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Retrospective Study

Title: Integrating *TYMS*, *KRAS* and *BRAF* testing in patients with metastatic colorectal cancer.

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1 What did this study explore?

This study explored the associations of *TYMS* polymorphisms, LOH, KRAS/BRAF mutations and clinicopathologic characteristics with the survival outcomes of patients with metastatic colorectal cancer (mCRC) treated with 1st line fluoropyrimidine-based chemotherapy.

2 How did the authors perform all experiments?

TYMS genotypes were identified with restriction fragment analysis PCR, while *KRAS* and *BRAF* mutation status was evaluated using real-time PCR assays on formalin-fixed paraffin-embedded tissues of patients with mCRC.

3 How did the authors process all experimental data?

All experimental data were processed anonymously by allocation of a number to each patient according to good clinical practice guidelines.

4 How did the authors deal with the pre-study hypothesis?

Potential resistance mechanisms to fluoropyrimidines for patients with mCRC that have been proposed by previous studies include TYMS gene amplification (Wang TL 2004) and the loss of heterozygosity (Uchida K 2004, Watanabe T et al 2001). Johnston PG 1995 et al and Ettienne MC et al 2002 showed that TYMS gene polymorphisms may be linked to survival outcomes of patients with mCRC while Mandola MV et al 2003 and Mandola MV et al 2004 found new polymorphisms that could affect the result (SNP G>C and polymorphisms in the TYMS 3'UTR). We sought to investigate if these polymorphisms impact independently survival outcomes, taking into consideration the KRAS and BRAF mutations in a multivariate manner.

5 What are the novel findings of this study?

Our study indicates that after taking into account the SNP G>C and LOH, only the polymorphisms in the *TYMS* 3'UTR, affecting the stability of mRNA, independently influenced the survival outcome for patients with mCRC treated with fluoropyrimidines-based chemotherapy. Genotypes that include del alleles, linked to *TYMS* mRNA instability, had better survival outcome.

These results suggest that *TYMS* polymorphisms could play a role in tailoring treatment of patients with mCRC but further prospective studies are needed to validate these.

Thank you again for accepting our manuscript for publication in the *World Journal of Gastroenterology*.

Sincerely yours,

Anastasios Ntavatzikos, MD, PhD student