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Title: Molecular profile in Paraguayan colorectal cancer patients, towards to a precision medicine strategy.

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Title: Molecular profile in Paraguayan colorectal cancer patients, towards to a precision medicine strategy.

Keywords: Colorectal cancer, mutational profile, microsatellite instability, precision medicine, Oncocarta.

Abstract:. Somatic mutation analysis and evaluation of microsatellite instability (MSI) have become mandatory for selecting personalized therapy strategies for advanced colorectal cancer and are not available as routine methods in Paraguay. The aims of this study were to analyze the molecular profile as well as the microsatellite status in a series of advanced colorectal patients from two public hospitals from Paraguay, to introduce these methodologies in the routine practice to guide the therapeutic decisions. Methods: 36 patients diagnosed with advanced colorectal from two referent public hospitals from Paraguay were recruited from May 2017 to February 2018. Sequenom Mass spectrometry, Oncocarta Panel V.1 was applied to analyze the mutational profile from FFPE samples. The microsatellite status was tested by immunohistochemistry. Results: The mean age of the patients was 52 years with a range from 20 to 74 years. Eighty three percent of the patients included in the study has advanced-stage tumors at the moment of the diagnosis. Sixteen patients (44.4%) were wild-type for all the oncogene regions analysed with the Oncocarta panel. 32 hot-spots mutations on seven oncogenes, among 20 patients (55.6 %), were identified, including KRAS, NRAS, BRAF, PI3KCA, FGFR, EGFR and PDGFRA. Five patients (14%) presented microsatellite instability. Conclusions: The immunohistochemical study for microsatellite status and the molecular profile analysis through Sequenom Mass Spectometry are feasible and useful methods, due to identify those patients candidates for targeted therapies and for the budgetary calculations of the National Health Plans.

Introduction:

Colorectal cancer (CRC) is the third most common cancer worldwide in terms of incidence, but also the second in terms of mortality with over 1.8 million new CRC cases and 881,000 deaths estimated to occur in 2018 [1].The highest CRC incidence rates are found in some parts of Europe (including Hungary, Slovenia, Slovakia, the Netherlands and Norway), Australia/New Zealand, Northern America and Eastern Asia. As an extent of westernization, CRC now ranks as the top five cancer in Latin-America, being the second and third leading cause of cancer death in South America and the Caribbean, respectively [2]. Paraguay occupies the position 19th within Latin-American countries with an incidence of 15 and 12.7 per 100.000 in men and women, respectively, and a mortality of 9 and 7.5 per 100.000 in men and women, respectively [1].

CRC in Latin-American countries is diagnosed at an advanced stage of the disease in almost 80% of cases, especially in those related with low socio-economic conditions [3]. Metastatic disease initially is not suitable for potentially curative resection. Therefore, advanced target-therapies, such as monoclonal antibodies (bevacizumab) or proteins (aflibercept) against vascular endothelial growth factor (VEGF), and against the epidermal growth factor receptor (EGFR), respectively, in combination with chemotherapy, should be considered in patients with metastatic CRC (mCRC), since they improve the outcome of mCRC patients [4-7]. However, the high costs associated with these targeted-therapies limits their application in developing countries, including Paraguay [8, 9].

The gold standard of the therapeutic strategy includes a multidisciplinary management and an early approach to the disease. For the advanced disease, chemotherapy associated with targeted therapies selected according to the pathological and molecular profile of the tumor, increase the median overall survival (OS) to around 30 months. Factors which may have contributed to this improvement in the OS includes: i) continuous and more exhaustive follow up, ii) improvements in the efficacy of systemic therapies, iii) inclusion of biomarker-based patient selection [10].

Somatic mutation analysis has become mandatory for selecting personalized therapies for CRC. Mutation profiling of the *RAS*/*BRAF* pathway could guide the selection of patients with potential

benefit from anti-EGFR therapies [5, 11, 12]. Mutations in *KRAS* or *NRAS* (expanded *RAS* analysis) predict a lack of response to EGFR-targeting monoclonal antibodies. Moreover, this targeted therapy has a detrimental effect in patients with *RAS*-mutant tumours, specifically when combined with an oxaliplatin-based cytotoxic backbone [13-15]. *BRAF* mutations (mainly V600E) are found in around 8%-12% of patients with mCRC included in clinical trials and are almost exclusively non-overlapping with other *RAS* mutations. *BRAF* mutations are a significant negative prognostic marker for patients with mCRC [16]. Moreover, two meta-analyses demonstrated that the benefit of EGFR antibody therapies was greater in patients with *RAS* wild-type/*BRAF* wild-type tumours than in those with *RAS* wild-type/*BRAF*-mutant tumours [11, 17]. Methods for molecular testing include Sanger sequencing, pyrosequencing, next generation sequencing (NGS) technology and mass spectrometry with different spectrum of advantages /disadvantages [18]. The mass spectrometry technique, matrix-assisted laser desorption/ionization-time of flight, is a cost-effective method that has been used to assess point mutations across different solid tumors [19, 20]. The Sequenom MassARRAY technology, in combination with a commercial kit called OncoCarta v1.0 was used to screen 238 somatic mutations across 19 oncogenes exploring somatic changes in oncogenes with known responses or resistance-targeted therapy.

The evaluation of microsatellite instability (MSI) in CRC through the immunohistochemical (IHC) study for mismatch repair proteins (MMR) expression has become mandatory in daily practice for various reasons. MMR deficiency is the main characteristic of the CMS1 group of the latest CRC consensus molecular classification [21]. This group of tumors is linked to specific clinicopathologic features with lower rates of response to chemotherapy and shorter disease-free survival periods after treatment. About 15% of CRC arise through the MSI pathway and most of these tumors are sporadic [22]. However, the IHC study for MMR proteins is recommended for the detection of the hereditary non-polyposis CRC syndrome (Lynch syndrome) accounting for 1% to 5% of all the cases [23]. Moreover, MSI is the only predictive biomarker approved by the FDA for the immune checkpoint blockade inhibitors therapy with pembrolizumab and nivolumab in gastrointestinal tumours. MMR deficient mCRC, represents approximately 4% of all mCRC cases and is characterized by very high levels of mutations. Extensive basic research and clinical trial efforts are underway to identify the optimal therapy combinations that are needed for this CRC subset.[24, 25]

The aim of this study was to characterize the underlying molecular changes associated to CRC in the Paraguayan population. For this purpose, we evaluated the histopathological features, the presence of common somatic mutations and the MMR proteins status in a cohort of

prospectively recruited Paraguayan patients, in order to incorporate these determinations in the Paraguayan Health Care System to guide therapeutics decisions.

Materials and Methods:

Patient selection and data collection

The design of the study was exploratory and prospective. A total of 36 consecutive and non-related CRC patients were recruited from May 2017 to February 2018 at the Medical Oncology Units from two public hospitals in Paraguay: Hospital de Clinicas (HC) and Instituto Nacional del Cancer (INCAN). Patient eligibility criteria included clinical and histological diagnoses of advanced CRC chemo-naive or in progression to a first line chemotherapy for the advanced disease, ECOG 0 or 1, and potential candidates to receive chemotherapy in combination with target therapies according to the clinical guidelines [26].

Clinical and pathological information, including age, sex, tumor location, histological grade and treatments were collected (Table 1). All study subjects gave written informed consent, and the study was approved by the Biomedical Research Institute INCLIVA and the Hospital de Clínicas-Paraguay Ethics Committee.

Formalin-fixed paraffin-embedded (FFPE) tissues were evaluated for their tumor content, and sections containing more than 30% tumor cells were selected by a dedicated pathologist. Genomic DNA was isolated from 4 unstained sections of 20 µm and diluted to a final solution of 10ng/µl. This was done using the QIAamp DNA FFPE tissue kit (QIAGEN). DNA concentration was quantified in samples by NanoDrop (NanoDrop Technologies, Wilmington, DE, USA).

Immunohistochemistry

Immunohistochemistry (IHC) assays were performed in the 36 colorectal cancer patients as we previously described [27]. The primary antibodies used were MLH1 (clone IR079, dilution 1:100, Dako), MSH2 (clone IR085, dilution 1:100, Dako), PMS2 (clone IR087, dilution 1:100, Dako) and MSH6 (clone IR086, dilution 1:100, Dako).

Tumours were considered positive if they present only nuclear staining, with or without cytoplasmic staining. Peritumoral lymphocytes, stromal cells and non-neoplastic epithelial cells were used as internal control. Only the complete loss of nuclear staining with positive internal control was classified as loss of miss-match repair (MMR) protein expression and was considered as evidence of microsatellite status instability (MSI). Normal expression was defined as the presence of nuclear staining in tumor cells, irrespective of the intensity.

Sequenom MassARRAY somatic mutation genotyping

The Sequenom MassARRAY and OncoCarta Panel v1.0 were used following the manufacturer's protocol (Sequenom, San Diego, CA, USA; (http://agenabio.com/oncocarta-panel)) as previously described [19]. The panel consisted of 24 multiplex assays capable of detecting 238 mutations in 19 oncogenes. This procedure was a rapid, cost-effective method of identifying key cancer driving mutations across a large number of samples because it avoided complex bioinformatic analyses and assays were performed within two days. The amount of DNA added to the polymerase chain reaction was 20 ng per reaction. DNA was amplified using the OncoCarta PCR primer pools. Unincorporated nucleotides were inactivated by shrimp alkaline phosphatase (SAP), and a single base extension reaction was performed using extension primers that hybridize immediately adjacent to the mutations and a custom mixture of nucleotides. Salts were removed by the addition of a cation exchange resin. Multiplexed reactions were spotted onto SpectroCHIP II arrays, and DNA fragments were resolved by MALDI-TOF on the Compact Mass Spectrometer (Sequenom, San Diego, CA). An additional customized panel was used for some of the samples as a quality control. Details regarding genes and hot-spot mutations analysed within the OncoCarta panel are provided within Supplementary Table 1.

Statistical analyses

Statistical analyses were carried out by IBM SPSS v 20.0. A *p value* of less than 0.05 was considered statistically significant. Comparison between clinical and pathologic patient's characteristics was done using the Chi squared test, the Fisher's exact test or the Wilcoxon rank test for qualitative and quantitative variables respectively prior assessment of normality using the Shapiro-Wilk test. Tumor-specific survival (TSS) was calculated from the time of diagnosis to the time of death because of tumor-related causes or until the last known follow-up. Survival curves were performed by using the Kaplan-Meier analysis compared thought the log-rank test.

Multivariate regression analysis was carried using Cox proportional hazards models with stepwise selection, including those variables significantly correlated with the survival probability on the univariate analysis. SPSS v20.0 was used to analyze the results.

Genomic data were analyzed using the Sequenom MassARRAY Typer Analyser 4.0 Software to visualize the mass spectra for mutations and to determine the frequency of mutant and wild-type alleles. The lower thresholds for mutation detection have been reported between 5-10% [28]. In order to reduce putative false positives, we set the threshold at 10%. More specifically, only mutations with frequencies higher than 10% were taken as positive results. Mutations were manually reviewed by use of visual and raw spectrum patterns. Two different personnel in the laboratory scored mutations, and no discrepancies were observed. Analyses were performed using IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp (IBM Corp. Released 2010).

Mutational Waterfall plot of the patients dataset was performed through visualization of the plot by cBioportal-OncoPrimer v1.18.0 (www.cbioportal.org/OncoPrimer) and Llolliplots have been draw with cBioportal-Mutation Mapper v1.18.0 (www.cbioportal.org/MutationMapper) [29, 30].

Results:

Patient characteristics

Seven patients (19%) from Hospital de Clínicas and 29 patients (81%) from INCAN were included in the study. The mean age of the patients was 52 years with a range from 20 to 74 years. Twenty-three patients were males (63.9%) and 13 were females (36.1%). Eighty three percent of the patients included in the study has advanced-stage tumors at the moment of the diagnosis with more tumors located in the left (61%, 22 cases) than in the right side (39%, 14 patients). All the patients received a first line chemotherapy. In addition, 9 of the patients received bevacizumab treatment.

Clinical characteristics of the patients are shown in Table 1 and Table supplementary 2.

Mutational analysis

The molecular characterization analysis showed 16 patients (44.4%) wild-type for all the oncogene regions analysed with the Oncocarta panel. We have been able to identified 32 hot-spots mutations on seven oncogenes among 20 patients (55.6 %). A total of 16 different oncogenic mutations were identified. Considering that the threshold of mutation detection with the technology applied is 10%, we observed a median average mutation load of 22.44% among all the samples, ranging from 8.5% up to 53,7%.

The most frequently mutated genes were *KRAS* in 11 tumours (7 with p.G12D, 3 with p.G12V and one with p.G13D), *PIK3CA* in 8 tumours (5 mutations in the hot-spot p.H1047R/Y, one in p.G.1049R, p.E542K and p.R88Q respectively), *NRAS* (all in p.G13D) and *BRAF* (2 in p.V600E, one in p.D594V and one in p.G469R) in 4 tumours. Seven out of 20 patients have two or more mutations. Four patients have co-occurrence mutations in *KRAS* and *PIK3CA*. Strikingly, two of the *KRAS/PIK3CA* mutated tumours carried also another mutation in *NRAS* (p.G13D). Low frequently mutated genes were *EGFR*, *PDGFRA* and *FGFR1* and variations in these genes appeared in co-ocurrence with mutations in the most frequently mutated genes mentioned above (see Figure 2). Plot with number of patients with mutations, frequency of mutated genes and co-ocurrences are presented in Figure 2. Full details of protein products of the mutated genes, specific mutations detected, its localisation in protein domain and their frequency are presented in Figure 3.

Immunohistochemistry of miss-matched repair (MMR) proteins

Five patients (14%) presented MSI. Three of them were younger than 50 years old and had family history of CRC. The other two MSI cases were 54 and 57 years old without any family history of cancer. Two patients presented lost of MSH2 and MSH6 expression (a 27 years old female and 41 years old one male). Clinical, pathological and molecular characteristics of patients with MMR protein expression alterations are shown in Table2 and Figure 4.

Clinical correlations of patients and survival data

There was a significant correlation between tumor location (right vs left) and age (> or < 50 years old) ($p<.0.05$). All cases of tumors located in the right colon were patients >50 years old. No other correlations between the clinical characteristics (gender, age, MSS status or mutation profile) were found significant.

The mean TSS at the moment of the analysis was 23.6 months (12-35 months). No differences were found in TSS according to the mutational status, gender, MSS status or the treatment administered (chemotherapy +/-bevacizumab). Survival curves are represented in Figure 5. However, a better survival trend to signification can be observed in relation with the following clinicopathological characteristics: patients without any mutation (candidates to anti-EGFR therapies), male patients, left side colon tumours, patients treated with antiangiogenics and patients with preserved MMR protein expression.

IHC and molecular profile analysis provided relevant information for a personalized medicine approach for all the cases. In our series, 45% of the patients had *RAS* wild-type tumors that could benefit from anti-EGFR therapies. Moreover, five patients with MSI profile, could benefit from immunotherapy with checkpoint inhibitors such as pembrolizumab or nivolumab.[31, 32]

Discussion:

Colorectal cancer (CRC) incidence rates vary widely, with 8-fold and 6-fold variations by world regions for colon and rectal cancer, respectively. Therefore, CRC could be considered a marker of socioeconomic development, as is seen in countries undergoing a major development transition, where incidence rates tend to rise uniformly with the increasing Human Development Index (HDI). [1] These rises in incidence—particularly the generational changes detected in most age-period-cohort analyses— point to the influence of dietary patterns, obesity and lifestyle factors. However, mortality rates are declining in more developed countries due to improvements in survival through the adoption of best practices in cancer prevention, early diagnosis through screening approaches and personalized treatments.[33] Actually, molecular characterization has become a useful and mandatory tool for a personalised medicine approach in CRC [10], however, screening programs are not available in all developing countries.

The situation in Paraguay is alarming, with a CRC incidence rising during the last 20 years for both sexes from a population rate /100.000 of 3.66 and 2.87 for males and females respectively in 1998 to 5.51 for males and 4.88 for females in 2015 [1]. Thus, there is an urgent need for the implementation of effective strategies at primary, secondary and third levels of prevention that could improve the results in Paraguay. For secondary prevention, the first steps have been made during 2018 with the implementation of CRC screening, however, it is still in a very initial stage. Regarding patients with advanced CRC, targeted therapies associated with chemotherapy improve the outcomes. [34, 35] Nevertheless, their high costs limit their availability and use in

Paraguay. Therefore, precision medicine through molecular testing is needed due to identify those patients candidates for targeted therapies and for the budgetary calculations of the National Health Plans.

The aim of our study was to characterize the underlying molecular changes associated to CRC in the Paraguayan population through MassARRAY technology, in order to incorporate these determinations into the Paraguayan Health Care System to guide therapeutics decisions. The Sequenom MassARRAY technology, in combination with a commercial kit called OncoCarta v1.0 was used to screen 238 somatic mutations across 19 oncogenes exploring somatic changes in oncogenes with known responses or resistance-targeted therapy. This methodology makes it possible for a medium-sized laboratory to analyse multiple key hotspot mutations rapidly (within 3 days) and without complex bioinformatics analysis tools at a moderate price. Although the limited number of patients included, our work is the first published data of advanced CRC in Paraguay. We found 45% of patients that could benefit from anti-EGFR therapies according to their mutational profile (*RAS* wild-type). Although, more than 80% of the patients recruited were diagnosed with advanced disease, we detected just above 55% of them with oncogenic mutations. From the 19 oncogenes evaluated, only 7 had mutations (including *KRAS*, *NRAS*, *BRAF*, *PI3KCA*, *PDGFRA*, *EGFR* and *FGFR*), which is in line with data reported in the COSMIC database and in previous studies. The largest CRC series of patients analysed by MassARRAY Oncocarta™ Panel included 239, 254 and 2299 patients [20, 28, 36, 37]. In a previous study from our group [19] mutations were detected in 48 out of 75 CRC cases (64.2%) using this technology. Specifically, mutations were found mainly in the *KRAS*, *PIK3CA* and *KIT* genes. In our experience, the MassARRAY technology in combination with the OncoCarta Panel successfully detected frequent cancer mutations in degraded DNA isolated from FFPE samples and covers up to 95% of known druggable markers. Thus, it provides an efficient mutation screening for clinical research trials and with high concordance with NGS technologies. Our results confirmed that MassARRAY technology is a rapid and effective method for identifying key cancer-driving mutations across a large number of samples, which allows for a more appropriate selection for personalized therapies, and could be a cost-effective method for the molecular profiling in Paraguay.

MSI in mCRC has a global frequency of 4%. As mentioned before, the analysis of MMR is relevant for the diagnosis of hereditary syndromes, as well as for the identification of biomarkers that would guide immunotherapy with immune checkpoint blockade inhibitors [38]. The analysis of MMR can be done through IHC and PCR techniques, both methods are available in Paraguay. Recently, checkpoint inhibitors have been included into the national drugs bank. In our series,

we detected MSI in five cases, two of them showing loss of MSH2 and MSH6, a pattern highly suggestive of Lynch syndrome. Those cases should be comprehensively analysed in genetic counselling units, in order to evaluate the presence of germline mutations. In our series, the young average age of presentation (just over 50 years), and the presence of MMR proteins loss of MSH2 and MSH6 in two cases (5%) highlights the importance of the urgent implementation of genetic counselling units in Paraguay.

Despite the low number of patients included in the study we were able to draw the mutation profile of CRC patients in Paraguay. In addition, the study would provide relevant clinical and molecular information to be included in Public Oncology Reference Hospitals of Paraguay, as well as the usefulness of Sequenom MassARRAY technology for the molecular profiling and the MSI testing to guide the therapeutics to guide the treatment of advanced CRC disease.

Table and Figure legends:

Table 1: Clinical and pathological characteristics of patients diagnosed with colorectal cancer.

Table 2: Clinical, pathological and molecular characteristics of patients with mismatch repair protein expression loss.

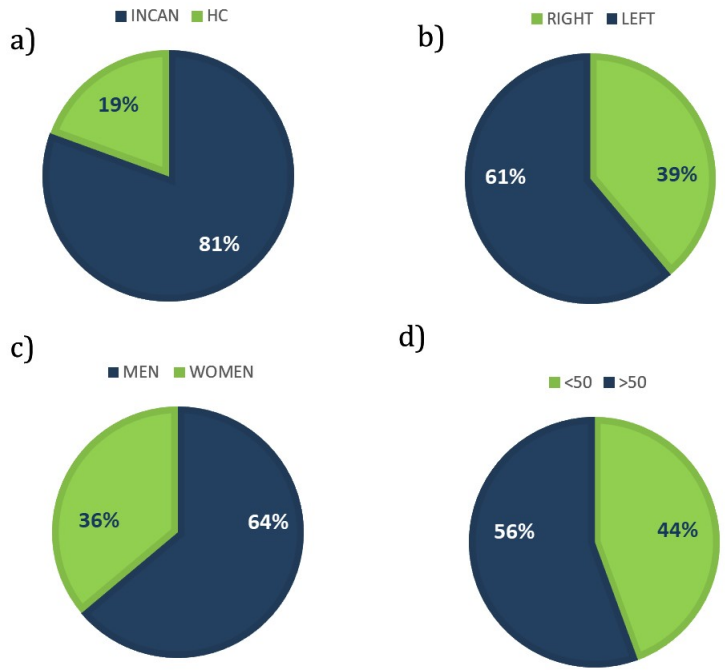

Figure 1: **Demographic characteristics of the serie**. a) Percentage of patients recruited from the two different participating centres: INCAN: Instituto Nacional del Cáncer HC: Hospital de Clínicas, b) Percentage of patients with right (green) and left (blue) location of tumor lesions, c) Distribution of men and women among the samples analyzed, d) Distribution of patients according to the age at the moment of diagnosis > or < 50 years old.

Figure 2: Mutational Waterfall plot of the patient's dataset. Data has been obtained by analyzing the Oncocarta™ v1.0 panel (MassARRAY® System by Agena Bioscience™). Visualization of the plot by cBioportal-OncoPrimer v1.18.0 [29, 30] (www.cbioportal.org/OncoPrimer). Colored squares mean the type of alteration detected: green indicates missense mutation whereas black identifies truncating mutation. All grey

squares identify one patient; when they are without any other color means that no alterations are present in the sample.

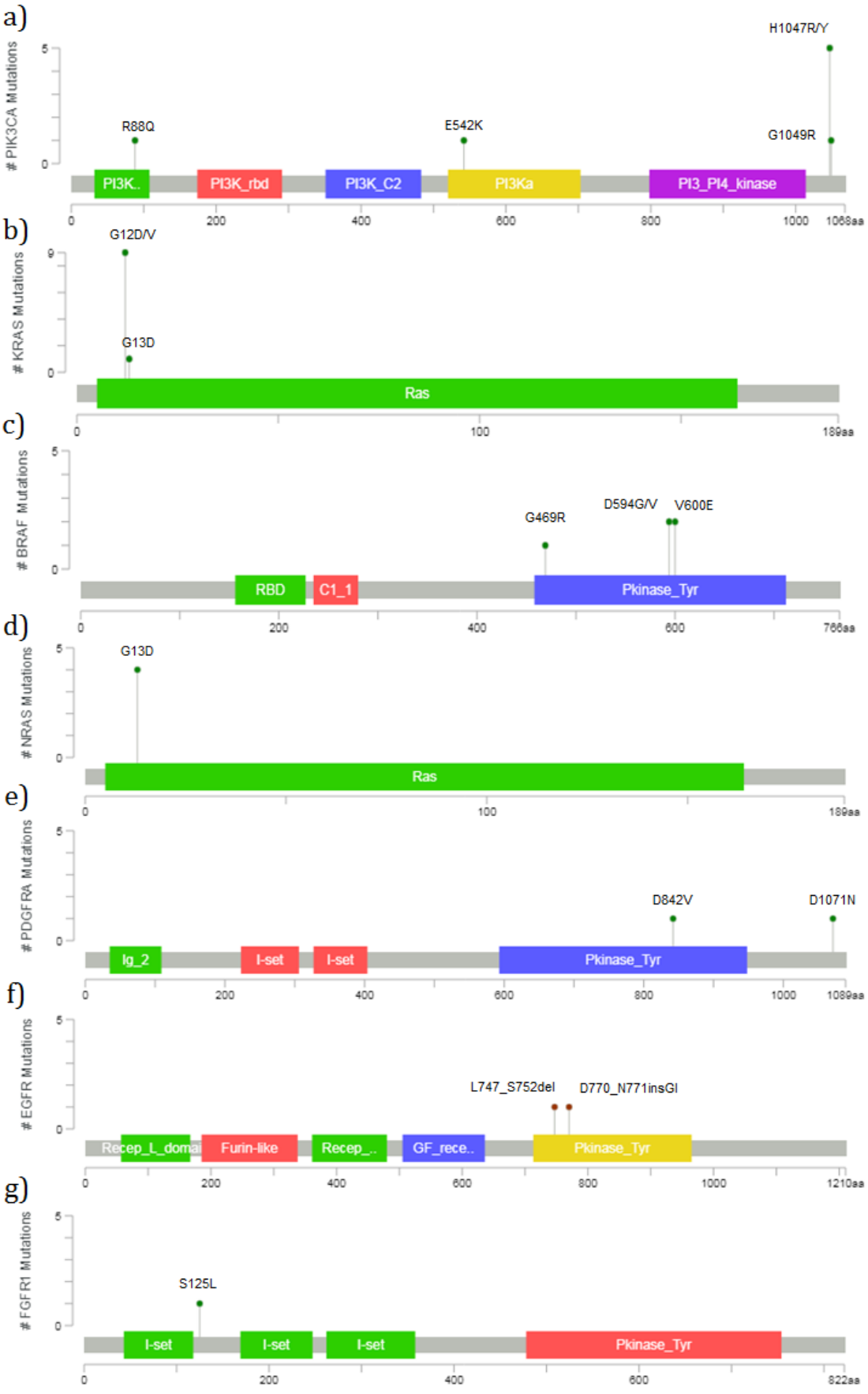
a)
PIK3CA Mutations
H1047R/Y
R88Q
E542K
G1049R
PI3K..
PI3K_rbd
PI3K_C2
PI3Ka
PI3_PI4_kinase
1068aa
b)
KRAS Mutations
G12D/V
G13D
Ras
189aa
c)
BRAF Mutations
G469R
D594G/V
V600E
RBD
C1_1
Pkinase_Tyr
766aa
d)
NRAS Mutations
G13D
Ras
189aa
e)
PDGFRA Mutations
D842V
D1071N
Ig_2
I-set
I-set
Pkinase_Tyr
1089aa
f)
EGFR Mutations
L747_S752del
D770_N771insGI
Recep_L_domai
Furin-like
Recep_..
GF_rece..
Pkinase_Tyr
1210aa
g)
FGFR1 Mutations
S125L
I-set
I-set
I-set
Pkinase_Tyr
822aa

Figure 3: Mapping of Mutations detected in seven oncogenes. Lolliplots have been draw with cBioportal-Mutation Mapper v1.18.0 (www.cbioportal.org/MutationMapper) [29, 30]. The plot identifies the different domains in each respective protein. The nature of the mutations and its position is shown. The number of times each mutation has been detected is shown with the left scale and is represented by the height of dot. A) *KRAS* , b) *PIK3CA*, c) *NRAS*, d) *BRAF*, e) *EFGR,* f) *PDGFRA* and g) *FGFR1*.

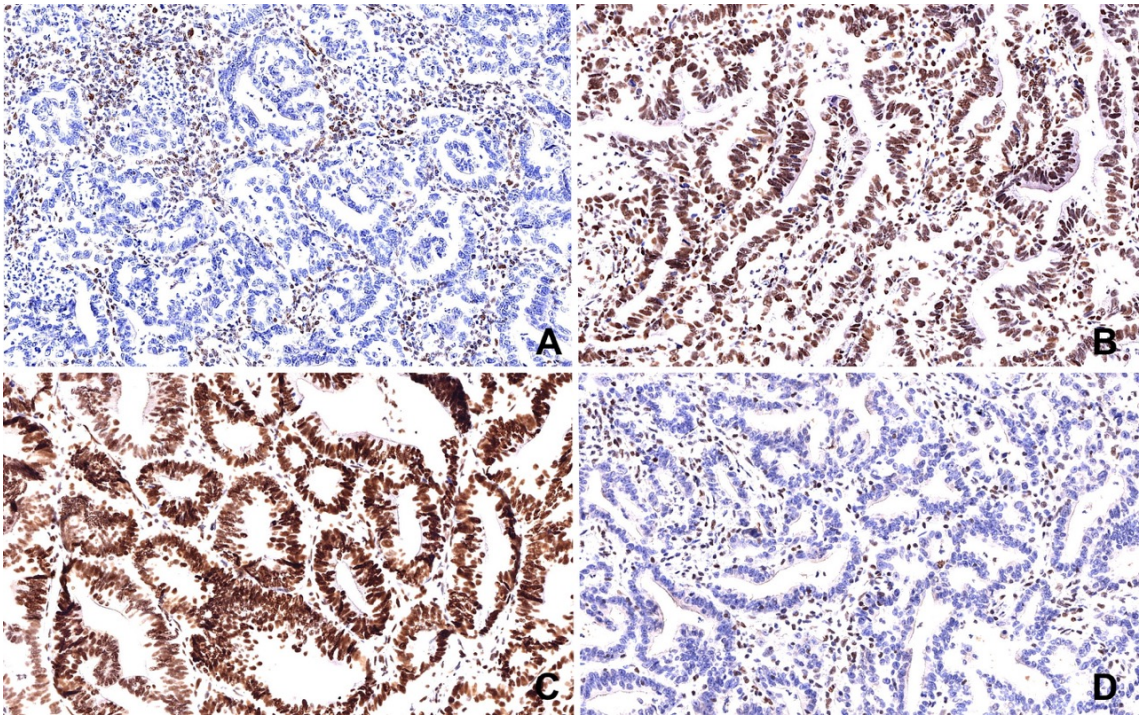

Figure 4: Immunohistochemical study of mismatch repair proteins expression. Complete loss of nuclear staining for MSH6 and MSH2 in tumor cells, with positive internal control in stromal lymphocytes and fibroblasts (A: MSH6 40X, D: MSH2 40X) Retained MLH1 and PMS2 nuclear expression in tumor cells (B: MLH1 40X, C: PMS2 40X).

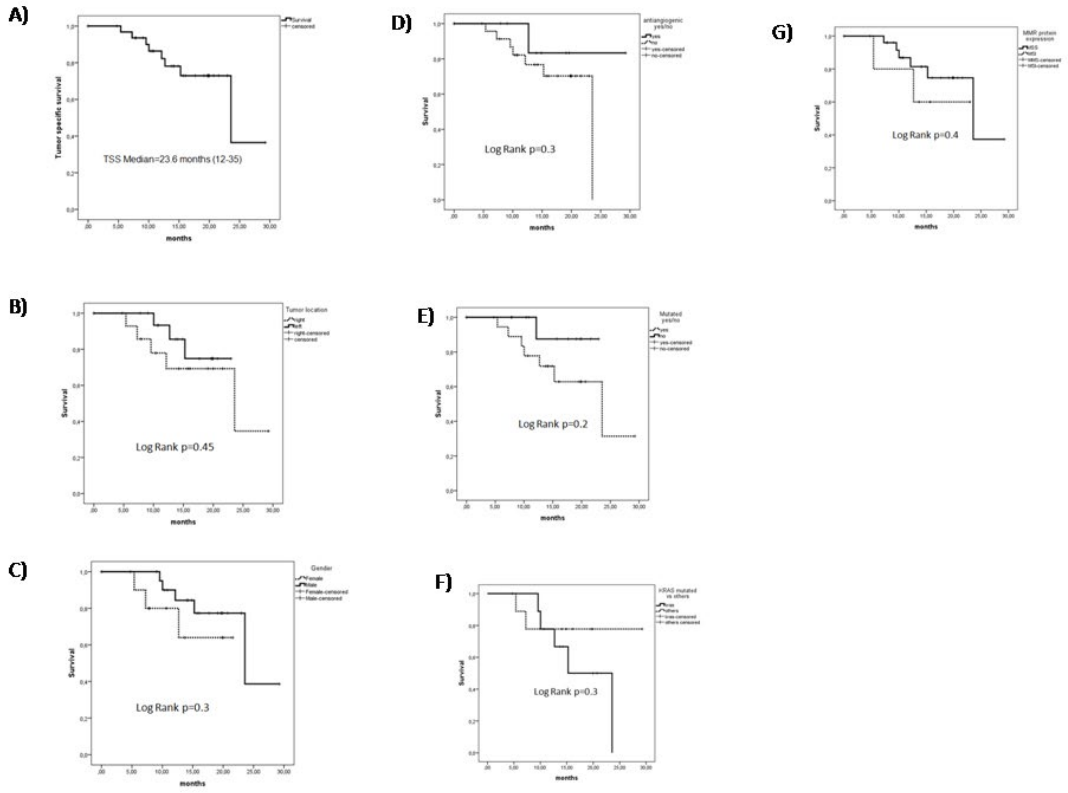

Figure 5: Kaplan Meier curves of tumor specific survival (TSS) of the colorectal cancer patients. **A)** Tumor specific survival of the all series. **B)** Tumor specific survival according to the tumor location (left vs right) **C)** Tumor specific survival according to the gender (males vs females). **D)** Tumor specific survival according to the treatment administered. (chemotherapy + antiangiogenics vs chemotherapy alone). **E)** Tumor specific survival according to the mutation profile (mutated/no mutated). **F)** Tumor specific survival according to the mutation profile: KRAS vs other mutations.

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Table 1- Clinical and pathological characteristics of patients diagnosed with colorectal cancer

Mean age (range)	52 (20-74)
Sex (%)	
Female	13 (36.1)
Males	23 (63.9)
Tumor location (%)	
Right	14 (39)
Left	22 (61)
Histology Grade (1-3)	
G1	1 (3)
G2	26 (72)
G3	9(25)
Mutation profile (%)	
ALL RAS WT	16 (44.4)
RAS/BRAF mutated	15 (41.6)
Other alterations	5 (14)
MSS status (%)	
MSS	29 (80.5)
MSI	5 (13.8)
Unknown	2 (5,7)
Familiar CRC / breast/ovarian(%)	7 (19.4)
Unknown	29 (80.6)
Clinical stage at diagnosis (%)	
Stage I-III	6 (16.7)
Stage IV	30 (83.3)
1st Line Treatments administered (%)	
5-FU + oxaliplatin/irinotecan	27 (75)
5-FU + oxaliplatin/irinotecan + Bevacizumab	9 (25)

Table 2. Clinical, pathological and molecular characteristics of patients with MMR protein expression alterations.

Gender	Age	Location	Molecular profile	MMR protein expression (IHC)	Family history (Bethesda or Amsterdam criteria)
Female	57	right	BRAF (11.0%)	PMS2 / MLH1 lost	-
Female	27	left	KRAS (25.7%)	PMS2 / MLH1 lost	+
Female	27	left	KRAS (29.0%)	MSH6 lost and MSH2 heterogeneous expression	+
Male	41	left	WT	MSH2 / MSH6 lost	+
Male	54	right	WT	PMS2 / MLH1 lost	unknown

Table supplementary 2: Clinical and Molecular characteristics of patients.

LAB CODE	CENTRE	GENDER	AGE YO	TUMOR LOCATION	GENE	MUT	%	MMR EXPRESSION
MTCYD1	INCAN	Male	< 50	Right		ALL WT		Normal
MT-CYD2	INCAN	Female	< 50	Left	KRAS	G12D	29,50%	MSH6 & MSH2 (-)
MT-CYD3	INCAN	Female	≥50	Left	BRAF	V600E	17,30%	Normal
MT-CYD4	INCAN	Male	< 50	Left	PIK3CA	G1049R	11.00%	NV
MT-CYD5	INCAN	Female	< 50	Left	KRAS	G12D	25,70%	PMS2 & MLH1(-)
MT-CYD6	HC	Male	≥50	Left	KRAS	G12V	31.20%	Normal
MT-CYD7	HC	Female	< 50	Left	KRAS	G13D	25,60%	Normal
MT-CYD8	HC	Male	< 50	Left	KRAS	G12D	24,20%	Normal
MT-CYD9	INCAN	Male	< 50	Right		ALL WT		Normal
MT-CYD10	INCAN	Male	< 50	Left	BRAF	V600E	18,80%	Normal
MT-CYD11	INCAN	Male	< 50	Left		ALL WT		Normal
MT-CYD12	HC	Female	≥50	Right	BRAF	D594V|G	11,20%	PMS2 & MLH1 (-)
MT-CYD13	HC	Male	≥50	Right	NRAS	G13D	25,60%	Normal
					PIK3CA	H1047R	11,80%	
					KRAS	G12D	26,80%	
MT-CYD14	HC	Female	≥50	Right	FGFR1	S125L	42,50%	Normal
					NRAS	G13D	25,90%	
MT-CYD15	HC	Male	< 50	Right	KRAS	G12V	16,40%	Normal
MT-CYD16	INCAN	Male	≥50	Right		ALL WT		Normal
MT-CYD17	INCAN	Female	≥50	Left		ALL WT		Normal
MT-CYD18	INCAN	Female	< 50	Left	KRAS	G12D	28,10%	NV
					PIK3CA	H1047R	15,10%	
MT-CYD19	INCAN	Male	< 50	Left	BRAF	G469R	24,90%	Normal
MT-CYD20	INCAN	Male	≥50	Left		ALL WT		Normal
MT-CYD21	INCAN	Female	≥50	Left	PIK3CA	R88Q	39,50%	Normal
					PIK3CA	H1047Y	13,60%	

					PDGFRA	D1071N	10,10%	
MT-CYD22	INCAN	Female	≥50	Right		ALL WT		Normal
MT-CYD23	INCAN	Male	≥50	Right	EGFR	D770_N771insG	33,80%	Normal
					NRAS	G13D	10,40%	
					PDGFRA	D842V	53,70%	
MT-CYD24	INCAN	Female	≥50	Right		ALL WT		Normal
MT-CYD25	INCAN	Female	< 50	Left	PIK3CA	H1047R	11,10%	Normal
					KRAS	G12V	8,50%	
					NRAS	G13D	9,50%	
					EGFR	L747_S752del, P753S	11,20%	
MT-CYD26	INCAN	Male	< 50	Left		ALLWT		MSH2 &MSH6(-)
MT-CYD27	INCAN	Male	≥50	Left	KRAS	G12D	34,00%	Normal
					PIK3CA	E542K	15,30%	
MT-CYD28	INCAN	Male	≥50	Right	PIK3CA	H1047Y	11,70%	Normal
MT-CYD29	INCAN	Male	≥50	Right		ALL WT		PMS2 & MLH1(-)
MT-CYD30	INCAN	Male	≥50	Left		ALL WT		Normal
MT-CYD31	INCAN	Male	≥50	Right	KRAS	G12D	41,50%	Normal
MT-CYD32	INCAN	Male	≥50	Left		ALL WT		Normal
MT-CYD33	INCAN	Male	≥50	Right		ALL WT		Normal
MT-CYD34	INCAN	Female	< 50	Left		ALL WT		Normal
MT-CYD35	INCAN	Male	< 50	Left		ALL WT		Normal
MT-CYD36	INCAN	Male	≥50	Left		ALL WT		Normal