Left-sided tumors and low expression of Mdm2 are independent favorable prognostic indicators of OS for stage II and III colorectal cancer.

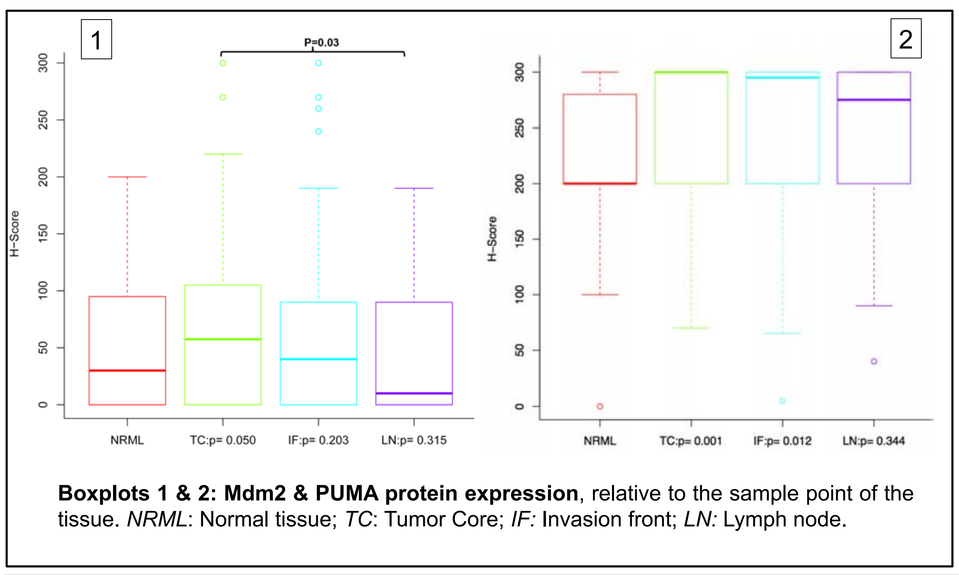
A. Mekras[1], M. Bobos[2], A. Vasilakou[1], D. Mekras[3], G. Mpasdanis[3], C. Atamer[1], A. Michalopoulos[3].

[1]Verbundkrankenhaus Bernkastel/Wittlich, [2]Aristotle University of Thessaloniki, [3]Aristotle University, General Hospital AHEPA

AIM: The aim of our study was to investigate, in tumors of patients who underwent surgery for colorectal cancer (CRC) stage II and III, the immunohistochemical (IHC) expression of a series of protein markers involved in apoptotic PUMA/p53 pathway, such as Mdm2, using the method of tissue microarrays (TMA) and correlated the results with clinicopathological characteristics and prognosis.

METHODS: The study included 130 patients with AJCC stage II and III who underwent surgery. Paraffin tissue blocks containing tumor, normal tissue and metastatic lymph node used for the creation of 7 TMA blocks. Specific antibodies involved in apoptotic PUMA/p53 pathway studied by IHC (PUMA, p53, Ki-67, BAD, BID, Bcl2 and MDM2).
The H-Score method was used for evaluation of IHC results. The median H-Score was used as a predetermined cut-off for each antibody. Positive samples were considered when the value of H-Score was equal to or greater than the median. Fisher's exact tests and Mann-Whitney or Kruskall-Wallis tests were used to test associations between protein expression and different categorical clinicopathological variables. Survival probabilities were estimated by the Kaplan-Meier method and compared using the log-rank test. For the multivariate analyses of the prognostic markers, model choice was performed using backward selection criteria with P<0.15, including in the initial step clinicopathological parameters.

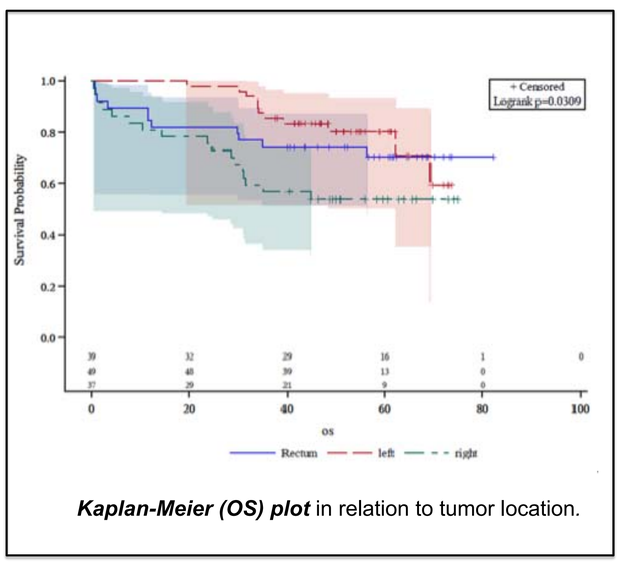
RESULTS: Of all patients, 39 (30%) had cancer in the right colon, 52 (40%) in the left colon, and 39 (30%) in the rectum. In 88 (67.7 %) cases administered postoperative chemotherapy, 40 (30.8%) received no treatment other than surgical excision of the tumor, while for 2 (1.5%) did not have any postoperative information. The median follow up time was 54 months (range: 1- 81) and the 3-year survival (CSS) was 70.8%. The majority of tumors showed moderate histologic differentiation (71.5%), 50% of patients was stage B2, while the remaining 50% was stage C1 and C2 according to Astler-Coller classification.



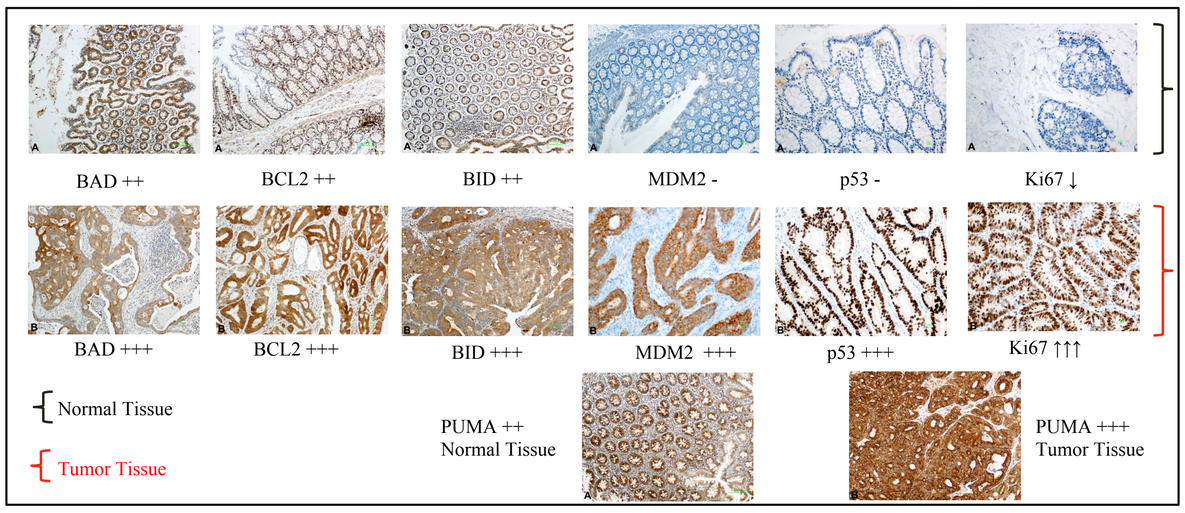
Boxplots 1 & 2: Mdm2 & PUMA protein expression, relative to the sample point of the tissue. *NRML*: Normal tissue; *TC*: Tumor Core; *IF:* Invasion front; *LN:* Lymph node.

		BAD			BCL2			BID			Ki-67			Mdm2			PUMA		
		H	L	P	H	L	P	H	L	P	H	L	P	H	L	P	H	L	P
BCL2	H	14 (50.0)	19 (48.7)	0.918															
	L	14 (50.0)	20 (51.3)																
BID	H	16 (57.1)	11 (27.5)	**0.014**	11 (34.4)	15 (44.1)	0.418												
	L	12 (42.9)	29 (72.5)		21 (65.6)	19 (55.9)													
Ki-67	H	17 (63.0)	14 (35.9)	**0.030**	17 (50.0)	15 (44.1)	0.627	15 (57.7)	16 (41.0)	0.188									
	L	10 (37.0)	25 (64.1)		17 (50.0)	19 (55.9)		11 (42.3)	23 (59.0)										
Mdm2	H	14 (51.9)	17 (43.6)	0.508	17 (53.1)	13 (40.6)	0.316	15 (55.6)	17 (42.5)	0.294	10 (32.3)	20 (60.6)	**0.023**						
	L	13 (48.1)	22 (56.4)		15 (46.9)	19 (59.4)		12 (44.4)	23 (57.5)		21 (67.7)	13 (39.4)							
PUMA	H	15 (55.6)	18 (46.2)	0.453	17 (51.5)	17 (50.0)	0.901	16 (64.0)	17 (42.5)	***0.092***	23 (71.9)	11 (32.4)	**0.001**	17 (56.7)	15 (45.5)	0.374			
	L	12 (44.4)	21 (53.8)		16 (48.5)	17 (50.0)		9 (36.0)	23 (57.5)		9 (28.1)	23 (67.6)		13 (43.3)	18 (54.5)				
p53	H	14 (51.9)	18 (47.4)	0.722	16 (48.5)	16 (48.5)	1.000	14 (53.8)	17 (44.7)	0.474	18 (58.1)	14 (40.0)	0.143	17 (56.7)	14 (42.4)	0.259	16 (48.5)	17 (53.1)	0.708
	L	13 (48.1)	20 (52.6)		17 (51.5)	17 (51.5)		12 (46.2)	21 (55.3)		13 (41.9)	21 (60.0)		13 (43.3)	19 (57.6)		17 (51.5)	15 (46.9)	

Correlation of **proteins expression** at the **IF** of the tumor.



Kaplan-Meier (OS) plot in relation to tumor location.



Multivariate Cox Analysis

Description	Hazart Ratio	WaldLower	WaldUpper	Wald's p-value
TNM_N N1 vs N0	0.451	0.113	1.804	0.2601
TNM_N N2 vs N0	2.843	0.897	9.013	0.0759
Tumor_Location_Rectum vs left colon	0.920	0.280	3.018	0.8902
Tumor_Location_Right vs left colon	3.407	1.104	10.510	0.0330
Invasion Yes vs No	2.866	0.985	8.333	0.0532
PUMA_p53_ high/high vs low/low	1.315	0.230	7.505	0.7579
PUMA_p53_high/low vs low/low	4.599	0.847	24.982	0.0772
PUMA_p53_ low/high vs low/low	2.674	0.382	18.729	0.3221
Ki-67_High vs Low	2.222	0.834	5.924	0.1104

Cancer Specific Survival (CSS)

Description	HazardRatio	WaldLower	WaldUpper	Wald's p-value
TNM_NN1 vs N0	0.818	0.288	2.323	0.7064
TNM_N N2vs N0	4.895	1.966	12.187	0.0006
Tumor_Location_Rectum vs left	5.053	1.510	16.913	0.0086
Tumor_Location_right vs left	2.629	1.025	6.743	0.0442
Adjuvant_chemo none vs folfox	7.356	2.677	20.213	0.0001
Adjuvant_chemo 5FU-ver vs folfox	1.098	0.123	9.800	0.9332
Adjuvant_chemo xelox vs folfox	0.066	0.008	0.582	0.0143
MDM2_High vs Low	2.001	1.018	5.634	0.1044
p53_ High vs Low	0.353	0.101	1.918	0.0159
Ki-67_ High vs Low	2.942	1.317	6.570	0.0085

Overall Survival (OS)

Statistically significant correlations emerged between BAD/BID (P=0.001), PUMA/BAD (P=0.018), PUMA/Ki-67 (P<0.0001) and p53/Mdm2 (P=0.033). There was a significant difference in expression of BAD, BID, PUMA, Ki-67 and p53 both in Tumor Core (TC), and the Invasion Front (IF) of the tumor compared to normal tissue (NRML). Correlations of protein markers expression with clinicopathological features showed statistically significant associations between Bcl-2 and tumor differentiation (P=0.036), BAD and tumor localization (P=0.011), p53 and the number of infiltrated lymph nodes (P=0.024), BID with the maximal tumor diameter and with lymphovascular invasion (P=0.002 and P=0.049 respectively), Mdm2 with maximal tumor diameter (P=0.015), and Ki-67 with mucinous adenocarcinomas (P=0.016). The univariate survival analysis showed significantly smaller CSS, DFS and OS for patients with lymphatic invasion (P=0.0031, P=0.0072 and P=0.0289) and >4 infiltrated lymph nodes (P=0.0016, P=0.0051 and P<0.0001). Lower OS, observed in patients aged >65 years (P=0.0109), in patients AJCC stage IIIC (P=0.0035), and with adenocarcinoma in the right colon (P=0.0309). In multivariate analysis as **independent unfavorable prognostic indicators for both DFS and the CSS** highlighted the lymphovascular invasion (HR=4.17 and H =2.72), >4 infiltrated lymph nodes (HR=1.39 and HR=1.79) and high Ki-67 labelling index (HR=3.20 and HR=2.36). In addition, **independent adverse predictor for DFS** was high expression of BAD (HR=2.97). For **OS** emerged as independent adverse prognostic indicators >4 metastatic lymph nodes (HR = 4.89), the location of the primary tumor in the rectum and right colon (HR = 5.05, HR = 2.63), no chemotherapy treatment (HR = 7.36), and high-expression of p53, Mdm2 and Ki-67 (HR = 0.35, HR = 2, and HR = 2.94). Finally, as an independent unfavorable prognostic value was highlighted the combination of high expression of PUMA with low expression of p53 in both OS and the CSS (HR: 2.42, and HR: 4.6).

CONCLUSION: We present significant differences in expression of apoptotic PUMA/p53 pathway between tumor and normal tissue, as well as, substantial associations among markers expression with clinicopathological features. Our results underline the reported in the literature difference in prognosis of RCRC and LCRC patients, while the observed Mdm2 overexpression in CRC denote the role of Mdm2 as potentially valuable target for molecular anti-cancer therapy. Larger studies are required to investigate the role of Mdm2 amplification and overexpression in therapeutic strategy for cancer treatment.

European Colorectal Congress 2019, 1-5 December St. Gallen, Switzerland