Table S1

Gene Name	Entrez Gene ID	Function	Fold increase or decrease/deviation			
			6BG/TMZ vs control	TMZ vs 6BG	6BG/TMZ vs 6BG	6BG/TMZ vs TMZ
Increase						
CDKN1A (p21,cip1)	1026	Cyclin-dependent kinase inhibitor	10.18 / 12 %	4.11 / 7 %	10.40 / 8 %	2.49 / 7 %
MDM2	4193	E3 Ubiquitin ligase	1.83/17%	[1]NTD	1.99 / 10 %	1.54 / 28 %
BAX_1	581	Apoptosis; [2]MOMP	2.19/2%	2.08 / 2 %	2.21 / 5 %	1.06 / 4 %
BAX_2	581	Apoptosis; MOMP	1.87%/2%	1.70 / 11 %	1.94 / 12 %	1.28 / 15 %
PUMA	27113	Pro-apoptotic BH3-only protein	3.07%/10%	2.10 / 7 %	2.97 / 3 %	1.35 / 9 %
FAS (CD95)	355	Death receptor	1.62 / 19 %	1.52 / 13 %	1.48 / 21 %	1.65 / 15 %
FOS	2353	Transcription factor	1.53%/12%	1.39 / 7 %	1.92 / 2 %	1.27 / 9 %
GM2A	2760	cytokine activity and defense response	1.85/8%	1.13 / 8 %	1.82 / 6 %	1.66 / 12 %
TNFSF9/ 4-1BB ligand	8744	Cell-mediated immune activation	2.91 / 42 %	1.70 / 13 %	2.37 / 13 %	1.07 / 8 %
Decrease						
HSPA5	3309	Stress response; cytoprotective	0.51/4%	1.19 / 5 %	0.61 / 0 %	0.51 / 3 %
HYOU1	10525	Stress response; cytoprotective	0.51/12%	1.14 / 7 %	0.79 / 5 %	0.73 / 2 %
HSP75	10131	Mitochondrial chaperone; cytoprotective	0.56/8%	1.08 / 8 %	0.65 / 11 %	0.59 / 4 %
LYAR	55646	Cell growth-regulator;nucle-olar protein	0.55 /16 %	1.11 / 6 %	0.64 / 6 %	0.51 / 12 %
no change						
P53	7157	Transcription factor; controller of cell survival and apoptosis	0.92 / 13 %	0.94 / 2 %	1.13 / 3 %	1.15 / 9 %
BCL2	596	Anti-apoptotic protein	0.69 / - %	1.03 / 23 %	1.02 / 25 %	1.10 / 17 %
TRAIL	8743	apoptosis via death receptors	0.93 / 7 %	0.89 / 7 %	1.09 / 5 %	1.24 / 15 %
FAS LIGAND	356	apoptosis via Fas receptor	[3]NE	NE	NE	NE

[1]no transcript detected (ND); [2]Mitochondrial outer membrane apoptosis (MOMP); [3]NE=not expressed

Treatment of MP cells

To look at early changes in gene expression, MP cells (40 x 10^6 per sample) derived from 3 pooled $CD34^+$ products were treated for 18 hours with control (vehicle), 6BG, TMZ, or 6BG/TMZ and cell pellets flash frozen. Total RNA were isolated at Miltenyi Biotec (Cologne, Germany) and bioinformatics analysis of four microarray datasets was performed by their Bioinformatics Group. The direct comparisons were: 6BG/TMZ vs Control, TMZ vs 6BG, 6BG/TMZ vs 6BG, 6BG/TMZ vs TMZ. A two-dye competitive hybridization of mRNAs derived from differently treated human cells in comparison to a reference mRNA derived from cells which underwent a different treatment was conducted. After treatment with two different drugs or a combination of both drugs, respectively, RNA was extracted from the cells and hybridized against the corresponding reference mRNA. As microarray platform, the PIQOR™ Cell Death Microarray with 494 probes was used. The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (16) and are accessible through GEO Series accession number GSE44122 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE44122).

Data Preprocessing

Each of the two-color microarray experiments has been performed relative to an individual reference derived from a control experiment. Genes, which were not detected in any of the four experiments, as well as genes showing no expression difference in any of the experiments (less than +/-1.07-fold expression difference) were removed from the list. A total of 353 genes was retained after this filtering step. The genes which are at least 1.5-fold differentially expressed compared to their reference are shown in Table 1.

Inter-Experiment Correlation Analysis

As an initial step in the analysis, the expression profiles of the four microarray data sets were subjected to a correlation analysis. The highest observed correlation coefficient (0.85) was between samples 6BG/TMZ vs Control and 6BG/TMZ vs 6BG, indicating that the two samples Control and 6BG used as controls are fairly similar in their expression profiles. The lowest correlation coefficient of -0.29 was assigned to the comparison of sample TMZ vs 6BG and 6BG/TMZ vs TMZ. The result of the correlation analysis suggests that samples control and 6BG samples have a rather similar gene expression pattern. There was no strong impact on the global expression profile of the MP cells treated with 6BG. TMZ alone influences the gene expression pattern of the treated cells much more than 6BG alone. The sample treated with TMZ has a rather similar gene expression pattern like the sample treated with the 6BG/TMZ, which is indicated by the anti-correlation between the samples 6BG/TMZ and TMZ vs 6BG.

Pathway Analysis

For pathway analysis, the most strongly regulated genes in the comparison of samples 6BG/TMZ, TMZ and 6BG versus control sample were chosen. The gene lists were subjected to a statistical analysis using Miltenyi Biotec's proprietary TreeRanker software, with which significant enrichments of annotations were searched within the gene sets. As annotation sources, databases containing information on Gene Ontology (GO) categories, protein sequence motifs, interaction data, complex membership and involvement in biological pathways were used. For the comparison of 6BG/TMZ with

control sample, the predominant annotation among the upregulated genes is ‘apoptosis’. The majority of the downregulated genes is assigned to heat shock proteins and proteins which bind unfolded proteins. In the artificially generated list comparing TMZ with control, the common annotation shared by most genes is also 'apoptosis' However, some of the genes upregulated here are also assigned to the mitochondria. The downregulated genes are related to anti-apoptotic processes. Finally, in the artificial comparison of 6BG with control, no genes rise over the threshold of 1.5-fold regulation. Summarizing, the analysis suggests, that the treatment with TMZ triggers the apoptotic p53 response in the MP cells. While p53 itself is not differentially regulated, the protein drives the cells in apoptosis by turning on apoptotic pathways via its target molecules.

In 6BG/TMZ-treated cells, while BAX 1 and BAX 2 expression was increased at the RNA level (Table 1) after overnight treatment, this did not translate into increases in bax protein expression in the presence of 6BG/TMZ at 3 days post-treatment (data not shown). RNA transcripts for the TNFSF9/4-1BB ligand protein were also increased in 6BG/TMZ-treated cells compared to other groups. However, flow cytometric analysis indicated that the TNFSF9/4-1BB ligand protein was not detected at the cell surface on control, 6BG, TMZ, or 6BG/TMZ treated cells (data not shown).