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PERSPECTIVE

Modulation of Cell State to Improve Drug Therapy

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Manipulation of cell state transitions – programs between cancer stem cells and differentiated cancer cells – may offer a means to enhance drug efficacy. A mathematical model that contrasted mutant IDH1 brain tumor growth under different temozolomide (TMZ) conditions showed tumor growth decreased in TMZ-resistant tumors under oscillating D2HG conditions, a mechanism to manipulate cell state. Future work is needed to complete mechanistic studies to expand and validate the simulations.

BACKGROUND

The majority of advances in anticancer drug therapy emanate from the drug discovery space – new molecules and new targets – rather than from new drug usage paradigms. Notwithstanding pharmacokinetic (PK) and pharmacodynamic (PD) input to design more efficacious and rational schedules, most drugs are administered in a uniform manner.

It is well appreciated that tumors consist of different cell types that possess a certain degree of plasticity.[1] Of particular interest is the transition between cancer stem cells and differentiated cancer cells that are linked to epigenetic reprograming.[1] By modulating cell state equilibria with drugs, cell survival programs may be disrupted and less durable, favoring drug activity.

Brain tumors provide a means to test the hypothesis that manipulation of cell state will improve drug therapy. Brain tumors possessing the mutant IDH1 enzyme – about 70% of grade II and III brain tumor patients – produce copious amounts of the oncometabolite, D-2-hydroxyglutarate (D2HG), that is responsible for the G-CIMP phenotype, methylated DNA and histones, that prevent stem cell differentiation to glioma cells.[2,3] A mainstay of drug therapy for brain tumors is the alkylating agent TMZ that invariably losses its effectiveness due to adaptive resistance. Thus, the interplay between D2HG and TMZ provided a viable means to test the merits of cell state modulation as a new strategy to improve drug therapy. Specifically, models were derived to simulate and compare different treatment scenarios; tumor growth under control, and TMZ-sensitive and TMZ-resistant conditions with and without cell state modulation.

CELL STATE MODEL

A model depicting quiescent (Q) cells, glioma stem (GS) cells, and glioma proliferating (GP) cells and transfer amongst these types was used to demonstrate the effects of cell state modulation (**Figure 1**). The model considered two intermediate states between GS and GP cells to capture the time-dependent progression. Cell proliferation of GS and GP cells is included as is death of each cell type. Mutant IDH1 brain tumors grow slowly,[4–6] which was used as a guide to set the growth characteristics over a 1-year time period. Thus, the control growth model consisted of three components – cell proliferation, cell state transfer, and cell death – that were cast as ordinary differential equations (ODEs) as follows:

$$\frac{dGP}{dt} = LgrowGP + \sum_{i=GS2,Q} (k_{iGP} i - k_{GPi} GP) - k_{Dead\text{-}Control} * GP \quad (1)$$

$$\frac{dGS0}{dt} = LgrowGS0 + \sum_{i=GP,Q} (k_{iGS0} i - k_{GS0i} GS0) - k_{Dead\text{-}Control} * GS0 \quad (2)$$

$$\frac{dQ}{dt} = \sum_{i=GS0,GP} (k_{iQ} i - k_{Qi} Q) - k_{Dead\text{-}Control} * Q \quad (3)$$

where LgrowGP and LgrowGS0 = logistic growth functions (cell proliferation),

Summation terms = first-order cell input and output functions (cell transfer), $k_{Dead\text{-}Control}$ = first-order cell death rate constants.

Full details on the model code and parameter values are provided in the **Supplementary Material**. The logistic growth functions are often used to characterize cell proliferation and have been previously used for TMZ in patients with brain tumors.[5,6] For simplicity, proliferation of GP and glioma stem state 0 (GS0) cells both used logistic growth functions. Cell death in the control model was attributed to physiological factors, such as limited blood flow, and the microenvironment, such as hypoxia, and again for simplicity, utilized equal first-order rate constants for each cell type.

Modeling tumor growth in the presence of TMZ utilized a previously developed hybrid physiologically based/PK model that introduced the idea of cell type-specific PK/PD models.[7] The patient-based model used a forcing function to characterize the systemic disposition of TMZ that yielded plasma concentrations that entered the brain tumor that consisted of vascular, interstitial fluid, and intracellular compartments. This model considered the pH-dependent intracellular conversion of TMZ to 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide to a methylating cation and the formation of DNA adducts, which were treated as a singular entity in both GP and GS0 cells. The model can be revised to depict unique DNA adducts, such as the lethal O6-methylguanine species, and kinetics related to DNA repair in each cell type; however,

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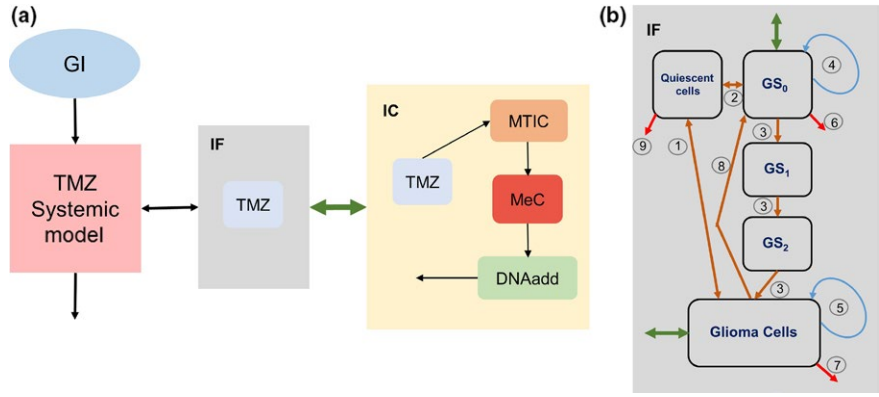

Figure 1 Pharmacokinetic (PK) and cell state model. (**a**) PK model of temozolomide (TMZ) used in all models except control. The model is analogous to that used previously.[7] Following oral administration TMZ is able to cross the blood-brain barrier and enter the brain tumor interstitial fluid (IF) and intracellular (IC compartments). In the IC glioma cell and glioma stem (GS_0) cell compartments TMZ is converted to 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (MTIC) then a methylating cation (MeC) and DNA adducts (DNAadd). (**b**) Cell state model that designates GS_0, two transition states (GS_1 and GS_2), glioma proliferating cells and quiescent cells. The brown arrows (1, 2, 3, and 8) represent cell transfers, blue arrows (4 and 5) cell proliferation, and red arrows (6, 7, and 9) cell death. The bidirectional green arrows in A and B link the PK and cell state models.

without additional data such assumptions were not made. Cell transfer rates were not altered in the presence of TMZ, and set to the same values used in the control model (see **Table S1**). TMZ not only affects cell proliferation but, in addition, activates apoptosis, and, thus, the cell death rate constants were uniformly accelerated 10-fold in each cell type relative to the control model. TMZ was administered as the standard 150 mg/m^2 daily × 5 schedule every 28 days for 12 cycles. The ODEs for the physiologically based/PK model for TMZ are provided in the **Supplementary Materials**. For all TMZ treatments, the TMZ-induced DNA adducts were linked to the control tumor growth model as follows:

$$\text{effDNAaddGP} = 1 - \left(\left(\frac{\text{DNAadd}}{\text{DNAaddMAX}}\right)^{\gamma_{GP}}\right) \tag{4}$$

$$\text{effDNAaddGS0} = 1 - \left(\left(\frac{\text{DNAadd}}{\text{DNAaddMAX}}\right)^{\gamma_{GS0}}\right) \tag{5}$$

$$\frac{d\text{GP}}{dt} = \text{LgrowGP}^{*}\text{effDNAaddGP} + \sum_{i=\text{GS2},Q} (k_{\text{iGP}} i - k_{\text{GPi}}\text{GP}) - k_{\text{Dead}-TMZ} * \text{GP} \tag{6}$$

$$\frac{\text{dGS0}}{dt} = \text{LgrowGS0}^{*}\text{effDNAaddGS0} + \sum_{i=\text{GP},Q} (k_{\text{iGS0}} i - k_{\text{GS0i}}\text{GS0}) - k_{\text{Dead}-TMZ} * \text{GS0} \tag{7}$$

where two functions, effDNAaddGP and effDNAaddGS0, are used to modify the control logistic growth rates (LgrowGP and LgrowGS0) in each proliferating cell type. These two functions are dependent on the DNA adduct concentrations produced normalized to the maximum possible DNA adduct concentration (DNAaddMAX) that was determined by assuming no DNA repair in one dosing cycle. The ratio of the DNA adducts to the maximum (DNAadd/DNAaddMAX) is raised to an exponent (γ_{GP}, γ_{GS0}) to allow greater flexibility in how the DNA adducts modulate the growth rates in each cell type; however, for the simulations they were set equal to one.

To account for TMZ resistance, the TMZ-sensitive cell proliferation and death functions were modified by semi-empirical time-dependent exponential functions (see **Supplementary Material**). Without additional data to specify resistance mechanisms, the exponential functions for cell proliferation were analogous in both GP and GS0 cells as were the exponential function for cell death applied to GP, Q, and GS0 cells (see **Table S1**). An exponential function to account for TMZ resistance has been previously used.[6]

A number of other assumptions – related to tumor size, compartment volumes, and cell fractions – were applied to set parameter values prior to conducting the simulations (see **Supplementary Material**). All models were developed with MIxplore (version 2016R1) model exploration and visualization program that is part of the Lixoft suite (Antony, France: Lixoft SAS, 2016).

The simulated tumor growth curves for the control, TMZ-sensitive and TMZ-resistant models are shown in **Figure 2a**. In the absence of TMZ, control brain tumor growth increased by about 50% over 1 year that is in agreement with a previous report.[4] The 50% reduction in tumor size, due to TMZ (blue curve, **Figure 2a**), over the 1-year period is consistent for patients with mutant IDH1 brain tumors.[4–6] The TMZ-resistant tumor growth shows an initial decline but, after about 100 days or 4 cycles of TMZ, starts to increase eventually reaching 0.067 L at 1 year or about a 139% increase relative to the TMZ-sensitive case.

CELL STATE MODEL WITH MODULATION

The premise that modulation of cell state can improve drug activity is predicated upon a means to change cell transfer rates among Q, GS cells, and GP cells, as shown in **Figure 1**. For mutant IDH1 brain tumors, we posit that D2HG intracellular concentrations can control cell state by its action on methylation; high D2HG concentrations favor GS cells and block differentiation and low D2HG concentrations favor Q and GP cells and differentiation. D2HG

is an endogenous oncometabolite produced in mutant IDH1 brain tumors. In lieu of specific PK/PD information that would characterize a drug concentration-response relationship between a mutant IDH1 inhibitor and D2HG concentrations, we used trigonometric functions – sine and cosine function – to simulate the time-dependent oscillations in D2HG concentrations that could be obtained through a multiple-dose regimen of a mutant IDH1 inhibitor. The functions are as follows:

$$\text{fD2HG} = M + A * \cos(\Omega * (t - \varphi)) \quad (8)$$

$$\text{gD2HG} = M + A * \sin(\Omega * (t - \alpha)) \quad (9)$$

where
M = amplitude modulator
A = amplitude
Ω = frequency
ϕ = phase shift for cosine function
α = phase shift for sine function

The oscillating functions fD2HG and gD2HG are treated as state variables, and when multiplied by the "base" cell transfer rate constants used in the control, TMZ-sensitive and TMZ-resistant models generate time-dependent cell transfer rates (see **Table S1**). The fD2HG and gD2HG functions are inverse from one another, which allowed the transfer rate constants to cycle in unison so all rate constants favoring differentiation are on the same cycle, whereas the rate constants favoring dedifferentiation are on the opposite cycle (see **Figure S1**). Untreated mutant IDH1 brain tumors are reported to produce D2HG concentrations of around 5 mM,[8] which are at the midpoint of each cycle, and conveniently permit a range between 0 and 10 within the cycle frequency. Mutant IDH1 inhibitors can substantially reduce D2HG tumor concentrations,[8] and, thus, the variations between 0 and 10 over the 7-day period are plausible.

The oscillations in the cell transfer rate constants were applied to both the TMZ-sensitive and TMZ-resistant models, as previously shown in **Figure 2a** (see **Figure 2b**). Except for the oscillations in the cell transfer rates, there were no other changes in any of the model parameters (see **Table S1**). The incorporation of the oscillations in the TMZ-sensitive case had a negligible effect (a decrease of 4%) on tumor growth over 1 year; however, introduction of the oscillations in the TMZ-resistant model produced a pronounced effect on tumor growth, a reduction of 24.7%, over 1 year.

The simulated models are meant to introduce cell state as a variable in drug therapy and to demonstrate within a mathematical framework, rather than a mechanistic one, that tumor growth can be manipulated. Kitano,[9] a forerunner of systems biology, considered a number of strategies to combat and control cancer robustness by decreasing heterogeneity to counteract functional redundancies and feedback loops. These ideas may translate to the current approach in that manipulation of cell state may limit heterogeneity and diversification and prevent durable cell stress responses. Cell stress caused by continued drug exposure initiates a trajectory of cell survival responses that favor the development of mutations and clonal behavior that yields heterogeneous resistant populations.[10] Oscillations in D2HG concentrations, and accordingly cell states, may mitigate the trajectory to drug resistance by limiting mutations and imprinting of cell survival programs, paradoxically through instability.

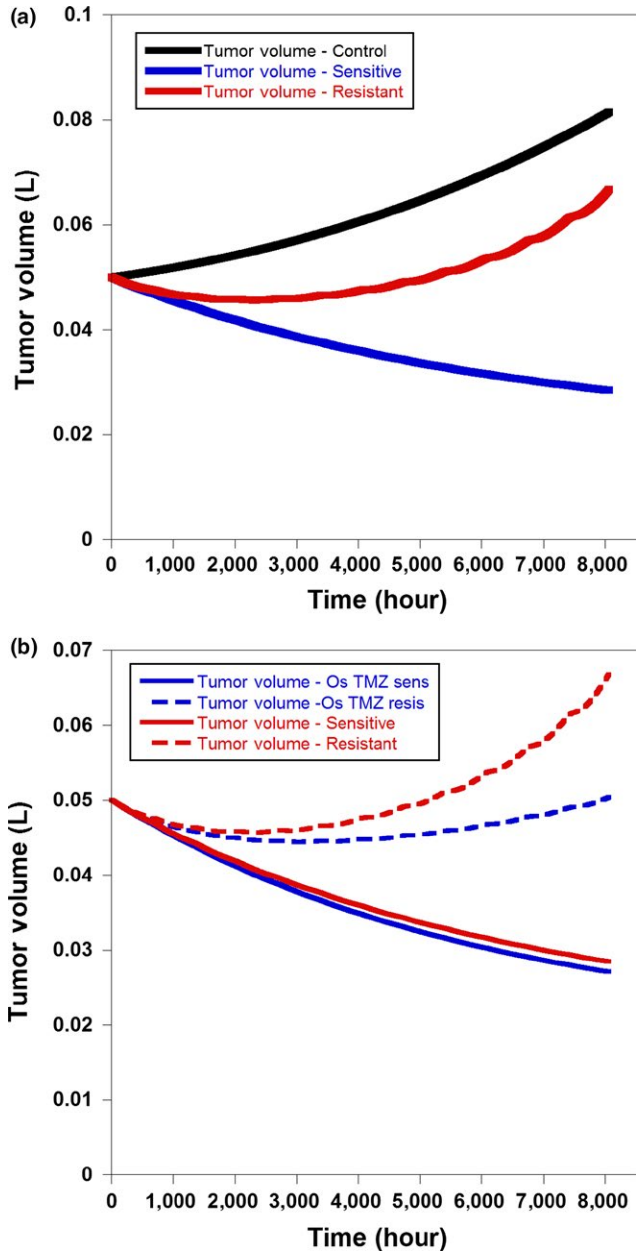

Figure 2 **(a)** Mutant IDH1 brain tumor volume in control (black), temozolomide-sensitive (blue), and temozolomide-resistant (red) conditions. The control and temozolomide (TMZ)-based curves were simulated from the ordinary differential equation (ODE) models described in the text and **Supplementary Material**. **(b)** Mutant IDH1 brain tumor volume in, temozolomide-sensitive (solid red), and temozolomide-resistant (dotted red) and the same with oscillations in cell transfer rate constants (blue) conditions. All tumor volume curves were simulated from the ODE models described in the text and **Supplementary Material**.

CONCLUSIONS

Plasticity among cancer cells is well recognized; however, the focus on the underlying biological control mechanisms have yet to appreciate how such processes may be manipulated for therapeutic advantage. The current simulation results suggest that manipulation of cell state – here using oscillating D2HG concentrations – can reduce tumor growth, and significantly so when resistance to TMZ occurs. Whether these data are mathematical nuances or can be mechanistically supported will require additional studies. Nonetheless, the idea of manipulating cell state is intriguing and may provide a tangible tool to improve drug therapy.

Supplementary Information

Supplementary information accompanies this paper on the *CPT: Pharmacometrics & Systems Pharmacology* website. (www.psp-journal.com)

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