



Engineering immune cells as dynamic therapies: An evolution-guided framework for temporally programmed CAR T cells

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Abstract

Genetically engineered immune cells have produced durable remissions in several B-cell malignancies, yet relapse, antigen escape, T-cell exhaustion, toxicity, and limited activity in solid tumors remain major barriers. Most current chimeric antigen receptor (CAR) T-cell products operate through a largely fixed functional program after infusion, while emerging strategies often address resistance by increasing potency or constitutively combining multiple functions. Such approaches may be incomplete because both tumor populations and therapeutic immune cells continue to change under treatment. This review evaluates the biological and engineering foundations of engineered immune-cell therapies and develops Adaptive Temporal Immune Programming (ATIP) as an evolution-guided framework for dynamic cell therapy. ATIP reframes CAR T-cell treatment as a control problem involving two evolving populations: the tumor and the therapeutic cells themselves. A single engineered product would contain independently controllable functional modules whose dominance changes sequentially in response to biomarkers or model-predicted escape dynamics rather than a fixed schedule. The framework distinguishes modules that alter tumor-clone selection from those that principally preserve CAR T-cell fitness and requires evidence that candidate modules impose distinct selection pressures before evolutionary steering is inferred. The central hypothesis is that appropriately timed changes in selective pressure could delay resistant-clone dominance while sequential expression reduces the functional burden of simultaneous multi-module activation. ATIP defines explicit assumptions, falsifiable predictions, failure criteria, and a staged experimental program progressing from barcoded mixed-clone cultures through patient-derived organoids and animal models to evolutionary optimal-control modeling. ATIP remains untested and is not evidence of clinical superiority. Its contribution is to shift immune-cell engineering from increasingly complex static therapeutic states toward testing whether living therapies can dynamically alter their functional state in response to the evolution they induce.

Keywords

CAR T-cell therapy, Adaptive cell therapy, Tumor evolution, Antigen escape, T-cell exhaustion, Synthetic biology, Programmable cell therapy, Cancer immunotherapy, Evolutionary therapy, Temporal control

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1. Introduction

1.1 Clinical promise and unresolved barriers

Genetic engineering has changed immune cells from naturally occurring effectors into programmable therapeutic agents. A chimeric antigen receptor combines an extracellular antigen-binding element with intracellular signaling domains so that a T cell can recognize a selected surface antigen without conventional major histocompatibility complex presentation (1). This design has produced substantial clinical benefit in several relapsed or refractory B-cell cancers. As examples, Tisagenlecleucel generated high remission rates in pediatric and young-adult B-cell acute lymphoblastic leukemia, and axicabtagene ciloleucel produced durable responses in aggressive large B-cell lymphoma (2, 3). Later randomized evidence showed that axicabtagene ciloleucel could outperform standard second-line therapy in an appropriate large B-cell lymphoma population (4). These results established engineered immune cells as clinically meaningful ‘living medicines’ rather than only experimental laboratory tools. CAR T-cell therapy can cause cytokine release syndrome and immune effector cell-associated neurotoxicity; patients generally require lymphodepleting chemotherapy before CAR T-cell infusion, adding treatment burden and immunosuppression; autologous manufacturing is time-consuming and expensive; and tumor control can fail through loss or reduction of the target antigen, insufficient trafficking, suppressive signaling, metabolic stress, and progressive T-cell dysfunction (5-9). These barriers are especially pronounced in solid tumors, where spatial heterogeneity and a hostile microenvironment

reduce the probability that a single fixed receptor program will remain effective across all tumor regions and over the entire course of treatment (6, 7, 10).

1.2 The conceptual gap: static immune programs in evolving tumors

A common strategy in immune-cell engineering is to address resistance by increasing receptor potency, expanding target recognition, or constitutively adding supportive functions. That approach is scientifically reasonable and has generated important technologies, but it may be incomplete. A tumor is not a fixed target. Treatment changes the relative fitness of antigen-positive and antigen-negative clones, alters signaling interactions in the microenvironment, and can favor cells that tolerate immune attack. Antigen escape after CAR T-cell exposure has been documented clinically and experimentally, including in glioblastoma and B-cell malignancies (7, 11). A therapy that applies the same dominant pressure throughout its functional lifetime may make the direction of selection more predictable, although this possibility has not been directly tested for temporally programmable CAR T-cell systems. Cancer evolutionary theory has shown that fixed maximum-intensity treatment can remove sensitive competitors and accelerate the expansion of resistant populations under some conditions (12-15). Those principles have been developed mainly for drugs rather than for living cell products. The specific gap addressed in this review is whether engineered immune cells could be designed not only to recognize tumors, but also to alter their dominant functional program over time in response to evidence of tumor adaptation. Existing inducible CARs,

pharmacologic pause systems, synthetic antigen-sensing circuits, and logic-gated receptors demonstrate that engineered T-cell activity can be controlled (16-22). However, those technologies have generally been developed for safety, dose control, or contextual recognition rather than as a coordinated, biomarker-guided rotation among distinct resistance-counteracting programs.

1.3 Review aims and conceptual framework

This review has two aims. First, it critically evaluates the biological platforms, gene-delivery methods, genome-editing technologies, clinical achievements, and failure mechanisms that define current immune-cell engineering. Second, it develops temporal modulation of CAR T-cell function into a testable framework termed Adaptive Temporal Immune Programming (ATIP). ATIP treats CAR T-cell therapy as an evolutionary control problem. Under this paradigm, a single cell product would contain several independently regulated modules, but only the module most relevant to the current tumor state would be dominant at a given interval. The schedule would be adjusted according to biomarkers or model-predicted escape dynamics instead of following a fixed weekly cycle. The framework is presented with explicit assumptions, engineering options, predicted benefits, failure conditions, and a staged experimental program so that it can be tested rather than accepted as a purely speculative idea.

2. Literature search and review approach

The literature used in this review was identified through targeted searches of PubMed and publisher websites and through examination of

the reference lists of major peer-reviewed articles. The source list was subsequently updated during revision, with the final literature check completed on August 23, 2026. Search combinations included “CAR T cell,” “CAR NK,” “TCR-engineered T cell,” “CRISPR immune cell,” “base-edited CAR,” “antigen escape,” “CAR T-cell exhaustion,” “tumor microenvironment,” “inducible CAR,” “small-molecule CAR switch,” “synthetic gene circuit,” “adaptive therapy,” “evolutionary game theory,” “optimal control,” “programmable cell therapy,” “smart CAR T-cell control,” “cybergenetics,” “inducible cytokine CAR,” and “cell-autonomous CAR control.” Priority was given to primary clinical studies, primary engineering studies, foundational mechanistic papers, and recent peer-reviewed reviews. Sources were retained only when they directly supported a statement used in the manuscript. The search was not designed as a systematic review, no meta-analysis was performed, and the proposed framework represents a hypothesis derived from integration of the cited evidence rather than a conclusion established by comparative clinical data. The final update specifically screened 2025-2026 literature for programmable cell therapies, synthetic gene-circuit translation, smart CAR T-cell control, conditional cytokine-armoring strategies, and cell autonomous regulation of CAR signaling (22-28). These sources were used to define engineering constraints and feasibility boundaries, not as evidence that ATIP itself has been validated.

3. Biological and engineering foundations

3.1 T cells and NK cells as therapeutic platforms

T cells are attractive engineering platforms because they can expand after antigen recognition, differentiate into effector and memory states, and persist after infusion. Their endogenous T-cell receptors recognize peptide-major histocompatibility complex complexes, whereas a CAR redirects recognition to a selected cell-surface molecule (1, 5). Persistence can support long-term surveillance, but persistent activation can also promote exhaustion or prolong toxicity. T-cell behavior is therefore both the principal advantage and one of the principal control problems of CAR therapy. Natural killer (NK) cells provide a complementary platform. They use germline encoded activating and inhibitory receptors, can kill stressed or major histocompatibility complex class I-low cells, and are less likely than donor T cells to initiate graft-versus-host disease. A clinical study of cord-blood-derived anti-CD19 CAR NK cells reported responses without the characteristic pattern of severe cytokine release syndrome, neurotoxicity, or graft versus-host disease seen with some CAR T-cell products; however, the study was small and did not establish broad comparative superiority (29). NK-cell sources may support off-the-shelf manufacturing, although expansion, persistence, cryopreservation, and tumor-microenvironment resistance remain important engineering challenges (29, 30).

3.2 CAR T cells, CAR NK cells, and TCR-engineered T cells

CARs are modular receptors. An antigen-binding domain, often a single-chain antibody fragment, is connected through a hinge and transmembrane region to signaling domains such as CD3-zeta and a co-stimulatory domain

(1, 5). CAR recognition is independent of peptide-major histocompatibility complex presentation, which is advantageous when tumors reduce antigen presentation. The limitation is that CARs generally require accessible surface targets and can damage normal tissues that express the same antigen. T-cell receptor-engineered T cells preserve peptide-major histocompatibility complex recognition and can therefore target intracellular proteins that are processed and displayed on the cell surface. Clinical studies have demonstrated antitumor activity against selected cancer-testis antigens, but the approach is restricted by human leukocyte antigen type and by the possibility of cross-reactivity with healthy tissues (31, 32). CAR NK-cell therapy combines CAR-directed recognition with NK-cell effector functions and may be suitable for donor-derived products, but its persistence and manufacturing characteristics differ from those of autologous CAR T cells (29, 30). These platforms cannot be described as universally interchangeable; each solves a different recognition, safety, and production problem.

3.3 Gene delivery and genome editing

Clinical CAR products commonly use integrating viral vectors because they can support efficient and durable expression. Their disadvantages include specialized manufacturing, cost, batch testing, and a low but consequential concern regarding insertional effects. Non-viral delivery by electroporation can introduce messenger RNA, transposon systems, or genome-editing complexes. Messenger RNA provides temporary expression, whereas targeted genome editing can place a transgene at a defined locus or

disrupt an inhibitory gene. Non-viral targeting of the T-cell receptor alpha constant locus has improved receptor regulation in preclinical studies, and CRISPR-edited T cells have entered early clinical testing (33-35). Conventional CRISPR-Cas9 produces a double-strand DNA break and relies on cellular repair, creating risks that include off target cleavage, chromosomal rearrangement, and variable insertions or deletions. Base editing can change selected nucleotides without a double-strand break, while prime editing can write a wider range of small sequence changes through a reverse-transcription mechanism (36, 37). Base-edited allogeneic CAR T cells have reached early clinical use in relapsed T-cell acute lymphoblastic leukemia, demonstrating the translational relevance of precise multi-gene editing while also emphasizing the need for long-term genomic surveillance (38). These technologies make multi-module immune cells technically more plausible, but every added edit increases characterization and manufacturing requirements.

4. Clinical achievements and mechanisms of failure

4.1 Hematologic malignancies: evidence that engineered cells can produce durable therapies
The strongest evidence for CAR T-cell efficacy originates from B-cell malignancies. In pediatric and young-adult B-cell acute lymphoblastic leukemia, tisagenlecleucel produced high overall remission rates after a single infusion, although severe adverse events occurred and not every remission remained durable (2). In refractory large B-cell lymphoma, axicabtagene ciloleucel produced substantial response rates,

and randomized second-line evidence later demonstrated improved event-free survival compared with standard care in a selected population (3, 4). These studies validate the central premise of immune-cell engineering: a modified cell can expand *in vivo*, eliminate disease, and persist after an equivalent chemotherapeutic infused dose would otherwise have disappeared. The same evidence also shows why a descriptive account of clinical success is not sufficient. Relapse still occurs, and the mechanism of relapse matters. Some tumors preserve the target antigen but outlast or suppress the infused cells; others reduce or eliminate target expression. The clinical problem is therefore not only initial cytotoxic potency but maintenance of useful function in a changing biological system (5-9).

4.2 Antigen escape and tumor heterogeneity

Antigen escape can occur through mutation, altered splicing, lineage switching, internalization, selection of pre-existing antigen-low cells, or adaptive reduction of target density (7). In glioblastoma, EGFRvIII-directed CAR T-cell exposure was followed by loss of the targeted antigen and evidence of an adaptive immunosuppressive response, illustrating how treatment can reshape both tumor-cell composition and the surrounding microenvironment (11). Multi-target CARs can reduce the probability that loss of a single antigen is sufficient for escape. Tandem targeting of HER2 and IL13R α 2, for example, reduced antigen-escape behavior in preclinical glioblastoma models (39). However, simultaneous multi-target recognition does not automatically solve non-antigenic resistance such as checkpoint signaling, transforming

growth factor- β exposure, hypoxia, nutrient deprivation, or T-cell exhaustion.

4.3 Suppressive signaling, metabolic stress, and exhaustion

Solid tumors expose immune cells to inhibitory ligands, suppressive cytokines, abnormal vasculature, low oxygen, acidity, competition for glucose and amino acids, and chronic antigen stimulation. These conditions can reduce proliferation, cytokine production, and cytotoxicity even when the CAR continues to recognize its target (5, 6, 10). Constitutive signaling from some CAR designs can also drive dysfunction. Importantly, exhaustion is not always an irreversible endpoint. Temporary interruption of CAR signaling produced epigenetic and functional recovery in experimental systems, supporting the idea that the timing of activity and rest can influence performance (19).

4.4 Toxicity, manufacturing, and access

Cytokine release syndrome and neurotoxicity can become severe and require specialized monitoring and treatment (8, 9). On-target, off-tumor injury remains a concern whenever the selected antigen is present on essential healthy tissue. Manufacturing introduces additional risk through variable starting material, vector production, cell expansion, release testing, shipping, and delays for patients with rapidly progressing disease. Allogeneic T-cell and CAR NK-cell approaches may shorten production time, but they introduce immune rejection, graft-versus-host disease, persistence, and gene-editing questions (29, 30, 38). A 2025 analysis of 1,580 [ClinicalTrials.gov](https://clinicaltrials.gov) CAR T-cell studies documented rapid global growth but substantial

geographic concentration of research activity; the same review identified complex manufacturing and very high treatment cost as accessibility barriers (40). These findings support a narrower conclusion that access and development capacity remain uneven rather than establishing universal access patterns across health systems.

5. Gap analysis: limitations of constitutive multi-function engineering

5.1 The prevailing optimization strategy

A common engineering response to resistance is to add another feature: a second antigen receptor, a cytokine, a checkpoint-resistant receptor, a metabolic support gene, or a logic gate. These additions can be valuable. Synthetic Notch circuits can require one antigen to induce a CAR against another, and newer logic-gated systems can separate recognition from signaling to improve contextual control (20, 21). Small-molecule-gated CARs, pharmacologic interruption with dasatinib, lenalidomide-regulated switches, and other chemical control systems demonstrate that activity can be externally adjusted (16-18, 22). The field therefore already contains many of the components needed for temporal control. The unresolved question is how those components should be scheduled. Constitutively expressing every support program may increase genetic size, metabolic demand, tonic signaling, cytokine exposure, and manufacturing complexity. It also leaves the tumor under a relatively stable combination of pressures. A fixed combination may be broader than a single mechanism, but it remains predictable after the tumor population has sampled that environment

long enough. This review therefore challenges the implicit assumption that simultaneous expression is always preferable to sequential expression.

5.2 CAR T-cell control strategies

The proposed framework distinguishes four designs that are often discussed as though they were equivalent. Their differences are summarized in Table 1.

Table 1. Comparison of static, constitutively armored, fixed-rotation, and adaptive temporal CAR T-cell strategies.

Strategy	Control Pattern	Principal Advantage	Principal Limitation
Conventional static CAR T cells	One dominant program remains active after infusion.	Lowest circuit complexity and strongest clinical precedent.	Applies a relatively constant selective pressure and may not respond to emerging resistance.
Constitutively armored CAR T cells	Multiple support functions are expressed simultaneously.	Targets several resistance mechanisms at the same time.	May increase circuit burden, tonic signaling, metabolic demand, toxicity, and cross-interference.
Fixed temporal rotation	Modules change according to a preset calendar.	Tests whether sequential expression reduces continuous signaling burden.	Timing may not correspond to the tumor's actual evolutionary state.
Adaptive Temporal Immune Programming	Modules change according to biomarkers or model-predicted escape dynamics.	Responds to changing tumor and immune states.	Requires reliable biomarkers, reversible switching, and a validated control model.

5.3 Evolutionary rationale for temporal control

The basic idea resembles resistance-management strategies in infectious disease, where changing treatment pressure may slow the rise of a resistant population. The comparison is only an analogy because tumors, microbes, and living CAR T-cell products behave differently. Adaptive cancer-therapy models are based on the related principle that treatment changes competition among tumor subpopulations. In some settings, the most resistant phenotype carries a fitness cost when treatment is absent; eliminating all sensitive competitors can therefore allow resistant cells to expand more rapidly (12). Preclinical and mathematical studies have shown that treatment schedules informed by tumor state can prolong

control compared with fixed maximum-intensity schedules in selected drug models (13-15). ATIP does not assume that the same result will automatically occur with CAR T cells. Instead, it uses those findings to generate a specific hypothesis: if distinct CAR T-cell modules create partially different selective environments, changing the dominant module before one escape state becomes established may delay clonal dominance, but this evolutionary effect requires direct evidence of different tumor-clone selection gradients. Modules that principally maintain CAR T-cell fitness may improve tumor control without changing the relative selection of resistant tumor clones. Temporal variation may also benefit the engineered cells. Sequential expression may reduce the burden

created by simultaneous cytokine secretion, checkpoint resistance, metabolic rewiring, and innate-cell recruitment, although this possibility has not yet been tested experimentally. Transient interruption of CAR signaling has restored function and remodeled exhaustion-associated states experimentally (19), and a 2026 cell-autonomous receptor-shedding system independently showed that feedback-limited CAR signaling can reduce exhaustion associated dysfunction in preclinical models (28). These findings support temporal control as a biologically plausible design principle without establishing that multi-module ATIP will improve outcomes. The framework therefore aims to control two evolving populations at once: the tumor population and the therapeutic-cell population.

6. Proposed framework: Adaptive Temporal Immune Programming

6.1 Core architecture

ATIP would begin with a baseline tumor-recognition system and a safety layer. The baseline CAR would provide antigen-dependent cytotoxicity. Because the support modules would be built into the initial product, the framework would not require repeated leukapheresis, repeated *ex vivo* engineering, or repeated lymphodepleting chemotherapy each time the dominant program changed. An independently available terminal safety switch would permit irreversible elimination of the infused cells if severe toxicity developed; it would remain accessible in every therapeutic state and would not be part of the repeating ATIP schedule. A distinct, reversible rest/pause program could temporarily suppress CAR

signaling and permit reactivation when prespecified recovery and safety criteria are met. Several other support modules would then be encoded in forms that could be activated, degraded, or paused independently.

A practical ATIP controller can be separated into three engineering layers: a sensor that measures an external drug or disease-associated cue, a processor that applies a predefined decision rule, and an actuator that changes the dominant CAR T-cell support program. Recent programmable-cell and synthetic-circuit literature uses this sense-compute-act architecture to distinguish sensing from information processing and therapeutic output (23-26). A 2026 primary study also demonstrated a compact, cell-autonomous activation-induced release (AIR) motif that rapidly and reversibly reduced surface-receptor expression after T-cell activation, showing that autonomous feedback can regulate CAR signaling without a continuously administered external command (28). This does not establish ATIP, but it strengthens the technical premise that engineered T cells can incorporate dynamic feedback. For an initial ATIP proof of concept, the processor should nevertheless remain clinician controlled so that the input, timing, dose, and stop decision can be audited.

The control technologies are not interchangeable. Small-molecule-assembled receptors and drug gated transcriptional systems offer reversible, dose-dependent external control, but their performance depends on inducer pharmacokinetics, tissue penetration, repeated dosing, and the possibility of background activity or interactions with other

medicines (16, 18, 22-24). Drug responsive degrons can regulate protein abundance rapidly, but incomplete degradation or variable intracellular drug exposure can blur the intended state transition. Pharmacologic pause systems such as dasatinib can suppress signaling without rebuilding the receptor, which is attractive for temporary rest or toxicity control, but they pause activity rather than selectively activating a different resistance-countering module (17, 19). Orthogonal receptor-adapter systems can retarget or titrate recognition by changing the administered adapter, although repeated adapter dosing adds pharmacokinetic and manufacturing dependencies (22, 23). Tumor responsive or logic-gated circuits can localize outputs and reduce reliance on repeated external commands, but autonomous designs are harder to validate and can fail if the sensed cue is heterogeneous or evolves (20, 21, 24-26). AIR illustrates a complementary autonomous strategy in which activation itself drives reversible receptor shedding; its compact design may reduce circuit burden, but it regulates receptor abundance rather than selecting among several distinct ATIP therapeutic programs (28).

These trade-offs suggest a staged design principle: early ATIP experiments should favor reversible, externally commanded switches with measurable on/off kinetics and low basal leakiness. Accordingly, the first experimental versions should use clinician-controlled external inputs because they are easier to audit and stop than fully autonomous circuits. More advanced versions could incorporate cell-intrinsic sensing after the relationships among biomarkers, tumor states, and module responses have been validated. Control could be implemented

through split receptors assembled by a small molecule, drug-responsive degrons, inducible promoters, pharmacologic pause systems, or orthogonal receptor-adapter pairs (16-18, 22). Synthetic Notch or other logic circuits could provide tumor-localized activation when the desired module should respond to a spatial cue rather than to a circulating biomarker (20, 21). The engineering objective would not be to switch every cell at exactly the same instant. Instead, the population-level output would need to change sufficiently and predictably to alter the dominant functional pressure while preserving safety. The key engineering specifications are therefore not merely whether a switch can turn on, but its dynamic range, response and washout time, reversibility after repeated cycles, orthogonality among modules, inducer bioavailability where applicable, population-level switching fidelity, and effects on CAR T-cell viability and persistence (22-28).

6.2 Candidate functional modules

The exact modules would depend on the tumor type and should be selected from mechanisms supported by preclinical evidence. The following set illustrates how the framework could be tested without claiming that these are the only or optimal choices. The biological mechanisms in Table 2 are evidence-supported design classes, whereas their use as sequential ATIP states, the proposed biomarkers, and the switch-away rules remain hypotheses to be tested. These modules must be classified by primary mechanism: innate-cell recruitment may change tumor-clone selection if recruited effectors recognize otherwise escaping clones; checkpoint countering, TGF- β resistance, and metabolic support principally maintain CAR T-

cell fitness unless paired tumor-lineage countering module. Recent work supports experiments establish a distinct selection effect; conditional cytokine armoring, programmable reversible rest principally regulates CAR T-cell context-dependent immune outputs, reversible functional recovery and toxicity. The terminal external control, and compact cell-autonomous elimination switch is an independent safety regulation, but does not validate the complete mechanism, not an adaptive resistance- ATIP sequence (22-28).

Table 2. Candidate ATIP functional modules, proposed triggers, intended functions, and switching criteria.

Module	Candidate Output	Possible Trigger	Intended function and reason to switch away
Checkpoint countering	Inducible PD-1/PD-L1 blockade, dominant-negative receptor, or switch receptor	Rising inhibitory-ligand expression or CAR T-cell dysfunction despite preserved target-antigen expression	Restore activity against checkpoint-dependent suppression. Switch if excessive cytokine output occurs, checkpoint dependence disappears, or another resistance state becomes dominant.
TGF- β resistance	Inducible dominant-negative TGF- β receptor signaling or another validated resistance design	Evidence of TGF- β -associated suppression and stromal remodeling	Preserve CAR T-cell function in a TGF- β -rich environment. Switch if the module provides no functional benefit, creates a growth disadvantage, or another resistance mechanism dominates.
Metabolic support	Tumor-specific program supporting mitochondrial fitness or resistance to hypoxia, acidity, adenosine, or nutrient restriction	Hypoxia or metabolic biomarkers, or evidence of CAR T-cell metabolic dysfunction	Support survival and cytotoxicity in metabolically hostile tumor regions. Switch if metabolic burden exceeds the benefit or the dominant escape state is not metabolic.
Innate-cell recruitment	Locally controlled chemokine or cytokine output designed to recruit or support NK cells or other innate effectors; this is not a second antigen-targeting receptor.	Falling target-antigen density or expansion of antigen-negative clones	Potentially alter tumor-clone selection through innate effectors that recognize cells escaping the baseline CAR. Test whether antigen-negative clones are actually depleted, rather than assuming recruitment changes selection. Switch if systemic inflammation develops, recruitment fails, or target-positive disease returns.
Reversible rest/pause state	Temporary, reversible CAR signaling suppression with measurable recovery and reactivation; no emergency cell elimination in this scheduled state.	Excessive activation, early manageable toxicity, or an exhaustion-associated phenotype, subject to prespecified clinical safety rules.	Reduce CAR signaling and allow functional recovery. Resume an appropriate therapeutic module only after predefined safety and recovery criteria are met; escalate to the independent terminal safety switch if required.

6.3 Biomarker-guided adaptive scheduling treated as the strongest therapeutic design. A fixed weekly rotation would be useful as an Tumors evolve at different rates, and different early engineering control, but it should not be regions of the same tumor may occupy different

states. ATIP therefore defines a switching decision as a response to evidence rather than to elapsed time. Candidate evidence includes circulating tumor DNA trends, target-antigen density from biopsy or imaging, soluble checkpoint or cytokine markers, tumor growth rate, CAR T-cell expansion, and the frequency of exhausted or dysfunctional CAR T-cell phenotypes in blood. Each marker has limitations. Circulating tumor DNA may not represent every tumor site, biopsies are spatially restricted and invasive, and immune biomarkers can change because of infection or other treatments. No biomarker set has yet been validated to identify all clinically relevant immune-evasion or resistance states that would be required for ATIP. Consequently, early ATIP studies should evaluate biomarker performance as a primary objective rather than assume that any candidate marker accurately represents the evolving tumor state. Discordance between measured biomarkers and actual tumor biology would represent a potential failure mode of the framework rather than merely a technical limitation. A decision algorithm would need to integrate multiple signals and quantify uncertainty instead of relying on a single threshold. A first-generation clinician-in-the-loop system could use predefined rules. For example, preserved target antigen with falling CAR T cell activity could trigger a checkpoint-countering module; rising antigen-negative burden could prompt evaluation of an innate-cell recruitment module, but only if prescreening shows that recruited effectors can control the emerging antigen-negative clones; and escalating inflammatory markers could trigger reversible rest when clinically appropriate; severe toxicity could independently trigger

terminal elimination. Later systems could use model-predictive control, in which a mathematical model is repeatedly updated with new measurements and selects the next module expected to minimize tumor burden, resistant-clone frequency, toxicity, and switching cost (15). The decision rule must be prospectively specified and experimentally validated to avoid *post hoc* schedule changes that cannot be interpreted.

To make adaptive scheduling experimentally interpretable, the first controller should use a prospectively specified state-estimation and switching rule rather than discretionary bedside changes. A practical rule would combine multiple measurements into a confidence score for each resistance state, require that a prespecified confidence threshold be crossed before changing modules, and impose a minimum dwell time unless a safety limit is exceeded. Hysteresis, defined as using a higher threshold to enter a state than to remain in it, could prevent rapid oscillation when biomarkers fluctuate near a cutoff. Safety rules would override efficacy rules: severe inflammatory toxicity would override efficacy rules and trigger the independent terminal elimination switch when prespecified criteria are met; reversible rest would remain an option only when clinically appropriate. If biomarker confidence were inadequate or two lesions produced conflicting signals, the controller would default to the previously validated state or a predefined safety state rather than infer a new program. These design features convert ATIP from a general idea of 'switching when the tumor changes' into a reproducible control policy whose thresholds, delays, error rates, and

failure modes can be measured prospectively (15, 23-26). The numerical values used for switching thresholds should not be selected arbitrarily. Instead, threshold candidates should be derived during preclinical studies by correlating biomarker measurements with independently validated resistance states and then prospectively tested in separate datasets. Threshold selection should balance sensitivity to emerging resistance against excessive switching caused by measurement noise or transient biological fluctuations. Subsequent organoid and animal studies should re-evaluate whether thresholds identified in one model remain valid in more complex systems. Minimum dwell times should likewise be determined empirically. Modules must remain active long enough to produce measurable biological effects, yet not so long that resistant populations become established before a switch

can occur. Early studies should therefore characterize response kinetics, signal persistence, and reversibility for each module and use those measurements to define the shortest effective dwell period. Sensitivity analyses should evaluate how controller performance changes across a range of dwell times because the optimal value will likely depend on tumor type, growth rate, and module mechanism.

6.4 Explicit assumptions and their consequences ATIP depends on explicit and implicit assumptions that must be stated before the framework can be evaluated. Table 3 identifies the principal biological, sensing, control, pharmacologic, and modeling assumptions, explains why each is plausible, and states how failure would change the interpretation of ATIP.

Table 3. Key assumptions of the ATIP framework, scientific justification for each assumption, and consequences if the assumption fails.

Assumption	Scientific Justification	Impact if False
A1. Tumors contain or can generate distinguishable resistance states.	Antigen loss, inhibitory signaling, and microenvironment-mediated resistance have been observed after immune therapy.	If states cannot be distinguished, the controller cannot reliably select an appropriate module.
A2. Modules create partially non-overlapping selective pressures and do not share complete cross-resistance.	Checkpoint resistance, TGF- β resistance, metabolic support, and innate-cell recruitment act through different mechanisms.	If resistance patterns are highly correlated, sequential switching would provide little advantage over constitutive therapy.
A3. At least some resistant states carry a context-dependent fitness cost.	Evolutionary control depends on trade-offs that prevent one resistant phenotype from dominating under every condition.	A universally fit resistant clone could expand regardless of switching, requiring combination therapy or alternative targeting.
A4. Biomarkers represent the clinically relevant tumor and immune state quickly enough.	Circulating tumor DNA, antigen measurements, cytokines, imaging, and immune-cell phenotypes may provide information about treatment response.	Delayed, inaccurate, or spatially biased biomarkers could cause selection of the wrong module.

A5. Switching can occur before the relevant escape population becomes irreversible or clinically dominant.	Early detection and intervention may alter clonal expansion before resistant disease becomes established.	If detection or switching is too slow, resistant clones may dominate before the new module becomes active.
A6. Module activation is reversible, sufficiently specific, and acceptably low in leakiness.	Existing drug-gated receptors, degrons, pause systems, and synthetic circuits demonstrate partial control over engineered-cell activity.	Irreversible or leaky activation could cause overlapping programs, toxicity, or uninterpretable results.
A7. External inducers achieve predictable exposure in relevant tissues without unacceptable interactions.	Small-molecule control systems can regulate engineered cells, but their operation depends on pharmacokinetics and tissue penetration.	Variable exposure could cause incomplete or inconsistent switching across cells and tumor sites.
A8. CAR T cells persist long enough to complete multiple switching cycles.	Some CAR T-cell products can expand and persist after infusion.	If the cells disappear early, temporal programming provides no meaningful advantage.
A9. Added engineering does not create prohibitive metabolic, genetic, or manufacturing burden.	Multi-component cell products are technically possible, although each additional element increases complexity.	Excessive burden could reduce cell viability, expansion, consistency, or manufacturability.
A10. The mathematical model captures the dominant biological relationships sufficiently well.	Evolutionary and control models can represent tumor subpopulations, treatment pressures, toxicity, and switching costs.	A poorly specified model could recommend ineffective or harmful module changes.
A11. Biomarker sampling occurs frequently enough to detect meaningful changes without responding primarily to noise.	Adaptive control requires repeated measurements and prespecified confidence thresholds.	Infrequent sampling may miss escape, while excessive sensitivity may cause unstable or unnecessary switching.

6.5 Central hypothesis and falsifiable predictions

The central hypothesis is that, in heterogeneous tumors, biomarker-guided sequential activation of mechanistically distinct CAR T-cell modules, including tumor-selection-modifying and CAR T-cell-fitness-supporting programs will delay expansion of resistant clones and reduce simultaneous multi-module expression and functional burden more effectively than either a conventional static CAR T-cell product or a constitutively multi-armored product, provided that switching occurs before an escape population crosses a critical frequency and the engineering burden remains tolerable. This hypothesis generates five falsifiable predictions:

1. Adaptive ATIP will delay time to dominance

of antigen-negative or suppressive tumor subpopulations compared with a single static CAR.

2. Adaptive switching will outperform a fixed calendar schedule only when the selected biomarkers accurately report the resistance state and when the controller acts before irreversible progression.

3. Sequential expression will reduce simultaneous transgene-expression burden, inflammatory output, or exhaustion-associated dysfunction compared with constitutive expression of all modules, without eliminating antitumor activity.

4. Excessive switching, poor population-level synchronization, or leaky module expression will reduce benefit and may perform worse than

a simpler CAR design.

5. If one resistance phenotype has no meaningful trade-off and is resistant to every module, ATIP will not prevent its expansion.

A decisive mechanistic test is whether the candidate modules actually impose different selection gradients on tumor populations. Before adaptive scheduling is tested, each candidate module should therefore be challenged against the same barcoded panel of resistant tumor states. The framework should advance its evolutionary-steering claim only if at least two tumor-selection-modifying programs yield meaningfully different clone-enrichment patterns and if at least one resistance state exhibits a measurable trade-off across those programs. In the same longitudinal cultures and at matched sampling times, CAR T-cell viability, expansion, cytotoxic function, cytokine output, and exhaustion-associated phenotypes alongside tumor-lineage frequencies should be measured. This paired design will distinguish true changes in tumor-clone selection from improved killing caused primarily by preservation of CAR T-cell fitness. Fitness-supporting modules must not be counted as independent evolutionary pressures without evidence of altered relative clone susceptibility.

Operationally, candidate modules should generate distinguishable susceptibility profiles across a barcoded panel of resistance phenotypes. If all tumor-selection-modifying modules repeatedly enrich the same resistant clone or produce highly correlated resistance patterns, evolutionary steering by adaptive scheduling would lack support even if fitness-supporting modules improve overall tumor

killing. Paired CAR T-cell functional readouts are needed to interpret the source of any apparent benefit. This pre-screen directly tests the evolutionary premise of ATIP: temporal control cannot slow escape if every module ultimately favors the same resistant population. The paired tumor-clone susceptibility and CAR T-cell functional-state matrices could then be used to parameterize the mathematical model and identify which module combinations warrant further investigation in organoid and animal studies. More broadly, these experiments would test whether the proposed modules create sufficiently distinct evolutionary pressures to justify adaptive temporal control as a resistance management strategy.

7. Testable research trajectory

7.1 Stage 1: modular engineering and mixed-population co-culture

The first stage should test engineering feasibility before attempting a complete adaptive product. Each module should be constructed and characterized separately for activation range, leakiness, reversibility, effect on viability, transgene burden, cytokine output, and recovery after repeated switching. A reduced proof-of-concept system with two modules is scientifically preferable to an unmanageable five-module design. For example, a checkpoint-countering state could be compared with an innate-recruiting state in co-culture with barcoded antigen-high, antigen-low, and checkpoint-dependent tumor populations. Experimental groups should include an unmodified T-cell control, a conventional static CAR, a constitutively dual-function CAR, a fixed-rotation product, and an adaptive ATIP

product. Allocation, assay processing, and outcome analysis should be blinded where feasible. Sample size should be determined by pilot variability and a prospectively defined primary endpoint, such as time to resistant-population dominance or area under the tumor-burden curve. Paired longitudinal endpoints should include total tumor killing, barcoded tumor-clone frequencies and enrichment, CAR T-cell expansion, viability, cytotoxicity, exhaustion-associated phenotypes, cytokine release, and module-switching fidelity at matched time points. A prespecified analysis should determine whether improved efficacy reflects altered relative clone selection, improved CAR T-cell fitness, or both. Switching fidelity should be quantified as the proportion of cells achieving the intended functional state, the time required to reach that state, and the degree of residual activity from previously active modules.

7.2 Stage 2: patient-derived organoids and microenvironment models

Three-dimensional tumor organoids and co-culture systems should then be used to introduce stromal cells, suppressive cytokines, hypoxia, and antigen heterogeneity. These models can test whether a schedule that succeeds in a well-mixed culture still succeeds when resistance is spatially organized. Longitudinal imaging, single-cell RNA sequencing, cell-surface antigen profiling, and lineage barcoding could measure how tumor and CAR T-cell states change before and after each switch. Organoid studies should also test whether the biomarker used by the controller accurately represents the dominant resistance state within the model. Agreement between biomarker classifications

and independently measured cellular states should be quantified prospectively because inaccurate state estimation could undermine adaptive scheduling even when the underlying therapeutic modules are effective.

7.3 Stage 3: animal models

Humanized or xenograft models should compare the same four principal strategies: static, constitutively multi-module, fixed temporal rotation, and adaptive ATIP. Primary endpoints should include survival or time to a predefined clinical endpoint, tumor burden, and frequency of resistant clones. Secondary endpoints should include CAR T-cell persistence, tissue infiltration, exhaustion, cytokine concentrations, off-tumor effects, and the distribution of active modules across tissues. A safety switch should be included from the start rather than added only after efficacy has been demonstrated. The adaptive rule should be locked before the confirmatory experiment. A pilot study may identify candidate thresholds, but the confirmatory study should use a prospectively specified algorithm. Interpretation of the framework would be weakened if the adaptive group were allowed to change schedules after viewing outcomes while the comparator groups remained fixed.

7.4 Mathematical modeling and optimal control

A mathematical model can formalize the decision problem. State variables could include the sizes of tumor subpopulations, antigen density, suppressive microenvironment activity, functional and exhausted CAR T-cell populations, and toxicity. Tumor-selection-modifying modules would have clone-specific susceptibility parameters, while fitness-

supporting modules would primarily modify CAR T-cell expansion, exhaustion, survival, or per-cell killing; the model must not assume that each support module independently changes tumor-clone selection. Reversible rest would reduce CAR signaling temporarily, and the independent terminal elimination switch would be represented as a noncycling safety override. Each scheduled module would also have an activation delay, cost, and probability of incomplete activation. The objective function could minimize weighted tumor burden, resistant clone frequency, cumulative inflammatory toxicity, and the number of switches. Evolutionary game theory can represent competition among tumor phenotypes, while dynamic programming or model-predictive control can select a module based on the current estimated state (15). The model should not be used as evidence that ATIP works. Its purpose is to identify conditions under which the hypothesis is plausible, reveal parameters that determine failure, and generate schedules for experimental testing. Sensitivity analysis should determine whether the proposed benefit depends on unrealistic biomarker precision, instantaneous switching, or extreme fitness costs. The model should additionally evaluate delayed biomarker reporting, incomplete module activation, measurement error, heterogeneous lesions with conflicting states, and controller failures. Benefits observed only under idealized assumptions should not be interpreted as support for biological feasibility. A framework that succeeds only under narrow idealized assumptions should not advance to complex animal experiments.

7.5 Decision criteria and falsification thresholds

ATIP should be considered unsupported if it fails to delay relapse or resistant-clone expansion compared with the best constitutive control, if sequential expression does not reduce functional burden, if biomarker-guided decisions are not reproducible, or if circuit complexity substantially reduces CAR T-cell expansion and persistence. A statistically significant reduction in short-term tumor burden alone would not be sufficient. The framework specifically predicts improved control of evolution over time, so longitudinal clonal and functional endpoints are required. Negative results would still be informative because they would identify whether the limiting problem is tumor evolution, therapeutic-cell engineering, biomarker measurement, or control timing.

A staged go/no-go framework would further prevent complexity from being mistaken for progress. A module pair should not advance from cell culture unless paired tumor-clone and CAR T-cell functional measurements identify whether any apparent benefit reflects altered selection or preserved immune-cell fitness, and unless repeated switching remains reversible, basal leakiness stays below a prospectively defined limit, and the adaptive arm improves the prespecified evolutionary endpoint without a clinically meaningful loss of CAR T-cell expansion or viability. Likewise, advancement should require evidence that biomarker defined states correspond to independently measured biological states more accurately than chance and with reproducible performance across experimental replicates. Demonstrating controllability without demonstrating state-identification accuracy would not support the central ATIP hypothesis. Organoid studies

should additionally require concordance between the controller's measured biomarker state and the dominant intratumoral state. Animal studies should proceed only if the same locked decision rule can operate without retrospective schedule adjustment and without excess systemic cytokine exposure or off-tumor injury. These criteria make failure informative and constrain later experiments to designs that have demonstrated both control fidelity and biological value.

8. Limitations, risks, and translational considerations

8.1 Scientific limitations

The proposed framework is derived from evidence across CAR engineering, tumor evolution, synthetic biology, and adaptive drug therapy; no cited study has tested the complete ATIP design. Drug-based adaptive therapy does not map perfectly onto living cells because CAR T cells proliferate, migrate, differentiate, and alter the microenvironment. Tumor resistance states may coexist rather than replace one another, making a single dominant module insufficient. Biomarker measurements may lag behind tumor evolution, and different lesions in the same patient may require conflicting programs. These limitations mean that ATIP, at present, should be evaluated as a research hypothesis, not as a recommended clinical protocol. A further limitation is control observability: the controller may have access only to indirect, delayed, spatially biased, or incomplete measurements of the underlying tumor state. Consequently, the biologically optimal module may not always be identifiable from clinically obtainable data. Another is

actuator latency: a biologically appropriate decision can still fail if drug delivery, gene-expression kinetics, or protein turnover changes the dominant module too slowly. These constraints should be treated as measurable failure modes rather than implementation details (23-26).

8.2 Engineering and safety risks

Encoding multiple inducible programs could exceed vector capacity, reduce expression consistency, increase manufacturing failure, or create unanticipated interactions among regulators. Repeated exposure to control drugs may have off-target effects, immunogenicity, or variable tissue exposure. Incomplete synchronization could produce a mixed therapeutic population in which several modules remain active simultaneously. Recent translational analyses of synthetic circuits emphasize that increasing circuit sophistication also increases validation, delivery, safety, and manufacturing burden; accordingly, ATIP should advance from two-state systems to higher-order controllers only when each additional state provides a measurable benefit over the simpler design (23-26). Compact autonomous control elements such as AIR may reduce some circuit-size burden, but they do not remove the need to validate state specificity, reversibility, and population-level behavior (28). Cytokine- or chemokine-based recruitment can generate systemic inflammation, and checkpoint resistance can remove physiological restraints that normally protect healthy tissue. An independent terminal elimination switch available in every therapeutic state, a separate reversible rest program, conservative activation limits, and tissue-localized outputs would

therefore be essential.

8.3 Clinical and ethical constraints

A biomarker-guided living therapy would require frequent testing, rapid data interpretation, and access to specialized cellular-therapy centers. Those requirements could worsen existing disparities unless monitoring and manufacturing are designed for broad implementation. Decision algorithms would also raise questions about validation, accountability, and how uncertainty is communicated during consent. Because the product could change state after infusion, patients would need to understand that exposure is not a single fixed intervention. Early trials should therefore prioritize transparent stopping rules, independent safety monitoring, and long-term follow-up for insertional, genomic, immune, and secondary-malignancy risks.

8.4 Evidence boundaries and interpretive limits

The literature supports four limited conclusions. First, engineered immune cells can produce durable clinical responses in selected hematologic malignancies (2-4). Second, tumors can escape through antigenic and non-antigenic mechanisms (5-7, 11). Third, CAR T-cell and other engineered-cell functions can be externally, synthetically, or cell-autonomously controlled in experimental systems, and recent 2025-2026 literature describes increasingly sophisticated programmable and context-responsive cell circuits (16-28). Fourth, adaptive treatment principles can improve control in selected drug-based models (12-15). The literature does not establish that temporal rotation of CAR T-cell support modules will improve human outcomes. Any claim of greater

safety, efficacy, or accessibility would therefore be premature.

9. Conclusion

Genetic engineering has established T cells and NK cells as programmable therapeutic platforms, yet durable control remains limited by tumor evolution, antigen escape, therapeutic-cell dysfunction, toxicity, and engineering and manufacturing constraints. The central challenge is therefore not simply how many functions can be incorporated into an engineered immune cell, but whether those functions can be deployed at biologically appropriate times as both the tumor and the therapeutic-cell population change. Adaptive Temporal Immune Programming (ATIP) reframes CAR T-cell therapy as this two-population control problem. Rather than constitutively expressing multiple resistance-countering functions, ATIP proposes a single product containing independently controllable states whose dominance is selected using measured or model-estimated biological information.

A critical distinction is that not every module should be assumed to impose a new evolutionary pressure on the tumor. Some modules may alter relative tumor-clone susceptibility, whereas others may primarily preserve CAR T-cell fitness, reduce exhaustion, or provide reversible rest. The evolutionary premise of ATIP therefore depends on demonstrating experimentally that candidate tumor-selection-modifying modules generate distinct selection gradients and exploitable trade-offs among resistant populations. Adaptive scheduling would additionally require sufficiently accurate biomarkers, reversible and

timely switching, persistent engineered cells, and a circuit burden compatible with cell function and manufacturing.

ATIP remains an untested framework rather than evidence of a superior therapeutic strategy.

Its value is that its central claims are falsifiable through direct comparison with static, constitutively multi-module, and fixed-rotation controls while simultaneously measuring tumor-clone dynamics and CAR T-cell functional state. If adaptive temporal control delays resistant-clone dominance while reducing the burden of simultaneous multi-module expression, it would support dynamic selective pressure as a design principle for engineered immune cells. If it does not, the resulting failure modes would help define the biological and engineering limits of evolutionary control in living therapies. Thus, the immediate contribution of ATIP is not a new clinical

treatment, but a testable framework for determining whether engineered immune cells can be designed to change their functional state in response to the evolution they themselves induce.

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