acute transcription and not including epigenetic measurement were excluded from the primary evidence map. The same principle was applied to conference abstracts with insufficient methodological information and to non-immune models with no direct mechanistic relevance. Outside the immune system, the success of epigenome editing work depended on the ability to demonstrate the functionality of the platform.

The author reviewed the records in two stages. Titles and abstracts were screened for any association of an epigenetic measurement or perturbation with a phenotype involving immune memory, training, tolerance or exhaustion. Subsequent full-text assessment evaluated cell source, timing of sampling, persistence after stimulus withdrawal, definition of the secondary challenge, epigenetic assay, functional endpoint, prospective structure, and use of regulator- or locus-specific perturbation. Screening was not independent. We aimed for a mechanistic synthesis that was transparent, not an estimation of a pooled effect.

The features used to evaluate study design, cell source, temporal structure, functional endpoints and perturbational evidence are presented in Table 1. The review was narrative and purposive and was conducted by one author. It was not registered as a systematic review. As such it does not claim to have formal study level risk of bias scoring, exhaustive retrieval counts, duplicate screening or pooled effect estimates as per the PRISMA flow diagram. Concept blocks, eligibility rules and appraisal criteria are reported to show the basis for selection and interpretation, not to suggest that the systematic review is complete.

Table 1: Evidence features used to appraise studies included in the narrative synthesis.

Evidence dimension	Preferred evidence	Criterion that upgrades confidence	Main residual threat
Molecular mechanism	Chromatin and expression measured in purified or single immune-cell populations	Locus- or regulator-specific perturbation changes immune function	Reverse causation; activation-induced marks
Memory durability	Longitudinal sampling before, after and well beyond the initiating stimulus	Persistence after stimulus withdrawal plus accelerated or altered recall	Transient activation misclassified as memory
Cell and tissue context	Sorted-cell, single-cell or spatial profiling with composition control	Replication across cohorts, tissues or model systems	Cell-mixture and microenvironmental confounding
Prediction	Prospective association with vaccine response, relapse or treatment outcome	External validation, calibration and added value over clinical predictors	Overfitting, batch effects and population shift
Translation	Target engagement, functional benefit and safety in a relevant model or trial	Biomarker-guided validation with selective delivery and durability monitoring	Global chromatin toxicity and off-target immune reprogramming

2.2 Novelty and added value of this review

But, rather than comparing epigenetic features across innate and adaptive lymphocyte memory as previous syntheses have done, more focused reviews have examined adaptive memory, trained immunity, inflammatory memory in tissues, or the persistence of trained epigenetic states [12, 16–19]. This review does not presume that these topics have not been studied. Its contribution is the application of one evidentiary scheme to five different enquiries: whether a regulatory feature is correlated with a state, predicts a subsequent response, contributes to functional recall, exerts causal control, or has shown clinical value. The same paradigm is used to study disease-associated inflammatory memory, exhaustion, trained and tolerated innate responses, and adaptive T- and B-cell memory. Thus, a durable chromatin feature is not a memory unless recall is assessed. A causal mechanism is not a therapeutic option unless immune safety and benefit are demonstrated.

The term memory is reserved for a state that persists beyond the completion of the primary response and that produces a measurable change in a predicted secondary response. Chromatin accessibility that is present without a recall experiment can be described with terms such as persistence or priming . A similar distinction is required for innate cells: training is defined as a return to baseline levels of activity prior to heterologous restimulation, and tolerance is defined as a less robust secondary response. If there is no recall design, a stable lineage shift is taken as differentiation and not memory [20].

3 CORE EPIGENETIC MECHANISMS IN IMMUNE CELLS

DNA methylation is a relatively stable epigenetic modification that involves the addition of a methyl group to cytosine at CpG sites. DNMT1 preserves methylation patterns established after DNA replication, whereas DNMT3A and DNMT3B establish new methylation. Enzymes in this group are involved in the repression of inappropriate gene programs, stabilisation of differentiation, and limitation of inflammatory activity in immune lineages. TET-family enzymes act in the opposite direction and oxidise 5-methylcytosine to 5-hydroxymethylcytosine. This promotes active or passive demethylation and can increase the accessibility of promoters or enhancers after activation and differentiation [21–24]. Thus, the balance between these two enzyme systems is key in the regulation of regulatory and effector T cells, B cell differentiation and the development of myeloid immune states.

Histone modifications allow a more dynamic level of control. Lysine acetylation is generally associated with active transcription and open chromatin. Transcription can be activated or repressed depending on the residue methylated. Promoters are enriched in H3K4me3, enhancers are enriched in H3K4me1, active enhancers are enriched in H3K27ac, and repression is often associated with H3K27me3 and H3K9me3. These marks are written, read and erased by families of enzymes including histone acetyltransferases, deacetylases, methyltransferases, demethylases, bromodomain proteins and Polycomb complexes [25–28]. These chromatin features allow the immune cell to transition from quiescence to activation, tolerance, exhaustion and memory.

ATP-dependent chromatin-remodeling complexes regulate DNA accessibility by repositioning nucleosomes and changing their local density. ISWI, CHD/NuRD, INO80 and SWI/SNF-family BAF complexes are all involved in inducible transcription and immune cell differentiation29,30. Regulatory RNA adds another layer of control. Non-coding RNAs can change mRNA stability, recruitment of chromatin machinery, strength of inflammatory signalling and transcriptional silencing. For example, miR-155 promotes immune differentiation and inflammatory activity, while miR-146a limits excessive Toll-like receptor and NF-κB signalling [31–35]. The molecular components, immune effects and evidence supporting these mechanisms are compared in Table 2 and assembled in Figure 2.

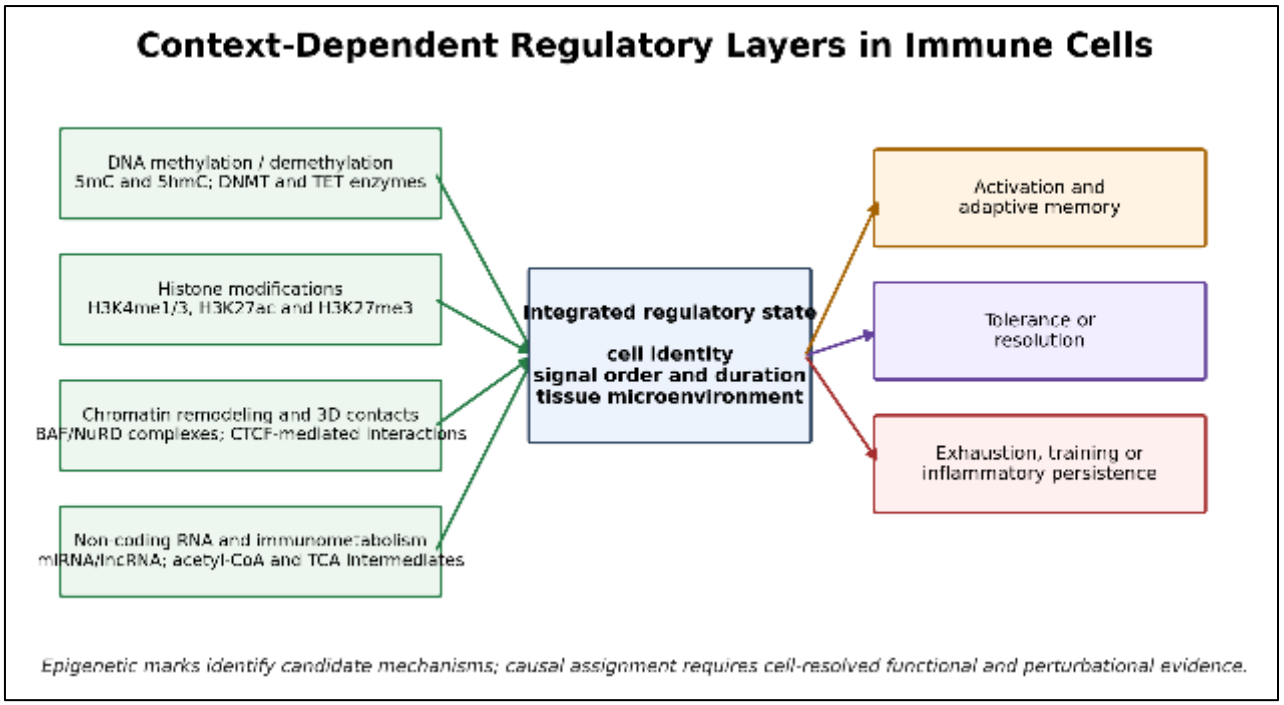


Figure 2: Context-dependent regulatory layers in immune cells. DNA modification, histone state, nucleosome remodeling, three-dimensional contacts and regulatory RNA interact with cell identity and signal order rather than acting as an isolated deterministic code.

Table 2: Principal epigenetic mechanisms and their documented immune effects.

Mechanism	Principal molecular components	Typical immune consequence	Representative evidence
DNA methylation / demethylation	DNMT1, DNMT3A, DNMT3B; TET1-3; 5mC and 5hmC	Stabilizes lineage identity; silences inappropriate genes; regulates tolerance and effector differentiation	[21–24, 36]
Histone acetylation and methylation	HATs, HDACs, HMTs, demethylases; H3K4me3, H3K27ac, H3K27me3, H3K9me3	Controls promoter/enhancer activity, inflammatory responsiveness, memory priming, and exhaustion	[25–28, 37, 38]
Chromatin remodeling	SWI/SNF/BAF, ISWI, CHD/NuRD, INO80 complexes	Regulates transcription-factor access during activation, differentiation, and antiviral responses	[29, 30]
Non-coding RNA regulation	miRNAs, lncRNAs and chromatin-associated regulatory RNA	Fine-tunes inflammatory signaling, cytokine production, and immune-cell subset differentiation	[31–35, 39]
3D genome organization	Chromatin loops, TADs, enhancer-promoter contacts	Coordinates long-range immune gene regulation during activation and differentiation	[7, 40, 41]

4 EPIGENETIC BASIS OF ADAPTIVE IMMUNE MEMORY

Adaptive recall quality is determined by factors other than the survival of antigen-specific cells. During the primary response, naive T cells reprogram DNA methylation and chromatin accessibility at loci regulating cytokine production, cytotoxic function, migration and transcription-factor activity. Open or poised regulatory regions allow some cells to survive the contraction phase. This configuration permits rapid expression of IFNG, TNF, IL2, GZMB, PRF1 and related effector genes upon re-stimulation with antigen [8, 36, 37, 42, 43]. In the framework used in this study, a map of accessibility or methylation is ranked as Level 1 evidence. When it predicts retrieval, it goes to Level 2, when it is linked to an immune function assay it goes to Level 3, and only when the relevant locus or regulator has been altered does it go to Level 4.

One possible explanation for the rapidity of T-cell recall is enhancer priming . Memory T cells can maintain H3K4me1-marked enhancers in a poised state and then acquire H3K27ac and transcriptional machinery soon after restimulation. This arrangement allows for a faster response than that of naïve cells [37], even when both populations are at resting baseline. A related principle in B cell memory is that the recall response is modulated by accessibility of loci involved in antigen presentation, survival, plasma cell differentiation and immunoglobulin regulation. Thus, adaptive memory is maintained by a regulatory system that works in concert with antigen specificity and not only receptor specificity.

4.1 Memory B-cell epigenetic states

The fate of an activated B cell is not determined only by the sequence of its antigen-receptor. Chromatin accessibility, transcription-factor occupancy, and metabolic state also determine if the cell goes back to the memory pool or moves on to antibody secretion. Prior stimulation is associated with the balance of BLIMP1 and BACH2 during recall as shown by evidence from epigenetic recording, which determines the fate of memory B-cells [44]. This experiment is indicative of a functional interpretation of chromatin state, as the recorded history was associated with a later cellular outcome and not an inference from a resting profile alone.

Memory B cells in different regulatory states. There is evidence that type I interferons can drive a memory subset with a unique epigenetic signature during chronic viral infection. This implies that the inflammatory milieu is capable of reconfiguring the compartment rather than just expanding or depleting a canonical memory population [45]. This state might increase responsiveness in one sense and decrease differentiation in another. However, one should not treat it as a net positive memory in itself. Typically, the regulatory regions used during recall are defined by the cell's identity, anatomical location and exposure history [12].

Interpretation is most convincing when a cell-resolved regulatory map is combined with a defined recall challenge, fate tracking, and an output such as antibody production or plasma-cell differentiation. Differences in chromatin measured in isolation are classified as Level 1. Evidence that a state measured prior to recall predicts the subsequent response supports Level 2 . Evidence that a persistent state is associated with altered differentiation or antibody output supports Level 3 . Level 4: Perturbation of the proposed enhancer, transcription factor, or chromatin regulator The intervention or biomarker should be prospectively validated in the target population for clinical utility.

4.2 Signal order and adaptive programming

The epigenetic mechanism is intimately related to the series of signalling events . The same cytokine, delivered after a different sequence of receptor inputs, can have a different outcome. Pre-exposure of cytotoxic lymphocytes to antigen-receptor engagement followed by IL-12 exposure facilitated adaptive programming, and cooperation of AP-1 factors achieved the redirection of IL-12-STAT4 binding. Terminal effector differentiation was promoted by IL-12 administration in the absence of the needed priming signal [46]. Thus, the relevant biological unit is the regulatory state that receives the downstream signal—not an isolated histone mark or transcription factor.

Signal-dependent programming is also limited by the genome topology. CTCF-dependent chromatin contacts that limit the generation of memory CD8+ T cells can influence lineage outcome, indicating that enhancer-promoter communication can influence lineage outcome even when using the same signalling pathways [41]. Signal order, cell state and three-dimensional architecture are then connected in that prior stimulation generates a regulatory context in which subsequent inputs act and chromatin organization determines which regulatory elements are interactable.

These results also have practical implications for the design of experiments. One cannot reconstruct the sequence of development of a regulatory state by sampling only at the end points. A better design would have an order of stimulation, would take samples before and after withdrawal of stimulus, would have a controlled recall interval, and would compare cells exposed to the same signals in a different order. To program causality from a temporal association, the transcription factor or chromatin interaction involved must be disrupted.

4.3 Exhaustion as an alternative branch

Tiredness is a clinically relevant outcome and distinct from traditional memory. Chronic infection or cancer results in a landscape of T cell epigenomic features characterised by accessible regions associated with exhaustion and less accessible effector-memory loci. The progenitor-like cells in the exhausted compartment show a greater degree of self-renewal and contribute more to the response to checkpoint inhibition than the terminally exhausted cells that are more tightly constrained by their chromatin state [47]. This distinction may explain why PD-1 blockade can yield substantial responses in certain settings but has limited effects once the exhaustion program is epigenetically established [38, 48]. Alternative trajectories of memory and exhaustion are illustrated in Figure 3, and compared across major immune-memory states in Table 3.

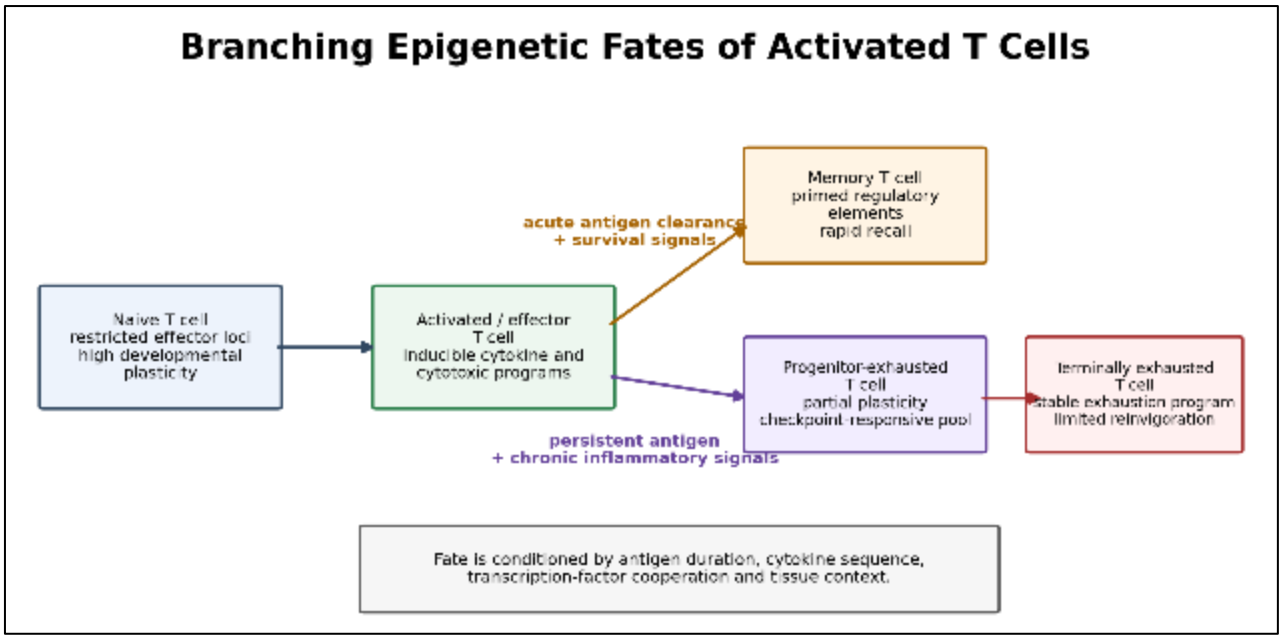


Figure 3: The fate forks of T cells post-activation. Activated cells can differentiate into durable memory, progenitor-exhausted or terminally exhausted populations depending on context cues and persistence of antigen. Thus, exhaustion is conceptualised as a separate regulatory process rather than as a stage that follows memory.

Table 3: Epigenetic configurations across major immune memory states.

Immune state	Epigenetic signature	Functional outcome	Therapeutic relevance
Naive T cell	Restricted effector-gene accessibility; developmental plasticity; quiescent metabolic state	Slow primary response requiring full activation and remodeling	Baseline for vaccine and immune-aging studies
Effector T cell	Open cytokine/cytotoxic loci; high H3K27ac at inducible enhancers	Rapid inflammatory or cytotoxic output but limited persistence	Useful for acute infection; may cause immunopathology
Memory T cell	Poised enhancers; accessible recall genes; retained transcription-factor networks	Fast recall with lower activation threshold	Goal of durable vaccines and adoptive cell therapies
Progenitor- and terminally exhausted T cells	Branch-specific accessibility; progenitor states retain renewal potential, whereas terminal states show more entrenched exhaustion programs	Heterogeneous dysfunction during persistent antigen exposure; limited reversibility in terminal states	Progenitor states are more responsive to checkpoint blockade than terminal states [47]
Trained monocyte/macrophage	H3K4me3/H3K27ac enrichment at inflammatory promoters/enhancers	Enhanced secondary response to heterologous stimulation	Protective against infection but risky in chronic inflammation

5 TRAINED IMMUNITY AND INNATE IMMUNE MEMORY

Following a primary stimulus, innate cells may retain a functional imprint that changes their response to a later challenge. The secondary response may be stronger (training) or weaker (tolerance), with the direction determined by stimulus identity and dose, exposure timing, cell source and tissue environment. Unlike adaptive memory, the trained response does not require rearranged antigen receptors. β-glucan, *Candida albicans*, BCG, oxidized LDL and dietary inflammatory stimuli have each been used to demonstrate such reprogramming in monocytes, macrophages, natural killer cells or myeloid progenitors [9–11, 49–53]. Chromatin alteration alone supports a Level 1 claim. A persistent

change demonstrated by heterologous recall in a controlled functional assay supports Level 3, whereas Level 4 requires perturbation of the responsible regulator or locus.

Metabolism and chromatin regulation are intimately connected in the development of trained immunity. A major challenge can alter the flux through the TCA cycle, cholesterol synthesis, glutaminolysis, oxidative phosphorylation and glycolysis. These alterations change the accessibility of acetyl-CoA, SAM, fumarate, succinate and other metabolites used by chromatin-modifying enzymes. Consequently, activating marks such as H3K4me3 and H3K27ac may remain at the promoters or enhancers of inflammatory genes, such as TNF, IL6, and IL1B [11, 49–51].

Evidence that training can begin in hematopoietic stem and progenitor cells has broadened the field beyond mature innate cells. BCG vaccination and inflammatory exposures can alter progenitors so that their myeloid descendants display enhanced antimicrobial activity. In this way, a systemic exposure may influence the composition and response potential of future immune-cell populations [13, 54–56]. The same plasticity can be harmful. Persistent or excessive training has been linked to atherosclerosis, metabolic inflammation and tissue injury [56, 57], so protective effects cannot be considered independently of inflammatory risk. Nor does a mechanistic signature of training establish clinical protection against a particular disease: BCG-Denmark failed to reduce COVID-19 risk relative to placebo in a randomized trial of health-care workers [58].

The inflammatory memory is not restricted to cells circulating in the blood. Changes in DNA and histone regulation, nucleosome positioning, and kinetics of response allow tissue-resident immune cells and non-immune stromal or parenchymal cells to remember past exposure. Such memory has been demonstrated directly by changes in the response to further tissue injury in skin epithelial stem cells, caused by previous inflammation [18, 59]. In the tissue the effect is context-dependent and should not be assumed to confer a generally beneficial training. Related studies in the central nervous system indicate that inflammatory experiences may be sustained in other immune and non-immune compartments [60]. Figure 4 distinguishes between peripheral reprogramming of mature cells and central programming of HSPCs, showing that both approaches could result in either benefit or harm.

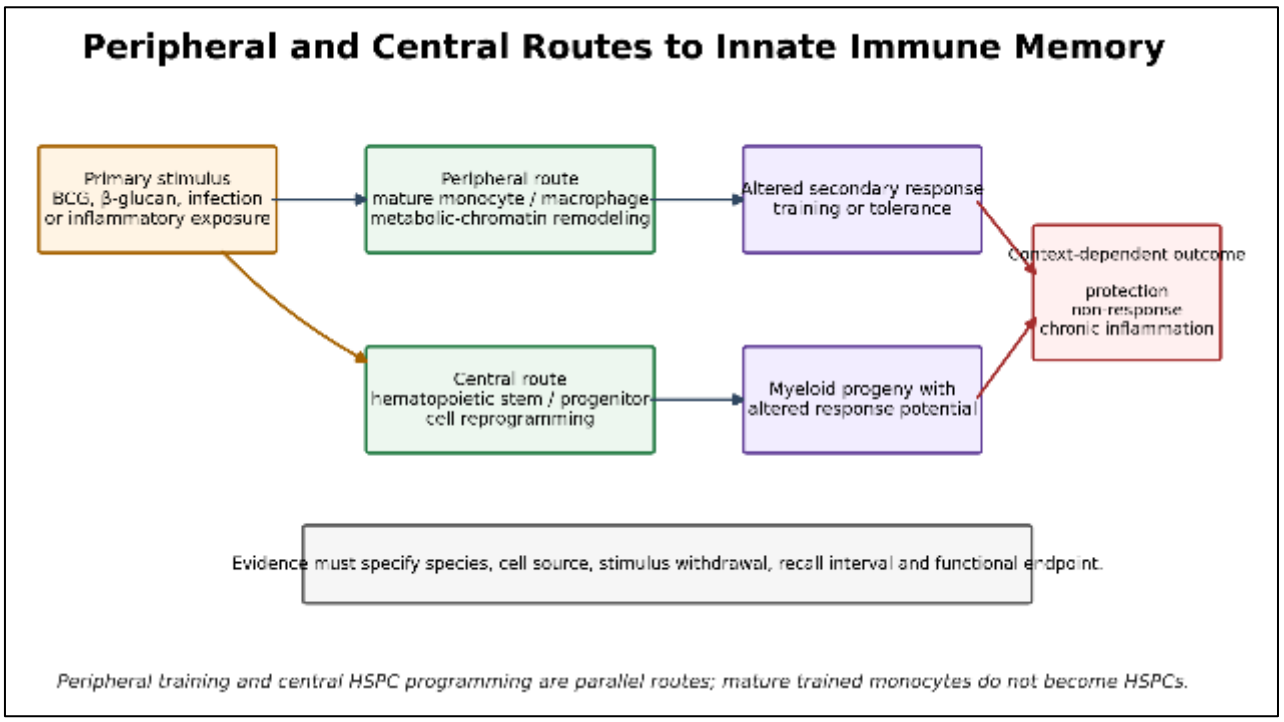


Figure 4: Parallel routes to trained or tolerized innate responses. Peripheral reprogramming in mature cells and central programming in hematopoietic stem and progenitor cells can alter later myeloid output, with benefit or harm determined by context.

6 EPIGENETIC DYSREGULATION IN IMMUNE-MEDIATED DISEASE

Human disease epigenomes are informative, but also overinterpreted. Systemic lupus erythematosus CD4+ T cells show DNA methylation changes that are related to autoreactive and interferon-related inflammatory pathways. Genome-wide methylation and twin-discordance studies support environmental and regulatory effects even when the inherited background is shared [61, 62]. Differences in chromatin accessibility between immune-mediated diseases may point to

candidate regulatory mechanisms but do not establish causality on their own [15]. Thus, most of these datasets are still at Level 1: the observed signal can be explained by cell composition, treatment or reverse causation. A pattern has to be prospectively replicated and perturbed before it can be considered predictive or causal.

Epigenetic regulation of both sides of the tumor-immune interaction promotes immune escape. DNA methylation and repressive chromatin in cancer cells may downregulate antigen-presentation machinery, interferon-response genes, or ligands necessary for immune recognition. At the same time, myeloid populations can develop suppressive programs that limit the effectiveness of antitumor responses and tumor-infiltrating lymphocytes can become exhausted. Thus, in addition to mutation, reversible or stable epigenetic states affect tumour immune evasion [38, 48, 63, 64].

A third environment in which regulatory memory may become maladaptive is that of chronic inflammatory disease. The Western diet and metabolic stress models induce durable myeloid changes favouring inflammatory readiness over resolution [16, 56, 57]. The practical question is not just whether memory is there, but whether it is protective, pathogenic or can be safely reset. Table 4 provides the most common regulatory abnormalities, rationale for intervention, and disease contexts, all subject to the evidence limitations described above.

Table 4: Epigenetic mechanisms in immune-mediated diseases and cancer immune evasion.

Disease context	Dominant epigenetic issue	Immune consequence	Potential intervention logic
Systemic lupus erythematosus	Altered DNA methylation and interferon-associated regulatory programs in T cells	Autoreactivity, inflammatory amplification, loss of tolerance	Candidate logic (not clinically validated): restore tolerance programs and evaluate methylation biomarkers
Rheumatoid arthritis / chronic inflammation	Persistent activation of stromal and myeloid enhancer landscapes [65]	Inflammatory memory and resistance to resolution	Candidate logic: test selective inhibition of pathogenic trained-like programs or inflammatory enhancers
Cancer immune evasion	Silencing of antigen-presentation and interferon-response programs; T-cell exhaustion epigenome	Reduced recognition by CD8+ T cells and poor checkpoint response	Context-dependent clinical strategy: combine epigenetic priming with checkpoint blockade or adoptive cells
Metabolic inflammation / atherosclerosis	Maladaptive training of myeloid cells after diet or lipid exposure	Sustained inflammatory readiness	Preclinical logic: interrupt NLRP3/metabolic-epigenetic feedback while monitoring host defense

7 THERAPEUTIC APPLICATIONS AND TRANSLATIONAL OPPORTUNITIES

7.1 Approved epigenetic drugs and immune effects

Most of the epigenetic drugs have been brought to the clinic through oncology, where they were recognised as druggable targets. These include DNA methyltransferases, histone deacetylases, mutant IDH enzymes, EZH2 and related chromatin regulators. The clinical role of hypomethylating therapy was established by azacitidine and decitabine. Conversely, vorinostat and romidepsin also show potential toxicity that may be related to broad HDAC inhibition. Another reason why tazemetostat is helpful is that approval in certain follicular lymphoma indications shows it is helpful in that setting; it does not show that immune memory has been therapeutically reprogrammed [66]. A Level 5 claim would need evidence of immune target engagement, a prospectively defined biomarker associated with benefit and acceptable immune safety.

The immune effects of these drugs will depend on the cellular compartment exposed. Chromatin-directed treatment may alter the composition of the tumour microenvironment, antigen presentation and chemokine output. DNMT inhibition may release endogenous retroviral transcripts and induce interferon signalling [14, 63, 64]. In different setups the result is not the same. Studies of immune-cell development and tumour progression have shown that the same mechanistic class can lead to different outcomes depending on the cell identity and disease context [39].

The maps of human diseases clearly show the limits of systemic treatment. Fibroblast-like synoviocytes derived from different anatomical sites in rheumatoid arthritis exhibit distinct epigenetic characteristics consistent with stromal heterogeneity in the regulation of pathogenic states [65]. Therefore, one cannot infer that correction of an unrelated

immune or stromal program is due to activity against a chromatin enzyme in a single tumour or cell population. Approved agents are valuable and clinically tested tools, but their use to alter immune memory would require cell-resolved biomarkers, dose-specific confirmation of target engagement and evidence that the desired immune response can be divorced from more general chromatin toxicity.

7.2 Combination epigenetic therapy and immunotherapy

There is a mechanistic rationale for combining immunotherapy with epigenetic therapy. Chromatin-directed agents may boost tumor-antigen presentation, trigger viral-mimicry responses and alter the microenvironment to be inflammatory. The ability of DNMT inhibitors to activate interferon signalling and to reveal endogenous retroviral elements provides the rationale to use them in combination with checkpoint blockade [63, 64]. Other agents may impact chemokines, suppressive myeloid programs or accessibility of effector genes. We do not know if chromatin opening is helpful in all tumours or immune compartments; these observations propose hypotheses that can be tested.

The schedule of treatment and the cellular selectivity are not secondary details. A more comprehensive effect on normal chromatin may also suppress T-cell proliferation or a beneficial immune state, in addition to enhancing the immunogenicity of tumour cells. Also priming before checkpoint blockade might be different than simultaneous or prolonged dosing. Combination studies should also measure target engagement in tumour and immune cells separately [14]. The sequence of treatment should be documented and each pharmacodynamic change should be correlated with the clinical outcome .

Therefore, a translational study should define a priori the cell population of interest, the chromatin or transcriptional biomarker to be evaluated and the expected direction of its alteration. Transient inflammation must be differentiated from durable reprogramming and immune toxicity must be actively followed up. In the absence of these elements, even when the combination of treatments is active, it is not possible to confidently attribute a clinical response to an epigenetic mechanism.

7.3 Modulating trained immunity

There are two separate therapeutic challenges within the framework of trained immunity. Vaccines or adjuvants can be used to boost protective heterologous responsiveness, while chronic inflammatory disease may require suppression of maladaptive myeloid-training. Multi-omics studies have identified post-vaccination cell states related to later trained responses and BCG has the capacity to reprogramme mature monocytes and haematopoietic progenitors [13,52,54]. This evidence supports both biological plausibility and functional training. But it doesn't guarantee that you are safe from all unconnected infections.

The BCG-Denmark trial provides a useful boundary for that interpretation. Although mechanistic evidence suggests that BCG can induce training, vaccination did not alter the risk of COVID-19 among health-care workers [58]. Hence, a clinical endpoint should be appropriate to the pathogen, population, duration of exposure and proposed route of protection. Prospective immune biomarkers can indicate that training has been done, but they can't replace the pre-defined disease outcome.

The converse risk is ongoing inflammatory training. Diet-induced metabolic reprogramming may lead to a maladaptive myeloid state that can trigger NLRP3-dependent innate immune reprogramming, as shown by experimental evidence [56]. It is vital to maintain antimicrobial defence and prevent cells from moving to tolerance in an effort to reverse such a program. Hence, a Level 5 claim would require monitoring specifically for impaired antimicrobial responsiveness or inflammatory injury, a priori biomarkers of target engagement and disease-specific randomised outcomes [11, 16, 19, 53].

7.4 Programmable epigenome editing

Locus-directed epigenome editing is one approach to circumvent the lack of selectivity often observed with systemic chromatin drugs . Modification of transcription at specific promoters or enhancers without generating a DNA double-strand break can be achieved by coupling catalytically inactive Cas9 to effectors like p300, DNMT3A, TET1 or KRAB. Initial evidence showed that individual regulatory elements can be activated or silenced by targeted chromatin modification upon recruitment of p300 and CRISPR-based repression [67, 68]. This principle was extended later by CRISPRoff, which generated transcriptional memory via targeted DNA methylation and repressive chromatin [69].

The question is not simply whether an edited state can be produced within the framework of translation. When required, it should be reversible, effective for the required length of time and limited to the correct immune-cell population. Genetic and epigenetic programs can be used synergistically to control a broad spectrum of cell functions, advancing the field of immune-cell engineering [70]. Experiments in primary human T cells have demonstrated this.

Accessibility atlases can help pinpoint transcriptional regulators, but nomination is not validation and direct perturbation is still needed before a candidate can be considered a causal control point [71].

Before clinical trials, it is essential to assess editing efficiency and persistence through cell division, immunogenicity, reversibility, off-target transcription, and delivery. Initially, ex vivo editing may be easier to control because the cells can be characterised before infusion. In vivo delivery further compounds the need for tissue and cell-type specific delivery. The goal is not maximal nor permanent activation, but the setting up of an immune program that is durable and manageable in case of an unintended state. Table 5 summarises the rationale and the main constraints of the strategies available today.

Table 5: Therapeutic strategies emerging from epigenetic immunology.

Strategy	Representative targets or tools	Immunological rationale	Major limitation
DNMT inhibition	Azacitidine, decitabine; DNMT1 depletion	Restore silenced immune genes; induce interferon/viral mimicry signaling	Global methylome effects; cytopenias; schedule sensitivity
HDAC inhibition	Vorinostat, romidepsin, class-selective HDAC inhibitors	Increase chromatin accessibility and antigen-presentation programs	Broad acetylation effects; immune-cell toxicity at high exposure
EZH2 / Polycomb targeting	Tazemetostat and other PRC2/EZH2-directed strategies [66]	Reverse H3K27me3-mediated silencing and reshape tumor immune recognition	Context-dependent effects on tumor and immune cells
Checkpoint + epigenetic combination	DNMTi/HDACi/EZH2i plus anti-PD-1/PD-L1/CTLA-4	Prime immune-cold tumors and improve antigen presentation	Optimal timing and biomarkers remain unresolved
Programmable epigenome editing	dCas9-p300, dCas9-DNMT3A, dCas9-TET1, CRISPRoff/on	Locus-specific activation or silencing without DNA double-strand breaks	Delivery, durability, reversibility, off-target chromatin effects
Trained-immunity modulation	BCG or adjuvant training; metabolic, IL-1 or NLRP3 pathway modulation	Enhance heterologous host defense or damp maladaptive myeloid training	Disease and context specificity; training-versus-tolerance uncertainty; inflammatory risk

8 STATE-OF-THE-ART TECHNOLOGIES

Modern epigenetic immunology is driven by high-resolution technologies. ChIP-seq maps histone modifications and transcription-factor occupancy; ATAC-seq measures chromatin accessibility; bisulfite-based methods profile cytosine modification, whereas dedicated oxidative or enzymatic approaches are needed to distinguish 5mC from 5hmC; Hi-C and capture-based methods resolve three-dimensional contacts and enhancer-promoter links; and single-cell multi-omics connects chromatin state to transcriptome and phenotype in individual immune cells [7, 40, 42, 72, 73].

Single-cell chromatin accessibility has transformed the field by revealing that apparently homogeneous immune populations contain multiple regulatory states. In tumors, scATAC-seq can distinguish exhausted, precursor-exhausted, memory-like, and tissue-resident T-cell states. In infection and vaccination, multi-omics can identify epigenetic predictors of cytokine responsiveness and trained immunity [13, 72, 73]. Recent chromatin-accessibility variation studies further suggest that regulatory variation helps explain immune-mediated disease risk beyond coding mutations [15].

Spatial epigenomics extends cell-resolved analysis by retaining tissue architecture and local microenvironmental context. Recent methods can profile chromatin accessibility in formalin-fixed, paraffin-embedded tissue, broadening access to clinically annotated archival cohorts; however, resolution, tissue chemistry and batch correction still constrain cross-study comparability [74].

No individual assay is likely to resolve causality. More informative studies will connect longitudinal samples with purified immune-cell populations, perturbation experiments, epigenome editing, spatial information and computational analysis. Machine learning may be useful when multi-omics data are sparse, but a predicted regulatory relationship still requires experimental testing—particularly when it is used to support a therapeutic recommendation.

Interpretability is also dependent on the study design. Establish cell-isolation procedures before analysis. Balance batches. Use spike-in or reference controls appropriate to the assay. Explicitly model donor and treatment effects. Independent cohorts and orthogonal functional assays are still needed. Computational integration can be used to rank potential regulatory elements, but perturbation is the only available means to determine whether an element is necessary, sufficient or just associated with the observed state. Table 6 compares the major outputs, strengths and limitations of the available technology platforms and Figure 5 shows how these steps can be integrated into a translational workflow.

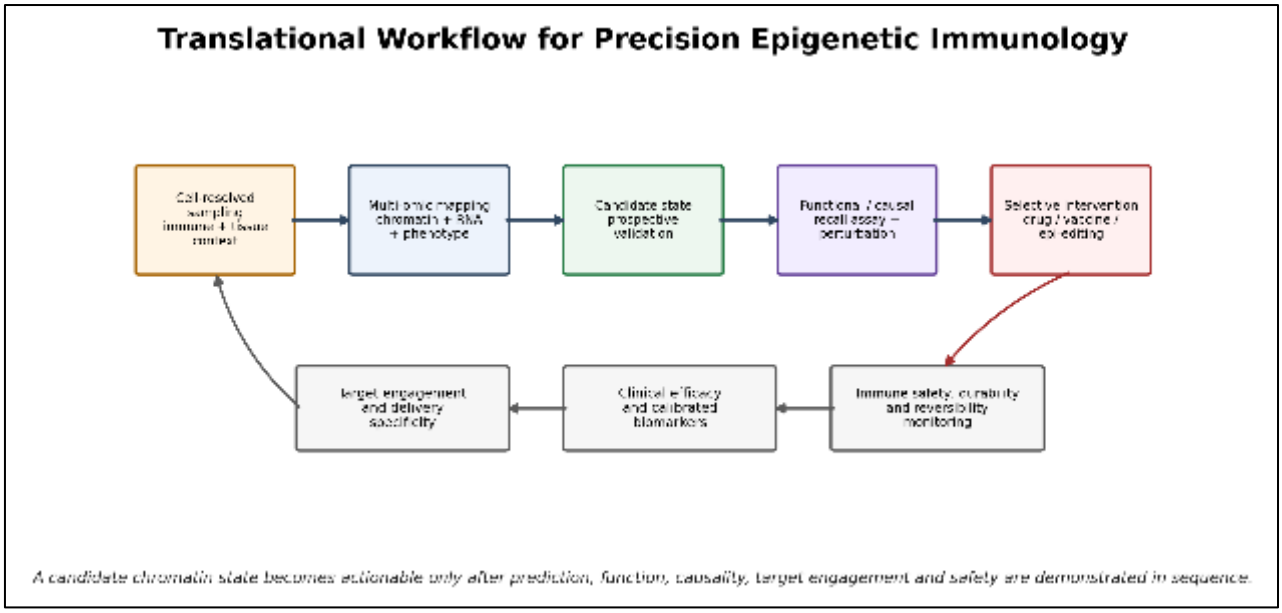


Figure 5: Translational workflow for immune epigenetics, integrating longitudinal sampling, cell-resolved profiling, prediction, orthogonal functional testing, causal perturbation, target-engagement measurement and safety assessment.

Table 6: Technology platforms for studying epigenetic immunity.

Platform	Main output	Strength	Caution
ChIP-seq / CUT&RUN / CUT&Tag	Histone marks and transcription-factor occupancy	Genome-wide enrichment or occupancy mapping	Antibody specificity and cell-number requirements
ATAC-seq / scATAC-seq	Open chromatin regions and regulatory accessibility	Sensitive mapping of promoters and enhancers; single-cell states	Accessibility does not prove regulatory causality
DNA methylome sequencing	CpG methylation profiles; 5hmC only with chemistry that distinguishes it from 5mC	Stable lineage and disease markers	Cell mixtures confound bulk profiles; conventional bisulfite does not distinguish 5mC from 5hmC
scRNA-seq + multi-omics	Transcriptome linked to chromatin or surface markers	Connects regulatory state to phenotype	Integration choices can bias clusters
CRISPR epigenome editing	Targeted activation or repression of selected loci	Tests causality and enables programmable therapy	Delivery and off-target monitoring are essential

Spatial epigenomics	Regulatory state in tissue context	Preserves microenvironmental context; spatial chromatin profiling is emerging [74]	Resolution, throughput, tissue compatibility and cost remain method-dependent

9 DISCUSSION

There are three perennial problems in the literature . Establishing causality is especially difficult, because an epigenetic mark may occur downstream of transcription, rather than at the transcriptional start. The interpretation of a modification may be context dependent: a modification that activates expression at one locus or in one lineage may have little effect, or be associated with repression elsewhere. This dependence on context makes the therapeutic process more complex. Selectivity is a prerequisite for clinical translation as systemic epigenetic agents can affect malignant cells, effector and regulatory lymphocytes, progenitors and non-immune tissues at the same time.

Progress will depend on the shift from association-based maps to perturbation studies defining functional immune regulatory elements. Longitudinal profiling in humans is also needed to determine how vaccination, infection, ageing, diet, the microbiome and treatment affect immune memory over time. Improved delivery systems should limit epigenetic modulators to the target immune population, and spatial epigenomic measurements should determine the effect of tissue location on immune identity. Together, these advances promise to revolutionise the field from descriptive atlases to precise regulation of the immune state.

9.1 Evidence grading and translational readiness

A single epigenetic difference does not provide enough evidence to claim translational readiness. The most informative studies are those that combine functional immune assays, chromatin and transcriptomic measurements and genetic or epigenome-editing perturbation with longitudinal sampling in purified or single cell populations. Primary cell studies have begun to directly associate T-cell function with regulatory elements and transcription factor programs, rather than infer their function solely from chromatin accessibility [46, 70, 71, 75]. This distinction is illustrated in Table 7, which assigns the highest level of inference to representative studies where this is justified by their design. The assignment concerns the claim under consideration: that longitudinal data can be used for prediction without identifying a causal component, and that targeted perturbation can demonstrate causal control without demonstrating clinical benefit or safety. Figure 6 therefore integrates predictive and functional evidence along complementary paths that make distinct contributions to mechanistic and translational evaluations.

Table 7: Classification of representative primary studies using claim-calibration framework. The level assigned is the strongest inference that the design of a study can support and should not be considered an overall rating of methodological quality.

Evidence level	Representative design and evidence	What the evidence supports	Main weakness if used alone
Level 1: descriptive	Human bulk methylome associations in systemic lupus erythematosus [61, 62]	Identifies reproducible candidate regulatory differences	Cannot establish whether a mark drives immune behavior; cell composition and reverse causation remain
Level 2: predictive	Longitudinal BCG multi-omics linked a baseline/post-vaccination state to later trained responses [13]	Shows that a measured state forecasts a later immune outcome	Prediction may remain confounded and requires external validation and calibration
Level 3: functional	Heterologous recall in controlled monocyte assays [49, 52]	Connects regulatory reprogramming to measurable immune output	May not identify the causal genomic element or establish disease-specific protection
Level 4: causal	Controlled signal order and STAT4–AP-1 cooperation altered cytotoxic-lymphocyte fate [46], while targeted CRISPRoff/on programming produced durable regulation and altered function in primary human T cells [70]	Supports causal control of a specified pathway or target in an immune-cell context	Does not by itself establish clinical benefit, delivery feasibility, durability or immune safety
Level 5: translational	No included study met all Level 5 criteria for deliberate immune-memory reprogramming	Would establish prospective benefit, immune target	Approved epigenetic drugs support their licensed indications but do not alone

		engagement and acceptable safety in the intended population	prove safe, biomarker-linked reprogramming of immune memory

Interpretive note: The framework assesses individual claims, not the articles as a whole. If a descriptive study is done very well it can be considered Level 1. Similarly, a claim is only considered to be at Level 5 when the intended population shows acceptable safety, immune target engagement and prospective benefit.

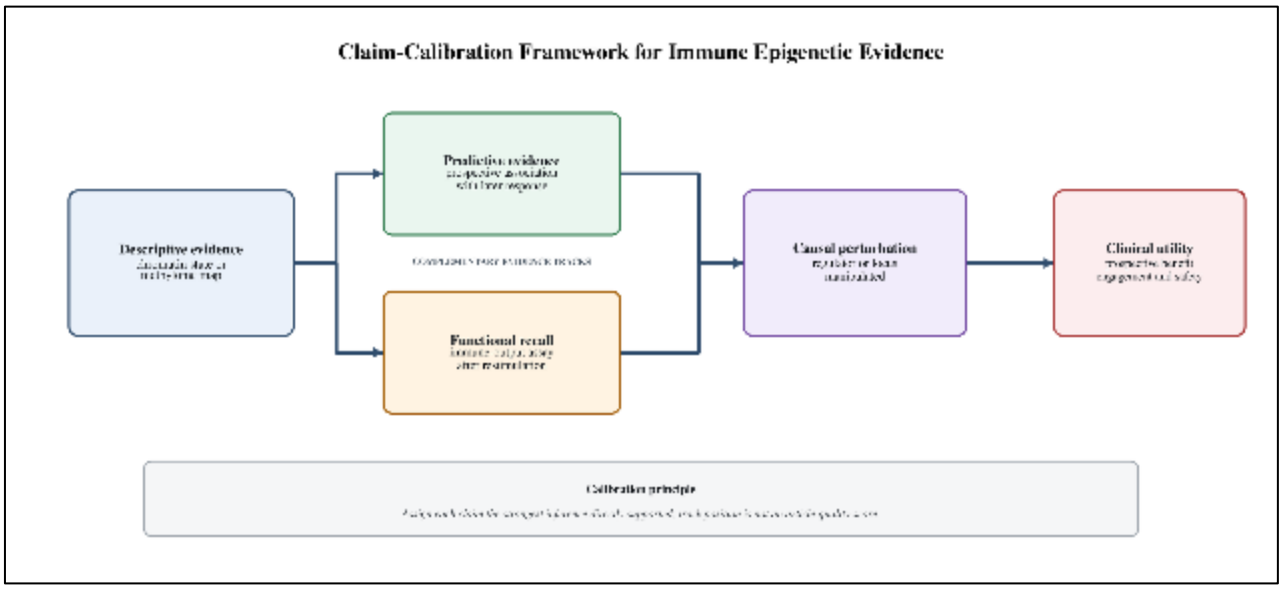


Figure 6: Framework for calibrating claims in immune epigenetics. Descriptive findings may be extended through either prospective prediction or functional-recall experiments. Perturbation strengthens causal interpretation, whereas a claim of clinical utility additionally requires prospective benefit, target engagement and acceptable safety. This framework is intended as a transparent interpretive guide, not as a validated risk-of-bias instrument.

9.2 Limitations of the current evidence base

Interpretation is limited by many features of the evidence that is currently available. Changes in cellular composition can be misinterpreted as epigenetic reprogramming when profiling bulk populations, and chromatin changes associated to activation may be transient rather than stable memory features. Many epigenetic drugs target multiple enzymes, cell types and genomic regions making it difficult to attribute a mechanism in pharmacological studies. The durability, reversibility, immune safety, and delivery efficiency still need to be further investigated, and programmable epigenome editing is currently in the pre-clinical or early-translational stage [14, 70, 71, 75]. For these reasons, this review does not include every altered mark as a therapeutic target and instead prioritises causal validation, cellular specificity and reproducibility over time.

9.3 Limitations of this review

The processes of screening titles and abstracts, selecting full texts, and assigning claims were not independent nor in duplicate, as this structured narrative review was conducted by a single author. The search covered a wide range of literature but was not intended to ensure exhaustive retrieval and supplementary citation chasing may have introduced ranking or selection bias. Cell populations, exposures, sampling intervals, epigenetic assays, and functional outcomes also varied making quantitative pooling inappropriate. Finally, the proposed framework has not been externally validated. It is the strongest inference that can be drawn from a study. Its predictive and functional tracks should not be seen as a strict hierarchy.

9.4 Integrative perspective and future priorities

Previous exposure to antigens, inflammation and the environment can alter the timing, magnitude and quality of a subsequent immune response, independent of any change in the underlying DNA sequence. The regulatory pathways of adaptive memory, trained innate immunity, tolerance, exhaustion and inflammatory persistence overlap but are biologically distinct states. Thus, the meaning of these signals is determined by the nature and tissue distribution of the

cells producing the signals, the sequence of signals, the return to basal levels, the duration of the signal after removal of the stimulus, and the presence of a defined secondary response [20].

Immune epigenetics is moving from descriptive atlases to experiments testing causality and programmable control. Counting the number of added chromatin maps alone is not a good measure of progress. The key questions are which regulatory elements can predict future behaviour, which are directly regulated and which can be safely modified. There are many ways to approach the problem and many study designs can be used. Single-cell and spatial methods can identify candidate states, perturbation can determine mechanism, and biomarker-guided trials can determine clinical value. Longitudinal studies can similarly be used to evaluate persistence. Interventions must be selective for the right cell type and locus, and must be reversible or otherwise controllable when necessary to be viable. Thus, one cannot assume that a causal mechanism is druggable . Similarly, one cannot equate persistent accessibility with memory. Programmable immune-epigenetic reprogramming is still at the preclinical stage, as long as immune safety and efficacy are established simultaneously.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of conflict of interest

The author declares no known competing interests.

Data availability

No new datasets were generated or analyzed during the preparation of this review.

Author contribution statement

Ban D.H. Al Khayali: conceptualization, literature investigation, evidence appraisal, visualization, writing—original draft, and writing—review and editing. The author approved the final manuscript and accepts responsibility for its content.

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