

Title: MUC16 mutations and prognosis in gastric cancer; a little goes a long way

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Gastric cancer is a global health problem; although incidence rates are declining, it remains the third most common cause of cancer death worldwide.<sup>1</sup> Gastric cancer patients with advanced disease have limited treatment options available, and most will live for less than two years, therefore exploration of gastric cancer disease biology is warranted to identify new targets.<sup>2</sup> Recent comprehensive molecular analyses have identified distinct subgroups of gastric cancer which may have therapeutic relevance, however with the exception of microsatellite unstable tumours the potential for genomically guided therapy has not been realised.<sup>3-5</sup>

In this edition of JAMA Oncology, Li and colleagues investigate the relationship between *MUC16* mutation, tumour mutation load (TML) and survival in patients with gastric adenocarcinoma.<sup>6</sup> Using data from the TCGA gastric cancer dataset and a second, smaller, Asian validation cohort, the investigators demonstrate that tumours which are *MUC16* mutant are more likely to have a higher tumour mutation burden ( $p < 0.001$ ). This association was independent of age, gender, and the presence of mutations in genes affecting genomic stability such as *BRCA1/2* and *POLE*. Independence from microsatellite instability (MSI) status could not be determined as MSI results were not available for every TCGA patient; however, the association between *MUC16* mutation and TML also appeared to be independent of derived mismatch repair deficiency signatures. Li et al then proceed to demonstrate that patients who have *MUC16* mutant tumours have longer median overall survival than patients with a *MUC16* wildtype tumour genotype. In TCGA patients with resected gastric cancer the median overall survival was 26.7 months for *MUC16* wildtype versus 46.9 months for *MUC16* mutant tumours respectively ( $p = 0.007$ ); similar results were demonstrated in the Asian dataset. This prognostic effect of *MUC16* mutation on overall survival also remained statistically significant when adjusted for confounding factors.

The findings presented by Li et al are interesting because if *MUC16* mutation is truly predictive of high TML this could have clinical implications. The most readily apparent use of *MUC16* mutation as a surrogate for TML would be to identify gastric cancer patients who

might benefit from immune checkpoint blockade. As only one in six patients with PD-L1 selected gastroesophageal cancer respond to anti-PD-1 therapy, better enrichment biomarkers are a priority.<sup>7</sup> Retrospective analysis across multiple cancer types and clinical trials demonstrates a significant correlation between tumour mutational load and response to PD-1 inhibition.<sup>8</sup> Prospectively in the Checkmate 577 study, non-small cell lung cancer patients with a high tumour mutation load ( $\geq 10$  mutations per megabase) had significantly superior progression free survival when treated with nivolumab and ipilimumab compared to chemotherapy, independent of PD-L1 expression.<sup>9</sup> The authors' transcriptomic results support the suggestion that *MUC16* mutation could predict for sensitivity to anti-PD-1 therapy; *MUC16* mutant gastric tumours are immunologically "hot". Most recently, tumour mutation load has been assessed using a commercially available next generation sequencing (NGS) panel.<sup>8,9</sup> However, if TML assessment were to be reduced to *MUC16* sequencing only as a surrogate for a larger NGS panel, precise quantification of the relationship between *MUC16* mutation and mutation rate per megabase in gastric cancer would be required to inform biomarker development.

Clinical limitations of the current work include the small size, restricted ethnic make-up, and heterogeneous nature of the validation cohort. The first major challenge to the scientific validity of the current work is the risk of a false positive association between *MUC16* mutation and high TML because of characteristics associated with *MUC16*. *MUC16* is a very large gene sited in an area with low replication timing. Regions of low replication timing are more likely to accumulate frequent mutations. Considering other relevant mutational co-variates, it is possible that *MUC16* has a mutation rate which is in line with what might be expected by chance. Secondly, correlation between *MUC16* mutation and high TML does not necessarily imply causation; a functional explanation for the link between *MUC16* mutation and high TML is absent. An alternative explanation for the improved long-term survival of *MUC16* mutant patients in this study may not relate directly to tumour mutation load. In an assessment of the tumour biology of long term survivors following pancreatic

tumour resection, neoantigens (but not non-antigenic mutations) in *MUC16* were identified four times more frequently in long term survivors than in patients who had shorter survival.<sup>10</sup> *In vitro*, MUC16 expression is associated with proliferation and metastatic potential in tumour cells, and MUC16 is also known to inhibit natural killer cell mediated lysis of tumours.<sup>11,12</sup> Thus, elimination of *MUC16* neoantigen presenting cancer cells via immune “pruning” could hypothetically leave a residual population of MUC16 non-expressing cells with reduced metastatic and immunosuppressive potential, which could be associated with longer survival; this could present an alternative explanation for the authors’ transcriptomic results on immune infiltrate. Data on *MUC16* neoantigen presentation are not provided in the current manuscript; this should be a priority for future research.

There is a paucity of evidence linking *MUC16* gene function to cancer, therefore it is unlikely that *MUC16* mutations are positively selected oncogenic drivers. This makes the hypothesis that *MUC16* mutations act through neoantigen presentation and immunoediting more plausible. Despite the challenges in understanding the relationship between *MUC16* mutation and TML, the relationship with prognosis appears to be robust. Although the results presented by Li and colleagues are preliminary and require further validation, they could provide a signpost for clinically relevant research in gastric cancer, or in view of the ubiquity of *MUC16* mutations, in other tumour types.

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