

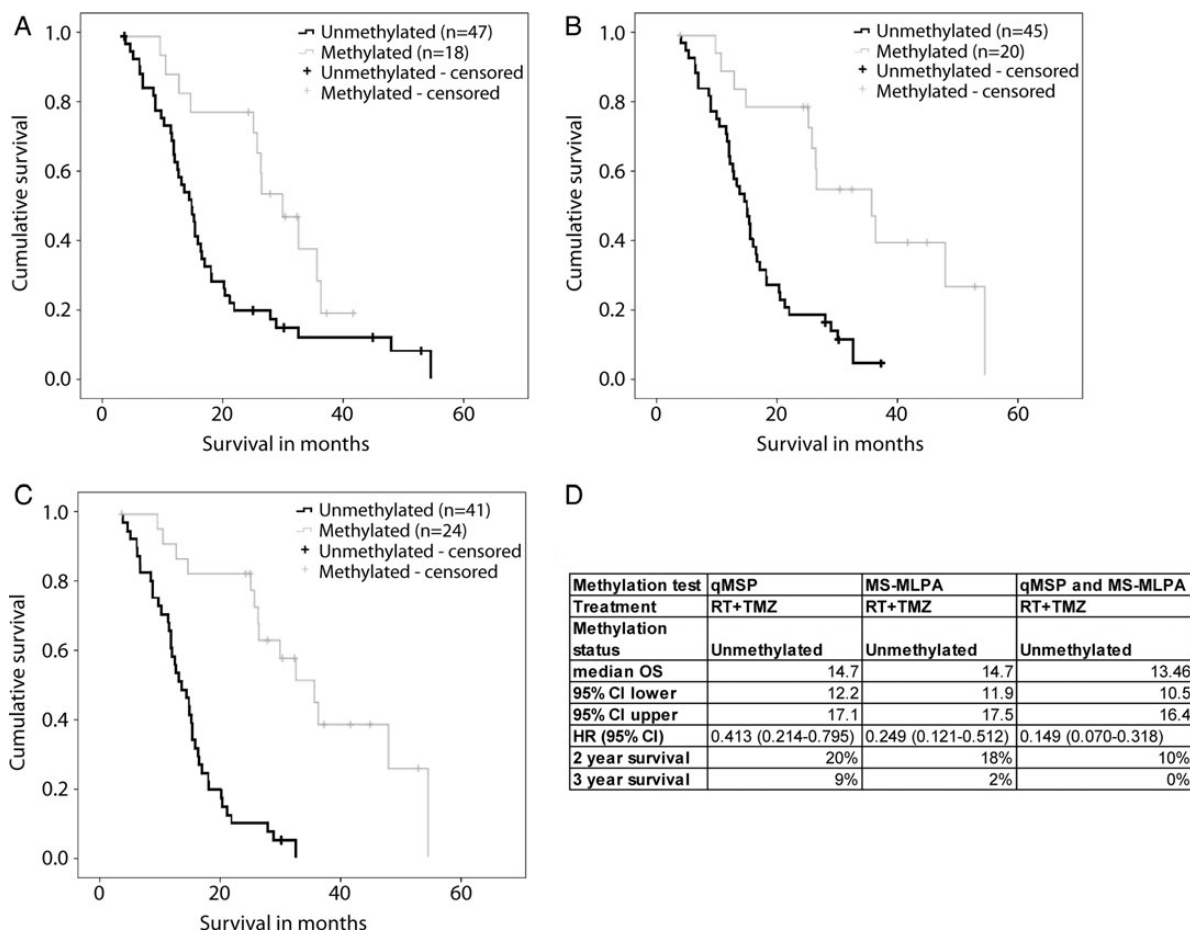
## Letter to the editor

### MGMT methylation in glioblastoma: tale of the tail

Promoter region methylation of O<sup>6</sup>-methylguanine DNA methyltransferase (MGMT) is a critical biomarker in neuro-oncology. Introduction of temozolomide (TMZ) and concomitant radiation as a standard of care (Stupp protocol) for glioblastoma multiforme (GBM) has improved survival for some patients.

Hegi et al. suggested that methylation status might be predictive of benefit from TMZ,<sup>1</sup> and multiple studies ever since have confirmed that MGMT methylation status is predictive of TMZ response.<sup>2</sup> However, debate persists regarding the best method for its evaluation. Consequently, MGMT status in the clinic has been underutilized, and TMZ remains a staple in caring for GBM patients regardless of their MGMT methylation status.

The MGMT promoter consists of 97 cytosine-guanine (CpG) dinucleotide sites, which can be grouped into 3 regions: R1, R2 and R3, where R1 is the most distal to the transcription



**Fig. 1.** Kaplan–Meier survival curves for MGMT methylated versus unmethylated patients. (A) MGMT methylation tested using qMSP (log-rank  $P = .006$ ). (B) MGMT methylation tested using MS-MLPA (log-rank  $P < .001$ ). (C) MGMT methylation determined using both MS-MLPA and qMSP (log-rank  $P < .001$ ). A sample was considered methylated if either of the test results was methylated (D). Survival statistics for MGMT unmethylated GBM patients using different testing techniques.

Received 13 October 2014; accepted 14 October 2014

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start site and R3 is adjacent to the transcription start site. In our previous study,<sup>3</sup> we comprehensively characterized methylation across the entire MGMT promoter and showed that all 3 regions correlate to mRNA expression, protein expression, and progression-free survival. The most widely used test that measures MGMT methylation is the qMSP test.<sup>4</sup> The qMSP test measures methylation in region R3, which is the most strongly correlated to patient outcome. However, the success of the test depends upon the co-methylation of at least 4 out of the 7 CpG sites in R3. Another test, the MS-MLPA, can measure methylation of 3 individual CpG sites, one in each of the aforementioned regions.<sup>5</sup> Hence, both tests are complementary in nature.

It has been suggested that GBM patients with and without MGMT methylation benefit from TMZ.<sup>1</sup> The tail in the unmethylated group (>3-year survival, Fig. 1A and B) seemingly indicated that even patients with unmethylated MGMT benefit from TMZ treatment. This survival tail in our cohort is consistent with other large-scale studies.<sup>2</sup> However, when we performed both qMSP and MS-MLPA tests together on 65 GBM samples, we observed increased detection sensitivity, and the tail in the unmethylated group disappeared (Fig. 1C and D). All 5 patients comprising the tail, when using just one of the tests, were indeed methylated by the other test. These data suggest that accurate MGMT characterization is grounds for questioning the alleged benefit of TMZ for all GBM patients and strengthen the case for needing new treatment regimens because the current standard of care does not provide benefit for MGMT-unmethylated patients.

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