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A Scoring System that Leverages Single Cell RNA-Sequencing Data to Predict Cell Therapy Outcome in Solid Tumor Treatment

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Abstract. Cancer, a prominent global health concern, involves the uncontrolled growth and dissemination of abnormal cells, forming tumors that can infiltrate nearby tissues and metastasize to distant sites. Early and precise tumor detection and treatments are crucial to impede their spread and mitigate disease progression. This study presents the development of a scoring system that harnesses single-cell RNA sequencing (scRNA-seq) data to predict the suitability of solid tumors, specifically glioblastoma (GBM) and lymphoma, for advanced cell therapies such as CAR-T, NK cell, and TIL therapies. By integrating key cellular factors—including tumor-infiltrating cells, immunosuppressive cells, and immune escape markers—this system provides a quantitative assessment of a tumor's potential responsiveness to these therapies. Validation with patient-derived samples has demonstrated the system's potential to improve precision in therapy selection, offering a promising tool for personalized oncology. This research not only aims to enhance the effectiveness of cell therapies for cancer patients but also lays the groundwork for applying similar methodologies to other cancer types, advancing the integration of precision medicine in cancer treatment.

Keywords: scoring system, single-cell RNA sequencing (scRNA-seq), cell therapies, cancer treatment.

1. Introduction

Cancer remains one of the leading causes of morbidity and mortality worldwide [1]. Cancer is a disease in which some of the body's cells grow uncontrollably and spread to other parts of the body. Tumors are areas of abnormal or damaged cells that multiply when they aren't supposed to. Cancerous tumors spread into, or invade, nearby tissues and can travel to distant places in the body to form new tumors.

Recently, single-cell RNA sequencing (scRNA-seq) has emerged as a potent tool in delineating the heterogeneity of tumors and providing crucial information as it can analyze the transcriptome at the single-cell level for millions of cells in a single study. It has been profoundly applied by scientists to understand heterogeneous tumors [2]. However, such tool's application remained within research entities instead of being applied clinically. Such technology is not widely used in clinical settings primarily due to its high cost, complexity of data analysis, and the current lack of standardized protocols for processing and interpreting the vast amounts of data it generates. Additionally, the technology requires sophisticated bioinformatics support and infrastructure that are not yet universally available in clinical laboratories [7].

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However, the special nature of single cell-RNA sequencing technology gives it a unique advantage when one contemplates how it can aid modern cancer treatment. Recently, one fast-developing new avenue of therapeutic intervention is cell therapy, cell therapy involves replacing the damaged or diseased cells with new, healthy cells. One example of cell therapy is CAR-T cell therapy, which uses genetically altered T cells to attack and destroy cancerous cells in the body with precise targeting [3]. Cell therapies have been tested against a variety of tumors with variable outcomes and limited explanation of why certain cell therapies work and others do not against specific tumors. Single cell-RNA sequencing data of the tumor might be able to answer this question as it allows us to model the interaction between groups of cells.

Regardless of the success of CART cell therapy in lymphomas and leukemias, treating solid tumors with cell therapy is a challenge that has been undertaken by researchers and clinicians worldwide. Thus, I have decided to create a framework for applying single-cell RNA-sequencing data of any solid tumor to predict how the specific tumor will respond to cell therapies. By evaluating the suitability of a patient's cancer for cell therapy based on scRNA-seq data, I hope to bring precision medicine technology one step closer to clinical reality. This research holds the potential to increase the success rate of cell therapies for cancer patients, ultimately reducing mortality rates and enhancing the development of cell therapies.

2. Methods

2.1. Study Workflow

To predict the efficacy of cell therapy in solid tumors, I developed a scoring system based on single-cell RNA sequencing (scRNA-seq) data. The system quantifies key factors, including tumor-infiltrating cells, immunosuppressive cells, immune escape markers, and cytokine levels, to evaluate a patient's suitability for cell therapy. Figure 1 outlines the overall study workflow, starting from the collection and processing of tumor samples to the final evaluation of cell therapy fitness.

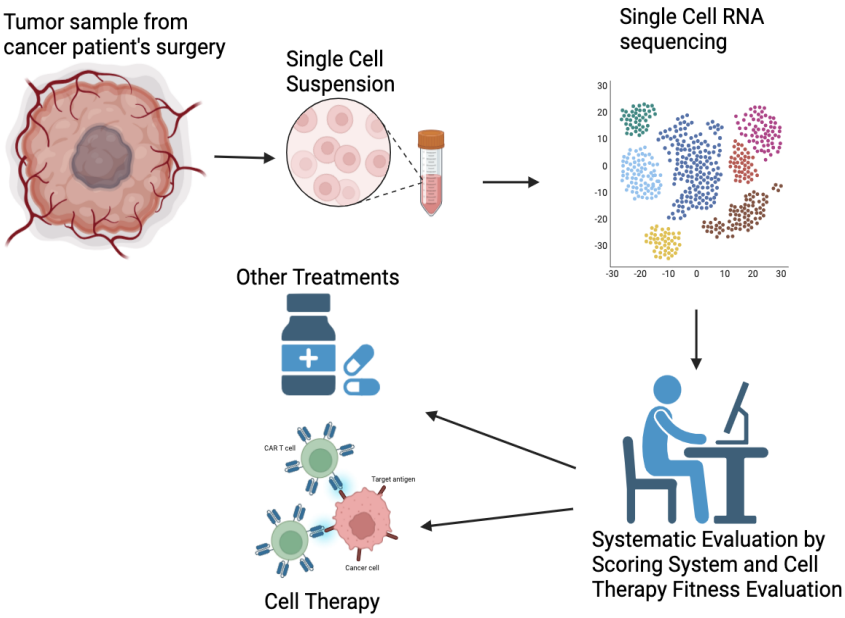

Figure 1. Flowchart of my study.

2.2. Scoring System Design

The scoring system was constructed by integrating and quantifying data from four key cellular factors relevant to cell therapy effectiveness:

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Tumor-infiltrating cells, which facilitate an immune response against cancer, were evaluated, with a higher percentage indicating better potential for cell therapy.

Immunosuppressive cells, which dampen the immune response and hinder therapy, were also considered, with a lower percentage being favorable for cell therapy.

Immune escape markers, which help cancer cells evade immune detection, were another critical factor; a lower percentage of these markers suggests a better chance of successful cell therapy.

Lastly, cytokine levels were assessed, with beneficial cytokines (e.g., IFNG, TNF) supporting immune activity and detrimental cytokines (e.g., IL10, TGFB1) suppressing it. A higher beneficial-to-detrimental cytokine ratio indicates better cell therapy fitness.

For each factor, the normal expression range was defined based on scRNA-seq data, and category scores were assigned using R-studio functions. For example, a tumor-infiltrating cell percentage within the 0-5% range was given a category score of 10, signaling optimal cell therapy fitness.

2.3. Single-cell RNA Sequencing Data Analysis

To evaluate my scoring system, I identified the published single-cell RNA seq data (Single-cell sequencing reveals the landscape of the human brain metastatic microenvironment by Song et al. 2023[4]). I found that they analyzed data from 5 patients with lung cancer or brain cancer who had a suspected brain metastasis. They used TTPSR to obtain fresh tissue samples and then they used a human tumor dissociation kit and GentleMACS protocols, followed by the removal of red blood cells (RBCs) through negative selection using CD235a microbeads (Miltenyi). They used a 10X Chromium single-cell capture chip. They also used an Illumina NextSeq500 instrument with a High Output 150 cycle kit.

After finding a source I analyzed the given data by running a read command[9]. Then, I created a Seurat object that would be the shortcut to the data so when I run the commands with that object name it will use the data from the sources found. I performed filtering, normalization, scaling, and identifying differentially expressed genes by using commands such as FindVariableFeatures, which identifies differentially expressed genes in the data set, and commands like FindNEighbors and FindClusters help us plot the UMAPs. Finally, I ran vlnplot to visualize how much each selected gene is expressed in the data.

2.4. Quantification of Characteristics for Scoring System

Then, using a print command, PercentageFeatureSet can also identify the different cells in each cluster. By using UMAP, we can map the clusters. Then I used findmarkers to name different genes in different clusters. Finally, by running the new cluster ID command we can assign a name to each cluster on the diagram. Commands like FeaturePlot and VlnPlot also help us plot the different genes. FindAllMarkers can also calculate and help identify the genes expressed in clusters. After finding different cells in each cluster we can research them to find the type of cells each cluster expresses.

2.5. Scoring Output Evaluation

After analyzing the patient's data, I validated it by rerunning our commands and also consulted other sources or professionals to confirm it. I used the analyzed data to determine the most effective therapy/treatment for the patient. Finally, I identified the treatment best suited for the patient and then conducted a similar experiment on other patients. This approach aims to enhance the quality of life for glioblastoma patients by providing more effective treatments and avoiding uncertainty associated with ineffective treatments. The methods for data collection included cancer reports and verified journals. R-studio was employed to study the data from the journals, and various packages were used to run commands such as vlnplot and plot the data. Additionally, the data was segmented into clusters, and by running specific commands, the expressed cell types in each cluster were identified to understand the cellular composition.

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3. Results

3.1. Identifying key factors that influence cell therapy effectiveness in solid tumors

The beginning of the scoring system composition is to identify the most relevant factors that would impact the potential effectiveness of cell therapies. Before a patient receives cell therapy, some expression information of the solid tumor is known through pathological examinations, such as CAR target expression and some variant expression information. Single-cell RNA-seq data analysis would not need to repeat on such examinations but can be investigated for an extremely holistic view of the tumor's local immune environment.

There are different cell therapies for treating brain tumors like CAR-T cell therapy, NK cell therapy, and TIL cell therapy. CAR-T cell therapy utilizes T cells from the patient's body that are modified to target the cancer antigens. NK cell therapy uses Natural Killer Cells that can kill without using antigen recognition. The NK cells are the native fighters against the tumor cells but the NK cells are less in number than T cells. TIL cell therapy uses T cells extracted from the tumor in the patient's body and expands them in a lab. This enables the T cells to infiltrate the tumors but they might be immunosuppressed because of the local immune environment.

CAR-T cell therapy is influenced by factors such as the amount of antigens in the tumor, poor trafficking, and the immunosuppressive microenvironment [5]. NK cell therapy can be influenced by factors like limited NK cell infiltration, short-term NK survival, and NK exhaustion. TIL cell therapy is limited by factors like the production time of TIL cells, TIL exhaustion, and finding proper TIL cells that can respond to tumors and are fit for expansion.

The final 4 factors that I chose were tumor-infiltrating cells, immunosuppressive cells, and the expression of immune escape markers. One main reason was how easily quantifiable each factor was based on the single-cell sequencing data. I also chose these 4 factors as the amount of tumor-infiltrating cells facilitates the immune response against the cancer. The immunosuppressive cells tune down the tumor microenvironment, which inhibits other immune cells from being activated. The immune escape makers are expressed in the cancer cells as an escape mechanism from the immune system. Together, these factors were the most easily quantifiable and affected cell therapies' effectiveness the most.

3.2. Scoring System Development

The determination of a patient's suitability for cell therapy was conducted by quantifying category scores based on the normal expression range of various factors. These factors were identified after analyzing clustered datasets and were selected for their compatibility with the scoring system and their ease of quantification. For both tumor-infiltrating cells and immunosuppressive cells, higher category scores indicate better fitness for cell therapy, though these scores vary across different ranges. Conversely, for immune escape markers and cytokine landscape, higher category scores denote poorer cell therapy fitness.

The scoring system operates by converting quantified data into category scores using R-studio functions. These functions assess a patient's cell therapy fitness based on gathered and analyzed data. The percentage cell counts are translated into category scores relative to the normal expression range. For instance, a 25% expression in a factor with a 0-50% expression range results in a category score of 5. These scores are then used to evaluate the patient's overall cell therapy fitness. These apply to all factors except the cytokine level, which itself is a reflection of the cell-therapy fitness of specific tumors' cytokine landscape.

I identified the 4 factors that affect the patient the most for cell therapy fitness by running a command that shows me the number of a certain type of cell expressed in the body to determine if the factor is quantifiable and if it has a large enough impact on how effective the cell therapy would be for the patient. This is done by analyzing each cluster and the different genes expressed in them to see what type of cell is the most easily quantifiable and also if their expression is good enough to construct a method to score them. Then I can run it in the scoring system I created to get a percentage that will help me determine if the patient has good cell therapy fitness. After analyzing the clustered dataset, I determined that tumor-

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infiltrating cells, cytokines, immunosuppressive cells, and the expression of immune escape markers were the 4 factors that affected the patient's cell therapy fitness the most. I used Treg (FOXP3, CD4, TIGIT, CD25) and MDSC (ARG1, NOS2, CD33, IL4R, CD11B) cells [6] to calculate the number of immunosuppressive cells in the microenvironment and found that 47 cells out of 1857 were either Treg or MDSC and converted that into a score of 2.5% which I then input into the scoring system to get the category score. This process is then repeated for the two other factors and for the number of tumor-infiltrating immune cells in the tumor tissue I calculated it using the CD8+ T cell, CD4+ T cell, Treg, B cells, NK cells, Dendritic cells, Macrophages, MDSC (myeloid-derived suppressor cells), and mast cells and found that 357 out of 1857 cells were tumor-infiltrating immune cells which were around 20% of the total cells. For the immune escape markers, I used markers PD-L1 (Programmed Death-Ligand 1), CTLA-4 (Cytotoxic T-Lymphocyte-Associated Protein 4), IDO (Indoleamine 2,3-Dioxygenase), and LAG-3 (Lymphocyte-Activation Gene 3) to calculate the number of escape markers and found that 148 out of 1857 cells were markers for tumor immune escape which was around 8% of the total cells. I looked for the percentage by running the cells in a command that gives me the number of each cell which I then use to convert the score into a percentage.

For cytokines, both beneficial (IFNG, TNF, CCL5, CXCL9, CXCL10, CD25) and detrimental (IL10, TGFB1, TGFB2, TGFB3) genes were identified [6]. The mean values for these beneficial and detrimental genes were calculated and summed. The detrimental score was then subtracted from the beneficial score, resulting in a possible range from 0 to 1. This score was scaled by subtracting the combined score from the minimum score, dividing by the difference between the maximum and minimum scores, multiplying by 9, and adding 1. The final cytokine score calculated was 6.32.

3.3. Real World Data Testing: A Comparative Study of a Glioblastoma and a Large Diffusive B-cell lymphoma Data clustering

For the clustering analysis, I used vlnplots first to determine the features of the data set from the patient after running a GBM command to read the data. Using cluster counts then helps me find the number of cells in each cluster which would help me determine each cluster's effect on the patient. A filtering process is used to eliminate false positive and false negative results, such as doublets and miscounts. After filtering, 1857 out of 2319 cells are used in the GBM Data Clustering Analysis. I used a normalized data command and a scale data command. The lognormalize command scales the data by a factor of 10000. I used the best method to find the top 2000 variable features (Figure 2.2). After examining the top 15 PCA heatmap results I decided to use the first 10 PCAs to continue my dimensional reduction and generate my final UMAP with a resolution of 0.5. Then using DimHeatMap allows me to run a DimPlot using a RunUMAP command which shows me the different clusters. Then by using GBM markers and then printing it, I can find all markers differentially regulated by each cluster compared to all others. This helps me find the different genes expressed in each cluster which I can then use to identify the types of cells each cluster represents. In my UMAP there were 8 clusters and I identified the clusters and named them using a find all markers command (Figure 2.3). I can see all the genes expressed in each cluster. There are 8 clusters in total. I named each cluster by running a GBM marker command. I then used Dimplot to visualize characteristic genes to help identify clusters. Cluster 0 is identified to be a glioma cancer cell because of the differential expression of CENPF, TOP2A, and UBE2C. Cluster 1 is identified to be monocytes because of the differential expression of CLDN4, HSPA5, and TRPS1. Cluster 2 is identified to be glioma cancer cells because of the differential expression of ATP5F1C, HMGN2, and H2AFJ. Cluster 3 is identified to be macrophages because of the differential expression of APOE, CCL3L1, and CCL3. Cluster 4 is identified to be cytotoxic T because of the differential expression of S100A9, S100A7, and LCN2. Cluster 5 is identified to be T-cells because of the differential expression of IGHG1, TSC22D3, and CXCR4. Cluster 6 is identified to be oligodendrocytes because of the differential expression of PLP1, MBP, and PTGDS. Cluster 7 is identified to be fibroblasts because of the differential expression of IGFBP7, FN1, and SPARC.

Using cluster counts in my lymphoma dataset helps me find the number of cells in each cluster which would help me determine each cluster's effect on the patient. A filtering process is used to eliminate

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false positive and false negative results, such as doublets and miscounts. After filtering, 7162 out of 7205 cells are used in the Lymphoma Data Clustering Analysis. I used a normalized data command and a scale data command. The lognormalize command scales the data by a factor of 10000. I used the best method to find the top 2000 variable features (Figure 2.4).

In my UMAP there were 11 clusters and I identified the clusters and named them using a find all markers command. I can see all the genes expressed in each cluster. I named each cluster by running a marker command. I can find all the genes expressed in each cluster and by using those genes I can find the type of cell each cluster represents. The cell types I identified for each cluster were memory T-cells for cluster 0, T follicular helper cells for cluster 1, NK cells for cluster 2, activated effector T cells for cluster 3, Tregs for cluster 4, Tregs for cluster 5, memory T-cells for cluster 6, NKT-cells for cluster 7, NKTregs for cluster 8, Cancerous b-cell cluster for cluster 9, and Cancerous b-cell cluster for cluster 10 (Figure 2.1).

Glioblastoma Dataset

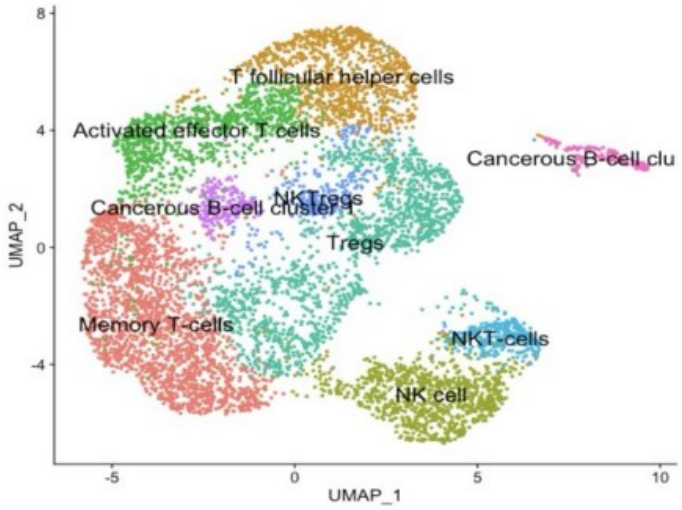

Figure 2.1. Clustered UMAP with cluster names

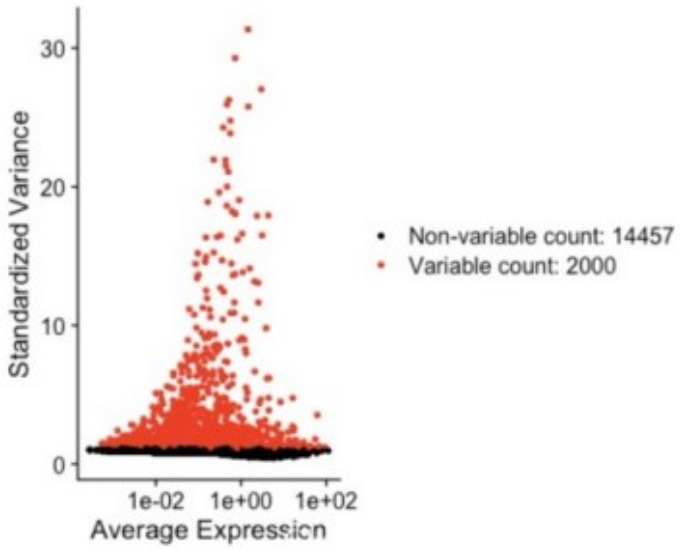

Figure 2.2. Expression of cells and variable counts

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Gene Variability in Single-Cell RNA-seq Data. This scatter plot illustrates the relationship between the average expression levels and the standardized variance of genes in a single-cell RNA-seq dataset.

Lymphoma Dataset

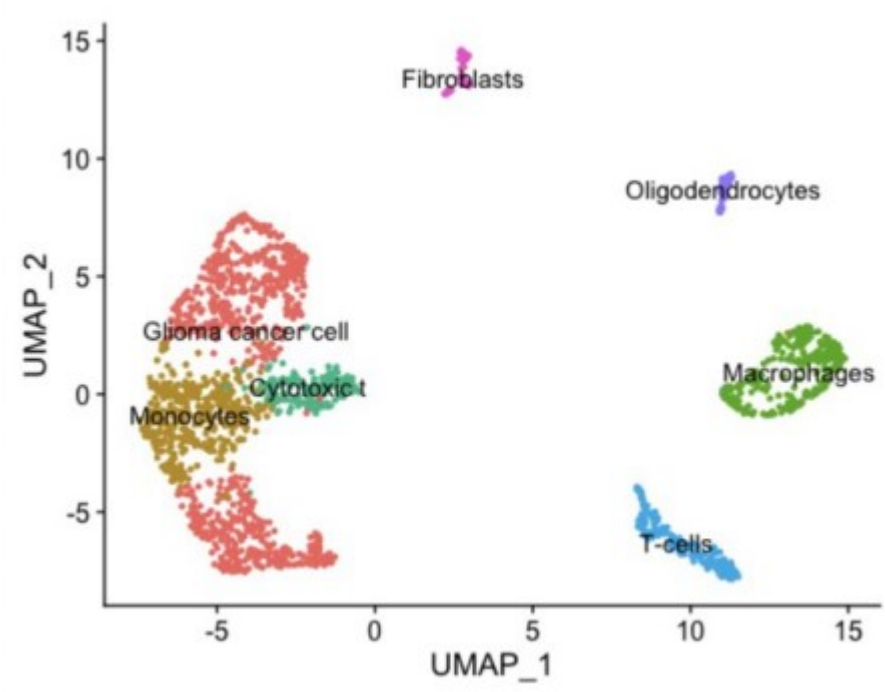

Figure 2.3. Clustered UMAP with cluster names

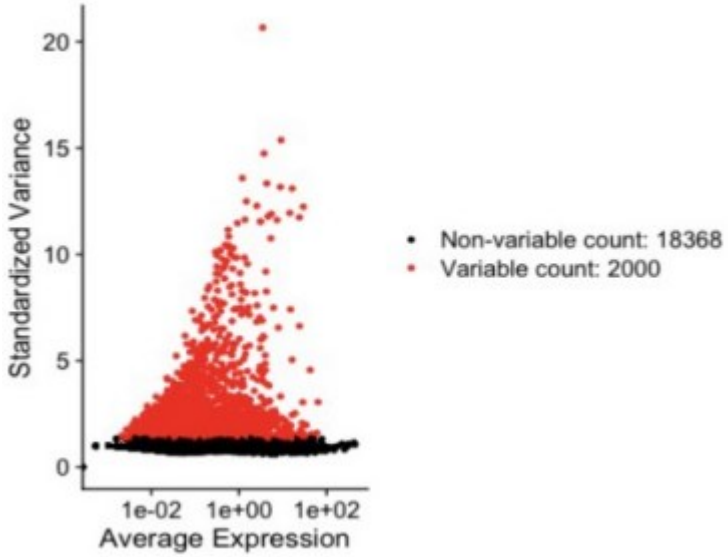

Figure 2.4. Expression of cells and variable counts

Figure 2. Clustered UMAPs and Single-Cell RNA-seq Data for Lymphoma and Glioblastoma datasets.

Gene Variability in Single-Cell RNA-seq Data. This scatter plot illustrates the relationship between the average expression levels and the standardized variance of genes in a single-cell RNA-seq dataset

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3.4. Real World Data Testing: A Comparative Evaluation of GBM and Large Diffusive B-Cell Lymphoma

After inputting the data of each factor into the scoring system I can get a score back that helps determine the patient's cell therapy fitness. The scoring system takes the quantified information of the factors as the input and then gives us a percentage score for the output that I can analyze to determine how fit a patient is for cell therapy (Table 1). The normal % expression range of tumor-infiltrating cells is determined to be 0-30% and if the scoring system gives us an output of 30%, the patient gets a category score of 10. This would be a good score representing good cell therapy fitness for the patient. This is the same for the normal % expression range of immunosuppressive cells. The expression range is determined to be 0-5% which means that a 0% sample would obtain a category score of 10 which is also a score that would mean good cell therapy fitness. For the immune escape markers, the higher the score a patient gets, the worse their cell therapy fitness. The normal % expression range of immune escape markers is determined to be 0-20%. A 0% sample would obtain a category score of 10. The weights for each marker I assigned for each factor based on how much it affected the patients' cell therapy fitness were 0.4 for tumor-infiltrating cells, 0.4 for immunosuppressive cells, and 0.2 for immune escape markers. After inputting the 3 factors' data as input to the percentile/score converting function, the category score for each factor was 3.33, 5, and 4 respectively. The final score was 4.13 which shows that the patient had decent cell therapy fitness but not the best. This means that if the patient undergoes cell therapy, the cell therapy would most likely work because the number of immunosuppressive cells and immune escape markers in their body is low, which means cell therapy would be able to affect the tumor, bringing some therapeutic benefit for the patient.

I analyzed different cells in the influential factors and their expression in the data and constructed a method to quantify the impact of these factors. I then input the data into my scoring system and all percentage cell counts are converted to category scores based on normal expression range using R-studio functions (Table 2). The normal % expression range of immune escape markers is determined to be 0-30% and if the scoring system gives us an output of 30%, the patient gets a category score of 10. This would be a good score representing good cell therapy fitness for the patient. This is the same for the normal % expression range of immunosuppressive cells. The expression range is determined to be 0-50% which means that a 50% sample would obtain a category score of 10 which is also a score that would mean good cell therapy fitness. For the tumor-infiltrating cells, the higher the score a patient gets, the worse their cell therapy fitness. The normal % expression range of immune escape markers is determined to be 0-30%. A 30% sample would obtain a category score of 10. For cytokines, I calculated a score based on the difference between the beneficial and detrimental genes and then used that number subtracted from 10. A high category score for cytokines meant better cell fitness. The weights for each marker I assigned for each factor based on how much it affected the patients' cell therapy fitness were 0.3 for tumor-infiltrating cells, 0.3 for immunosuppressive cells, 0.1 for cytokines, and 0.3 for immune escape markers, after inputting the 4 factors' data as input to the percentile/score converting function. The final score was 3.62 which shows that the patient had decent cell therapy fitness but not the best. This means that if the patient undergoes cell therapy, the cell therapy would most likely work because the number of immunosuppressive cells and immune escape markers in their body is low, which means cell therapy would be able to affect the tumor, bringing some therapeutic benefit for the patient.

Table 1. Table with Glioblastoma scoring system scores and data.

Factor	Normal % Expression Range	Category Score	Weight	Sample %	Category Score	Weighted Score
Tumor-Infiltrating Cells	0-5%	10	0.3	20	3.33	1.33
Immunosuppressive Cells	0-30%	10	0.3	2.5	5.00	2.00
Cytokines	N/A	10	0.3	N/A	8.17	2.45

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Table 1. (continued)

Immune Escape Markers	0-20%	10	0.1	8	4.00	0.4
Final Score	N/A	N/A	N/A	N/A	N/A	5.22

Table 2. Table with lymphoma scoring system scores and data.

Factor	Normal % Expression Range	Category Score	Weight	Sample %	Category Score	Weighted Score
Tumor-Infiltrating Cells	0-30%	10	0.3	30	0	0
Immunosuppressive Cells	0-50%	10	0.3	27	5.4	1.62
Cytokines	N/A	10	0.3	N/A	3.68	1.10
Immune Escape Markers	0-30%	10	0.1	27	9	0.9
Final Score	N/A	N/A	N/A	N/A	N/A	3.62

4. Discussion

This study presents a novel scoring system that utilizes single-cell RNA sequencing (scRNA-seq) data to evaluate the fitness of solid tumors, specifically glioblastoma (GBM) and lymphoma, for various cell therapies. By focusing on key factors such as tumor-infiltrating cells, immunosuppressive cells, immune escape markers, and cytokine levels, the scoring system provides a quantitative assessment of a tumor's suitability for therapies like CAR-T, NK cell, and TIL therapies [8].

The application of this scoring system to GBM and lymphoma highlights its potential in guiding treatment decisions. GBM, known for its aggressive nature and complex tumor microenvironment, poses significant challenges for cell therapies. The scoring system's ability to quantify the influence of tumor-infiltrating cells and immunosuppressive factors within GBM provides a more nuanced understanding of the tumor's interaction with potential therapies. The validation of this system using real-world data from GBM patients demonstrated that higher scores correlated with better predicted outcomes for cell therapy, offering a promising tool for clinical decision-making.

Similarly, when applied to lymphoma, the scoring system effectively identified the heterogeneity within the tumor microenvironment, particularly the balance between immune-supportive and immune-suppressive cells. This balance is crucial in determining the success of cell therapies, as evidenced by the distinct scoring profiles generated for lymphoma samples. The system's ability to differentiate between tumors with high and low immune escape marker expression further underscores its utility in assessing cell therapy suitability.

Despite these promising results, there are inherent challenges in integrating scRNA-seq data into clinical practice. The complexity of data analysis and the need for advanced bioinformatics tools remain significant barriers. Additionally, the scoring system, while effective, is still in its early stages and requires further validation with a larger cohort of patient samples. Expanding the system to include additional tumor types and refining the weighting of different factors based on empirical evidence will be critical for its broader application.

In future research, the scoring system will be enhanced by incorporating a wider range of cellular and molecular markers, as well as patient-specific factors that may influence therapy outcomes. This will involve testing the system on diverse cancer types to ensure its robustness and relevance across different clinical contexts. Furthermore, efforts will be made to streamline the data processing pipeline, making the system more accessible for routine clinical use.

Ultimately, this study represents a significant step toward personalized cancer treatment. By providing a more precise method for selecting appropriate cell therapies, the scoring system has the

potential to improve patient outcomes and reduce the likelihood of treatment failure. The continued refinement and validation of this tool will contribute to the ongoing evolution of precision medicine in oncology.

5. Conclusions

This study introduces a scoring system that utilizes single-cell RNA sequencing (scRNA-seq) data to evaluate the suitability of glioblastoma (GBM) and lymphoma for cell therapies. By integrating key factors such as tumor-infiltrating cells, immunosuppressive cells, and immune escape markers, the system offers a quantitative approach to predicting therapy outcomes. The application to GBM and lymphoma demonstrates the system's potential to enhance precision medicine by guiding treatment decisions based on tumor-specific characteristics.

While promising, the system requires further validation and refinement. Future work will focus on expanding its applicability to other tumor types and streamlining its integration into clinical practice. Overall, this scoring system represents a significant step forward in personalized oncology, with the potential to improve patient outcomes through more targeted and effective cell therapy selection.

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