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Review

Revisiting Temozolomide's role in solid tumors: Old is gold?

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Abstract

Temozolomide is an imidazotetrazine with a long history in oncology especially for the high grade malignant glioma and metastatic melanoma. However, last year's new indications for its use are added. Its optimum pharmacodynamic profile, its ability to penetrate the blood-brain barrier, the existence of methylation of MGMT in solid tumors which enhances its efficacy, the identification of new agents that can overcome temozolomide's resistance, the promising role of temozolomide in turning immune cold tumors to hot ones, are leading to expand its use in other solid tumors, giving oncologists an additional tool for the treatment of advanced and aggressive neoplasms.

Keywords: temozolomide; solid tumors

1. Introduction: General principles of action

The chemical formula for temozolomide, also known as CCRG 81045, M & b 39831, SCH 52365, and NSC 362856, is 8-carbamoyl-3-methylimidazo-[5,1-d]-1,2,3,5-tetrazin4(3H)1. The U.S. Food and Drug Administration approved temozolomide (Scheder Corporation's Temodar® capsules) on March 15, 2005, for the concurrent treatment of adult patients with newly diagnosed glioblastoma multiforme (GBM) with radiation therapy and later as a maintenance treatment. In 1999, temozolomide (TMZ) was granted expedited approval for the management of adult patients suffering from refractory anaplastic astrocytoma. The NCCN recommendations also recommend temozolomide for the treatment of advanced or metastatic melanoma (1). The NCCN guidelines state that temozolomide-based therapy is also recommended for the management of advanced pancreatic neuroendocrine tumors. Soft tissue sarcomas with non-specific histologies can also be treated with TMZ as an active single agent. Furthermore, temozolomide activity combined with irinotecan is recognized by NCCN recommendations

as a second-line therapy for Ewing's sarcomas (relapsed/refractory or metastatic disease). Temozolomide is a prodrug that breaks down spontaneously into the reactive intermediate 5-(3-methyl-1-triazeno)imidazole-4-carboxamide (MTIC) in solution at physiological pH. MTIC is an alkylating chemical that methylates adenine at the N3 position as well as guanine at the N7 and O6 positions. A perpetual cycle of DNA base mismatch and repair is brought on by these methyl adducts, culminating in strand breaks and cellular death [2, 3, 4, 5]. It is believed that the most common cytotoxic adduct is methylation of guanine at the O6 site [6, 7]. The primary lesion caused by MTIC methylation of guanine's O6-position mismatches with thymine in double-stranded DNA (O6G-T) during the first cell cycle following therapy. Recurrent GT-mismatches cause mismatch repair, which is induced by this mismatch and can lead to double strand breaks or a secondary lesion. This secondary lesion is a location created by defective mismatch repair, which halts replication and causes sister chromatid exchange, tertiary lesion formation, or other abnormalities [8–12]. Therefore, tumor cells die as a result of tertiary lesions created by improper mismatch repair rather than the main lesions brought on by TMZ. Other methods of action have also been examined and investigated. Two days after treatment, TMZ can cause a prolonged G2-M arrest mediated by p53 and p21WAF1/Cip1, with most cells senescing over ten days, while a small percentage of cells undergo apoptosis. On the other hand, in p53 deficient cells, TMZ causes a transient G2-M arrest along with little alterations in p53 or p21WAF1/Cip1 expression [13]. Both on its own and in conjunction with other compounds, TMZ has a number of benefits. These features include the capacity to penetrate the central nervous system, stability in acidic environments that for total oral absorption, and quick and widespread tissue dispersion. Prodrug of 5-(3-methyltriazen-1-yl)imidazole-4-carboximide [5,14], the active species that methylates DNA (7), TMZ is similar to DTIC. Only hepatic p450 metabolism, which is extremely erratic and changeable, can activate DTIC, although TEM's metabolic activation happens spontaneously and fully at physiological pH [5, 7]. Furthermore, TMZ is effective against a wide range of conditions, such as melanoma, mycosis fungoides, and recurrent high-grade astrocytomas [5, 15–18]. In Mer-human brain tumor xenografts resistant to BCNU (bis-chloroethylnitrosourea), TMZ also exhibits action [19].

In this review we aimed to investigate the uses of temozolomide in solid tumors, excluding melanoma and brain tumors, in which its value is recognized and broadly accepted.

2. Search strategy and selection criteria

The references for this review were found by searching PubMed and PMC between 1980 and November 2023 using the keywords "temozolomide," "temodal," and "solid tumors." Reviewing was limited to papers published in English and French. Originality and relevance to the wide scope of this Review were the guiding principles in the creation of the final reference list. Using the same method, Clinicaltrials.gov's ongoing and completed trials were found (Table 1).

3. How to measure MGMT function

The methylation-specific polymerase chain reaction test (MS-PCR) is used in most investigations on MGMT (methyl-guanine methyl transferase) promoter methylation [20–22]. Using immunohisto-chemistry to determine the MGMT gene's function is an additional approach [23–25]. Nonetheless, there is inconsistent information regarding both MGMT immunoreactivity and MGMT promoter methylation [26–28]. If only FFPE tissue is available, immuno-histochemistry is a more dependable approach than MS-PCR. Nonetheless, there is disagreement on the significance of MGMT-immunoreactivity, particularly in light of its correlation with the methylation state of the MGMT promoter [26–28,29, 30]. Under some circumstances, it has been demonstrated that extensive MGMT promoter methylation correlates with MGMT gene expression [31]. However, a negative MGMT-immunostaining did not correspond with a specific promoter methylation status, which may indicate that MGMT protein expression is not always coupled with MGMT promoter methylation. In addition to promoter methylation, gene deletion or mutation can result in a reduction of protein production, which is one of the several methods of gene silencing that have been reported. Furthermore, as MGMT is an inducible protein [29,32,33], a lack of immunoreactivity at the time of diagnosis may not indicate that the protein has the capacity to operate as intended. Such therapy may be expected to be responsive in tumors with low or no MGMT levels because of MGMT being epigenetically silenced by methylation of CpG islands in the promoter region [20]. Figure 1 shows the frequency of MGMT promoter methylation in various tumor types. Lesions brought on by chemotherapy do not heal and cause cytotoxicity and apoptosis. Numerous investigations have looked into the relationship between the methylation status of the MGMT promoter and how tumors react to alkylating drugs, such as carmustin, lomustine, and temozolomide [20, 21, 34]. After receiving TMZ therapy, patients with methylation

MGMT promoters for glioblastoma multiforme fared better than those without such a promoter. This supports the theory that the tumor's vulnerability to alkylating drugs is correlated with MGMT inactivation caused by aberrant promoter methylation [34].

Table 1. Trials with Temozolomide combinations in solid tumors.

INVESTIGATORS	CLINICAL TRIAL ID	ENROLLING PATIENTS (E) OR COMPLETED (C)	COMBINED MOLECULE	PHASE	TYPE OF STUDY	ENDPOINT	CHILDREN (UNTIL 18Y) (C) OR ADULT (A)	NEOPLASM	TEMODAL DOSE AND SCHEDULE
Bhardwaj Dessai, MD,	NCT01193140	C	VELIPARIB	2	Non-Randomized, Open Labeled	SAFETY	A	SOLID TUMORS	Dose orally once daily for 5 days, consecutively, every cycle
Jeffrey A. Sosman, MD	NCT00512798	C	BORTEZOMIB	1,2	Non-Randomized, Open Label, Single Group Assignment	DOSE AND EFFICACY	A	SOLID TUMORS, MELANOMA	PHASE 1: 50, 75mg/m2 PHASE 2: 75 mg/m2 po daily, during weeks 2-8 (42 days) of every 9-week course.
Matthew Taylor, MD, Antonio Omuro, MD	NCT01107522	E	carboxyamidotriazole orotate (CTO)	1	Interventional	SAFETY, TOLERABILITY, AND THE MAXIMUM TOLERATED DOSE/RECOMMENDED PHASE II DOSE	A	Glioblastoma, Recurrent Malignant Gliomas,Solid Tumors	ARM B: orally at fixed dose of 150 mg/m2 daily for Days 1-5 /28dARM C: po 75 mg/m2/d during RT, then at 150mg/m2 for 1-5 d of Cycle 1, and then up to 200 mg/m2 1-5d of subsequent cycles
Jennifer Eads, MD	NCT00892385	E	METHOXYAMINE	1	Interventional, Open Label, Single Group Assignment	SAFETY/EFFICACY	A	Advanced solid tumours	Tem per os/d 1-5d. every 28 days
Rochelle Bagatell	NCT01141244	C	TEMSIROLIMUS, IRINOTECAN	1	Interventional, Open Label, Single Group Assignment	SAFETY	C	RECURRENT OR REFRACTORY SOLID TUMOURS	temsirolimus IV over 30 minutes on days 1 and 8 or on days 1, 8, and 15 and temozolomide PO and irinotecan hydrochloride PO on days 1-5
Shivaani Kummar, M.D.	NCT01851369	E	TRC102	1	Interventional, Open Label, Single Group Assignment, Non-randomized	SAFETY, EFFICACY	A	ADVANCED SOLID TUMORS OR LYMPHOMAS	TEM po 1-5days
Jana Portnow, MD	NCT00544284	C	BORTEZOMIB	1	Interventional, Open Label, Single Group Assignment, Non-randomized	SAFETY	A	REFRACTORY SOLID TUMORS, BRAIN CNS TUMORS, LYMPHOMA	TEM po 1-5days
Zev Wainberg	NCT02049593	E	(PARP) inhibitor BMN-673, OR IRINOTECAN ALONE	1	Interventional, Open Label, Non-randomized	SAFETY, DOSE ESCALATION	A	ADVANCED SOLID TUMORS	TEM po 1-5days
Lars M. Wagner, MD, John P. Perentesis, MD	NCT00138216	C	VINCRISTINE, IRINOTECAN	1	Interventional, Open Label, Single Group Assignment	SAFETY, DOSE ESCALATION	C & A TILL 21Y	BRAIN AND CNS TUMORS, SOLID TUMORS	TEM po 1-5days
Eisai Medical Services	NCT01127178	C	(PARP) Inhibitor E7016	1	Interventional, Open Label, Single Group Assignment	SAFETY, DOSE ESCALATION	A	ADVANCED SOLID TUMORS AND GLOMAS	TEM po 1-5days
Pamela Z New, M.D.	NCT01736800	E	TOPOTECAN	2	Interventional, Open Label, Single Group Assignment	SAFETY/EFFICACY	A	SOLID TUMORS WITH CNS METASTASES	TEM po 1-5days
Lionel D. Lewis, MD	NCT00014261	E	PEG-interferon alfa-2B	1	Interventional	EFFICACY, DOSE ESCALATION	A	Refractory And/Or Advanced Solid tumors	TEM on days 1-7 and 15-21
Elizabeth Fox, MD, Holly Meany, MD	NCT00303940	C	TALABOSTAT	1	Interventional	SAFETY, DOSE ESCALATION	C	RELAPSED OR REFRACTORY BRAIN TUMORS, SOLID TUMORS	TEM po 1-5days
Katherine Warren, MD	NCT00020150	C	O6-benzylguanine	1	Interventional	EFFICACY	C & A (UP TO 21Y)	SOLID TUMORS	TEM po 1-5days
Damon Reed, M.D., Jonathan Gill, M.D.	NCT01528046	E	Metformin, Irinotecan, Vincristine	1	Interventional, Open Label, Single Group Assignment	SAFETY/EFFICACY	C	RECCURENT REFRACTORY SOLID TUMORS	TEM:100 mg/m^2/day PO Days 1-5
Rajkumar Venkatramani, MD	NCT00993044	C	Irinotecan, Vincristine, Bevacizumab	1	Interventional, Open Label, Single Group Assignment	SAFETY	C	REFRACTORY SOLID TUMORS	TEM:100 mg/m^2/day PO Days 1-5
Eli Lilly and Company	NCT01284335	C	LY573636-sodium	1	Interventional, Open Label,Non-Randomized	SAFETY, EFFICACY	A	ADVANCED SOLID TUMORS	200 mg/m2 administered orally on days 1-5 of a 28 day cycle
Brian Turpin,	NCT00786669	C	Bevacizumab,vincri	1,2	Interventional, Open	SAFETY,	C & A (UP TO	RELAPSED OR	00 mg/m2/day po on

INVESTIGATORS	CLINICAL TRIAL ID	ENROLLING PATIENTS (E) OR COMPLETED (C)	COMBINED MOLECULE	PHASE	TYPE OF STUDY	ENDPOINT	CHILDREN (UNTIL 18Y) (C) OR ADULT (A)	NEOPLASM	TEMODAL DOSE AND SCHEDULE
D.O.			stine, irinotecan		Label, Single Group Assignment	EFFICACY	30Y)	REFRASCTORY SOLID TUMORS	Days 1-5 every 3 weeks for up to 6 cycles
Sponsor's Medical Expert, MD	NCT00920595	C	CEP-9722	1	Interventional, Open Label, Single Group Assignment	SAFETY, EFFICACY	A	ADVANCED SOLID TUMORS	TEMO 150 mg/m2/day on Days 1-5
Regina Jakacki	NCT00077454	C	ERLOTINIB	1	Interventional, Open Label, Single Group Assignment	SAFETY	A	RECCURENT/REFRACTORY SOLID TUMORS	TEM po 1-5days
Judith M. Ford, MD, PhD	NCT00012116	C	NO	2	Interventional, Open Label, Single Group Assignment	EFFICACY	A	ADVANCED SOLID TUMORS WITH BRAIN METS	once a day for 6 weeks followed by 4 weeks of rest/ Daily dose: 75mg/m2.
Eric Schafer	NCT02116777	C	PARP INHIBITOR BMN-673	1,2	Interventional, Open Label, Single Group Assignment	SAFETY, EFFICACY, DOSE ESCALATION	C& A (UP TO 30 Y)	REFRACTORY OR RECURRENT MALIGNANCIES	TEMO PO QD on days 2-6/28days
Stanton L. Gerson, MD	NCT00003567	C	Mutant MGMT Gene Transfer Into Human Hematopoietic Progenitors, O6-Benzylguanine, carmustine	1	Interventional, Open Label, Single Group Assignment	SAFETY, EFFICACY	A	ADVANCED SOLID TUMORS-NON-HODGKIN LYMPHOMAS	Four weeks after the completion of BG and carmustine, patients receive TEMO IV over 1 hour every 4 weeks for up to 5 courses,
Cynthia E. Herzog, MD	NCT00492141	C	liposomal 9-Nitro-20-(S)-Camptothecin (L9-NC) by aerosol	1,2	Interventional, Open Label, Single Group Assignment/ Non-Randomized	SAFETY, EFFICACY	A	EWING'S SARCOMA AND SOLID TUMORS INVOLVING THE LUNG	100 mg/m^2 oral/day for Cycle 2 Days 1-5.
Merck Sharp & Dohme Corp.	NCT00960063	C	SCH 717454, Irinotecan	1/1B	Non-randomized, open-label, dose-escalation study	SAFETY	C&A (UP TO 21Y)	SOLID TUMORS	TEMO 100 mg/m2/day on Days 1-5
Henry S. Friedman, MD	NCT00005952	C	given with peripheral stem cell transplantation	1,2	Interventional	SAFETY, DOSE ESCALATION	C	MALIGNANT GLIOMAS, RECURRENT CNS TUMORS, SOLID TUMORS	oral temozolomide daily for 5 consecutive days
Ruth Plummer, Prof	NCT01618136	E	Polymerase (PARP) Inhibitor E7449	1,2	Non-randomized, open-label, dose-escalation study	SAFETY, DOSE ESCALATION, EFFICACY	A	ADVANCED SOLID TUMORS, OR B-CELL MALIGNANCIES	150 mg/m2/d TMZ administered orally, once daily for 5 days /28d
Volker W. Stieber, MD	NCT00049361	C	WBRT and Thalidomide	2	Interventional, Open Label	EFFICACY	A	SOLID TUMORS WITH BRAIN METS	Beginning on the day before the first radiation treatment, patients receive oral thalidomide once daily and oral temozolomide once daily for 21 days.
Thomas H. Davis, MD	NCT00005812	C	NO	2	Intereventional, Open Label, Single Group Assignment	SAFETY/EFFICACY	A	LEPTOMENINGEAL METASTASES FROM SOLID TUMORS OR LYMPHOMA	Oral temozolomide 75 mg/m2/day for 6 weeks, followed by 4 week break
William H Meyer, MD	NCT00222443	C	IRINOTECAN, VINCRISTINE, VANTIN	1	Intereventional, Open Label, Parallel Assignment, Non-Randomized	SAFETY/EFFICACY	C&A (up TO 21Y)	RECURRENT SOLID TUMORS OR LYMPHOMAS	Temozolomide is given by mouth one hour prior to each daily irinotecan dose days 1-5 of each cycle. 100 mg/m2/day.
Robert Bukowski	NCT00401180	C	DOCETAXEL	1	Intereventional, Open Label, Single Group Assignment	SAFETY, DOSE ESCALATION	A	METASTATIC SOLID TUMORS	orally daily for 3 weeks (escalating doses of 75 to 100 mg/m(2))
Bhardwaj Desai, MD	NCT00526617	C	ABT-888	1	Intereventional, Open Label, Single Group Assignment	SAFETY	A	SOLID TUMORS, etastatic melanoma (MM), BRCA deficient breast, ovarian, primary peritoneal, or fallopian tube cancer, and hepatocellular carcinoma (HCC).	-
Sanofi	NCT00422682	C	BSI-201	1B	Intereventional, Open Label, Parallel Assignment	SAFETY/EFFICACY	A	ADVANCED SOLID TUMORS	-
Merck Sharp & Dohme Corp.	NCT01294735	C	MK-4827	1	Intereventional, Open Label, Parallel Assignment	SAFETY/EFFICACY	A	ADVANCED CANCER	-

INVESTIGATORS	CLINICAL TRIAL ID	ENROLLING PATIENTS (E) OR COMPLETED (C)	COMBINED MOLECULE	PHASE	TYPE OF STUDY	ENDPOINT	CHILDREN (UNTIL 18Y) (C) OR ADULT (A)	NEOPLASM	TEMODAL DOSE AND SCHEDULE
Shivaani Kummar, M.D	NCT01827384	E	OR EVEROLIMUS OR CARBOPLATIN OR TRAMETINIB DMSO OR ABT-888 OR MK-1775	2	Intereventional, Open Label, Parallel Assignment, Non-Randomized	EFFICACY	A	ADVANCED SOLID TUMORS	-
Fa-Chyi Lee, MD	NCT00249964	C	PACLITAXEL, CARBOPLATIN	1	Intereventional, Open Label, Single Group Assignment, Non-Randomized	EFFICACY, DOSE ESCALATION	A	SOLID TUMORS	starting dose of temozolomide at 75 mg/m2/day from day 2 to day 6, a total of 5 days/21days
Sajeel Chowdhary, MD, Jade Homsi, MD	NCT00437957	C	VALPROIC ACID, RT	1	Intereventional, Open Label, Single Group Assignment	SAFETY/EFFICACY	A	SOLID TUMORS WITH BRAIN METS	75 Mg/m2/day for all Cohorts
Michael J Pishvaian, MD, PhD	NCT01051596	C	ABT-888	1	Open Label, Single Group Assignment, Non-Randomized	EFFICACY	A	ADVANCED COLORECTAL CANCER	150 mg/m2 once a day on Days 1-5 of each 28-day cycle
Pamela Kunz	NCT01824875	E	CAPECITABINE	2	Interventional, Randomized, Parallel Assignement, Open Label	EFFICACY	A	ADVANCED PANCREATIC NEUROENDOCRINE TUMORS	ARM A:TEMO PO QD on days 1-5./28d, ARM B: TEMO PO QD on days 10-14/28d
Suman Malempati	NCT01055314	C	ETOPOSIDE, VINCRISTINE, IRINOTECAN, IFOSFAMIDE, DOXORUBICIN, CYCLOPHOSHAMIDE, DACTINOMYCIN, RT	1	Interventional, Randomized, Open Label, Parallel Assignment	SAFETY/EFFICACY	C& A (up to 49 y)	METASTATIC RHABDOMYOSARCOMA	TEMO PO on days 1-5 of weeks 1, 4, 20, 23, 47, and 50.
Morris D. Groves, MD	NCT00515788	C	intrathecal liposomal cytarabine (DepoCyt)	1	Intereventional, Open Label, Single Group Assignment	SAFETY/EFFICACY	A	SOLID TUMORS, LYMPHOMA WITH NEOPLASTIC MENINGITIS	100 mg/m2 po daily for 7 days every 14 days.
Philipp Hoffmanns, MD, PhD	NCT02231762	E	Lanreotide Autogel 120 mg	1	Intereventional, Open Label, Single Group Assignment	EFFICACY	A	Progressive Gastro-entero-pancreatic Neuroendocrine Tumours (GEP-NET) G1/G2	TEMO PO 150 mg/m2 per day for 5 days in the first month. 200 mg/m2 per day for 5 days in months 2, 3, 4, 5 and 6.
Carlos Gamboa-Vignolle, MD	NCT01015534	C	WBRT	2	Interventional, Randomized, Parallel Assignement, Open Label	EFFICACY	A	SOLID TUMORS WITH BRAIN METS	1h before each fraction of whole brain irradiation, 200 mg on Monday, Wednesday, Friday; 300 mg on Tuesday, and Thursday.
Merck Sharp & Dohme Corp	NCT00034697	C	RT	2	Double-blind Interventional, Randomized, Parallel Assignement, Open Label	SAFETY/EFFICACY	A	NSCLC WITH BRAIN METS	-
Hoffmann-La Roche	NCT00811993	C	R1507	1	Interventional, Randomized, Parallel Assignement, Open Label	SAFETY	A	ADVANCED MALGINANT NEOPLASMS	AS PRESCRIBED
Oana C Danciu, M.D	NCT03332355	C	PAC-1	1	Interventional Single group assignment	SAFETY, DOSE ESCALATION	A	ADVANCED SOLID TUMOR OR HEMATOLOGIC MALIGNANCY (LIMITED TO LYMPHOMA)	150 MG/M2 DOSE OF TEMOZOLOMIDE GIVEN FOR THE 5 DAYS STARTING AT DAY 8 OF CYCLE 1
Wen-Jen Hwu, MD, PhD,	NCT00005815	C	Thalidomide	1,2	Interventional	SAFETY, DOSE ESCALATION	A	METASTATIC MALIGNANT MELANOMA THAT IS CONSIDERED UNRESECTABLE STAGE III OR IV OCULAR, MUCOSAL, OR CUTANEOUS MELANOMA	ESCALATING DOSES OF TEMOZOLOMIDE UNTIL THE MAXIMUM TOLERATED DOSE (MTD) IS DETERMINED
Novartis Pharmaceuticals	NCT05429502	E	Ribociclib (LEE011) in Combination With Topotecan	1,2	Randomized parallel assignment	SAFETY/EFFICACY	C&A (up TO 21Y)	Neuroblastoma, Medulloblastoma, High-grade glioma, Malignant rhabdoid tumor,	TEMOZOLAMIDE ADMINISTERED AT THE STANDARD DOSE GIVEN TO NEUROBLASTOMA PATIENTS

INVESTIGATORS	CLINICAL TRIAL ID	ENROLLING PATIENTS (E) OR COMPLETED (C)	COMBINED MOLECULE	PHASE	TYPE OF STUDY	ENDPOINT	CHILDREN (UNTIL 18Y) (C) OR ADULT (A)	NEOPLASM	TEMODAL DOSE AND SCHEDULE
								Rhabdomyosarcoma	
María Angeles Vaz, M.D	NCT03466450	E	Glasdegib (SHH pathway inhibitor)	1,2	Phase Ib/II, multicentric, non-randomized, open label	SAFETY/EFFICACY	A	GBM	TMZ at 75 mg/m2 /d concurrently with RT for a maximum of 42 days. At 4 weeks after RT completion, patients will start taking TMZ at 150 mg/m2/d for the first 5 days of a 28-day cycle. If first cycle is well tolerated, patients will receive TMZ at 200 mg/m2/d for the first 5 days of every subsequent 28-day cycle for another 5 cycles.

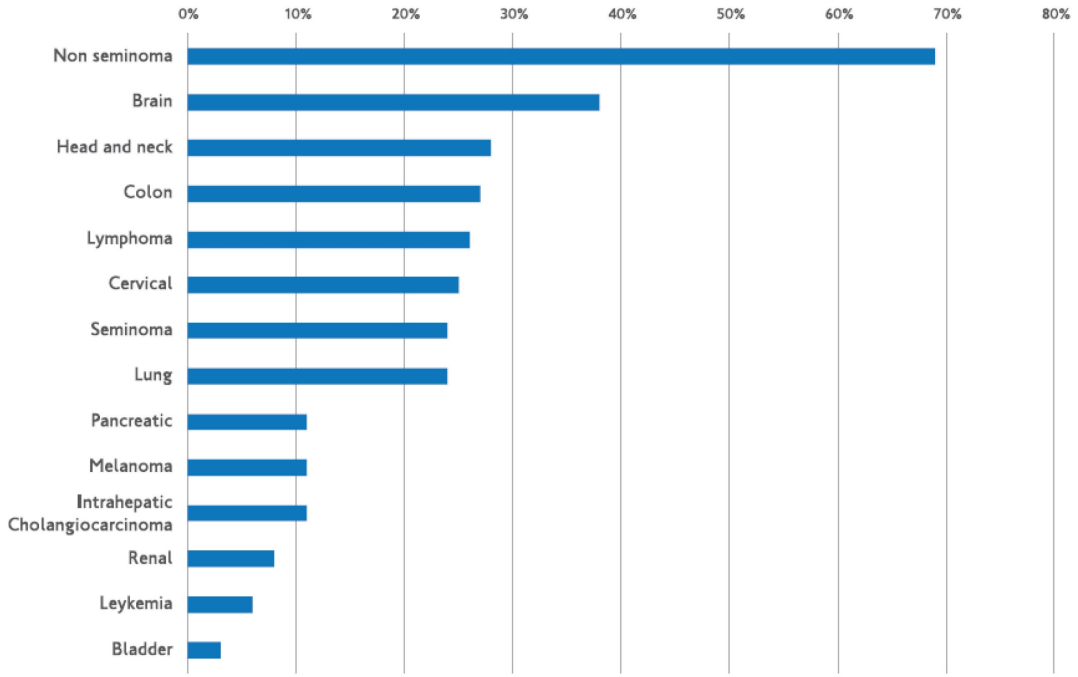

Figure 1. Frequency of MGMT promoter methylation in different solid tumors. Markus Christmann *et al.* Bernd Kaina O(6)-Methylguanine-DNA methyltransferase (MGMT) in normal tissues and tumors: enzyme activity, promoter methylation and immunohistochemistry Biochim Biophys Acta. 2011 Dec;1816(2):179-90.

4. Progress in methods of detection of MGMT promoter methylation status

While methylation-specific PCR, pyroseq-uencing, or methylation arrays are recommended for the detection of MGMT promoter methylation, the European Society of Medical Oncology no longer recommends immunohistochemistry, despite the fact that it was once the basis method for MGMT methylation detections [35, 36]. On the other hand, pyrosequence can be very informative in evaluating the proportion of MGMT methylation, which can predict the volume response and prognosis of patients with residual GBM [37–39,40]. It is recommended to use a biological cutoff of 10% or 21% of the receiver operating characteristic. Other approaches that show promise for MGMT promoter methylation detection include endonuclease-resistant DNA methylation quantification, Lab on Chip compatible isothermal amplification, and two probe quantification of MSB [40–42]. In terms of defining the ideal cutoff, research indicates that, for CpG sites 74–78, a cutoff of 9% is preferable to a higher cutoff of 28% or 29% [38]. Furthermore, it appears that a PSQ score of 10% for MGMT promoter methylation can classify patients into a "transition zone" or "gray area" since it may increase their susceptibility to TMZ treatment [43]. Additionally, advances in radiomics techniques are being made in an effort to provide a noninvasive, preoperative method of MGMT promoter methylation detection [44–49]. Ultimately, significant advance-ments have also been made in the examination of the

MGMT methylation status in peripheral blood and cerebral fluid [50–51].

5. Resistance to temozolomide

The DNA repair protein O6-alkylguanine-DNA alkyltransferase (AGT), which eliminates the methyl adduct from the O6 position of guanine, mediates resistance to temozolomide. By acting as a substrate and moving its benzyl group to the active site of AGT, O6-benzylguanine (O6BG), a modifying agent, inactivates and depletes AGT. For TMZ-induced methylating cytotoxicity to occur, an MMR repair mechanism must be functional. A weak MMR pathway will cause the alkylation damage to be tolerated. Melanomas are less common than brain malignancies in this regard. Furthermore, as observed in melanomas with bcl-2 overexpression, tumor cells' capacity to evade apoptosis is another element that may contribute to temozolomide resistance [52]. In a study looking at glioblastoma chemoresistance, Beier et al. came to the conclusion that MGMT protein expression is linked to a high level of TMZ resistance in cancer stem cells (CSC). Furthermore, the authors observed that neurosphere-forming cells lacking MGMT expression were vulnerable to TMZ when examined in depth. Additionally, they discovered that inconsistent experimental outcomes could arise from varying TMZ timings and dosages. Additionally, they noted that environmental conditions, such as hypoxia in the glioblastoma's core, could be a component in the CSC's resistance to TMZ. They came to the conclusion that TMZ resistance is impeded by a number of signaling pathways, including those of Shh, IGF-1/PI-3 kinase, NOTCH, and STAT3 [53]. For TMZ to induce harmful double strand breaks, the mismatch repair system must be functional. Therefore, TMZ resistance was mediated by changes in the main, critical component of the mismatch repair mechanism, mutS homolog 6 (MSH6), particularly in recurrent GBM following TMZ-based radiochemotherapy [54, 55]. A fraction of GBM recurrences following radiation therapy and TMZ treatment had inactivated mutations in the mismatch repair gene MSH6, which results in the loss of MSH6 immunostaining. During TMZ treatment, loss of MSH6 was associated with tumor development [55, 56]. TMZ resistance was linked to MSH6 inactivation and mutation in GBMs after TMZ therapy, both in vitro and in vivo [55]. The double strand breaks that cause cell death are inhibited by a well-established resistance mechanism. Mutations affecting the apoptotic cascade, which carries out double stranded break-induced apoptosis, as well as p53 and Poly(ADP-ribose)polymerase (PARP) signaling, are among the signaling cascades involved [57,58]. Glutathione-S-transferase is a protein that contributes to chemoresistance but is not as well understood. Figure 2 shows methods for overcoming the resistance to temozolomide.

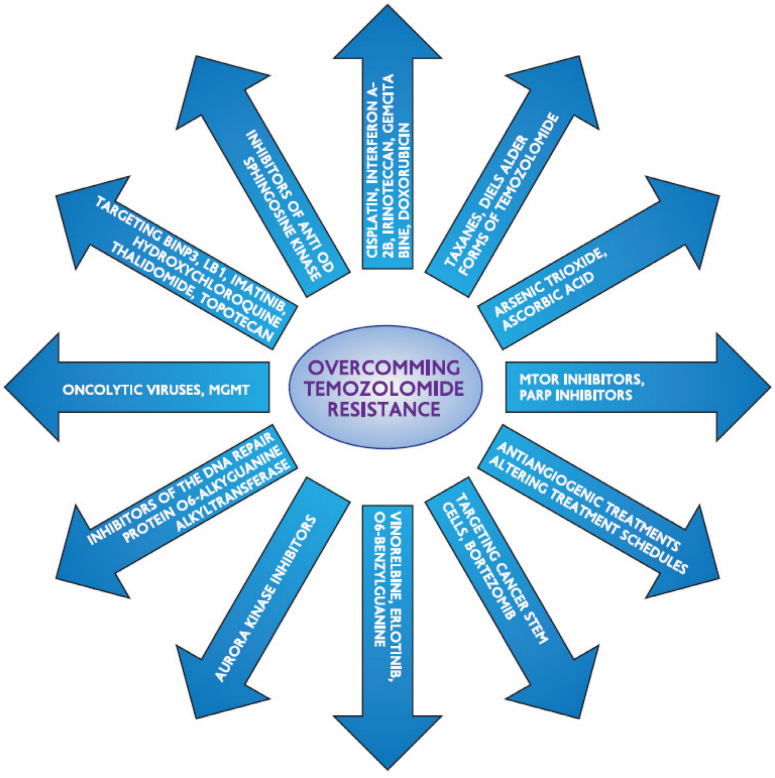

Figure 2. Strategies to overcome temozolomide's resistance.

6. Temozolomide's efficacy in solid tumors other than brain and melanoma

6.1. Colorectal cancer

The removal of alkyl groups from guanine's O6-position is carried out by the DNA repair gene O6-methylguanine-DNA methyltransferase (MGMT). If dormant, it could contribute to the initial stages of colorectal cancer by raising the rate of mutations, especially G-to-A point mutations in the KRAS gene [59, 60]. The MGMT-encoded protein fixes DNA damage caused by alkylating chemicals in a variety of tumor types [61,62]. Hypermethylation of CpG island in MGMT promoter is linked to epigenetic silencing of MGMT during colorectal carcinogenesis [63]. Reduced DNA-repair of O6-alkylguanine adducts is caused by this transcriptional gene suppression, which increases chemosensitivity to alkylating drugs, especially dacarbazine and its oral prodrug temozolomide [64]. 32 patients with advanced chemorefractory colorectal cancer with MGMT promoter methylation were enrolled in our phase 2 research. In 4-week cycles, the patients received treatment with TMZ at a dose of 150 mg/m2/day for 5 days in a row. The course of treatment was followed until the condition worsened, or at least six cycles. At 12%, the objective response rate reached the pre-established threshold for activity that showed promise. There was a median of 1.8 months for progression-free survival and 8.4 months for overall survival. When compared to patients with any RAS or BRAF mutation, those with KRAS, BRAF, and NRAS wild-type CRC demonstrated a significantly greater response (44% versus 0%; P = 0.004) (65). In order to investigate the effectiveness of TMZ in conjunction with pegylated liposomal doxorubicin for the treatment of brain metastases from different solid tumors, Caraglia et al. carried out a phase 2 trial. A total of 36.8% (95% CI:19.1–59.2) of the 19 patients in the cohort had a complete response (CR), four had a partial response (PR), and three had a complete response (CR). This response rate exceeded the research design's target activity. The primary diagnosis in three cases was colorectal cancer. Of them, two (67%) reported giving only a partial answer [66]. The idea of using temozolomide as an induction treatment that sensitizes patients with MSS and MGMT-silenced CRC to later use of immunotherapy was supported by three trials by Gonzalez et al., the MAYA trial, and the Arethusa trial, as we will analyze later in our review. This opened the door for a strategy that turns immune cold tumors into hot ones [67–69].

6.2. Neuro-endocrine tumors, melanoma

Temozolomide was prescribed to patients with malignant endocrine tumors because to the comparable mechanisms of action between dacarbazine and TMZ. In mice, TMZ has been shown to be less harmful than dacarbazine [5]. There was no significant difference in the safety of the drugs between TMZ and dacarbazine in a randomized phase III research conducted on patients with advanced metastatic melanoma [1]. After treatment with oral TMZ, there was a greater systemic exposure (area under the curve) to the parent drug and its active metabolite, 5-(3-methyl-triazeno)imidazole-4-carboximide, compared to dacarbazine administered intravenously. The most frequent toxicities associated with TMZ were mild to severe nausea and vomiting that could be treated with ease, as well as a noncumulative temporary myelosuppression [1]. Additionally, patients' health-related quality of life was enhanced with TMZ therapy. Furthermore, it has been demonstrated that TMZ is effective in glioblastoma patients and enhances survival when combined with radiation therapy in this context [70]. Interesting outcomes of TMZ in patients with endocrine malignancies were documented in two investigations [71, 72]. The effectiveness and safety of TMZ in treating patients with malignant digestive endocrine tumors were evaluated by Mairie et al. TMZ given at doses of 200 mg/m2 daily for 5 days every 28 days resulted in the disease stabilization of 81% of patients in their cohort of 21 patients with metastatic well-differentiated digestive endocrine tumors [73]. In a phase II trial, the combination of thalidomide plus TMZ was found to have an overall radiologic response rate of 25% over a median of 13.5 months for the treatment of metastatic neuroendocrine tumors [71]. A 2006 American Society of Clinical Oncology meeting abstract featured a retrospective analysis of TMZ and capecitabine combined treatment for pancreatic neuroendocrine tumors. The study found that a median of 9.5 months was experienced by 6% of patients who experienced a full response and 53% who experienced a partial response [74]. A phase II study with TMZ plus bevacizumab, presented at the same meeting, revealed an overall response rate of 14% [75]. The lack of benefit from this treatment in some NETs, and in carcinoids specifically, may be explained by the dependency of TMZ response on poor MGMT expression. Kulke et al. evaluated 76 patients who were getting temozolomide-based therapy in a retrospective manner. About 33% of patients with pancreatic NETs (11/35 patients) had a radiographic response (determined by RECIST criteria), but 0% of patients with carcinoid tumors

(0/38) had a radiographic response (P<0.001). Complete lack of MGMT expression appeared to characterize patients with pancreatic NET (5/8 pancreatic NET and 0/13 carcinoid tumors) who benefit significantly from temozolomide in 21 available specimens [76]. Ekeblad et al. looked at 36 patients with advanced neuroendocrine tumors to see if TMZ was effective. Of the patients, 14% had a radiologic response, while 53% had stable illness [72]. Hirohata et al. investigated the function of DNA mismatch repair protein (MSH6) as a response biomarker in patients receiving TMZ treatment for pituitary cancer and atypical pituitary adenomas. They discovered a positive correlation between the TMZ response and the immunopositivity of MSH6 [77]. Based on its method of action and advantageous toxicity profile, the CAPTEM regimen is currently frequently utilized in clinical practice, particularly for G2-G3 NETs. [78,79]. Only metastatic or unresactable GEP-NENs G3 with a Ki-67 >20% and <55% treated with CAPTEM were included in a recent single-arm phase II trial. The results indicated a significant improvement in PFS and OS in NETs compared to NEC (9.3 months versus 3.5 months, P = 0.005, not reached versus 6.2 months, P = 0.004). Furthermore, CAPTEM is the recommended course of action for patients with well-differentiated G3 NETs, as evidenced by the decreased ORR (14.3% versus 34.8%, P = 0.393) and DCR (42.9% against 87.0%, P = 0.033) in NEC patients compared to NETs G (31). In 144 patients with advanced low or intermediate grade pNETs, one of the most recent randomized phase II trials (E2211) compared temozolomide monotherapy to CAPTEM, establishing CAPTEM as the standard chemotherapy for advanced pNETs. Despite the absence of a statistically significant difference in ORR (33.3% for CAPTEM vs. 27.8% for TEM, p = 0.47) between the two treatment modalities, the combination was linked to a considerably longer median PFS (22.7 vs. 14.4 months) than TEM monotherapy [81]. It is noteworthy that the ORR for NENs treated with CAPTEM was greater than the ORR for the majority of licensed therapies (≈ 30%). The best order of treatment is still up for debate because there hasn't been a prospective, randomized clinical trial contrasting CAPTEM with single-agent tyrosine kinase inhibitors [82–84]. In a recent review, Arrivi et al. draw the conclusion that, while pNETs have more robust efficacy data, which has led to the widespread adoption of the CAPTEM regimen in cancers of the pancreas, CAPTEM appears to be a safe and effective treatment for patients with advanced well-moderately differentiated NENs of the gastrointestinal tract, the lung, and those of unknown origin [85].

6.3. Breast Cancer

Because temozolomide is an oral medication that can pass across the blood-brain barrier and has been effective in treating other tumor sites, it was an intriguing chemical to investigate for the treatment of metastatic breast cancer. Furthermore, as a cytotoxic alkylating agent it is chemically different compared to other drugs used to treat breast cancer. Due to these factors, the NCIC – Clinical Trials Group looked into TMZ's effectiveness in treating women whose breast cancer had spread and had previously received chemotherapy. To increase the likelihood of a response, a treatment plan of 150 mg/m2 on days 1-4 every two weeks (normal doses [86] every two weeks instead of every four weeks) was selected. Other phase II studies have investigated the activity of TMZ in patients with brain metastases, including those secondary to breast cancer [87–89]. It has been demonstrated that TMZ in conjunction with cisplatin (CDPP), which decreases the DNA repair enzyme MGMT similarly to temozolomide, causes partial responses (PR) in breast cancer patients' extracranial and brain regions [87]. In a phase II trial run by the Hellenic Cooperative Oncology Group, six out of fifteen women who were included achieved partial remission (PR) by using 150–200 mg/m2 on days 1–5 every 28 days with 75 mg/m2 of CDDP on day 1, including four patients who had progressed after receiving whole brain radiation therapy in the past. The same group's earlier phase II research [88], which assessed TMZ alone, was unable to show any improvements in patients with breast cancer. In a third research, 10 patients with breast cancer exhibited no response, whereas 4 individuals had stable brain disease for 8 weeks [89]. The first investigation of single-agent TMZ in patients with breast cancer is this phase II trial. In order to ascertain the effectiveness and toxicity of TMZ in patients with metastatic breast cancer, Trudeau et al. carried out a phase 2 research in which a cohort of nineteen patients was administered a dosage dense regimen of 150 mg/m2 on days 1–7 and 15–21 in a 28-day cycle. These people with severely pretreated metastatic breast cancer, including brain metastases, did not show any response to TMZ [90]. Hoffman et al. described the cases of two women with diffuse CNS metastases from breast cancer. Following irradiation of the symptomatic areas, TMZ 100 mg/m2 day 1–5/7 was administered in combination with intrathecal liposomal Ara–C every 2-4 weeks. Both patients' neurological symptoms and cerebrospinal fluid (CSF) cytology improved and stabilized over several months. After diagnosis, the patients lived for 10 and 17 months respectively, showing no symptoms of brain damage [91]. The results of a phase I clinical trial

in a cohort of women with metastatic HER2+ breast cancer to the brain following treatment with SRS or WBRT were published by Jenkins et al. in a relatively recent paper. Subsequently, the patients were administered a low-dose metronomic temozolomide together with an appropriate HER2-targeted systemic drug, T-DM1, to prevent brain metastases. Toxicities were mostly of low grade. Out of twelve patients, only two experienced new parenchymal brain metastases after an average follow-up of 9.6 months. The administration of temozolomide for the secondary prevention of brain metastases is supported for the first time by this trial [92].

6.4. Lung Cancer

Since temozolomide may pass across the blood-brain barrier in both animal and human models, it has demonstrated efficacy against brain metastases from a range of solid cancers, including NSCLC [88,89] [93]. Moreover, TMZ has demonstrated some efficacy in treating NSCLC as a second-line treatment [94]. Brain metastases are relatively common in NSCLC patients—nearly 20% at diagnosis and 40% at autopsy [95, 96]. Since TMZ may be able to treat or prevent brain metastases, it may be a great option for these individuals. In a group of 31 NSCLC patients who had previously received treatment, Kouroussis et al. investigated the effectiveness of TMZ. Three patients (10%) had stable illness, and two patients (6.5%; 95% CI: -2.2 to 15.1%) had a partial response. The 1-year survival rate was 22.5%, the median survival time was 3.3 months, and the median time to progression was 2.4 months [97]. TMZ did not exhibit any effect in NSCLC patients with or without brain metastases in an EORTC phase II investigation [98]. Research on TMZ in patients with small cell lung cancer (SCLC) has a solid history. In SCLC, alkylating drugs are effective when used alone [99]. Brain metastases are prevalent in this condition, and TMZ passes the blood–brain barrier [100]. MGMT is abnormally methylated in SCLC [64, 101]. Lastly, SCLC has reported anecdotal reactions to TMZ [102]. In order to find out how effective TMZ was for patients with relapsed sensitive or refractory small cell lung cancer, Pietanza et al. carried out a phase II research. After one or two previous chemotherapy regimens, patients with disease progression were given TMZ at a dose of 75 mg/m2/d for 21 days within a 28-day cycle. In susceptible individuals, there was one CR and ten PRs [ORR, 23%; 95% confidence interval (CI), 12%–37%]. In the refractory cohort, two PRs were seen (ORR, 13%; 95% CI, 2%–38%). The ORR for second and third-line treatments, respectively, was 19% (95% CI, 7%–36%) and 22% (95% CI, 9%–40%). A CR or PR was present in 38% of patients with target brain lesions (95% CI: 14%–68%). In comparison to patients with unmethylated MGMT, a higher proportion of methylated MGMT cases (38% vs. 7%; P= 0.08) exhibited a reaction [103]. Research has shown that when TMZ and WBRT were used together to treat patients with brain metastases from non-small cell lung cancer, the combination showed a greater response rate and a longer progression-free survival time [104]. WBRT+TMZ can raise the ORR for brain metastases of NSCLC, according to a recent meta-analysis by Han et al. [105]. However, there is an increased risk of treatment-associated grade III/IV hematological toxicity and gastrointestinal damage when compared to WBRT alone.

6.5. Prostate Cancer

Disappointing findings were found in a phase II research on TMZ and prostate cancer [106]. The existence of aneuploid cell fractions, which provide a wide range of cells from extremely sensitive to medication resistant, may be one of the causes of this [107]. Higher local TMZ concentrations were realized as a result of efforts to enhance this unsatisfactory state; these concentrations are adequate to kill cells regardless of inherent cellular sensitivity and cell DNA index. In order to restructure the TMZ for intervention, Braun et al. ligated it to a peptide-based carrier system known as TMZ-BioShuttle. The carrier is modular in nature, consisting of a transmembrane transporter (CPP) coupled to a cleavably-bound nuclear localization sequence (NLS) that was associated with TMZ. Following enzymatic cleavage within the cytoplasm and separation from the CPP, the TMZ-BioShuttle transmembrane passage and intracytoplasmic delivery of the TMZ into the cell nucleus are made possible by the NLS sequence. The hormone-refractory prostate cancer serves as an example of how this TMZ-BioShuttle may enhance treatment alternatives [108,109]. Hussain et al. recently evaluated the safety and effectiveness of TMZ and veliparib (ABT-888), low dose oral PARP inhibitors, in patients with metastatic castration-resistant prostate cancer (mCRPC) who had received prior docetaxel treatment. Thirteen patients had stable PSA, ten had PSA advancement, and two had a verified PSA response (8.0 %; 95% CI: 1.0–26.0) [110].

6.6. Sarcomas

TMZ possesses anti-sarcoma properties similar to dacarbazine [111–113]. Therefore, it might be helpful in treating metastases as well as primary control of sarcoma radiosensitization. In recurrent Ewing's sarcoma and desmoplastic small round cell tumors (DSRCT), Anderson et al. confirm a strong

response rate that may even be higher than that documented in the literature [114–116]. Compared to conventional regimens involving ifosfamide or cyclophosphamide, the combination of TMZ with irinotecan is less immunosuppressive [117]. Given that it has been demonstrated that lymphocyte recovery—defined as an absolute lymphocyte count of more than 500 on day 15 following the first round of chemotherapy—is linked to a noticeably greater survival rate in Ewing's sarcoma, this may be particularly noteworthy in this case [118]. Additionally, dacarbazine, commonly known as TMZ, has been used with other medications, such as doxorubicin liposomes [120] and gemcitabine [119]. Temozolomide showed an objective response rate (ORR) of 18% when administered to patients with previously treated unresectable or metastatic leiomyosarcoma; 27% of patients experienced disease stabilization [121]. Another phase II trial demonstrating an overall response rate of 15.5% involved 45 patients with soft-tissue sarcoma. Out of 11 patients with gynecologic leiomyosarcoma, 5 showed these responses [122]. Noh et al. used mouse xenograft models and uterine sarcoma cell lines to assess the anticancer effects of cabozatinib, temozolomide, and their combination. They discovered that in uterine sarcoma cell lines and xenograft mice models, including PDX, cabozatinib and temozolomide together provide synergistic anticancer effects. These findings call for additional research in a phase 1 clinical trial.

6.7. Pediatric Tumors

Recent years have seen the completion of several TMZ trials on pediatric cancers. The Children's Cancer Group (CCG) carried out a phase I clinical trial with TMZ in children and young adults with recurrent solid tumors [124]. The study's maximum tolerated dose (MTD) was 180 mg/m2/day for five days for patients who had previously received radiation therapy and 215 mg/m2/day for participants who had not received prior craniospinal irradiation (CSI). There was little evidence of major negative effects. Subsequent phase II research revealed TMZ activity in medulloblastoma and high-grade gliomas [125], among other forms of brain tumors. Sio et al. looked into the use of as a single agent in juvenile solid tumors that had relapsed or were resistant. For five days, the medication was given to patients who had previously received autologous bone marrow transplantation (ABMT) or craniospinal irradiation (CSI) at a dose of 215 mg/m2/day or 180 mg/m2/day, respectively. In our series, the objective response-rate (CR, PR, or MR) was 13.4% (1.9% CR, 3.8% PR, and 7.7% MR); 38.4% of patients experienced SD, and 48% had PD [126]. 39 patients (median age B13 years; 14 pretreated with high-dose chemotherapy, craniospinal irradiation, or having bone marrow involvement) with refractory or recurrent solid tumors were evaluated by Geoerger et al. The patients received cisplatin treatment, followed by oral TMZ for five days every four weeks at dose levels of 80 mgm_2/150 mgm_2 day_1, 80/200, and 100/200, respectively. A total of 38 patients were eligible for toxicity evaluation (median 2, range 1-3). Two neuroblastomas, one brain stem glioma, and two malignant gliomas all showed partial responses. After five days of TMZ treatment, the median MGMT activity in PBMCs dropped; low MGMT activity was associated with a higher degree of thrombocytopenia. Combinations of cisplatin and temozolomide are well tolerated and do not cause any more harm than single-agent therapies [127]. In 46 children with resistant solid tumors, Jakachi et al. performed a phase I and pharmacokinetic investigation of the epidermal growth factor receptor (EGFR) inhibitor erlotinib as a single drug and in combination with TMZ. Nineteen months were spent in stable condition for one patient with neurocytoma, twenty-three and twenty-four months were spent on study for two patients with neuroblastoma, and one patient with myoepithelioma saw a mixed response [128]. In addition to being well tolerated, TMZ and irinotecan have been shown to be effective against a number of pediatric solid tumors, such as neuroblastoma [131] and Ewing sarcoma [129, 130]. Wagner et al. examined the effectiveness of bevacizumab in combination with vincristine, oral irinotecan, and TMZ (VOIT Regimen) in pediatric patients with recurrent solid tumors or brain tumors. Tolerability was increased by reducing TMZ from 150 to 100 mg/m2/day; treatment with this reduced TMZ dose was practical and easy to administer as outpatient therapy. Even though Ewing sarcoma showed responses, it was uncertain whether adding bevacizumab would be beneficial [132]. Temozolomide has been reported to be a successful treatment in a number of patients with metastatic PPGL (phaeochromocytoma/paraganglioma), according to two small studies [133,134] and several case reports [135]. It has been demonstrated that patients with germline SDHB mutations responded more favorably to temozolomide [133,134].

7. Increasing the efficacy and overcoming the resistance of temozolomide in tumors

In order to increase the effectiveness of temozolomide, numerous tactics are being used that attempt to attack MGMT in various ways. Exosome-mediated circWDR62, miR-214-5p, and lncRNA

UCA1/miR-182-5p have been shown to enhance resistance mechanisms to temozolomide [135,137]. Patients with advanced hepatocellular carcinoma have been treated with temozolomide plus a MAPK/ERK inhibitor (U0126) because this combination increases the susceptibility of HCC cells to TMZ and down-regulates MGMT expression by blocking the MAPK/ERK signaling pathway [138]. By ubiquitinating and degrading MGMT, TRIM72 improved the sensitivity of dacarbazine treatment [139], hence reinstating the resistance mechanism of dacarbazine treatment in uveal melanoma. Ultimately, it has been demonstrated that NCT503, Tubeimoside-I, GNA13, Pyrviniumpamoate, DEC1, METTL3, and MMR improve GBM sensitivity to TMZ treatment by controlling MGMT [140–146].

8. Parp-inhibitors and temozolomide combination

Preclinical data suggests that in MGMT-silenced tumors, this could improve tumor cell death [147–149]. The multifaceted enhanced TMZ sensitivity of tumors with a PARPi takes use of PARP inhibitor activity in delaying the start of HR-mediated recovery [148]. Additionally, the combination of temozolomide with PARP inhibitor sensitivity depends on "PARP trapping" on DNA, suggesting that olaparib is a molecule that can work in concert with temozolomide [148–151]. Cechini et al. discovered that the combination of temozolomide and Olaparib was well tolerated by patients suffering from colorectal cancer, and that it did demonstrate anticancer effectiveness in a subgroup of patients whose tumors showed MGMT promoter hypermethylation, reduced MGMT protein expression, and enhanced CD8+ effector TILs. [152]. O6-methylguanine (O6MeG), one of the several methyl adducts produced when exposed to temozolomide, makes up a small percentage of these adducts but is the main cytotoxic lesion that seriously hinders DNA replication because thymine is inserted in opposition to methylguanine [153,154]. DNA mismatch repair (MMR), BER, the enzyme alkylpurine-DNA-N-glycosylase (APNG), or O6-methylguanine DNA methyltransferase (MGMT) can all be used to treat the O6MeG lesion. Temozolomide sensitivity is dependent on the expression of MGMT, APNG, and BER proteins in addition to MMR status [154]. In a recent study, Drxheimer et al. investigated the potentiation of DNA-damaging drugs by pharmacologic modulation of DNA repair pathways using Multicellular Spheroids, an in vitro model of human solid tumors composed of malignant cells, endothelial cells, and mesenchymal stem cells. They discovered that when temozolomide and the PARP inhibitors olaparib and talazoparib were combined, there were clear synergistic effects [155]. This is consistent with earlier preclinical research that showed temozolomide and PARP inhibitor worked synergistically in ten glioblastoma multiforme cancer stem cell lines [156]. The more common N7MeG (N7-methylguanine) and N3MeA (N3-methyladenine) adducts are repaired by the BER pathway in a process that needs PARP activity, whereas MGMT reverses O6MeG lesions caused by cytotoxic temozolomide [157]. Therefore, unrepaired and potentially fatal temozolomide-induced N7MeG and N3MeA lesions result from the suppression of PARP-mediated BER, which increases temozolomide cytotoxicity. Additionally, it has been shown that PARP's PARylation of MGMT is essential for the repair of O6MeG adducts, hence strengthening PARP's involvement in the BER and MGMT-mediated DNA repair of temozolomide-induced DNA damage [158]. The temozolomide/PARP inhibitor combination has been and is still being evaluated in clinical trials for glioblastoma, SCLC, renal cancer, Ewing sarcoma, rhadomyosarcoma, and advanced stage rare cancers (NCT04434482, NCT04603365, NCT01858168), based on the encouraging results in preclinical cancer models [159–161].

9. Combining temozolomide with immunotherapy; is it an enhancer?

In addition to the direct effects on tumor cells discussed above, TMZ has also been demonstrated to have immunoregulatory qualities. Like all other chemotherapy drugs, TMZ also has a variety of effects on the immune system, but maybe the most significant one is that it modifies the characteristics of immune cells, particularly the ratio of Treg cells to T-cells and the proliferation of T-cells [162]. Since TMZ has been the most commonly used treatment for GBM patients, the most research has been done on the immunological changes it makes to the tumor microenvironment (ME) and in a systematic manner. The most frequently reported systemic immunological response is lymphopenia. Numerous investigations have revealed a considerable decline in lymphocytes, particularly B-cells and CD4+ T-cells, and to a lesser extent, CD8+ T-cells [162, 163]. From a therapeutic standpoint, these systemic effects of TMZ have been examined in a few clinical trials where cellular immunotherapy drugs were also provided with TMZ. One of them used TMZ as an adjuvant in conjunction with a peptide vaccination targeting the EGFRvIII mutant version of the epidermal growth factor receptor. This trial's underlying mechanism relates to the immune-stimulatory effects it likely possesses, aside from its cytotoxic qualities, as it has been demonstrated to increase the tumor cells'

susceptibility to T-cell death and phagocytosis. This happens as a result of TMZ's upregulation of calreticulin (CRT) surface expression and its forced release of high-mobility group 1 protein (HMGB1). Additionally, both molecules have danger-associated molecular pattern molecules (DAMP) and function as DC and macrophage stimulants. These qualities have the potential to increase immune responses against tumors, hence reducing the need for additional adjuvants. In vivo research has demonstrated favorable results in terms of long-term survival [163-165]. According to the previously described research, there was a notable increase in the number of regulatory T-cells in response to the tumor cells, indicating that TMZ was more than just a chemotherapeutic drug [164]. TMZ has an impact on GBM immunological ME as well. Since the GBM ME is known to be extremely immunosuppressive due to its excretion of IL-11, which improves the tumor cells' ability to evade the immune system through the STAT3-MYC pathway [165]. Tregs, myeloid-derived suppressor cells (MDSCs), and macrophages are the immunosuppressive components that make up the ME. As was previously mentioned, TMZ systematically reduces the amount of Tregs. However, TMZ alone is unable to counteract the immunosuppressive characteristics of ME and change its immunological characteristics. Overall, TMZ continues to play a significant role in the treatment of GBM, either as an immune system modulator or as a cytotoxic agent. The combination of TMZ with other immunotherapy agents, such as immune checkpoint inhibitors, is an area that warrants further investigation and further study [162,165].

In a similar direction, Morano et al. carried out the phase II MAYA trial in patients with metastatic colorectal cancer, attempting to take advantage of the immunomodulatory benefits of temozolomide. The MAYA trial concluded by presenting data regarding the function of temozolomide as an immune-sensitizing drug for immune-cold mCRCs and MSS that are chosen based on MGMT silencing and disease control during temozolomide priming. In patients with microsatellite-stable (MSS) and O6-methylguanine–DNA methyltransferase (MGMT)–silenced metastatic colorectal cancer (mCRC), the researchers administered two cycles of temozolomide first as an immunosensitizer, and then a combination of low-dose ipilimumab and nivolumab. There was evidence of clinical benefit in this patient group, thereby proving the theory that temozolomide priming followed by a combination of low-dose ipilimumab and nivolumab may produce long-lasting clinical benefit in MSS and MGMT silenced mCRC [166]. Researchers included colorectal cancer patients with O6-methylguanine-DNA-methyltransferase (MGMT)-deficient tumors that were also MMR-proficient and RAS-mutant in the Arethusa trial, a proof of concept trial that involved priming therapy with TMZ. Following TMZ therapy, a unique mutational signature and elevated TMB were found by analysis of tissue samples and circulating tumor DNA (ctDNA). MMR genes showed several changes in the nucleotide context preferred by the TMZ signature, and in 94% (16/17) of the patients, the p.T1219I MSH6 variant was found in the ctDNA and tissue. After receiving pembrolizumab treatment, a subgroup of patients whose tumors had the TMZ mutational profile, elevated TMB, and the MSH6 mutation had stable disease [167]. The proof of concept for temozolomide's possible function in converting immunological "cold" tumors to "hot" ones, where immunotherapy may then be therapeutically advantageous, was made feasible by these two trials (Figure 3).

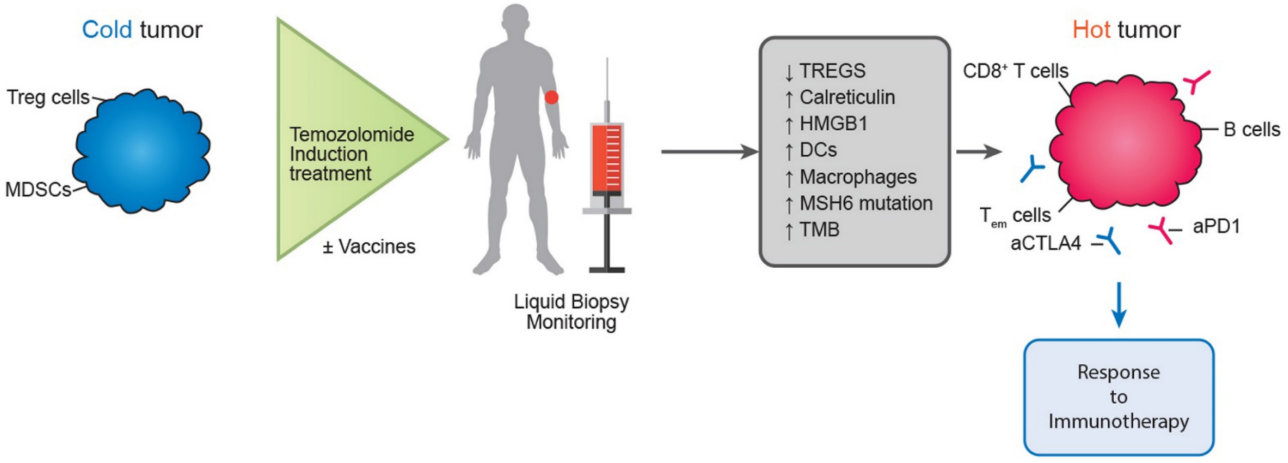

Figure 3. Potential future role of temozolomide in turning immune "cold" tumors to "hot" ones.

10. Combining Temozolomide with Radiotherapy

The current standard treatment for patients with newly diagnosed GBM involves targeted radiation therapy together with chemotherapy, followed by additional cycles of chemotherapy as per the Stupp regimen. This approach was established by the EORTC-NCIC phase 3 trial around twenty years ago [71,168]. RT and TMZ cause an accumulation of DNA damage in the form of single-stranded breaks (SSBs) or double-stranded breaks (DSBs) leading to tumor cell death. Hegi et al. found that the impact on survival was particularly significant in glioblastoma patients with MGMT promoter methylation when combining radiation with temozolomide. Patients with glioblastoma and unmethylated MGMT promoters did not have improved survival when temozolomide was added to radiation treatment. The 2-year survival rates for the four patient groups, categorized by unmethylated and methylated MGMT promoters and treated with either radiation alone or radiotherapy combined with temozolomide, are 2%, 14%, 23%, and 46%, respectively [21]. The effect of prolonged adjuvant TMZ treatment (more than 6 cycles) on survival results has been a topic of debate with no agreement on the best duration of adjuvant TMZ therapy [169, 170]. MGMT gene promoter methylation is used as a predictive marker for response to alkylating TMZ chemotherapy. Some oncologists are extending adjuvant TMZ treatment beyond the standard 6 cycles, up to 12 or even 24 cycles, based on personal or institutional preferences, despite the lack of solid scientific evidence regarding its efficacy and safety. In order to further enhance the activity of the combination of Temozolomide and radiotherapy, an interesting phase 1 trial is underway, that intracranially administers γδ T cells modified to be temozolomide resistant so as to be active, concurrent with temozolomide and radiotherapy, a strategy called "Drug Resistance Immunotherapy"

11. Conclusions

Temozolomide has exhibited activity to various solid tumors. Due to its advantageous pharmaco-dynamic profile and to new combinations that overcome the phenomenon of chemoresistance, broaden its use as an active molecule in advanced cancers where effective treatments are on demand.

Competing Interests

The authors have declared that no competing interest exists.

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