Making drugs from T cells: Quantitative Analysis of CAR-T Pharmacology

FOCIS Cancer Immunity & Immunotherapy Course
June 20, 2023
Daniel Kirouac
Pharmacometrics: Quantitative Pharmacokinetics & Pharmacodynamics (PKPD)

Pharmacokinetics (PK): Dose-Exposure

- C\textsubscript{max}: maximal concentration
- T\textsubscript{max}: time at C\textsubscript{max}
- AUC: Area under the Curve
- CL: Clearance rate (~half-life\textsuperscript{-1})

Pharmacodynamics (PD): Exposure-Response

- Effect = E\textsubscript{max} \cdot \left( \frac{C^k}{C^k + EC50^k} \right)
- Therapeutic index: EC50(efficacy) – EC50(tox)

PKPD: Dose regimen optimization

How do we apply these quantitative metrics to adoptive T cell therapy?
Adoptive T cell therapy: what drives exposure/response?

Distribution
• Where do T cells go?
• Does proliferation/expansion occur in tissues or blood?

Cell Expansion
• Memory vs. exhaustion phenotype...sometimes
• Intrinsic proliferative capacity of the cells
• CAR design & expression
• Patient cytokine levels
• Tumor burden

Contraction & Clearance/Persistence
• Memory cell generation following antigen clearance
• Competition from host T cells for ‘space’
• Allogeneic elimination (host vs. graft)

Anti-tumor efficacy & toxicity (CRS)
• Exposure (Cmax / AUC)
• Intrinsic cytotoxic potency
• CAR design & expression
• Tumor Microenvironment inflammatory/anti-inflammatory signals
• Tumor homing/penetration**


Adoptive T cell therapy: what drives exposure/response?

Outline

1. What pharmacometrics predict patient response?
   - *Empirical* pharmacokinetic (PK) modelling

2. What cell-intrinsic properties of the CART product underly the wide clinical variability?
   - *Mechanistic* PKPD modelling of Tcell:tumor interactions
   - *Machine learning* model for predicting response

3. What patient-intrinsic factors mediate response?
   A. T cell bio-distribution*
   B. Tumor inflammation
   C. Lympho-depletion regimen & patient response
   D. Host vs. Graft (allogeneic clearance)

1. What CAR-T pharmacometrics predict response?
CAR-T pharmacokinetic ("cellular kinetics") model
Developed for Kymriah (TISAGENLECLEUCEL-T) BLA

Empirical model quantifies PK curves

**Math**

\[
\begin{align*}
\text{Expansion} & : T_{\text{max}} \\
\text{Decline} & : T_{\text{max}} \\
\text{Effector} & : p \\
\text{Memory} & : \alpha \\
\end{align*}
\]

\[
\begin{align*}
\text{Expansion} & : \frac{p}{k_{+}+k_{-}} \\
\text{Decline} & : \frac{p}{k_{-}} \\
\text{Effector} & : \frac{p}{k_{+}} \\
\text{Memory} & : \frac{p}{k_{-}} \\
\end{align*}
\]

PK simulations vs. clinical data

Internal model simulations

**Model parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>THETA (mean)</th>
<th>ETA (variance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax</td>
<td>24000 (counts/ug)</td>
<td>0.65</td>
</tr>
<tr>
<td>Tmax</td>
<td>9.3 (day)</td>
<td>0.38</td>
</tr>
<tr>
<td>foldX (Cmax/C_0)</td>
<td>3900</td>
<td>2.4</td>
</tr>
<tr>
<td>Fb (fraction Tm at tmax)</td>
<td>0.0079</td>
<td>0.8</td>
</tr>
<tr>
<td>Alpha (contraction)</td>
<td>0.16 day⁻¹</td>
<td>0.91</td>
</tr>
<tr>
<td>Beta (persistence)</td>
<td>0.0032 day⁻¹</td>
<td>0.86</td>
</tr>
</tbody>
</table>

CAR-T exposure-response analyses
Abecma in Multiple Myeloma

Inter-individual variability (IIV) washes out dose-responses
Kymriah in DLBCL

Impossible to dose-optimize (current)-CARTs

2. What cell-intrinsic properties underly clinical variability and response?


“Toggle switch” model structure and assumptions

T cell differentiation **toggle switch**

- Low antigen ($B_A$) levels
  - $T_M$ self-renewal
  - $T_M$ regeneration from $T_E$
- High antigen ($B_A$) levels
  - $T_M$ differentiation
  - $T_E$ proliferation
  - $T_E$ exhaustion ($T_X$)
- $T$ effectors kill B-cells
- N cell divisions within $T_E$ compartment

*Toggle switch* model structure and assumptions

- $T_M$: memory T cells
- $T_E$: effector T cells
- $T_X$: exhausted T cells
- B: B cells (tumor)
- $B_A$: B cell antigen

![Diagram showing the toggle switch model with nodes $T_M$, $T_E$, $T_X$, $B_A$, and B cells, and arrows for inhibition and stimulation.](image-url)

Kymriah (Tisagenlecleucel): CD19-targeted CAR-T approved for the treatment of B-cell lymphomas

Model training data: Kymriah in Chronic Lymphoblastic Leukemia
PKPD profiles, CAR-T product transcriptomes and immuno-phenotypes vs. response

Population mean PKPD: Kymriah in Chronic Lymphoblastic Leukemia (CLL)

*mean ± std, digitized from publication
CR=8, PR =5, NR=25

CR = Complete Response
PR = Partial Response
NR = Non-Response

Can we recapitulate the pharmacokinetics & tumor dynamics (PKPD) based on T cell biology?

What kinetic parameters / molecular features distinguish robust vs. poor responding patients?

Pre-infusion CAR-T transcriptomes
CR=5, PR =5, NR=21

Pre-infusion CAR-T immunophenotype

Model development and validation workflow

**Conceptual model of T cell biology**
- Toggle switch circuit
  - $T_N$, $T_H$, $T_K$, $T_P$, $T_D$, $B_1$
  - Red: inhibition, Blue: stimulation

**Mechanism-based dynamical model**
- Math
- Executable code

**Clinical Training Data**
- Kymriah in CLL: PKPD separated by response
- Abecma in MM: Phase1 escalation
  - What determines CR/PR/NR? What makes an effective dose?

**Genomic “Validation” Data**
- ssRNAseq: CR vs. NR classifier
- ssRNAseq: CR vs. NR in ALL
- Bulk RNAseq: CR vs. NR in CLL
  - How do model parameters relate to cell populations and pathways?

**Clinical Validation Data**
- Kymriah in B-ALL: Quantification of IIV
- Yescarta in LBCL: Covariates of response
  - What parameters underly IIV? Do simulations predict response?
Model calibration & analysis
What features (model parameters) separate clinical outcomes?

**Parameter Analysis**

- Tmem prolif & death rates
- Cytotoxic potency

**Model calibration**

- CR
- PR
- NR

**What differentiates CR vs. NR?**

1. Heightened memory cell turnover ($\mu_M, d_M$
2. Heightened cytotoxic potency (TK50)
3. Little difference in Tmem/Texh frequency

---

*Assume Dose = $10^8$ cells, Tumor burden = $10^{10}$ cells (median reported); Estimate parameters using PSO: simulations represent 90% confidence intervals

‘Validation’ of model inferences via single-cell transcriptomes
Mathematical inferences assessed in an additional blood cancer: Acute Lymphoblastic Lymphoma

T cell composition (memory vs. exhausted cells) does not substantially vary by response category

scRNAseq: Kyrmiah in ALL annotated by Response

T cell population frequencies by response category

Cell-intrinsic differences


T memory cells from NR patients display intrinsic functional deficits analogous to T cell exhaustion

scRNAseq: Kyrmiah in ALL annotated by Response

T cell population frequencies by response category


CAR-T clinical response prediction
Are pre-infusion CAR-T transcriptomes predictive of clinical response (CR vs. NR)?

Machine learning workflow

Model training & validation: Repeat 2,500X: 40:60 test:train splits

Lage P, small N problem: the central challenge in biomedical genomics
CAR-T clinical response prediction
Are pre-infusion CAR-T transcriptomes predictive of clinical response (CR vs. NR)?

Predictive accuracy of response classification using 60:40 train:test splits

**Kymriah in ALL** (Bai 2022)
- **Accuracy** = 80%
- Tmem, Tex: CITESeq data
- CR = 5; NR/RL = 7

**Kymriah in LBCL** (Haradhvala 2022)
- **Accuracy** = 80%
- Tmem, Tex: ProjecTILS*
- CR = 6; NR = 7

**Yescarta in LBCL** (Haradhvala 2022)
- **Accuracy** = 71%
- Tmem, Tex: ProjecTILS
- CR = 11; NR/PR = 8

Functional attributes predictive of clinical outcomes are CART-cell-intrinsic & indication-agnostic
Transcriptome > ‘gold standard’ immunophenotyping
CAR-T clinical response prediction
What transcriptional signatures are predictive of CAR-T response?

**CAR-T Response Score-card**

<table>
<thead>
<tr>
<th></th>
<th>Kymriah in CLL</th>
<th>Kymriah in ALL</th>
<th>Kymriah in LBCL</th>
<th>Yescarta in LBCL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Score</strong></td>
<td>Complete</td>
<td>Non-durable</td>
<td>Complete</td>
<td>Non-durable</td>
</tr>
<tr>
<td><strong>Frequency</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Accuracy</strong></td>
<td>90%</td>
<td>80%</td>
<td>80%</td>
<td>71%</td>
</tr>
</tbody>
</table>

**P:** PROGENy
**F:** Fraietta 2018
**H:** Hallmark
**R:** Reactome
**A:** Albert 2018
3. Patient-intrinsic factors mediating response

A. T cell biodistribution
B. Tumor Inflammation
C. Response to Lympho-depletion & host-T cell competition
D. Host vs. Graft response (allogeneic elimination)
3A. Adoptive T cell Biodistribution

Where do CAR-Ts go once administered? What happens in tissues vs. Blood?

**Pharmacokinetics & biodistribution of radio-labelled T cells in mice**

- Whole blood
- Lungs
- Heart
- Spleen
- Liver
- Tumor
- Bone
- TDN
- IGLN

**Pharmacology ‘accounting’ in man vs. mouse**

- Kymriah in B-ALL
  - ER ~ 100
  - ER ~ 5
  - ER ~ 1/4

- Kymriah in NALM6 xenograft mice
  - ER ~ 1/60

**Question:** Where do the majority of CARTs distribute?

**Question:** Where does the ‘action’ happen (tissue vs. blood)?

*BC = Biodistribution Coefficient. = AUC of T cells in tissue vs. blood

Majority of administered T cells distribute to lungs, spleen, liver, kidney & lymph nodes.

Q: Where do the majority of CARTs distribute?

Q: Where does the ‘action’ happen (tissue vs. blood)?

*ER = Expansion Ratio. How many cells do you detect at Cmax per infused? = Cmax*Vblood / Dose

3B. Tumor inflammation and CAR-T response
Yescarta (CD19-CART) in DLBCL: ZUMA-1 trial

‘Immunoscore’ (Tumor inflammation) is the most significant patient-intrinsic predictor of CART response

Immunoscore (Tumor inflammation) also drives Cmax

Q: How would pre-existing TILs influence CAR-T expansion?
3C. Lympho-depletion intensity & response
via IL7 availability?

Lymphodepletion intensity drives CART expansion

PFS vs. lymphodepletion

\[ P = .05 \]

Lymphodepletion intensity drives IL7 expression

Q: How does Lympho-depletion intensity affect CAR-T expansion and peak IL7 concentration?
Q: Can we *mimic* intense-LDT via cytokine support?

*60 vs. 30 mg/kg cyclophosphamide, CD19 CART therapy in NHL

Q: What is the mechanism underlying T cell competition for limited 'space'?
3D. Host vs. Graft response (allogeneic elimination)
Host T cells actively clear (allogenic) T cell grafts

UCART19 in B-ALL: The first reported allogeneic CAR-T clinical data
CD19-CART, allogeneic (healthy donor-derived) T cells, TRAC⁻

Allogeneic Elimination

UCART19 vs. Kymriah
CART Pharmacokinetics

Host T cell reconstitution ~ UCART19 exposure

- Deeper LDT & slower T cell reconstitution ~ greater allogenic CART exposure

Q: How would additional gene edits (i.e. MHC-knock out) affect allo-clearance rates


The next frontier: iPSC-derived CAR-Ts

FT819: The first reported clinically tested iPSC-derived CART
CD19-CART, allogeneic (iPSC-differentiated) T cells, TRAC-/-

• Both robust cell expansion + persistence (AUC) is required for clinical activity

Q: Why are (FT819) iPSC-CARTs incapable of persistence - *Cell intrinsic* deficit vs. *allogeneic*-clearance?


PK data digitized from ASH poster
*n=1 patient*

FT819 vs. Kymriah
CART Pharmacokinetics

AUC vs. Durable Response

Probability of Response:
B-cell reduction to 'normal' at 1 yr
1. Empirical PKPD models

- Cmax predicts response
- High variability makes dose-optimization infeasible

2. Mechanistic modelling & machine learning

- Product intrinsic-proliferation of memory cells is important for clinical response
- Predictive features are buried in CART transcriptomes

3. Patient-intrinsic effects

- Biodistribution, inflammatory state, lympho-depletion response, and Host vs Graft affect PK and response

Mathematical models can enable CAR-T design, optimization and data interpretation
Quantitative data is required to translate measurements to kinetic parameters
Thank You!

**Vancouver, BC**
✓ Developmental immunology  
✓ Systems Biology and T cell pharmacology

**Seattle, WA**
✓ Protein and genome engineering  
✓ Translational sciences  
✓ Cancer biology

**Toronto, ON**
✓ GMP iPSCs and gene editing  
✓ T cell manufacturing  
✓ QA/QC

Avisek Deyati, Jordan Sicherman, Cole Zmurchok, Peter Zandstra, Chris Bond, Gregory Block, Irja Elliott Donaghue
What value does modelling bring to drug development?

The biological mechanisms underlying experimental data are often complex and non-intuitive.

The number of possible experiments to conduct is infinite.
3D. Host vs. Graft response (allogeneic elimination)
Host T cells actively clear (allogenic) T cell grafts

UCART19 in B-ALL: The first reported allogeneic CAR-T clinical data
CD19-CART, allogeneic (healthy donor-derived) T cells, \( TRAC^{--} \)

Cmax predicts response

Host T cell reconstitution limits CAR-T expansion

Initial expansion (Cmax) predicts response for multiple CAR-Ts
Clearance does not (for autologous products)


Cell Kinetic model to data from 7 CART trials (Jansen)
Model-based insights into clinical response:
cell dose & tumor burden

Predicted covariates of response: Cmax vs. Tumor Burden
Virtual Populations vs. Yescarta in LCBCL (ZUMA-1)


Mechanism-based models can predict biological processes underlying clinical observations
Lympho-depletion intensity & response via IL7 availability?

Cyclophosphamide (Cy) vs. Cy + Fludarabine (Flu): CD19-CART therapy in B-ALL


High vs. Low-intensity Cy+Flu: CD19-CART therapy in NHL


Q: How does Lympho-depletion intensity affect CAR-T expansion and peak IL7 concentration?

Q: Can we *mimic* intense-LDT via cytokine support?

*60 vs. 30 mg/kg cyclophosphamide

3B. Tumor inflammation and CAR-T response
Yescarta in DLBCL: ZUMA-1

T cell inflamed tumors ~ improved survival

Immunoscore is the most significant “co-variate”
Cox-regression (statistical) model

Q: How would pre-existing TILs influence CAR-T expansion?