REVIEW

# Can high intensity focused ultrasound facilitate immunomodulation in glioblastoma multiforme?

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**Glioblastoma Multiforme (GBM, Astrocytoma grade-IV) is the most common primary malignant brain tumour in adults and unfortunately the most deadly. Patients with GBM exhibit a deficient anti-tumor immune response. Immunotherapy is rapidly becoming one of the pillars of anti-cancer therapy. GBM has not received similar clinical successes as of yet, a fact which may be attributed to its relative inaccessibility, its poor immunogenicity, or any of the many other immune mechanisms known to be inactivated in these tumor cells. Focused Ultrasound (FUS) is emerging as a promising treatment approach. The effects of FUS on the tissue are not merely thermal. Reported FUS-induced acoustic cavitation which carries both mechanical and molecular implications as well as FUS induced immunomodulation play important roles. This is a concise research highlights on a comprehensive report by the same group. We separately discuss the different pertinent immunosuppressive mechanisms harnessed by GBM and the immunomodulatory effects of FUS. The three modes of FUS action can all be assigned a molecular final common pathway of immunomodulation. Thermal ablation induced immune effects, microbubbles effects in disrupting the BBB and introducing antigens and drugs to the tumor milieu as well as FUS induced molecular effects are discussed. The effect of FUS on the pro-inflammatory cytokines secretion profile, the stress response, the intra-tumoral immune-cells populations, dendritic cells activity moderation and FUS induced increased cytotoxic cells potency are all discussed. A conceptual synopsis of the synergistic treatment of GBM utilizing FUS and immunotherapy is presented. The interaction of multiple approaches harnessing immune-components and circumventing immunosuppressing mechanisms may herald a new era in the fight against GBM.**

***Keywords:*** Focused ultrasound; FUS; GBM; immunomodulation; Synergistic Immunotherapy

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## Introduction

Glioblastoma Multiforme (GBM, Astrocytoma grade-IV) is the most common primary malignant brain tumour in adults and unfortunately most deadly. The classical histological characterization of GBM is clearly becoming

**Table 1. GBM related Immune-evasion and immunosuppression mechanisms, review***

| Ref. | Proposed Mechanism | | | Comments |
|---|---|---|---|---|
| 27 | Allelic loss of #10q. | Disruption of two tumor suppressor genes in this locus (i.e. DBMT1, PTEN). | | Decreased rate of infection in patients with this allelic loss<br>Impaired antitumor immunity and impaired systemic immunity leading to bacterial infections. |
| 28 | | PTEN and protein kinase B | PTEN loss increases B7-H1 expression, and peripheral anergy. | |
| 34<br>33<br>35 | Altered mitogenic pathways | PI3-K / PTEN. | Dominant $Th_2$ type cytokines release, supporting anergy and tolerance to the tumor | Tolerance and anergy to the tumor cells |
| | | p16/pRb/CDK4,<br>p53/ MDM2/ p14ARF,<br>EGFRvIII<br>PDGF | | |
| 30<br>31<br>32 | Increased $T_{reg}$ (CD4+FoxP+ T cells) population | >2.5-fold increase<br>Increased $T_{reg}$ cells in TIL's of human GBM.<br>Increased $T_{reg}$ and microglia in GBM. | | The frequency of T-regs was shown to correlate directly with in vitro suppression of T cell activation. |
| 40<br>29 | Immunosuppressive cytokines release | Interferon-γ<br>IL-10<br>TGFβ | | Supporting development of tolerance and anergy to the tumor cells |
| 36 | MHC-I downregulation | tumor's ability to down-regulate or express low levels of class-I MHC | hiding the tumor cells presence from the cellular arm of the immune system | |
| 37<br>38 | HLA-G expression | aberrant expression of this non-classical MHC-I molecule, structurally related to classical MHC class Ia (HLA-A, -B, -C). | Render cells resistant to direct alloreactive lysis, and inhibits the alloproliferative response.<br>Prevents efficient priming of cytotoxic T cells. | |
| 39 | Anti-Apoptosis | Upregulation of anti-apoptotic proteins (i.e. Survivin), rendering cells immortal and unresponsive to normal death signals | | |

Abbreviations: PTEN - Phosphatase tensin, DBMT - Deleted brain malignant tumor, PI3-K - phosphatidylinositol 3'-kinase signaling pathway, EGFRvIII - Epidermal growth factor receptor variant III, PDGF - Platelet-derived growth factor receptor, TIL's-Tumor Infiltrating lymphocytes. *based on Cohen-Inbar *et al* [1]

less valid with respect to its prognostic significance serving as somewhat of a wastebasket category. Multiple molecular subsets of GBM are now known, carrying different prognostic horizons [1]. Despite standard of care treatment, the median survival of a patient harboring a GBM is less than 2 years, a grim figure which changed very little in the past decades, proving resistant to most developments and revolutions incurred on modern medicine [2-4]. The unique nature of GBM and its inherent challenging features was evident as early as 80 years ago. Early reports of GBM patients who endured a post-operative surgical-site infection who surprisingly exhibited longer survival sparked an interest in many clinicians, suspecting an important role for the immune system both in disease progression as well as in tumor triumph. Since these initial pivotal observations, with developing techniques and widespread interest, multiple studies were put forward describing different molecular immunosuppressive mechanisms taking place in GBM cells and microenvironment, claiming these to be the dominant key events (table 1) [1, 5]. Unfortunately, things are not as straightforward or simple, and both arms of the immune system are known to be hampered in GBM, as do many other anatomical barriers, micro-environmental conditions and features unique to tumors within the central nervous system, once termed as immune-privileged [27-28].

Continuous-wave (CW) high intensity focused ultrasound (HI-FUS) is emerging as a promising treatment approach. It is the only noninvasive thermal technique that allows for real-time imaging of the treatment progress using MR-Thermometry [41]. Yet, the effects of FUS on the tissue are not merely thermal, shown to induce mechanical acoustic cavitation, carrying both mechanical and molecular implications and also modulate the host antitumor immune responses (table 2) [5]. We present a short report of research highlights capturing the essence of a paper we recently published [5]. We will briefly discuss different pertinent immunosuppressive mechanisms harnessed by GBM and the immunomodulatory effects of FUS.A potential conceptual synopsis of the two is presented. As discussed, the synergistic treatment of GBM utilizing HIFU and immunotherapy has molecular evidence to support it. For ease of grasping, we will divide our discussion to GBM immune-evasion and immune-suppressing mechanisms, FUS-mediated immunomodulation and a synopsis of these two.

## GBM mediated immune-evasion

Mounting an effective brain anti-GBM immune response requires that certain requirements are met. GBM cells

**Table 2. Focused Ultrasound Immunomodulatory effect – Literature Review**

| Ref. | Indication | Immunologic Effect | | | | |
|---|---|---|---|---|---|---|
| | | Mechanism | | | | Comments |
| 6 | Breast | $CD4^+/CD8^+$ inversion | CD3+ increase | NK cell stimulation | Increased apoptotic markers | Increased TIL's$^{Þ}$, NK-cells and CD4+/CD8+ inversion. Increased expression of apoptotic Fas-L, Granzyme-B, Perforin+ TIL's |
| 7 | Pancreatic cancer | | | | | $CD4^+/CD8^+$ inversion and $CD3^+$ increase in 10 patients ($NP^{€}$) |
| 8 | $OS^{£}$ (6), $HCC^{α}$ (5), $RCC^{β}$ (5) | | | | | Increased $CD4^+$ and inversion of the $CD4^+/CD8^+$ |
| 9 | Choroidal Melanoma | | | | | 2/3 patients reverted the ratio from abnormal levels |
| 10 | HCC | | | | Resistance to tumor re-challenge | Increased $CD4^+$ and inversion of the $CD4^+/CD8^+$ |
| 11 | $NB^{∞}$ | | | | | Resistance to tumor re-challenge |
| 12 | Prostate | | | Anti-Inflammatory Cytokines decrease | | Sonicted tumor cells downregulate STAT-3 (less proliferation of immature DCs), decreased T-regulatory population in the spleen and tumor draining lymph nodes. |
| 13 | HCC (13), Sarcoma (2) | | | | | Decreased serum levels of: VEGF, TGF-β1, TGF-β2. |
| 14 | Breast | $HSP^{π}$ | | | | HSP-70 and epithelial membrane antigen showed 100% expression in the tumor debris. Cytokines found in the tumor milieu: TGF-β1 (57%), TGF-β2 (70%), IL-6 (48%), IL-10 (61%), VEGF (30%) |
| 15 | Prostate cancer | | Pro-Inflammatory Cytokines increase | | | Increased expression of HSP-72, HSP73, GRP75, GRP78 Increased release of IL-2, IFNγ, TNFα Decreased release of IL-4, IL-5, IL-10 |
| 16 | $CRC^{γ}$ | | | $DC^{δ}$ and $MPS^{ε}$ activation | | ATP and HSP-60 release from CRC cells. DC and MPS activation (mechanical more than thermal) Enhanced IL-12 and TNFα secretion. |
| 17 | HCC | Increased $CTL's^{Ш}$ activity | | | | Increased IFNγ and TNFα secretion and CTL TIL's. |
| 18 | HCC | | | | | IFNγ and TNFα increased secretion. |
| 19 | CRC | | | | Resistance to tumor re-challenge | The mechanical FUS effect is better than the thermal effect in DC activation. Increased CTL's activity and IFNγ secreting cells. |
| 20 | HCC | | | | | |
| 21 | Melanoma | | | | | Increased CTL's cytotoxicity, no increased risk of metastases after HIFU. |
| 22 | Breast | | | $DC^{δ}$ and $MPS^{ε}$ activation | | Increased activation and infiltration of DC's and MPS. Increased expression of CD80, CD86 in sonicated tumors. |
| 23 | Melanoma | | | | | |
| 24 | NA | $HSP^{π}$ | | | | Peak HSP-70 expression at 6-48 hours after sonication, persisting for 96 hours. |
| 25 | Melanoma, Fibroma, $SCC^{Σ}$ | | | | | HSP-70 expression induced at a lower temperature than heat stress alone. |
| 26 | Prostate (5), Bladder $TCC^{**}$ (4) | | | | | HSP-27 increased expression, most notably 2-3 hours after sonodynamic ablation. The effect is still evident 5-8 days post sonication. |

ΩNumber of patients, ζNot applicable, usually refers to pre-clinical studies, ∞Neuroblastoma, *Cluster of Differentiation, €Not statistically Significant, £Osteosarcoma, πHeat Shock Proteins, **Transitional cell carcinoma, αHepatocellular Carcinoma, βRenal Cell Carcinoma, γColorectal Carcinoma, δDendritic Cells, εMononuclear phagocyte system (i.e. macrophages), ШCytotoxic (CD8+) T-lymphocytes, ΣSquamous Cell Carcinoma, ÞTumor infiltrating lymphocytes. ⁺Based on Cohen-Inbar *et al* [5]

developed mechanisms to evade or block its development at multiple steps (Table 1) [1, 5]. Tumor associated target antigens must be sufficiently different from self-antigens, avoiding the development of immune-tolerance and anergy to self (and consequently to the tumor) on the one hand or the development of an auto-immune response on the other. Tumor cells must express major histocompatibility complex (MHC) molecule in adequate numbers to present antigens to Cytotoxic T-cells (CTL's) in order to mount a specific CTL's effector mediated response. The activated effector CTL's should maintain their potency and activity during migration through involved brain parenchyma and its resident cells, as well as during the interaction with the tumor cells. A local inflammatory response should than be instigated and properly regulated. The multitude of immunosuppressive mechanisms (both active and passive) as well as immune-evasion techniques attributed to GBM cells are summarized in Table 1 [1, 5]. These mechanisms independently, support the evolution of anergy and tolerance to the tumor. Of note, the complex interplay between the different mechanisms stated is complex and largely unknown.

**FUS-mediated immunomodulation**

FUS exerts its effect on the tumor cells utilizing three complementary "modes" of action: thermal ablation, acoustic cavitation and immunomodulation. The third mechanism employs the uniform low-level heating of a region of interest not killing the cells [14, 42-43]. The three modes of action can be assigned a molecular final common pathway of immunomodulation. Thermal ablation results in two complimentary effects, i.e. the release of immunogenic cellular antigenic debris [44] into the interstitial space activating Dendritic cells (DC's) [18], as well as inducing the surviving tumor cells to up-regulate danger signals such as heat shock proteins (HSP) and adenosine tri-phosphate (ATP), both highly potent activators of innate immunity [16, 25, 45]. Mechanical cavitation was shown to facilitate better BBB penetration for drugs, antigens and immune cells as well as results in lysis related tumor debris [46-49]. The FUS microbubble (MB) induced BBB-disruption effects last several hours and can be localized to the tumor region, prior to returning to the pre-FUS state [50]. FUS-MB was reported to increase the intra-tumoral concentrations of delivered liposomal doxorubicin [51], temozolomide [52], interleukin-4 [53], nanoparticles, DNA, plasmid vectors, and antibodies [54-55], and IL-12 [56].

Pulsed-mode FUS with increased negative pressures was shown to boost the systemic antitumor immune response through multiple mechanisms. Table 2 [5] presents a brief overview of key preclinical and clinical studies, per different tumor type, segregated based on the proposed FUS-induced immunomodulatory effect. FUS was shown to support and amplify an anti-tumor immune response, prolong overall survival and protect from growth of new tumors when re-challenged (Table 2) [5]. One should note that all immune-modulating effects discussed hereafter and presented in table-2 were described on multiple tumor types, not restricted to studies conducted in the CNS or on GBM cell lines or tissue samples. The assumption that these effects are tumor type independent, assigned only to FUS, were not validated objectively.

FUS mediated immune effects can be grouped into its effects on cytokines and the stress response, its effects on peripheral and intra-tumoral immune cell populations, FUS mediated augmentation of Dendritic cell activity or a more general, increased CTL's potency and FUS mediated resistances to tumor re-challenge. The latter refers to lengthened survival and immunomodulatory effects of FUS noted in different reports but lacking a proven exact molecular mechanism [11, 20, 23]. HSP's are known potent immune-stimulants, able to bind tumor peptide antigens and enhance tumor cell immunogenicity [57-62]. FUS was shown to up-regulate the expression of HSP70 both in-vitro and ex-vitro [16, 24, 25]. An increased HSP-70 expression was detected on the surviving cell membrane of 23 patients with breast cancer treated with HIFU ablation[14] FUS was shown to enrich the TIL's population in immune-potent pro-inflammatory potent anti-tumor effector cells in human breast cancer specimens [6, 63], posterior uveal melanoma [9], pancreatic carcinoma [7], osteosarcoma [8], hepatocellular carcinomas (HCC) [8], and Renal Cell Carcinoma (RCC) [8]. FUS was shown to enhance the infiltration capabilities and activity of dendritic cells (DCs) [36, 40] as well as other antigen presenting cells [22] in the treated tumor, leading to an increased expression of costimulatory molecules and enhanced secretion of IL-12 (via DCs) and TNF-α (macrophages) [16]. Zhang *et al* [20] demonstrated that tumor debris induced by FUS could serve as an effective immunogenic vaccine. Increased CTL's Potency and effector function after FUS, reported as increased IFNγ and TNFα secretion [17-19] or increased direct CTL's mediated cytotoxicity [21] serves as another avenue of immunomodulation.

**Synopsis& Future directions**

The complexity of interacting immune-evasion and immunosuppressing mechanisms dysregulated in GBM cells, mechanisms modulated by FUS, as well as tumor specific and patient (i.e. immune system) specific mechanisms is largely unknown. A theoretical action-reaction scheme is presented in previous comprehensive report [5]; connecting certain known GBM-evasion mechanisms with the FUS induced counter response. One should note that a single FUS mediated effect may influence multiple immune mechanisms and vice versa. There seem to be a theoretical basis for the effectiveness of FUS immunomodulation, synergistically supporting various immunotherapeutic approaches in overcoming many of the GBM mediated immune-resistance mechanisms. Future research still needs to be done to both dissect the different FUS-induced molecular and immunological mechanisms at play as well as to optimize the FUS treatment method.

**Conclusions**

No single treatment modality will cure GBM. In recent years, immunotherapy has come to the forefront of anti-cancer therapy. While some cancer types have been amenable to immunotherapeutic approaches, GBM has not received similar clinical successes, likely due to its poor immunogenicity and for its location in the immunologically distinct CNS. We briefly review FUS-induced immunomodulation, which can be harnessed to current and developing immunotherapies approaches. These research

highlights of a broader report by our group[5] serve to better define the essence of new findings and existing gaps in our understanding. Further study to the synergistic collaboration of different therapeutic approaches and the elaborate molecular immune interplay will shed light on this formidable challenge.

## Conflicting interests

The authors have declared that no conflict of interests exist.

## Abbreviations

APCs: antigen-presenting cells; ATP: Adenosine triphosphate; BBB: blood brain barrier; CD: cluster of differentiation; CRC: colorectal adenocarcinoma; CTLs: cytotoxic T cells; CW: continuous-wave; DCs: dendritic cells; EGFR: epidermal growth factor receptor; FUS: focused ultrasound; GBM: glioblastoma multiforme; HCC: hepatocellular carcinomas; HIFU: high intensity focused ultrasound; HLA: human leukocyte antigen; HSP: heat shock proteins; IFN: interferon; IL: interleukin; LPS: lipopolysaccharide; MB: microbubbles; MHC: major histocompatibility complex; PTEN: phosphatase tensin; RCC: renal cell carcinoma; TCR: T cell receptor; TH: T helper cell; TILs: tumor-infiltrating lymphocytes; TNF: tumor necrosis factor.

### References

1. Cohen-Inbar O, Zaaroor M. Immunological Aspects of Malignant Gliomas. CJN 2016, In press.
2. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, *et al*. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005; 352:987-996.
3. Weller M, van den Bent M, Hopkins K, Tonn JC, Stupp R, Falini A, *et al*. EANO guideline for the diagnosis and treatment of anaplastic gliomas and glioblastoma. Lancet Oncol 2014; 15:e395-e403.
4. Or cohen-inbar. Focused Neurosurgery book. Jaypee Brothers Publishers, 2015.
5. Cohen-Inbar O, Xu Z, Sheehan JP. Focused ultrasound-aided immunomodulation in glioblastoma multiforme: a therapeutic concept. J Ther Ultrasound 2016;4:2.
6. Lu P, Zhu XQ, Xu ZL, Zhou Q, Zhang J, Wu F. Increased infiltration of activated tumor-infiltrating lymphocytes after high intensity focused ultrasound ablation of human breast cancer. Surgery 2009; 145:286-293.
7. Wang X, Sun J. High-intensity focused ultrasound in patients with late-stage pancreatic carcinoma. Chin Med J 2002; 115:1332-1335.
8. Wu F, Wang ZB, Lu P, Xu ZL, Chen WZ, Zhu H, *et al*. Activated anti-tumor immunity in cancer patients after high intensity focused ultrasound ablation. Ultrasound Med Biol 2004; 30:1217-1222.
9. Rosberger DF, Coleman DJ, Silverman R, Woods S, Rondeau M, Cunningham-Rundles S. Immunomodulation in choroidal melanoma: reversal of inverted CD4/CD8 ratios following treatment with ultrasonic hyperthermia. Biotechnol Ther 1994; 5:59-68.
10. Zhou P, Fu M, Bai J, Wang Z, Wu F. Immune response after high-intensity focused ultrasound ablation for H22 tumor. Journal of Clinical Oncology, ASCO 2007 Annual Meeting Proceedings (Post-Meeting Edition) 2007; 25: 21169.
11. Yang R, Reilly CR, Rescorla FJ, Sanghvi NT, Fry FJ, Franklin TD Jr, *et al*. Effects of high-intensity focused ultrasound in the treatment of experimental neuroblastoma. J Pediatr Surg 1992; 27:246-250; discussion 250-251.
12. Huang X, Yuan F, Liang M, Lo HW, Shinohara ML, Robertson C, *et al*. M-HIFU inhibits tumor growth, suppresses STAT3 activity and enhances tumor specific immunity in a transplant tumor model of prostate cancer. PLoS One 2012; 7:e41632.
13. Zhou Q, Zhu XQ, Zhang J, Xu ZL, Lu P, Wu F. Changes in circulating immunosuppressive cytokine levels of cancer patients after high intensity focused ultrasound treatment. Ultrasound Med Biol 2008; 34:81-87.
14. Wu F, Wang ZB, Cao YD, Zhou Q, Zhang Y, Xu ZL, *et al*. Expression of tumor antigens and heat-shock protein 70 in breast cancer cells after high-intensity focused ultrasound ablation. Ann Surg Oncol 2007; 14:1237-1242.
15. Kramer G, Steiner GE, Gröbl M, Hrachowitz K, Reithmayr F, Paucz L, *et al*. Response to sublethal heat treatment of prostatic tumor cells and of prostatic tumor infiltrating T-cells. Prostate 2004; 58:109-120.
16. Hu Z, Yang XY, Liu Y, Morse MA, Lyerly HK, Clay TM, *et al*. Release of endogenous danger signals from HIFU-treated tumor cells and their stimulatory effects on APCs. BiochemBiophys Res Commun 2005; 335:124-131.
17. Xia JZ, Xie FL, Ran LF, Xie XP, Fan YM, Wu F. High-intensity focused ultrasound tumor ablation activates autologous tumor-specific cytotoxic T lymphocytes. Ultrasound Med Biol 2012; 38:1363-1371.
18. Deng J, Zhang Y, Feng J, Wu F. Dendritic cells loaded with ultrasound-ablated tumour induce in vivo specific antitumour immune responses. Ultrasound Med Biol 2010; 36:441-448.
19. Hu Z, Yang XY, Liu Y, Sankin GN, Pua EC, Morse MA, *et al*. Investigation of HIFU-induced anti-tumor immunity in a murine tumor model. J Transl Med 2007; 5:34.
20. Zhang Y, Deng J, Feng J, Wu F. Enhancement of antitumor vaccine in ablated hepatocellular carcinoma by high-intensity focused ultrasound. World J. Gastroenterol 2010; 16:3584-3591.
21. Xing Y, Lu X, Pua EC, Zhong P. The effect of high intensity focused ultrasound treatment on metastases in a murine melanoma model. BiochemBiophys Res Commun 2008; 375:645-650.
22. Xu ZL, Zhu XQ, Lu P, Zhou Q, Zhang J, Wu F. Activation of tumor-infiltrating antigen presenting cells by high intensity focused ultrasound ablation of human breast cancer. Ultrasound Med Biol 2009; 35:50-57.

23. Liu F, Hu Z, Qiu L, Hui C, Li C, Zhong P, *et al*. Boosting high-intensity focused ultrasound-induced anti-tumor immunity using a sparse-scan strategy that can more effectively promote dendritic cell maturation. J Transl Med 2010; 8:7.

24. Kruse DE, Mackanos MA, O'Connell-Rodwell CE, Contag CH, Ferrara KW. Short-duration-focused ultrasound stimulation of Hsp70 expression in vivo. Phys Med Biol 2008; 53:3641-3660.

25. Hundt W, O'Connell-Rodwell CE, Bednarski MD, Steinbach S, Guccione S. In Vitro Effect of Focused Ultrasound or Thermal Stress on HSP70 Expression and Cell Viability in Three Tumor Cell Lines. Acad Radiol 2007; 14:859-870.

26. Madersbacher S, Gröbl M, Kramer G, Dirnhofer S, Steiner GE, Marberger M. Regulation of heat shock protein 27 expression of prostatic cells in response to heat treatment. Prostate 1998; 37:174-181.

27. Aghi MK, Batchelor TT, Louis DN, Barker FG 2nd, Curry WT Jr. Decreased rate of infection in glioblastoma patients with allelic loss of chromosome 10q. J Neurooncol 2009; 93:115-120.

28. Parsa AT, Waldron JS, Panner A, Crane CA, Parney IF, Barry JJ, *et al*. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. Nat Med 2007; 13:84-88.

29. Hao C, Parney IF, Roa WH, Turner J, Petruk KC, Ramsay DA. Cytokine and cytokine receptor mRNA expression in human glioblastomas: evidence of Th1, Th2 and Th3 cytokine dysregulation. Acta Neuropathol 2002; 103:171-178.

30. Fecci PE, Mitchell DA, Whitesides JF, Xie W, Friedman AH, Archer GE, *et al*. Increased regulatory T-cell fraction amidst a diminished CD4 compartment explains cellular immune defects in patients with malignant glioma. Cancer Res 2006; 66:3294-3302.

31. El Andaloussi A, Lesniak MS. An increase in CD4+CD25+FOXP3+ regulatory T cells in tumor-infiltrating lymphocytes of human glioblastoma multiforme. Neuro Oncol 2006; 8:234-243.

32. See AP, Parker JJ, Waziri A. The role of regulatory T cells and microglia in Glioblastoma associated immunosuppression. J Neurooncol 2015; 123:405-412.

33. Choe G, Horvath S, Cloughesy TF, Crosby K, Seligson D, Palotie A, *et al*. Analysis of the phosphatidylinositol 3'-kinase signaling pathway in glioblastoma patients in vivo. Cancer Res 2003; 63:2742-2746.

34. Ermoian RP, Furniss CS, Lamborn KR, Basila D, Berger MS, Gottschalk AR, *et al*. Dysregulation of PTEN and protein kinase B is associated with glioma histology and patient survival. Clin Cancer Res 2002; 8:1100-1106.

35. Louis DN, Holland EC, Cairncross JG. Glioma classification: a molecular reappraisal. Am J Pathol 2001; 159:779-786.

36. Yang L, Ng KY, Lillehei KO. Cell-mediated immunotherapy: a new approach to the treatment of malignant glioma. Cancer Control 2003; 10:138-147.

37. Pistoia V, Morandi F, Wang X, Ferrone S. Soluble HLA-G: Are they clinically relevant? Semin Cancer Biol 2007; 17:469-479.

38. Wiendl H, Mitsdoerffer M, Weller M. Hide-and-seek in the brain: a role for HLA-G mediating immune privilege for glioma cells. Semin. Cancer Biol 2003; 13:343-351.

39. Das A, Tan WL, Teo J, Smith DR. Expression of survivin in primary glioblastomas. J Cancer Res Clin Oncol 2002; 128:302-306.

40. Gomez GG, Kruse CA. Mechanisms of malignant glioma immune resistance and sources of immunosuppression. Gene Ther Mol Biol 2006; 10:133-146.

41. Kennedy JE. High-intensity focused ultrasound in the treatment of solid tumours. Nat Rev Cancer 2005; 5:321-327.

42. Skitzki JJ, Repasky EA, Evans SS. Hyperthermia as an immunotherapy strategy for cancer. Curr OpinInvestig Drugs 2009; 10:550-558.

43. Partanen A, Yarmolenko PS, Viitala A, Appanaboyina S, Haemmerich D, Ranjan A, *et al*. Mild hyperthermia with magnetic resonance-guided high-intensity focused ultrasound for applications in drug delivery. Int J Hyperthermia 2012; 28:320-336.

44. Wu F. Heat-Based Tumor Ablation: Role of the Immune Response. Adv Exp Med Biol 2016; 880:131-153.

45. Hundt W, Steinbach S, Burbelko M, Kiessling A, Rominger M, O'Connell-Rodwell CE, *et al*. Induction of luciferase activity under the control of an hsp70 promoter using high-intensity focused ultrasound: combination of bioluminescence and MRI imaging in three different tumour models. Technol Cancer Res Treat 2011; 10:197-210.

46. Frenkel V, Li KC. Potential role of pulsed-high intensity focused ultrasound in gene therapy. Future Oncol 2006; 2:111-119.

47. Hwang JH, Brayman AA, Reidy MA, Matula TJ, Kimmey MB, Crum LA. Vascular effects induced by combined 1-MHz ultrasound and microbubble contrast agent treatments in vivo. Ultrasound Med Biol 2005; 31:553-564.

48. Ferrara K, Pollard R, Borden M. Ultrasound microbubble contrast agents: fundamentals and application to gene and drug delivery. Annu Rev Biomed Eng 2007; 9:415-447.

49. Vestweber D. Relevance of endothelial junctions in leukocyte extravasation and vascular permeability. Ann N Y Acad Sci 2012; 1257:184-192.

50. Sheikov N, McDannold N, Vykhodtseva N, Jolesz F, Hynynen K. Cellular mechanisms of the blood-brain barrier opening induced by ultrasound in presence of microbubbles. Ultrasound Med Biol 2004; 30:979-989.

51. Aryal M, Vykhodtseva N, Zhang YZ, Park J, McDannold N. Multiple treatments with liposomal doxorubicin and ultrasound-induced disruption of blood-tumor and blood-brain barriers improve outcomes in a rat glioma model. J Control Release 2013; 169: 103-111.

52. Liu HL, Huang CY, Chen JY, Wang HY, Chen PY, Wei KC. Pharmacodynamic and therapeutic investigation of focused ultrasound-induced blood-brain barrier opening for enhanced temozolomide delivery in glioma treatment. PLoS One 2014; 9:e114311.

53. Yang FY, Wong TT, Teng MC, Liu RS, Lu M, Liang HF, *et al*. Focused ultrasound and interleukin-4 receptor-targeted liposomal doxorubicin for enhanced targeted drug delivery and antitumor effect in glioblastoma multiforme. J Control Release 2012; 160:652-658.

54. Diaz RJ, McVeigh PZ, O'Reilly MA, Burrell K, Bebenek M, Smith C, *et al*. Focused ultrasound delivery of Raman

nanoparticles across the blood-brain barrier: Potential for targeting experimental brain tumors. Nanomedicine 2014; 10:1075-1087.

55. Alonso A. Ultrasound-induced blood-brain barrier opening for drug delivery. Front Neurol Neurosci 2015; 36:106-115.
56. Chen PY, Hsieh HY, Huang CY, Lin CY, Wei KC, Liu HL. Focused ultrasound-induced blood-brain barrier opening to enhance interleukin-12 delivery for brain tumor immunotherapy: a preclinical feasibility study. J Transl Med 2015; 13:93.
57. Pockley AG. Heat shock proteins as regulators of the immune response. Lancet 2003; 362:469-476.
58. Fučíková J, Bartůňková J, Špíšek R. The Concept of Immunogenic Cell Death in Antitumor Immunity. Klin Onkol 2015; 28:48-55.
59. Muth C, Rubner Y, Semrau S, Rühle PF, Frey B, Strnad A, *et al*. Primary glioblastoma multiforme tumors and recurrence: Comparative analysis of the danger signals HMGB1, HSP70, and calreticulin. Strahlenther Onkol 2015;
60. Wang X, Ji J, Zhang H, Fan Z, Zhang L, Shi L, *et al*. Stimulation of dendritic cells by DAMPs in ALA-PDT treated SCC tumor cells. Oncotarget 2015
61. Todryk SM, Gough MJ, Pockley AG. Facets of heat shock protein 70 show immunotherapeutic potential. Immunology 2003; 110:1-9.
62. Bajzert J, Stefaniak T. Heat shock protein HSP60 and the perspective for future using as vaccine antigens. PostepyHig Med Dosw (Online) 2015; 69:1149-1168.
63. Higgins JP, Bernstein MB, Hodge JW. Enhancing immune responses to tumor-associated antigens. Cancer Biol Ther 2009; 8:1440-1449.