Título: ON THE ROAD TO IMPROVE GLIOBLASTOMA THERAPY FOLLOW-UP. IMMUNE MICROENVIRONMENT: WHAT IS BEHIND THE MRSI-BASED NOSOLOGICAL IMAGES?

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Resumen: Glioblastoma (GB) is an aggressive brain tumour with poor survival. Improvements in both therapeutic and follow-up strategies are urgently needed.
In this work, we described an oscillatory pattern of response to Temozolomide (TMZ) detected through magnetic resonance spectroscopic imaging (MRSI)-based machine learning approaches when treating GL261 GB tumour-bearing C57BL/6j mice with the immune respectful Immune-Enhancing Metronomic Schedule (IMS), that consists in an every 6-days TMZ administration. These results suggest that host immune response has a relevant role in therapy response/escape in GL261 tumours under IMS-TMZ therapy since the oscillatory pattern length matches with immune cycle length, which would be being sampled by MRSI-derived nosological images. Furthermore, we observed that IMS-TMZ therapy produced significant improvement in mice survival, outperforming standard TMZ treatment. Moreover, during IMS-TMZ treatment, tumours from some mice (n=6) fully regressed and remained undetectable without further treatment for one month. These animals were considered "cured" and a GL261 re-challenge experiment was performed, with no tumour reappearance in 5 out of 6 cases.

Considering that 1) the tumour microenvironment is a key element in GB evolution and response to therapy, 2) the glioma-associated microglia/macrophage (GAM) population constitutes the most abundant non-tumour cell type within the GB, and 3) the Programmed Death Factor 1 ligand (PD-L1) and its receptor (PD-1) are one of the mechanisms mediating immunosuppression in GB, we analysed the GAM polarisation and PD-L1 gene expression within the glioblastoma microenvironment. We observed that GAM polarisation towards an anti-tumour phenotype prevailed in TMZ treated tumours compared to control tumours. Moreover, PD-L1 expression was higher in treated tumours and its expression level was correlated with the antitumour GAM phenotype. GAMs can represent up to 30-40% of the GB tumour volume, which leads us to think that the oscillatory change in the GAMs population could be one of the key causes for the differential MRSI-detected pattern, allowing this to act as immune system activity biomarker.

Matrix Metalloproteinases (MMPs) and 'A Disintegrin and Metalloproteinase' (ADAMs) are involved in tumour microenvironment regulation and are associated with microglia/macrophage functions in GB. Their analyses could provide insight into the molecular signature of these cells and might be a predictor of GB patients' survival. We analysed tumours from IMS-TMZ treated mice either responding or non-responding to treatment, as well as relapsing and vehicle-treated tumours, in order to characterise the different subpopulations of GAMs and PD-L1 gene expression, as well as the molecular profile of different protease genes, known to be associated with GB progression (ADAM8, ADAM10, ADAM17, MMP9 and MMP14).

We found an increase in GAM infiltration and its shift to antitumour phenotypes, as well as ADAM8, ADAM10, ADAM17, MMP14 and PD-L1 genes increased, in mice responding to IMS-TMZ therapy compared to control, relapsing and non-responding tumours. On the opposite, the antitumoural phenotype was significantly increased in actively proliferation situations, supporting tumour progression. Finally, the MMP9 gene expression level was found to increase in non-responding tumours in comparison with other groups.

It has been described the crucial role of the different lymphocytes (Th cells, Tc cells, Treg cells and NK cells) in cancer growth and response to treatment. We analysed these cells in responding samples from GL261 tumour-bearing mice treated with IMS-TMZ and vehicle. Tc, Th, Treg and NK percentage populations increase in IMS-TMZ treated responding mice.

All these results support the idea that the host immune system activation is closely related to the beneficial outcome reached from IMS-TMZ therapeutic schedules and could at least partially explain spectral pattern changes observed by the MRSI-based biomarker. Further studies will be needed in order to clarify additional molecular/cellular elements explaining the MRSI-based findings.