Peer Review

Liang, Xiwen. 2026. “CAR T-Cell Therapy Failure in Solid Tumors as a Problem of Temporal Signal Integration.” *Journal of High School Science* 10 (1): 76–87. https://doi.org/10.64336/001c.155592

Your paper has listed the challenges but has not framed them from a systems integration perspective. I suggest the following change in the title, with the following sections.

“CAR-T Failure in Solid Tumors as a Problem of Temporal Signal Integration”

Sections

1.The Hidden Assumption: Static Antigen Availability

2.CAR Activation Requires Sustained Signal Integration

3.Metabolic Constraint Shortens Effective Signaling Time

4.Logic Gates Break Under Asynchronous Antigen Exposure

The Design Implication will be Synchronizing CAR Exposure in Time. Your central perspective will be “If you do not measure or control antigen exposure time relative to CAR signaling kinetics and metabolic capacity, your CAR design will fail — regardless of affinity, logic, or target choice.” This is testable and forward looking. If you frame your perspective in this manner, with relevant references and in-depth discussion, it will be publishable in the Journal.

You can modify your abstract to

“Chimeric antigen receptor (CAR) T-cell therapy has transformed treatment of hematologic malignancies but remains largely ineffective against solid tumors. Traditional explanations invoke antigen heterogeneity, immunosuppressive microenvironments, or CAR specificity. We argue these are symptoms of a deeper, unifying issue: a temporal mismatch between transient antigen accessibility, CAR signaling integration, and metabolic constraints in the tumor microenvironment (TME). Current CAR designs assume static antigen presentation and unlimited signaling capacity, which rarely occurs in vivo. Recognizing CAR activation as a time-dependent, energy-limited process reframes observed failures and suggests new strategies to improve efficacy in solid tumors.”

Here’s a brief summary of the sections. Expand on the discussion and provide adequate references with a thorough search of the literature.

1.The Hidden Assumption: Static Antigen Availability

Most CAR designs assume that if a target antigen is expressed at sufficient density, T-cells will recognize and eliminate tumor cells. This underlies affinity tuning, logic-gated CARs, and neoantigen-targeting strategies. However, antigen expression does not guarantee antigen accessibility, nor does it imply temporal persistence.

In solid tumors, antigens can be shielded by protein folding, membrane clustering, or extracellular matrix, or trafficked in a cell-cycle–dependent manner. Two tumor cells with identical surface antigen levels can differ dramatically in effective exposure to CARs. Yet most preclinical studies treat antigen density as static, ignoring the temporal fragmentation of exposure.

CAR activation is not instantaneous; it requires sustained receptor engagement to integrate intracellular signals. Intermittent antigen presentation may initiate partial signaling insufficient to trigger full cytotoxicity. In this framework, antigen heterogeneity is both a spatial and temporal phenomenon, producing windows of opportunity often too brief for T-cell activation.

2.CAR Activation as a Time-Integrating Process

CAR signaling is often described as a binary switch, but activation involves sequential phosphorylation, signal amplification, and transcriptional reprogramming. Successful effector function requires signals to persist long enough to surpass intracellular thresholds.

Transient or incomplete antigen encounters can result in subthreshold activation, inducing dysfunction or exhaustion without killing tumor cells. Increasing CAR affinity cannot compensate for short exposure durations. This reframing explains why tumor cells “escape” CAR therapy: activation thresholds are never met, despite sufficient antigen density.

3.Metabolic Constraints Shorten Effective Signaling

Signal integration is energetically costly. Solid tumors create nutrient-depleted, hypoxic, and acidic environments that limit T-cell metabolic capacity. Competition for glucose, amino acids, and oxygen with tumor and suppressive myeloid cells shortens the effective window for signaling. Metabolic limitations make partial CAR activation more likely, accelerating exhaustion. Thus, failures often attributed to immunosuppression or antigen heterogeneity may in fact reflect energy-limited signal integration. Enhancing CAR signaling without accounting for metabolic constraints risks increasing dysfunctional activation states.

4.Logic-Gated CARs Fail Under Asynchrony

Logic-gated CARs, designed to reduce off-tumor toxicity, require simultaneous presence of multiple antigens. These designs assume synchronous antigen expression, rarely true in solid tumors. Asynchronous or temporally fluctuating antigens may generate incomplete signals, reducing efficacy or inducing partial activation.

This explains why complex CAR circuits that perform in vitro often fail in vivo. Logic gating does not overcome heterogeneity without temporal alignment, emphasizing the need to consider when antigens are available, not just which antigens are present.

5.A Unifying Failure Mode: Temporal Mismatch

Antigen heterogeneity, metabolic suppression, and logic-gated failures can be traced to a single underlying problem: CAR T-cells operate under static assumptions in dynamic, resource-limited systems.

Efficacy depends on the alignment of three time-dependent variables:

Antigen accessibility windows

Duration of CAR signaling integration

Metabolic capacity to sustain signaling

When these are mismatched, therapy fails predictably, regardless of affinity tuning, logic gating, or antigen selection. This framework unifies many disparate observations in solid tumor CAR research.

6.Design Implications: Synchronizing CARs in Time

Improving CAR therapy requires explicit incorporation of temporal and metabolic dynamics. Potential strategies include:

Time-synchronized CAR delivery: dosing aligned with maximal antigen accessibility

Conditional activation systems: delaying signaling until sustained antigen engagement occurs

Metabolic priming: buffering TME or engineering T-cells to extend energy availability

These approaches shift the design question from “What antigen to target?” to “When and for how long should CAR signaling occur?”, emphasizing the temporal dimension as critical for efficacy.

7.Conclusions

CAR T-cell failures in solid tumors are not solely due to antigen choice, immune suppression, or toxicity. Recasting CAR activation as a time-dependent, energy-limited signal integration process provides a unifying explanation for diverse failure modes.

This perspective is actionable: it prioritizes experimental measurements of temporal antigen accessibility, CAR activation kinetics, and T-cell metabolic status. Aligning these factors may enable next-generation CAR designs that succeed where current approaches fail, offering a conceptual framework to move beyond hematologic malignancies toward durable solid tumor therapy.

By focusing on temporal dynamics and metabolic capacity, CAR research can shift from incremental optimizations to mechanistically informed strategies, ensuring that engineered T-cells act when and where they are needed most.

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Manuscript Title (Original): *Engineering CAR T-cell Therapy for Solid Tumors_ Current Advancements and Next-Generation Approaches*

Manuscript Title (Revised): *CAR T-cell Therapy Failure in Solid Tumors as a Problem of Temporal Signal Integration*

Reviewer Comment 1

“Your paper has listed the challenges but has not framed them from a systems integration perspective. I suggest the following change in the title, with the following sections.
“CAR-T Failure in Solid Tumors as a Problem of Temporal Signal Integration” Sections

1. The Hidden Assumption: Static Antigen Availability
2. CAR Activation Requires Sustained Signal Integration
3. Metabolic Constraint Shortens Effective Signaling Time
4. Logic Gates Break Under Asynchronous Antigen Exposure

The Design Implication will be Synchronizing CAR Exposure in Time. Your central perspective will be “If you do not measure or control antigen exposure time relative to CAR signaling kinetics and metabolic capacity, your CAR design will fail — regardless of affinity, logic, or target choice.” This is testable and forward looking. If you frame your perspective in this manner, with relevant references and in-depth discussion, it will be publishable in the Journal.”

Edits

1. Title revision—changed to *CAR T-cell Therapy Failure in Solid Tumors as a Problem of Temporal Signal Integration*

2. Overall Conceptual Reframing

- The manuscript has been reorganized around the central thesis that CAR T-cell failure in solid tumors arises from a temporal mismatch between antigen accessibility, CAR signaling integration kinetics, and metabolic capacity, rather than isolated factors such as antigen choice or immunosuppression
- The idea suggested by reviewer—failure occurs if antigen exposure time is not aligned with signaling and metabolic constraints—is now explicitly stated and revisited throughout the manuscript

3. New Section Structure

With the revised structure, but organized slightly, specifically with “Logic Gates Break Under Asynchronous Antigen Exposure” integrated in section 3. The break down of sections with its according subsections are as follow:

- 3. The Hidden Assumption: Static Antigen Availability
  - 3.1 Antigen Density and Spatial Heterogeneity
  - 3.2 Current Solutions and Their Limitations
  - 3.3 Proposed Research Pathways: Integrating Accessibility and Dynamics
- 4. CAR Activation as a Time-Integrating Process
  - 4.1 CAR Signaling is Sequential and Kinetically Constrained
  - 4.2 Subthreshold Activation and Dysfunctional Signaling States
  - 4.3 Proposed Research Pathways: Integrating Temporal Mismatch
- 5. Metabolic Constraints Shorten Effective Signaling
  - 5.1 CAR Signaling as an Energy-dependent Process
  - 5.2 Metabolic Stress Causes Incomplete Integration and Exhaustion
  - 5.3 Metabolism as a Determinant of Temporal Alignment
  - 5.4 Proposed Research Pathways: Integrating Metabolism
- 6. Design Implications: Synchronizing CARs in Time Outlined
- 7. Conclusion

Reviewer Comment 2

“You can modify your abstract to…”

Edits

The abstract has been rewritten to closely follow the reviewer's proposed version, with minor edits such as adding a preview of the sections I'll be talking about in the paper.

Reviewer Comment 3

Here's a brief summary of the sections. Expand on the discussion and provide adequate references with a thorough search of the literature.

The Hidden Assumption: Static Antigen Availability (summary)

Most CAR designs assume that if a target antigen is expressed at sufficient density, T-cells will recognize and eliminate tumor cells. This underlies affinity tuning, logic-gated CARs, and neoantigen-targeting strategies. However, antigen expression does not guarantee antigen accessibility, nor does it imply temporal persistence.

In solid tumors, antigens can be shielded by protein folding, membrane clustering, or extracellular matrix, or trafficked in a
cell-cycle–dependent manner. Two tumor cells with identical surface antigen levels can differ dramatically in effective exposure to CARs. Yet most preclinical studies treat antigen density as static, ignoring the temporal fragmentation of exposure.

CAR activation is not instantaneous; it requires sustained receptor engagement to integrate intracellular signals. Intermittent antigen presentation may initiate partial signaling insufficient to trigger full cytotoxicity. In this framework, antigen heterogeneity is both a spatial and temporal phenomenon, producing windows of opportunity often too brief for T-cell activation.

Edit

The Hidden Assumption: Static Antigen Availability

Current CAR T-cell strategies (affinity tuning, CAR density modulation, combinatorial targeting, and neoantigen approaches) assume that antigen expression equals antigen accessibility, which is a critical oversimplification. Increasing affinity or receptor density cannot compensate for spatial or temporal inaccessibility and instead increases off-target effects, tonic signaling, and T-cell exhaustion. Combinatorial and logic-gated CARs improve specificity but further reduce effective tumor recognition when required antigens are transiently inaccessible. Neoantigen targeting remains limited by tumor heterogeneity, immune selection, and fluctuating antigen presentation. Overall, existing approaches focus on *what* to target while neglecting *when and where* antigens are accessible. Future research should shift toward quantifying epitope accessibility and mapping temporal antigen dynamics to better align CAR
T-cell activity with functional antigen availability.

Reviewer comment 4

CAR Activation as a Time-Integrating Process

CAR signaling is often described as a binary switch, but activation involves sequential phosphorylation, signal amplification, and transcriptional reprogramming. Successful effector function requires signals to persist long enough to surpass intracellular thresholds.

Transient or incomplete antigen encounters can result in subthreshold activation, inducing dysfunction or exhaustion without killing tumor cells. Increasing CAR affinity cannot compensate for short exposure durations. This reframing explains why tumor cells
"escape" CAR therapy: activation thresholds are never met, despite sufficient antigen density.

Edit

CAR Activation as a Time-Integrating Process (summary)
Many CAR T-cell strategies also assume that antigen recognition instantly triggers cytotoxicity, treating CAR activation as an on–off switch. In reality, CAR signaling is sequential and kinetically constrained, requiring sustained or repeated antigen engagement to
integrate intracellular signals and cross activation thresholds. In solid tumors, antigen encounters are often brief or fragmented, producing subthreshold signaling that fails to induce killing while promoting dysfunctional states such as early exhaustion. This explains why tumors with adequate antigen expression can evade CAR T-cell killing despite apparent accessibility. To address this temporal mismatch, future research should quantify signaling kinetics, decay rates, and minimum engagement times using live-cell imaging, real-time signaling analysis, and computational modeling, explicitly integrating time-dependent signal accumulation into CAR design and evaluation.

Reviewer comment 5

Metabolic Constraints Shorten Effective Signaling
Signal integration is energetically costly. Solid tumors create nutrient-depleted, hypoxic, and acidic environments that limit T-cell
metabolic capacity. Competition for glucose, amino acids, and oxygen with tumor and suppressive myeloid cells shortens the effective window for signaling.

Metabolic limitations make partial CAR activation more likely, accelerating exhaustion. Thus, failures often attributed to immunosuppression or antigen heterogeneity may in fact reflect energy-limited signal integration. Enhancing CAR signaling without accounting for metabolic constraints risks increasing dysfunctional activation states.

Edit

Metabolic Constraints Shorten Effective Signaling (summary)

A third major limitation in CAR T-cell therapy is metabolic competition within the solid tumor microenvironment. CAR activation is energy-dependent, but nutrient scarcity, hypoxia, and acidity in tumors shorten the time CAR T-cells can sustain signaling. This leads to incomplete signal integration, promoting partial activation and exhaustion rather than cytotoxicity. Metabolic stress thus creates a feedback loop that progressively reduces CAR T-cell function, even when antigen exposure is adequate. Addressing this requires integrating metabolic measurements with signaling and antigen dynamics, and developing strategies that enhance metabolic resilience or extend the duration of metabolically supported CAR signaling.

Reviewer comment 6

Logic-Gated CARs Fail Under Asynchrony

Logic-gated CARs, designed to reduce off-tumor toxicity, require simultaneous presence of multiple antigens. These designs assume synchronous antigen expression, rarely true in solid tumors. Asynchronous or temporally fluctuating antigens may generate
incomplete signals, reducing efficacy or inducing partial activation.

This explains why complex CAR circuits that perform in vitro often fail in vivo. Logic gating does not overcome heterogeneity without temporal alignment, emphasizing the need to consider when antigens are available, not just which antigens are present.

Edit

This section is included in section 3: “The Hidden Assumption: Static Antigen Availability”

Reviewer comment 7

A Unifying Failure Mode: Temporal Mismatch

Antigen heterogeneity, metabolic suppression, and logic-gated failures can be traced to a single underlying problem: CAR T-cells operate under static assumptions in dynamic, resource-limited systems.

Efficacy depends on the alignment of three time-dependent variables:

Edit

I combined the analysis and the according suggested improvement/ future research pathways within each section (after I addressed the temporal mismatch).

Reviewer comment 8

Design Implications: Synchronizing CARs in Time

Improving CAR therapy requires explicit incorporation of temporal and metabolic dynamics.
Potential strategies include: Time-synchronized CAR delivery: dosing aligned with maximal antigen accessibility
Conditional activation systems: delaying signaling until sustained antigen engagement occurs
Metabolic priming: buffering TME or engineering T-cells to extend energy availability

These approaches shift the design question from “What antigen to target?” to “When and for how long should CAR signaling occur?”, emphasizing the temporal dimension as critical for efficacy.

Edit

In this section, I included a brief summary of the proposed pathways I talked about previously in each sections:

1. Time-synchronized CAR Delivery

2. Conditional Activation Systems: Gated CAR T-cells that delay activation until sufficient antigen is available, not just density.

3. Metabolic Enhancement and Support

Reviewer comment 9

Conclusions

CAR T-cell failures in solid tumors are not solely due to antigen choice, immune suppression, or toxicity. Recasting CAR activation as a time-dependent, energy-limited signal integration process provides a unifying explanation for diverse failure modes.

This perspective is actionable: it prioritizes experimental measurements of temporal antigen accessibility, CAR activation kinetics, and T-cell metabolic status. Aligning these factors may enable next-generation CAR designs that succeed where current approaches fail, offering a conceptual framework to move beyond hematologic malignancies toward durable solid tumor therapy.

By focusing on temporal dynamics and metabolic capacity, CAR research can shift from incremental optimizations to mechanistically informed strategies, ensuring that engineered T-cells act when and where they are needed most.

Edit

Conclusion similar to suggested version, with minor stylistic edits.

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Thank you for addressing my comments. The manuscript now becomes a valuable contribution since it introduces a new temporal framework for CARs in solid tumors. However, many of your claims overreach. Some additional inconsistencies and suggestions are identified and listed below.

1.“Most current CAR T-cell strategies implicitly assume static antigen availability and instantaneous T-cell activation.” Instead of this, write "Many conventional CAR T-cell strategies are evaluated under simplified conditions that treat antigen expression and encounter as effectively constant during functional assays, and they rarely account explicitly for temporal variability of antigen presentation or the duration of antigen engagement required for full T-cell activation. "

2." Two tumor cells with identical nominal antigen expression may differ drastically in CAR accessibility due to protein folding, membrane clustering, or shielding by the extracellular matrix." Instead of this, write "Even cells with similar measured antigen levels may differ in how accessible those antigens are to CAR T-cells, influenced by extracellular matrix structures and cell surface context that can impede physical access.”

3.“Many tumor-associated antigens are produced or moved during certain phases of cell division.” Instead of this, write "“Some antigens exhibit dynamic changes in expression associated with cellular processes, suggesting that antigen presentation could vary over time, though this has not been comprehensively mapped in vivo for many tumor types.”

4.“Intermittent antigen encounters produce subthreshold activation, inducing partial signaling or exhaustion without effective cytotoxicity.” Instead of this, write “Intermittent or brief antigen encounters may fail to sustain the signal strength required for full activation; when occurring repeatedly in suppressive microenvironments, such suboptimal stimulation could contribute to dysfunctional T-cell states alongside other stressors such as chronic antigen exposure and metabolic constraints.”

5.“Metabolic resources are sufficient, signaling cascades can be maintained long enough to integrate antigen-derived inputs and surpass activation thresholds.” Instead of this, write “When metabolic resources such as glucose, amino acids, and oxygen are available, CAR T-cells have greater capacity to sustain effector functions; in contrast, nutrient depletion and acidotic conditions typical of solid tumor microenvironments can undermine intracellular signaling cascades and T-cell persistence.”

6.“Lactate accumulation is almost never discussed, even though due to the Warburg Effect, the pH … drops continuously.”, instead of this, write “Although glucose competition and amino acid scarcity are frequently examined, other metabolic factors such as lactate accumulation and extracellular acidity also influence T-cell function in solid tumors and merit further attention.”

7.“Time-synchronized CAR Delivery … aligns CAR T-cell dosing with periods of maximal antigen availability … maximizing therapeutic effectiveness.” Instead of this, write “One conceptual strategy is to explore whether aligning CAR T-cell dosing with periods of enhanced antigen expression in tumors could improve engagement, though identifying such windows and demonstrating clinical benefit remains untested.”

8.“Conditional Activation Systems … prevent premature signaling during transient antigen exposure.” Instead of this, write “Conditional activation systems, such as synNotch or logic-gated CARs, aim to improve specificity by requiring defined combinations of signals before full activation; future designs might incorporate features that additionally favor sustained rather than transient antigen engagement.”
9.Include this verbiage in the conclusion (with appropriate pruning and rewording, in the abstract as well). This shows that your framework is not merely theoretical but clinically achievable.
“Although direct real-time monitoring of CAR T-cell signal integration in patients remains a technological frontier, a convergence of in vitro real-time assays, biosensor imaging, non-invasive in vivo tracking, and quantitative modeling provides a robust toolkit for probing temporal dynamics. These emerging capabilities make the temporal mismatch framework conceptualized in this paper not merely theoretical but experimentally testable and clinically relevant.” In addition, include the following in the abstract “A central priority for future studies should be the development and validation of robust temporal monitoring tools for CAR T-cell antigen engagement and signaling integration, both in preclinical models and clinical trials.”
10.Please check language, grammar, sentence structure and composition throughout the manuscript.

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Reviewer comment 1
many of your claims overreach.

Edit
I read through and took out words such as “most,” “ensures,” “prevent,” ect and replaced by words such as “many,” “often,” “may,” ect.; reframed sentence structures and phrases to avoid overstatement all together (in addition to the following suggested edits from the reviewer below)

The manuscript now avoids overreach.

Reviewer comment 2
Some additional inconsistencies and suggestions are identified and listed below.

5. “Most current CAR T-cell strategies implicitly assume static antigen availability and instantaneous T-cell activation.” Instead of this, write "Many conventional CAR T-cell strategies are evaluated under simplified conditions that treat antigen expression and encounter as effectively constant during functional assays, and they rarely account explicitly for temporal variability of antigen presentation or the duration of antigen engagement required for full T-cell activation. "

Edit
Integrated verbatim into the according sentence.
Reviewer comment 3
6. " Two tumor cells with identical nominal antigen expression may differ drastically in CAR accessibility due to protein folding, membrane clustering, or shielding by the extracellular matrix." Instead of this, write "Even cells with similar measured antigen levels may differ in how accessible those antigens are to CAR T-cells, influenced by extracellular matrix structures and cell surface context that can impede physical access.”
7. Edit
Integrated with slight edits of stylistic choices and details: Even cells with similar measured antigen levels may differ in how
accessible those antigens are to CAR T-cells, influenced by extracellular matrix structures and cell surface context, such as protein folding or membrane clustering, that can impede physical access, creating spatial heterogeneity (4).

Reviewer comment 4

8. “Many tumor-associated antigens are produced or moved during certain phases of cell division.” Instead of this, write "“Some antigens exhibit dynamic changes in expression associated with cellular processes, suggesting that antigen presentation could vary over time, though this has not been comprehensively mapped in vivo for many tumor types.”

Edit
Integrated verbatim except “for many tumor types” part.

Reviewer comment 5
9. “Intermittent antigen encounters produce subthreshold activation, inducing partial signaling or exhaustion without effective cytotoxicity.” Instead of this, write “Intermittent or brief antigen encounters may fail to sustain the signal strength required for full activation; when occurring repeatedly in suppressive microenvironments, such suboptimal stimulation could contribute to dysfunctional T-cell states alongside other stressors such as chronic antigen exposure and metabolic constraints.”

Edit
Integrated verbatim
Reviewer comment 6
10. “Metabolic resources are sufficient, signaling cascades can be maintained long enough to integrate antigen-derived inputs and surpass activation thresholds.” Instead of this, write “When metabolic resources such as glucose, amino acids, and oxygen are available, CAR T-cells have greater capacity to sustain effector functions; in contrast, nutrient depletion and acidotic conditions typical of solid tumor microenvironments can undermine intracellular signaling cascades and T-cell persistence.”

Edit
Used the format, though made edits of stylistic choices and extra details for comprehensive paper: When metabolic resources such as glucose, amino acids, and oxygen are available, CAR T-cells have greater capacity to sustain effector functions (20). In contrast, nutrient depletion and acidotic conditions typical of solid tumor microenvironments, such as amino acid scarcity and acidic pH due to tumor metabolism and competition, can undermine intracellular signaling cascades and T-cell persistence (21,22,23).
Reviewer comment 6
11. “Lactate accumulation is almost never discussed, even though due to the Warburg Effect, the pH … drops continuously.”, instead of this, write “Although glucose competition and amino acid scarcity are frequently examined, other metabolic factors such as lactate accumulation and extracellular acidity also influence T-cell function in solid tumors and merit further attention.”

Edit
Integrated verbatim
Reviewer comment 7
12. “Time-synchronized CAR Delivery … aligns CAR T-cell dosing with periods of maximal antigen availability … maximizing therapeutic effectiveness.” Instead of this, write “One conceptual strategy is to explore whether aligning CAR T-cell dosing with periods of enhanced antigen expression in tumors could improve engagement, though identifying such windows and demonstrating clinical benefit remains untested.”
13. Edit
Used the idea suggested, edit the paragraph to the following: Time-synchronized CAR Delivery. This conceptual strategy is to explore whether aligning CAR T-cell dosing with periods of enhanced antigen expression in tumors could improve engagement. Rather than relying on continuous or

arbitrary infusion schedules, temporally optimized delivery may ensure CARs encounter their targets during windows when signaling integration is achievable, potentially maximizing cytotoxic potential while reducing wasted cellular effort.
Identifying such windows and demonstrating clinical benefit, however, remains untested.

Reviewer comment 8
14. "Conditional Activation Systems … prevent premature signaling during transient antigen exposure." Instead of this, write "Conditional activation systems, such as synNotch or logic-gated CARs, aim to improve specificity by requiring defined combinations of signals before full activation; future designs might incorporate features that additionally favor sustained rather than transient antigen engagement."

Edit
Used the idea suggested, edit the paragraph to the following: Conditional Activation Systems, such as synNotch or logic-gated CARs, aim to improve specificity by requiring defined combinations of signals before full activation; future designs could incorporate features that additionally favor sustained rather than transient antigen engagement.

Reviewer comment 9
15. Include this verbiage in the conclusion (with appropriate pruning and rewording, in the abstract as well). This shows that your framework is not merely theoretical but clinically achievable. "Although direct real-time monitoring of CAR T-cell signal integration in patients remains a technological frontier, a convergence of in vitro real-time assays, biosensor imaging, non-invasive in vivo tracking, and quantitative modeling provides a robust toolkit for probing temporal dynamics. These emerging capabilities make the temporal mismatch framework conceptualized in this paper not merely theoretical but experimentally testable and clinically relevant." In addition, include the following in the abstract "A central priority for future studies should be the development and validation of robust temporal monitoring tools for CAR T-cell antigen engagement and signaling integration, both in preclinical models and clinical trials."

Edit
Integrated both sections to abstract and conclusion.

Reviewer comment 10
Please check language, grammar, sentence structure and composition throughout the manuscript.

Edit
Fixed incoherent/ sentences that are not smooth for the entire paper after all the edits made above.