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Editorial

MGMT testing always worth an emotion

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The methylation status of the *MGMT* promoter has been the first predictive biomarker in neuro-oncology and predicts benefit of glioblastoma patients from temozolomide (TMZ) treatment[1] that remains the mainstay of the current standard of care. This biomarker has dramatically changed the design of clinical trials for glioblastoma and since, it serves as stratification factor or is used for patient selection in trials for glioblastoma[2]. However, outside clinical trials the *MGMT* methylation status had less impact on management of GBM patients, likely due to the fact that there are no alternative treatment choices and the perceived uncertainty of test results, given standardized tests are not widely available.
To clarify the situation of the validity of the numerous methods that have been published over the years, a systematic analysis has been performed evaluating the prognostic value of such tests best predicting overall survival in GBM patients treated with TMZ, published in a Cochran report[3]. The most relevant findings of this evaluation are summarized and commented in this issue[4].

Here a few considerations of methodological and mechanistic aspects: Methods evaluating the methylation status of the *MGMT* promoter aim at a binary output of methylated versus unmethylated *MGMT* that should predict whether the gene is active. If the promoter is methylated, the gene is silenced and will not be expressed, while in the unmethylated state the gene can be activated, rendering the tumor proficient for DNA repair and thereby conferring resistance to TMZ treatment. However, unlike a mutation that is present or absent – the regulatory CpG methylation extends throughout the promoter and beyond, while only methylation of certain CpGs have an effect on gene silencing. Two regions have been identified that when methylated show a particularly high association with suppressing *MGMT* gene expression[5]. A fraction of the CpGs located in one of these two areas is therefore interrogated as a proxy by most of the assays. Different methods usually interrogate distinct subsets of CpGs, which is a confounding factor when comparing technology. Furthermore, the same technology may interrogate different CpGs, and/or summarize the raw values in different ways, and/or use different strategies to define cut-offs. While the results using different methods maybe comparable when using the same samples, validation in independent datasets, or data on assay reproducibility are often missing.

The study reports on the comparison of three frequently used methods for determining the *MGMT* methylation status, comprising methylation specific-PCR, methylation specific pyrosequencing, and surprisingly immunohistochemistry for the MGMT protein. The study included 32 independent cohorts comprising 3474 GBM patients treated with TMZ. In addition, 190 studies using a single method were described. The sobering results suggest that pyrosequencing may be slightly more prognostic than methylation specific PCR, or MGMT immunohistochemistry. No firm statements on the best choices of CpGs, or best cut-offs could be made. This laudable effort to help guide through the plethora of tests available is limited by the fact that comparisons between technologies were generally performed in relatively small datasets of less than 100 patients, and often without validation of the defined cut-offs in independent datasets, or correction for other clinically relevant prognostic factors, thereby limiting the statistical power and generalizability. The good news is that most patients will be classified the same way by most tests, as methylation is highly correlated between CpGs that are interrogated by most methods. However, the patients with values close to the cut-off(s) will be classified inconsistently by

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different tests. This is most likely due to heterogeneous methylation across the CpG sites that may affect different tests in varying degrees[6]. The extent of non-neoplastic cell invasion also influences the results particularly in these patients[7]. This has implications for patient management. The interpretation of the results will depend on the risk-dependent treatment choices: If omission of TMZ is to be proposed, e.g. in the context of novel treatments in a clinical trial[2], the result needs to ensure that the *MGMT* promoter is unmethylated, in order not to withhold a potentially effective treatment[8]. While in frail patients, where the choice is between TMZ or RT, a clearly methylated result is necessary to opt for TMZ only[9].

Taken together, a best method has not been identified, and the most accurate cut-off is not defined. Considering the nature of *MGMT* methylation, this may never be achieved. Nonetheless, it is of importance that the evaluation of the *MGMT* promoter methylation status is established using validated tests for best patient care. It is of note, epigenetic biomarkers based on promoter methylation of genes are less commonly used in precision medicine than mutation analyses, but a number of markers have been implemented in other cancer types and some certified tests are available, including for *MGMT*[10].

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