

## BACKGROUND

Several clinical trials have demonstrated that expression of O<sup>6</sup>-methylguanine-DNA methyltransferase (MGMT) is an independent prognostic factor in predicting the response to alkylating agents in patients with high grade astrocytoma<sup>1-2</sup>. However, the current methods to evaluate MGMT expression - immunohistochemistry and methylation-specific PCR may show discordant results and thus hamper reliable stratification of patients. Inconsistencies in the results have been attributed to MGMT expression in the non-neoplastic cell component of tumours which includes lymphocytes, vascular endothelial cells, and macrophages/microglia<sup>3</sup>.

Tumour-infiltrating microglia may account for up to 40% of cells in glioblastomas<sup>4</sup> but no study has examined the impact of microglia on the determination of MGMT status.

Aim of this study was to investigate the expression of MGMT in tumour-infiltrating microglia in cases of high grade astrocytoma.

## PATIENT SELECTION

Thirty adults patients (age range 29-84 years, 17 males) with supratentorial high grade astrocytoma (10 WHO grade III and 20 WHO grade IV) who underwent surgical debulking and received radiotherapy and chemotherapy with temozolomide were selected from the BTRC Brain Tumour Registry at Charing Cross Hospital.

## MATERIALS AND METHODS

Immunohistochemical stains were performed on formalin fixed paraffin embedded tissue sections to detect MGMT (Millipore, Ltd., 1:400) and Iba1 (WAKO, Ltd., 1:750) expression.

Results were evaluated quantitatively using a computer-based image analysis system (ImagePro Plus, Media Cybernetics, Inc.). Images of ten fields at 20x magnification were captured and the overall number of MGMT positive cells and Iba1 immunostained microglia were counted. Endothelial cells and lymphocytic infiltrates were excluded from the analysis. Double immunofluorescence was utilized to visualise MGMT/Iba1-immunoreactive cells.

## RESULTS

All cases contained a population of MGMT negative microglia (Fig. 1). A considerable variability between cases was seen.

Colocalisation studies using immunofluorescence confirmed the presence of MGMT-negative microglial cells and highlighted the variability between cases ranging from 20 to up to 60% (Fig. 2).

In 3 anaplastic astrocytomas and 5 glioblastomas, quantitative analysis showed that tumour-infiltrating microglia outnumbered the whole population of MGMT positive cells confirming the presence of a subset of MGMT negative microglial cells (Fig. 3).

## REFERENCES

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4. Graeber MB, Scheithauer BW, Kreutzberg GW. Microglia in brain tumours. *Glia* 2002 40:2: 252-59

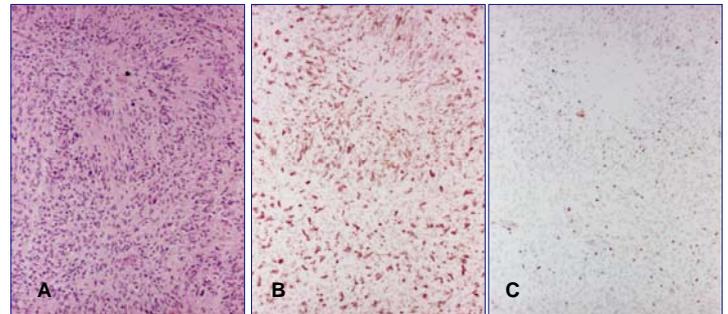


Figure 1: This GBM shows a considerable proportion of MGMT negative microglial cells (same field) (A, HE) (B, Iba1) (C, MGMT). Original magnification 10x.

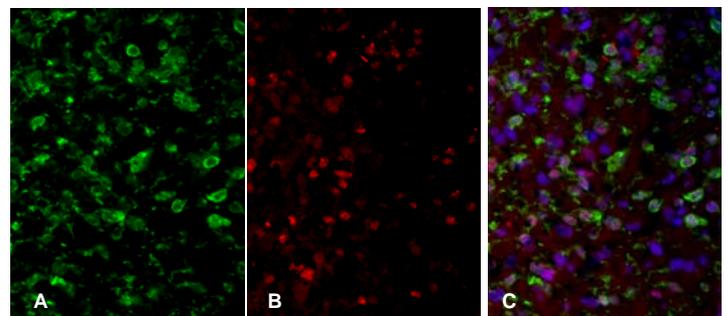


Figure 2: Double immunofluorescence in this example of GBM reveals a few MGMT-negative microglial cells highlighting the variability between cases (A, green, Iba1) (B, red, MGMT) (C, overlay image with counterstained nuclei). Original magnification 20x

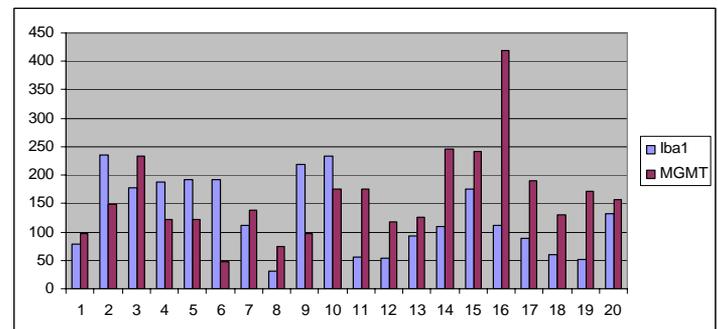


Figure 3. Graph demonstrating number of MGMT and Iba1 expressing cells in the 20 GBMs. Six cases show higher expression of Iba1 than MGMT, suggesting the presence of MGMT-negative microglial cells.

## CONCLUDING REMARKS

- A proportion of microglial cells in high grade astrocytomas does not express MGMT suggesting that silencing of the MGMT gene may occur in non-neoplastic cells.
- Variable MGMT expression by microglia may hamper the overall evaluation of MGMT status in anaplastic astrocytomas and glioblastomas. Immunostaining for Iba1 may help to better assess MGMT status.
- MGMT-negative microglia may be susceptible to alkylating agents.
- The presence of an MGMT-negative population of microglial cells raises the possibility that the reduction in size of high grade astrocytomas following treatment with temozolomide may also be secondary to the reduction of microglia.