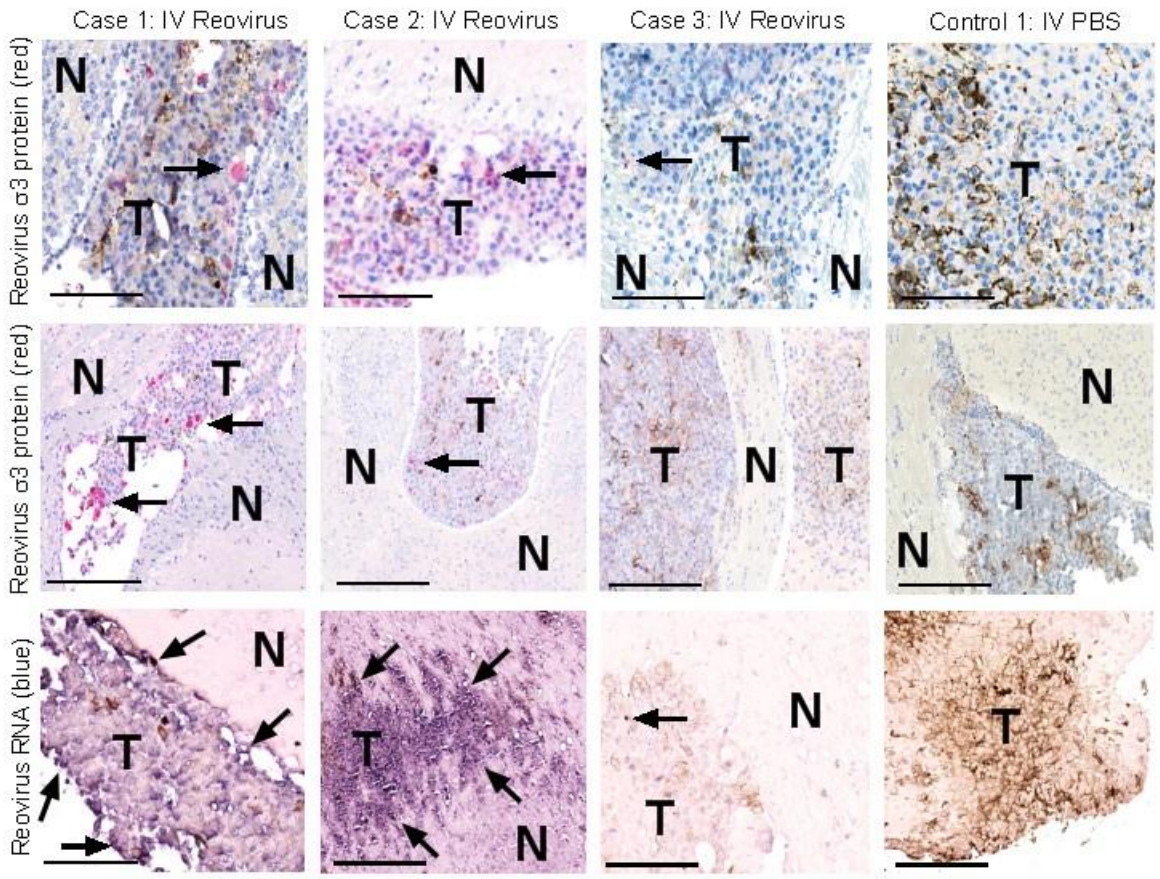
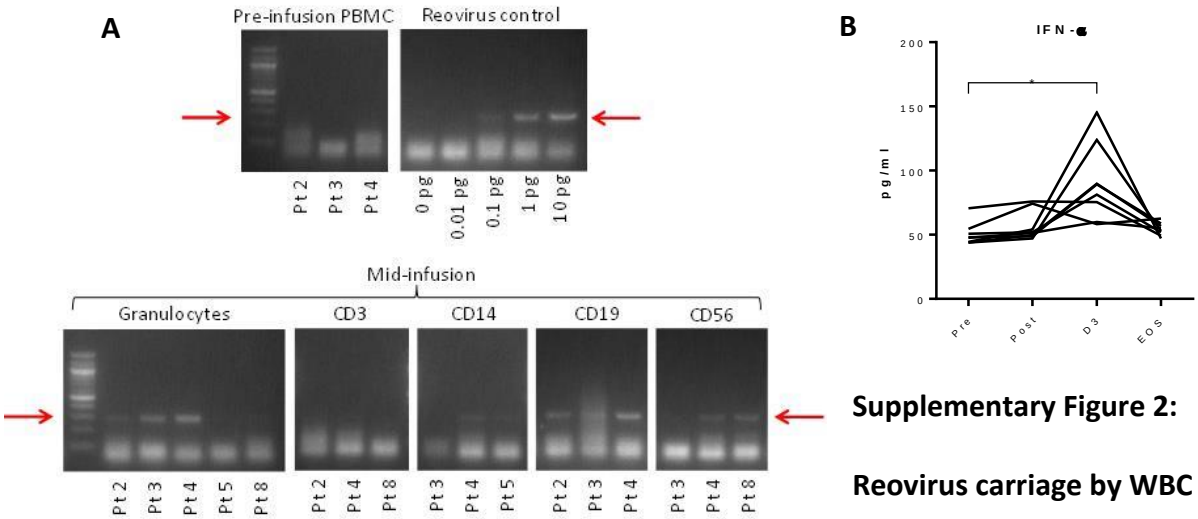
Supplementary Materials:



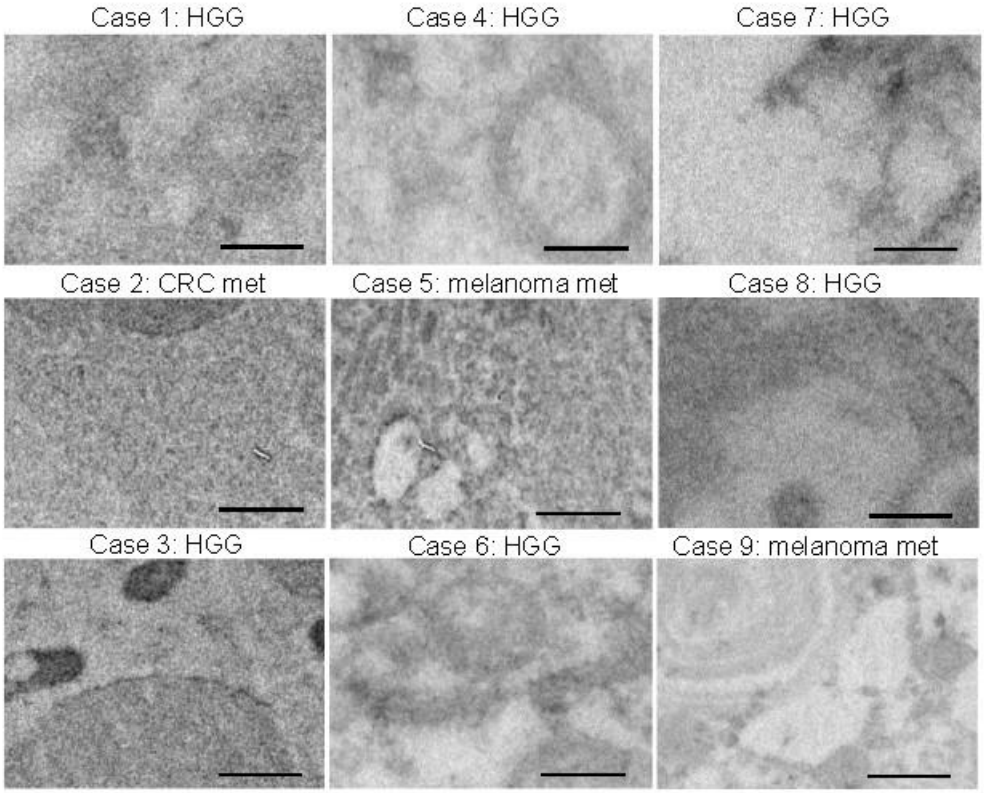
Supplementary Figure 1: Selective i.v. delivery of reovirus to intracranial melanoma in immunocompetent mice

Representative IHC for reovirus σ3 capsid protein (top two rows, red) and ISH for reovirus RNA (bottom row, blue), using tumor sections from C57/BL6 mice implanted intracranially with B16 melanoma (tumor melanin is brown) and treated with a single injection of i.v. reovirus or PBS. 'N' corresponds to normal brain tissue, and 'T' represents tumor. Arrows point to positive cells/positive areas of tissue. Top and bottom row scale bars = 30 µm. Middle row scale bars = 120 µm.



Supplementary Figure 2: Reovirus carriage by WBC subsets and changes in serum IFN-α

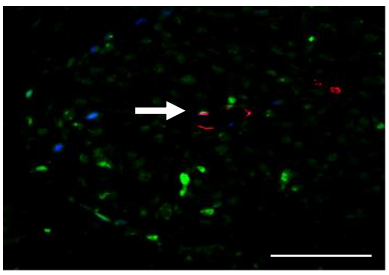
A) RT-PCR for reovirus σ3 gene using whole RNA derived from mid-infusion WBC subsets. PBMC subsets were sorted by positive bead selection. RNA derived from pre-infusion PBMCs served as a negative control, and a dilution series of purified reovirus RNA served as a positive control. 100 bp DNA ladder shown on the left. PCR product = 288 bp (indicated by red arrows). **B)** ELISA for IFN-α from patient serum taken immediately before reovirus (Pre), immediately after reovirus (post), two days after reovirus (D3), and at the end of study (EOS). * $P<0.05$. Each line represents serum IFN-α concentrations from a single patient.



Supplementary Figure 3: Secondary antibody-only control immunogold-TEM images from trial patient brain tumors

Trial patient tumor immunogold-TEM images stained using gold-conjugated secondary antibody only. Scale bar = 200 nm.

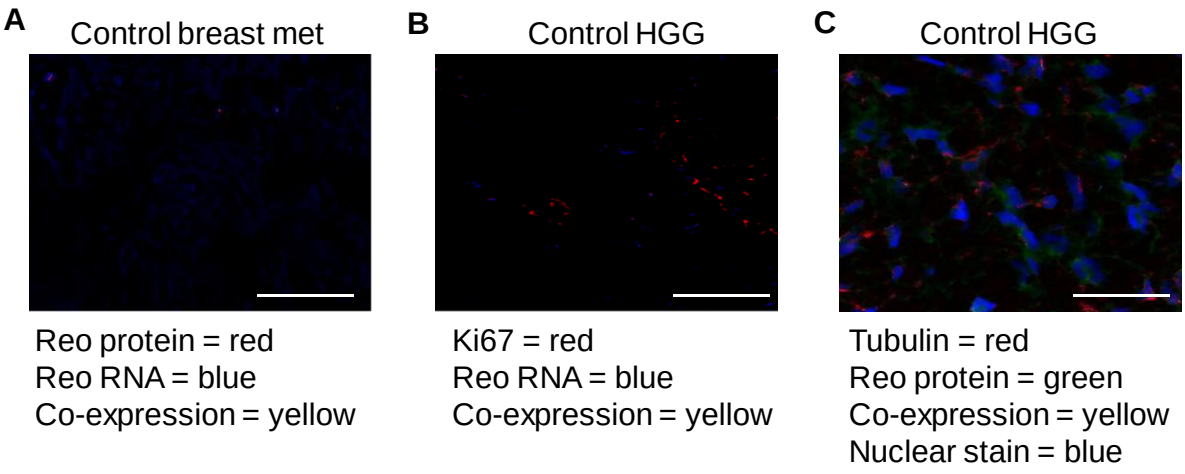
Case 9: Melanoma met



Reo protein = green
CD31 = red
Co-expression = yellow (arrow)

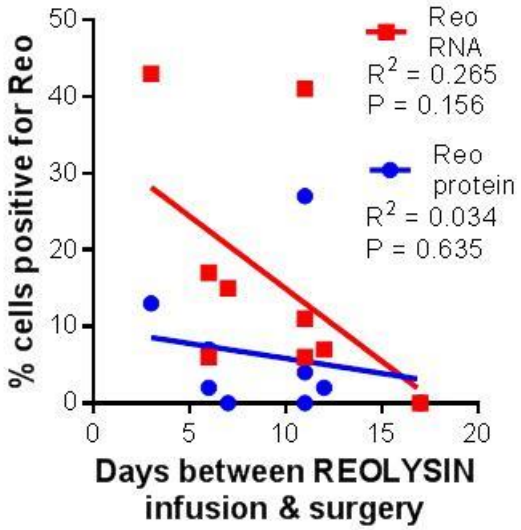
Supplementary Figure 4: Reovirus protein expression in endothelial cells

Representative IF from the tumor of trial patient nine. Scale bar = 40 µm.



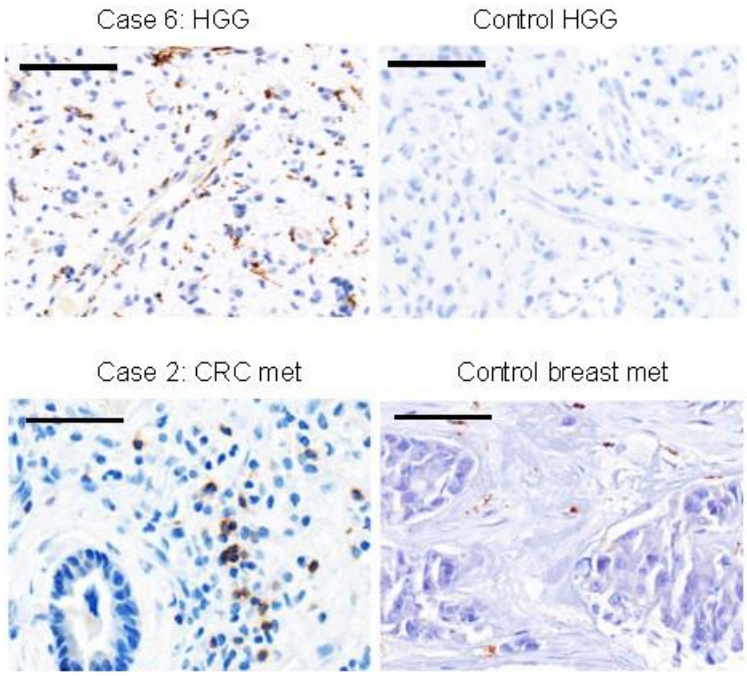
Supplementary Figure 5: Negative controls for reovirus co-expression

A) Representative IF from control breast metastasis. Scale bar = 40 µm. **B)** Representative IF from control HGG. Scale bar = 40 µm. **C)** Representative IF from control HGG. Scale bar = 20 µm.



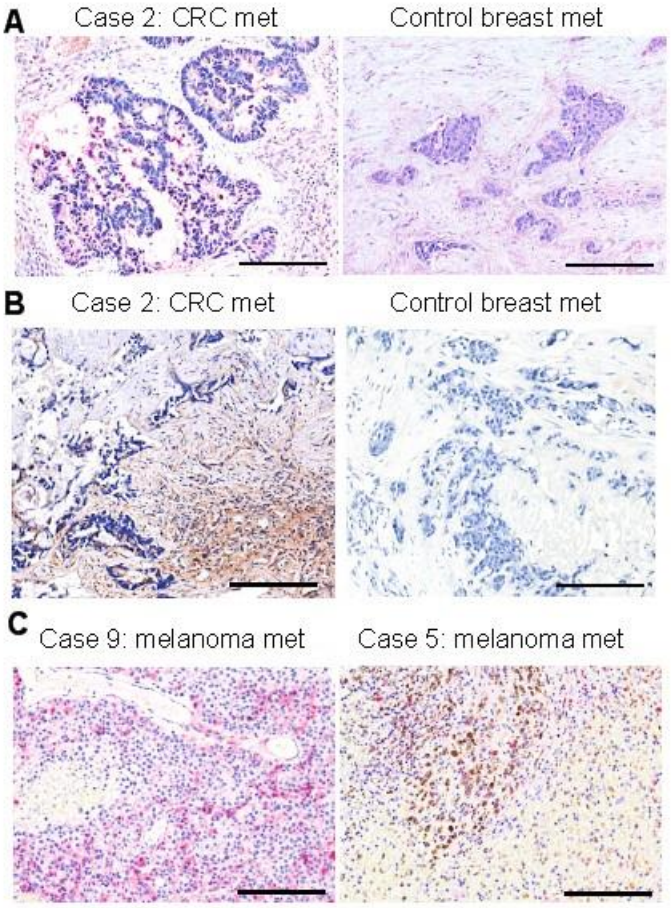
Supplementary Figure 6: Correlation of reovirus RNA/protein with time between infusion and surgery

Scatter plot and line of best fit, correlating the percentage of tumor cells positive by IHC for reovirus RNA or σ3 protein against the number of days from reovirus infusion to surgery.



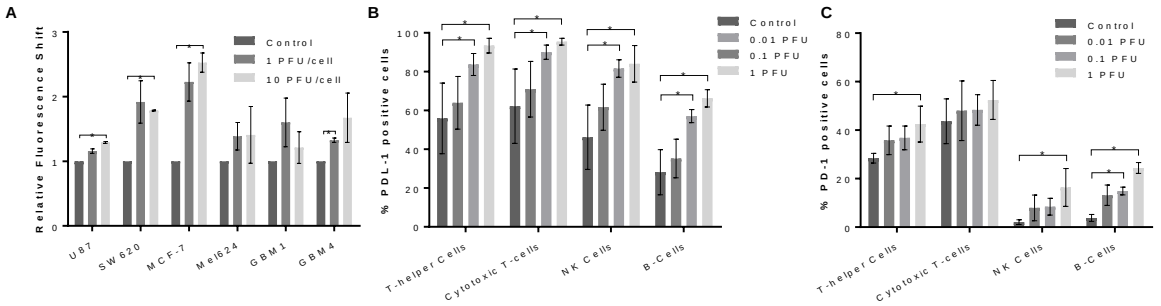
Supplementary Figure 7: CD68 tumor-infiltrating cells

Representative trial and control patient tumor sections stained for CD68 (brown) by IHC. Top row scale bar = 30 µm. Bottom row scale bar = 20 µm.



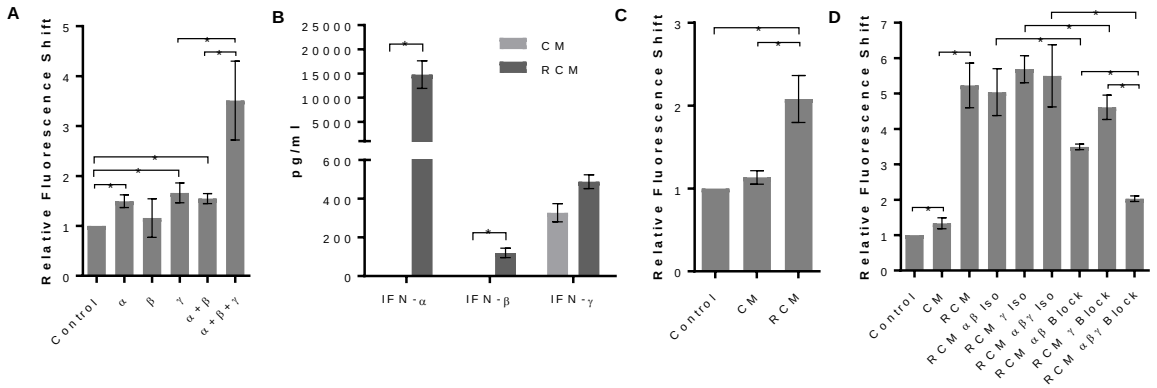
Supplementary Figure 8: Expression of cleaved caspase 3 and PD-L1 in brain tumor metastases after reovirus stimulation

A) Representative trial and control patient brain metastasis sections stained for cleaved caspase 3 (red) by IHC. Scale bars = 40 µm. **B)** Representative trial and control patient brain metastasis sections stained by IHC for PD-L1 (brown). Scale bars = 40 µm. **C)** Representative IHC sections of melanoma brain metastases stained for PD-L1 (red) from patient nine (41 % of cells positive for reovirus RNA) and patient five (11 % of cells positive for reovirus RNA). Note: brown is melanin. Scale bars = 40 µm.



Supplementary Figure 9: In vitro expression of PD-L1 and PD-1 in human-derived cell lines and healthy donor PBMCs after reovirus stimulation

A) Flow cytometry for PD-L1 on GBM1, GBM4, MCF-7, SW620, U87, and Mel624 cells after stimulation with 1 or 10 PFU/cell reovirus for 48 hours. **B)** Flow cytometry for PD-L1 on control patient-derived PBMC subsets after 24 hours of incubation of PBMCs with reovirus at the indicated PFU per cell. **C)** Flow cytometry for PD-1 on healthy donor PBMC subsets after 24 hours of incubation of PBMCs with reovirus at the indicated PFU per cell. Bars represent the mean of at least 3 PBMC donors, with standard deviation. * $P<0.05$.



Supplementary Figure 10: In vitro expression of PD-L1 on GBM1 cells after purified interferon or conditioned medium stimulation

A) Flow cytometry for PD-L1 on GBM1 cells after stimulation with combinations of purified interferon-α/β/γ for 24 hours, each at 100 pg/ml. **B)** ELISA for interferons secreted from fresh ex vivo HGG single-cell suspensions after control (CM) or reovirus treatment (RCM). **C)** Flow cytometry for PD-L1 on GBM1 cells after stimulation with ex vivo HGG-derived CM or RCM for 24 hours (at a concentration of 1:4 of conditioned medium to native medium). **D)** Flow cytometry for PD-L1 on GBM1 cells after stimulation using PBMC-derived CM or RCM for 24 hours (at a concentration of 1:4 of conditioned medium to native medium) with blockade of interferon-α+β/γ/ α+β+γ or equivalent isotope controls. Bars represent the mean of at least 3 repeats or CM/RCM donors, with standard deviation. * $P<0.05$.

Supplementary Table 1: Participant baseline clinical characteristics, grade 3 / 4 adverse events, and survival after reovirus infusion

Participant	Age	Baseline histology	Previous brain cancer therapy	Days to surgery	Grade 3/4 adverse events	PFS (days)	OS (days)
1	59	HGG	1.Surgery & TMZ chemoradiotherapy	6	Lymphopenia	40	128
2	72	Colorectal cancer	Nil	3	Lymphopenia; postural hypotension	77	335
3	45	HGG	1.Surgery & radiotherapy; 2.PCV; 3.Surgery	11	Lymphopenia; neutropenia	355	1043
4	48	HGG	1.Surgery & radiotherapy; 2.PCV 3.TMZ	7	Lymphopenia	395	431
5	64	Melanoma	Nil	11	Nil	977	1079
6	65	HGG	1.Surgery & TMZ chemoradiotherapy	6	Lymphopenia	158	469
7	65	HGG	1.Surgery & TMZ chemoradiotherapy; 2.CCNU; 3.Bevacizumab	17	Lymphopenia	83	118
8	66	HGG	1.Surgery & TMZ chemoradiotherapy	12	Nil	172	561
9	74	Melanoma	Nil	11	Nil	211	532

Days to surgery: number of days between reovirus infusion and surgery; HGG: high-grade glioma; TMZ: temozolomide; CCNU: lomustine; PCV: procarbazine, CCNU, and vincristine; PFS: progression-free survival (days from reovirus infusion to radiological progression); OS: overall survival (days from reovirus infusion to death).

Supplementary Table 2: Change in plasma inflammatory cytokines and chemokines after i.v. reovirus infusion

Inflammatory cytokine	Day 3 (fold change relative to baseline)	Standard error of difference in fold change	P-value
IL-3	1.375	0.05901	1.78E-05
IL-18	1.889	0.156	5.52E-05
M-CSF	1.873	0.09376	2.25E-07
MIF	20.07	7.325	0.020851
β-NGF	1.375	0.05901	1.78E-05
Chemokine			
CXCL1	1.708	0.1768	0.001306
HGF	2.015	0.2347	0.004947
IL-16	2.977	0.4357	0.000465
CCL7	1.185	0.03485	0.00011
CCL4	1.329	0.07656	0.000743
CXCL12	1.479	0.1193	0.001279

Supplementary Table 3: Presence of reovirus protein and RNA in resected brain tumors

Participant	IHC σ3 protein (%)	σ3 protein distribution	ISH RNA (%)	EM	qRT-PCR
1	7	100% tumor	17	+	-
2	13	95% tumor, 5% endothelial	43	+	+
3	4	94% tumor, 6% endothelial	6	+	-
4	0	-	15	+	+
5	0	-	11	+	Unavailable
6	2	Too few positive cells	6	+	+
7	0	-	0	+	+
8	2	Too few positive cells	7	+	Unavailable
9	27	96% tumor, 4% endothelial	41	+	-

% positive cells indicate the percent of tumor cells positive for reovirus RNA or σ3 protein, using the InForm system.

Supplementary Table 4: Ki67, cleaved caspase 3, immune cell infiltration, and PD-1 / PD-L1 expression in resected trial and control brain tumors

Participant	Dex (mg)	Ki67 (%)	Cleaved Casp 3	PD-L1	PD-1	CD8	CD68
Reo 1	8	18.3	1+	2+	2+	Weak	2+
Reo 2	2	29.8	2+	2+	2+	2+	3+
Reo 3	0	0.1	2+	2+	2+	Weak	2+
Reo 4	0	0.7	0	0	1+	Weak	1+
Reo 5	2	7.7	2+	1+	2+	3+	2+
Reo 6	2	13.2	2+	2+	2+	Weak	2+
Reo 7	0	4.4	0	Weak	0	0	3+
Reo 8	0	22.8	1+	1+	2+	3+	3+
Reo 9	8	32.6	2+	3+	2+	3+	3+
Control 1	0	ND	Rare + cells	0	0	0	1+
Control 2	0	0.5	0	0	0	0	Weak
Control 3	2	9.1	0	0	0	Weak	1+
Control 4	2	19.6	Rare + cells	0	0	Weak	Weak
Control 5	8	61.3	0	0	0	0	Weak
Control 6	0	ND	0	0	Weak	1+	1+

Dex: dose of dexamethasone in milligrams on the day of surgery.

Antigen staining was scored as follows: 0 = no + cells; weak = signal in <10 % of target cells;

1+ = signal in from 10 to 24 % of target cells; 2+ = signal in from 25 % to 50 % of target cells;

3+ = signal in >50 % of target cells. ND: not detected.

Supplementary Table 6: Enriched biological processes within genes differentially expressed between control and trial GBM tumors

Category name	Ratio of enrichment	adj-P value
Cellular response to chemical stimulus	3.58	0.0000336
Negative regulation of viral transcription	70.04	0.0000502
Regulation of cell communication	2.73	0.0002
Positive regulation of programmed cell death	5.66	0.0003
Negative regulation of cellular metabolic process	2.99	0.001
Cytokine activity	7.53	0.0129

adj-P: P value adjusted for multiple tests

Gene ontology chart available at:

http://www.bioinformatics.leeds.ac.uk/~bs06lw/Samson_et_al/DAG_1453455821.html