Table W1. Summary of Clinical and Molecular Data of 73 Primary GBMs at First Surgery.

Patient No.	Age (Years)/Sex	KPS	RPA Class	Surgical Resection	Ki67 (%)	EGFRvIII	MGMT	PTEN	Overall Survival (Months)
1	54/F	80	IV	P	40	+	M	+	35
2	72/F	40	VI	T	40	–	M	+	3.5
3	42/F	90	III	T	15	+	M	+	75
4	69/M	80	IV	T	40	+	M	+	12.5
5	56/M	50	VI	T	15	–	UM	–	2
6	47/M	70	IV	P	35	–	M	–	12.5
7	56/M	90	IV	T	20	+	UM	+	12
8	66/M	90	IV	T	40	–	UM	–	12
9	51/F	50	V	T	50	–	UM	+	11
10	75/F	50	V	P	20	+	UM	–	5.5
11	61/M	60	VI	T	40	–	UM	–	9
12	56/M	60	V	T	25	+	M	–	23
13	61/M	50	VI	T	30	–	UM	+	2
14	59/F	60	V	T	50	+	UM	+	10
15	77/F	50	V	T	20	+	UM	+	6.5
16	30/F	90	III	T	50	+	M	+	55
17	77/M	90	V	T	60	+	UM	–	9
18	69/M	70	V	T	50	–	M	+	4
19	72/F	80	V	T	5	+	UM	–	7.5
20	76/M	90	V	P	8	–	UM	+	7.5
21	62/F	80	V	T	20	+	UM	+	6
22	47/M	90	III	T	30	–	M	–	7
23	49/M	70	IV	P	15	+	M	+	36
24	64/F	50	VI	P	35	–	M	+	6
25	53/F	70	V	T	25	–	UM	+	15
26	67/F	50	V	T	20	–	UM	–	5
27	58/F	80	V	P	25	+	UM	+	2
28	51/F	50	V	T	20	–	UM	–	21
29	68/M	80	V	T	15	+	UM	+	24
30	64/F	60	VI	T	10	+	M	+	19
31	55/M	80	V	P	30	+	M	+	7
32	46/M	60	IV	P	10	–	M	–	6.5
33	72/M	40	V	T	20	–	UM	+	11
34	54/F	60	V	T	5	+	UM	+	18
35	48/F	70	IV	T	15	–	M	+	6
36	58/M	60	V	T	10	–	UM	+	10.5
37	51/M	90	IV	P	25	–	UM	+	12.5
38	73/M	50	VI	T	25	–	UM	–	2
39	66/M	90	V	P	10	–	UM	–	11
40	59/M	80	V	T	35	+	UM	+	6
41	64/M	80	V	T	10	–	M	+	14
42	74/M	60	VI	T	15	–	M	–	5.5
43	50/M	90	IV	T	30	–	M	–	11
44	62/F	60	VI	T	35	+	M	–	19
45	20/M	90	III	T	20	+	M	–	27.5
46	70/F	60	V	T	15	+	UM	+	8
47	70/M	50	VI	T	20	–	M	+	9
48	66/M	30	VI	T	25	–	M	+	3.5
49	52/F	60	V	T	20	+	M	–	26
50	64/M	90	IV	T	15	–	M	–	15
51	53/M	80	V	T	30	+	UM	+	11
52	61/M	90	IV	T	15	–	M	–	22
53	58/M	70	V	T	35	+	UM	–	12.5
54	56/F	90	IV	T	25	+	UM	–	12
55	52/M	60	VI	T	50	+	UM	+	10
56	67/M	70	V	T	30	–	M	–	7.5
57	75/F	30	VI	T	50	–	UM	–	2
58	48/M	80	IV	T	50	+	M	–	4
59	67/F	60	V	T	20	+	UM	–	20
60	71/F	60	V	P	20	–	M	+	3
61	76/F	60	V	P	25	+	M	–	4
62	52/M	90	IV	T	30	–	UM	+	20
63	69/F	40	VI	T	25	+	UM	–	3
64	48/M	30	IV	T	25	–	UM	–	2
65	69/F	60	VI	T	25	–	UM	+	11
66	52/M	60	V	P	20	–	UM	+	19
67	58/M	40	VI	T	30	–	UM	+	8
68	55/M	50	V	T	50	–	UM	+	2
69	68/M	50	VI	T	25	+	UM	–	8
70	80/M	70	V	T	25	+	M	+	4.5
71	33/M	50	IV	T	20	+	M	+	65
72	67/F	60	V	T	10	–	UM	+	10
73	59/M	70	IV	T	10	+	M	+	18

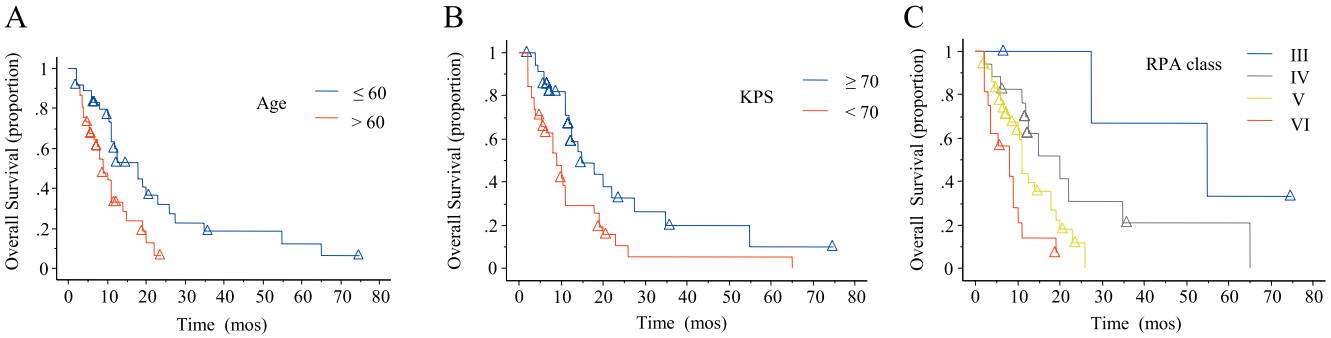

Figure W1. Kaplan-Meier survival curves of 73 GBM patients stratified by age, KPS, and RPA class. (A) Age 60 years or younger (blue line) conferred a favorable survival advantage ($P = .0069$). (B) KPS score of 70 or higher (blue line) was related with longer OS ($P = .0035$). (C) RPA classes III-IV (blue and gray lines) were associated with longer OS ($P = .0007$). There were no differences in survival times between the RPA class V (yellow line) and VI (red line).

Table W2. Summary of Clinical and Molecular Data of 14 Recurrent GBMs.

Patient No.	KPS	RPA Class	Time for Recurrence (Months)	Ki67 (%)	EGFRvIII	MGMT	PTEN	Survival after Recurrence (Months)
1	70	V	28	40	+	M	+	7
12	60	V	18	20	+	M	–	5
16	80	IV	48	50	+	UM	+	7
23	70	IV	26	12	+	UM	+	10
28	50	V	17	25	–	M	–	4
30	60	VI	13	10	+	M	+	6
43	80	V	9	30	–	M	–	2
50	90	IV	8	25	–	M	–	7
51	80	V	7	40	+	UM	+	4
52	80	V	13	20	–	M	–	9
55	60	VI	8.5	40	+	UM	+	1.5
59	60	V	17	20	+	UM	–	3
71	50	IV	58	30	+	M	+	7
73	60	V	12	25	+	M	+	6

Table W3. Multivariate Analysis for OS.

Covariate	*b*	SE	*P*	Exp(*b*)	95% CI of Exp(*b*)
EGFRvIII	−0.8330	0.3346	**.0128**	0.4347	0.2264-0.8349
Age	0.7596	0.3218	**.0182**	2.1374	1.1412-4.0033
KPS	−0.8524	0.3069	**.0055**	0.4264	0.2344-0.7757
Ki67	0.8817	0.2993	**.0032**	2.4150	1.3472-4.3292
MGMT status	−0.3326	0.3334	.3186	0.7171	0.3743-1.3739
PTEN expression	0.1591	0.3018	.5979	1.1725	0.6509-2.1120
Resection	0.0679	0.4316	.8749	1.0703	0.4613-2.4832

In **bold** the statistically significant $P(P < .05)$.

Table W4. Molecular Profile of Parent GBM and Neurosphere Cell Cultures.

No.	GBM Neurosphere Cultures								
	Parent GBM Tissue			Culture A			Culture B		
	EGFRvIII	MGMT	PTEN	EGFRvIII	MGMT	PTEN	EGFRvIII	MGMT	PTEN
1	Positive	M	Normal	Negative	UM	Normal	Positive	UM	Normal
2	Positive	M	Normal	Positive	M	Normal	Negative	M	Normal
3	Negative	UM	Low	Negative	M	Low	Negative	UM	Low

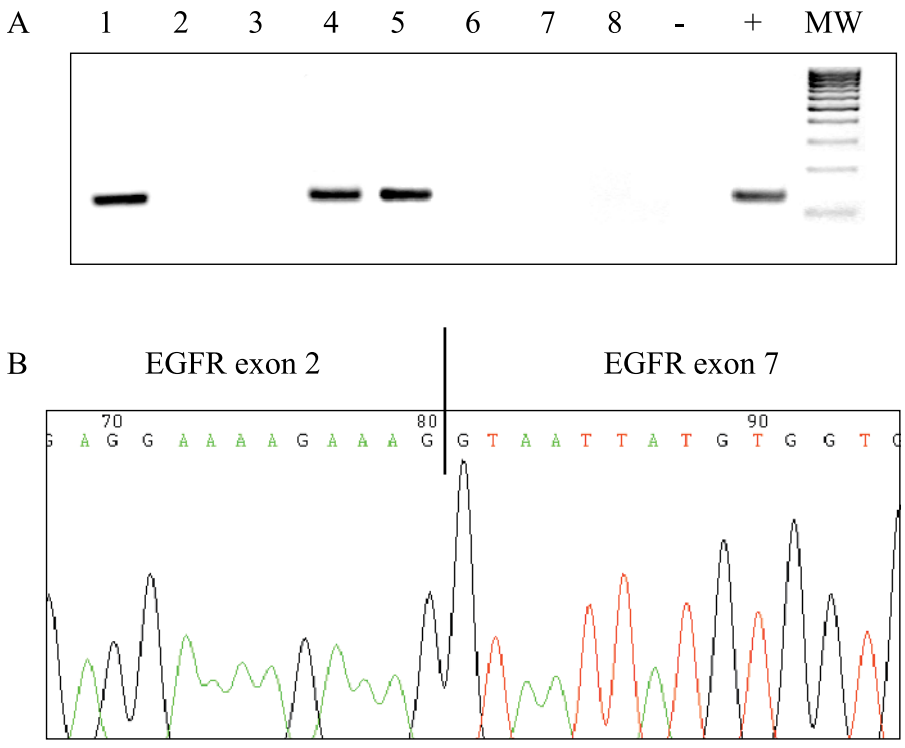

Figure W2. RT-PCR assessment for EGFRvIII on selected regions of formalin-fixed paraffin-embedded tumor specimens. (A) EGFRvIII RT-PCR performed on eight GBMs representative cases. The EGFRvIII is present only in samples 1, 4, and 5. Water was used as negative control (−) and plasmid containing EGFRvIII as positive control (+). MW indicates molecular weight marker. (B) Partial sequence of EGFR cDNA showing the deletion of EGFRvIII.

Table W5. Summary of Studies on EGFRvIII Expression and Prognosis of GBM Patients.

Author, Year	No. Cases	Technique	Results	Proposed Mechanism
Feldkamp et al., 1999*	12	IHC, RT-PCR, Western blot analysis	Worse prognosis (ns)	None
Shinojima et al., 2003[†]	87	IHC	Worse prognosis (s)	EFGR amplification
Aldape et al., 2004[‡]	105	IHC, RT-PCR	No prognostic value, worse prognosis in AAs	EGFRvIII as marker of GBM-like cells in AAs
Heimberger et al., 2005[§]	196	IHC	Worse prognosis for patients surviving >1 year	Cell proliferation (ns), ependymal involvement (ns)
Liu et al., 2005[¶]	160	RT-PCR	No prognostic value	Older age in AAs
Heimberger et al., 2005[#]	54	IHC	No prognostic value	None
Mellinghoff et al., 2005**	50	IHC, RT-PCR, Western blot analysis	Better prognosis in the erlotinib arm	PTEN coexpression
Pelloski et al., 2007[††]	509	IHC	Worse prognosis (s)	None
Viana-Pereira et al., 2008[‡‡]	27	IHC	No prognostic value	None
Brown et al., 2008[§§]	81	IHC	No prognostic value	None
van den Bent et al., 2009[¶¶]	49	IHC	No prognostic value, worse prognosis in the erlotinib arm	None
Thiessen et al., 2010[##]	16	Real-time PCR	No prognostic value	None
Reardon et al., 2010***	20	IHC	No prognostic value	None

AA indicates anaplastic astrocytoma; IHC, immunohistochemistry; ns, not significant; s, significant.

*Feldkamp MM, Lala P, Lau N, Roncari L, and Guha A (1999). Expression of activated epidermal growth factor receptors, Ras-guanosine triphosphate, and mitogen-activated protein kinase in human glioblastoma multiforme specimens. *Neurosurgery* **45**, 1442–1453.

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