# Study of IDO1 gene expression in histological variants of colorectal carcinoma

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**Background:** Conventional carcinomas (CCs) which represent most colorectal carcinomas (CRCs) have no alteration in microsatellite instability (MSI) and are classified as microsatellite stable tumors (MSS) while tumors with high-grade of microsatellite instability (MSI-H) has been associated with proximal-located sporadic CRC showing MLH1 promoter methylation and BRAF mutation. MSI-H tumors present more lymphocytic infiltrate than CCs. MSI-H tumors are considered as one end-point of the so-called serrated polyp pathway. In contrast, another CRC from this pathway, the serrated adenocarcinoma (SAC), which is more frequently KRAS mutated, usually microsatellite stable (MSS) and has no lymphocytic infiltrate, is associated with a bad prognosis. Patients with MSI-H tumors have been reported to get some benefits from immunotherapy treatments while CC and SACs patients do not obtain any benefits. These differences are believed to be due to the differences in the microsatellite instability (MSS or MSI tumors). The aim of this study is to determine the IDO gene expression in the different subtypes of colorectal carcinomas

**Materials and methods:** Colorectal carcinoma samples from Hospital General Universitario Santa Lucia (Cartagena) have been classified for cancer type, age, MSS status, T;N;M. RNAs from CCs, SACs and hmMSI-H tumors were extracted with the miRNeasy kit (ref:217004, Qiagen) and used for validation by qPCR. The retrotranscriptase reaction was performed from a total of 1 µg of DNAseI-treated RNA using the DyNAmo cDNA synthesis Kit (ref:F470L) provided by Thermo Scientific (Rockford, IL). Five µl of 1:5 diluted cDNA was added to the qPCR reaction containing 12.5µl 2X QuantiTect SYBR Green PCR Kit (ref:204145, Qiagen) and 300nM of each primer in a total volume of 25µl. qPCR was performed on a 7500F real time PCR system by Applied Biosystems (Foster City, CA, USA) according to the instruction manual and following the standard protocol. Primers used were IDO1, PDL-1, CTLA4 and GAPDH as housekeeping gene.

**Results:** As it is showed in Fig 1, IDO1 expression is higher in MSI-H tumors than in MSS tumors (n=11, p=0,126) but it does not reach statistically significant and do not correlationate with PD-L1 (n=11; p=0,662) and CTLA-4 (n=11; p=0,755) expression.

In Fig 2, it is analyzed PD-L1 and CTLA4 expression in CC, SAC and MSI-H tumors in the same samples as IDO1, there is a small coincidence between the patterns of IDO1(n=11; p=0,208) and PD-L1 (n=11; p=0,166) expression and no correlation with CTLA4 (n=11; p=0316) expression.

Preliminary results of more samples of CTLA4 and PD-L1 expression (n=39) show CTLA4 expression is higher in SAC than in CC or MSI-H tumors (p=0,44) while the difference in PD-L1 expression is not statistically significant among the tumors (p=0,906). The difference between MSS and MSI-H tumors was also analyzed but no statistically differences were found (CTLA4 p=0,317; PD-L1 p=0,460).

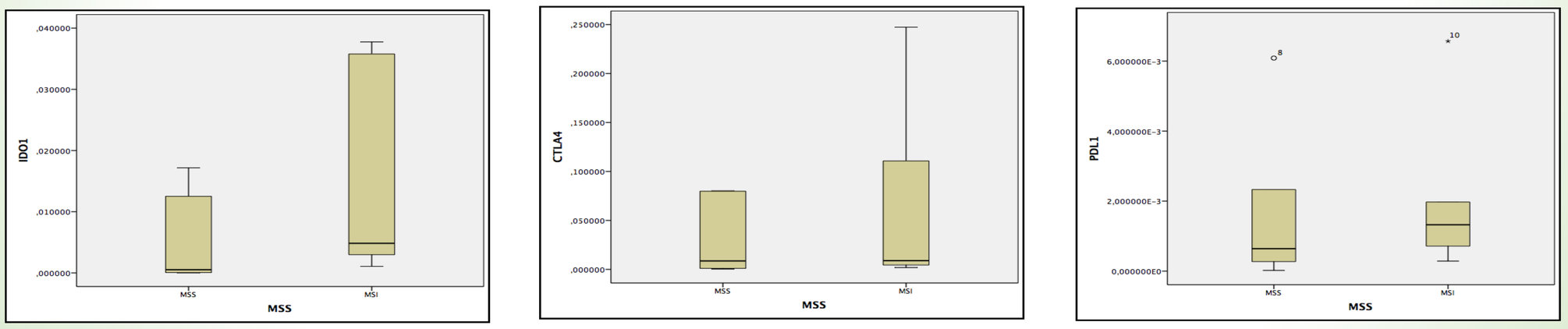


**Fig1 : Comparison of IDO1, CTLA4 and PD-L1 expression between MSS and MSI colon tumors**

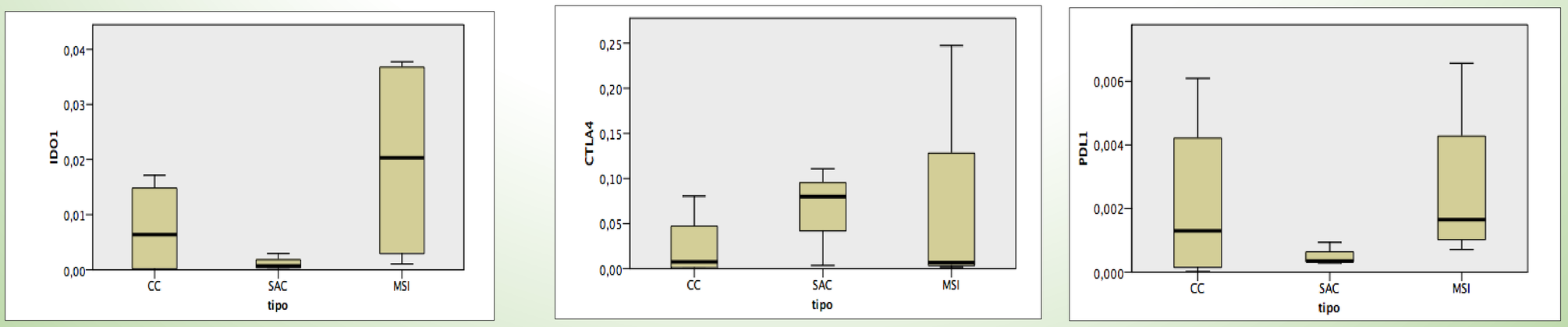


**Fig2 : Comparison of IDO1, CTLA4 and PD-L1 expression in the different subtypes of colon tumors**

**Conclusions:** After this preliminary result, it could be interesting to analyze IDO1 expression in order to decide the best strategy in each tumor. We are working to increase the number of analyzed samples to confirm this preliminary result.

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