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The Effectiveness of Multidrug Chemotherapy with Olaparib, Temozolomide and Oxaliplatin Compared to Radiotherapy in Glioblastoma Multiforme in Vitro Models and Human Fibroblasts

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Research Article

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Abstract

Introduction

: Glioblastoma multiforme (GBM) remains one of the most treatment-resistant central nervous system (CNS) tumors. This study evaluated the efficacy of multidrug repositioning combining oxaliplatin (OXA), olaparib (OLA), and temozolomide (TMZ), administered alone or in combination with photon radiotherapy, in GBM cell lines as *in vitro* models.

Methods

U118 MG and U87 MG cells, as well as control human fibroblasts (hFib) from a healthy donor, were treated with OXA (50–200 µM), OLA (1-100 µM), and TMZ (10–100 µM), alone and in combination. Cell viability was assessed after 72 hours using the MTS assay, and apoptosis/necrosis was quantified using a fluorescent apoptosis, necrosis, and healthy cell quantification kit. Treatments were tested with (2 Gy, 4 Gy) and without irradiation.

Results

Radiotherapy significantly enhanced the cytotoxic effects of all drugs, with the strongest reductions in viability observed for multidrug combinations. The addition of 2 Gy and 4 Gy irradiation markedly enhanced the activity of OLA + TMZ, OXA + TMZ, and OLA + OXA, leading to substantial loss of GBM cell viability. The greatest synergistic response has been observed with the triple drug combination (OLA + OXA + TMZ) plus radiotherapy, which produced extensive necrosis across all GBM models used.

Conclusions

The combination of OXA, OLA, and TMZ, especially when combined with photon radiotherapy, demonstrates potential synergistic cytotoxicity in GBM while sparing healthy fibroblasts. These results support the use of multidrug combination chemotherapy with radiotherapy as a promising and potentially safer treatment strategy for GBM.

INTRODUCTION

Glioblastoma multiforme (GBM) is the most common and aggressive brain tumor, with a high mortality rate and a five-year survival of only 7.2% [1, 2]. Standard management includes surgical resection followed by radiotherapy (RT) combined with temozolomide (TMZ), yet its limited efficacy underscores the need for more effective therapeutic strategies [3, 4]. Glioma stem-like cells (GSCs) have been identified as a key factor in GBM resistance, exhibiting high self-renewal, metastatic potential, and efficient DNA repair. These cells overexpress DNA damage response proteins, such as checkpoint kinase

1 (CHK1) and poly(ADP-ribose) polymerase 1 (PARP-1), which contribute to chemoresistance and radioresistance [1, 3, 4]. TMZ, a first-line chemotherapy agent for GBM, induces cytotoxicity via DNA methylation, creating lesions such as O6-methylguanine (O6-MeG), which trigger DNA double-strand breaks (DSBs) and tumor cell death through the mismatch repair (MMR) pathway [1, 3]. GBM frequently acquires resistance via MMR deficiency or overexpression of O6-methylguanine-DNA methyltransferase (MGMT), which repairs O6-MeG lesions [5].

Other chemotherapeutic agents, such as oxaliplatin (OXA), a third-generation platinum compound, induce DNA cross-links and DSBs while presenting lower systemic toxicity compared to earlier platinum drugs [6, 7]. However, tumor cells can evade cytotoxicity via homologous recombination and non-homologous end-joining repair pathways [8, 9]. PARP inhibitors, such as olaparib (OLA), interfere with DNA repair by trapping the PARP-DNA complex, leading to accumulation of DSBs and cell death. OLA has proven efficacy in tumors with homologous recombination deficiency (HRD), including certain isocitrate dehydrogenase (IDH) mutant GBM variants, and can enhance the cytotoxicity of alkylating agents even in HR-proficient cells [10, 11]. Oxaliplatin has been primarily studied as a radiosensitizer in combination with radiotherapy in glioblastoma multiforme models, where it has been shown to potentiate radiation-induced cytotoxicity [12, 13]. Combination including radiotherapy and drugs, such as TMZ or PARP inhibitors, without including OXA in multi-agent radiotherapy regimens has already been studied [10, 14]. In this research, OXA has been combined with repositioning chemotherapy (TMZ and OLA) at lower doses and radiotherapy. While other studies show only combinations of OXA with drugs or OXA alone with radiotherapy [1, 12].

Radiotherapy remains a cornerstone of glioblastoma (GBM) management. In this study, in vitro irradiation was performed using a Cobalt-60 (Co-60) source [15]. GBM cells were exposed to clinically relevant doses of 2 Gy and 4 Gy [16]. This experimental framework allowed further evaluation of potential synergistic interactions between radiotherapy and chemotherapy, including whether combined treatments enhance tumor cell killing while reducing toxicity compared to monotherapies [10, 11, 15, 16].

MATERIALS AND METHODS

1. Cell lines and cell culture

The following GBM cell lines were used as preclinical *in vitro* models [17, 18]. U118 MG (Cat. No. HTB-15), and U87 MG (Cat. No. HTB-14) were obtained from the American Type Culture Collection (ATCC).

A healthy donor human fibroblast line (hFib) served as a control [1]. Before the fragment of skin was taken, the participant signed written informed consent; the study protocol was reviewed and approved by the Human Bioethical Committee of the Regional Medical Board in Kraków (No. 163/KBL/OIL/2023). Details on cell lines and culturing are provided in the Supplementary Information (SI).

2. Chemiotherapeutics

TMZ (Cat. No. T2577) and OXA (Cat. No. PHR1528) were obtained from Sigma-Aldrich. The following synthesis of OLA was conducted following the literature data [19, 20], with minor modifications, which are described in detail in the SI.

3. Irradiation with a Cobalt-60 (Co-60) beam and dosimetry

The irradiation was performed using a Cobalt-60 (Co-60) source (Theratron 780E, Best Theratronics; activity 236.7 TBq on November 2, 2018) at the Institute of Nuclear Physics, Polish Academy of Sciences (IFJ PAN), Krakow, Poland. Photon beam dosimetry was conducted using a UNIDOS Webline electrometer (PTW-Freiburg, T10021) in combination with a Farmer-type ionization chamber (PTW-Freiburg, TM30010-1). Details on irradiation with a Co-60 beam are provided in the SI.

4. Cell Viability Assessment of OLA, TMZ, OXA, and their Combination

Cell viability of TMZ, OLA, and OXA and their combinations with radiotherapy was evaluated using GBM cell lines seeded in 96-well plates at a density of 4×10^3 cells per well. Cells were treated with increasing concentrations of each drug individually or in combination with radiotherapy or alone as detailed in the SI.

5. Cell death analysis

Cells (U118, U87, and human fibroblasts) were seeded in 6-well plates and following attachment, exposed to chemotherapeutic agents at various concentrations are provided in the SI. Immediately after drug addition, the plates were exposed to radiation using a cobalt-60 source (Theratron 780E, Best Theratronics), according to the manufacturer's certificate. Irradiation was delivered as single doses of 2 Gy and 4 Gy. Detailed procedures and representative images are provided in the SI.

6. Statistical analysis

Cells were subjected to drug treatment, radiotherapy (at single doses of 2 Gy or 4 Gy), or combined treatments. Cell viability was assessed 72 hours after treatment using the MTS assay, with absorbance measured at 490 nm.

Statistical analyses were conducted using GraphPad Prism version 10.6.1 (892) for Windows (GraphPad Software, Boston, Massachusetts, USA). The data are presented as mean ± standard deviation (SD) of at least three replicates. All data were tested for the assumptions of normality (the Shapiro-Wilk test). Two-way ANOVA of the variance was performed by the Tukey test. Statistical significance is indicated by asterisks (* $p < 0.05$; **$p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

Error bars in all graphs represent the standard deviation of at least three replicates. Both chemotherapeutic treatments and irradiation doses (2 Gy and 4 Gy) were included in the statistical comparisons, allowing assessment of monotherapy and combined effects.

RESULTS

1. Chemistry

OLA was resynthesized using literature-based conditions with slight modifications [19]. Briefly, the carboxylic acid component was first activated with O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU) and then coupled with Boc-protected piperazine. Subsequent Boc deprotection afforded compound **2**, which was finally acylated with cyclopropanecarbonyl chloride to yield OLA. This synthetic sequence provided isolated yields of 60–91%. The structure and purity of the final compound were confirmed by chromatographic and spectroscopic analyses, as detailed in the SI (Fig. 1).

2. Cell viability

To assess the effects of OXA, OLA, TMZ, and their combinations on cell viability, two human GBM cell lines were used: U87 (grade IV), U118 (grade III/IV). As a non-cancerous control, normal human fibroblasts (hFib) were used, obtained from a healthy donor. Details on cell lines and culturing are provided in SI (Fig. 2).

A two-way ANOVA using Type III sums of squares was conducted to examine the effects of radiation doses and treatment groups on cell viability. Unequal sample sizes were handled using Type III SS. The main effects of radiation were statistically significant, while treatment group as well as interaction between OLA 1 μM and OLA 100 μM or OXA 50 μM and OXA 100 μM or TMZ 10 μM and TMZ 100 μM were non-significant. Treatment group was analyzed as a categorical factor due to non-equivalent drug concentrations between compounds.

The interaction between radiation and treatment group was not significant ($F(_{2, 30}) = 0.5599$, $p = 0.5771$), indicating that the effect of radiation on cell viability was consistent across treatment conditions. There was a significant main effect of radiation dose ($F(_{2, 30}) = 11.85$, $p = 0.0002$), demonstrating that radiation significantly altered U87 cell viability. In contrast, the main effect of treatment group was not significant ($F(_{1, 30}) = 2.348$, $p = 0.1360$), suggesting that drug treatment alone did not significantly affect cell viability under the tested conditions.

Post hoc Tukey comparison was performer separately for each drug to examine the interaction between various drug concentrations and radiation doses. For OLA, radiation significantly reduced cell viability at higher concentration (100 μM), while lower concentration (1 μM) showed non-significant effect. For OXA, significant differences were observed between radiation doses at 50 μM. Last drug, TMZ at each of the concentrations showed non-significant effect with tendency to decrease of viability. These analyses

demonstrate that the response to radiation depends on drug concentration within each compound (Fig. 3).

Cell viability of the U118 cell line was analyzed as a function of radiation doses and treatment groups using a two-way ANOVA with Type III sums of squares. All three effects – the main effects of radiation doses and treatment groups, as well as their interaction – were statistically significant.

A significant interaction between radiation and treatment group was observed ($F(_{14, 187}) = 13.25$, $p < 0.0001$), indicating that the response to radiation differed across treatment conditions. There was a significant main effect of radiation ($F(_{2, 187}) = 247.9$, $p < 0.0001$), with increasing radiation doses significantly reducing cell viability. A significant main effect of treatment groups were also observed ($F(_{7, 187}) = 55.15$, $p < 0.0001$), demonstrating that different treatment conditions significantly influenced cell viability. Treatment groups were analyzed as a categorical factor due to non-equivalent drug concentrations between compounds.

Post hoc Tukey comparisons were performed separately for each drug to examine the interaction between drug concentration and radiation dose. For OLA, radiation significantly reduced cell viability at both concentrations (100 μM and1 μM). For OXA, radiation significantly reduces cell viability at lower concentrations (50 μM and 100 μM), while at higher concentration no changes were observed. For TMZ, reduction of cell viability with radiation were observed at both concentrations (10 μM and 100μM) (Fig. 4).

A two-way ANOVA using Type III sums of squares was conducted to examine the effects of radiation doses and treatment groups, and their interaction on fibroblast viability. All three effects – the main effects of radiation and treatment group, as well as their interaction – were statistically significant. Treatment groups were analyzed as a categorical factor due to non-equivalent drug concentrations between compounds. A significant interaction between radiation doses and treatment groups was observed ($F(_{14, 48}) = 4.837$, $p < 0.0001$), indicating that the response to radiation differed across treatment conditions. There was a significant main effect of radiation ($F(_{2, 48}) = 156.7$, $p < 0.0001$), with higher radiation doses significantly reducing fibroblast viability. The main effect of treatment groups were also significant ($F(_{7, 48)} = 18.79$, $p < 0.0001$), demonstrating that different treatment conditions significantly affected cell viability.

Post hoc Tukey comparisons were performed separately for each drug to examine the interaction between drug concentrations and radiation doses. For OLA, radiation significantly reduced cell viability at both concentrations (1 μM and 100 μM). Second drug, OXA showed significant decrease in cell viability between radiation doses in concentration 50 μM, while in 100 μM and 200 μM no differences were observed. For TMZ, radiation significantly reduced cell viability at both concentrations (10 μM and 100 μM) (Fig. 5).

Cell viability in U87 cells was analyzed using a two-way ANOVA with radiation doses and drug treatment groups as factors (Type III sums of squares). No significant interaction between radiation doses and drug treatments was observed ($F_{(6, 34)}$ = 2.012, p = 0.0911), indicating that the effect of radiation on cell viability was largely independent of drug treatment groups. The main effect of radiation dose did not reach statistical significance ($F_{(2, 34)}$ = 3.141, p = 0.0560), although a trend toward reduced viability with increasing radiation dose was observed. In contrast, a significant main effect of drug treatment was detected ($F_{(3, 34)}$ = 3.146, p = 0.0376). Post hoc test followed by Tukey was conducted and indicate significant differences between radiation doses in group with triple combinations of drugs.

Cell viability in U118 cells was examined using a two-way ANOVA with radiation dose and drug treatment as factors. Interaction between radiation dose and drug treatment was not statistically significant ($F_{(6, 24)}$ = 1.262, p = 0.3112). A significant main effect of radiation dose was observed ($F_{(2, 24)}$ = 8.389, p = 0.0017), as well as drug treatment ($F_{(3, 24)}$ = 6.707, p = 0.0019).

In fibroblast group, cell viability was analyzed using a two-way ANOVA with radiation dose and drug treatment as factors. No significant interaction between radiation and drug treatment was detected ($F_{(6, 33)}$ = 1.329, p = 0.2721). Radiation doses have significant main effect on fibroblast viability ($F_{(2, 33)}$ = 22.36, p < 0.0001) as well as drug treatment gropus($F_{(3, 33)}$ = 6.762, p = 0.0011). Post hoc Tukey comparisons were performed and showed significant differences between radiation doses within the group treated with 50 μM OXA + 50 μM OLA and in group 50 μM OXA + 50 μM OLA + 50 μM TMZ.

3. Cell death analysis

Two-way ANOVA revealed a significant interaction between drug treatment (OXA, OLA, TMZ) and radiation (2 Gy, 4 Gy) for all tested cell lines, indicating that the cellular response (viability and death profiles) significantly differed between drug-only and combined treatment groups (Fig. 6). Cells were subjected to drug (OXA, OLA, TMZ) treatment, radiotherapy (single doses of 2 Gy or 4 Gy) or combined treatments after 72 hours. The impact of TMZ, OXA, and/or OLA with radiotherapy on the type of cell death was assessed in two commercially available GBM cell lines (U118MG, U87MG). The human fibroblast line (hFib) obtained from a healthy donor was used as controls. The results for the compared groups were as follows: hFib, F70,216 = 408.5.0, p < 0.0001; U118, F70,216 = 787.9, p < 0.0001; and U87, F70,216 = 1224, p < 0.0001 (Table 1).

Table 1

Effect of TMZ, OXA, and/or OLA on the type of cell death in glioblastoma cell lines (U118 MG, U87 MG – ATCC) and human fibroblasts (hFib, control, from a healthy donor).

Anti-cancer drug		Early and late apoptosis, necrosis and lid live cells presented as [%] after 72 hours for each cell line								
	Type of cells	hFib			U118			U87		
	Radiotherapy [Gy]	0	2	4	0	2	4	0	2	4
Control, without anti-cancer drug	live cells	100	0	0	98	0	0	100	5	10
	Early apoptosis	0	91	0	2	64	32	0	18	19
	Late apoptosis	0	9	28	0	34	52	0	31	35
	necrosis	0	0	72	0	2	16	0	46	36
OLA 100μM	Live cells	8	15	0	0	0	0	0	0	0
	Early apoptosis	47	45	22	98	0	0	13	20	0
	Late apoptosis	45	40	78	2	25	0	87	63	72
	Necrosis	0	0	0	0	75	00	0	17	28
TMZ 100μM	Live cells	0	0	0	32	0	0	0	0	0
	Early apoptosis	54	0	0	34	0	0	48	0	0
	Late apoptosis	46	100	10	34	100	50	52	6	0
	Necrosis	0	0	90	0	0	50	0	94	100
OXA 50μM	Live cells	74	0	0	0	0	0	0	0	0
	Early apoptosis	24	0	0	32	0	0	46	0	0
	Late apoptosis	2	100	100	68	20	0	54	0	0
	Necrosis	0	0	0	0	80	100	0	100	100
100μM	Live cells	45	0	0	0	0	0	0	0	0
	Early apoptosis	55	0	0	0	0	0	0	0	0

		Late apoptosis	0	60	0	55	10	5	100	0	0
		Necrosis	0	40	100	45	90	95	0	100	100
	200μM	Live cells	0	0	0	0	0	0	0	0	0
		Early apoptosis	20	0	0	2	0	0	5	0	0
		Late apoptosis	51	32	0	59	0	0	90	0	0
		Necrosis	29	68	100	39	100	100	5	100	100
OLA 50μM + TMZ 50μM		Live cells	42	10	0	0	0	0	0	0	0
		Early apoptosis	41	38	0	14	0	0	5	0	0
		Late apoptosis	17	52	100	86	0	0	95	11	0
		Necrosis	0	0	0	0	100	100	0	89	100
OLA 50μM + OXA 50μM		Live cells	60	10	0	0	0	0	0	0	0
		Early apoptosis	22	35	13	5	0	0	5	63	0
		Late apoptosis	18	55	87	95	5	0	95	37	30
		Necrosis	0	0	0	0	95	100	0	0	70
OLA 50μM + OXA 50μM + TMZ 50μM		Live cells	57	0	0	9	0	0	0	0	0
		Early apoptosis	20	0	0	46	0	0	24	0	0
		Late apoptosis	23	50	35	45	20	0	76	0	0
		Necrosis	0	50	65	0	80	100	0	100	100

Treatment with 100 μM OLA induced apoptosis in hFib (early apoptosis: 47%, 45%, 22% vs. late apoptosis: 45%, 40%, 78%, respectively) without evidence of necrosis. In contrast, GBM cell lines showed no viable cells; necrosis predominated in U118 cells at 2 and 4 Gy (75%, 100%) and in U87 cell line at 2 and 4 Gy (17%, 28%), (see Table 1).

In contrast, treatment with 100 μM TMZ caused 90% necrosis in hFib cells at 4 Gy, 50% in U118 and 100% in U87 cell lines. At 2 Gy, necrosis was observed only in the U87 cell line (94%) (see Table 1). Treatment with 50 μM OXA without radiation induced early and late apoptosis in hFib cells (24% and 2%, respectively), with no necrosis observed. However, at 200 μM OXA, hFib cells exhibited necrosis (29%)

and late apoptosis (51%), while U118 and U87 cells showed necrosis (39% and 5%, respectively) without radiotherapy. In the presence of 2 Gy and 4 Gy radiotherapy, the following effects were observed: after 72 hours of treatment with 200 μM OXA, complete necrosis (100%) were detected in both U118 and U87 cells, at both radiation doses.

Combination treatments with 50 μM OLA and 50 μM TMZ or 50 μM OLA and 50 μM OXA at 2 Gy and 4 Gy predominantly induced apoptosis without necrosis in hFib cell line. At both doses: 2 Gy and 4 Gy, the combination of 50 μM OLA and 50 μM TMZ led to necrosis in U118 (100%, 100%, respectively) and in U87 (89%, 100%, respectively). A similar pattern was observed for combination 50 μM OLA and 50 μM OXA in U118 cell line (95%, 100% necrosis at 2 Gy and 4 Gy). In contrast, hFib and U87 cells treated with 50 μM OLA and 50 μM OXA showed early and late apoptosis following irradiation at 2 Gy. In hFib cells, early and late apoptosis exhibited in 35% and 55% respectively whereas in U87 cells these values were 63% and 39%, respectively (see Table 1.).

The three-drug combination 50 μM OLA, 50 μM TMZ, and 50 μM OXA resulted in early and late apoptosis without necrosis in all cell lines (20% and 23% in hFib, 46% and 45% in U118, 24% and 76% in U87). Following irradiation, necrosis was observed in GBM cells lines and in hFib cells, at 2 and 4 Gy, necrosis reached 50% and 65% in hFib cells, 80% and 100% in U118 cells, and 100% at both doses in U87 cells (see Table 1).

DISCUSSION

In this study, the cytotoxic effects of alkylating drugs OXA with TMZ administered alone or in combination with the PARP inhibitor OLA were assessed in GBM cell lines and human fibroblasts in combination with photon radiotherapy [1, 21, 22]. The results showed that increasing therapeutic complexity through multidrug combinations and concomitant ionizing radiation was associated with a progressive decrease in GBM cell viability compared to hFib [1, 15, 22, 23].

Similar responses of U118 and U87 cell lines to drugs and radiotherapy combination were visualized in Figs. 2, 3, 5, and illustrating the modulatory role of MGMT expression in drug resistance. U118 cells, characterized by high MGMT expressions, showed limited sensitivity to monotherapies but clearly responded significantly to combination therapies including OLA [17, 18, 24, 25]. In contrast, U87 cells showed greater sensitivity to OLA + TMZ and to the triplet OLA + TMZ + OLA without RT [1]. Zajac-Grabiec et al. [1] also reported a synergistic effect of OLA with TMZ in the GBM models, especially in the U87, H4, and U118 cell lines [1]. OLA enhances the effect of TMZ, but not OXA, in glioma cell lines irrespective of MGMT status, as shown in previous reports [1, 2, 4]. In the present study, the drugs used alone and in combinations: OLA and TMZ or OXA and TMZ or OXA and OLA, as well as the triplet preparation of OXA, OLA and TMZ administrated with radiotherapy (2Gy, 4Gy), showed synergistic effects.

In Fig. 5, enhanced activity is observed for the combination regimens, with higher cytotoxicity being caused by OLA + TMZ, OXA + TMZ, and OLA + OXA following radiotherapy compared to monotherapy,

while the most pronounced reduction in cell viability is observed with the triple combination (OLA + OXA + TMZ) in all GBM models. These findings support the conclusion to confirm that synergistic accumulation of cytotoxic DNA damage results from the simultaneous inhibition of multiple DNA repair pathways. Specifically, DNA alkylation is induced by TMZ with OXA, whereas OLA, thereby preventing the removal of single-strand breaks and promoting their conversion into lethal double-strand breaks (DSBs) during replication [10, 25]. This impairment of DNA repair is considered to underline the enhanced efficacy of multidrug regimens following photon therapy [11, 26].

Moreover, the efficacy of all drugs OLA, TMZ, and OXA, alone was enhanced by radiotherapy. As shown in Fig. 2–4 (single drug) and Fig. 5 (drug combinations), both 2 Gy and 4 Gy increased cytotoxicity. This effect is likely attributable to increased susceptibility of tumor cells to radiation-induced DSBs when DNA repair pathways are pharmacologically impaired. The synergic interaction between chemotherapy and radiotherapy was found to be significantly stronger in U118 and U87 cells than in hFib cells, supporting the notion that GBM-specific genomic instability is associated with an increased dependence on DNA repair mechanisms that are selectively targeted by OLA, OXA, and TMZ [1, 15, 16].

The results of this study demonstrated a differential response between hFib cells as a control and glioblastoma multiforme cell lines (U118 and U87) to treatment with OLA, TMZ, OXA, their combinations, and radiotherapy. In particular, in this study, hFib cells predominantly underwent apoptosis, whereas GBM cells responded mainly with necrosis, especially when combined with ionizing radiation (2 Gy, 4 Gy). These findings underscore the sensitivity of GBM cells to combined cytotoxic stress and the relative resistance of hFib cells to necrotic cell death (see Table 1).

Treatment with 100 µM OLA, particularly after irradiation, induced an apoptotic response in hFib cells, with no evidence of necrosis. In contrast, GBM cells subjected to the same treatment did not exhibit viability or early apoptosis, and necrosis predominated the cell death profile (see Table 1).

Furthermore, treatment with 100 µM TMZ caused significant necrosis of hFib cells only at the higher radiation dose (4 Gy). GBM cell lines showed greater susceptibility at the 2 Gy dose, particularly U87 cells, which showed 94% necrosis. The increased sensitivity of GBM cells demonstrated a synergistic interaction between DNA alkylation by TMZ and radiation-induced DNA damage, which impairs DNA repair pathways frequently mutated in GBM [15, 16, 23].

OXA treatment showed a dose-dependent response in hFib cells, with 50 µM predominantly inducing apoptosis without necrosis. In contrast, treatment with 100 and 200 µM OXA resulted in both necrosis and late apoptosis, particularly following irradiation. Notably, 200 µM OXA combined with 2 or 4 Gy caused 100% necrosis in both GBM cells. These findings support the observation that radiotherapy enhances the cytotoxic effects of OXA, particularly in tumor cells. Furthermore, when hFib cells were exposed to 50 µM OLA and TMZ combined with irradiation, apoptosis remained the dominant mechanism of cell death, with no necrosis detected. This indicates that at lower drug concentrations, hFib cells maintained controlled cell death pathways, even under combined chemotherapy and photon

irradiation [21, 26]. In contrast, complete necrosis was observed in GBM cells under the same treatment conditions, indicating a higher sensitivity to combined chemotherapy and radiotherapy [15, 16].

In this study, OLA combined with TMZ or OXA induced apoptosis without necrosis in hFib cells, even following irradiation. In U118 and U87 cell lines, the same combinations caused extensive necrosis at doses of 2 Gy and 4 Gy, with U118 cells exhibiting almost complete necrosis and U87 cells displaying a mixed apoptotic-necrotic profile during chemotherapy and radiotherapy. These results indicate that hFib cells retained their capacity for programmed cell death after multiple drug administration, whereas GBM cells lost this regulatory control after exposure to combined stressors [1, 11].

The three-drug combination (OLA, TMZ, OXA) induced apoptosis without evidence of necrosis in all cell lines in the absence of photon irradiation. However, following irradiation necrosis occurred in all cell types. Moreover, hFib as a control group demonstrated a different viability profile, as shown in Fig. 2, 3, 4, 5, and Table 1. Despite a moderate reduction in viability, cytotoxicity was significantly lower than that observed in GBM cell lines, even under multidrug treatment or in the presence of radiotherapy. [27, 28]. These findings highlight the potential of multidrug repositioning strategies (OXA, OLA) in combination with TMZ and photon therapy may bring a breakthrough, which will ultimately enable more effective and safer pharmacotherapy for patients [27, 29].

Although our results are preliminary, the observed synergistic effect of alkylating agents with combination of OLA and radiotherapy supports the proposal of a novel chemotherapy strategy for GBM. Further investigation of the synergistic effects of repositioning drugs (OXA, OLA) combined with TMZ or modern radiotherapy, such as FLASH, may lead to therapeutic breakthroughs that will ultimately enable more effective and safer pharmacotherapy for patients. Therefore, *in vitro* and *in vivo* molecular studies of OXA and OLA combined with TMZ and various types of radiotherapy in GBM in different models (i.e., 2D and 3D) are necessary and will be conducted in the next part of this ongoing project.

Abbreviations

CHK1
checkpoint kinase 1
CNS
central nervous system
DCM
dichloromethane
DMF
dimethylformamide
DMSO
dimethyl sulfoxide
DSBs
double–strand breaks

GBM
glioblastoma multiforme
GSCs
glioma stem–like cells
HATU
hexafluorophosphate azabenzotriazole tetramethyl uronium
HBTU
O–benzotriazole–N,N,N',N'–tetramethyl–uronium–hexafluoro–phosphate
HCL
hydrochloric acid
HD
helical
hFib
human fibroblast line
HPLC
high–performance liquid chromatography
HRD
homologous recombination deficiency
IC_{50}
half maximal inhibitory concentration
IDH
isocitrate dehydrogenase
MGMT
O6–methylguanine–DNA methyltransferase
MMR
mismatch repair
MTS
3–(4,5–dimethylthiazol–2–yl)–5–(3–carboxymethoxyphenyl)–2–(4–sulfophenyl)–2H–tetrazolium
NI
nicotinamide
OLA
olaparib
OXA
oxaliplatin
O6
MeG–O6–methylguanine
PARP1
poly(ADP–ribose) polymerase 1
PH
phosphate

RT
radiotherapy
SI
supplementary information
TBTU
O–(benzotriazol–1–yl)–N,N,N',N'–tetramethyluronium tetrafluoroborate
TEA
triethylamine
TMZ
temozolomide

Declarations

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Author contributions:

Anna Zając-Grabiec*, conceptualization, data curation, funding acquisition, investigation, resources, methodology, roles/writing - original draft, writing - review & editing.

Anna Czopek*, investigation, data curation, visualization, methodology, funding acquisition, roles/writing - original draft, writing - review & editing.

Filip Michałkiewicz, software, formal analysis, validation, visualization.

Tomasz Skóra, investigation.

Beata Biesaga, supervision.

Monika Bania, investigation.

Krzysztof Łukowicz, investigation

Marta Bałamut, investigation, methodology

Dawid Krzempek, investigation, methodology

Dominik Wiśniewski, investigation, methodology.

Paula Ajersch, formal analysis, validation, visualization.

Justyna Miszczyk, funding acquisition

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Human Bioethical Committee of the Regional Medical Board in Kraków (No. 163/KBL/OIL/2023, 06.07.2023). Written informed consent was obtained from the participant involved in the study before the fragment of skin was taken.

Ethics declarations

Ethics Approval and Consent to Participate. The study was conducted in accordance with the guidelines of the Declaration of Helsinki and approved by the Human Bioethical Committee of the Regional Medical Board in Kraków (Approval No. 163/KBL/OIL/2023, dated 06.07.2023). Written informed consent was obtained from the participant prior to the collection of skin fragments.

Consent for publication

Written informed consent for publication was obtained from the participant.

Data available with manuscript or supplementary information:

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format, they are available from the corresponding author upon reasonable request.

References

1. Zając-Grabiec A, Czopek A, Pazdan K, Jończyk J, Michałkiewicz F, Skóra T, et al. In vitro repositioning therapy with olaparib, temozolomide and oxaliplatin in glioblastoma cell lines: U118, U87, U251, H4 and human fibroblasts. Pharmacol Rep. 2025;77(6):1716–26. https://doi.org/10.1007/s43440-025-00783-w. Epub 2025 Sep 3. PMID: 40900233; PMCID: PMC12647329.
2. Wu W, Klockow JL, Zhang M, Lafortune F, Chang E, Jin L, et al. Glioblastoma multiforme (GBM): An overview of current therapies and mechanisms of resistance. Pharmacol Res. 2021;171:105780. https://doi.org/10.1016/j.phrs.2021.105780. Epub 2021 Jul 21. PMID: 34302977; PMCID: PMC8384724.
3. Hwang K, Lee JH, Kim SH, Go KO, Ji SY, Han JH, et al. The Combination PARP Inhibitor Olaparib With Temozolomide in an Experimental Glioblastoma Model. Vivo. 2021;35(4):2015–23. https://doi.org/10.21873/invivo.12470.
4. Ahmed SU, Carruthers R, Gilmour L, Yildirim S, Watts C, Chalmers AJ. Selective Inhibition of Parallel DNA Damage Response Pathways Optimizes Radiosensitization of Glioblastoma Stem-like Cells. Cancer Res. 2015;75(20):4416–28. https://doi.org/10.1158/0008-5472.CAN-14-3790.

5. Wang K, Chen D, Qian Z, Cui D, Gao L, Lou M. Hedgehog/Gli1 signalling pathway regulates MGMT expression and chemoresistance to temozolomide in human glioblastoma. Cancer Cell Int. 2017;17:117. https://doi.org/10.1186/s12935-017-0491-x.
6. Li X, Cheng Y, Yang Z, Ji Q, Huan M, Ye W, et al. Glioma-targeted oxaliplatin/ferritin clathrate reversing the immunosuppressive microenvironment through hijacking Fe2 + and boosting Fenton reaction. J Nanobiotechnol. 2024;22:93. https://doi.org/10.1186/s12951-024-02376-w.
7. Xu K, Chen Z, Cui Y, Qin C, He Y, Song X. Combined olaparib and oxaliplatin inhibits tumor proliferation and induces G2/M arrest and γ-H2AX foci formation in colorectal cancer. Onco Targets Ther. 2015;8:3047–54. https://doi.org/10.2147/OTT.S89154.
8. Andreidesz K, Koszegi B, Kovacs D, Vantus VB, Gallyas F, Kovacs K. Effect of Oxaliplatin, Olaparib and LY294002 in Combination on Triple-Negative Breast Cancer Cells. Int J Mol Sci. 2021;22(4):2056. https://doi.org/10.3390/ijms22042056.
9. Roberts NB, Alqazzaz A, Hwang JR, Qi X, Keegan AD, Kim AJ, et al. Oxaliplatin disrupts pathological features of glioma cells and associated macrophages independent of apoptosis induction. J Neurooncol. 2018;140(3):497–507. https://doi.org/10.1007/s11060-018-2979-1.
10. Schaff LR, Kushnirsky M, Lin AL, Nandakumar S, Grommes C, Miller AM, et al. Combination Olaparib and Temozolomide for the Treatment of Glioma: A Retrospective Case Series. Neurology. 2022;99(17):750–55. https://doi.org/10.1212/WNL.0000000000201203.
11. 't Hart E, Bianco J, Bruin MAC, Derieppe M, Besse HC, Berkhout K, et al. Radiosensitisation by olaparib through focused ultrasound delivery in a diffuse midline glioma model. J Control Release. 2023;357:287–98. https://doi.org/10.1016/j.jconrel.2023.03.058.
12. Benzina S, Debomy F, Bergerat JP, Denis JM, Gueulette J, Dufour P, et al. The cytotoxicity of high-linear energy transfer radiation is reinforced by oxaliplatin in human glioblastoma cells. Cancer Lett. 2007;28(1):54–62. https://doi.org/10.1016/j.canlet.2007.02.001.
13. Charest G, Sanche L, Fortin D, Mathieu D, Paquette B. Glioblastoma treatment: bypassing the toxicity of platinum compounds by using liposomal formulation and increasing treatment efficiency with concomitant radiotherapy. Int J Radiat Oncol Biol Phys. 2012;1(1):244–9. https://doi.org/10.1016/j.ijrobp.2011.10.054.
14. Stefan D, Lesueur P, Lequesne J, Feuvret L, Bronnimann C, Castéra M, et al. Olaparib, Temozolomide, and Concomitant Radiotherapy for Partially Resected or Biopsy-Only Glioblastoma First-Line Treatment: Results from the OLA-TMZ-RTE-01 Phase I Study. Clin Cancer Res. 2025;1(7):1212–22. https://doi.org/10.1158/1078-0432.CCR-24-2974.
15. Healy BJ, van der Merwe D, Christaki KE, Meghzifene A. Cobalt-60 Machines and Medical Linear Accelerators: Competing Technologies for External Beam Radiotherapy. Clin Oncol (R Coll Radiol). 2017;29(2):110–5. 10.1016/j.clon.2016.11.002.
16. Walter Y, Hubbard A, Benoit A, Jank E, Salas O, Jordan D, et al. Development of In Vitro Assays for Advancing Radioimmunotherapy against Brain Tumors. Biomedicines. 2022;10(8):1796. https://doi.org/10.3390/biomedicines10081796. PMID: 35892697; PMCID: PMC9394411.

17. Lesueur P, Chevalier F, Austry JB, Waissi W, Burckel H, Noël G, et al. Poly-(ADP-ribose)-polymerase inhibitors as radiosensitizers: a systematic review of pre-clinical and clinical human studies. Oncotarget. 2017;8(40):69105–124. https://doi.org/10.18632/oncotarget.19079.
18. Gradwohl G, Ménissier de Murcia JM, Molinete M, Simonin F, Koken M, Hoeijmakers JH, et al. The second zinc-finger domain of poly(ADP-ribose) polymerase determines specificity for single-stranded breaks in DNA. Proc Natl Acad Sci U S A. 1990;87(8):2990–4. https://doi.org/10.1073/pnas.87.8.2990.
19. Menear KA, Adcock C, Boulter R, Cockcroft XL, Copsey L, Cranston A, et al. 4-[3-(4-cyclopropanecarbonylpiperazine-1-carbonyl)-4-fluorobenzyl]-2H-phthalazin-1-one: a novel bioavailable inhibitor of poly(ADP-ribose) polymerase-1. J Med Chem. 2008;51(20):6581–91. https://doi.org/10.1021/jm8001263.
20. Xu Q, Chen J, Wang Z, Zang Y, Li G, Zhu F, et al. Two-step flow synthesis of Olaparib in microreactor: Route design, process development and kinetics research. Chem Eng J. 2023;471(3):144304. https://doi.org/10.1016/j.cej.2023.144304.
21. Bian X, Piipponen M, Liu Z, Luo L, Geara J, Chen Y, et al. Epigenetic memory of radiotherapy in dermal fibroblasts impairs wound repair capacity in cancer survivors. Nat Commun. 2024;15(1):9286. https://doi.org/10.1038/s41467-024-53295-1.
22. Hill RP, Kaspler P, Griffin AM, O'Sullivan B, Catton C, Alasti H, et al. Studies of the in vivo radiosensitivity of human skin fibroblasts. Radiother Oncol. 2007;84(1):75–83. https://doi.org/10.1016/j.radonc.2007.05.025.
23. Tokuyama Y, Mori K, Isobe M, Terato H. Comparison of mutation spectra induced by gamma-rays and carbon ion beams. J Radiat Res. 2024;65(4):491–9. https://doi.org/10.1093/jrr/rrae050. PMID: 38940734; PMCID: PMC11262859.
24. Helleday T, Petermann E, Lundin C, Hodgson B, Sharma RA. DNA repair pathways as targets for cancer therapy. Nat Rev Cancer. 2008;8(3):193–204. https://doi.org/10.1038/nrc2342.
25. Xu K, Chen Z, Cui Y, Qin C, He Y, Song X. Combined olaparib and oxaliplatin inhibits tumor proliferation and induces G2/M arrest and γ-H2AX foci formation in colorectal cancer. Onco Targets Ther. 2015;8:3047–54. https://doi.org/10.2147/OTT.S89154.
26. Nickoloff JA, Taylor L, Sharma N, Kato TA. Exploiting DNA repair pathways for tumor sensitization, mitigation of resistance, and normal tissue protection in radiotherapy. Cancer Drug Resist. 2021;4(2):244–63. https://doi.org/10.20517/cdr.2020.89. Epub 2021 Jun 19. PMID: 34337349; PMCID: PMC8323830.
27. Shaw R, Basu M, Karmakar S, Ghosh MK. MGMT in TMZ-based glioma therapy: Multifaceted insights and clinical trial perspectives. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research. 2024;187(3):119673. https://doi.org/10.1016/j.bbamcr.2024.119673
28. Liu J, Jiang J, Hui X, Wang W, Fang D, Ding L. Mir-758-5p Suppresses Glioblastoma Proliferation, Migration and Invasion by Targeting ZBTB20. Cell Physiol Biochem. 2018;48(5):2074–83. https://doi.org/10.1159/000492545.

29. Schatz J, Ladinig A, Fietkau R, Putz F, Gaipl US, Frey B, et al. Normofractionated irradiation and not temozolomide modulates the immunogenic and oncogenic phenotype of human glioblastoma cell lines. Strahlenther Onkol. 2023;199(12):1140–51. https://doi.org/10.1007/s00066-022-02028-8.

Figures

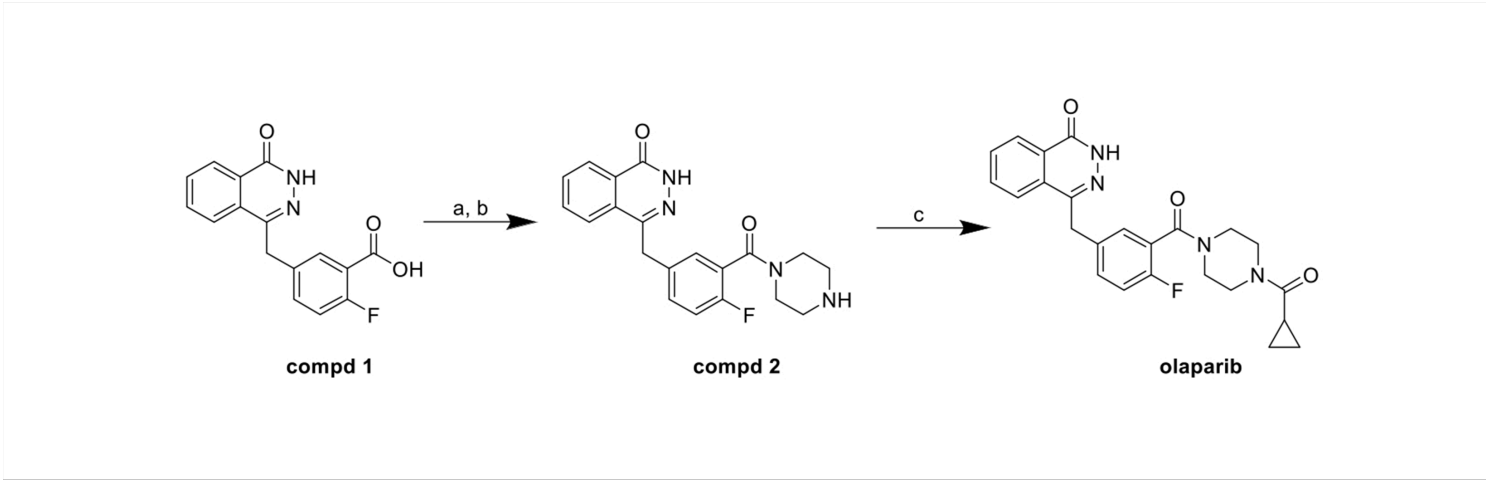


Figure 1

Resynthesis of Olaparib. Reagents and conditions; a: N-boc-piperazine, TBTU, TEA, DMF, 24 h, room temperature, b: HCl (aq), ethanol, c: cyclopropanecarbonyl chloride, TEA, DCM, 30 min., 0 ºC. 2-Fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoic acid (**1**) was commercially available.

TMZ and OXA were commercially available and used as received for biological studies.

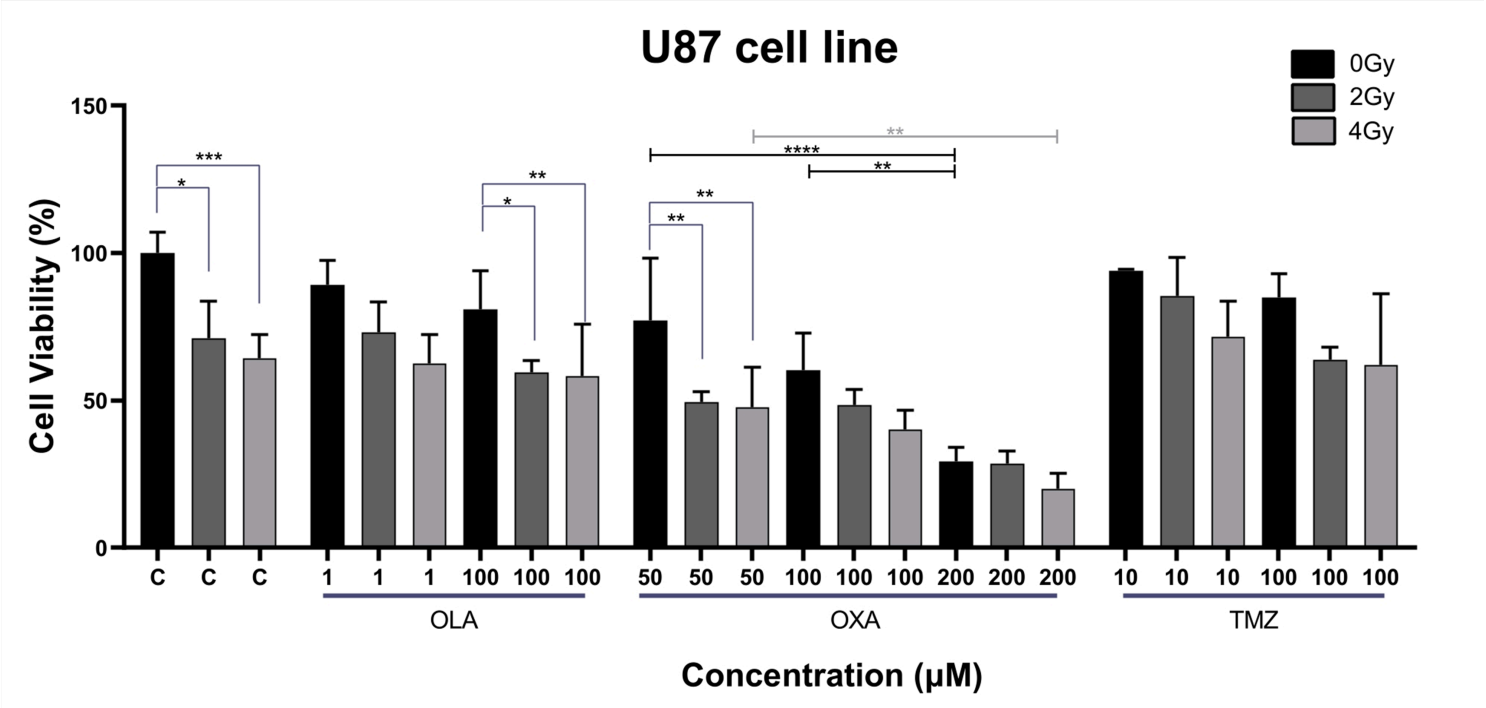


Figure 2

Cell viability of the U87 cell line with different drug concentrations with different doses of photon radiation. Results are expressed as mean ± SD. * p<0.05; **p<0.01; *** p<0.001; **** p<0.0001 denote statistically significant group differences. C – control; TMZ – temozolomide; OLA – olaparib; OXA – oxaliplatin; Gy – Grey.

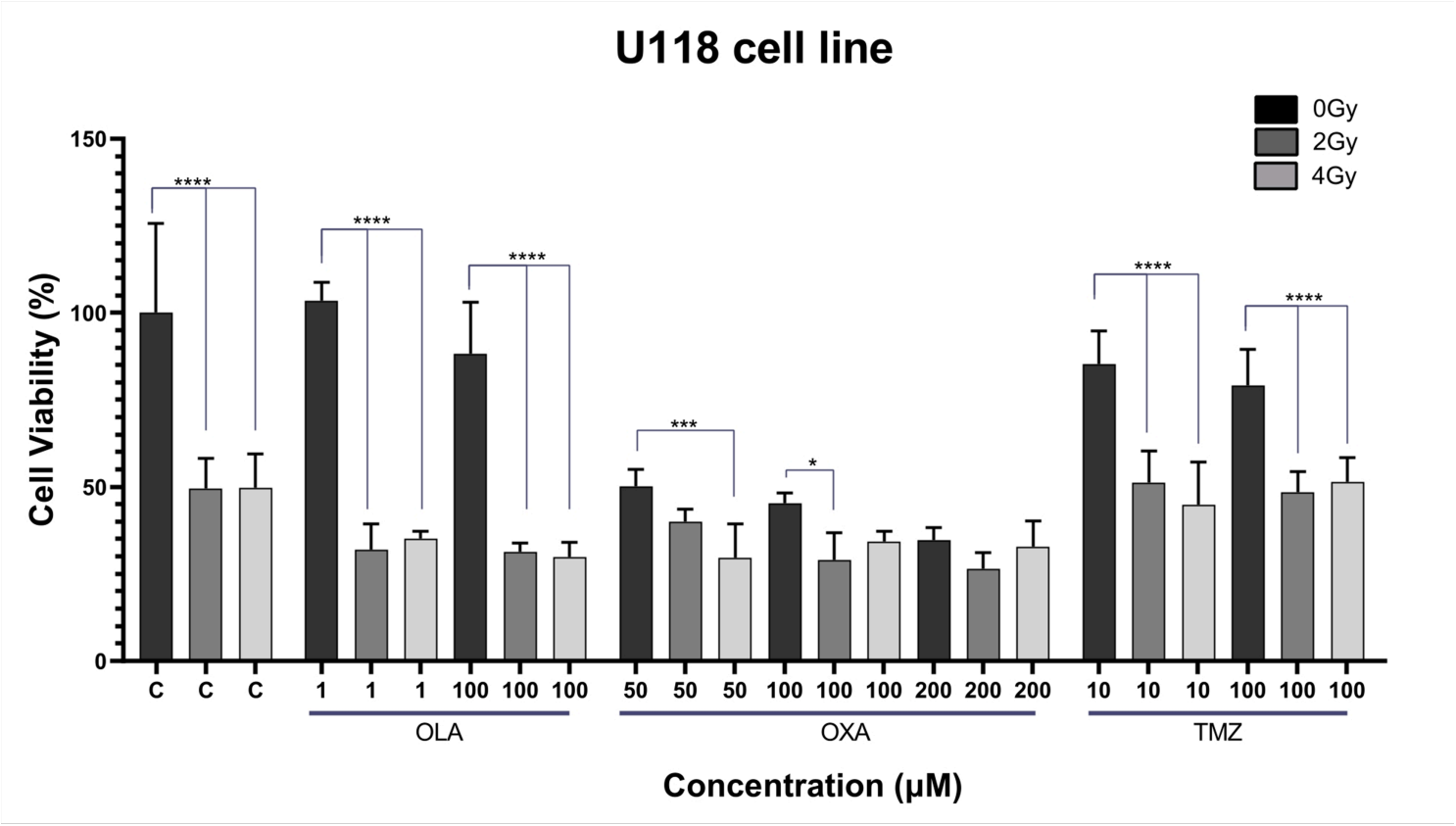


Figure 3

Cell viability of the U118 cell line with drug at different concentrations with different doses of radiation. Results are expressed as mean ± SD. * p<0.05; *** p<0.001; **** p<0.0001 denote statistically significant group differences. C – control; TMZ – temozolomide; OLA – olaparib; OXA – oxaliplatin; Gy – Grey.

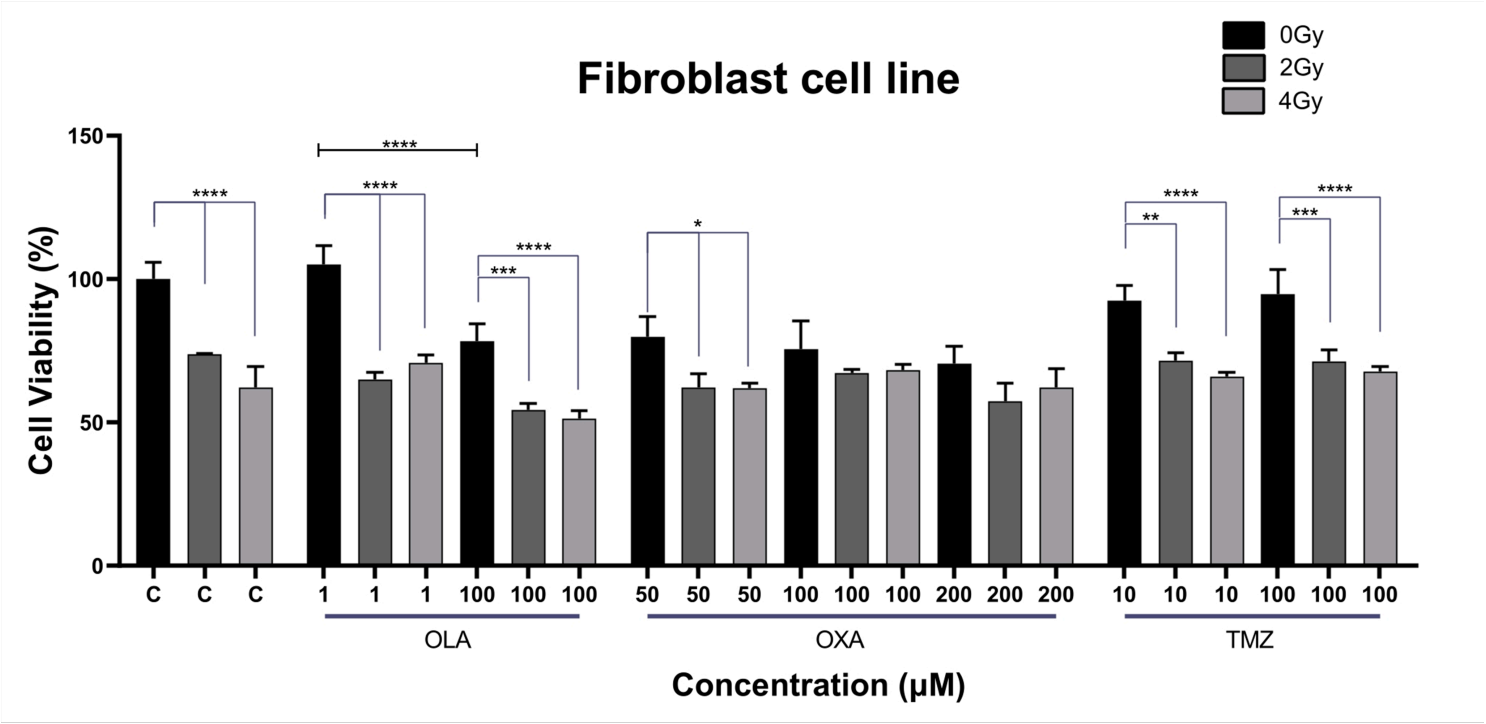


Figure 4

Cell viability of fibroblast cell line with different drug concentrations with different doses of radiation. Results are expressed as mean ± SD. * p<0.05; **p<0.01; *** p<0.001; **** p<0.0001 denote statistically significant group differences. C – control; TMZ – temozolomide; OLA – olaparib; OXA – oxaliplatin; Gy – Grey.

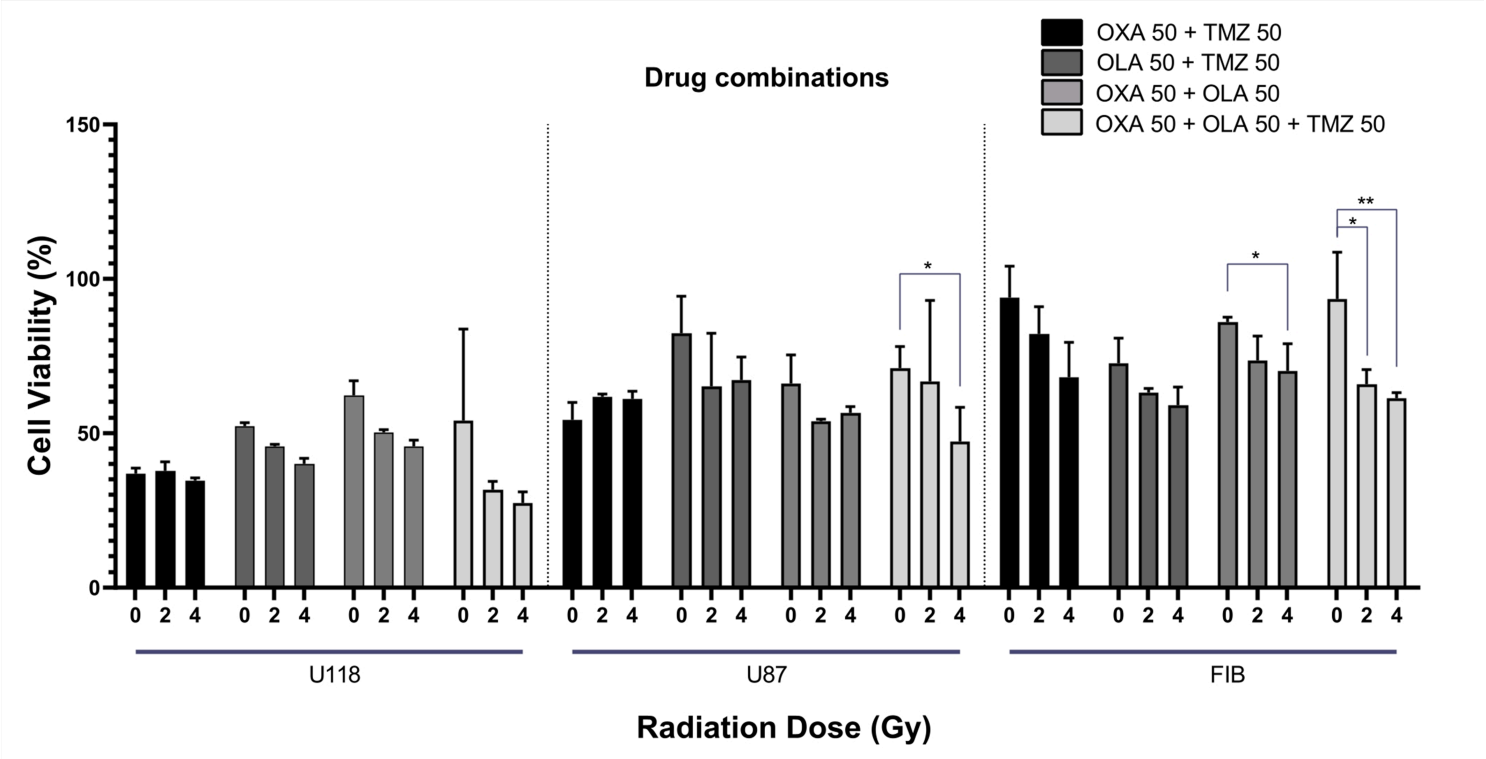


Figure 5

Cell viability of three cell lines: U 118, U 87, and fibroblast, using drugs in the following combinations: 50 μM OLA with 50 μM TMZ, or 50 μM OXA with 50 μM TMZ, or 50 μM OXA with 50 μM OLA, and in a triplet 50 μM OXA+50 μM OLA+50 μM TMZ in combination with different radiation doses (0, 2, 4 Gy). Results are expressed as mean ± SD. C – control; TMZ – temozolomide; OLA – olaparib; OXA – oxaliplatin; Gy – Grey.

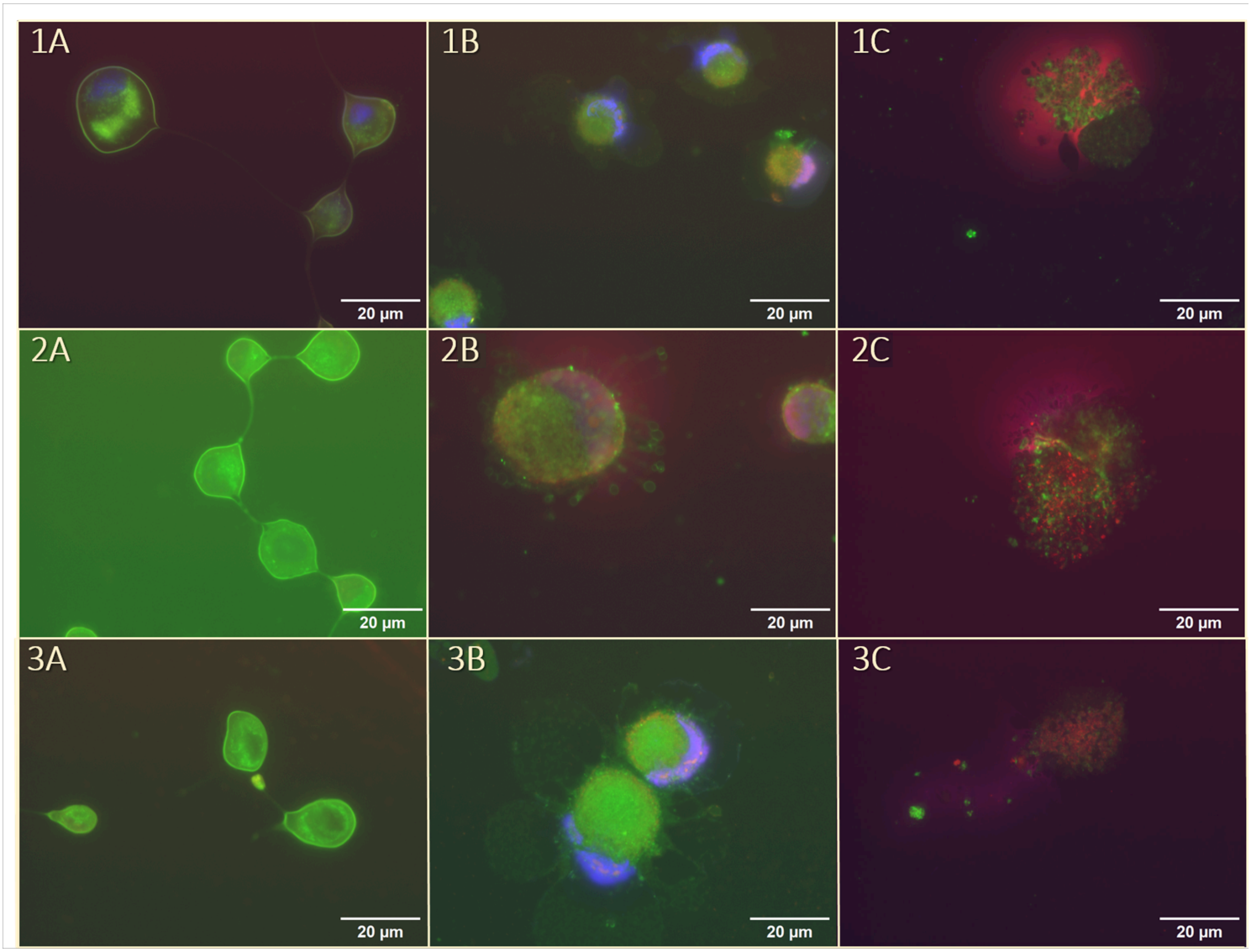


Figure 6

Type of cell death of three cell lines: U118, U87, and hFib. Fluorescence microscopy analysis of apoptotic and necrotic cells after 72 h of treatment.
Cells were stained with Annexin V-FITC and Ethidium Homodimer III (Biotium) and analyzed using a Zeiss Axio Imager Z2 microscope. Fig. 1A- hFib live cells, Fig. 1B- hFib apoptosis, Fig.1C- hFib necrosis, Fig. 2A- U87 GBM live cells, Fig. 2B- U87 GBM apoptosis, Fig. 2C- U87 GBM necrosis, Fig. 3A- U118 GBM live cells, Fig. 3B- U118 GBM apoptosis, Fig. 3C- U118 GBM necrosis.

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