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Article

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Posted Date: September 7th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10763447/v1

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Additional Declarations: No competing interests reported.

From Transcriptomics to Game-Theoretic Models of Treatment Resistance in Glioblastoma Organoids

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Abstract

Integrating patient-specific data with mechanistic mathematical models is essential for developing personalized cancer therapies. Here, we present a data-driven systems biology framework that combines transcriptomic analysis, machine learning, and game-theoretical modeling to predict evolutionary responses to irradiation in patient-derived glioblastoma organoids. Supervised and unsupervised learning approaches identify relevant cancer cell populations and quantify temporal changes in their composition from bulk transcriptomic data. These estimates parameterize replicator dynamics to infer transient evolutionary trajectories and evolutionary stable strategy (ESS) compositions. Our analyses reveal that hypoxia is the primary driver of short-term responses to irradiation, while exerting a smaller influence on long-term evolutionary outcomes. Furthermore, supervised approaches provide the most accurate estimates of evolutionary dynamics when therapy resistance is represented as a qualitative trait. This integrative framework demonstrates how transcriptomic data can inform mechanistic models of tumor evolution, supporting patient-specific treatment optimization and advancing data-driven mathematical oncology.

Keywords: Qualitative resistance, evolutionary dynamics, game theory, transcriptomics, glioblastoma, patient-derived organoids, radioresistance

1 Introduction & Motivation

A major challenge in treating advanced cancers is the emergence of therapy induced resistance [1, 2, 3, 4, 5, 6, 7, 8, 9]. Game-theoretic models of cancer and its treatment have led to the development of evolutionary therapies, also known as adaptive or Darwinian therapies, which aim to prevent therapy resistance in cancer cells by anticipating its evolution [10, 11, 12, 13, 14, 15, 16, 17, 18]. These therapeutic strategies have shown promising results, improving both patient survival and quality of life in multiple clinical trials (e.g. NCT02415621, NCT03511196, NCT05651828, NCT03543969, NCT05393791 (ANZadapt), NCT05080556, NCT04343365, NCT05080556).

One trial where evolutionary therapies have been shown to be effective is the trial in metastatic castrate-resistant prostate cancer by Zhang et al [19]. Here the prostate-specific antigen (PSA) was used

as a proxy for the tumor size in the course of the treatment [19]. Due to of the lack of information on the composition of the tumors, possible initial tumor compositions were estimated from in-vitro measurements and expected interactions among cancer cells of different types [20, 10, 21]. This has led to 22 different possible parameterizations of the model, differing in their predictions regarding proportion of therapy resistant cells in the evolutionary stable equilibrium. These 22 cases were clustered into 3 different groups: best-responder, responder, and non-responder [20, 10, 22, 23]. In simulations, Zhang et al's protocol was shown to be more effective than the maximum tolerable dose corresponding to the standard of care for all these parameterizations [10, 22, 23, 24]. A finding that was also confirmed in the trial itself [19, 21]. In the absence of detailed information on tumor composition, the treatment protocol was designed such that it was effective for all possible initial tumor compositions. These different compositions had to be considered as tumors with the same size but different composition will likely respond differently to the same treatment [6, 24]. Therefore, more precise information on the tumor composition in the course of treatment is needed to design even more effective evolutionary therapies [25, 2].

A rough estimate of tumor composition may be made from cancer cells' response to initial treatment application, as proposed in [26]. Another way that will be explored here is to utilize transcriptomics data from patient-derived organoids to model and predict how the proportions of different cancer cell types change over time in response to the treatment [27, 28, 29].

Ideally, single cell technologies would be used to identify tumor composition, however even with the steep decrease in costs of these techniques, they remain costly not only in monetary terms but also in expertise needed to handle the vast amount of complex data these techniques produce [30]. If costs either in processing time/effort or money were to remain high this would prevent their routine use in the clinic, especially in scenarios where patients need long term regular monitoring to track the status of their tumor.

Therefore, here we will focus on techniques to obtain this information from bulk sequencing data, where data is obtained from many cells simultaneously using standard omics techniques, such as transcriptomics [31]. These deconvolution methods come in two distinct classes, supervised - where the training set is furnished with the known proportions of the cell types, or the profile of each cell type is known in advance and unsupervised - where nothing is assumed about the proportions nor the profiles of the cell types in advance of the analysis [32]. In this paper we test a range of approaches from each class.

We compare supervised and unsupervised methods on the data from patient-derived glioblastoma organoids to obtain cell profiles and proportions for the cell types present in glioblastoma [29, 27]. The organoids provide a platform on which we can test potential treatments and observe the treatment effects *in vitro*. With organoids, where the total tumor cell population is kept constant, we can track how treatment impacts the cell type composition over time without needing invasive biopsies. The tumor burden change over time can be tracked in the patient blood biomarker and imaging data. This makes organoids an ideal testing ground for the game-theoretic models. We therefore, after identifying different cell types in organoids and how their proportions change over time in response to radiotherapy, we fit the most classical game-theoretic model, replicator dynamics, to this time series data. We hereby provide a methodology to utilize bulk data for estimating cancer evolutionary dynamics, which will then lead to better evolutionary therapies.

2 Results

2.1 Unsupervised methods

As explained in Section 4.2, when applying the BSS model to define a deconvolution problem there is no prerequisite on prior knowledge regarding cell type signatures. In the absence of this information, one has to start the deconvolution by determining the number of cell types in the cancer mixture and only then determine their profiles and proportions. Here we created several simulated data sets and tested them with different NMF algorithms [33, 34, 35, 36, 37]. We used the measures as are detailed in methods to evaluate performance of these algorithms. These indicators on the simulated dataset mainly pointed to the actual value of the factoring rank. To disentangle the tumor mixture in glioblastoma, determine the number of component cancer cell types and determine their profiles, we compared results of the different NMF algorithms on the TCGA collection 4.1.1. We concluded that the measured gene expression values consist of three clusters, and therefore we used rank 3 of the factorization. Subsequently, we approximated gene profiles of the clusters found in the tumor mixture. We then identified the proportion of each subtype (designated 1, 2 and 3) in the mixture before treatment was applied and tracked their changes in time after irradiation.

Cell type	Pathway	Adjusted p-value
1	Cytokine-cytokine receptor interaction	$2.86 \cdot 10^{-9}$
	Viral protein interaction with cytokine and cytokine receptor	$1.40 \cdot 10^{-7}$
	Staphylococcus aureus infection	$1.37 \cdot 10^{-5}$
2	Salmonella infection	$2.10 \cdot 10^{-5}$
	Endocytosis	$5.53 \cdot 10^{-5}$
	MicroRNAs in cancer	$6.41 \cdot 10^{-5}$
	Cell cycle	$1.76 \cdot 10^{-4}$
	Human T-cell leukemia virus 1 infection	$2.02 \cdot 10^{-4}$
	Protein processing in endoplasmic reticulum	$2.37 \cdot 10^{-4}$
	Amyotrophic lateral sclerosis	$4.72 \cdot 10^{-4}$
3	Neuroactive ligand-receptor interaction	$1.26 \cdot 10^{-19}$
	Olfactory transduction	$5.99 \cdot 10^{-6}$

Table 1: KEGG pathways with an adjusted $p < 0.001$ after over-representation analysis using ConsensusPathDataBase [38] with the genes from the NMF derived cell-type profiles and a background list of all genes used to generate the profiles

The changes in the proportions observed while the total population of cancer cells remains constant led us to a hypothesis that there are three types of cells in the mixture: The first type is sensitive to treatment (proportion in the mixture decreases), the second type is treatment-resistant (proportion in the mixture increases) and the for the last one the proportions remains unchanged with treatment.

To test this for each cell type obtained through NMF, we calculated Log2FoldChange (LFC) with respect to the other two cell types (one expression value subtracted from the other) for every gene. We then defined a threshold (LFC > 2). If the LFC was above 2 for both cases (LFCs calculated with respect to the other cell types), the gene was assigned to the signature of the corresponding cell type. We found that type 1, type 2 and type 3 signatures consist of 408, 15566, and 987 genes, respectively.

Taking these signature lists we adopted an over-representation approach [38], looking for which KEGG pathways are over-represented in the list of genes each signature. The most significant pathways (adjusted $p < 0.001$) from the KEGG database are shown in Table 1. Here we can see that the significant pathways in type 1 cells are related to infections, indicating genes involved in immune processes are important for those cells, thus type 1 may be immune cells. The corresponding processes could be then radiation-induced immunogenic cell death [39]. For type 2 cells we see some cancer pathways and cell cycle, indicating that the genes defining the type 2 cell profile are related to the tumor, typically proliferative cancer cells. Finally, for type 3 cells we see that the neuroactive ligand-receptor interaction pathway is the most significant hit, indicating that we are finding genes relating to more normal neuronal cells.

As a further analysis we compared these genes to the genes from the meta-modules suggested by Neftel et al. We found that these were mostly covered by the type 2 cell type signature from the unsupervised model, as shown in Table 2, confirming as seen above that only one of the three types identified in our unsupervised analysis corresponds to proliferative tumor cells.

	MES1	MES2	AC	OPC	NPC1	NPC2	G1/S	G2/M
V1	0/50	0/50	0/39	0/50	0/50	0/50	0/29	0/45
V2	43/50	46/50	36/39	42/50	45/50	41/50	22/29	41/45
V3	0/50	0/50	0/39	0/50	0/50	1/50	0/29	0/45

Table 2: Overlap of signature genes between Neftel's meta-modules and cell types obtained through NMF (overlapping genes / total number of genes in the given Neftel's meta-module). Signature genes for meta-modules have been made available by Neftel et al [40].

2.2 Supervised methods

In the supervised methods cell type proportions were inferred from microarray data using Instaprism [41]. The corresponding cell type proportions are shown for patient-derived organoids from three patients under two different conditions (4 and 10 Gy irradiation) both untreated and at 3 time points after treatment (Figure 1). For each patient, the first timepoint is pre-exposure and shared between the two radiation conditions. The next three timepoints diverge due to differences in radiation doses.

This supervised deconvolution method only showed changes in the two types of mesenchymal like cells, the hypoxia-dependent and hypoxia-independent ones, MES-like 1 and 2 respectively in Neftel et al. scheme [40]. All other cell types as annotated by this method only changed proportions marginally upon radiation and are therefore omitted here. Moreover, the model reported predictions indicate that more than 85% of all organoids (at all timepoints) consist of mesenchymal-like cells while the remaining 15% belong to other four cell types present in Neftel's dataset.

For the organoid of the first patient we observe a slight decline in the fraction of hypoxia-independent cells and an increase for hypoxia-dependent fraction post-radiation (Figure 1). Furthermore, a lower dose of radiation shows a greater effect on changes in cell-type portions directly after the irradiation.

Patient 2's organoid also shows different effects of the different radiation doses with a larger change in relative proportions at a low radiation dose. However interestingly, different dynamics seem to be observed over longer time scale between the two radiation doses. A lower radiation dose appears to lower the proportion of hypoxia dependent cells, where as a higher dose increases the proportion of this cell type. With the opposite dynamics are observed for the hypoxia independent celltype.

The most prominent changes in cell populations were recorded for patient 3. There is a clear trend of hypoxia-dependent cells overtaking hypoxia-independent cells as the primary cell type already at 24 hours post irradiation.

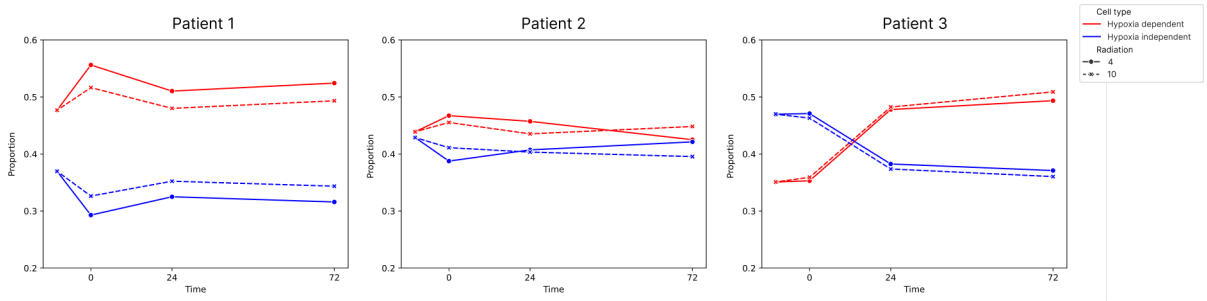


Figure 1: Population dynamics of mesenchymal-like cells in patient-derived glioma organoids under two radiation conditions with deconvolution predictions. Hypoxia-independent cells are MES-like 1 and Hypoxia-dependent cells are MES-like 2 in Neftel's scheme [40]. Results averaged over 100 deconvolution iterations, error below 0.001.

The dynamics in MES-like cell type could be caused by the up regulation of hypoxia-response genes in the hypoxia-independent cell population. This would lead to a transition from one cell type to another without any changes in population size. Similar dynamics could also be explained through different resistance to irradiation. If hypoxia-dependent cell types showed higher probability of survival (fitness) upon irradiation, as suggested by Wang et al. [42], we would observe a similar effect with a decrease in a total number of tumor cells due to apoptosis or other forms of cell death [43]. An alternative hypothesis revolves around the mechanism by which radiation works to cause DNA damage and cell death. This is achieved through the generations of reactive oxygen species, in hypoxic environments the necessary reactive oxygen can not be generated, so any cells surviving in hypoxic environments are naturally radiation resistant. This fits with previous observations that cells in the hypoxic core of glioblastoma organoids are resistant to radiation even when the cells in the normoxic environment at the edge of the organoid are not [29].

2.3 Game theoretic model

As discussed earlier, comparison of the type profiles found by unsupervised methods with the meta-models proposed by Neftel et al. showed that most genes are covered by cell signatures from one of the three types [40]. Thus, the assumption that the unsupervised methods result in different cancer cell types appears to be incorrect, hence the game theoretic model was only fitted to the results obtained by the supervised methods.

The results obtained with the model showed that organoids are mainly composed of mesenchymal cells. In order to simplify the tumor dynamics model, a replicator population was considered, which consists of two types of mesanchymal cells: hypoxia-independent (MES-like 1) and hypoxia-dependent (MES-like 2). Given two cell types in a population, the payoff matrix A has dimension 2×2, and its elements a_{ij} represent the probability of reproduction of cell type i when interacting with type j. As

adding any constant to any column of the fitness matrix A does not change the dynamics, a_{11} and a_{22} are set to zero. To determine the true frequency dynamics defined by the fitness matrix, the remaining matrix parameters are fitted using available measurements in both of the environments created by treatment initialization.

The fitness matrix, A is assumed to have the following form:

$$\begin{array}{c} \\ \text{MES 1} \\ \text{MES 2} \end{array} \begin{array}{c} \begin{array}{cc} \text{MES 1} & \text{MES 2} \end{array} \\ \begin{bmatrix} 0 & a_{12} \\ a_{21} & 0 \end{bmatrix} \end{array}$$

For the found matrices, the mean squared error (MSE) between the available measurements and the fitted replicator dynamics is no more than 0.001 for each patient in both environments. The plots in Figure 2 show the predicted dynamics of the two types (MES-like 1 and MES-like 2) and the relevant measurements. Horizontal lines in the figures represent evolutionary stable strategies (ESSs). The fitted values for a_{12} and a_{21} of the fitness matrix are shown in Table 3.

Patient	Radiation (Gy)	Fitness matrix	ESS
1	4	$\begin{bmatrix} 0 & 0.84 \\ 1.37 & 0 \end{bmatrix}$	(0.62 ; 0.38)
	10	$\begin{bmatrix} 0 & 0.96 \\ 1.34 & 0 \end{bmatrix}$	(0.58 ; 0.42)
2	4	$\begin{bmatrix} 0 & 0.02 \\ 0.01 & 0 \end{bmatrix}$	(0.45 ; 0.55)
	10	$\begin{bmatrix} 0 & 0.96 \\ 1.06 & 0 \end{bmatrix}$	(0.52 ; 0.47)
3	4	$\begin{bmatrix} 0 & 0.15 \\ 0.22 & 0 \end{bmatrix}$	(0.57 ; 0.43)
	10	$\begin{bmatrix} 0 & 0.13 \\ 0.18 & 0 \end{bmatrix}$	(0.58 ; 0.41)

Table 3: Fitted parameters of matrix A and calculated ESSs for each patient with two types MES 1 and MES 2.

One of the characteristic features of advanced tumors is hypoxia, which is closely related to tumor radioresistance, as it can promote tumor progression and lead to poor clinical outcomes [44, 45]. In each two of the patients, regardless of radiotherapy dose, the fitted model predicts in the long-term a majority of hypoxia-dependent cells, while both observed cancer cell types coexist at equilibrium. However, interestingly for patient 2 the dose of radiation seems to control if the hypoxia dependent or independent cells are present as the majority when ESS is reached. Where hypoxia-independent cells are more prevalent when a low radiation dose (4 Gy) is applied.

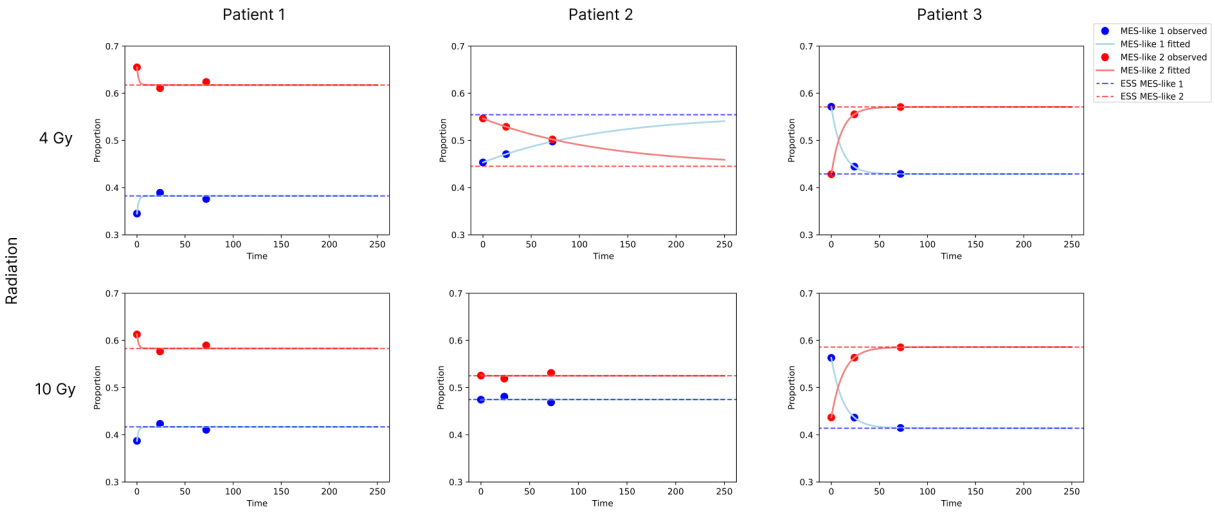


Figure 2: Fitted replicator dynamics equations vs. the measurements and ESS. Points indicate predicted proportion of MES-like 1 and 2. Solid lines show trajectory of the fit of the replicator dynamics whereas the dashed lines indicate the calculated ESS.

3 Discussion

Glioblastoma is a universally fatal disease for which there are no curative treatments and all patients eventually relapse after radiation and temozolomide chemotherapy. Treatment-induced resistance in cancer is thought to be caused by stochastic genetic or epigenetic driven phenotypes that preexist in small populations of tumour cells or evolve during the course of treatment [46, 47, 6, 13, 48, 49]. Tumor markers that predict the emergence of treatment refractory subpopulations in human tumors before and during the course of standard treatment may be exploited to adapt treatment and prevent relapse. Here we used machine-learning techniques to estimate how proportions of relevant glioblastoma cell types change over time under treatment from transcriptomics organoid data, to give insight in the changes in these populations as well as the different methods to decern these.

To identify different cell subpopulations, we utilized both unsupervised and supervised machine learning methods. The unsupervised methods are attractive to researchers as they do not require any assumptions about the number of cell types present in the mixture, nor the profiles of said types. However, this makes them trickier to work with and less immediately interpretable. To overcome this shortcoming in interpretability, we selected genes with a high-fold change between their values in different profiles and compared them to both genes seen in signatures of published single cell analyses and genes from known pathways in standard pathway databases. Perhaps unsurprisingly, we found that the patient tumor samples were deconvoluted into immune cells, cycling cancer cells and healthy neuronal cells. This led to issues extrapolating from tumor profiles obtained *in vivo* to those *in vitro*, as not all cell types seen *in vivo* will be expected to be present in the *in vitro* situation. In the patient-derived organoids, for instance, immune cells, neurons, and blood vessels are missing.

A secondary issue with the unsupervised methods is the need to determine the number of cell types present *a priori*. If no assumptions about this are made, then extra steps are needed to estimate the optimal number of types empirically from the data. In our experience, both in this work and earlier unpublished work, this step does not generally yield a clear result when working with real datasets.

We found the supervised method more interpretable, as this immediately allows us to extract and interpret key information about the expected cell types present in the mixture; a key advantage over the unsupervised methods.

However, there are difficulties extrapolating from single cell data to bulk data, as unlike in single cell sequencing one sequence in bulk sequenced data does not have to correspond to one cell [30]. Thereby making extra caution needed interpreting small changes in the relative proportions found by the deconvolution. Moreover, the supervised analysis relies on appropriate single-cell reference datasets for training, the generation of which is costly and limits their widespread availability.

Another limitation of this study is that tumors have spatial aspects, for instance hypoxic cells are more common in the center/core of the tumor where the supply of oxygen is limited (diffusion-limited hypoxia) or in the neighborhood of defective vessels (acute hypoxia). The spatial aspects could be analysed through

single-cell imaging data, for instance [50, 51], or through taking separate samples for bulk analysis from the core and the rim [52]. These data points could then be used in spatial game-theoretic models to predict further spatio-temporal response. Cells could then be assumed to only interact with the cells in their local neighborhood, so local dynamics would become important, and tumors are not assumed to be well-mixed [20]. In case of using a replicator dynamics model, one could assume that different regions correspond to different fitness matrices, as done in [53], because cells' chances for proliferation and survival will vary with conditions in their neighborhood.

Our results from the supervised analysis suggest that all three of our analyzed organoids contain mainly mesenchymal cells, with some being the MES-like 2 hypoxia-dependent cells and others being the MES-like 1 hypoxia-independent cells. As the cell profiles we trained on were based on Neftel's data [40] they can be directly compared to the results from Shakya et al [52] where they showed that the hypoxic organoid core mimicked the MES-like 2 profile. However, in their work they saw that the other cells in the organoid, in particular the cells in the organoid rim, expressed genes from the AC-like or OPC-like profiles. One key difference between their work and our approach is that we directly train a predictor of the cell type proportions from their single cell data, whereas they use the meta-modules of genes seen to be consistently differentially-expressed in these cells, this difference could potentially lead to these anomalies and should be investigated further. One hypothesis here could be that as we observe less presence and variability in proportions of these cell types that they play a lesser role in radioresistance of glioblastoma organoids than the mesenchymal like cells that we describe here.

For prediction of the long-term response in organoids we fitted the identified proportions of different cell types to replicator dynamics [54, 55], which model classic and likely the most simple evolutionary dynamics. While the fit of data to replicator dynamics was very good, we could use different game-theoretic dynamics, such as Darwinian or adaptive dynamics [56, 57, 58]. As the total cell volume in organoids considered in the experiments carried out here was kept constant, to use these different dynamics we would need to assume that the population equilibrium was reached and fit only the evolutionary dynamics. Alternatively, we could combine volumetric data from the patients to fit the population dynamics, similarly as we did in [59, 60, 61], while fitting the evolutionary dynamics that are very difficult to estimate in vivo from the patient-derived organoids. An advantage of this approach could be that we would not need to assume that there is a fixed number of possible cell types with a fixed profile, but instead could trace radioresistance as an evolving trait, similarly to others modeling evolving traits responding to treatment [13, 62, 63]. Once the used model is validated, we could optimize the treatment, for example using the Stackelberg evolutionary game framework [62, 64, 65].

While our game-theoretic models predicted coexistence of the two different tumor types in organoids, we did not collect additional measurements to validate whether these predictions are correct. This was also not the main goal of our study; rather we wanted to demonstrates all steps needed for utilization of transcriptomics data in predictive game-theoretic modeling. Validation of our predictions and comparing the fit and predictive capabilities of different game-theoretic dynamics will be our natural next step. More time series data will be collected to perform both data fitting and validation. Once we have a validated game-theoretic model of the radiotherapy response in organoids, we can predict the impact of alternative treatments (including their timing and dosing), in order to delay/predict therapy resistance in the corresponding patients. A huge advantage of organoids is that these predictions can be then validated through real experiments. This is because in many studies organoids have been shown to retain major patient characteristics that determine treatment resistance [66, 67, 27, 28]. As such, we can utilize game-theoretic models validated through organoids to improve treatment in real patients.

Further it should be noted, that the decrease in the hypoxia-independent cells in response to radiation seen in our results for patient 3 is expected, as radiation-resistance is known to be related to hypoxia e.g. [68, 44, 45] and therefore the hypoxia-dependent cells (MES-like 2) can be expected to be more radio-resistant.

Our results show co-existence of the two mesenchymal like cell types described when ESS is reached and the hypoxia dependent type, thought to be most radiation-resistance, is predicted to be the majority at that time point. The fact that in patient 3 the more radiation-resistance can take over from the sensitive cells after ration that finished, might point to a gain of resistance, increasing fitness in these resistant populations. Interestingly radiation of a lower dose in patient 2 gives an opposite result. Here a lower dose of radiation leads to a higher fraction of more radiosensitive cells, which could point to a strong cost of resistance for the MES-like 2 cells. One hypothesis potentially explaining these differences could be due to small genetic differences present between these patients. In glioblastoma several different resistance mechanisms have been described and these findings could point to a different resistance mechanism being activated based on radiation dose and inter-patient differences potentially leading to these different

outcomes [69, 70]. However more research focusing on the effect of these different resistance mechanisms on cell fitness and their correlation to radiation dosing is needed.

Despite its limitations, this study shows that it is possible to apply both supervised and unsupervised deconvolution techniques to bulk gene expression data from tumors and to use the information obtained on the changes in cell type proportions over time to fit game-theoretic models of the tumor eco-evolutionary response to treatment. This is an important step forward in the development and application of these models and their use with real data predicting both ecological and evolutionary cancer dynamics and designing evolutionary therapies, i.e. therapies that anticipate and forestall treatment-induced resistance in cancer cells.

For the next steps we note that the TGCA dataset contains many different sets of measurements on the same patients. In this work we have only made use of the transcriptomics data, given all these measurements are made on the same tumors, which can be assumed to be composed of the same types, one direction for further research is learning to leverage the additional information available in other measurement sets from these tumors to improve the deconvolution overall and to improve the fit and predictive capabilities of game-theoretic models.

We believe that using cancer data in a mathematical model and adapting treatment strategies to the resulting predictions has the potential to revolutionize personalized medicine. When facing such a heterogeneous formation as cancer, one must apply a therapy adapted to the targeted malignancy. Our ultimate goal is to provide a process which, using the available information about the tumor composition and its measurements combined with a game-theoretic model, will simplify the selection of a better cancer treatment strategy.

4 Methods

4.1 Datasets

In this work we make use of several datasets, which we describe below.

4.1.1 TGCA Glioblastoma dataset

The Cancer Genome Atlas (TCGA) is a publicly available resource containing molecular data for over 10000 specimens, from at least 33 different types of cancer and metadata linking this information to clinical data for the patients [71]. We used the TCGA-GBM project, which focuses on glioblastoma samples. We used 168 samples with available RNA-SEQ data. For each sample we retrieved the FPKM-UQ normalised RNA-SEQ. These files provide measurements for over 60000 RNA sequences per sample.

4.1.2 Neftel's single-cell data

We utilized Neftel et al.'s single-cell dataset acquired through full-length scRNA-SEQ (Smart-seq2) [40]. We downloaded this dataset from NIH Gene Expression Omnibus (GEO: GSE131928). It contains pre-processed single cell RNA-seq expression profiles of adult and paediatric IDH-wildtype Glioblastomas from 28 tumors with an average of 5730 genes detected per cell. From the initial 5730 genes, 289 were selected for cell type distribution analysis based on meta-module signatures. scRNA-SEQ data were complemented with metadata from Broad Institute Single Cell Portal (Study: Single cell RNA-seq of adult and pediatric glioblastoma) containing meta-module (NPC1, NPC2, MES1, MES2, OPC, AC) scores for 7930 cells. Cells were categorized according to their highest corresponding meta-module scores.

4.1.3 Organoid data

The patient-derived organoids were grown as per the protocol in [29] and then treated with either 4 Gy or 10 Gy irradiation. Samples were collected pre-treatment, directly post-treatment, and at 24 and 72 hours post-treatment. RNA was isolated using Nucleospin RNA (Macherey-Nagel) according to the manufacturers protocol. RNA concentration and integrity was confirmed using 2100 Bioanalyzer (Agilent Technologies). RNA was hybridized to affymetrix human microarrays by Eurofins (Denmark). All conditions were tested in GBM organoids derived from 3 patients.

4.2 Unsupervised methods

For the unsupervised methods, we focus on non-negative matrix factorisation (NMF), a method which describes the data matrix as a product of two matrices, one describing the cell type proportions and the other describing the profiles of each cell type. This assumes that the data can be approximated simply by taking a weighted sum of the cell type profiles where the weights are the proportions of each cell type present [72].

The deconvolution of cancer cells can be seen as a specific case of the blind source separation (BSS) issue [73], i.e. separating a group of source signals from measurements of mixed signals. A cancer tumor, as a heterogeneous mixture of different cell types, can be analysed using a linear BSS model, as this model does not require knowledge of any predefined cell type signatures and can therefore be used to formulate the deconvolution problem. Whilst there are various methods which have been applied for unsupervised deconvolution of bulk cell profiles [36, 74, 75], in earlier unpublished work we showed that Non-Negative matrix factorisation (NMF) is by far the most promising approach that agrees with the literature findings [73, 75]. Therefore, here we focused on the family of non-negative matrix factorisation-based approaches.

In this study we applied several NMF algorithms [33, 34, 35, 36, 37]. The number of cell types selected should correspond to the NMF rank for which the best results are obtained. To evaluate the obtained results, we used several classic indicators (Cophenetic coefficient [33], Dispersion coefficient [37], Residual Sum of Squares [76], Silhouette Coefficient [77]), whose values indicated the optimal factorization rank.

4.3 Supervised methods

For the supervised approaches we have applied a method called InstaPrism [41]. InstaPrism is reference-based deconvolution framework that is used determine the cellular composition of glioblastoma patient-derived organoids from microarray profiles [41]. As a reference, we rely on single-cell profiles and a blueprint suggested by Neftel et al. that assigns cells into four meta-modules, each characterized by a unique gene expression signature and genetic alterations [40].

4.3.1 Instaprism

InstaPrism is an optimized implementation of BayesPrism, designed to improve the computational efficiency of Bayesian deconvolution while preserving its analytical rigor [78, 41]. Similar to BayesPrism, InstaPrism models bulk RNA-seq deconvolution as a topic modeling problem, where bulk RNA-seq reads correspond to words, samples to documents, and cell states to topics, leveraging prior information from scRNA-seq data to estimate cell-type proportions and gene expression profiles. The primary methodological advancement in InstaPrism is the replacement of BayesPrism's computationally intensive Gibbs sampling with a fixed-point algorithm, significantly reducing computational runtime from hours to seconds while maintaining comparable accuracy. The algorithm processes bulk expression data using a precompiled reference matrix derived from scRNA-seq, employs vectorized calculations to estimate cell-type fractions and gene expression, and outputs a compressed representation to optimize memory efficiency. Additionally, InstaPrism includes built-in reference profiles curated for various cancer types, facilitating streamlined and reproducible deconvolution analyses. By offering a computationally efficient alternative to BayesPrism without compromising accuracy, InstaPrism enables scalable and robust cell-type deconvolution, making it particularly well-suited for large-scale transcriptomic studies.

4.4 Game-theoretic model of glioblastoma

Changes in population characteristics such as size and density of cancer cells are associated with cancer ecological dynamics, while changes in heritable cell lineage characteristics (quantitative traits) or frequency of cancer cell types (qualitative traits) are considered evolutionary dynamics. This eco-evolution in the tumor can be captured with a use of various mathematical models. As the total cell population in organoids remains constant through the experiment, we analyze only changes in evolutionary dynamics. We employ a widely applicable approach to integrate the time scales of evolutionary dynamics: replicator dynamics [54, 55]. As a fundamental game-theoretic framework, replicator dynamics has been extensively used to model various biological processes, including selection in population genetics.

Given a finite set of cancer strategies $\mathcal{T}$, here understood as a set of cell types, their fitness matrix and their proportions at time t, the dynamics of cells of the same type can be described by deriving the

following dynamics:

$$\dot{x}_i = x_i \left((Ax)_i - x^T Ax \right), \tag{1}$$

where x_i denotes the proportion of type $i \in \mathcal{T}$, and consequently the column vector of proportions of all types is denoted by $\mathbf{x}$. The $n \times n$ fitness matrix $A = (a_{ij})$ defines the fitness of the considered cell types. If the entries of this matrix are between 0 and 1, each value a_{ij} defines the probability that a focal cell of type $i \in \mathcal{T}$ will produce an offspring of its own type when interacting with a cell of type $j \in \mathcal{T}$. We can calculate the so-called evolutionary stable strategies (ESSs) of A, which maximize the fitness of the entire population and can not be invaded by a rare mutant with a different strategy. These ESSs may correspond to the attractors of the replicator dynamics (1). Here the replicator dynamics are calculated for one patients organoid.

Author Contributions

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Rachel Cavill - Conceptualization, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing
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Acknowledgements

We would like to thank Dr. Jafar Rezaei for his valuable feedback that improved quality of this work.

This research was supported by European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 955708, the Dutch National Foundation projects ENWPR.020.006, and OCENW.KLEIN.277 and VI.Vidi.213.139, Dutch Cancer Society (KWF 11698) and funding from StopHersenTumoren.

Conflict of interest/Competing interests

The authors declare no conflict of interest.

Data and code availability

The data analyzed during the current study is available along side the code used for analysis at `https://github.com/LouiseMerle/Transcriptomics.git`.

Ethics statement

All data used in this study were de-identified prior to access, and no additional ethical approval was required for this analysis.

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