

## Postoperative Adjuvant Dendritic Cell – Based Immunotherapy in Patients with Relapsed Glioblastoma Multiforme

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**Abstract Purpose:** To investigate the therapeutic role of adjuvant vaccination with autologous mature dendritic cells (DC) loaded with tumor lysates derived from autologous, resected glioblastoma multiforme (GBM) at time of relapse.

**Experimental Design:** Fifty-six patients with relapsed GBM (WHO grade IV) were treated with at least three vaccinations. Children and adults were treated similarly in three consecutive cohorts, with progressively shorter vaccination intervals per cohort. Feasibility and toxicity were assessed as well as effect of age, extent of resection, Karnofsky Performance Score, and treatment cohort on the progression-free (PFS) and overall survival (OS) using univariable and multivariable analysis.

**Results:** Since the prevaccine reoperation, the median PFS and OS of the total group was 3 and 9.6 months, respectively, with a 2-year OS of 14.8%. Total resection was a predictor for better PFS both in univariable analysis *and* after correction for the other covariates. For OS, younger age and total resection were predictors of a better outcome in univariable analysis but not in multivariable analysis. A trend to improved PFS was observed in favor of the faster DC vaccination schedule with tumor lysate boosting. Vaccine-related edema in one patient with gross residual disease before vaccination was the only serious adverse event.

**Conclusion:** Adjuvant DC-based immunotherapy for patients with relapsed GBM is safe and can induce long-term survival. A trend to PFS improvement was shown in the faster vaccination schedule. The importance of age and a minimal residual disease status at the start of the vaccination is underscored.

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In spite of multimodal therapy, including maximal safe neurosurgical resection followed by radiochemotherapy and maintenance chemotherapy, malignant or high-grade gliomas (HGG) continue to have a dismal prognosis (1). Prognosis after relapse is even worse (2, 3), with a median progression-free survival (PFS) of 2 months, and virtually all patients being dead 18 months after the relapse. Even in pediatric patients with relapsed HGG, the prognosis is poor (4).

The rationale, the preclinical, and the early clinical evidence to develop dendritic cell (DC)-based immunotherapy for HGG has been reviewed by our research group (5, 6) and by others (7–14). Vaccination is done with autologous mature monocyte-derived *ex vivo* generated DC loaded with autologous tumor lysate. The characteristics and *in vitro* function of the vaccine have been analyzed (15, 16). We described some early clinical experience on feasibility and toxicity that we obtained in a small group of patients (17, 18).

For the implementation of immunotherapy in clinical practice for patients with relapsed HGG, an observational prospective cohort comparison trial, HGG-IMMUNO, was designed, in which adjuvant autologous DC vaccination is done in patients with relapsed HGG after maximal new resection of the relapsed tumor. Except for the time window in cohort A, which was the

starting-up cohort, each cohort is defined by a minimum of 20 patients with relapsed HGG included and a time window of ~15 months. Within the global HGG-IMMUNO trial concept, each cohort serves as historical control for the next cohorts. The concept of such prospective cohort comparison trial to improve therapeutic efficacy for HGG has already been published earlier (19). In HGG-IMMUNO, the primary end point of each cohort is feasibility and toxicity of DC vaccination (18). The secondary end points are shifts in PFS and overall survival (OS) as indications for a potential stepwise improvement of the efficacy of the immunotherapy by modifications of the vaccination characteristics in the consecutive cohorts of patients with relapsed HGG. The HGG-IMMUNO trial was approved by the ethics committees of the universities of Leuven (Belgium) and Würzburg (Germany). In this article, we report on the clinical data from the 56 patients with histologically proven relapsed glioblastoma multiforme (GBM; WHO grade IV) who were included and treated in cohorts A, B, and C.

## Patients and Methods

**Patients.** For inclusion in HGG-IMMUNO, patients must be aged >3 y, (without upper age limit) and must have suffered from a relapsed HGG, proven after new histopathologic examination of the tumor tissue at time of relapse. Sterile dry-frozen tumor material must be stored immediately after resection. Magnetic resonance imaging (MRI) must be done within 72 h after resection. The patient must give written informed consent. Exclusion criteria in HGG-IMMUNO are ongoing need for corticosteroids, a history of autoimmunity, immune deficiency, or a positive serology for HIV, Hepatitis B and C, and TPHA (syphilis).

From a total group of 72 patients with relapsed HGG, who were treated according to HGG-IMMUNO between October, 1st 2000 and October, 1st 2006, 56 patients had relapsed GBM as defined by the criteria of the WHO classification (20). For all patients ages <21 y, who were treated for their primary tumor according to HIT-GBM protocols of the German Society of Pediatric Oncology and Hematology, central pathology review was done. For all adult patients, at least two, independent pathologic reports were obtained confirming the diagnosis. The patients had initial treatment with at least surgery and external beam radiotherapy of the primary tumor. The vast majority of patients (51 of 56) had already received (several types of) chemotherapy at an earlier stage of their disease. After a new intentional maximal, safe resection, and an anticipated rapid weaning from corticosteroids, patients were included after obtaining written informed consent.

The extent of surgery before vaccination was judged on an early postoperative MRI ( $T_1$ -weighted spin-echo images before and after gadolinium enhancement) done within 72 h after surgery. Only if the neurosurgeon reported total resection and if no contrast enhancement was visualized on the early postoperative MRI scan, the resection was considered to be total. All other situations were defined to be less than total resections. All patients were without corticosteroids at least 1 wk before the harvesting of peripheral blood mononuclear cells (PBMC) for *ex vivo* DC differentiation.

**Vaccination.** In all, except six patients, PBMCs were obtained by leukapheresis and were kept frozen. For each vaccination, part of the PBMC was thawed. In six patients from cohort A, PBMC were isolated from fresh blood samples for preparing each vaccination (18). Immature monocyte-derived autologous DCs were generated and loaded with autologous whole tumor cell lysate as described (15, 17, 18); after mechanical homogenization, the tumor lysate was exposed to 6 snap freeze-thaw cycles and irradiated to 60 Gray. Tumor cell death was verified using Trypan Blue Exclusion assay. At time of loading, rTNF- $\alpha$  (Strathmann Biotec AG), rIL-1 $\beta$  (Strathmann Biotec AG), and

PGE2 (Prostin; Pharmacia) were added in a final concentration of 120 mg/mL, 120 ng/mL, and 20  $\mu$ g/mL, respectively.

After maturation, the cells were injected intradermally in the upper arms according to a predefined vaccination schedule: In cohort A, DC were given at week 1 and 3 and then further each 4 wk. In cohort B, 5 DC vaccinations were given at a 2-wk interval and then further each 4 wk. In cohort C, 4 weekly DC vaccinations were given, and boosts were done with intradermal injections of 1.5 mg autologous tumor lysate.

**Patient assessment.** Patients were followed clinically, biochemically, and radiologically during the vaccination period and each 3 mo afterwards, or earlier, upon clinical indication. Adverse events were scored according to the National Cancer Institute Common Toxicity Criteria version 2.0. Progression was defined as any volume increase of >25% of a residual tumor in patients that were all off steroids during and after the vaccination, or the appearance of any new contrast-enhancing tumoral lesion on MRI (21). Tumor volume was calculated using the algorithm  $A \times B \times C/2$  with  $A$  being the largest tumor diameter on MRI and  $B$  and  $C$  being the two perpendicular diameters (18).

**Skin test.** When enough tumor material was available, delayed type hypersensitivity (DTH) responses were tested using intradermal injections of 100  $\mu$ L inactivated tumor lysate and 100  $\mu$ L vaccine vehicle solution (negative control) before vaccination and after at least two vaccinations. After 48 and 72 h, redness and induration were assessed by an independent observer. DTH responses were judged as positive if the average perpendicular measurement of the reaction exceeded 5 mm.

**Statistical analysis.** PFS and OS curves were constructed using Kaplan-Meier estimates. All survival periods were calculated from the time of reoperation at the moment of relapse. Log-rank tests were done to compare survival curves. Univariable statistical analyses were done using Prism software (Prism3; GraphPad Software, Inc.). Using the statistical package SAS (version 9.1), Cox regression models have been used to study, for OS and PFS, the relation with a set of predictors. For the Karnofsky index, a distinction has been made between three levels: 100 to 90, 80 to 70, and  $\leq 60$ . A multiple Cox regression model has been build with age, total resection, Karnofsky index, and cohort as predictors. Age has been assessed as a linear covariate and, after looking at the data, categorized with a cutoff of 35 y.  $P$  values are considered significant when smaller than 0.05. All reported  $P$  values are two sided.

## Results

**Patients.** Among all patients with relapsed HGG who were treated according to HGG-IMMUNO in the first three cohorts, only the group of 56 patients with GBM, WHO grade IV (36 males and 20 females), were included for this analysis, with all WHO grade III lesions being excluded. Twenty patients with relapsed GBM were treated in cohort A, 16 patients come from cohort B, and 32 patients from cohort C. Median age was 45 years (range, 7-77 years). Age distribution was similar in the three cohorts, although a trend occurred of more children being treated in cohort A versus cohort C.

Before the reoperation for vaccination, all patients had undergone surgery and radiotherapy, and 51 from 56 patients had been treated with one or more chemotherapeutics before the relapse. At presentation, the median Karnofsky Performance Status was 80 (range, 50-100). Surgery was done in all patients before vaccination and was determined to be a total resection in 27 patients. At the time of progression during or after immunotherapy, possible rescue therapy was at the physician's discretion.

**Vaccine preparation and characterization.** For each vaccine preparation, immature DC were differentiated out of thawed PBMC taken by leukapheresis (50 patients) or freshly isolated PBMC (6 patients from cohort A). A median of  $12 \times 10^6$  (range,  $1-51.75 \times 10^6$ ) immature DC were loaded with a

median of 200  $\mu\text{g}$  (minimum 13  $\mu\text{g}$ , maximum 200  $\mu\text{g}$ ) lysate proteins, measured by Coomassie Blue Staining (22). A median of  $6 \times 10^6$  (range,  $0.7\text{-}25.7 \times 10^6$ ) mature DC were injected per vaccine. The phenotype of the mature DC was determined by fluorescence-activated cell sorting as illustrated in our first report (18). In Table 1, an overview of the phenotype of the DC products is depicted. Based on the phenotypical characteristics and the typical morphology with cytoplasmic veils, as recently illustrated by our group (6), DC were defined as early mature. For the patients in cohort A, a median of 5 (range, 3-7) DC vaccinations were administered; in cohort B, a median of 6 (range, 3-9) autologous DC vaccinations were administered; and in cohort C, four weekly DC vaccinations were administered in all patients, followed by a median of 2 (range, 0-6) monthly boost vaccinations using the autologous lysate without DC.

**Survival analysis.** In Fig. 1, PFS and OS curves are shown for the complete group of patients with a relapsed GBM ( $n = 56$ ). The median follow-up is 15.8 months (range, 11.3-70.4 months). The median PFS and OS of the total group was 3 respectively 9.6 months. OS at 12, 24, and 36 months was, respectively, 37.4%, 14.8%, and 11.1%. PFS at 12 months after the reoperation was 10.7%.

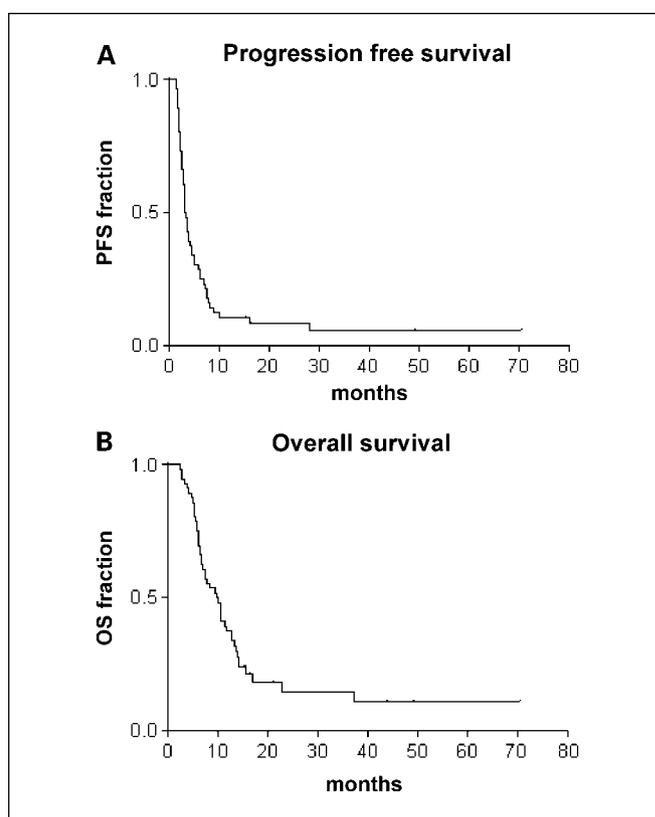
Age 35 years or less was a predictor of better overall but not PFS in univariable analysis (Fig. 2) with a median OS of 15.4 months for the younger patients versus 7.5 months for the patients above 35 years [ $P = 0.012$ ; hazard ratio (HR), 0.46; 95% confidence interval (CI), 0.24-0.84]. In univariable analysis, total resection was a predictor of better PFS but not OS, although a clear trend was found also for OS (Fig. 3). Median PFS for patients with total resections before vaccination was 4.5 months versus 2.5 months for patients with incomplete resections ( $P = 0.014$ ; HR, 0.51; 95% CI, 0.28-0.86). Karnofsky Performance Score was not a good predictor of outcome in this group. After correction for the other covariates, only total resection was an independent predictor of better PFS ( $P = 0.018$ ; HR, 0.48; 95% CI, 0.26-0.88; Table 2) and age above 35 years showed a strong trend to worse OS, without reaching statistical significance ( $P = 0.062$ ; HR, 2.51; 95% CI, 0.955-6.613; Table 3). There was a trend for better survival in

**Table 1.** DC phenotype characteristics

|         | Median | IQR   |
|---------|--------|-------|
| CD86%   | 80.74  | 27.63 |
| HLA DR% | 93.48  | 9.97  |
| CD14%   | 2.38   | 5.48  |
| CD3%    | 0.12   | 1.19  |
| CD1a%   | 12.08  | 23.61 |
| CD25%   | 11.84  | 25.03 |
| CD83%   | 26.38  | 32.15 |
| CD80%   | 42.45  | 48.74 |
| CD19%   | 0      | 0.26  |

NOTE: The phenotype of freshly cultured autologous monocyte-derived mature DC loaded with autologous GBM tumor cell lysate was analyzed by fluorescence-activated cell sorting. Cells were gated by scatter, and the percentage positivity of the respective markers, compared with the negative isotype control, was determined. Nonparametric distribution of the data for each marker: median and IQR (IQR, percentile 75-25).

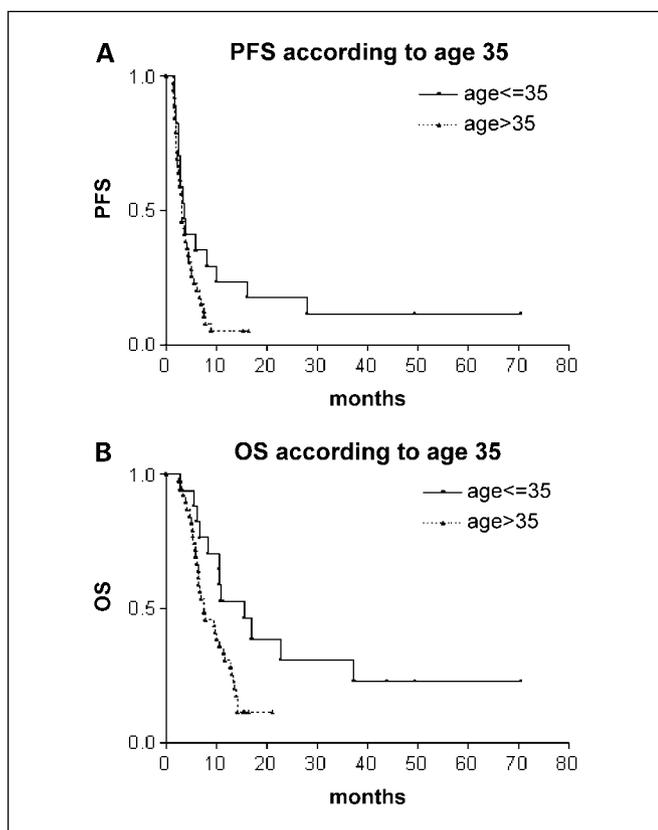
Abbreviations: CD, cluster of differentiation; HLA DR, human leukocyte antigen DR; IQR, interquartile range.



**Fig. 1.** A, PFS since reoperation is depicted for all vaccinated patients with recurrent GBM ( $n = 56$ ). Median PFS, 3 mo. B, OS since reoperation is depicted for all vaccinated patients with recurrent GBM ( $n = 56$ ). Median OS, 9.6 mo. 2-y survival, 14.8%.

cohort C. In addition, we looked at only the adult patients ( $\geq 21$  years) with relapsed GBM as a subgroup (Fig. 4) and found a significantly improved PFS in this subgroup of adult patients of cohort C, which might have been partially blurred by the trend of more children being treated in cohort A.

**Adverse events.** In general, only mild adverse events were seen, except for a strong, repetitive, vaccination-induced grade IV neurotoxicity (stupor) in a patient with a fairly large residual tumor after reoperation and before vaccination. Corresponding imaging showed this to be caused by repetitive vaccine-induced perilesional edema making the administration of steroids unavoidable. Grade II transient hematologic toxicity was seen in two patients, a transient increase in focal neurologic signs in six patients (hemiparesis in two and dysphasia in four patients), headache in nine patients (one transient lymphocytic, nonviral meningitis), vomitus in two patients, flu-like symptoms in three patients, increase in the frequency of epileptic seizures in four patients, fatigue in seven patients, myalgia in the shoulders with or without arthralgia in three patients, postoperative subdural hygroma in two patients, and an intratumoral hemorrhage in progressive tumor tissue in two patients. Temporary, vaccine-induced redness with or without itching and swelling at the injection sites was the rule in all patients. Several very rare adverse events were noted only once and included confusion, epididymitis, a burning sensation at the craniotomy scar, concentration deficit, dizziness, tremor, pneumonia, cerebrovascular accident, and a metastasis of the GBM in the lumbar spine and lungs.



**Fig. 2.** A, PFS since reoperation is depicted for vaccinated patients with recurrent GBM who are ages 35 y or younger ( $n = 17$ ) and for those older than 35 y ( $n = 39$ ).  $P = 0.107$ ; HR, 0.63 (95% CI, 0.34-1.11). B, OS since reoperation is depicted for vaccinated patients with recurrent GBM who are ages 35 y or younger ( $n = 17$ ) and for those older than 35 y ( $n = 39$ ).  $P = 0.012$ ; HR, 0.46 (95% CI, 0.24-0.84).

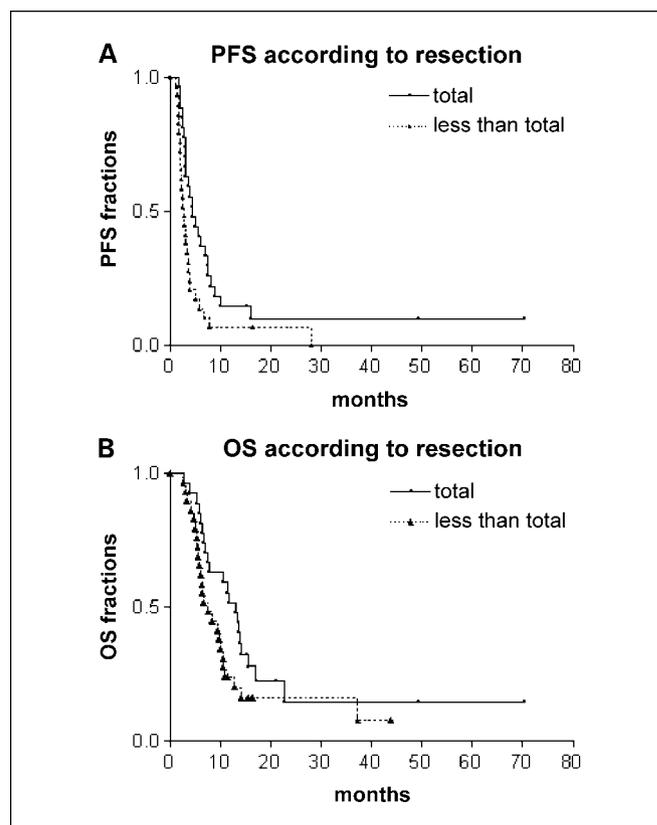
**Skin test.** Skin tests for DTH response could be done in those patients from whom enough tumor material was available. In none of the patients, general immune suppression was clinically suspected. Skin tests at diagnosis were done in 21 patients: 5 patients from cohort A, 5 patients from cohort B, and 11 patients from cohort C. The tests were positive in four, two, and three patients, respectively. Regarding the different risk factors, there was no difference in age or extent of resection between the 9 patients with positive DTH response versus the 12 patients with negative DTH response at diagnosis. Of these 21 patients, a second skin test after at least 2 vaccines was done in 2, 3, and 7 patients, respectively. In total, seven skin tests remained negative at both time points, two tests were negative at diagnosis and became positive after vaccination, two tests were positive at both time points, and in one patient with rapid progressive disease, a positive test became negative after vaccination. In four other patients, a skin test was only done later on during the immunotherapy, all being positive. There was no correlation between the results of the 9 of 21 positive skin tests at diagnosis or 9 of 17 positive skin tests after at least two vaccinations and survival (both PFS and OS).

## Discussion

We present our clinical experience in a large cohort of patients with relapsed GBM who were treated after new surgery with adjuvant immunotherapy based on autologous mature

DC loaded with autologous tumor cell lysate, and show that several young patients could obtain an unexpected long-term tumor control. The utmost importance of a minimal residual disease burden before vaccination is underscored in the finding that a total resection before vaccination is the only predictor of a better PFS, which is still significant after correction for the other covariates: age, Karnofsky Performance Score, and cohort. By changing the adjuvant vaccination schedule, a trend of improved PFS could be shown for the fastest DC vaccination schedule with lysate boosting. Of note, vaccination treatment was done in an ambulatory setting without major side effects.

Although inclusion and referral bias is unavoidable, no age limits were set nor did we do an active selection based on performance status in this large group of patients. The patients were treated with adjuvant autologous DC vaccines, using autologous tumor cell lysate as a source of antigens to load the DC. HGG-IMMUNO is conceived as a cohort comparison trial, in which three consecutive cohorts of patients were treated using only slightly different, empirical schedules of autologous DC vaccination after intentionally gross total resection of the recurrent HGG. We confirm the feasibility and low toxicity of the vaccination treatment, as has been documented in our previous reports (17, 18) and by other studies that were reviewed (7-14). Moreover, a global analysis of this large group of relapsed GBM patients shows promising results in terms of median OS but especially in terms of the percentage long-term



**Fig. 3.** A, PFS because reoperation is depicted for patients with recurrent GBM undergoing total ( $n = 27$ ) or less than total ( $n = 29$ ) resections before vaccination.  $P = 0.014$ ; HR, 0.51 (95% CI, 0.28-0.86). B, OS since reoperation is depicted for patients with recurrent GBM undergoing total ( $n = 27$ ) or less than total ( $n = 29$ ) resections before vaccination.  $P = 0.07$ ; HR, 0.59 (95% CI, 0.31-1.05).

**Table 2.** Results from the full multivariable model for OS

|                 | HR    | LL    | UL    | P           |
|-----------------|-------|-------|-------|-------------|
| Cohort          |       |       |       | <b>0.60</b> |
| Cohort A vs C   | 0.744 | 0.26  | 2.13  | 0.58        |
| Cohort B vs C   | 1.3   | 0.615 | 2.748 | 0.49        |
| Cohort A vs B   | 0.573 | 0.185 | 1.772 | 0.33        |
| Total resection | 0.629 | 0.335 | 1.18  | 0.15        |
| Karnofsky       |       |       |       | <b>0.14</b> |
| k ≤ 80          | 2.218 | 1.006 | 4.89  | 0.048       |
| k ≤ 60          | 0.717 | 0.298 | 1.724 | 0.46        |
| Age (y)         |       |       |       |             |
| Age >35         | 2.513 | 0.955 | 6.613 | 0.062       |

NOTE: For the Karnofsky score, a division in three categories (≤60, 70 and 80, 90, and 100) is considered. As such, two HR estimates are reported. The first indicating the change in hazard when comparing patients with a Karnofsky index of 70 or 80 and patients with a Karnofsky index higher than 80. The second indicating the change in hazard comparing patients with a Karnofsky index lower than or equal to 60 and patients with a Karnofsky index of than 70 or 80. *P* values in bold refer to the effect of a predictor with more than 1 degree of freedom.

Abbreviations: LL/UL, lower and upper limit of the 95% CI for the HR.

survivors: starting from the reoperation for the vaccination, median OS was 9.6 months, and 2-year and 3-year survival was 14.8% and 11.1%, respectively; median OS being in the upper range of some selected previously run trials and the latter, long term survival at two, and three years after relapse, clearly comparing favorable to any large study for recurrent HGG (2). Treatment with temozolomide for recurrent GBM resulted in a median OS of 6 months (3). One might argue that the reoperation accounts for the observed survival benefit. However, comparing the data from patient groups with similar prognostic features reported in studies from Bleeher et al. (23) and Brem et al. (24) suggests that resection as such does not improve the median OS of 7 months of HGG patients at time of relapse, compared with chemotherapy alone (2). The use of local chemotherapeutics in the resection cavity of reoperated patients resulted in a median OS of only 3 months (25). Barker et al. (26) reported a median OS of 8 months after resection of relapsed GBM combined with individual additional treatments. Only in patients treated with 1,3-bis(2-chloroethyl)-1-nitrosourea plus immunotherapy with IFN- $\alpha$ , previous surgery might have had a positive prognostic effect (27).

This series is unique in terms of the wide range of the age of patients involved in this trial: unlike most large trials, we applied the same treatment to children and adults with relapsed GBM and provide, for the first time, data on immunotherapy with autologous DC for children with relapsed GBM. Furthermore, HGG-IMMUNO provided us with the unique possibility to perform a survival analysis, in which age was included as a covariate. For the OS analysis, age ≤35 years is a clear predictor of better outcome. This finding underscores the possible different cytogenetics and biological behavior of the tumor at different ages but also the different potential of DC vaccination as a tool for active specific immunotherapy in different age

categories. This will be utterly important for age stratification processes in further randomized trials being conceived. After correcting for the other covariates, however, younger age tended to be related with better OS but did not reach a statistical significance.

The extent of resection (scored as total or less than total) significantly influences PFS, even after correction for the other covariates. In fact, "intentional macroscopic complete resection" was a prerequisite for treatment because of the need of a rapid weaning from steroids postoperatively, the need to obtain enough tumor material to make the lysate, safety issues, and the largely documented immune suppressive potency of bulky tumor (28–30). As a consequence, patients only undergoing a biopsy could not be included in the study. These findings underscore the importance of a good surgical resection, for relapsed HGG patients aimed to receive immunotherapy, and confirms finally what has already been shown in patients with newly diagnosed GBM (31, 32). This also implies, however, that extent of resection should be included as a possible predictor of outcome in the design of (any) trial dealing with relapsed HGG.

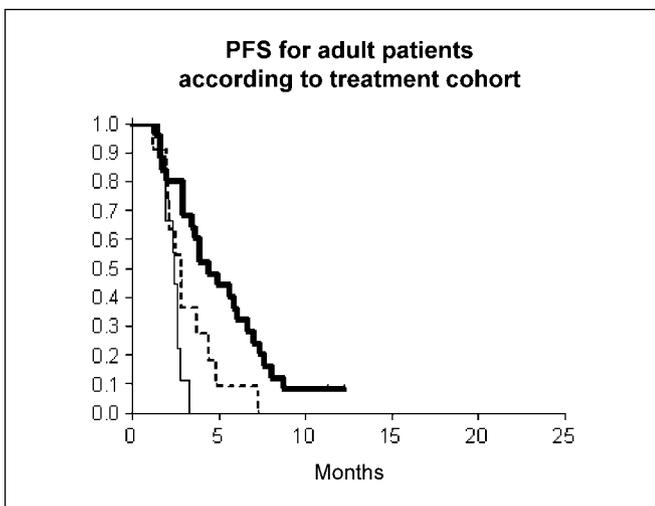
If disease progression was diagnosed after DC vaccination, median OS after disease progression was still 4 months (range, 0.8–38.3 months) with 25% of patients surviving 7 months or longer after the diagnosis of new progressive disease. This remarkable finding might be an argument of "disease modulation" or induction of a "slower progression" as normally seen in these patients. Alternatively, some patients who were again treated at this stage with chemotherapy might have shown an improved chemoresponsiveness after vaccination as has been suggested for HGG (33, 34) or as a general treatment paradigm in oncological disease (35, 36). This issue will be subject of further research.

In our approach, early mature autologous DC loaded with autologous tumor cell lysate were injected. Most immunotherapy protocols consist now of mature DC instead of immature DC. The latter DC phenotype has been suggested to induce tolerance rather than immune response (37, 38). Earlier experiences already suggested the improved efficacy of mature DC over immature DC for HGG immunotherapy (39).

**Table 3.** Results from the full multivariable model for progression-free survival

|                 | HR    | LL    | UL    | P     |
|-----------------|-------|-------|-------|-------|
| Cohort          |       |       |       | 0.141 |
| Cohort A vs C   | 1.259 | 0.455 | 3.487 | 0.66  |
| Cohort B vs C   | 2.141 | 1.008 | 4.548 | 0.048 |
| Cohort A vs B   | 0.588 | 0.188 | 1.837 | 0.36  |
| Total resection | 0.482 | 0.263 | 0.881 | 0.018 |
| Karnofsky       |       |       |       | 0.50  |
| k ≤ 80          | 1.396 | 0.677 | 2.876 | 0.37  |
| k ≤ 60          | 0.628 | 0.272 | 1.451 | 0.28  |
| Age (y)         |       |       |       |       |
| Age >35         | 1.908 | 0.77  | 4.732 | 0.16  |

NOTE: In univariable models, only the presence of total resection turns out to be significant (*P* = 0.016). HR, 0.506; 95% CI, 0.291 to 0.880. This effect remains significant, irrespective other predictors are added in the model.



**Fig. 4.** PFS for adult patients ( $\geq 21$  y) since reoperation is depicted for patients with relapsed GBM treated in cohort A (thin full line; median PFS, 2.4 mo;  $n = 9$ ), in cohort B (dotted line; median PFS, 2.8 mo;  $n = 11$ ), and in cohort C (thick full line; median PFS, 4.4 mo;  $n = 26$ ).  $P = 0.0008$ .

There was a large range of amount of mature DC administered to the patients. Even within a single patient, a variable amount of DC were injected per vaccine. In the 11 phase I/II trials published thus far and reviewed (6), the amount of DC applied per vaccine ranged from  $10^6$  to  $10^8$ . A dose-response phenomenon could not be shown thus far, supporting the paradigm that the antitumoral immune response rather displays an on/off function (40).

Early mature autologous DC were injected intradermally in an empirical schedule slightly different for the three cohorts. In cohort C, four induction vaccines were administered weekly, followed by monthly boost vaccines consisting of the autologous tumor cell lysate alone. The reason to shorten the interval between vaccines, from 4 weeks over 2 weeks to 1 week, was due to our clinical impression of the delay between start of vaccination and generation of an objective antitumoral immune response. The latter was observed in some patients as a transient contrast enhancement on MRI, which occurred several months after vaccination (17). We hypothesized that the immune suppressive capacities from the growing residual tumor cells (28–30) could interfere with the installation of the systemic immune responsiveness induced by immunization with vaccinations at long interval. These clinical observations might be of general importance for designing future vaccination strategies. In cohort C, after four induction DC vaccines, boosts were administered with lysate only. The rationale to change toward such approach was based on an orthotopic glioma mouse model, in which multiple vaccinations with DC induced cell-mediated immunity but did not elicit optimal long-term

survival. In contrast, injection of DC for the priming, followed by boosts with tumor cell lysate alone generated the most effective antitumor effects. This vaccination methodology allowed better cytotoxic T-lymphocyte responses and also triggered an antitumor humoral response (41). Moreover, in renal cell carcinoma, the immunogenicity and survival benefit of intradermal tumor cell lysate administration has been shown (42).

The immune monitoring of the patients in this trial was restricted to skin tests. These tests were done when enough tumor proteins were available. Nine of 21 patients had a positive test before any vaccination. This result might reflect the presence of an ongoing, indolent immune responsiveness in some patients as has been described recently (43). Overall, the clinical outcome of our patients was not linked to the presence of a positive DTH response at diagnosis or during vaccination, which underscores the importance of developing a more elaborated immunomonitoring. These findings are illustrative for the difficulty to find a good correlation between immune responsiveness as surrogate marker and clinical antitumoral efficacy, especially if whole tumor cell lysates are used as a source of antigens (44, 45). Moreover, DC vaccination can induce a lot of antigen-independent immune responses that are hard to detect with the current antigen-specific immune monitoring techniques (46). Nevertheless, Yamanaka et al. (39) did find an improved OS in vaccinated patients showing a positive DTH response.

In conclusion, we present clinical data on 56 patients with relapsed GBM, who were treated according to HGG-IMMUNO, a prospective cohort-comparison trial of autologous DC vaccination as an adjuvant therapy after reoperation. The patient group covers a wide range of ages of representative patients with relapsed GBM (WHO grade IV), all treated according to a similar protocol. The unexpected long-term progression-free survival observed in some younger patients, the substantial numbers of patients being alive 2 and 3 years after the treatment for the relapse, as well as the trend for improved PFS by fastening the vaccination schedule in univariate analysis, provide strong evidence for the efficacy of DC vaccination to induce tumor control. The importance of a macroscopically total resection before vaccination, as the only statistically significant predictor of better PFS after correction for the other covariates, underscores the importance of a minimal residual disease status, required for a successful implementation of DC-based immunotherapy. Moreover, the clinical results of this adjuvant postoperative DC vaccination program underscore the growing awareness that improving the outcome for patients with GBM requires an additional emphasis on discovering novel therapies implemented in soundly designed trials of adjuvant therapies (47).

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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