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FOCUS | LETTERS
SUPPLEMENTARY INFORMATION -5

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Neoadjuvant nivolumab modifies the tumor immune microenvironment in resectable glioblastoma

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Supplementary Table 1: main inclusion and exclusion criteria of the protocol.

INCLUSION CRITERIA	EXCLUSION CRITERIA
Age ≥ 18 years and ECOG of 0-1.	Prior malignancies except basal cell carcinoma or scaly skin, cervical carcinoma in situ adequately treated or other tumors treated curatively without recurrence for 3 or more years.
Ability to provide informed consent and express their desire to fulfill all protocol requirements during the study period.	Suspected or known autoimmune disease. Subjects with vitiligo, type I diabetes mellitus, residual hypothyroidism due to autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger were permitted to enroll.
Patients with histologically confirmed glioblastoma, either requiring salvage surgery to treat relapsed disease or undergoing primary resection.	Previous treatment with a PD-1, P-DL1 or targeted therapy.
Patients in whom surgery can be safely delayed for a minimum period of 2 weeks following the administration of the first dose of nivolumab.	Positive serology for HIV, hepatitis B (HBsAg) or hepatitis C.
The patient should, in the investigator's opinion, be able to comply with all clinical trial requirements.	Conditions requiring systemic treatment with either corticosteroids (>10 mg daily prednisone equivalents), with the exception of control of cerebral edema, or other immunosuppressive medications within 14 days of study drug administration. Inhaled or topical steroids and adrenal replacement doses > 10 mg daily prednisone equivalents were permitted in the absence of active autoimmune disease.
Negative pregnancy test for women of childbearing age and adequate contraceptive measures.	Pregnant or lactating women

Supplementary Table 2: summary of patient characteristics.

Patient characteristics	n=29 patients
Age at diagnosis (years), median (range)	54 (33-73)
Gender, n (%)	Male = 19 (65.5%) Female = 10 (34.5%)
GBM cohort, n (%)	Primary = 3 (10.4%) Relapsed = 26 (89.6%)
Number of previous treatments, n (%)	0 = 3 (10.4%) 1 = 12 (41.3%) ≥2 = 14 (48.3%)
Tumor characteristics	
MGMT methylation status n (%)	Methylated = 10 (34.5%) Unmethylated = 14 (48.3%) Unknown = 5 (17.2%)
IDH mutation status n (%)	Not Mutated = 26 (89.6%) Mutated = 3 (10.4%)
EGFR amplification status n (%)	Amplified = 11 (38%) Not Amplified =12 (41.3%) Unknown = 6 (20.7%)
Treatment characteristics	
Neoadjuvant nivolumab, n (%)	29 (100%)
Surgery, n (%)	29 (100%)
Extent of tumor resection, n (%)	Complete = 14 (48.3%) Nearly Complete = 4 (13.7%) Partial = 11 (38%)
Number of Nivolumab doses after surgery, median (range)	7.2 (1-50)
Status at end of study, n (%)	Alive = 3 (10.4%) Death from tumor = 22 (75.9%) Death from other cause= 4 (13.7%)
Subsequent treatment, n (%)	No treatment = 15 (51.7%) BVZ (alone or in combination) = 9 (34.6%) Other= 4 (13.7%)

*excluding patients with primary diagnosis

Supplementary Table 3: individual patient characteristics.

Patient number	Cohort	Gender	Age	Previous treatment	Nivolumab presurgery	Resection	MGMT promoter	IDH1 mutation	EGFR amplification	Number of nivolumab doses post-surgery	Status	Subsequent treatment	PFS pre nivolumab (days)	PFS (days)	OS (days)
001	Recurrent	M	41	Stupp/S+BVZ	Yes	Complete	Methylated	No	No	3	DT	No	235	65	84
003	Recurrent	M	49	Stupp	Yes	Partial	Methylated	IDH1 R132	No	19	DT	BVZ	2310	322	353
004	Primary	F	65	N/A	Yes	Complete	Methylated	No	No	50	A	NA	NA	+876	+876
005	Recurrent	F	38	Stupp/F	Yes	Partial	Unmethylated	No	Yes	3	DT	BVZ	297	101	179
007	Recurrent	M	42	Stupp	Yes	Complete	Unmethylated	No	Yes	3	DO	No	257	130	130
008	Recurrent	M	71	stupp	Yes	Complete	Methylated	No	No	5	DT	No	167	93	282
009	Recurrent	M	73	stupp	Yes	Partial	Methylated	No	No	2	DT	No	403	556	556
010	Recurrent	M	51	Stupp+DC/Lytix V	Yes	Nearly Complete	Methylated	No	Yes	4	DT	RT	319	78	119
011	Recurrent	F	57	Stupp/S+PVC	Yes	Partial	Unmethylated	No	Yes	7	DT	No	161	119	238
012	Recurrent	M	60	Stupp/Lytix V	Yes	Partial	Methylated	No	No	2	DT	No	84	83	207
013	Recurrent	M	65	S+TMZ	Yes	Partial	Unmethylated	No	Yes	2	DT	No	134	42	42
014	Primary	M	73	N/A	Yes	Partial	Unmethylated	No	UK	3	DT	UK	NA	124	313
015	Recurrent	M	32	Stupp/F+IRIN+BVZ	Yes	Partial	Unmethylated	No	No	3	DO	No	313	91	91
017	Recurrent	F	41	Stupp	Yes	Complete	UK	No	UK	1	DT	TMZ	144	35	158
018	Recurrent	F	64	Stupp/BVZ	Yes	Complete	UK	IDH1 R132	UK	4	DT	BVZ + IRIN	264	71	527
019	Recurrent	M	67	Stupp/S+BVZ	Yes	Complete	Unmethylated	No	Yes	6	DT	BVZ	226	105	207
020	Recurrent	M	59	Stupp/TMZ+BVZ	Yes	Nearly Complete	Unmethylated	No	No	11	DO	No	91	231	231
021	Recurrent	F	57	Stupp	Yes	Partial	Unmethylated	No	No	8	DT	No	481	159	159
022	Primary	F	33	N/A	Yes	Complete	Methylated	IDH1 R132	No	35	A	NA	N/A	+733	+733
023	Recurrent	M	59	Stupp	Yes	Complete	UK	No	Yes	8	DT	No	223	165	191
024	Recurrent	M	59	Stupp/BVZ	Yes	Nearly	Unmethylated	No	No	3	DT	No	210	82	162

						Complete									
025	Recurrent	M	45	S/Stupp	Yes	Partial	Methylated	No	No	7	DT	No	456	125	219
026	Recurrent	M	53	Stupp/F	Yes	*	Methylated	No	Yes	0	C	UK	122	14	14
027	Recurrent	M	54	Stupp / Lytix V+ F + IRIN / BVZ	Yes	Nearly Complete	Unmethylated	No	UK	4	DT	BVZ	259	85	228
028	Recurrent	F	54	Stupp	Yes	Complete	Unmethylated	No	Yes	5	DT	No	283	221	221
029	Recurrent	M	43	Stupp	Yes	Complete	UK	No	Yes	6	A	BVZ	230	123	+769
030	Recurrent	M	41	Stupp/S+F	Yes	Partial	Unmethylated	No	Yes	3	DT	BVZ	159	77	343
032	Recurrent	F	61	Stupp	Yes	Complete	UK	NO	UK	4	DT	BVZ + IRIN	239	75	236
033	Recurrent	F	66	Stupp	Yes	Complete	Unmethylated	No	Yes	4	DO	No	186	156	156
034	Recurrent	M	66	Stupp	Yes	Complete	Methylated	NO	UK	3	DT	BVZ	239	160	182

M: male. F: female. Stupp: Stupp´s regimen (surgery followed by radiation +temozolamide). S: surgery. F: fotemustine, BVZ: bevacizumab. Lytix V: oncolytic adenovirus. PVC: procarbazine, lomustine and vincristine. IRIN: irinotecan. TMZ: temozolamide. UK: unknown. DT: death from tumor. A: alive. DO: death from other cause. TTP: Time to progression. C: censured (lost to follow-up). NE: non-evaluable. NA: not applicable. Nivo: nivolumab. UK: unknown (due to paucity of sample). OS: overall survival. PFS: Progression-free survival.

Supplementary Table 4: nivolumab related toxicity.

Adverse Events (n=30patients)	Grade 1	Grade 2	Grade 3
Liver function tests	1 (3%)	1 (3%)	1 (3%)
Rash	1 (3%)	-	-
Anemia	-	1 (3%)	-
Autoimmune primary hyperthyroidism	-	1 (3%)	-
Tumor bleeding	-	-	2 (6%)
Total number of patients	**2 (6%)**	**3 (9%)**	**3 (9%)**

Supplementary Table 5: clinical characteristics of patients included in the control group.

N.code	Gender	Age	Most recent prior treatment	Interval between surgeries (months)	Subsequent treatment	PFS post salvage surgery (months)	OS post salvage surgery (months)	MGMT methylation	IDH mutation	EGFR amplification
1	male	44	Stupp	57	CT - BVZ, RT	4	7	Unmethylated	No	No
2	male	28	CT + BVZ	10	No	1	1	Unmethylated	No	UK
3	male	54	Stupp	11	CT	1	9	Unmethylated	No	UK
4	male	65	Stupp	11	UK	3	3	Unmethylated	No	No
5	male	71	Stupp	24	RT, BVZ	6	6	Methylated	No	Yes
6	female	45	Stupp	19	CT + BVZ	3	3	Methylated	No	No
7	male	68	Stupp	5	CT+BVZ	6	11	Unmethylated	No	No
8	female	70	Stupp	6	BVZ	23	29	Methylated	No	UK
9	female	39	Oncolytic virus, CT	3	BVZ	2	3	Unmethylated	No	No
10	female	62	Stupp	7	CT	41	41	Methylated	No	No

Stupp: Stupp´s regimen (surgery followed by radiation + temozolamide). RT: radiation therapy. CT: chemotherapy. BVZ: bevacizumab. UK: unknown.

Supplementary Table 6: Monoclonal antibodies used for flow cytometry

Material	Vendor	Catalog #
CD127-AF488	Biolegend	351314
CD4-PerCP-Cy5.5	Biolegend	317428
CD16-FITC	BD Bioscience	555406
CD56-PE	BD Bioscience	345612
CD3-PerCP-Cy5.5	Biolegend	300430
HLADR-PE-Cy7	Biolegend	307616
CD8-APC	BD Bioscience	555369
CD4-PB	Biolegend	300521
CD45-V500	BD Bioscience	560777
CD33-FITC	Biolegend	303304
CD11b-PerCP-Cy5.5	Biolegend	301327
CD45-PE-Cy7	Biolegend	304016
CD14-BV421	Biolegend	301830
CD15-BV510	Biolegend	323027
HLADR-FITC	BD	347400
CD69-PE	Biolegend	310906
PD1-PerCP/EF710 (Clone MIH4)	Ebioscience	46-9969-42
PD1-PerCPCy5.5 (Clone EH12.2H7)	Biolegend	329913
CD3-PE-Cy7	Biolegend	317333
CD137-APC	Biolegend	309810
HLADR-APC	Biolegend	307610
CD8-BV510	Biolegend	301048
CD25-APC	BD	340907
CD3-V450	BD	580365
CD16-PE	Biolegend	302008
mIgG1-APC	Biolegend	400121
mIgG1-PE	Biolegend	400112
IgG1 PerCP-Cy5.5	Biolegend	400150
IgG1 k-FITC	Biolegend	400110
IgG1 k PerCPEF710	EBiosciencec	46-4714-82
Zombie	Biolegend	54423106