CARTopiaX: Extending a Next-Generation Platform for Computational Cancer Biology

CARTopiaX project description

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Overview

Chimeric Antigen Receptor T-cell (CAR T) therapy has revolutionized immunotherapy, particularly in treating hematological malignancies. However, its effectiveness in solid tumors remains limited due to challenges such as poor tumor infiltration, immunosuppressive microenvironments, and T-cell exhaustion. Computational modeling has become a crucial tool for optimizing CAR T-cell therapy, as it allows researchers to predict outcomes, explore various treatment strategies, and guide experimental research.

CARTopiaX [1] is an advanced agent-based model developed to address this challenge, that implements the mathematical framework proposed in the Nature paper “In silico study of heterogeneous tumour-derived organoid response to CAR

T-cell therapy”[2]. Built on BioDynaMo [3], a high-performance, open-source platform for large-scale and modular biological modeling, CARTopiaX enables detailed exploration of complex biological interactions, hypothesis testing, and data-driven discovery within solid tumor microenvironments.

This project aims to extend the capabilities of CARTopiaX by replicating cutting-edge *in vitro* assays, thereby strengthening a framework capable of simulating a broader spectrum of biological phenomena. By enhancing the model's fidelity, we provide a robust platform for *in silico* hypothesis testing in CAR T-cell therapy. Ultimately, this expansion seeks to accelerate scientific discovery by streamlining the transition from theory to clinic, significantly reducing the prohibitive costs and time traditionally associated with exhaustive wet-lab experimentation.

Background

CAR T-cell therapy [4] is a type of immunotherapy in which a patient’s own T cells are genetically engineered to recognize and attack cancer cells, and are then reinfused into the patient’s body. This therapy has proven highly effective for leukemia and other hematological malignancies. Consequently, the literature contains numerous robust mathematical models, typically based on differential equations, that simulate CAR T-cell treatment in blood cancers, such as [5], [6], and [7], the latter being based on the well-known predator-prey Lotka-Volterra equations.

In contrast, CAR T-cell therapy has shown more limited success in the treatment of solid tumors due to the unique characteristics of the tumor microenvironment. These include limited tumor infiltration, T-cell exhaustion, and immunosuppressive mechanisms [8]. Moreover, far fewer computational models exist for these scenarios, highlighting the need for new modelling approaches that allow researchers to better study and potentially overcome these microenvironmental barriers. In this context, as argued in [9], Agent-Based Models (ABMs) are particularly suitable for studying and understanding the complex local dynamics of tumor microenvironments, as emergent phenomena arise from modelling individual entities as agents governed by relatively simple behavioral rules.

Some of the most relevant state-of-the-art ABMs in this area are [10], [11], and [2]. The latter is particularly important because CARTopiaX implements its mathematical framework in order to reproduce its core results. In its current state, CARTopiaX models CAR T-cell treatment applied to a heterogeneous tumor-derived organoid system and includes the following main components:

- Two agents:
    - **Tumor cells**, which express different levels of oncoproteins and collectively form a spherical 3D organoid. Cells with higher oncoprotein expression are more proliferative but are also more susceptible to CAR T-cell killing.
    - **CAR T-cells**, which move across the extracellular matrix toward tumor cells in order to attack them.
- Two diffused chemical fields:
    - **An immunostimulatory factor** secreted by tumor cells that guides CAR T-cells through chemotaxis.
    - **Oxygen**, supplied from the container walls, which is necessary to maintain cancer cell viability and proliferation.
- Physical and numerical components:
    - Mechanical interactions modelling repulsion and adhesion forces between cells.
    - A chemical diffusion solver based on the Thomas algorithm [12].

With these components, CARTopiaX is able to reproduce the general trends observed in wet-lab experiments. However, in order to establish CARTopiaX as a practical framework for researchers studying CAR T-cell therapy, further development is needed. In this work, I propose extending the model to replicate additional biological phenomena, both providing new functionalities to users, and demonstrating the adaptability of CARTopiaX to emerging research challenges in this field.

Studied Phenomena

With the purpose of extending the model so that it can replicate observations from wet-lab studies, several state-of-the-art *in vitro* experiments provide datasets that

could be used to modify and calibrate the Agent-Based Model. Incorporating any of these datasets would require extending the current model with additional functionalities in order to represent the biological phenomena studied in the experiments. Most of these studies involve several biological mechanisms and multimodal datasets, meaning that multiple extensions would need to be implemented at the same time. The following list summarizes some relevant types of extensions and the literature supporting them; in many cases, a single study involves several of these new functionalities. These extensions can be naturally organized according to the core components of Agent-Based Models (ABMs): **Agents**, **Microenvironment** and **Rules**.

- **Agents:** A major line of extensions focuses on increasing the diversity and biological realism of the agents represented in the model.
    - First, many studies incorporate different types of immune cells, often referred to as **PBMCs** (Peripheral Blood Mononuclear Cells), including dendritic cells and other immune populations. Some of these cells are capable of recognizing and attacking tumor cells, while others regulate immune responses or present antigens to activate **T cells**. Importantly, many studies measure treatment with several kinds of T cells and **CAR T cells**, such as [21], [16], [17], [18], [19], [20], [22], [30], [31], [32] and [33].

      In addition, some models include **macrophages**, immune cells that engulf and remove dead or damaged cells through phagocytosis. Macrophages contribute to tissue homeostasis and shape the immune microenvironment by clearing cellular debris and releasing signaling molecules that influence inflammation and immune activation. Such mechanisms appear in studies such as [13], [23], [27] and [32].

      Some studies also consider **Myeloid-Derived Suppressor Cells (MDSCs)**, which reduce the effectiveness of anti-tumor immune responses by secreting immunosuppressive factors. These cells accumulate in the tumor microenvironment and inhibit T-cell activation, proliferation, and cytotoxic activity through the release of suppressive cytokines, reactive oxygen species, or metabolic inhibitors. This

suppression can significantly reduce the therapeutic effectiveness of CAR T-cells in solid tumors, as shown in [31].

- **Tumor heterogeneity** is another key aspect addressed by many works. This requires representing tumor cells with diverse biological properties. For example, some cells within the organoid may **not express the target antigen** recognized by CAR T cells and therefore cannot be attacked, as described in [21], [13], [20] and [18]. Other studies examine **antigen re-expression over time**, where tumor cells temporarily lose antigen expression [14]. Additionally, some studies research different cancers or even organoids with several tumoral subpopulations that **differ in the expressed antigens** as explored in [33], [19], and [34].
- Finally, some models incorporate **healthy stromal cells present in the tumor microenvironment**. Fibroblasts, for instance, are structural cells that produce extracellular matrix components and can physically hinder CAR T-cell movement, as in [17] and [22]. Similarly, **Cancer-Associated Fibroblasts (CAFs)**, which are fibroblasts reprogrammed by the tumor to support its growth, can create barriers that limit immune cell infiltration, as shown in [23] and [35].

- **Microenvironment:** Another major group of extensions concerns the representation of the tumor microenvironment and its biochemical and physical properties.
  - First, **almost all models require the implementation of additional chemical fields** that diffuse through the simulation grid and influence cellular behaviour. These chemical species may also serve as observable quantities for comparison with experimental measurements obtained in wet-lab assays. In particular, **cytokines** are a key family of molecules frequently included in these models. Cytokines are small signaling proteins released by immune or tumor cells that mediate intercellular communication and regulate immune activity, including recruitment, activation, and suppression of immune responses. Cytokine release syndrome (CRS), resulting from rapid immune activation induced by CAR T cells, is the most significant treatment-related toxicity, making it a key aspect to include in the

model [43]. Cytokines are commonly measured experimentally as indicators of immune activation or suppression, as in [16], [18], [19], [20], [15], [21], [29], [35] and [36].
In addition, some studies focus on **immunosuppressive factors** such as TGF-β or IL-10, which inhibit immune responses and contribute to tumor immune evasion, as shown in [17], [26], [27], [30], [31] and [32].

- Oxygen distribution is another critical component of the microenvironment. **Hypoxia** refers to regions within the tumor where oxygen concentration becomes very low due to limited diffusion and high metabolic consumption by tumor cells. These spatial gradients strongly influence tumor growth, immune cell behavior, and treatment response, as explored in [25], [17], [22], [34], and [37]. Some models also incorporate **vascular vessels** that act as sources of nutrients and oxygen diffusion into the tissue, as in [17].
- The **physical properties of the extracellular matrix (ECM)** also play a crucial role. The ECM is a complex network of proteins and polysaccharides surrounding cells in tissues. When it becomes dense or stiff, it can act as a physical barrier that limits CAR T-cell infiltration. Properties such as **medium viscosity and hydrogel barriers** can significantly affect chemotactic migration, infiltration capability, and proliferation of immune cells. These mechanisms are explored in [34], [32], [35], [17], [20], [25], [28], [30] and [29].
- Several studies also investigate **strategies to enhance CAR T-cell infiltration into rigid tumor organoids** by modifying the microenvironment. For example, some experiments use chemicals such as **nattokinase** [24], which can degrade components of the ECM and improve immune cell penetration. Other approaches, such as in [34], propose **heat-induction strategies** that temporarily alter the tumor microenvironment to facilitate CAR T-cell infiltration.

- **Rules**: Finally, many extensions discussed in the previous sections inherently introduce additional rules governing cell behaviour and state transitions in order to replicate the associated referenced studies. These rules emerge directly from the interplay between the diversity of agents and the properties

of the microenvironment, thereby defining the dynamic logic of the system and the resulting behaviours within the ABM framework.

- A representative example is **hypoxia-induced necrosis**, where oxygen deprivation leads to uncontrolled cell death. This rule directly links microenvironmental conditions with cellular fate and plays a key role in shaping tumor structure.
- Beyond this, several other important rules arise from the discussed extensions. Immunosuppressive cytokines and cells lead to **immunosuppression mechanisms** that reduce CAR T-cell activation, proliferation and cytotoxicity. Tumor heterogeneity introduces **antigen-dependent recognition and killing**, as well as the possible **antigen loss and re-expression dynamics**, enabling immune evasion.
- Additional immune populations imply rules governing **activation, exhaustion and regulation**, including antigen presentation, signaling-mediated responses and suppression by other immune cells. Macrophages further introduce **phagocytosis** and cytokine-mediated feedback.
- Microenvironmental structure gives rise to rules affecting **migration and infiltration**, where dense ECM, fibroblasts, and CAFs constrain movement, while cytokine gradients drive **chemotaxis**.
- Finally, interventions such as ECM degradation or heat-induced changes define rules that **dynamically modify tissue permeability** and immune cell access to the tumor.

In addition to implementing these model extensions, all of these studies would require **calibration of both existing and newly introduced model hyperparameters** in order to reproduce the experimental observations obtained from *in vitro* data. Among all these possibilities, the final choice of which extensions to implement would depend on two main factors: the expected scientific impact in the area of the resulting model and the practical feasibility given the available resources, including the quality, resolution, and completeness of the experimental datasets from the studies that agree to provide their data.

Model Fit & Hyperparameters tuning

In order to reproduce the dynamics observed in the selected *in vitro* experiments, the parameters of the Agent-Based Model (ABM) must be calibrated so that the simulated outputs match the empirical measurements reported in the corresponding studies. This process involves defining quantitative measures of similarity between the simulation results and the experimental data and then searching for the set of model parameters that minimizes the discrepancy.

The first step is to define **fitness functions** that measure how closely the simulation reproduces the biological observations. These metrics are computed by comparing time-series generated by the model with the corresponding measurements reported in the wet-lab experiments. Typical observables that can be used include:

- The **number of tumor cells over time**, which reflects tumor growth or reduction during treatment.
- The **number of CAR T-cells**, which may change due to proliferation, exhaustion, or death.
- The **concentration of specific chemicals or signaling molecules**, such as cytokines or other factors measured in the experiments.

The discrepancy between simulation and experimental data can be measured using common error metrics such as **Mean Squared Error (MSE)** or **Root Mean Squared Error (RMSE)** across time points. These metrics provide a single scalar value representing the level of fit between the model and the empirical observations.

A key difficulty in calibrating ABMs is their high computational cost. A full simulation of the model takes several hours to execute, and the parameter space may contain many variables such as proliferation rates, diffusion coefficients, chemotaxis strengths, or cell-cell interaction parameters. Performing a naive exhaustive search over this space would therefore be computationally infeasible. For this reason, parameter optimization must rely on **search strategies that minimize the number of model evaluations**.

Consequently, I suggest the following optimization methods, as they are particularly well suited to **computationally expensive simulations**, since they prioritize

evaluations in the most promising regions of the parameter space rather than sampling it uniformly.

- A simple approach for tuning independent parameters that have a monotonic influence on the output is **binary search**. This method iteratively narrows the interval of possible values by evaluating the model at the midpoint of the interval and selecting the half of the interval that produces results closer to the desired outcome. Binary search is especially effective when calibrating single parameters such as proliferation rates, diffusion coefficients, or decay constants, provided that the relationship between the parameter and the observed metric is approximately smooth and monotonic. In such cases, this approach can significantly reduce the number of model evaluations compared with grid search.
- For higher-dimensional parameter spaces, **Bayesian optimization** provides a more powerful strategy. Bayesian optimization builds a probabilistic surrogate model of the objective function (in this case, the fitness score measuring agreement between simulation and experimental data). Instead of directly evaluating the expensive simulation at many points, the method first fits a surrogate statistical model, such as a Gaussian process regression model as in the ABM [40], or a Random Forest as in the ABMs [38], [39], to approximate the relationship between the model parameters and the objective function. An acquisition function is then used to determine which parameter configuration should be evaluated next. This function balances exploration (testing regions of the parameter space with high uncertainty) and exploitation (refining regions where good results have already been observed). By iteratively updating the surrogate model after each simulation run, Bayesian optimization can identify optimal parameter regions with a relatively small number of expensive evaluations.
- Another suitable family of methods are **Evolutionary Algorithms**, which are population-based optimization techniques inspired by biological evolution. These algorithms maintain a population of candidate parameter sets, which are iteratively improved through operations such as selection, mutation, and recombination. At each generation, candidate solutions are evaluated using the fitness function, and those producing better results are more likely to be

retained and combined to form new candidate solutions. Evolutionary algorithms are particularly effective in large and high-dimensional search spaces, where gradient information is unavailable and the objective function may be noisy or highly non-linear, as is typically the case in stochastic ABMs. Because they explore multiple regions of the parameter space simultaneously, they are relatively robust to local optima and can handle complex interactions between parameters. Examples of their application to complex simulation-based models are discussed in [41] and [42].

In addition, to reduce computation time during the parameter search, I propose the following strategies.

- First, **early termination of unpromising simulations** can be implemented. During execution, simulations whose intermediate outputs already deviate strongly from the experimental data can be stopped prematurely. This prevents wasting computational resources on parameter configurations that clearly produce unrealistic behavior.
- Second, **reduced-complexity simulations** can be used during the initial stages of the search. The model may be executed with reduced spatial resolution, fewer cells, or shorter simulated time spans. These faster simulations allow the optimizer to identify promising regions of the parameter space before running the full high-fidelity model.
- Third, **sequential calibration of parameters** can be applied. Instead of tuning all parameters simultaneously, groups of parameters can be calibrated in stages. For example, parameters controlling tumor growth may first be fitted using simulations without CAR T-cells, after which immune-related parameters can be tuned.
- Additionally, for some specific parameters, **small controlled simulations** can be designed in order to test them independently from the rest of the system. For instance, to tune the attachment forces between a CAR T-cell and a cancer cell, it is sufficient to run a minimal simulation containing only these two cells and adjust the relevant parameters until the desired interaction behavior is obtained. The target behaviour can be guided by quantitative measurements reported in the literature for these specific interactions.

- Importantly, many **hyperparameters may remain unchanged**, since CARTopiaX is already calibrated to reproduce the results of a published model [2] that captures realistic biological dynamics. Examples include mechanical repulsion forces between cells or the oxygen diffusion coefficient, which have already been validated in previous work.
- Finally, because ABMs are stochastic, each parameter configuration should ideally be evaluated multiple times, and the fitness score can be defined as the average error across several simulation runs. However, since simulations are computationally expensive, during the early stages of the optimization the fitness can be **approximated using a single Monte Carlo run** for each parameter configuration. In the final stages, once reasonably accurate parameters have been identified, the results can be validated by performing multiple runs with different random seeds.

High-Level Implementation Schedule

The final schedule can only be agreed between the mentors and the selected candidates once announced, in addition the implementation plan highly depends on the chosen biological phenomena to extend CARTopiaX. However this is a realistic hypothetical schedule of how I would organize the derivables as a candidate.

Week	Activities	Deliverable
Week 1 (June 15-21)	Studying relevant state-of-the-art in vitro studies, contact authors for potential data sharing/collaboration. Write Compiler Research initial project blog post.	List of potential datasets and collaborators, notes on access and permissions. Compiler Research Blog Post.
Week 2 (June 22-28)	Select target study, obtain dataset and clean it, analyze experimental design and observables	Chosen study, dataset received, plan for model extension

Week 3 (June 29 - July 5)	Define required model extensions based on study: e.g., chemical fields, tumor heterogeneity, specific immune cells	Detailed list of necessary extensions, design plan for implementation
Week 4 (July 6-12)	Implement the first set of required extensions in CARTopiaX (Agents, Microenvironment, Rules)	Preliminary model updates integrated, ready for initial tests
Week 5 (July 13-19)	Run initial simulations to check feasibility of replicating study results. Keep extending the model	First simulation outputs, feedback on implementation
Week 6 (July 20-26)	Finish adding new features and install BioDynaMo and CARTopiaX on a High-performance computing cluster (HPC cluster)	Preliminary extended but unfitted model installed in a HPC
Week 7 (July 27 - Aug 2)	Midterm presentation, show definitive plan for full replication	Midterm Slides and Definitive Replication Plan
Week 8 (Aug 3-9)	Implement higher-dimensional optimization: Bayesian Optimization or Evolutionary Algorithms	Optimization method implementation
Week 9 (Aug 10-16)	Initiate model parameters calibration (binary search for simple parameters) to start fitting experimental data with simple scenarios	Initial calibration results, metrics of fit
Week 10 (Aug 17-23)	Finish reducing the hyperparameters to be tuned as much as possible by tuning some of them independently with simple simulations	Small validation reports, updated simulation results

Week 11 (Aug 24-30)	Apply the implemented higher-dimensional optimization (Bayesian Optimization or Evolutionary Algorithms) with early stopping on unrealistic parameter-sets simulations.	Progress by fitting in the configured HPC and good initial values for further optimization.
Week 12 (Sept 1-7)	Full executions for parameter values determination.	Better fit of the model
Week 13 (Sept 8-14)	Run multiple stochastic simulations to assess robustness, determine final parameter set	Model fit replicating the wet-lab data
Week 14 (Sept 15-21)	Slightly changes on the final fitted parameters to study their effect on the model outcomes	Sensitivity analysis for a future paper publication
Week 15 (Sept 22-28)	Code cleanup, integrate Clang-Tidy/Clang-Format for consistent style	Formatted and clean code to be released
Week 16 (Sept 29 - Oct 5)	Generate documentation with Doxygen, write tutorials/examples for users	Doxygen documentation, updated README
Week 17 (Oct 6 - Oct 12)	Add unit and integration tests, integrate GitHub Actions for automated testing	Small tests that are key to validate future Pull Requests in the open-source repository
Week 18 (Oct 13 - Oct 17)	Prepare Final Presentation Slides and submit wrap-up report	Final Presentation and Blog Wrap-up

Conclusion

In this work, I have outlined a comprehensive strategy to extend the CARTopiaX platform within BioDynaMo, enabling it to replicate a broader range of in vitro observations relevant to CAR T-cell therapy in solid tumors. By implementing additional agents, microenvironmental factors and rules, the model is expected to capture critical dynamics that influence therapeutic outcomes. Coupled with robust parameter calibration strategies, this framework provides a powerful, flexible tool for in silico hypothesis testing, laying the groundwork for impactful scientific insights.

My interdisciplinary skill set makes me particularly well-suited to carry out this project. With a dual degree in Mathematics and Computer Engineering, I operate at the intersection of quantitative modeling and computational implementation. My training with the Môlab (Mathematical Oncology Laboratory) group at the University of Cadiz provided a strong foundation in oncology and computational biology, deepening my understanding of the relevant biological context. Crucially, as the author of the CARTopiaX repository, I am uniquely positioned to extend and enhance the model effectively. This combination of technical expertise, biological insight, and firsthand experience ensures that the proposed extensions are both theoretically rigorous and practically achievable.

If the proposed extensions and calibrations prove successful, the enhanced CARTopiaX model will not only serve as a reliable platform for exploring CAR T-cell therapies but also has the potential to generate a high-impact publication in computational cancer biology. By bridging experimental data with predictive modeling, this work could significantly accelerate the translation of in silico findings into actionable insights for experimental and clinical research.

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