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Survival gain in glioblastoma patients treated with dendritic cell immunotherapy is associated with increased NK but not CD8⁺ T cell activation in the presence of adjuvant temozolomide

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conditions.^{19,35,36} On day 15 after surgery, leukapheresis and basal clinical, radiological and immune testing were performed. Delayed-Type Hypersensitivity (DTH) skin reactions, injecting Ag purified tuberculin as control and 10 mg of inactivated tumor lysate, were tested before and after vaccinations 1-4. The first 4 vaccinations with tumor lysate loaded DC were performed every two weeks, from week 9 to 15. After the fourth vaccine, MRI was performed. Vaccinations 5 and 6 were spaced one month (week 19 and 23, respectively). The last vaccine dose (the 7th) was on week 31. At each vaccine injection, clinical and immune monitoring was performed. From the end of immunotherapy on, MRI, clinical and immune monitoring were continued every 2 months. The 1st, 5th, 6th and 7th vaccines contained 10 million DC; the 2nd, 3rd and 4th vaccines 5 million DC. Adjuvant TMZ started immediately after 3rd vaccination and continued for 6 cycles (Fig 1A).

MRI and response evaluation. Patients underwent conventional contrast enhanced MRI (see Supplementary Data for detailed radiological protocol) within two days after surgery, within two days before the first vaccination, every two months, or in case of clinical worsening. Tumor volumes were determined on the 3D post gadolinium T1 weighted images by manually outlining the enhancing portion of the lesion in MRIcro (http://www.mricro.com). To calculate the total enhancing volume of the tumor, the number of enhancing voxels was multiplied by the voxel size. Disease progression was defined according to RANO criteria.³⁷

Immune monitoring. Immune monitoring was performed on the whole blood of each patient before, during and after DC vaccinations. The immune responses were assessed before the treatment, after each vaccination and at the end of the treatment until tumor recurrence. Eight patients were re-operated (Pt 2, 8, 9, 10, 14, 15, 16, 24). No adequate/sufficient material was obtained from surgery of Pt 2 and 17; Pt 24 underwent surgery in another institute. Tumor infiltrating immune cells were isolated by tumor specimens obtained from Pt 8, 9, 10, 14 and 15 using human Tumor Dissociation Kit in combination with GentleMACS (Miltenyi Biotec). Antibodies, staining for effector activation, memory status formation and real time PCR protocols are reported in Supplementary Data.

Statistical analyses. The ratio of the mean of vaccinations (2nd to 7th)/baseline values (V/B ratio) of absolute count and frequency of NK cells, CD8⁺ and CD4⁺ T cells for each patient was calculated, and the median of all of the observations was used as the cut off value to separate patients into the "low" or "high" groups. The threshold able to separate patients with "low" or "high" V/B ratio and having the best sensitivity and specificity, was defined using Receiver Operating Characteristic (ROC) curves. PFS was calculated from first surgery until disease progression and death/last follow-up, if censored. Overall Survival (OS) was calculated from surgery to death due to any cause or last follow-up (censored). Kaplan-Meier analysis was used to estimate PFS and OS. The log rank test assessed differences in progression or survival in patients with different immunological or clinical parameters.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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