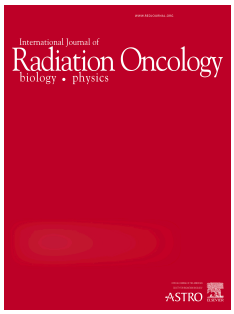
Journal Pre-proof



Prediction of Outcomes with a Computational Biology Model in Newly Diagnosed Glioblastoma Patients Treated with Radiation Therapy and Temozolomide

Rifaquat Rahman, MD, Lorenzo Trippa, PhD, Stephanie Alden, BA, Geoffrey Fell, MSc, Taher Abbasi, MBA, M.S., Yatin Mundkur, M.S., Neeraj K. Singh, B. Tech, Anay Talawdekar, M. Tech, Zakir Husain, M. Tech, Shireen Vali, PhD, Keith L. Ligon, MD, PhD, Patrick Y. Wen, MD, Brian M. Alexander, MD, MPH

PII: S0360-3016(20)31134-2

DOI: https://doi.org/10.1016/j.ijrobp.2020.05.010

Reference: ROB 26344

To appear in: *International Journal of Radiation Oncology • Biology • Physics*

Received Date: 1 February 2019

Revised Date: 16 April 2020

Accepted Date: 7 May 2020

Please cite this article as: Rahman R, Trippa L, Alden S, Fell G, Abbasi T, Mundkur Y, Singh NK, Talawdekar A, Husain Z, Vali S, Ligon KL, Wen PY, Alexander BM, Prediction of Outcomes with a Computational Biology Model in Newly Diagnosed Glioblastoma Patients Treated with Radiation Therapy and Temozolomide, *International Journal of Radiation Oncology • Biology • Physics* (2020), doi: https://doi.org/10.1016/j.ijrobp.2020.05.010.

Prediction of Outcomes with a Computational Biology Model in Newly Diagnosed Glioblastoma Patients Treated with Radiation Therapy and Temozolomide

Rifaquat Rahman, MD[1], Lorenzo Trippa, PhD[2], Stephanie Alden, BA[3], Geoffrey Fell, MSc[2], Taher Abbasi, MBA, M.S. [4], Yatin Mundkur, M.S.[4], Neeraj K. Singh, B. Tech[5], Anay Talawdekar, M. Tech[5], Zakir Husain, M. Tech[5], Shireen Vali PhD[4], Keith L. Ligon MD, PhD[6], Patrick Y. Wen, MD[7], Brian M. Alexander, MD, MPH[1]

Affiliations

1. Department of Radiation Oncology, Dana-Farber/Brigham and Women's Cancer Center, Harvard Medical School, Boston, MA
2. Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Harvard School of Public Health, Boston, MA.
3. Harvard Medical School, Boston, MA.
4. Cellworks Group, Inc., San Jose, CA.
5. Cellworks Research India Pvt. Ltd., Bengaluru, India.
6. Department of Oncologic Pathology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA.
7. Center for Neuro-Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA.

Corresponding Author:

Brian M. Alexander, MD, MPH

Harvard Medical School, Armenise 109D

210 Longwood Ave

Boston, MA 02115

Phone: 617-732-6313

Fax: 617-975-0932

bmalexander@lroc.harvard.edu

Running Title: Predictive simulation modeling in GBM

Presentations: This study has not been previously presented.

Conflict of Interest Disclosures:

Brian Alexander reports personal fees from AbbVie, Bristol-Myers Squibb, Precision Health Economics, and Schlesinger Associates and employment at Foundation Medicine. He reports research support from Puma, Eli Lilly, Celgene.

Patrick Wen reports personal fees from Abbvie, Agios, Astra Zeneca, Blue Earth Diagnostics, Eli Lilly, Genentech/Roche, Immunomic Therapeutics, Kadmon, Kiyatec, Merck, Puma, Vascular Biogenics, Taiho, Tocagen, Deciphera, VBI Vaccines and research support from Agios, Astra Zeneca, Beigene, Eli Lily, Genentech/Roche, Karyopharm, Kazia, MediciNova, Merck, Novartis, Oncoceutics, Sanofi-Aventis, VBI Vaccines

Keith L. Ligon reports personal fees from Travera LLC, Bristol Myers Squibb, research support from Bristol Myers Squibb, Amgen, X4, Deciphera, Tragara, Eli Lilly, Novartis. He holds equity and is a founder of Travera LLC.

The following authors are employed by Cellworks, Inc: Taher Abbasi and Shireen Vali. Yatin Mundkur is also an employee of Cellworks Group, Inc.

The following authors are employed by Cellworks Research India Pvt, a wholly owned subsidiary of Cellworks Group Inc., San Jose, CA: Neeraj K. Singh, Anay Talawdekar, Zakir Husain

Funding Statement: Internal funds were used for expenses associated with this study. Cellworks Group Inc. provided computational biology model testing for patients with internal funding.

Statistical Analysis: Lorenzo Trippa, PhD and Brian Alexander, MD PhD were responsible for statistical analysis.

Prediction of Outcomes with a Computational Biology Model in Newly Diagnosed Glioblastoma Patients Treated with Radiation Therapy and Temozolomide

Running Title: Predictive simulation modeling in GBM

Abstract

Purpose: Precision medicine has been most successful in targeting single mutations, but personalized medicine using broader genomic tumor profiles for individual patients is less well-developed. We evaluate a genomics-informed computational biology model (CBM) to predict outcomes from standard treatments and to suggest novel therapy recommendations in glioblastoma (GBM).

Methods and Materials: In this retrospective study, 98 patients with newly diagnosed GBM undergoing surgery followed by radiation therapy and temozolomide at a single institution with available genomic data were identified. Incorporating mutational and copy number aberration data, a CBM was used to simulate the response of GBM tumor cells and generate efficacy predictions for radiation therapy (RTeff) and temozolomide (TMZeff). RTeff and TMZeff were evaluated for association with overall survival (OS) and progression-free survival in a Cox regression model. To demonstrate a CBM-based individualized therapy strategy, treatment recommendations were generated for each patient by testing a panel of 45 CNS-penetrant FDA-approved agents.

Results: High RTeff scores were associated with longer survival on univariable analysis (UVA) (P<0.001), which persisted after controlling for age, extent of resection, performance status, MGMT and IDH status (P=0.017). High RTeff patients had a longer OS compared to low RTeff patients (median 27.7 vs. 14.6 months). High TMZeff was also associated with longer survival on UVA (P=0.007) but did not hold on multivariable analysis, suggesting an interplay with MGMT status. Among predictions of the three most efficacious combination therapies for each

patient, only 2.4% (7 of 294) of two-drug recommendations produced by the CBM included TMZ.

Conclusions: CBM-based predictions of RT and TMZ effectiveness were associated with survival in newly diagnosed GBM patients treated with those therapies, suggesting a possible predictive utility. Furthermore, the model was able to suggest novel individualized monotherapies and combinations. Prospective evaluation of such a personalized treatment strategy in clinical trials is needed.

Introduction

Glioblastoma (GBM) is the most common primary adult brain cancer, and it is associated with a poor prognosis (1). Despite aggressive multimodality therapy with surgery, radiation therapy (RT), and temozolomide (TMZ), GBM continues to be associated with poor survival and limited therapeutic progress (2). Molecular profiling and genome-wide analyses have provided a window into the intricate complexity underlying GBM tumor biology (3).

As the number of targeted therapies expand, genetic tumor profiling has become more accurate, affordable, and available (4). The increasing amount of tumor genomic sequencing has raised the possibility of personalized medicine where treatments can be tailored to each patient's tumor (5). Harnessing the wealth of genomic and molecular data from tumors to tailor appropriate therapy has been challenging but has the potential to revolutionize the management of oncology patients (5, 6). In this context, there is a need for novel approaches to streamline clinical decision-making to identify patients most likely to benefit from a particular therapy through the lens of tumor genetics (7–9).

Predictive simulation modeling is an emerging technology that has garnered interest for GBM in recent years (10). In this evolving paradigm, a computational simulation avatar is created via genomic profiling information derived from tumor tissue, allowing a digital library of therapies with varying combinations and doses to be tested on an individual avatar disease network map (11). Using a computational biology model (CBM), simulations of complex biological mechanisms and key signaling pathways can be used to predict the expected tumor response to various therapies for each patient based on their tumor genomics. While there are currently no computational models that have successfully translated to the clinic, preliminary evidence suggests immense potential across various cancers, including GBM (9, 10).

In this study, we hypothesized that a GBM CBM model-based prediction of RT and TMZ efficacy would be prognostic for progression-free survival (PFS) and overall survival (OS) in patients receiving these therapies as part of standard of care treatment. We then utilized the CBM platform to screen a panel of FDA-approved drugs to illustrate the potential to suggest an individualized treatment strategy based on tumor genomic profiles.

Material and Methods

Patients and Genomic Assays

This retrospective study was approved by the institutional review board at XXXX. Somatic mutational profiling was performed with specific consent as clinical research testing and approved by the XXXX IRB. The XXXX cohort consisted of adult patients $\geq$ 18 years old with a newly diagnosed GBM treated at our institution 2005-2014 with available clinical molecular profiling. Brain tumor tissue was acquired as a part of initial surgery for patients at time of initial presentation. Samples were obtained from surgical procedures with selection of viable samples by a pathologist within 30 minutes to 1 hour of surgical removal. Samples were verified to contain viable tumor tissue at least 50% by volume and were stored frozen at -80 degrees Celsius or fixed in formalin using standard procedures. Tests were performed within the Cytogenetics and Molecular Diagnostics Divisions of the XXXX, a CLIA-certified laboratory environment. Each patient had sequencing data and whole genome copy number data generated and available from the clinical medical record. Sequencing was generated by at least 1 of 2 clinical sequencing assays: OncoMap (12), a mass spectrometry–based mutation genotyping covering 471 mutations from 41 cancer genes (version 4); or OncoPanel (13), a targeted exome sequencing platform covering 275 cancer genes and 91 select introns across 30 genes to detect somatic mutations,

copy number alterations, and structural rearrangements. Copy data was generated using Agilent array CGH (SurePrint 1M resolution array) to call amplification, losses, deletions, focal (<10 Mb) and broad (>10Mb) variables for a subset of CNS cancer related genes and arm level aberrations (see Supplementary Text for complete list of genomic data).

O^6-DNA methylguanine-methyltransferase (MGMT)–promoter methylation status in this cohort was generally assessed using methylation-specific polymerase chain reaction (MS-PCR). Clinical, demographic, pathologic, and follow-up data were collected retrospectively from the medical record.

Details of the CBM and in Silico Modeling Approach

The modeling system used in this study is an updated version of a previously detailed model (14–17). Briefly, the CBM, developed by XXXX contains information acquired from a variety of data sources, including studies on cell receptors, signaling pathways, pathway signaling intermediates, transcription factors, activation factors, and enzyme kinetics. Simulation experiments and analyses using the predictive tumor model, constituting a dynamic and comprehensive representation of signaling and metabolic pathways, is used for testing and validation. To ensure accuracy of computational simulation models, published data has been aggregated through manual scientific review to maintain quality of input information and resolve contradictory datasets.

The simulation model includes representations of key signaling pathways underlying growth factor signaling, cell cycle regulation, tumor metabolism, oxidative stress, epigenetics, protein homeostasis, DNA damage repair, and apoptosis. The current version of the model

includes more than 3,000 genes, 2,500 unique biomarkers and 85,000 functional interactions associated with signaling pathways associated with cancer. This comprises comprehensive and extensive coverage of the kinome, transcriptome, proteome and metabolome.

Details on model derivation, experimental support, *in vitro* model validation, drug effect simulation, and creation of simulation avatars has been previously described (14, 17). Further information about the CBM, including how genomic data is interpreted and analyzed, is available in the Supplementary Material. Using trained disease models, ten anti-cancer drugs have been previously tested *in silico* on eight patient-derived GBM cell lines in a blinded prospective study, and 76% of CBM predictions agreed with *in vitro* experimental results (14). The CBM has been updated and expanded to include prediction of efficacy of radiation and temozolomide (see Supplementary Figure 1 for a schematic representation of drug effect modeling). Training and testing sets show high correlation between predictions of therapy efficacy of therapies (including radiation and temozolomide, respectively) in GBM with *in vitro* experimental findings and clinical outcomes in tested datasets (see Supplementary Table 1).

The CBM was used to test RT and TMZ on a *computational avatar* for each included patient to obtain an efficacy score for each intervention (e.g. RTeff and TMZeff, respectively). A patient treated with RT and TMZ produced both a RTeff and TMZeff score, while a patient treated with TMZ alone produced a TMZeff score only. RTeff and TMZeff represented the simulated impact on tumor growth due to each respective therapy, and they were analyzed as a continuous variable. These parameters were not available at time of treatment, and patients received therapy at discretion of treating physicians.

Drug Screening

A pre-specified list of 45 FDA-approved drugs were used in simulations with the CBM to generate treatment recommendations based on the therapy most likely to be efficacious for each patient in the XXXX cohort. While 170 drugs have been modeled in the XXXX platform, 45 drugs were chosen based on evidence of crossing the blood-brain barrier and preclinical or clinical evidence of activity in GBM (Supplementary Table 2). Single agent treatment and two-drug combination therapies were evaluated.

Statistical analysis

To ensure appropriate blinding, investigators generating treatment efficacy predictions had access to deidentified genomic data but did not have access to clinical outcomes data. All statistical analyses were done by investigators that were not affiliated with the development of the CBM. Progression was determined retrospectively for the XXXX cohort through clinical note assessments integrating imaging and clinical status. Univariable (UVA) and multivariable (MVA) Cox proportional hazards modeling was used to identify predictors of PFS and OS. Actuarial estimates of PFS and OS were calculated using the Kaplan–Meier method. Statistical significance was set at $P<0.05$, and all tests were two-sided. Analyses were performed using RStudio (version 1.1.383) running R (version 3.4.2) with the survival package (18). An overview of the study design of our retrospective study is presented in Supplementary Figure 2.

Results

Patient characteristics

The XXXX cohort included a total of 98 patients, and patient characteristics are summarized in Table 1. The majority (93%) of patients were diagnosed 2011-2014. OncoPanel data was available for 68 patients, OncoMap data was available for 16 patients, and aCGH data was available for 79 patients (49 patients with OncoPanel had aCGH data and all 16 patients with OncoMap had aCGH data). The median age was 60 years with 46% females. The majority of patients had a KPS $\geq$ 70 (82%) and were IDH1/2 wildtype (96%). With respect to MGMT promoter methylation status, MGMT promoter was methylated or partially methylated for 46% of patients, unmethylated in 41% of patients and unknown in 13% of patients. While 94% patients (91 of 98) were treated with RT and TMZ, there were 5 patients treated with radiation alone, and 2 patients treated with temozolomide alone.

CBM efficacy predictions for radiation therapy and temozolomide

CBM predictions were made using genomic data to generate efficacy scores, representative of a computational prediction of percentage change of tumor growth from therapy. The median value of RTeff was 11.4% (range 2.1%-66.3%), and the median value of TMZeff was 27.3% (range 0.55%-86.1%). RTeff and TMZeff were correlated (r=0.763). RTeff was correlated with neither age (r= -0.018) nor KPS (r= 0.096). By t-test, there was no difference in RTeff based upon MGMT promoter methylation status (mean 15.7% in unmethylated MGMT patients vs. 18.8% in methylated MGMT patients, *P*=0.28), extent of resection (dichotomized, 18.5% vs. 15.5%, *P*=0.28) or p53 mutation status (16.5% vs. 18.6%, *P*=0.59). There was, however, a significant difference in TMZeff based upon MGMT promoter methylation status; mean TMZeff was 22.8%

in unmethylated patients and mean TMZeff was 39.1% in methylated patients (P<0.001). There was no difference in TMZeff based upon neither extent of resection nor p53 mutation status.

Overall survival analysis

Median overall survival (OS) for all patients was 18.7 months. Table 2 summarizes regression results. Factors significant on UVA for association with OS included age (HR 1.05, 95%CI 1.02-1.08, P=0.008) and MGMT methylation promoter status (HR 0.56, 95%CI 0.32-0.96, P=0.036). We dichotomized extent of resection (subtotal resection or biopsy vs. gross total resection, HR 1.65, 95%CI 0.99-2.77, P=0.055), RT dose (RT dose < 5940cGy vs. ≥ 5940cGy, HR 2.10, 95%CI 0.90-4.86, P=0.08), and KPS (KPS ≥ 70 vs. < 70, HR 0.50, 95%CI 0.20-1.27, P=0.15), but these were not significant on UVA.Both the RTeff score (HR 0.95, 95%CI 0.93-0.98, P<0.001) and TMZeff score (HR 0.98, 95%CI 0.97-0.99, P=0.007) were associated with OS. In dichotomizing RTeff by the median value (11.4%), there was a significant (P=0.002 by log-rank) difference in survival in patients with high RTeff (median 27.7 months) vs. low RTeff (median 14.6 months; HR 0.42, 95%CI 0.24-0.72; Figure 1).

On MVA, age (adjusted hazard ratio [AHR] 1.07, 95%CI 1.04-1.11, P<0.001), extent of resection (AHR 2.53, 95%CI 1.20-5.33, P=0.015), MGMT promoter methylation status (AHR 0.46, 95%CI 0.22-0.98, P=0.04), and RTeff (AHR 0.95, 95%CI 0.90-0.99, P=0.017) were associated with OS. MVA included 84 patients with complete data available. There was no significant interaction between age and KPS (P=0.20; Supplementary Table 3).

Progression-free survival analysis

Median progression-free survival (PFS) for all patients was 9.7 months. The only clinical or molecular variable significant on UVA for association with PFS was MGMT promoter methylation status (HR 0.49, 95%CI 0.37-0.92, *P*=0.02). RTeff was associated with PFS (HR 0.98, 95%CI 0.96-0.999, *P*=0.046), while TMZeff showed a trend towards an association but did not meet criteria for statistical significance (HR 0.99, 95%CI 0.98-1.00, *P*=0.052). KPS (HR 1.09, 95%CI 0.40-3.0, *P*=0.88) and RT dose (HR 1.58, 95% CI 0.72-3.48, *P*=0.26) were not associated with PFS. On MVA, MGMT promoter methylation status (AHR 0.45, 95%CI 0.24-0.83, *P*=0.01) was significantly associated with PFS (Supplementary Table 3). RTeff (AHR 1.00, 95%CI 0.97-1.02, *P*=0.74) and TMZeff (AHR1.00, 95%CI 0.98-1.01, *P*=0.67) were not significant in MVA.

MGMT subgroup analysis

We performed an exploratory analysis evaluating TMZeff as a predictor of OS in MGMT methylated and unmethylated patients since there are no known predictors of TMZ efficacy in unmethylated patients (19). TMZeff did not have a statistically significant association with OS in unmethylated (n=38) MGMT patients (HR 0.98, 95%CI 0.96-1.00, *P*=0.10) or methylated (n=46) MGMT patients (HR 0.99, 95%CI 0.97-1.01, *P*=0.518).

Recommended therapies based upon CBM-derived predictions

We examined the top three single agent and two-drug combination regimens that were predicted to be most efficacious for each patient by CBM (Supplementary Table 4). For illustrative purposes, Table 4 demonstrates the top predicted combinations based upon CBM-generated efficacy scores for three patients. Patients were not treated with these agents, and this was an exploratory analysis to illustrate the potential in applying this CBM to individualize treatments for patients. Further study would be necessary to correlate efficacy outcomes for these agents with clinically relevant outcomes. Among single agent therapies, nelfinavir was most frequently predicted to be most efficacious; it was the top prediction for 26% of patients. Lomustine (20%), everolimus (14%), cabozantinib (13%), and TMZ (10%) were the next most common top prediction for single agent therapy for patients.

When considering two-drug combination therapies, the combination of cabozantinib and nelfinavir was most often predicted to be the most efficacious regimen; it was the top prediction for 20% of patients. Lomustine and nelfinavir (13%), cabozantinib and lomustine (11%), afatinib and everolimus (7%), nelfinavir and TMZ (7%), afatinib and nelfinavir (6%), and everolimus and lomustine (6%) were the next most common top prediction for two-drug combination therapy for patients.

The most common therapy predictions of single agent and combination regimens that appeared in top three therapy recommendations for each patient are listed in Table 3. Only 6.5% (19 of 294) of single agent therapy predictions and 2.4% (7 of 294) of two-drug combination regimens included TMZ as part of recommended therapy.

Discussion

Precision medicine approaches frequently characterize patients using genomic profiling to find biomarkers predictive of therapeutic response. Despite the wealth of genomic information generated for GBM, however, targetable driver alterations have not been found in a manner similar to other cancers (20). Furthermore, using genomic information in totality to individualize therapy is less well developed than the experience with single driver variants. In our study, we showed that a CBM-based prediction for RT and TMZ efficacy was associated with longer survival in GBM patients treated with these therapies. These findings are particularly striking since there are only a few known genomic factors associated with OS in GBM (Table 2) and there is a paucity of genomic predictors of RT response across cancers. While a truly predictive claim would require evidence of a differential association with treated and untreated patients (few patients with newly diagnosed GBM do not receive RT), our findings are consistent with a possible predictive utility of a CBM-based approach.

Despite evidence that the benefit of RT in patients is not uniform (21), there is limited data on how genomic differences can be associated with radiosensitivity (22). Prior *in vitro* studies have indicated significant variation in radiation sensitivity across and within cell line lineages with genomic features associated with variability (23). Supporting this notion, radiosensitivity index (RSI) (24) and the related genome-based model for adjusting radiotherapy dose (GARD) (25), using The Cancer Genome Atlas GBM patient cohort have previously described a genomics-derived parameter associated with OS . Of note, high RSI was associated with MGMT methylation (26), which suggests some overlap in the prognostic value of RSI and MGMT methylation. In contrast, RTeff in our study did not differ between methylated or unmethylated MGMT patients and remained an independent predictor of OS on MVA. Since RSI and GARD only used expression data from ten specific genes, they did not incorporate the

breadth of genomic data, biological mechanisms and signaling pathways captured by our CBM-based approach. The incorporation of comprehensive genomic information and accounting of known clinical variables likely explain the higher magnitude of stratification of patient survival seen with RTeff in our study (HR 0.42, 95%CI 0.24-0.72) relative to these prior studies.

The identification of patients more or less likely to benefit from RT is important in the management of GBM patients, particularly with continued interest in dose escalation (27, 28), such as the ongoing NRG-BN001 phase 3 trial (NCT02163135). Our CBM-driven approach has value as a prognostic factor, which could be useful in stratification of patients in such trials. Furthermore, if there is a predictive component to RTeff, clinical trials employing radiation dose escalation or de-escalation may help further characterize this relationship.

TMZeff was also associated with OS on UVA. In contrast to RT, there is already a molecular factor (MGMT promoter methylation) predictive for TMZ treatment efficacy. Indeed, high TMZeff was associated with MGMT methylation (mean TMZeff 39.1% vs. 22.8% in methylated vs. unmethylated MGMT patients, respectively; $P<0.001$), suggesting that the CBM may identify genomic changes more likely to be associated with MGMT methylation. The interplay of genomic changes and MGMT methylation status warrants further study. While TMZ is more effective in MGMT methylated patients (29, 30), there is still a small absolute benefit in MGMT unmethylated patients (30).While limited by subgroup sample size, MGMT unmethylated tumors (n=38, 10 patients with high TMZeff) had more heterogeneous scores (HR 0.98, 95%CI 0.96-1.00, $P=0.10$) that could potentially be leveraged to determine which patients with MGMT unmethylated tumors might still respond to TMZ. . While the CBM-based approach yields largely redundant information as MGMT status, this exploratory subgroup analysis suggests that there may be some non-overlapping explanatory power which could help identify unmethylated MGMT

patients most likely to benefit from TMZ added to RT. Further study is needed, as such findings would have important implications for the management of unmethylated MGMT patients, since these patients are increasingly enrolling onto trials in which TMZ is omitted.

Molecular tumor boards have been proposed as a solution to bring precision medicine into clinical practice, but clinicians and associated experts need additional tools to effectively analyze and interpret multiplexed genomic data (6). With a CBM incorporating comprehensive genomic inputs beyond just driver genes, many different therapeutic interventions can be "tested" simultaneously *in silico,* a potentially useful tool for clinical decision support. To explore the feasibility of using a CBM to individualize therapy, we screened a pre-specified list of FDA approved drugs on each patient's computational avatar in the XXXX cohort. The result was a large degree of heterogeneity in treatment recommendations, and more variation than might be expected based on evaluation of single genes commonly altered in GBM (9). Nelfinavir and cabozantinib were the most frequent single agent therapies predicted to be most efficacious, and their combination was the most commonly recommended two-drug regimen. Both nelfinavir and cabozantinib have been evaluated in a limited capacity in unselected GBM patients (31–33), and our CBM suggests that there is a subset of patients that may preferentially benefit from these therapies, potentially in combination. While these results are provocative, patients in this study did not receive these screened therapies, and further study is necessary to associate our CBM predictions in patients receiving a predicted treatment with clinically meaningful outcomes. Future study would also allow for clarification of the relationship between the magnitude of the predicted efficacy score and clinical outcome, including the significance and validity of the CBM-mediated ranking procedure that provides recommendations of most likely effective therapies for a specific patient (Table 4).

Our findings highlight the potential of developing tools to individualize therapy based upon genomic data. Moving forward, strategies for genomics-informed treatment must be tested in a rigorous manner, and there is limited experience of this in GBM (9). To evaluate CBM-based treatment decision-making, newly diagnosed GBM patients could be randomized to standard therapy or an experimental arm where genomic data is incorporated into a CBM to predict the most efficacious adjuvant therapy. In this paradigm, instead of a single experimental agent, patients on the experimental arm would receive the CBM-predicted therapy or combination of therapies. This design creates challenges in trial conduct, including access for multiple non-FDA approved agents, but it can serve as a viable framework to test treatment decision-making strategies and actualize genomics-driven individualized cancer care.

There are several limitations in our study. We have presented clinical validation of the previously generated model in one institutional cohort; while the model has been trained and tested in relatively small databases (Supplementary Table 1), additional analyses in other datasets would be valuable. The CBM predictions represent point predictions of efficacy that are not 100% accurate, and we provided 95% confidence intervals of RTeff and TMZeff in their association with OS and PFS. Furthermore, genomic profiling data in was heterogenous in our cohort with different sequencing platforms (i.e. OncoPanel and OncoMap) permitted. Given the paucity of newly diagnosed GBM patients receiving neither TMZ nor RT in our dataset, a truly predictive relationship could not be determined. More generally, the predictive utility of the CBM model would be better tested in randomized trials with varying treatment groups. A larger sample size would be helpful to better evaluate the use of TMZeff in MGMT-defined subgroups of patients.

Conclusion

We showed that CBM-based predictions of therapeutic response to standard therapies in newly diagnosed GBM were associated with survival and that the same model could generate individualized treatment recommendations. The use of a CBM approach based on mathematical modeling represents a promising strategy towards genomics-driven personalized medicine that merits further investigation.

References

1. Wen PY, Kesari S. Malignant Gliomas in Adults. *N. Engl. J. Med.* 2008;359:492–507.

2. Stupp R, Mason WP, van den Bent MJ, *et al.* Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N. Engl. J. Med.* 2005;352:987–996.

3. Aubry M, de Tayrac M, Etcheverry A, *et al.* From the core to beyond the margin: a genomic picture of glioblastoma intratumor heterogeneity. *Oncotarget*. 2015;6:12094–12109.

4. van der Velden DL, van Herpen CML, van Laarhoven HWM, *et al.* Molecular Tumor Boards: current practice and future needs. *Ann. Oncol. Off. J. Eur. Soc. Med. Oncol.* 2017;28:3070–3075.

5. Garraway LA, Verweij J, Ballman KV. Precision oncology: an overview. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* 2013;31:1803–1805.

6. Van Allen EM, Wagle N, Levy MA. Clinical analysis and interpretation of cancer genome data. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* 2013;31:1825–1833.

7. Garraway LA. Genomics-driven oncology: framework for an emerging paradigm. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* 2013;31:1806–1814.

8. Verma M. Personalized Medicine and Cancer. *J. Pers. Med.* 2012;2:1–14.

9. Byron SA, Tran NL, Halperin RF, *et al.* Prospective Feasibility Trial for Genomics-Informed Treatment in Recurrent and Progressive Glioblastoma. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* 2018;24:295–305.

10. Deisboeck TS, Zhang L, Yoon J, *et al.* In silico cancer modeling: is it ready for prime time? *Nat. Clin. Pract. Oncol.* 2009;6:34–42.

11. Kobayashi SS, Vali S, Kumar A, *et al.* Identification of myeloproliferative neoplasm drug agents via predictive simulation modeling: assessing responsiveness with micro-environment derived cytokines. *Oncotarget*. 2016;7:35989–36001.

12. MacConaill LE, Campbell CD, Kehoe SM, *et al.* Profiling critical cancer gene mutations in clinical tumor samples. *PloS One*. 2009;4:e7887.

13. Wagle N, Berger MF, Davis MJ, *et al.* High-throughput detection of actionable genomic alterations in clinical tumor samples by targeted, massively parallel sequencing. *Cancer Discov.* 2012;2:82–93.

14. Pingle SC, Sultana Z, Pastorino S, *et al.* In silico modeling predicts drug sensitivity of patient-derived cancer cells. *J. Transl. Med.* 2014;12:128.

15. Doudican NA, Kumar A, Singh NK, *et al.* Personalization of cancer treatment using predictive simulation. *J. Transl. Med.* 2015;13:43.

16. Drusbosky L, Medina C, Martuscello R, *et al.* Computational drug treatment simulations on projections of dysregulated protein networks derived from the myelodysplastic mutanome match clinical response in patients. *Leuk. Res.* 2017;52:1–7.

17. Jiang P, Mukthavaram R, Chao Y, *et al.* In vitro and in vivo anticancer effects of mevalonate pathway modulation on human cancer cells. *Br. J. Cancer*. 2014;111:1562–1571.

18. Therneau TM, until 2009) TL (original S->R port and R maintainer. *survival: Survival Analysis*. 2018.

19. Hegi ME, Stupp R. Withholding temozolomide in glioblastoma patients with unmethylated MGMT promoter—still a dilemma? *Neuro-Oncol.* 2015;17:1425–1427.

20. Letai A. Functional precision cancer medicine—moving beyond pure genomics. *Nat. Med.* 2017;23:1028–1035.

21. Eschrich S, Zhang H, Zhao H, *et al.* Systems biology modeling of the radiation sensitivity network: a biomarker discovery platform. *Int. J. Radiat. Oncol. Biol. Phys.* 2009;75:497–505.

22. Kamran SC, Mouw KW. Applying Precision Oncology Principles in Radiation Oncology. *JCO Precis. Oncol.* 2018:1–23.

23. Yard BD, Adams DJ, Chie EK, *et al.* A genetic basis for the variation in the vulnerability of cancer to DNA damage. *Nat. Commun.* 2016;7:11428.

24. Eschrich SA, Pramana J, Zhang H, *et al.* A gene expression model of intrinsic tumor radiosensitivity: prediction of response and prognosis after chemoradiation. *Int. J. Radiat. Oncol. Biol. Phys.* 2009;75:489–496.

25. Scott JG, Berglund A, Schell MJ, *et al.* A genome-based model for adjusting radiotherapy dose (GARD): a retrospective, cohort-based study. *Lancet Oncol.* 2017;18:202–211.

26. Ahmed KA, Chinnaiyan P, Fulp WJ, *et al.* The radiosensitivity index predicts for overall survival in glioblastoma. *Oncotarget*. 2015;6:34414–34422.

27. Khan L, Soliman H, Sahgal A, *et al.* External beam radiation dose escalation for high grade glioma. *Cochrane Database Syst. Rev.* 2016:CD011475.

28. Yanagihara TK, Saadatmand HJ, Wang TJC. Reevaluating stereotactic radiosurgery for glioblastoma: new potential for targeted dose-escalation. *J. Neurooncol.* 2016;130:397–411.

29. Smrdel U, Popovic M, Zwitter M, *et al.* Long-term survival in glioblastoma: methyl guanine methyl transferase (MGMT) promoter methylation as independent favourable prognostic factor. *Radiol. Oncol.* 2016;50:394–401.

30. Hegi ME, Diserens A-C, Gorlia T, *et al.* MGMT gene silencing and benefit from temozolomide in glioblastoma. *N. Engl. J. Med.* 2005;352:997–1003.

31. Alonso-Basanta M, Fang P, Maity A, *et al.* A phase I study of nelfinavir concurrent with temozolomide and radiotherapy in patients with glioblastoma multiforme. *J. Neurooncol.* 2014;116:365–372.

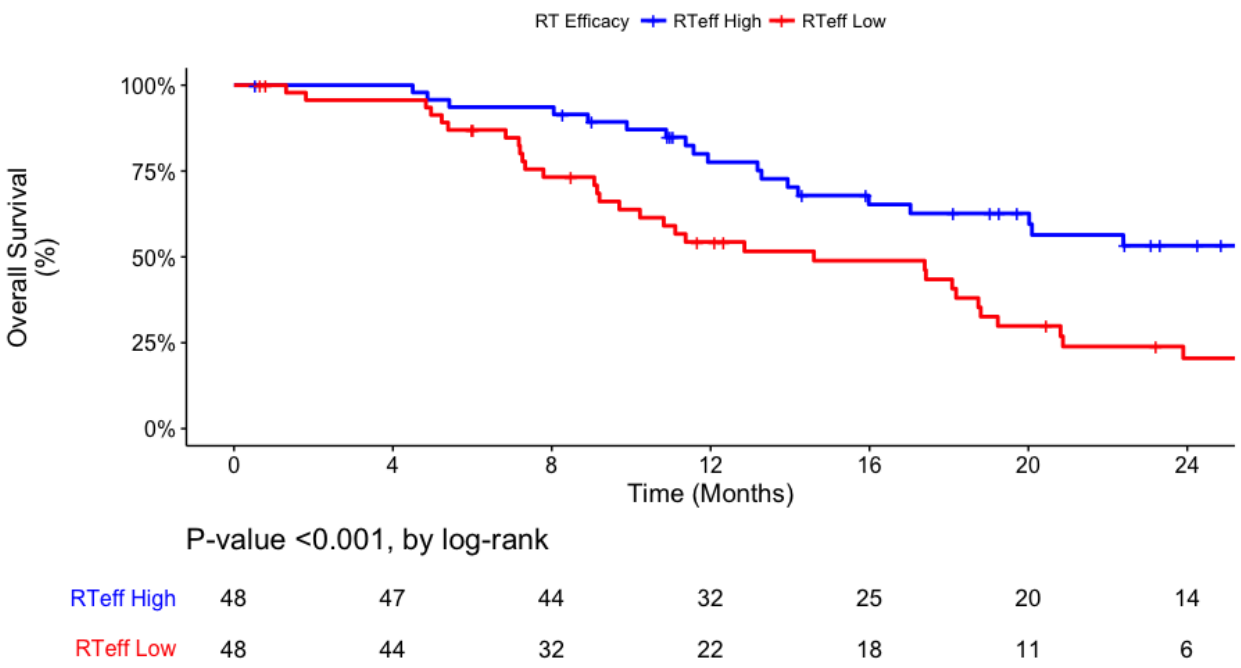
32. Wen PY, Drappatz J, de Groot J, *et al.* Phase II study of cabozantinib in patients with progressive glioblastoma: subset analysis of patients naive to antiangiogenic therapy. *Neuro-Oncol.* 2018;20:249–258.

33. Cloughesy TF, Drappatz J, de Groot J, *et al.* Phase II study of cabozantinib in patients with progressive glioblastoma: subset analysis of patients with prior antiangiogenic therapy. *Neuro-Oncol.* 2018;20:259–267.

Figure Captions

Figure 1: Kaplan Meier estimates of overall survival (OS) for patients with high RTeff compared to patients with low RTeff. High RTeff was associated with improved OS (P<0.001 by log-rank).

Figure 2: Kaplan Meier estimates of overall survival (OS) for patients with high TMZeff compared to patients with low TMZeff, stratified by unmethylated (uMGMT) and methylated (mMGMT) MGMT promoter status. TMZeff did not have a statistically significant association with OS in unmethylated patients (n=39, P=0.10 by log-rank) nor methylated patients (n=47, P=0.52 by log-rank).



RTeff High	48	47	44	32	25	20	14
RTeff Low	48	44	32	22	18	11	6