Abstract 167 Figure 3  GBM patient-derived BVax
GBM patient-derived BVax promote anti-tumor CD8+ T cells. (A) Paired fresh peripheral blood and tumor were collected from newly diagnosed GBM patients (n=4). BVax were generated and pulsed with tumor lysates and co-cultured with autologous eFluor450-labeled CD8+ T cells. CD8+ T-cell activation was assessed by cell proliferation (Efluor450 fluorescence dilution measured as expansion index) and intracellular expression of GzmB. (B and C) Paired samples from primary GBM IDH WT (case NU 02120, B) and recurrent GBM IDH WT (NU02265, C). BVax-activated autologous CD8+ T cells were obtained as shown in (A) and tested for their ability to kill autologous glioma cells. Cell killing measurement were taken periodically for 12.5 hours using the IncuCyte S3 Live Cell Analysis System. Statistical significance is depicted as ns: no statistically significant, *p<0.05, **p<0.01, ***p<0.001.

Abstract 167 Figure 4  BVax produce anti-tumor IgG
BVax produce tumor-reactive antibodies with therapeutic effect. (A) Schema of BVax-derived serum immunoglobulin (Ig) obtained. BNaive, BAct and BVax-derived IgG were tested for IgG subtype (top) and their reactivity to B KO tumor bearing-brains (bottom). Top: Diagram representing the distribution of different Ig subtypes from serum antibodies derived from BNaive, BAct and BVax. Ig subtype measurement of serum samples was performed by ELISA, and mean total Ig concentration is shown in the bottom of the diagram (mg/ml). The experiment was performed in 7 mice/group. Bottom: B-cell subsets IgG reactivity was measured by immunofluorescence. Serum samples were incubated on tumor-bearing brains sections from B KO. Binding IgG was detected using anti-mouse IgG Cy5 (red) secondary antibody. Nuclei was detected using DAPI (blue), and myeloid cells were evaluated by using anti-mouse CD11b AF488 antibody (green). Bars represent 100 mm. Shown, a representative experiment of serum obtained in 4 mice/group, performed twice independently. (B) BNaive, BAct and BVax were generated from GL261 overexpressing ovalbumin (GL261-OVA) tumor-bearing mice. B cells were allowed to produce antibodies in GL261-OVA-bearing B KO. Serum samples were collected and IgG were purified and tested for their reactivity against OVA peptide SIINFEKL by ELISA. Semi-quantitative measurement is shown as optical density (O.D). Serum from B-cell deficient mice and C57BL/6 SIINFEKL-immunized mice were used as negative and positive control respectively (n=4/group). (C) Purified IgG were tested for their ability to kill autologous glioma cells. Experimental groups received either BNaive-derived IgG (BNaive IgG, blue line) or BVax-derived IgG (BVax IgG, pink line). The experiment was performed using n=10 mice/group. Statistical significance is depicted as ns: no statistically significant, *p<0.05, **p<0.01, ***p<0.001.

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In conclusion, BVax tackles GBM immunosurveillance escape by using both cellular (CD8+ T-cell activation) and humoral (anti-tumor antibody production) immunity. Our study provides an efficient alternative to current immunotherapeutic approaches that can be readily translated to the clinic.

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