Online resource 1. Supplementary Information

Suppl. Table S1 Inclusion and exclusion criteria for the protocol.

Below criteria includes criteria for both patients allocated to surgery + treatment (Arm A) and treatment only (Arm B).

Inclusion criteria

1. Pathologically confirmed GBM (including all histologic variants).

2. Age ≥ 18 years.

3. Evidence of radiological (MRI-scan) measurable recurrent progressive GBM evaluated by the Response Assessment in Neuro-Oncology [RANO] criteria.

4. In arm B measurable disease according to the RANO guidelines, within 14 days of starting treatment. Measurable disease after surgery on arm A is not required with radiographic evidence of recurrent disease after treatment with temozolomide and radiotherapy.

5. An interval of at least 4 weeks between prior radiotherapy or chemotherapy and enrolment on this protocol.

6. Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) 0-2.

7. Life expectancy, in the opinion of the investigator > 3 months.

8. Written informed consent obtained prior to any screening procedures. Patients must be willing and able to comply with the protocol and aware of the investigational nature of this study.

9. Patients must have adequate bone marrow function and organ function within 2 weeks of study treatment as defined by the following laboratory criteria.

a. Hematopoietic function: total white blood cell count (WBC) ≥ 3000/mm³, absolute neutrophil count (ANC) ≥ 1500/mm³, platelet count ≥ 125,000/mm³; hemoglobin ≥ 9g/dL

b. Hepatic function: bilirubin < 1.5 times the upper limit of normal (ULN) (excluding Gilberts Syndrome, for which bilirubin must be < 4 times ULN), ALAT < 2.5 times ULN.

c. Renal function: serum creatinine < 1.5 ULN or estimated creatinine clearance of ≥ 50 ml/min, calculated using the formula of Cockcroft and Gault.

d. APTT and INR < normal limit

10. All female patients and partners of childbearing potential must agree to use adequate birth control during study treatment and for 5 months after the last dose of study drug and have a negative serum pregnancy test at screening. Acceptable methods of contraception are oral, implantable, or injectable contraceptives, contraceptive patch, intrauterine device, or a sexual partner who is surgically sterilized or post-menopausal.

11. Fertile males must be willing to employ adequate means of contraception during study treatment and for 7 months after the last dose of study drug.

12. Archived paraffin-embedded tissue (approximately 10 unstained slides or a tumor block) must be available for confirmation of tumor diagnosis and correlative studies.

13. Patients in the surgical arm (Arm A) must be predicted pre-operatively to have sufficiently sized recurrent tumor to allow for 500 mg of enhancing tumor and 300 mg of non-enhancing tumor to be resected.

14. Patients must be on a stable or decreasing dose of corticosteroids (or none) for at least 5 days prior to MRI and maximum of a dose of 20 mg prednisolone per day at enrollment of the study.

Exclusion criteria

1. Patients must not have significant medical illness that in the investigator's opinion cannot be adequately controlled with appropriate therapy or would compromise the patient's ability to tolerate this therapy.

2. Co-medication that may interfere with study results, e.g. immuno-suppressive agents other than corticosteroids (equivalent to max dose of 20 mg prednisolone per day) and stable for at least 5 days prior to day 1;

3. Any condition (medical, social, psychological), which would prevent adequate information and follow-up.

4. Any other active malignancy or previous malignancies within the last 5 years, except, adequately treated basal or squamous cell carcinoma of the skin, or carcinoma in situ.

5. Uncontrolled hypertension (systolic blood pressure (BP) > 150 mmHg and/or diastolic BP > 100 mmHg), unstable angina, congestive heart failure (CHF) of any New York Heart Association (NYHA) classification, serious cardiac arrhythmia requiring treatment (exceptions: atrial fibrillation, paroxysmal supraventricular tachycardia), history of myocardial infarction within 6 months of enrollment.

6. Clinically significant peripheral vascular disease

7. Evidence of bleeding diathesis, coagulopathy or taking ASA, NSAIDs or clopidogrel.

8. Patients with coagulation problems and medically significant bleeding in the month prior to start of treatment (e.g., peptic ulcer, epistaxis, spontaneous bleeding).

9. Major surgical procedure, open biopsy, or significant traumatic injury within 28 days prior to day 0, anticipation of need for major surgical procedure during the curse of the study.

10. Minor surgical procedures, fine needle aspirations or core biopsies within 7 days prior to day 0.

11. History of abdominal fistula, gastrointestinal perforation, or intra-abdominal abscess within 6 months prior to day 0.

12. Known active hepatitis A, B or C infection; or known to be positive for HCV RNA or HBsAg (HBV surface antigen); hepatitis testing is not required.

13. Known HIV infection; HIV testing is not required.

14. Active infection requiring parenteral systemic antibiotics.

15. Administration of a live, attenuated vaccine within 4 weeks before first dose of Nivolumab prior to surgery in Arm A or Cycle 1 Day 1 (Arm A and B) or anticipation that such a live attenuated vaccine will be required during the study. Influenza vaccination should be given during influenza season only (approximately October to March). Patients must not receive live, attenuated influenza vaccine (e.g., FluMist) within 4 weeks before first dose of Nivolumab prior to surgery in Arm A or Cycle 1 Day 1 (Arm A and B) or at any time during the study.

16. Severe infections within 4 weeks prior to Cycle 1 Day 1, including but not limited to hospitalization for complications of infection, bacteremia, or severe pneumonia.

17. Received oral or intravenous (IV) antibiotics within 2 weeks prior to Cycle 1 Day 1. Patients receiving prophylactic antibiotics (e.g., for prevention of a urinary tract infection or chronic obstructive pulmonary disease) are eligible.

18. Any other diseases, metabolic dysfunction, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that would contraindicate the use of an investigational drug;

19. Dementia or altered mental status that would prohibit informed consent.

20. History of organ allograft.

21. History or risk of autoimmune disease, including but not limited to systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, vascular thrombosis associated with antiphospholipid syndrome, Wegner´s granulomatosis, Sjogren's syndrome, Bell´s palsy, Guillain-Barre syndrome, multiple sclerosis, vasculitis, or glomerulonephritis.

22. History of idiopathic pulmonary fibrosis, pneumonitis (including drug induced), organizing pneumonia (i.e., bronchiolitis obliterans, cryptogenic organizing pneumonia, etc.), or evidence of active pneumonitis on screening chest CT scan. History of radiation pneumonitis in the radiation field (fibrosis) is permitted.

23. Pregnant or breast-feeding women.

24. Prior treatment with PD-1/PD-L1 inhibitors.

25. Known hypersensitivity to any of the components of Nivolumab or Bevacizumab.

26. Investigational therapy (defined as treatment for which there is no regulatory authority; within 28 days prior to Cycle 1 Day 1.

27. Any approved anti-cancer therapy, including chemotherapy or hormonal therapy, within 3 weeks prior to Cycle 1 Day 1, with the following exceptions: a. Hormone-replacement therapy or oral contraceptives

28. Treatment with systemic immunosuppressive medications including, but not limited to cyclophosphamide, azathioprine, methotrexate, thalidomide, and anti-TNF agents within 2 weeks prior to Cycle 1, Day 1. The use of inhaled corticosteroids and mineralocorticoids (e.g., fludrocortisone) for patients with orthostatic hypotension or adrenocortical insufficiency is allowed.

29. Concurrent therapy with approved or investigational anticancer therapeutics.

30. Body weight significantly below ideal body weight in the opinion of the investigator.

Suppl. Table S2. Patient characteristics, MRI and [^{18}F]FET PET and OS

ID	Sex	Age	IDH mut	MGMT meth.	Steroid at incl.	Series bev/niv	CEV (ml) baseline	CEV (ml) follow-up	CEV (%)	MTV (ml) baseline	MTV (ml) follow-up	MTV (%)	TBR_{mean} baseline	TBR_{mean} follow-up	TBR_{mean} (%)	TBR_{max} baseline	TBR_{max} follow-up	TBR_{max} (%)	PRM (ml) plus	PRM (ml) minus	RANO MRI	RANO PET	Non-enh.. T2/T2FLAIR	OS months
Non-responders (OS <11 months)																								
1	F	66	-	+	no	8	1.3	0.3	-77	2.8	0	-100	1.9	1.5	-20	2.5	1.5	-39	0	2.7	SD	CR	Stable	10.6
7	M	48	-	+	no	4	23	27.5	20	72.4	84.3	16	2.5	2.6	4	5.2	4.7	-10	52.2	19.5	PD	SD	Incr.	6.5
11	F	51	-	+	no	2	23.1	58.7	154	43.9	.	.	.	.	.	5.2	.	.	.	.	PD	.	Incr.	2.2
15	M	60	-	-	12.5 mg	2	11.6	0.6	-95	29.2	.	.	.	.	.	2.5	.	.	.	.	PR	.	Incr.	4.9
23	F	64	-	-	15 mg	4	3.4	0.6	-82	5.1	0.05	-99	1.8	1.7	-8	2.4	1.7	-30	0	5	PR	PR	Decr.	7.3
24	M	37	+	+	no	1	7.2	.	.	51.9	.	.	.	.	.	4.6	.	.	.	.	.	.	.	1.4
25	M	50	-	-	no	2	2.3	13.8	500	59.1	232	293	1.9	1.8	-4	3.1	3.2	4	188.3	25.4	PD	PD	Incr.	2.3
27	M	37	-	-	no	2	6.1	2.5	-59	59.3	50	-16	2.7	2.2	-18	6.7	5.2	-21	4.3	47.5	SD	PR	Incr.	3.6
28	M	65	-	+	12.5 mg	4	3.5	2.3	-34	11.1	11.3	2	2.1	2.2	3	3.8	3.9	3	6.3	3.1	SD	SD	Decr.	3.5
34	M	38	+	+	no	4	5.2	0.5	-90	37.4	71.5	91	1.8	2	10	2.4	3.3	36	49.0	21.3	PR	PD	Stable	6.3
36	M	55	-	-	no	4	17.2	6.3	-63	49.4	22.1	-55	2.1	1.9	-8	3.4	3.1	-8	12	42.1	PR	PD	Decr.	4.8
40	M	41	-	-	15 mg	4	7.5	34.4	359	24	129.2	438	2.1	2.3	11	4.2	4.4	5	121.6	3.1	PD	PD	Incr.	3.1
43	M	49	-	+	no	4	31.7	23	-27	76.1	53.4	-30	2.3	2.3	3	4	4.7	19	22.8	56.3	PD	PD	Decr.	5.9
Responders (OS>11 months)																								
4	F	57	-	+	12.5 mg	8	49.6	5.1	-90	111	7.2	-94	1.9	1.7	-9	3.2	2.1	-34	0.7	105.5	PR	PR	Decr.	14.1
9	M	55	+	+	no	10	9	1.4	-84	54.8	14.4	-74	2.2	1.9	-14	4.5	2.9	-36	0	51.2	PR	PR	Decr.	18.8
21	M	54	-	-	no	4	3.8	2.8	-26	18.7	6.6	-65	2.1	2	-4	3.8	2.9	-23	1.3	14.9	SD	PR	Decr.+Stable	13.2
29	F	23	+	+	no	4	0.2	2	900	1.1	2.1	91	2	1.8	-9	2.8	2.3	-19	1.2	0.3	PD	PD	Incr.	15.4
31	F	54	+	-	no	8	4.3	0.6	-86	7.4	0.7	-91	2	1.7	-12	2.9	2	-31	0	7.3	PR	PR	Decr.	13.9
41	F	64	-	+	no	15	2.5	0.3	-88	7.4	0.6	-92	1.8	1.7	-9	2.6	1.8	-30	0	6.2	PR	PR	Decr	24.8
42	M	45	-	-	no	6	5.1	5.2	2	18	12.5	-31	2.3	2.2	-5	4.6	4.1	-12	3.7	11.6	SD	SD	Decr	16.4
Groups median or n																								
Non-responders		50	2	7	4	4	7.2	4.4	-47	43.9	51.7	-7	2.1	2.1	0	3.8	3.6	-3	17.4	20.4	4/3/5[c]	3/2/5[c]	3/4/5[d]	4.8
Responders		54	3	4	1	8	4.3	2	-84	18	6.6*[b]	-74	2	1.8*[b]	-9	3.2	2.3*[b]	-30*[a]	0.7*[a]	11.6	4/2/1[c]	5/1/1[c]	6/0/1*[d]	15.4*[a]

Abbreviations: IDH mut isocitrate dehydrogenase mutated; MGMT meth methylguanine-DNA-methyltransferase methylated; bev bevacizumab, niv nivolumab; CEV contrast enhancing volume; MTV [^{18}F]FET tumour active volume; TBR_{mean} mean [^{18}F]FET tumour to background ratio; TBR_{max} maximal [^{18}F]FET tumour to background ratio; PRM parametric response mapping; RANO response assessment in neuro-oncology; OS overall survival; CR complete response; PR partial response; SD stable disease; PD progressive disease.

Footnotes: *[a]p<0.05 responders vs non-responders, *[b]p<0.05 follow-up vs baseline, [c] CR or PR/SD/PD, [d] decrease/stable/increase

Suppl. Table S3. Patient characteristics, and imaging metrics at baseline and follow – summary statistics and univariate Cox and ROC analysis

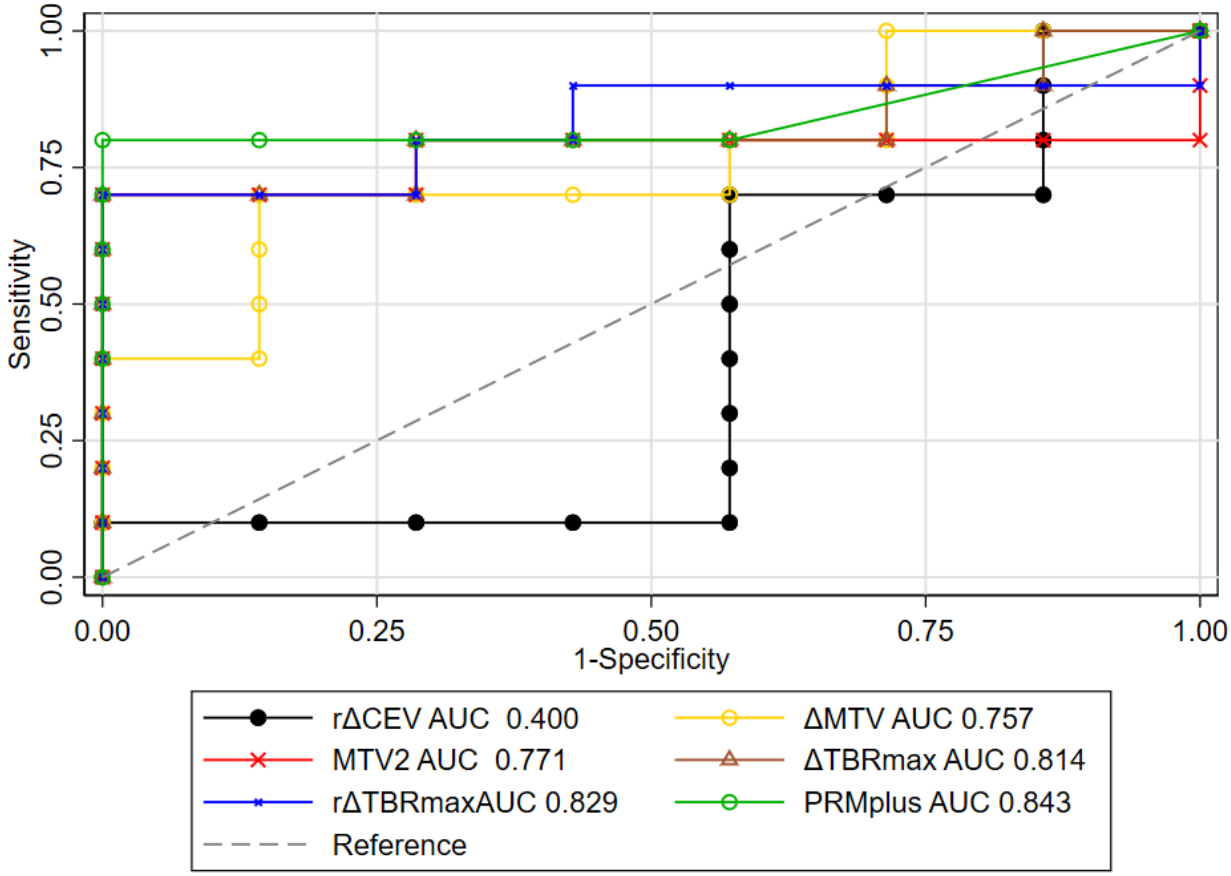
	Summary				Overall survival		ROC	
	All (n=20)	Non responders (n=13)	Responders (n=7)	p-val	HR	p-val	AUC	p-val
Clinical								
IDH mut, n (%)	5 (25)	2 (15.3)	3 (42.9)	(0.290)	0.695	(0.492)	0.363	(0.227)
MGMT meth, n (%)	11 (55)	7 (53.9)	4 (57.1)	(1.000)	0.639	(0.350)	0.484	(0.894)
Multifocal, n (%)	6 (30)	5 (38.5)	1 (14.3)	(0.354)	2.037	(0.173)	0.621	(0.228)
Steroid use, n (%)	5 (25)	4 (30.8)	1 (14.3)	(0.613)	1.707	(0.325)	0.582	(0.399)
PS 1, n (%)	2 (10)	2 (15.3)	0	(0.521)	2.121	(0.350)	0.577	(0.140)
Age, years	52.5 [23;66]	50 [37;66]	54 [23;64]	(0.103)	0.987	(0.490)	0.473	(0.849)
Baseline								
CEV (ml)	5.7 [0.2;49.6]	7.2 [1.3;31.7]	4.3 [0.2;49.6]	(0.405)	1.007	(0.671)	0.615	(0.435)
MTV (ml)	33.3 [1.1;111]	43.9 [2.8;76.1]	18.0 [1.1;111]	(0.285)	1.006	(0.395)	0.648	(0.329)
TBR_{max}	3.57 [2.36;6.67]	3.82 [2.36;6.67]	3.22 [2.56;4.62]	(0.782)	1.288	(0.245)	0.538	(0.776)
TBR_{mean}	2.08 [1.8;2.69]	2.08[1.8;2.69]	2.02 [1.84 2.31]	(1.000)	2.249	(0.493)	0.450	(0.741)
Follow-up								
CEV (ml)	2.5 [0.3;58.7]	4.4 [0.3;58.7]	2.0 [0.3;5.2]	(0.290)	1.073	**(0.002)**	0.649	(0.256)
MTV (ml)	12.5 [0;232]	51.7 [0;232]	6.6 [0.6;14.4]†	(0.064)	1.031	**(0.002)**	0.771	**(0.041)**
TBR_{max}	3.11† [1.51;5.24]	3.59 [1.51;5.24]	2.29 [1.78;4.06]†	(0.097)	1.682	**(0.038)**	0.743	(0.078)
TBR_{mean}	1.92 [1.51;2.56]	2.09[1.51;2.56]	1.84 [1.7;2.2] †	(0.283)	4.633	(0.115)	0.657	(0.263)
Change								
ΔCEV (ml)	-2.2 [-44.5;35.6]	-2.0 [-11;37.6]	-2.2 [-44.5;1.8]	(0.612)	1.048	(0.106)	0.571	(0.610)
ΔMTV (ml)	-5.5 [-104;173]	-1.3 [-27.3;173]	-6.8 [-104;1]	(0.079)	1.023	**(0.014)**	0.757	**(0.037)**
ΔTBR_{max}	-.0.56 [-1.63;0.86]	-0.09 [-1.43;0.86]	-0.86[-1.63;-0.53]	(0.032)	2.807	**(0.020)**	0.814	**(0.005)**
ΔTBR_{mean}	-0.14 [-0.47;0.24]	-.0.003 [-0.47;.24]	-0.17 [-0.31;-0.08]	(0.097)	39.65	(0.067)	0.743	(0.071)
Relative change								
rΔCEV (%)	-59 [-94.8;900]	-46.7 [-94.8;500]	-84.4 [-89.7;900]	(0.612)	1.000	(0.709)	0.571	(0.648)
rΔMTV (%)	-30.6 [-100;438]	-6.9 [-100;438]	-73.7 [-93.5;90.9]	(0.143)	1.007	**(0.006)**	0.714	(0.129)
$r\Delta TBR_{max}$ (%)	-18.9 [-38.8;0.35.7]	-2.76 [-0.38.8;37.7]	-30.5 [-36.3;-12.1]	**(0.025)**	1.034	**(0.012)**	0.829	**(0.003)**
$r\Delta TBR_{mean}$ (%)	-7.8 [-20.3;11.4]	-0.5 [-20.3; 11.4]	-8.6 [-14;-3.8]	(0.097)	1.084	**(0.045)**	0.743	(0.071)
PRM metrics								
PRM_{plus} (ml)	3.7 [0;188]	17.4 [0;188]	0.7 [0;3.7]	**(0.018)**	1.040	**(0.003)**	0.843	**(0.001)**
$rPRM_{plus}$ (%)	18.8 [0;92.3]	35.5 [0;92.3]	0.6 [0;54.5]	(0.093)	1.026	**(0.014)**	0.743	(0.054)
PRM_{minus} (ml)	14.9 [0.3;106]	20.4 [2.7;56.3]	11.6 [0.3;106]	(1.000)	1.000	(0.958)	0.500	(1.000)
$rPRM_{minus}$ (%)	67.6 [2.4;100]	41.9 [2.4;98]	86.1 [13.6;100]	(0.172)	0.985	**(0.050)**	0.300	(0.154)
PRM_{net} (ml)	-6.2 [-105;163]	0.3 [-43.2;163]	-7.9 [-105;0.9]	(0.079)	1.019	**(0.019)**	**0.757**	**(0.040)**
$rPRM_{net}$ (%)	-50.6 [-100; 89.9]	-6.4 [-98;.89.9]	-86.1 [-100;40.9]	(0.143)	1.010	**(0.029)**	0.714	(0.122)

Suppl. Table S4. MRI and PET RANO response assessment in subset with PET follow-up

	All	Responder			Cox OS		ROC	
		No	Yes	p-value	HR	p-value	AUC	p-value
PET RANO measurable disease	n=17	n=10	n=7					
MRI-RANO 2.0								
Response (yes/no), n	7/10	3/7	4/3	0.359	0.435	0.132	0.364	0.284
Progression (yes/no), n	5/12	4/6	1/6	0.338	2.241	0.153	0.629	0.236
Combined [a], n	7/5/5	3/3/4	4/2/1	0.604	1.717	0.090	0.671	0.188
PET-RANO 1.0								
Response [b] (yes/no), n	8/9	3/7	5/2	0.153	0.407	0.092	0.293	0.084
Progression (yes/no), n	6/11	5/5	1/6	0.604	2.824	0.062	0.679	0.104
Combined [a,b], n	8/3/6	3/2/5	5/1/1	0.178	1.670	0.077	0.693	0.143
MRI RANO measurable disease	n=15	n=9	n=6					
MRI-RANO 2.0								
Response (yes/no), n	7/8	3/6	4/2	0.231	0.393	0.109	0.333	0.215
Progression (yes/no), n	3/12	3/6	0/6	0.338	4.305	0.058	0.667	0.046
Combined [a], n	7/5/3	3/3/3	4/2/0	0.329	2.333	0.040	0.722	0.069
PET-RANO 1.0								
Response [b] (yes/no), n	8/7	3/5	5/1	0.084	0.327	0.054	0.250	0.034
Progression (yes/no), n	4/11	4/5	0/6	0.092	8.165	0.017	0.722	0.011
Combined [a,b], n	8/3/6	3/2/5	5/1/1	0.178	2.367	0.030	0.741	0.061

Abbreviations PRM parametric response mapping; RANO response assessment in neuro-oncology; OS overall survival, ROC receiver operating characteristics; AUC area under curve.

Footnotes: [a] response/stable/progression; [b] response includes both complete response (n=1) and partial response.



Suppl. Figs S1. ROC curves for prediction of response. ROC curves for parameters with ROC AUC significantly ($p<0.05$) different from 0.5: metabolically active at follow-up (MTV2) and absolute change from baseline (ΔMTV), absolute (ΔTBR_{max}) and relative ($r\Delta TBR_{max}$) change in maximal tumour-to-background ratio, and progressive volume (PRM_{plus}) identified by parametric response mapping. ROC curve for percent change in contrast enhancing volume (rΔCEV) for patients with also PET follow-up (n=17) is shown for comparison. ROC AUC are not significantly different, except for PRM_{plus} vs rΔCEV ($p=0.04$).