REDEFINING SYNCHRONOUS COLORECTAL CANCER FROM THE BASE OF CLONALITY.

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ABSTRACT

Background:.

Materials and methods:

Results:

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INTRODUCTION

Synchronous colorectal cancer (SCRC) refers to more than one primary colorectal carcinoma (CRC) detected in a single patient at the time of diagnosis (1). From the beginning of its clinical definition was differentiated from Metachronous CRC (MCRC) according to the interval of diagnosis: these lasts were defined being diagnosed at a minimal interval of 6 months after the initial CRC, while SCRCs should be as higher as within 6 months after the initial diagnosis (2). SCRC accounts for about 1.1-8.1% of newly diagnosed CRCs (1). There are some already-known entities, hereditary forms of CRC, in which tumor co-occurrence is frequently shown, such as Lynch syndrome (LS) or familial adenomatous polyposis (FAP), and patients with inflammatory bowel disease and serrated polyps/hyperplastic polyposis are also known to have a higher risk of developing SCRC (1). Nevertheless, these situations only account for around 10% (3).

To date, the specific approaches to understand the molecular basis of SCRC have been mainly focused on the main colorectal carcinogenetic pathways, apart from the already known proportion of hereditary CRC forms developing SCRC (e.g. Lynch Syndrome (LS), Familial Adenomatous Polyposis (FAP)). SCRC patients seem to have a higher proportion of microsatellite instability (MSI) as well as the CpG island methylator phenotype (CIMP) tumors than single-tumor patients [4-6]. A hypothetical correlation between the molecular basis of SCRC and the location of the tumors in the colon has also been pointed out: Right-sided SCRC may be related with CIMP-High genotypes and LS, whereas left-sided SCRC seems to be related with the chromosomal instability (CIN) pathway and low-penetrance genes; SCRC throughout the entire colon seems to be related with the CIN pathway and germline mutations in *APC* or *MUTYH* [7]. In the same direction, our group, taking as a starting point the MSI/CIMP statuses of SCRC, defined 4 molecular groups which interestingly showed a certain

correspondence with the molecular classification of CRC described by Ogino and Goel (8), showing a link with the location of the tumors in the same individual (paired tumors) (9).

But more interesting talking about SCRC should be the possibility that the paired tumors could share concordance genetic routes and a same genetic origin, acquiring at the beginning some similar somatic alterations, showing therefore a likely clonal origin. Comparative analysis of SCRC have been carried out frequently, specially focused on Microsatellites or CIMP, or punctual mutations, with disagreeing results (10-12). Recently, some studies have been converging in the idea of a possible clonal origin of some subsets of tumors, as bilateral breast cancer, or SCRC, using mutational concordance or clonality analysis using copy number profiles (13-15). In those studies results showed that in both cases paired tumors were in origin different, but their limited sample size makes that the conclusions should be taken with caution. In this direction, finding at least a subset of tumors fulfilling these features should have not only therapeutical implications (treating different or similar paired tumors in the same patient), but also imply changes in the intrinsic definition of SCRC.

In our study, we try to find out the presence of a clonal origin in paired SCRC, at least in a part of them, converging in cases without already known hereditary molecular basis (nowadays sporadic or familial aggregation forms), in which clinical implications are already defined. We carried out a comprehensive Comparative Genomic Hybridization array (aCGH), and confirmed the results with the use of whole-exome sequencing of the main genes related with CRC. Moreover, we categorized the subsequent groups, also according to the tumors location, trying to find out differential phenotypes with clinical implications.

RESULTS

Main sample and clonality analysis.

Clonality groups.

Two tumors that originally evolved from the same "clone" of cancer cells will thus show some somatic changes that are identical. Consequently, comparison of the DNA profiles for the extent of similarities in the patterns of somatic changes is a powerful strategy for determining the diagnosis of a new tumor as independent or as a clone of the original primary (16). In this point, we carried out the analysis by using aCGH to study Copy Number Alterations (CAN) landscape of all these tumors. So, the LR2 evaluate the clonal relatedness of the initially fifty-three pairs (106 paired tumors) of tumors and found a significantly higher LR2 value for 20 SCRC pairs (40 paired tumors). However, no obvious relationships were observed for the other thirty-three SCRC (66 paired tumors) (P value <0.05). (Supplementary Table 1). The first group were defined as Monoclonal, and the second therefore as Polyclonal, being shown in Figure 1a and 1b one example of each. Only one case should be removed because it was a newly-diagnosed LS case. It was classified as Monoclonal, with the tumors located in the right colon, fulfilling Amsterdam type II criteria, and developing years later a metachronous CRC. Both paired tumors showed both MSI, and CIMP-low and High, correlatively. Clinico-pathological and familial features of the rest of the whole group are shown in Table 1.

The most frequent changes detected in the 19 Monoclonal cases (38 samples) with more than 50% of cases were: 20q13.2 (71%), 12p13.1 (66%), 8q24.3, 12p13.2, 13q22.1, 13q34, 20p11.2 (61%) and losses on 1p36.22, 1p35.3, 18p11.32 (61%), 1p21.3, (58%), 1p13.2, 9q34, and 16p13 (53%) (Supplementary Table 2). On the other hand, in relation with the 33 Polyclonal SCRC (66 samples), the most frequent gains were in 8q24.22,

20q13 (67%), 7q36, 20q12 (64%), 7p11.2, 8q23 (62%), 8q12, 20p11 (61%), 12p13 (59%), 7q31.2, 13q34 (56%) 13q22 (55%), 3q36.31, 7p21.1 and 12q14.1 (50%). Most frequent losses were in 18p11.32 (62%), 1p35.3 (58%), 1p36.21 (53%) 1p35.1 and 15q25 (50%) (Supplementary Table 3). Comparatively, both Monoclonal and Polyclonal SCRC showed differential SCNA profiles: Gains on 3p13 (55%), 5p15, 6p25 18p11.31 (53%), and losses on 9q34 (53%) were more frequent in Monoclonal, while Gains on 8q22.2 (50%), 8q13.3 and 13q32.2 (47%) were most frequent in Polyclonal SCRC ($p<0.05$ Fischer test) (Supplementary Table 4).

The MIP methodology by Oncoscan allows us to analyse simultaneously CNA and LOH regions. Comparing both clonality groups profiles (CNV and LOH), no significantly differences between ratio of LOH and total number of LOH were found (Table? ¿Data not shown?). The same happened with the degree of genomic instability of tumors between both groups.

Confirmation of clonality using whole-exome analysis by NGS.

In our series, we used mutational concordance to confirm our clonality results, as other publications published as their main role (14). In Table 2A are shown the mutational status of all cases, showing in "blue" color those paired cases with the same pathogenic mutations. Nine cases within Monoclonal group (9/19, 47%) showed at least one paired tumors with the same pathogenic mutation: Four cases with three different same-mutated genes paired tumors; one with two; and four with one. Apart from *KRAS*, with four cases, the most frequently mutated genes were *APC* and *SMAD4*, with four and three respectively. Only three Polyclonal cases showed same mutation in paired tumors (3/33, 9%), all of them in *KRAS,* and one also in *BRAF*.

Indirect signs of clonality confirmation.

Other possible indirect signs that could be used as a marker of clonality could be CIMP and stage-at-diagnosis, comparing paired tumors (Table 2B). According to the first issue, 14 cases out of 19 showed the same CIMP in both paired tumors (74%), with the other five showing no distant CIMP (CIMP-0 and the other CIMP-low or CIMP-low with CIMP-High). On the other hand, Polyclonal cases showed a slightly different distribution: 21 out of 33 showed concordance between paired tumors (64%), nine with no-distant CIMP, but also 3 cases showed a high CIMP-Mismatch.

Nevertheless, talking about paired tumors staging, within Monoclonal group, 14 out of 19 showed the same stage at diagnosis (74%), with two more almost equivalent (grey color), while only 12 out 33 cases (36%), with two more almost equivalent.

Clonal and location SCRC categories.

Groups and correlative regions.

The Monoclonal group was divided according to the tumors locations in Monosegmental (MM) and Pancolonic (MP). The first one was composed by ten cases (19% of the total), while the MP by nine cases (17%). The colon distribution in MM was four right-colon cases and six left-colon cases. The Polyclonal group was identically divided: 19 cases were Polyclonal Monosegmental (PM) (37%) and 14 cases showed a Pancolonic distribution (PP) (27%). In this category, PM showed and important preference by left colon location (17 cases, 89%).

Beginning with MM group, the most frequent gains were on 20q13 (85%) and 12p13 (75%) and deletion on 1p21 (85%), 1p36 and 1p13 (75%) (Supplementary Table 5). MP category was characterized by gains on 13q22 (67%) 4p16 and 8q24.3 (61%). These group presented the less number of genomic changes compared with the other three

(Supplementary Table 6). Differential Genomic regions between both Monoclonal categories were deletion on 1p21, 1p13 and gains on 1q24, 8q24, all of them for MM group, and only gain of 14q11.2, with lower proportions, was one of the few for MP group (Supplementary Table 7).

According to Polyclonal categories, PM were characterized by gains on 7q36.2, 20q13 and deletion on 18p11 (68%) (Supplementary Table 8), while for PP the most frequently changes were detected gains on 8q24 and 7p11 (Supplementary Table 9). Most frequent differential statistical regions between both groups within Polyclonal cases were gains on 7q36 and deletion on 1p36, for PM group. (Supplementary Table 10).

Mutational status.

As it was mentioned before, Monoclonal cases showed most of the number of same-mutation genes paired tumors, and everyone different from *KRAS* and *BRAF* same-mutation paired tumors (Table 2A). To MM cases belonged six (60% of the group), showing three of them a threesome of same-mutation genes. MP cases showed 3 cases (33%), with one case with a couple of same-mutation genes. *APC* gene was the most frequent with the same mutation in both paired tumors (4 cases), without counting *KRAS*. It should be underlined the important proportion of *APC* mutations in MM and PM groups (70% and 42% of all tumors, respectively), compared with Pancolonic categories, Monoclonal and Polyclonal (33% and 32%). *KRAS* mutations appeared homogenously in all groups, while remarkably, *TP53* was scarcely mutated, with a 25% in MM cases. At a global sight through other genes, MM were cases showing mutations in genes related with cell-adhesion, angiogenesis and invasiveness (*CTNNB1*, *ERBB2*, *PDGFRA*) while PM were cases showing mutations in control and cellular damage (*ATM*, *PTEN*).

Main carcinogenetic pathways distribution and analysis of CRC hereditary forms.

Only three from the 53 original cases showed MSI at least one of the tumors. One of which should be removed because it was a newly-diagnosed LS case (*MSH2* mutation). It was classified as MM, with both tumors located in the right colon, fulfilling Amsterdam type II criteria, and developing later a MCRC. Both paired tumors showed both MSI, and CIMP-low and High, correlatively. The two others showed MSI due to MLH1 promoter Hypermethylation and/or *BRAF* mutations, being defined as MM and PM. According to the CIMP, CIMP-High status showed a high prevalence within SCRC (Table 2B). Listing by order the four groups (MM, MP, PM, and PP), the proportion of CIMP-High tumors was 65%, 44%, 45%, and 50%, but focused on CIMP-High paired tumors, the order exhibited a decreasing link: 50%, 44%, 37% and 29%.

Although an exclusion criterion was FAP, we took those cases showing an identical pathogenic mutation in *APC* in both paired tumors and we carried out the analysis of APC mutation in normal colonic tissue to exclude PAF or *APC* mosaicisms. None of the four cases with the same *APC* mutation in paired tumors showed pathogenic mutations, thus both entities were excepted. *MYH* was carried out as well (40 cases out of the 52, 77%), and only two cases showed mutation c.1187G>A (p. Gly396Asp). One of them in homozygosis, with the SCRC at an age of 71, being defined as PM (left colon), with 41 mixed-type associated polyps during follow-up, and no familial cancer history, developing as well a MCRC one year later. The other one showed the mutation in heterozygosis, with the SCRC with 77 y/o, classified as MM (left colon), with 17 also mixed-type associated polyps. Although no familial cancer history was shown, the patient developed multiple primary neoplasms: a urothelial tumor (71 y/o) and a MCRC (80 y/o), in addition to the SCRC.

Phenotypes and clinical implications.

Comparative results rendering clinic-pathological and familial features between the four defined categories are shown in Table 1. Specific differences arose for each one. Firstly, MM showed a high proportion of mucinous tumors (37.5%), with the least mean number of SCRC as well as associated polyps, and displaying the worst prognosis both related with recurrence and mortality (Figures 3A and 3B). Then MP, with the youngest age at diagnosis and largest mean-number of associated polyps, with a comparatively intermediate mean number of SCRC, and showing the best prognosis and an important familial cancer component. Moreover, PM seemed to be a "frontier" group, with proximal features to MP, except from the sporadic characteristic. Lately, PP showed also mucin component (almost 40%), with the highest mean-number of SCRC (4.6) compared with the mean-number of polyps (7.7), rendering a bad prognosis and pure sporadic landscape.

DISCUSSION

Tumor multiplicity is widely recognized as a feature of genetic predisposition for the development of neoplasms (17). Although the heterogeneous phenotype of SCRC has been widely discussed, the disease remains poorly understood. Comparing the genetic patterns of synchronous lesions may provide important knowledge about the biology of these tumors. But more interesting should be the possibility that the paired tumors could share concordance genetic routes and a same genetic origin, acquiring at the beginning some similar somatic alterations, showing therefore a likely clonal origin. We have defined at least a 36% of SCRC. In this direction, finding at least a subset of tumors fulfilling these features should have not only therapeutical implications (treating different or similar paired tumors in the same patient), but also in the intrinsic definition of SCRC. There are only two recent studies analyzing this, showing both an apparent

intertumor heterogeneity within the same patient (14, 15). Compared with our, they analyzed smaller samples (10 and 15 vs our 52), from a genetic point of view (concordance of paired-mutated genes, whole exome sequencing), while we developed a different strategy, comparing the DNA profiles for the extent of similarities in the patterns of somatic changes as a powerful strategy for determining the diagnosis of a new tumor as independent or as a clone of the original primary. Moreover, those previous studies tried to define clonality as concordance and high proportions of cells genes mutations, fact that we believe difficult due to the high heterogeneity that CRC acquire during development. Our test statistic is the total number of loci at which concordant mutations occur on the same parental allele, with higher values providing more evidence in favor of a clonal origin for the two tumors. The test is shown to have high power for detecting clonality for plausible models of the alternative (clonal) hypothesis, and for reasonable numbers of informative loci, preferably located on distinct chromosomal arms (18). The concordance of gene mutations in the paired tumors, together with some other indirect aspects, as CIMP concordance or most of same-stage paired tumors have helped us to outline the clonality or similarity between paired tumors found by the statistic method.

The relationship between colon location and CRC differential clinical and molecular features has been widely established (19-21), and the same seems to arise in SCRC (2, 7, 9). The division of SCRC into categories according to their clonality and the location in the colon of the tumors within the same patient defined as not only important prognostic subclasses, highlighting MM and PP, with an important mucinous component, but also possible differential influence of the “field” effect within SCRC (23, 24). Other interesting point was the few cases of Right colon SCRC, mainly monoclonal, in comparison with the higher rate of Left colon SCRC and, on the

contrary, Polyclonal, maybe confirming the predisposition to settle in the right colon certain type of colon cancers different from the usual sporadic ones, characteristic of left colon cancers.

Many studies point out the importance of an environmental field effect that promotes multiple colorectal tumors, being possible causes an important exposure to a carcinogen or a genetic predisposition for cancer development in a single patient (4, 14, 15, 23). Cereda et al. showed a possible mixture of both, exposition and genetic predisposition, in the basis of SCRC (14), joining inherited damaging alterations of immune-related genes with the consequent inflammatory conditions, all of which stimulate tumorigenesis (14). One interesting aspect of our results in agreement with this point of the "field" effect is the different proportion of malignization of the associated polyps presented by each category. While MM and PP showed proportions of number of SCRC/number of polyps around one of each two polyps, the other two (MP and PM) were around 1 of each 5. This, together with the fact that the MP group presented an important familial cancer component, may convert MP cases as the ones with the lowest influence of a "field" effect, and PM category, with an important predisposition to the left colon, as a sum of random stochastic events, due to the frequency of CRC in the general population. The "field" effect should have a higher influence within PP through the entire colon developing heterogenous tumors, while in MM cases could be adding all variables, "field" effect, familial, individual, and specific topographic factors.

Our findings point out the importance of differentiate clonality within SCRC to distinguish them from a prognostic point of view, but also at the time of therapeutic management, with the need to perform the analysis of both tumors, especially in cases of polyclonal tumors. The microbiome and molecular alterations in normal mucosa within SCRC patients' analysis should be next steps to clarify the exact role of both

environmental and individual predisposition within SCRC. Moreover, the studies that appear in last years in relation to the growing need to analyze clonality in SCRC, determine the need to carry out more extensive studies, and perhaps, if the existence of some "clonal" cases is confirmed, the possibility of redefining the SCRC not only from a clinical chronological point of view.

MATERIAL AND METHODS

Patients, samples and data collection. Initially, a total of 53 individuals diagnosed with SCRC were consecutively collected from January 2006 at the 12 de Octubre University Hospital (Madrid). We defined a CRC as SCRC when 2 or more histologically distinct colorectal tumors were identified in the same patient at the same time or in a period less than six month after the first diagnosis. Tumor relapse was defined either as regrowth at the anastomosis site (± 5cm) or as the detection of new metastatic disease. Metachronous CRC was defined as a secondary tumor occurring outside the anastomosis area more than 6 months after surgery. For each patient, we performed molecular analysis of the two tumors with the highest percentage of neoplastic material, and used the most advanced neoplasia for establishing staging. Tumor location was defined according to previous publication (9), and therefore patients were initially classified into 3 categories according to the anatomical location of the tumors. Thus, (1) “right colon” was defined as the colocation of the synchronous tumors at the right side of the large intestine (cecum, ascending colon, hepatic flexure, and first portion of the transverse colon); (2) “left colon” as the colocation at the left side of the large intestine (second portion of the transverse colon, splenic flexure, descending colon, sigmoid colon, and rectum); and (3) “entire colon” as the location of

the synchronous tumors at different sides of the colon. And finally (1) or (2) cases were defined as “Monosegmental”, while (3) cases as “Pancolonic”.

Family history of cancer and clinicopathological information was obtained for each patient with a mean follow-up of 74 months after surgery. We considered local recurrence, severely dysplastic tumors and a diagnosis of inflammatory bowel disease, LS or FAP as exclusion criteria. All patients (or a first degree relative in case of death of the index case) provided written consent, and the study was approved by the Ethics Committee of our Institution.

DNA isolation and MSI and CIMP characterization of the tumors. Two tissue specimens were obtained from each index case (the two highest stages at diagnosis). Microscopic inspection of paraffin-embedded samples was performed by a pathologist, and samples with more than 70% of tumor cells in the neoplastic material were considered adequate for further analysis. The protocol for DNA isolation was as previously reported (25).

We used the Bethesda panel to assess the MSI status, and considered two or more altered markers as a positive result (26). Moreover, MSI tumors were firstly analyzed for the *BRAF* V600E mutation and hypermethylation of the *MLH1* gene promoter to confirm their sporadic nature, as previous described, and when negative, they were therefore screened for germline mutations in the MMR genes *MLH1*, *MSH2*, *MSH6* and *PMS2* (27). For the assessment of CIMP, we investigated the methylation status of the promoter regions of *CACNA1G*, *CDKN2A*, *CRABP1*, *IGF2*, *MLH1*, *NEUROG1*, *RUNX3* and *SOCS1*. Each patient was categorized as CIMP-High, CIMP-Low or CIMP-0 depending on whether their simultaneous tumors showed ≥5/8, 2/8 to 4/8, or 0/8 to 1/8 methylated promoters, respectively. Patients with different CIMP status in their paired

tumors were categorized as CIMP-MM (mismatching). The procedures for the evaluation of MSI and CIMP have been previously described (27).

MYH ANALYSIS.

MUTYH hotspots screening included the three most common mutations in our population: c.536A>G, p.(Tyr179Cys), rs34612342; c.1187G>A, p.(Gly396Asp), rs36053993 and c.1227_1228dupGG, p.(Glu410Glyfs*43), rs587780078 (RefSeq NM_001128425.1, NP_001121897.1, dbSNP). This screening was carried out using high resolution melting (HRM) analysis on a LightCycler 96 System (Roche). Positive profiles were sequenced using Sanger dideoxy method to identify the variant.

Copy number alterations by aCGH and clonality analyses.

We performed OncoScan FFPE Assay to asses copy number and loss heterozygosity on the paired samples corresponding with patients with SCRC. Oncoscan FFEPE Assay, provided by Affymetrix, is a platform based on Molecular Inversion Probe technology which using small amounts of DNA from FFPE samples. Genomic DNA was quantified using Picogreen protocol (Quant-iT PicoGreen dsDNA Products, Invitrogen, P-7589). The OncoScan FFPE Assay Kit was used according to the manufacturer's instructions (Affymetrix). A GeneAmp PCR system 9700 Thermal Cycler (Life Technologies) was used from the anneal stage to the denaturation stage. The digest DNA target was hybridized on OncoScan array (Affymetrix) and incubated at 49ºC in the Genechip Hybridization oven 640 (Affymetrix) for 17h at 60rpm. OncoScan arrays were then washed in a GeneChips Fluidics Station 450 (Affymetrix) using OncoScan stain and wash reagents according to the manufacturer's instructions (Affymetrix). The

microarrays were finally scanned on a GeneChip scanner 3,000 (Affymetrix). Data QC analysis was performed with the OncoScan Console software (Affymetrix). CNV events were called using the normalized data using Nexus Express for OncoScan 3.1 (Affymetrix). Applying the Affymetrix OSCHP-TuScan allowed to known %aberrant cells and overall ploidy, as well as copy number events and %LOH for each sample.

Weighted log2 ratios from Chas console (Affymetrix) were also processed using the copynumber R package (28). This package performs a pre-processing step of detection and modification of extreme values through a method called Winsorization (29), and a single sample segmentation step using Piecewise Constant Fitting (PCF) algorithms. Gamma value, which is the penalty for each discontinuity in the curve, was set to 40, and the minimum number of probes allowed in each segment was set to 5. Copy Number frequency plots were constructed using this package, setting the log2 ratio threshold for gains and losses to 0.1 and −0.1, respectively. The grade of Genome Instability was performed as previously reported (25).

With respect to CNV, we used the R software package Clonality (30), which uses tumor copy number profiles at the probe level, to determine whether two tumors from the same patient were clonally or origin independent using a likelihood ratio 2 (LR2) statistic (quantifying the odds that the two tumors are clonal). To run Clonality, we used DNA copy to create a copy number array object. The copy number array object was used as input for Clonality.

We further enhanced the analysis by using a second algorithm termed “GISTIC” for Genomic Identification of Significant Targets in Cancer, which identifies functionally significant CNAs by giving more weight to high copy gains and homozygous losses (amplitudes) that may be functionally relevant to the successful evolution of the cancer genome (31, 32).

Next Generation Sequencing

Ion torrent PGM library preparation. An Ion Torrent adapter-ligated library was generated using the Ion AmpliSeq Library Kit 2.0 and the Ion AmpliSeq Cancer Hotspot Panel version 2 (Thermo Fisher Scientific, Rev. B.0; MAN0006735). Briefly, 2μL of 5X Ion AmpliSeq™ HiFi mix, 2μL of 5X Ion AmpliSeq™ Primer Pool and 5ng of gDNA per reaction were mixed together and amplified following the temperature conditions provided by the manufacturer. Then, primer sequences were partially digested by adding 1μL of FuPa Reagent and loaded in a thermal cycler under the conditions detailed in the user guide. Finally, each library was marked with a unique adapter provided in Ion Xpress™ barcode adapters 1-96 Kit (Life Technologies) in a reaction mixture containing 2μL of Switch Solution, 1μL of diluted barcode and 1μL of DNA Ligase, also under the temperature conditions provided by the manufacturer.

After AMPure bead (Beckman Coulter, Brea, CA, USA) purification, the concentration of the library (in a 100-fold dilution) was determined using the Ion Library TaqMan quantitation assay kit (Thermo Fisher Scientific) in a 7500 Real-Time PCR System (Thermo Fisher Scientific, Foster City, CA) Each sample was run in a minimum of two replicates.

Emulsion PCR. Sample emulsion PCR and enrichment were performed using the Ion PGMTM Template OT2 200 Kit and the Ion One TouchTM 2 System (Life Technologies). We followed the manufacturer's instructions except for the concentration of the pooled libraries which in this work was set at 9pM.

Sequencing on the Ion torrent PGM platform. All barcoded samples were sequenced using the Ion PGMTM Hi-QTM Sequencing Kit (Life Technologies) in an Ion Torrent PGM instrument (Life Technologies) with Ion 318™ v2 chips (Life Technologies).

Chip loading procedure was performed according to the user guide for the Ion PGMTM Hi-QTM Sequencing Kit (Life Technologies). A maximum of 16 samples were loaded on a single chip per sequencing run.

Bioinformatics processing and data analysis. Base calling and alignment to the human genome (hg19) were executed with the Torrent Suite Software v.4.0 using the variant caller plugin. Variants were annotated using Ion Reporter and each mutation was verified in the Integrative genome viewer (IGV) from the Broad Institute (http://www.broadinstitute.org/igv/) (33).

Statistical Analysis. Continuous variables were expressed as mean values plus/minus standard deviation (SD), and categorical variables were expressed as number of cases and their percentage. Comparison of categorical variables was done using Pearson's Chi Square (χ^2) test, and Student's t independent samples test and U Mann—Whitney tests for continuous variables, as appropriate. For comparisons between more than two groups, analysis of variance (ANOVA) (for normal distributions) or the Kruskal-Wallis test (for nonparametric distributions) were used. The Kaplan—Meier method (log-rank test) was used to assess the relationship between CNAs and overall survival (OS) and disease-free survival (DFS). Statistical analysis was performed using SPSS version 23.0 (IBM), and differences were considered statistically significant when the p-value was <0.05.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

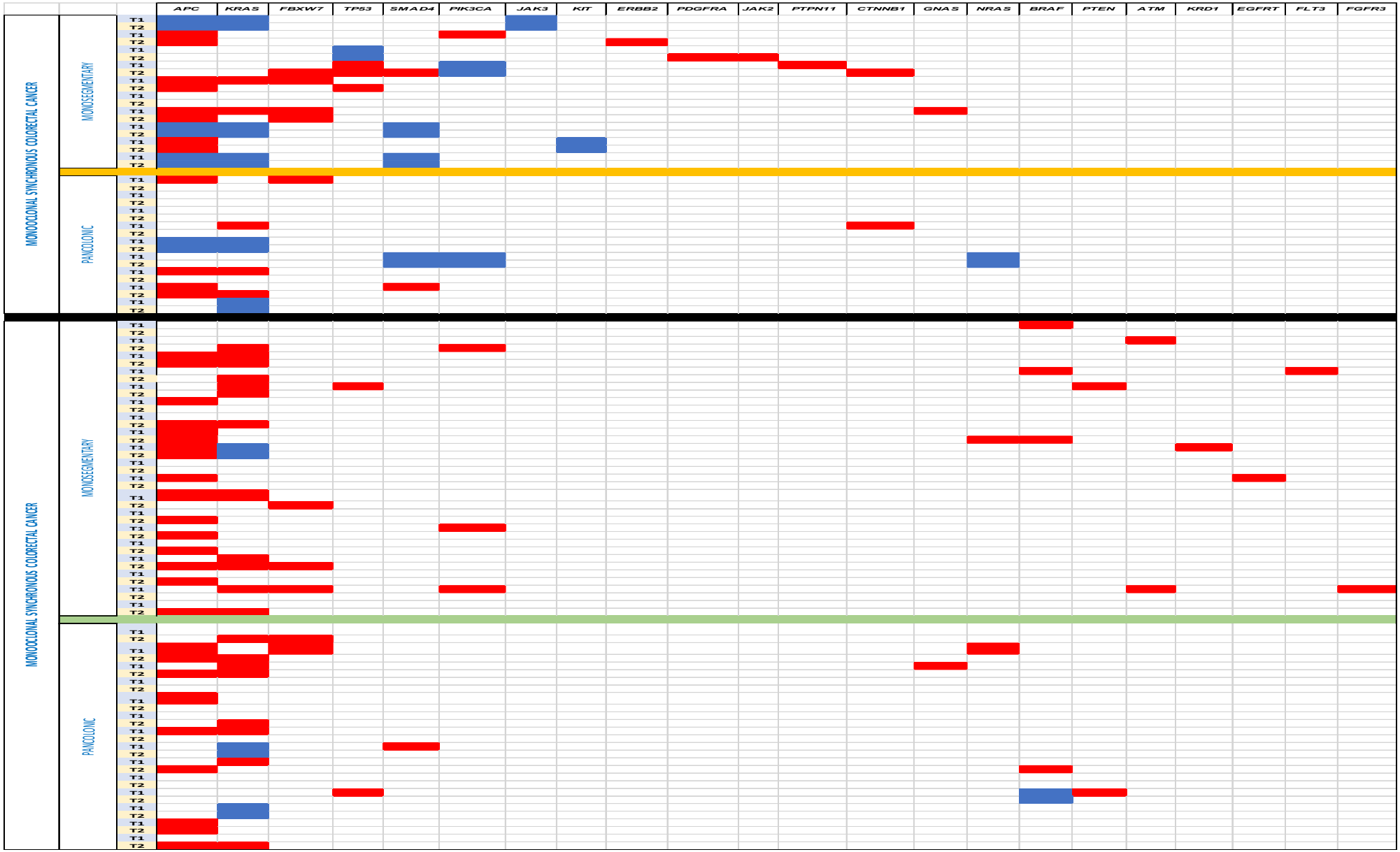
Table 1. Comparison and description of the clinicopathological and molecular features of the subgroups categorized according to the clonality and the anatomic location of the tumors in the colon.

	Total SCRC	MM	MP	PM	PP	p-value[1]
No. of patients	52 (100)	10 (19.2)	9 (17.3)	19 (36.5)	14 (27)	-
Average age of onset	71.1 [9.9]	73.5 [8.5]	65 [12.6]	69.6 [9.6]	72.4 [9.8]	0.2[2]
Sex:						0.4
Male	35 (67.2)	8 (80)	4 (44.4)	13 (68.4)	10 (71.4)	
Female	17 (32.8)	2 (20)	5 (55.6)	6 (31.6)	4 (28.6)	
Colon location:						0.08*
Right colon	6 (11.5)	4 (40)		2 (10.5)		
Left colon	23 (44.2)	6 (60)		17 (89.5)		
Entire colon	23 (44.2)					
T. differentiation[4]						0.3
High	22 (52.4)	3 (37.5)	3 (75)	9 (55.6)	7 (53.8)	
Moderate	18 (42.8)	5 (62.5)	1 (25)	8 (44.4)	4 (30.8)	
Low	2 (4.8)	0	0	0	2 (15.4)	
Mucin production[4]	9/42 (21.4)	3/8 (37.5)	0/4 (0)	1/17 (6)	5/13 (38.5)	0.07
"Signet ring" cells[4]	2/42 (4.8)	0 (0)	0 (0)	1/1 (6)	1/13 (8)	0.8
Astler-Coller stage						0.5
A	16 (30.8)	2 (20)	6 (66.6)	5 (26.3)	3 (21.4))	
B	23 (44.2)	6 (60)	2 (22.2)	9 (47.4)	6 (42.9)	
C	10 (19.2)	1 (10)	1 (11.)	4 (21.1)	4 (28.6)	
D	3 (5.8)	1 (10)	0 (0)	1 (5.3)	1 (7.1)	
Number of SCRCs	3.2 [2.7]	2.4 [0.5]	3.2 [1.5]	2.5 [0.7]	4.6 [4.7]	0.09[2]
Associated polyps						
Yes	49 (94.2)	8 (80)	9 (100)	18 (95)	14 (100)	0.2
Mean.	10.1 [12.4]	5.3 [5.3]	15.6 [16.8]	11.7 [15.2]	7.7 [6.3]	0.2[2]
Type:						0.7
Adenomatous.	27 (65.1)	5 (62.5)	5 (55.5)	8 (44.4)	9 (64.3)	
Hyperplastic/mixed	22 (44.9)	3 (37.5)	4 (44.4)	10 (55.5)	5 (35.7)	
Metachronous CRC.	8 (15.7)	3 (30)	0 (0)	4 (22)	1 (7)	0.2
Recurrence	8 (15.7)	3 (30)	1 (9)	4 (28.6)	0 (0)	0.2
Global Mortality/relat	24 (46)/7 (13)	8 (80)/ 3 (30)	1 (9)/ 0	6 (43)/ 2(14)	9 (63)/ 2 (14)	**0.006**
DFS (months)	68.1 [41.1]	51.4 [39.1]	99.2 [18.7]	68.7 [39]	59.4 [47.6]	**0.05[2]**
OS (months)	75.3 [37.2]	61.1 [38.5]	100.2 [19.2]	78.5 [33.1]	65.1 [44.1]	0.09[2]
Familial cancer history						**0.009**
Sporadic	43 (82.7)	7 (70)	4 (44.4)	18 (94.7)	14 (100)	
Familial aggregation	8 (15.4)	3 (30)	4 (44.4)	1 (5.3)	0 (0)	
Amsterdam II positive	1 (1.9)	0 (0)	1 (11.2)	0 (0)	0 (0)	

Data shown in parenthesis represent percentages. Data shown in brackets represent standard deviation. [1]Statistical comparison was performed using Pearson's Chi Square test (χ^2). [2]Statistical comparison was performed using analysis of variance (ANOVA). [4]Percentages shown are based on varying total numbers as some cases were excluded because only one biopsy was taken (stage D), or because tumors were severely dysplastic with "in situ" carcinoma and it was not possible to study any other characteristic. CIMP: CpG island methylator phenotype. DFS: Disease-free survival. HGD: High-grade dysplasia. LS: Lynch syndrome. MM: Mismatching. MSI: Microsatellite instability. MSS: Microsatellite stability. No.: Number. NS: Not significant. OS: Overall survival. SCRC: Synchronous colorectal cancer. T.: Tumor.

Table 2A and 2B.

CIMP: CpG island methylator phenotype. DFS: Disease-free survival. LS: Lynch syndrome. MM: Mismatching. MSI: Microsatellite instability. MSS: Microsatellite stability. No.: Number. NS: Not significant. OS: Overall survival. SCRC: Synchronous colorectal cancer. T.: Tumor.


APC
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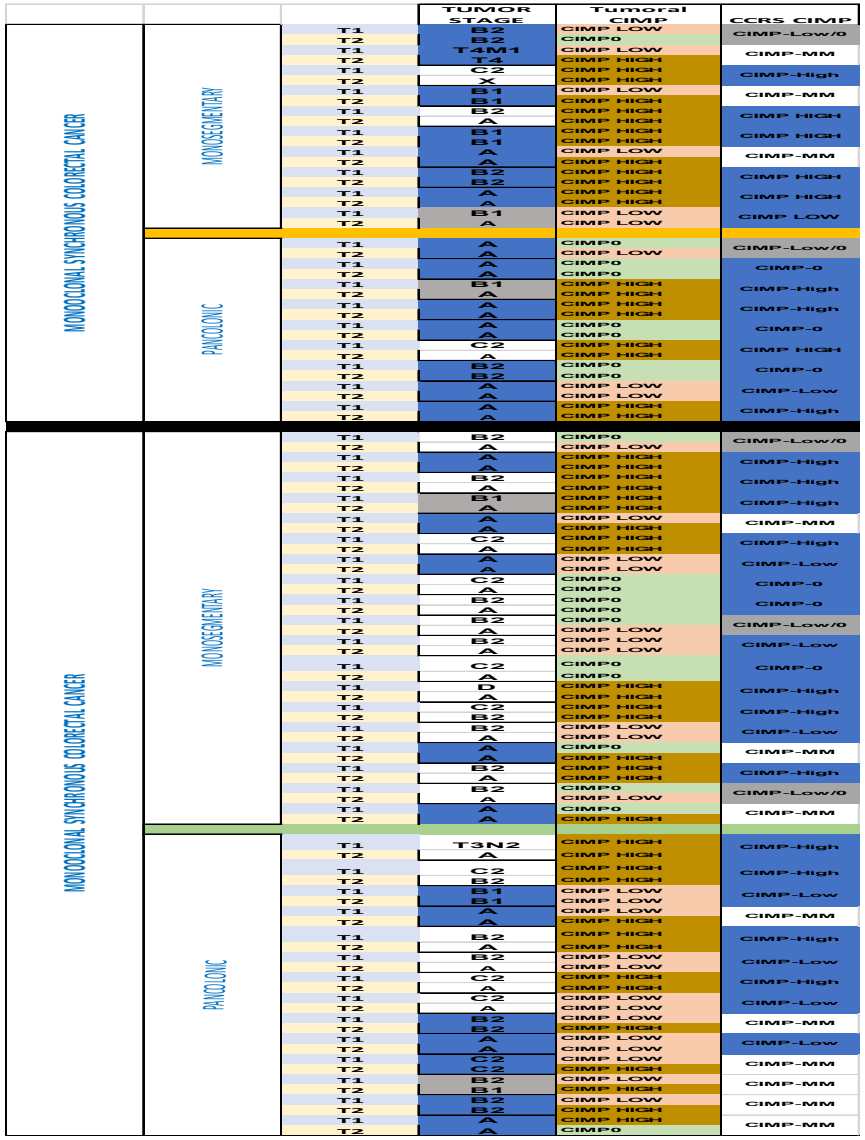
FBXW7
TP53
SMAD4
PIK3CA
JAK3
KIT
ERBB2
PDGFRA
JAK2
PTPN11
CTNNB1
GNAS
NRAS
BRAF
PTEN
ATM
KRD1
EGFRT
FLT3
FGFR3
MONOCLONAL SYNCHRONOUS COLORECTAL CANCER
MONOSEGMENTARY
PANCOLONIC
MONOCLONAL SYNCHRONOUS COLORECTAL CANCER
MONOSEGMENTARY
PANCOLONIC



			TUMOR STAGE	Tumoral CIMP	CCRS CIMP
MONOCLONAL SYNCHRONOUS COLORECTAL CANCER	MONOSEGMENTARY	T1	B2	CIMP LOW	CIMP-Low/0
		T2	B2	CIMP0	
		T1	T4M1	CIMP LOW	CIMP-MM
		T2	T4	CIMP HIGH	
		T1	C2	CIMP HIGH	CIMP-High
		T2	X	CIMP HIGH	
		T1	B1	CIMP LOW	CIMP-MM
		T2	B1	CIMP HIGH	
		T1	B2	CIMP HIGH	CIMP HIGH
		T2	A	CIMP HIGH	
		T1	B1	CIMP HIGH	CIMP HIGH
		T2	B1	CIMP HIGH	
		T1	A	CIMP LOW	CIMP-MM
		T2	A	CIMP HIGH	
		T1	B2	CIMP HIGH	CIMP HIGH
		T2	B2	CIMP HIGH	
		T1	A	CIMP HIGH	CIMP HIGH
		T2	A	CIMP HIGH	
		T1	B1	CIMP LOW	CIMP LOW
		T2	A	CIMP LOW	
	PANCOLONIC	T1	A	CIMP0	CIMP-Low/0
		T2	A	CIMP LOW	
		T1	A	CIMP0	CIMP-0
		T2	A	CIMP0	
		T1	B1	CIMP HIGH	CIMP-High
		T2	A	CIMP HIGH	
		T1	A	CIMP HIGH	CIMP-High
		T2	A	CIMP HIGH	
		T1	A	CIMP0	CIMP-0
		T2	A	CIMP0	
		T1	C2	CIMP HIGH	CIMP HIGH
		T2	A	CIMP HIGH	
		T1	B2	CIMP0	CIMP-0
		T2	B2	CIMP0	
		T1	A	CIMP LOW	CIMP-Low
		T2	A	CIMP LOW	
		T1	A	CIMP HIGH	CIMP-High
		T2	A	CIMP HIGH	
MONOCLONAL SYNCHRONOUS COLORECTAL CANCER	MONOSEGMENTARY	T1	B2	CIMP0	CIMP-Low/0
		T2	A	CIMP LOW	
		T1	A	CIMP HIGH	CIMP-High
		T2	A	CIMP HIGH	
		T1	B2	CIMP HIGH	CIMP-High
		T2	A	CIMP HIGH	
		T1	B1	CIMP HIGH	CIMP-High
		T2	A	CIMP HIGH	
		T1	A	CIMP LOW	CIMP-MM
		T2	A	CIMP HIGH	
		T1	C2	CIMP HIGH	CIMP-High
		T2	A	CIMP HIGH	
		T1	A	CIMP LOW	CIMP-Low
		T2	A	CIMP LOW	
		T1	C2	CIMP0	CIMP-0
		T2	A	CIMP0	
		T1	B2	CIMP0	CIMP-0
		T2	A	CIMP0	
		T1	B2	CIMP0	CIMP-Low/0
		T2	A	CIMP LOW	
		T1	B2	CIMP LOW	CIMP-Low
		T2	A	CIMP LOW	
		T1	C2	CIMP0	CIMP-0
		T2	A	CIMP0	
		T1	D	CIMP HIGH	CIMP-High
		T2	A	CIMP HIGH	
		T1	C2	CIMP HIGH	CIMP-High
		T2	B2	CIMP HIGH	
		T1	B2	CIMP LOW	CIMP-Low
		T2	A	CIMP LOW	
		T1	A	CIMP0	CIMP-MM
		T2	A	CIMP HIGH	
		T1	B2	CIMP HIGH	CIMP-High
		T2	A	CIMP HIGH	
		T1	B2	CIMP0	CIMP-Low/0
		T2	A	CIMP LOW	
		T1	A	CIMP0	CIMP-MM
		T2	A	CIMP HIGH	
	PANCOLONIC	T1	T3N2	CIMP HIGH	CIMP-High
		T2	A	CIMP HIGH	
		T1	C2	CIMP HIGH	CIMP-High
		T2	B2	CIMP HIGH	
		T1	B1	CIMP LOW	CIMP-Low
		T2	B1	CIMP LOW	
		T1	A	CIMP LOW	CIMP-MM
		T2	A	CIMP HIGH	
		T1	B2	CIMP HIGH	CIMP-High
		T2	A	CIMP HIGH	
		T1	B2	CIMP LOW	CIMP-Low
		T2	A	CIMP LOW	
		T1	C2	CIMP HIGH	CIMP-High
		T2	A	CIMP HIGH	
		T1	C2	CIMP LOW	CIMP-Low
		T2	A	CIMP LOW	
		T1	B2	CIMP LOW	CIMP-MM
		T2	B2	CIMP HIGH	
		T1	A	CIMP LOW	CIMP-Low
		T2	A	CIMP LOW	
		T1	C2	CIMP LOW	CIMP-MM
		T2	C2	CIMP HIGH	
		T1	B2	CIMP LOW	CIMP-MM
		T2	B1	CIMP HIGH	
		T1	B2	CIMP LOW	CIMP-MM
		T2	B2	CIMP HIGH	
		T1	A	CIMP HIGH	CIMP-MM
		T2	A	CIMP0	

FIGURES.

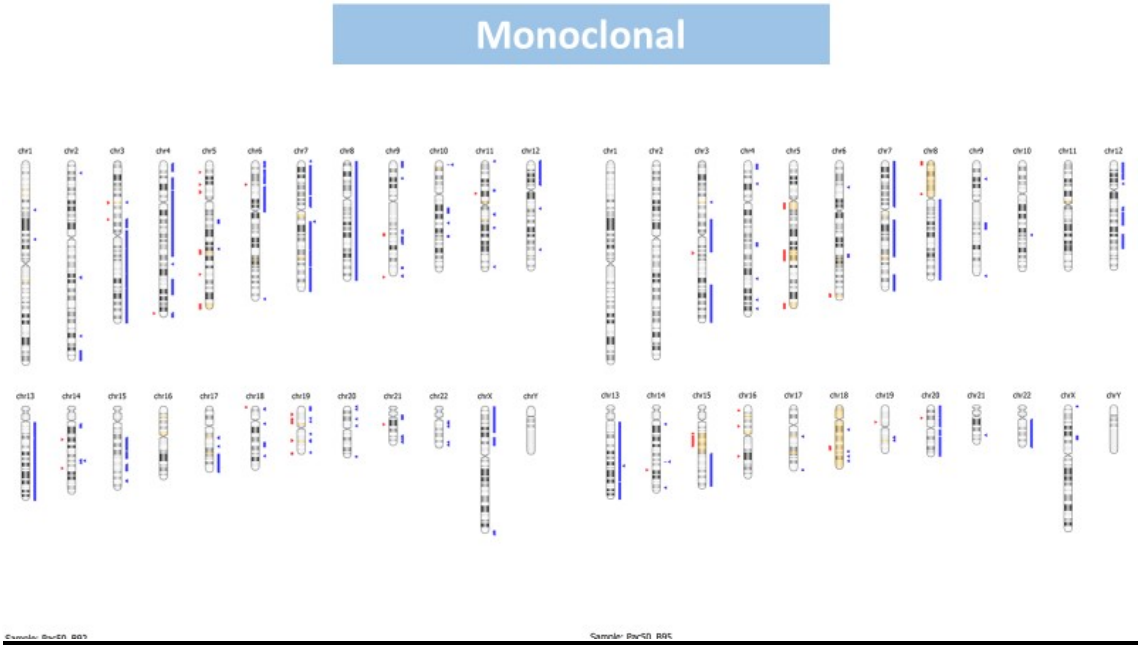


Figure 1a. Two paired tumours of a case defined as Monoclonal by aCGH.

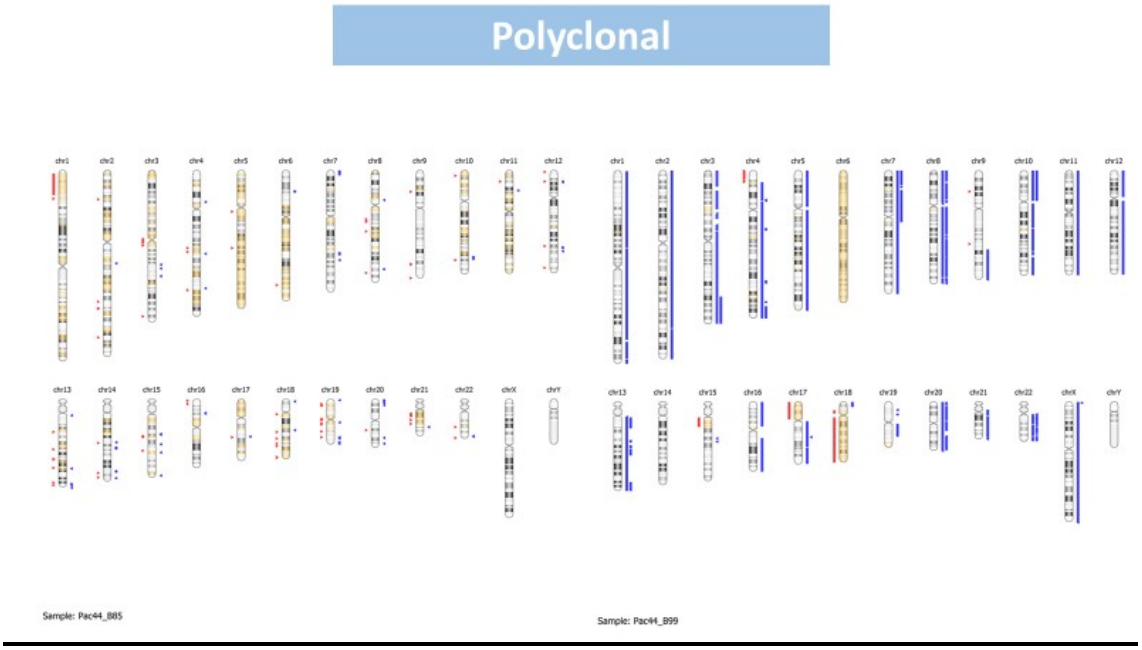
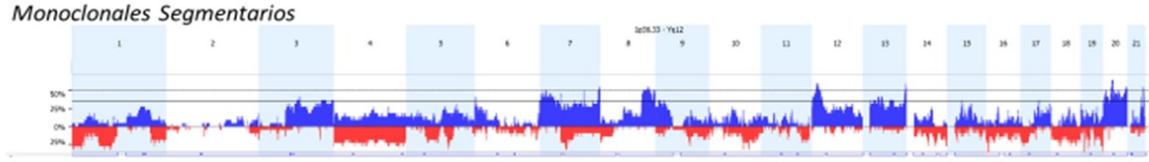
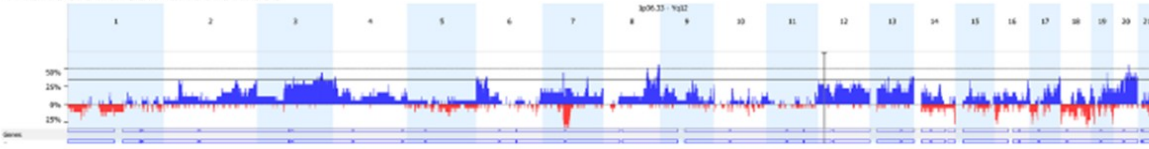


Figure 1b. Two paired tumours of a case defined as Monoclonal by aCGH.

Monoclonales Segmentarios

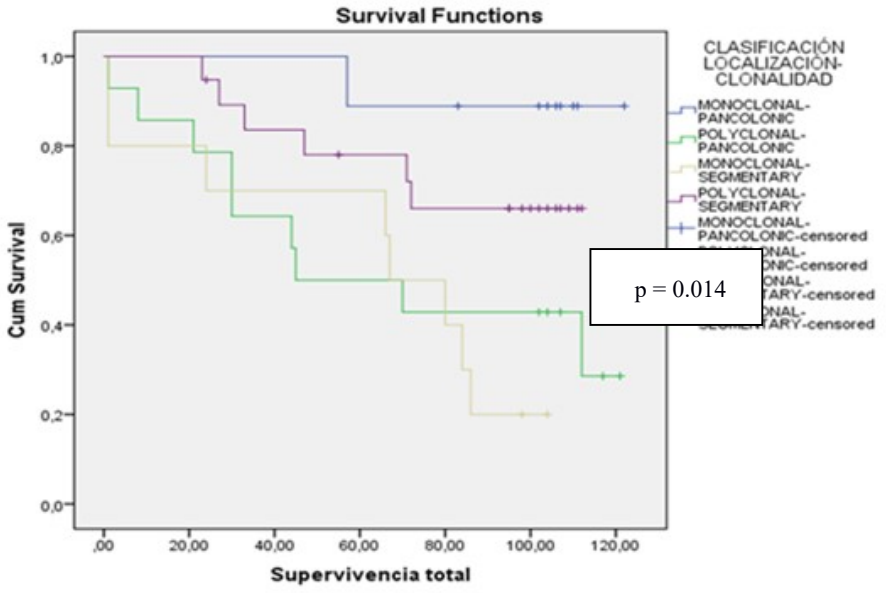


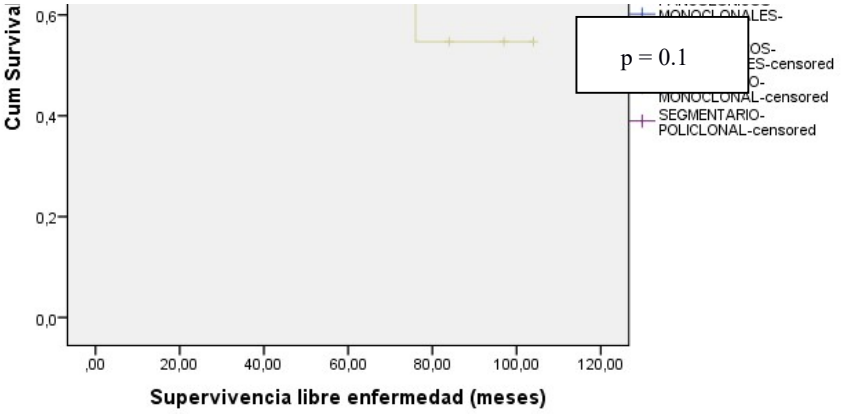
Monoclonales Pancolonicos



Policlonales Segmentarios

Policlonales Pancolónicos





SUPPLEMENTARY TAI

according to their Copy N

	Case	Sample1	S			
	1	B1	B			
	3	B5	B6	145.2965	2665552000	0.00235849
	13	B26	B27	24.32845	5224887	0.00562409
	23	B49	B50	321.9103	7.1621E+13	0.00036284
	29	B61	B62	56.82849	56.82849	0.03338171
	37	B77	B78	3.180847	94.63356	0.03029753
MONOCLONAL CASES	47	B92	B95	1.894864	246715.2	0.00834543
	48	B94	B96	42.50469	4196563000	0.00181422
	51	B103	B106	10914	3.1809E+10	0.00126996
	52	B107	B108	0.3218853	551228.7	0.00780116
	10	B19	B20	7.37E+00	7.37E+00	0.04934688
	11	B21	B22	5.08E+01	1.16E+03	0.02050073
	20	B43	B44	1.41E+01	3.16E+00	0.06259071
	24	B51	B52	3.63E-01	8.32E+01	0.03102322
	27	B57	B58	3.02E+01	8.38E+03	0.01578374
	33	B69	B70	6.86E+01	4.25E+11	0.00090711
	34	B71	B72	9.41E+02	1.69E+13	0.00036284
	40	B100	B83	1.00E+01	1.01E+03	0.02122642
	45	B102	B89	2.31E+05	4.35E+15	0.00036284
POL ICL	4	B7	B8	2.23E-01	2.23E-01	0.14713353
	6	B11	B12	5.98E-04	5.98E-04	0.62880987

8	B15	B16	7.18E-03	1.25E-01	0.17271408
14	B28	B29	1.53E-02	1.13E-03	0.56204644
15	B30	B31	1.10E-01	2.06E-02	0.2857402
16	B39	B40	8.69E-04	4.96E-05	0.84687954
17	B118	B36	4.66E-03	4.66E-03	0.40765602
18	B37	B38	8.41E-03	2.19E-03	0.48385341
19	B41	B42	3.90E-01	3.90E-01	0.11992017
21	B45	B46	5.78E-04	1.27E-03	0.5446299
22	B47	B48	5.14E-04	5.14E-04	0.64296081
25	B53	B54	1.36E-04	1.89E-02	0.29063861
31	B65	B66	1.03E-01	4.17E-02	0.23730044
35	B73	B74	1.42E-02	4.98E-02	0.22605225
41	B101	B84	1.73E-02	1.73E-02	0.29644412
44	B104	B88	5.10E-02	2.15E+00	0.06748911
46	B105	B90	1.52E-02	1.52E-02	0.30914369
49	B93	B97	1.23E-03	1.23E-03	0.55152395
50	B122	B98	2.90E-03	2.90E-03	0.45609579
2	B3	B4	1.53E-02	1.47E-02	0.3100508
5	B10	B9	1.90E-02	6.49E-04	0.62354862
7	B13	B14	3.83E-01	2.70E+00	0.06422351
9	B17	B18	3.85E-01	2.34E-04	0.72024673
12	B23	B24	2.39E-02	3.14E-02	0.25453556
26	B55	B56	1.78E-01	8.94E-02	0.19502903
30	B63	B64	2.43E-02	1.08E-01	0.17815675
32	B67	B68	1.57E-03	6.44E-03	0.37971698
36	B75	B76	5.31E-03	5.31E-03	0.39658926
38	B79	B80	7.90E-04	9.09E-05	0.79644412
39	B81	B82	1.79E-03	6.09E-01	0.09978229
42	B85	B99	1.52E-03	2.66E-04	0.71135704
43	B86	B87	1.49E-05	1.42E-04	0.76197388
53	B109	B110	2.09E-04	3.53E-05	0.86701742

LR: Likelihood Ratio.

LR2: Likelihood ratio 2, quantifies the odds that the two tumors are clonal

Supplementary Table 2. Recurrent genomic alterations in Monoclonal Synchronous Colorectal Cancer.

Change	Chr	Cytoband	Start	End	q values	Tumors	%	Putatives genes					
Amplification	chr20	20q13.2	52186785	52304107	7,7074E-23	27	71	ZNF217					
Amplification	chr12	12p13.1	12845688	13104999	8,7236E-07	25	66	**CDKN1B**	GPR19	GPRC5A	DDX47	GPRC5D	APOLD1
Amplification	chr8	8q24.3	142073847	142429472	0,000015589	24	63	GPR20	DENND3	SLC45A4			
Amplification	chr12	12p13.2	11870769	12039752	4,1169E-06	23	61	**ETV6**					
Amplification	chr13	13q22.1	73539854	73986627	6,3971E-15	23	61	**KLF5**	PIBF1				
Amplification	chr13	13q34	112666365	115169878	0,00061722	23	61	**TFDP1**	ATP4B	F7	F10	GAS6	LAMP1
Amplification	chr20	20p11.23	19665256	20032997	0,00028865	23	61	NAA20	CRNKL1	RIN2	SLC24A3		
Amplification	chr20	20p11.21	22276300	22757959	0,0010468	23	61	FOXA2	LINC00261	LOC284788			
Amplification	chr20	20q13.12	42924922	43123027	0,78151	23	61	HNF4A	TTPAL	FITM2	R3HDML	MIR3646	
Deletion	chr1	1p36.22	11340473	11721692	5,4306E-17	23	61	FBXO2	PTCHD2				
Deletion	chr1	1p36.21	14105299	15489816	0,000014374	23	61	KAZN	C1orf126				
Deletion	chr1	1p35.3	29533081	31184217	8,3539E-11	23	61	PTPRU					
Deletion	chr18	18p11.32	809912	2548100	8,3624E-10	23	61	ADCYAP1					
Amplification	chr1	1p32.1	59245265	59285474	9,6037E-18	22	58	**JUN**					
Amplification	chr3	3q13.13	107919862	108567922	0,94844	22	58	DZIP3	HHLA2	MYH15	TRAT1	IFT57	KIAA1524
Amplification	chr3	3q26.2	168837737	169096899	0,0018589	22	58	**MECOM**					
Amplification	chr8	8q24.3	145008707	145069677	0,00073701	22	58	GRINA	PLEC	PARP10			
Amplification	chr13	13q34	110188242	110447636	8,4619E-10	22	58	ATP4B	F7	F10	GAS6	LAMP1	GRK1
Amplification	chr19	19q13.11	33524070	33550588	2,7793E-08	22	58	RHPN2					
Deletion	chr1	1p21.3	95709940	98681347	0,0055657	22	58	**DPYD**	PTBP2				
Amplification	chr3	3p13	71049002	71266884	0,23232	21	55	**FOXP1**					
Amplification	chr3	3q21.3	127449562	127726736	0,00046561	21	55	MGLL	KBTBD12				
Amplification	chr7	7p11.2	55189547	55202597	1,4582E-33	21	55	**EGFR**					
Amplification	chr8	8q12.2	61419729	61922458	0,000013838	21	55	RAB2A	**CHD7**				
Amplification	chr11	11q24.3	128231470	128393419	1,7375E-23	21	55	**ETS1**					
Amplification	chr12	12p12.3	15786388	15956939	0,019294	21	55	EPS8					
Amplification	chr12	12q21.33	89702070	89938589	0,041743	21	55	DUSP6	GALNT4	POC1B	POC1B-GALNT4		
Amplification	chr13	13q33.2	106659101	107272113	4,5578E-06	21	55	EFNB2	ARGLU1	LINC00460			
Amplification	chr20	20p11.21	24994290	25339323	0,70646	21	55	ENTPD6	PYGB	ABHD12	VSX1	ACSS1	LOC284798
Amplification	chr20	20q13.12	45854488	46278181	0,0019868	21	55	NCOA3	ZMYND8				
Amplification	chr20	20q13.31	56044263	56240496	4,2713E-10	21	55	PCK1	PMEPA1	ZBP1	CTCFL		
Amplification	chr4	4q35.2	187598457	187664374	1,0733E-18	20	53	**FAT1**					
Amplification	chr5	5p15.33	1	2053238	0,0038951	20	53	**TERT**	**PDCD6**	NDUFS6	SDHA	SLC6A3	SLC9A3
Amplification	chr6	6p25.2	1637742	3033093	0,0020842	20	53	SERPINB1	GMDS	NQO2	SERPINB6	SERPINB9	WRNIP1
Amplification	chr7	7p15.3	24802000	26247113	0,019353	20	53	**HNRNPA2B1**	**NFE2L3**	CBX3	OSBPL3	CYCS	NPVF
Amplification	chr7	7q11.23	73165451	73261246	4,8733E-08	20	53	CLDN4	CLDN3	WBSCR27			

Amplification	chr7	7q36.3	158271643	158660922	1,1004E-08	20	53	PTPRN2	NCAPG2	WDR60	ESYT2	MIR595	
Amplification	chr8	8q23.1	109991638	111117737	0,077761	20	53	**PKHD1L1**	TRHR	EBAG9	KCNV1	SYBU	ENY2
Amplification	chr10	10p15.1	3812241	3949084	2,471E-36	20	53	**KLF6**					
Amplification	chr18	18p11.31	2979829	3770468	0,0025749	20	53	TGIF1	MYOM1	DLGAP1	LPIN2	MYL12A	MYL12B
Amplification	chr20	20q12	39650599	39727694	1,1365E-06	20	53	**TOP1**					
Amplification	chr21	21q22.3	42811396	43938672	5,5982E-12	20	53	**TMPRSS2**	MX1	TFF1	TFF2	TFF3	ABCG1
Deletion	chr1	1p13.2	112239322	112945981	2,5053E-07	20	53	KCND3	DDX20	FAM212B			
Deletion	chr9	9q34.3	140998977	141213431	0,000064294	20	53	TUBBP5	FAM157B				
Deletion	chr16	16p13.3	5142284	8626081	0,00002022	20	53	RBFOX1	TMEM114				
Amplification	chr3	3q26.31	171461612	171898475	0,0014993	19	50	PLD1	FNDC3B	TMEM212			
Amplification	chr3	3q29	193515521	193867078	0,00005946	19	50	HES1					
Amplification	chr4	4p16.1	6696461	6971604	3,6619E-19	19	50	**ABLIM2**	SH3TC1	AFAP1	AFAP1-AS1		
Amplification	chr6	6p25.1	6655523	6926070	0,000076821	19	50						
Amplification	chr7	7q31.2	116409778	116458045	2,801E-11	19	50	**MET**					
Amplification	chr8	8q24.21	128999641	130128117	0,000037173	19	50	PVT1					
Amplification	chr8	8q24.22	133713111	134615749	0,000019805	19	50	**NDRG1**	ST3GAL1	SLA	TG	WISP1	PHF20L1
Amplification	chr9	9q34.3	139404896	139656705	0,0013121	19	50	**NOTCH1**	AGPAT2	EGFL7	SNHG7	LCN8	FAM69B
Amplification	chr12	12p13.31	6288835	6635273	1,0507E-07	19	50	CD9	CD27	LTBR	SCNN1A	VAMP1	TNFRSF1A
Amplification	chr13	13q14.2	48969516	48989936	0,0028954	19	50	**RB1**	LPAR6				
Amplification	chr14	14q24.1	68405245	68930664	1,5663E-10	19	50	**RAD51B**					
Amplification	chr15	15q26.1	90551004	91147511	0,0025749	19	50	**IDH2**	**CRTC3**	IQGAP1	SEMA4B	CIB1	GABARAPL3
Amplification	chr17	17q21.32	46672717	46707988	5,4258E-20	19	50	HOXB6	HOXB7	HOXB8	HOXB9	LOC404266	
Amplification	chr17	17q21.33	48702095	48893817	1,2306E-09	19	50	ABCC3	CACNA1G	LUC7L3	LINC00483	ANKRD40	
Amplification	chr17	17q24.3	70370889	70659502	2,42E-12	19	50	SLC39A11	LINC00511	LOC100499467			
Deletion	chr3	3p21.31	50389478	50615538	7,4817E-08	19	50	CACNA2D2	TMEM115	C3orf18			
Deletion	chr5	5q31.2	135691679	136971076	6,4897E-06	19	50	SPOCK1					
Deletion	chr16	16p13.12	12661478	14017380	6,9053E-09	19	50	CPPED1	SHISA9	MIR4718			
Deletion	chr16	16p11.2	32137966	34197194	0,0022206	19	50	TP53TG3	SLC6A10P	LOC390705	HERC2P4	LINC00273	TP53TG3C

Supplementary Table 3. Recurrent genomic alterations in Polyclonal Synchronous Colorectal Cancer.

Change	Chr	Cytoband	Start	End	q values	Tumors	%	Putative Genes					
Amplification	chr8	8q24.22	133713111	134615749	1.98E-05	44	67	ST3GAL1	SLA	TG	**WISP1**	**NDRG1**	PHF20L1
Amplification	chr8	8q24.3	142073847	142429472	1.56E-05	44	67	GPR20	DENND3	SLC45A4			
Amplification	chr20	20q13.12	45854488	46278181	0.0019868	44	67	NCOA3	ZMYND8				
Amplification	chr20	20q13.2	52186785	52304107	7.71E-23	44	67	ZNF217					
Amplification	chr20	20q13.31	56044263	56240496	4.27E-10	44	67	PCK1	PMEPA1	ZBP1	CTCFL		
Amplification	chr8	8q24.21	128999641	130128117	3.72E-05	43	65	PVT1					
Amplification	chr20	20q13.12	42924922	43123027	7.82E-01	43	65	HNF4A	TTPAL	FITM2	R3HDML		
Amplification	chr7	7q36.3	158271643	158660922	1.10E-08	42	64	PTPRN2	NCAPG2	WDR60	ESYT2		
Amplification	chr20	20q12	39650599	39727694	1.14E-06	42	64	**TOP1**					
Amplification	chr7	7p11.2	55189547	55202597	1.46E-33	41	62	**EGFR**					
Amplification	chr8	8q23.1	109991638	111117737	7.78E-02	41	62	TRHR	EBAG9	KCNV1	SYBU	ENY2	NUDCD1
Amplification	chr8	8q24.3	145008707	145069677	7.37E-04	41	62	GRINA	PLEC	PARP10			
Amplification	chr20	20q13.13	48792592	49133279	1.31E-03	41	62	CEBPB	**PTPN1**				
Deletion	chr18	18p11.32	809912	2548100	8.36E-10	41	62	ADCYAP1					
Amplification	chr8	8q12.2	61419729	61922458	1.38E-05	40	61	RAB2A	**CHD7**				
Amplification	chr8	8q24.13	126172341	126409667	6.25E-08	40	61	NSMCE2					
Amplification	chr20	20p11.21	22276300	22757959	1.05E-03	40	61	FOXA2					
Amplification	chr20	20q13.33	60541887	61594432	0.060469	40	61	COL9A3	LAMA5	NTSR1	PSMA7	RPS21	TAF4
Amplification	chr12	12p13.2	11870769	12039752	4.12E-06	39	59	**ETV6**					
Amplification	chr12	12p13.1	12845688	13104999	8.72E-07	39	59	**CDKN1B**	GPR19	GPRC5A	DDX47	GPRC5D	APOLD1
Amplification	chr20	20p11.23	19665256	20032997	2.89E-04	39	59	NAA20	CRNKL1	RIN2	SLC24A3		
Amplification	chr7	7q36.2	154664675	155798939	7.06E-01	38	58	**DPP6**	EN2	HTR5A	INSIG1	**SHH**	PAXIP1
Amplification	chr20	20p11.21	24994290	25339323	7.06E-01	38	58	ENTPD6	PYGB	ABHD12	VSX1	ACSS1	

Deletion	chr1	1p35.3	29533081	31184217	8.35E-11	38	58	PTPRU					
Amplification	chr7	7q31.2	116409778	116458045	2.80E-11	37	56	**MET**					
Amplification	chr13	13q34	110188242	110447636	8.46E-10	37	56	ATP4B	F7	F10	GAS6	LAMP1	GRK1
Amplification	chr7	7q31.2	117064626	117372202	6.64E-06	36	55	CFTR	CTTNBP2	ASZ1			
Amplification	chr8	8q24.12	120928956	121937244	0.008956	36	55	SNTB1	COL14A1	MTBP	MRPL13	DEPTOR	
Amplification	chr12	12p12.3	15786388	15956939	1.93E-02	36	55	EPS8					
Amplification	chr13	13q22.1	73539854	73986627	6.40E-15	36	55	**KLF5**	PIBF1				
Amplification	chr12	12p13.31	6288835	6635273	1.05E-07	35	53	CD9	CD27	LTBR	SCNN1A	VAMP1	TNFRSF1A
Amplification	chr12	12q13.11	48283288	48317968	1.79E-11	35	53	VDR					
Amplification	chr20	20q11.21	30755699	30983019	0.11002	35	53	PLAGL2	KIF3B	POFUT1	TSPY26P	**ASXL1**	
Deletion	chr1	1p36.21	14105299	15489816	1.44E-05	35	53	KAZN					
Amplification	chr7	7p15.2	27168014	27250376	2.80E-11	34	52	HOXA4	HOXA5	HOXA6	**HOXA7**	**HOXA9**	HOXA10
Amplification	chr8	8q24.21	128150653	128561430	2.68E-02	34	52	POU5F1B					
Amplification	chr13	13q31.1	79623692	81759787	0.0038951	34	52	SPRY2	NDFIP2	RBM26	RBM26-AS1		
Deletion	chr1	1p36.22	11340473	11721692	5.43E-17	34	52	FBXO2	PTCHD2				
Amplification	chr3	3q26.31	171461612	171898475	0.0014993	33	50	PLD1	FNDC3B	TMEM212			
Amplification	chr7	7p21.1	16687543	17225606	2.27E-05	33	50	AGR2	TSPAN13	BZW2	AGR3		
Amplification	chr8	8q22.2	101174187	101582666	0.0025603	33	50	SPAG1	RNF19A	ANKRD46			
Amplification	chr12	12q14.1	59215534	59511652	0.023305	33	50	**CDK4**	TSPAN31	MARCH9			
Amplification	chr12	12q21.33	89702070	89938589	4.17E-02	33	50	DUSP6	GALNT4	POC1B	POC1B-GALNT4		
Amplification	chr12	12q23.3	106903322	107159404	3.64E-06	33	50	RFX4	POLR3B				
Deletion	chr1	1p35.1	33911648	35165737	1.62E-04	33	50	ZSCAN20	CSMD2	HMGB4			
Deletion	chr15	15q25.2	83732906	84327770	1.63E-05	33	50	BNC1	SH3GL3	HDGFRP3	TM6SF1	MIR4515	

Supplementary Table 4. Summary of the statistically significant differential alterations between both clonality groups

					Monoclonal SCRC (n=38)		Polyclonal SCRC (n=66)							
Change	**chr**	**Cytoband**	**star**	**End**	**cases**	**%**	**cases**	**%**	**Putatives Genes**					
Amplification	chr1	1p13.3	110221640	110250718	17	45	15	23	GSTM1	GSTM2				
Amplification	chr1	1p13.2	114280718	114475349	12	30	8	12	**PTPN22**	AP4B1	PHTF1	RSBN1	DCLRE1B	HIPK1
Amplification	chr1	1q25.2	175910533	176249548	13	34	11	17	RFWD2	SCARNA3				
Amplification	chr1	1q31.2	192888346	193462868	14	35	12	18	**CDC73**	hsa-mir-1278	TROVE2	B3GALT2	GLRX2	UCHL5
Amplification	chr2	2p21	42228536	42459287	16	43	14	21	**EML4**	PKDCC				
Amplification	chr2	2p21	43319720	43508204	15	40	12	18	ZFP36L2	THADA				
Amplification	chr2	2q24.1	157979281	158354713	16	42	15	23	CYTIP	GALNT5	ERMN			
Amplification	chr3	3p13	71049002	71266884	21	**55**	16	24	**FOXP1**					
Amplification	chr5	5p15.33	1	2053238	20	**53**	22	33	**TERT**	**PDCD6**	hsa-mi4277	NDUFS6	SDHA	SLC6A3
Amplification	chr5	5p13.1	39395398	39637564	18	48	19	29	DAB2					
Amplification	chr5	5q11.2	58741492	58981178	12	32	9	14	PDE4D					
Amplification	chr5	5q13.3	73283474	74971845	12	32	9	14	HEXB	HMGCR	ENC1	COL4A3BP	NSA2	FAM169A
Amplification	chr5	5q23.2	127140487	127314580	15	40	9	14						
Amplification	chr5	5q31.3	142176207	142266412	11	29	8	12	**ARHGAP26**					
Amplification	chr6	6p25.2	1637742	3033093	20	**53**	21	32	SERPINB1	GMDS	NQO2	SERPINB6	SERPINB9	WRNIP1
Amplification	chr6	6p25.1	6655523	6926070	19	**52**	19	29						
Amplification	chr6	6p21.33	30693817	30813687	12	32	10	15	IER3	FLOT1				
Amplification	chr7	7q32.3	130502279	130767115	8	21	28	**42**	hsa-mir-29b-1	FLJ43663	MIR29A	MIR29B1	LOC646329	
Amplification	chr8	8q13.3	71088549	71278585	11	29	31	**47**	**NCOA2**					
Amplification	chr8	8q22.2	101174187	101582666	11	29	33	**50**	SPAG1	RNF19A	ANKRD46	MIR4471		
Amplification	chr10	10q21.2	63613871	63835691	17	45	18	27	**ARID5B**					
Amplification	chr10	10q23.31	89617345	90778966	13	34	12	18	**FAS**	**PTEN**	ACTA2	LIPF	RNLS	STAMBPL1

Amplification	chr11	11q13.5	76190213	76338578	14	37	10	15	C11orf30					
Amplification	chr11	11q12.3	62068686	62394880	9	24	6	9	**AHNAK**	EEF1G	ROM1	SCGB1A1	MTA2	GANAB
Amplification	chr13	13q32.3	99818725	100051333	10	26	31	**47**	hsa-mir-623	**GPR183**	GPR18	UBAC2	MIR623	FKSG29
Amplification	chr15	15q26.1	90551004	91147511	19	50	19	29	**IDH2**	**CRTC3**	IQGAP1	SEMA4B	CIB1	GABARAPL3
Amplification	chr16	16p12.1	27168122	27358947	15	40	14	21	IL4R	JMJD5	NSMCE1	FLJ21408		
Amplification	chr17	17p13.1	7063556	7530746	8	22	5	8	**CTDNEP1**	hsa-mir-324	ACADVL	ASGR1	CD68	CHRNB1
Amplification	chr17	17q24.3	70370889	70659502	19	50	19	29	SLC39A11	LINC00511				
Amplification	chr18	18p11.31	2979829	3770468	20	**53**	16	24	TGIF1	MYOM1	DLGAP1	LPIN2	MYL12A	MYL12B
Amplification	chr18	18q21.1	45425968	45716892	12	32	10	15	**SMAD2**	ZBTB7C				
Amplification	chr21	21q22.2	40090624	40403594	14	37	13	20	ETS2	LINC00114				
Amplification	chr22	22q13.1	38535289	38587383	10	26	3	5	PLA2G6					
Deletion	chr3	3p26.2	1	3848506	16	40	14	21	IL5RA	CHL1	CNTN6	TRNT1	CRBN	CNTN4
Deletion	chr3	3p22.2	35832878	37032295	9	24	6	9	STAC	TRANK1	DCLK3			
Deletion	chr3	3p13	68586523	71249674	17	45	16	24	**MITF**	hsa-mir-3136	TMF1	UBA3	ARL6IP5	FRMD4B
Deletion	chr4	4q13.3	71893865	73926669	14	37	11	17	GC	SLC4A4	ADAMTS3	NPFFR2		
Deletion	chr5	5q22.2	112179558	112784006	18	47	17	26	MCC	SRP19	REEP5	TSSK1B	DCP2	
Deletion	chr8	8q24.22	135811617	140632500	17	45	12	18	**FAM135B**	**COL22A1**	LOC286094	MIR30B	MIR30D	hsa-mir-30d
Deletion	chr9	9q34.3	140998977	141213431	20	**53**	15	23	TUBBP5	FAM157B				
Deletion	chr14	14q22.3	57740794	58722538	16	42	16	24	C14orf105	ACTR10	NAA30	C14orf37	SLC35F4	
Deletion	chr16	16p12.3	19883083	20446959	16	42	14	24	GP2	UMOD	GPR139	PDILT		
Deletion	chr16	16p11.2	32137966	34197194	16	42	14	24	TP53TG3	SLC6A10P		HERC2P4	LINC00273	TP53TG3C
Deletion	chr16	16p13.12	12661478	14017380	20	50	21	32	CPPED1	SHISA9	MIR4718			
Deletion	chr16	16q22.2	70833950	71887380	8	20	4	6	**HYDIN**	AP1G1	CALB2	TAT	ZNF19	ZNF23
Deletion	chr19	19q13.31	44721491	45121035	10	26	7	11	ZNF180	ZFP112	ZNF229	ZNF235	ZNF285	CEACAM20
Deletion	chr19	19q13.42	53522101	54234352	17	45	15	23	**ZNF331**	**ZNF765**	hsa-mir-518a-1	ZNF415	ZNF665	ZNF347
Deletion	chr22	22q11.23	24314259	24408077	4	10	21	32	GSTT1	GSTT2	GSTTP1	LOC391322	GSTTP2	
Deletion	chr22	22q12.3	33446938	35467198	3	8	22	33	LARGE	LOC100506195	MIR4764			

SUPPLEMENTARY TABLE 5. Most frequent alterations in Monoclonal Monosegmentary Synchronous Colorectal cancers.

Change	chr	Cytoband	Start	End	q values	cases	%	Putatives Genes					
Amplification	chr20	20q13.2	52186785	52304107	7,71E-23	17	85	ZNF217					
Deletion	chr1	1p21.3	95709940	98681347	0,0055657	17	85	**DPYD**	hsa-mir-137	PTBP2	MIR137HG	MIR137	FLJ31662
Amplification	chr12	12p13.2	11870769	12039752	4,12E-06	15	75	**ETV6**					
Amplification	chr12	12p13.1	12845688	13104999	8,72E-07	15	75	**CDKN1B**	hsa-mir-614	hsa-mir-613	GPR19	GPRC5A	DDX47
Deletion	chr1	1p36.22	11340473	11721692	5,43E-17	15	75	FBXO2	PTCHD2				
Deletion	chr1	1p13.2	112239322	112945981	2,51E-07	15	75	KCND3	DDX20	FAM212B	LOC100506343		
Amplification	chr12	12p12.3	15786388	15956939	1,93E-02	14	70	EPS8					
Amplification	chr20	20p11.23	19665256	20032997	2,89E-04	14	70	NAA20	CRNKL1	RIN2	SLC24A3		
Amplification	chr20	20q13.31	56044263	56240496	4,27E-10	14	70	PCK1	PMEPA1	ZBP1	CTCFL		
Deletion	chr18	18p11.32	809912	2548100	8,36E-10	14	70	ADCYAP1	LINC00470				
Amplification	chr1	1p32.1	59245265	59285474	9,60E-18	13	65	**JUN**	LOC100131060				
Amplification	chr3	3q13.13	107919862	108567922	9,48E-01	13	65	DZIP3	HHLA2	MYH15	TRAT1	IFT57	KIAA1524
Amplification	chr3	3q21.3	127449562	127726736	4,66E-04	13	65	MGLL	KBTBD12				
Amplification	chr7	7p15.3	24802000	26247113	0,019353	13	65	**HNRNPA2B1**	**NFE2L3**	hsa-mir-148a	CBX3	OSBPL3	CYCS
Amplification	chr8	8q24.3	142073847	142429472	1,56E-05	13	65	GPR20	DENND3	SLC45A4	LOC731779		
Amplification	chr12	12p13.31	6288835	6635273	1,05E-07	13	65	CD9	CD27	LTBR	SCNN1A	VAMP1	TNFRSF1A
Amplification	chr13	13q34	110188242	110447636	8,46E-10	13	65	ATP4B	F7	F10	GAS6	LAMP1	GRK1
Amplification	chr20	20p11.21	22276300	22757959	1,05E-03	13	65	FOXA2	LINC00261	LOC284788			
Amplification	chr20	20q13.12	42924922	43123027	7,82E-01	13	65	HNF4A	TTPAL	FITM2	R3HDML	MIR3646	
Deletion	chr1	1p36.21	14105299	15489816	1,44E-05	13	65	KAZN	C1orf126				
Deletion	chr1	1p35.3	29533081	31184217	8,35E-11	13	65	PTPRU					
Deletion	chr9	9q34.3	140998977	141213431	6,43E-05	13	65	TUBBP5	FAM157B				
Amplification	chr1	1q24.2	168743953	169136832	9,62E-06	12	60	ATP1B1	NME7	MGC4473			
Amplification	chr3	3q26.2	168837737	169096899	1,86E-03	12	60	**MECOM**					
Amplification	chr5	5p15.33	1	2053238	0,0038951	12	60	**TERT**	**PDCD6**	hsa-mir-4277	NDUFS6	SDHA	SLC6A3
Amplification	chr7	7p21.1	16687543	17225606	2,27E-05	12	60	AGR2	TSPAN13	BZW2	AGR3		
Amplification	chr7	7p11.2	55189547	55202597	1,46E-33	12	60	**EGFR**					
Amplification	chr7	7q31.2	116409778	116458045	2,80E-11	12	60	**MET**					
Amplification	chr7	7q36.2	154664675	155798939	7,06E-01	12	60	**DPP6**	EN2	HTR5A	INSIG1	SHH	PAXIP1
Amplification	chr7	7q36.3	158271643	158660922	1,10E-08	12	60	hsa-mir-595	PTPRN2	NCAPG2	WDR60	ESYT2	MIR595

Amplification	chr8	8p11.23	37374653	37917939	0,000174	12	60	ADRB3	EIF4EBP1	ERLIN2	PROSC	GPR124	BRF2
Amplification	chr8	8q24.13	126172341	126409667	6,25E-08	12	60	NSMCE2					
Amplification	chr8	8q24.21	128150653	128561430	2,68E-02	12	60	POU5F1B	LOC727677				
Amplification	chr11	11q24.3	128231470	128393419	1,74E-23	12	60	**ETS1**					
Amplification	chr13	13q34	112666365	115169878	0,00061722	12	60	**TFDP1**	ATP4B	F7	F10	GAS6	LAMP1
Amplification	chr14	14q24.1	68405245	68930664	1,57E-10	12	60	**RAD51B**					
Amplification	chr15	15q26.1	90551004	91147511	2,57E-03	12	60	**IDH2**	**CRTC3**	IQGAP1	SEMA4B	CIB1	GABARAPL3
Amplification	chr17	17q24.3	70370889	70659502	2,42E-12	12	60	SLC39A11	LINC00511	LOC100499467			
Amplification	chr18	18p11.31	2979829	3770468	2,57E-03	12	60	TGIF1	MYOM1	DLGAP1	LPIN2	MYL12A	MYL12B
Amplification	chr19	19q13.11	33524070	33550588	2,78E-08	12	60	RHPN2					
Amplification	chr20	20p11.21	24994290	25339323	7,06E-01	12	60	ENTPD6	PYGB	ABHD12	VSX1	ACSS1	LOC284798
Amplification	chr20	20q12	39650599	39727694	1,14E-06	12	60	**TOP1**					
Amplification	chr20	20q13.12	45854488	46278181	0,0019868	12	60	NCOA3	ZMYND8	LOC100131496			
Amplification	chr21	21q22.3	42811396	43938672	5,60E-12	12	60	**TMPRSS2**	MX1	TFF1	TFF2	TFF3	ABCG1
Deletion	chr1	1p13.2	114523466	114939943	0,00015954	12	60	KCND3	DDX20	FAM212B	LOC100506343		
Deletion	chr3	3p21.31	50389478	50615538	7,48E-08	12	60	CACNA2D2	TMEM115	C3orf18			
Deletion	chr4	4q13.3	71893865	73926669	0,022569	12	60	GC	SLC4A4	ADAMTS3	NPFFR2		
Deletion	chr14	14q32.33	105978538	106388980	3,77E-03	12	60	LINC00226					
Deletion	chr16	16p13.3	5142284	8626081	2,02E-05	12	60	RBFOX1	TMEM114				
Deletion	chr16	16p13.2	9306668	10480845	4,39E-01	12	60	**GRIN2A**	MIR548X				
Deletion	chr17	17p11.2	21447016	25637342	1,05E-01	12	60	FLJ36000	MTRNR2L1	MIR4522			
Amplification	chr3	3p13	71049002	71266884	2,32E-01	11	55	**FOXP1**					
Amplification	chr3	3q25.1	151873983	152256743	6,30E-02	11	55	MBNL1	LOC401093	TMEM14E			
Amplification	chr3	3q26.31	171461612	171898475	0,0014993	11	55	PLD1	FNDC3B	TMEM212			
Amplification	chr3	3q26.32	176807172	177119142	2,14E-02	11	55	**TBL1XR1**					
Amplification	chr3	3q29	193515521	193867078	5,95E-05	11	55	HES1	LOC647323	LOC100128023			
Amplification	chr4	4q35.2	187598457	187664374	1,07E-18	11	55	**FAT1**					
Amplification	chr5	5q31.1	135203514	135506705	0,0021649	11	55	**SLC25A48**	hsa-mir-886	IL9	LECT2	SMAD5	TGFBI
Amplification	chr6	6p25.2	1637742	3033093	2,08E-03	11	55	SERPINB1	GMDS	NQO2	SERPINB6	SERPINB9	WRNIP1
Amplification	chr6	6p21.1	43617555	43815363	2,01E-11	11	55	VEGFA	MRPS18A	RSPH9			
Amplification	chr7	7p14.3	29273987	29663844	0,033166	11	55	CHN2	PRR15				
Amplification	chr7	7q31.2	117064626	117372202	6,64E-06	11	55	CFTR	CTTNBP2	ASZ1			
Amplification	chr8	8q12.2	61419729	61922458	1,38E-05	11	55	**CHD7**	RAB2A	LOC100130298			
Amplification	chr8	8q23.1	109991638	111117737	7,78E-02	11	55	**PKHD1L1**	TRHR	EBAG9	KCNV1	SYBU	ENY2
Amplification	chr8	8q24.21	128999641	130128117	3,72E-05	11	55	hsa-mir-1208	hsa-mir-1207	PVT1	MIR1206	MIR1207	MIR1208

Amplification	chr8	8q24.22	133713111	134615749	1,98E-05	11	55	**NDRG1**	ST3GAL1	SLA	TG	WISP1	PHF20L1
Amplification	chr8	8q24.3	145008707	145069677	7,37E-04	11	55	hsa-mir-661	GRINA	PLEC	PARP10	MIR661	
Amplification	chr10	10p15.1	3812241	3949084	2,47E-36	11	55	**KLF6**					
Amplification	chr10	10q21.2	63613871	63835691	1,02E-14	11	55	**ARID5B**					
Amplification	chr13	13q14.2	48969516	48989936	2,90E-03	11	55	**RB1**	LPAR6				
Amplification	chr13	13q22.1	73539854	73986627	6,40E-15	11	55	**KLF5**	PIBF1				
Amplification	chr13	13q33.2	106659101	107272113	4,56E-06	11	55	EFNB2	ARGLU1	LINC00460			
Amplification	chr17	17q21.32	46672717	46707988	5,43E-20	11	55	HOXB6	HOXB7	HOXB8	HOXB9	LOC404266	
Amplification	chr17	17q21.33	48702095	48893817	1,23E-09	11	55	ABCC3	CACNA1G	LUC7L3	LINC00483	ANKRD40	
Amplification	chr19	19p13.3	1236397	1265924	6,69E-09	11	55	**C19orf26**	ATP5D	MIDN			
Amplification	chr20	20p12.3	5488645	5799407	0,05314	11	55	GPCPD1	C20orf196				
Amplification	chr20	20p12.2	10562659	11565455	0,0023524	11	55	**PLCB4**					
Amplification	chr20	20q13.13	48792592	49133279	1,31E-03	11	55	**PTPN1**	CEBPB	LOC284751			
Amplification	chr20	20q13.33	60541887	61594432	0,060469	11	55	**SS18L1**	hsa-mir-133a-2	hsa-mir-3195	COL9A3	LAMA5	NTSR1
Amplification	chr21	21q22.2	40090624	40403594	1,19E-01	11	55	ETS2	LINC00114				
Deletion	chr1	1p36.32	2583273	3046462	1,51E-01	11	55	hsa-mir-4251	ACTRT2	FLJ42875	MIR4251		
Deletion	chr1	1p22.1	91990919	92419268	0,041298	11	55	TGFBR3	HSP90B3P				
Deletion	chr14	14q22.3	57740794	58722538	0,0054865	11	55	C14orf105	ACTR10	NAA30	C14orf37	SLC35F4	
Deletion	chr15	15q25.2	83732906	84327770	1,63E-05	11	55	BNC1	SH3GL3	HDGFRP3	TM6SF1	MIR4515	
Deletion	chr16	16p13.12	12661478	14017380	6,91E-09	11	55	CPPED1	SHISA9	MIR4718			
Deletion	chr16	16p11.2	32137966	34197194	2,22E-03	11	55	hsa-mir-1826	TP53TG3	SLC6A10P	LOC390705	HERC2P4	LINC00273
Amplification	chr1	1q22	156078756	156102475	1,23E-09	10	50	LMNA					
Amplification	chr1	1q31.2	192888346	193462868	0,011731	10	50	**CDC73**	hsa-mir-1278	TROVE2	B3GALT2	GLRX2	UCHL5
Amplification	chr1	1q32.1	203207521	203318143	8,59E-14	10	50	**MDM4**	**PIK3C2B**				
Amplification	chr1	1q32.1	205146461	205762138	0,00019122	10	50	**BTG2**	FMOD	LOC730227			
Amplification	chr1	1q42.3	234649706	235276288	3,23E-06	10	50	TOMM20	IRF2BP2	LINC00184	LOC100506795	LOC100506810	
Amplification	chr2	2p25.3	1	86337	1,57E-25	10	50	FAM110C					
Amplification	chr2	2q13	113849838	114525192	0,00061722	10	50	hsa-mir-4267	NPHP1	MALL	RGPD5	LIMS3	LOC440894
Amplification	chr3	3q13.11	105396141	105642918	0,010927	10	50	**CBLB**					
Amplification	chr3	3q21.1	122377977	122595776	1,23E-05	10	50	PARP14	HSPBAP1	DIRC2			
Amplification	chr3	3q26.33	182350797	182513350	0,081244	10	50	ATP11B					
Amplification	chr5	5p15.2	14106137	15029265	0,0071768	10	50	TRIO	FAM105A	ANKH	FAM105B	LOC100130744	MIR4637
Amplification	chr5	5q15	95936429	96359911	0,0049389	10	50	CAST	LNPEP	ERAP1	ERAP2		
Amplification	chr5	5q23.2	127140487	127314580	4,49E-07	10	50	[FLJ33630]					

Amplification	chr5	5q34	159418491	160356605	0,013254	10	50	hsa-mir-146a	hsa-mir-3142	FABP6	TTC1	PTTG1	SLU7
Amplification	chr6	6p25.1	6655523	6926070	7,68E-05	10	50	[LY86]					
Amplification	chr6	6q27	168111239	168599919	4,49E-07	10	50	**MLLT4**	KIF25	C6orf123	FRMD1	MLLT4-AS1	HGC6.3
Amplification	chr7	7p22.1	6398652	6435900	8,69E-05	10	50	**RAC1**					
Amplification	chr7	7p15.2	27168014	27250376	2,80E-11	10	50	**HOXA7**	**HOXA9**	**HOXA11**	**HOXA13**	hsa-mir-196b	HOXA4
Amplification	chr7	7q11.23	73165451	73261246	4,87E-08	10	50	CLDN4	CLDN3	WBSCR27			
Amplification	chr7	7q11.23	75896467	77509873	0,20576	10	50	**PTPN12**	HSPB1		YWHAG	ZP3	FGL2
Amplification	chr7	7q21.2	92224326	92489812	0,00061722	10	50	**CDK6**					
Amplification	chr7	7q22.3	104536784	104717516	1,93E-06	10	50	MLL5	LHFPL3	LOC723809	LOC100216545	LOC100216546	
Amplification	chr8	8p23.1	8137084	8385546	1,65E-03	10	50	SGK223					
Amplification	chr9	9p21.3	20356742	20625874	0,0004646	10	50	**MLLT3**	MIR4473	MIR4474			
Amplification	chr9	9p21.2	27328887	27670082	0,0045975	10	50	IFNK	MOB3B	C9orf72			
Amplification	chr9	9q34.3	139404896	139656705	1,31E-03	10	50	**NOTCH1**	hsa-mir-126		AGPAT2	EGFL7	SNHG7
Amplification	chr12	12q13.11	48283288	48317968	1,79E-11	10	50	VDR					
Amplification	chr12	12q21.33	89702070	89938589	4,17E-02	10	50	DUSP6	GALNT4	POC1B	POC1B-GALNT4		
Amplification	chr16	16p12.1	27168122	27358947	1,80E-04	10	50	IL4R	JMJD5	NSMCE1	FLJ21408		
Amplification	chr17	17q24.1	63511571	64039897	1,72E-02	10	50	AXIN2	CEP112				
Amplification	chr19	19p13.11	18288899	18480170	0,0002875	10	50	hsa-mir-3188	JUND	PDE4C	RAB3A	IFI30	LSM4
Amplification	chr20	20p12.2	9123559	9384012	0,065195	10	50	**JAG1**	C20orf94	LOC339593			
Amplification	chr20	20p12.1	17504102	18005112	9,48E-01	10	50	BFSP1	RRBP1	DSTN	SNX5	OVOL2	C20orf72
Amplification	chr21	21q22.12	36222863	36673572	2,57E-01	10	50	**RUNX1**	RUNX1-IT1				
Deletion	chr1	1p35.1	33911648	35165737	1,62E-04	10	50	hsa-mir-552	ZSCAN20	C1orf94	CSMD2	HMGB4	LOC402779
Deletion	chr1	1q21.2	147826659	149815535	1,67E-09	10	50	FCGR1A	HIST2H2AA3	HIST2H4A	NBPF14	FAM91A2	HIST2H3C
Deletion	chr4	4q35.1	182068857	185273482	0,0064971	10	50	hsa-mir-1305	DCTD	ING2	CLDN22	CDKN2AIP	ODZ3
Deletion	chr5	5q31.2	135691679	136971076	6,49E-06	10	50	SPOCK1					
Deletion	chr5	5q33.1	150579351	151047492	0,00018108	10	50	**FAT2**	GM2A	SLC36A2	SLC36A1	SLC36A3	
Deletion	chr11	11q13.3	69489243	69931046	2,06E-05	10	50	**FGF19**	FGF3	FGF4			
Deletion	chr14	14q32.33	106436197	106942649	2,48E-01	10	50	LINC00226					
Deletion	chr15	15q11.2	1	83215162	1,05E-01	10	50	**ADAM10**	**B2M**	**BUB1B**	**CLK3**	**LTK**	**MYO9A**
Deletion	chr16	16p12.3	19883083	20446959	7,21E-01	10	50	GP2	UMOD	GPR139	PDILT		
Deletion	chr17	17p13.1	7529764	7571856	0,00012473	10	50	ATP1B2	SHBG				
Deletion	chr18	18q11.2	22010422	28586619	0,17559	10	50	**SS18**	**ZNF521**	hsa-mir-302f	AQP4	CDH2	TAF4B
Deletion	chr18	18q21.32	56719437	57568692	0,0012857	10	50	GRP	LMAN1	RAX	SEC11C	CCBE1	CPLX4
Deletion	chr19	19p12	22013964	23407184	0,00036162	10	50	**ZNF99**	**ZNF208**	ZNF492	ZNF257	ZNF98	ZNF676

SUPPLEMENTARY TABLE 6. **Most frequent alterations in Monoclonal Pancolonic Synchronous Colorectal cancers.**

Change	Chr	Cytoband	Start	End	q values	cases	%	Putative Genes					
Amplification	chr13	13q22.1	73539854	73986627	6,3971E-15	12	67	**KLF5**	PIBF1				
Amplification	chr4	4p16.1	6696461	6971604	3,6619E-19	11	61	**ABLIM2**	hsa-mir-95	SH3TC1	AFAP1	AFAP1-AS1	
Amplification	chr8	8q24.3	142073847	142429472	1,5589E-05	11	61	GPR20	DENND3	SLC45A4	LOC731779		
Amplification	chr8	8q24.3	145008707	145069677	0,00073701	11	61	hsa-mir-661	GRINA	PLEC	PARP10	MIR661	
Amplification	chr12	12q21.33	89702070	89938589	0,041743	11	61	DUSP6	GALNT4	POC1B	POC1B-GALNT4		
Amplification	chr13	13q34	112666365	115169878	0,00061722	11	61	**TFDP1**	ATP4B	F7	F10	GAS6	LAMP1
Deletion	chr1	1p36.21	14105299	15489816	1,4374E-05	10	56	KAZN	C1orf126				
Deletion	chr1	1p35.3	29533081	31184217	8,3539E-11	10	56	PTPRU					
Amplification	chr3	3p13	71049002	71266884	0,23232	10	56	**FOXP1**					
Amplification	chr3	3q26.2	168837737	169096899	0,0018589	10	56	**MECOM**					
Amplification	chr4	4p16.3	2594979	3582650	0,28498	10	56	**RGS12**	ADD1	GRK4	HTT	HGFAC	LRPAP1
Amplification	chr7	7q11.23	73165451	73261246	4,8733E-08	10	56	CLDN4	CLDN3	WBSCR27			
Amplification	chr8	8q12.2	61419729	61922458	1,3838E-05	10	56	**CHD7**	RAB2A	LOC100130298			
Amplification	chr12	12p13.1	12845688	13104999	8,7236E-07	10	56	**CDKN1B**	hsa-mir-614	hsa-mir-613	GPR19	GPRC5A	DDX47
Amplification	chr13	13q33.2	106659101	107272113	4,5578E-06	10	56	EFNB2	ARGLU1	LINC00460			
Amplification	chr19	19q13.11	33524070	33550588	2,7793E-08	10	56	RHPN2					
Amplification	chr20	20p11.21	22276300	22757959	0,0010468	10	56	FOXA2	LINC00261	LOC284788			
Amplification	chr20	20q13.12	42924922	43123027	0,78151	10	56	HNF4A	TTPAL	FITM2	R3HDML	MIR3646	
Amplification	chr20	20q13.2	52186785	52304107	7,7074E-23	10	56	ZNF217					
Amplification	chr1	1p32.1	59245265	59285474	9,6037E-18	9	50	**JUN**	LOC100131060				
Amplification	chr3	3q13.13	107919862	108567922	0,94844	9	50	DZIP3	HHLA2	MYH15	TRAT1	IFT57	KIAA1524
Amplification	chr4	4p16.1	7778525	8256011	0,00023465	9	50	S100P	KIAA0232	CNO	TBC1D14	MRFAP1L1	
Amplification	chr4	4q21.1	74814267	78022903	0,030047	9	50	**SHROOM3**	AREG	ART3	BTC	SCARB2	EREG
Amplification	chr4	4q35.2	187598457	187664374	1,0733E-18	9	50	**FAT1**					

Amplification	chr5	5p13.1	39395398	39637564	0,78151	9	50	DAB2					
Deletion	chr5	5q22.2	112179558	112784006	0,00087075	9	50	MCC	SRP19	REEP5	TSSK1B	DCP2	
Deletion	chr5	5q31.2	135691679	136971076	6,4897E-06	9	50	SPOCK1					
Amplification	chr6	6p25.2	1637742	3033093	0,0020842	9	50	SERPINB1	GMDS	NQO2	SERPINB6	SERPINB9	WRNIP1
Amplification	chr6	6p25.1	6655523	6926070	7,6821E-05	9	50	[LY86]					
Amplification	chr7	7p11.2	55189547	55202597	1,4582E-33	9	50	**EGFR**					
Deletion	chr7	7q11.22	70743034	70807983	5,7955E-09	9	50	PMS2P4	AUTS2	STAG3L4			
Amplification	chr8	8q11.23	53168383	53361210	0,19745	9	50	ST18					
Amplification	chr8	8q23.1	109991638	111117737	0,077761	9	50	**PKHD1L1**	TRHR	EBAG9	KCNV1	SYBU	ENY2
Deletion	chr8	8q24.22	135811617	140632500	0,0098959	9	50	**COL22A1**	**FAM135B**	hsa-mir-30d	KHDRBS3	LOC286094	MIR30B
Amplification	chr9	9q21.33	87311716	87574008	0,0051249	9	50	**NTRK2**					
Amplification	chr9	9q34.3	139404896	139656705	0,0013121	9	50	**NOTCH1**	hsa-mir-126	AGPAT2	EGFL7	SNHG7	LCN8
Amplification	chr10	10p15.1	3812241	3949084	2,471E-36	9	50	**KLF6**					
Amplification	chr11	11q24.3	128231470	128393419	1,7375E-23	9	50	**ETS1**					
Amplification	chr12	12q23.3	106903322	107159404	3,6439E-06	9	50	RFX4	POLR3B	LOC100287944	LOC100505978		
Deletion	chr13	13q12.11	1	19757954	3,2266E-12	9	50	**TUBA3C**	PHF2P1	ANKRD20A9P	LINC00442		
Amplification	chr13	13q31.1	79623692	81759787	0,0038951	9	50	SPRY2	NDFIP2	RBM26	RBM26-AS1		
Amplification	chr13	13q34	110188242	110447636	8,4619E-10	9	50	IRS2					
Deletion	chr18	18p11.32	809912	2548100	8,3624E-10	9	50	ADCYAP1	LINC00470				
Amplification	chr20	20p11.23	19665256	20032997	0,00028865	9	50	NAA20	CRNKL1	RIN2	SLC24A3		
Amplification	chr20	20p11.21	24994290	25339323	0,70646	9	50	ENTPD6	PYGB	ABHD12	VSX1	ACSS1	LOC284798
Amplification	chr20	20q13.12	45854488	46278181	0,0019868	9	50	NCOA3	ZMYND8	LOC100131496			

SUPPLEMANTARY TABLE 7. Differential alterations between Segmentary and Pancolonic Monoclonal Synchronous Colorectal Cancer

Change	Chr	Cytoband	Start	End	MM (n=20)		MP (n=18)							
					Nº	%	Nº	%						
Deletion Peak	chr1	1p21.3	95709940	98681347	17	85	5	28	**DPYD**	hsa-mir-137	PTBP2	MIR137HG	MIR137	FLJ31662
Deletion Peak	chr1	1p13.2	112239322	112945981	15	75	5	28	KCND3	DDX20	FAM212B			
Amplification	chr1	1q24.2	168743953	169136832	12	60	4	22	ATP1B1	NME7	MGC4473			
Amplification	chr8	8q24.21	128150653	128561430	12	60	5	28	POU5F1B	LOC727677				
Deletion Peak	chr1	1p13.2	114523466	114939943	12	60	5	28	SYT6					
Deletion Peak	chr4	4q13.3	71893865	73926669	12	60	2	11	GC	SLC4A4	ADAMTS3	NPFFR2		
Deletion Peak	chr16	16p13.2	9306668	10480845	12	60	5	28	**GRIN2A**	MIR548X				
Amplification	chr5	5q31.1	135203514	135506705	11	55	2	11	**SLC25A48**	hsa-mir-886	IL9	LECT2	SMAD5	TGFBI
Amplification	chr20	20p12.3	5488645	5799407	11	55	2	11	GPCPD1	C20orf196				
Amplification	chr21	21q22.2	40090624	40403594	11	55	3	17	ETS2	LINC00114				
Deletion Peak	chr1	1p36.22	11340473	11721692	11	55	4	22	FBXO2	PTCHD2				
Amplification	chr1	1q22	156078756	156102475	10	50	3	17	LMNA					
Amplification	chr1	1q32.1	205146461	205762138	10	50	3	17	**ELK4**	**SLC45A3**	hsa-mir-135b	CDK18	RAB7L1	TMCC2
Amplification	chr5	5q15	95936429	96359911	10	50	1	6	CAST	LNPEP	ERAP1	ERAP2		
Amplification	chr5	5q34	159418491	160356605	10	50	2	11	hsa-mir-146a	hsa-mir-3142	FABP6	TTC1	PTTG1	SLU7
Amplification	chr9	9p21.2	27328887	27670082	10	50	3	17	IFNK	MOB3B	C9orf72			
Deletion Peak	chr4	4q35.1	182068857	185273482	10	50	2	11	hsa-mir-1305	DCTD	ING2	CLDN22	CDKN2AIP	ODZ3
Amplification	chr5	5q31.3	139460237	140086190	9	45	1	6	CD14	HBEGF	HARS	IK	NDUFA2	PFDN1
Amplification	chr5	5q31.3	142176207	142266412	9	45	2	11	**ARHGAP26**					
Amplification	chr1	1q23.3	164569855	164645696	8	40	2	11	**PBX1**					
Amplification	chr1	1q32.2	207179783	207574824	8	4	1	6	C4BPA	C4BPB	CD55	PFKFB2	YOD1	C1orf116
Amplification	chr22	22q13.1	38535289	38587383	8	40	2	11	PLA2G6					
Deletion Peak	chr6	6q26	160670283	163148165	8	40	2	11	**C6orf118**	**QKI**	PDE10A	PACRG	LOC285796	DKFZp451B082
Deletion Peak	chr12	12q12	34175509	39687753	8	40	1	6	ALG10B	CPNE8				

Deletion Peak	chr19	19q13.2	41310223	41622614	8	40	2	11	**CYP2A7**	CYP2A6	CYP2A13	CYP2B6	CYP2B7P1	CYP2G1P
Amplification	chr6	6q22.33	128397550	128886848	7	35	1	6	**PTPRK**					
Amplification	chr14	14q21.3	50315104	50566924	7	35	1	6	ARF6	NEMF	C14orf183	C14orf182		
Amplification	chr22	22q12.2	31433520	31717798	7	35	1	6	**PIK3IP1**	LIMK2	SMTN	INPP5J	PLA2G3	RNF185
Deletion Peak	chr2	2q11.1	89110856	95539832	7	35	0	0	LOC90499	ACTR3BP2	GGT8P	LOC654342	ANKRD20A8P	MIR4436A
Deletion Peak	chr2	2q11.2	100094800	101451520	7	35	1	6	**AFF3**	CHST10	PDCL3	NMS	LONRF2	
Deletion Peak	chr12	12q24.11	111121120	111802274	7	35	1	6	**CCDC63**	MYL2	PPP1CC	CUX2	LOC100131138	
Deletion Peak	chr16	16q22.2	70833950	71887380	7	35	1	6	**HYDIN**	AP1G1	CALB2	TAT	ZNF19	ZNF23
Deletion Peak	chr17	17q21.31	41063467	41197273	7	35	1	6	IFI35	RPL27	RND2	VAT1	AARSD1	RUNDC1
Deletion Peak	chr4	4p11	48900986	52715939	6	30	0	0	CWH43					
Deletion Peak	chr15	15q13.3	1	51330697	5	25	0	0	**ATP10A**	**B2M**	**BUB1B**	**C15orf2 (NPAP1)**	**C15orf23 (KNSTRN)**	**C15orf55 (NUTM1)**
Deletion Peak	chr4	4p15.2	26486910	26863840	2	25	0	0	TBC1D19					
Deletion Peak	chr6	6q22.31	119803029	129207953	2	25	0	0	**PTPRK**	**STL**	hsa-mir-588	hsa-mir-3144	FABP7	GJA1
Amplification	chr14	14q11.2	1	20400579	1	5	6	33	OR4K5	OR11H2	OR4N2	OR4K2	POTEG	OR11H12
Amplification	chr10	10q22.1	72100685	73148814	0	0	4	22	**PRF1**	EIF4EBP2	NODAL	PCBD1	SGPL1	KIAA1274
Deletion Peak	chr22	22q11.23	24314259	24408077	0	0	4	22	GSTT1	GSTT2	GSTTP1	LOC391322	GSTTP2	

SUPPLEMANTARY TABLE 8. Most frequent alterations in Polyclonal Monosegmentary Synchronous Colorectal cancers.

Change	Chr	Cytoband	start	End	q values	cases	%						
Amplification	chr7	7q36.2	154664675	155798939	7.06E-01	26	68	**DPP6**	EN2	HTR5A	INSIG1	SHH	PAXIP1
Amplification	chr7	7q36.3	158271643	158660922	1.10E-08	26	68	hsa-mir-595	PTPRN2	NCAPG2	WDR60	ESYT2	MIR595
Amplification	chr20	20q13.12	42924922	43123027	7.82E-01	26	68	HNF4A	TTPAL	FITM2	R3HDML	MIR3646	
Amplification	chr20	20q13.12	45854488	46278181	0.0019868	26	68	NCOA3	ZMYND8				
Amplification	chr20	20q13.2	52186785	52304107	7.71E-23	26	68	ZNF217					
Deletion Peak	chr18	18p11.32	809912	2548100	8.36E-10	26	68	ADCYAP1	LINC00470				
Amplification	chr20	20q13.31	56044263	56240496	4.27E-10	25	66	PCK1	PMEPA1	ZBP1	CTCFL		
Amplification	chr7	7q31.2	117064626	117372202	6.64E-06	24	63	**MET**					
Amplification	chr8	8q24.21	128999641	130128117	3.72E-05	24	63	hsa-mir-1208	hsa-mir-1207	PVT1	MIR1206	MIR1207	MIR1208
Amplification	chr8	8q24.22	133713111	134615749	1.98E-05	24	63	**NDRG1**	ST3GAL1	SLA	TG	WISP1	PHF20L1
Amplification	chr20	20p11.21	22276300	22757959	1.05E-03	24	63	FOXA2	LINC00261	LOC284788			
Amplification	chr20	20q12	39650599	39727694	1.14E-06	24	63	**TOP1**					
Amplification	chr8	8q24.3	142073847	142429472	1.56E-05	23	61	GPR20	DENND3	SLC45A4	LOC731779		
Amplification	chr20	20p11.23	19665256	20032997	2.89E-04	23	61	NAA20	CRNKL1	RIN2	SLC24A3		
Amplification	chr20	20q13.13	48792592	49133279	1.31E-03	23	61	**PTPN1**	CEBPB	LOC284751			
Deletion Peak	chr1	1p36.23	8066915	8926867	1.36E-03	23	61	**ERRFI1**	RERE	SLC45A1			
Deletion Peak	chr1	1p36.21	14105299	15489816	1.44E-05	23	61	KAZN	C1orf126				
Deletion Peak	chr1	1p35.3	29533081	31184217	8.35E-11	23	61	PTPRU					
Amplification	chr7	7p11.2	55189547	55202597	1.46E-33	22	58	**EGFR**					
Amplification	chr7	7q31.2	116409778	116458045	2.80E-11	22	58	CFTR	CTTNBP2	ASZ1			
Amplification	chr8	8q23.1	109991638	111117737	7.78E-02	22	58	**PKHD1L1**	TRHR	EBAG9	KCNV1	SYBU	ENY2
Amplification	chr12	12p13.2	11870769	12039752	4.12E-06	22	58	**ETV6**					

Amplification	chr12	12p13.1	12845688	13104999	8.72E-07	22	58	**CDKN1B**	hsa-mir-614	hsa-mir-613	GPR19	GPRC5A	DDX47
Amplification	chr12	12q13.11	48283288	48317968	1.79E-11	22	58	VDR					
Amplification	chr20	20p11.21	24994290	25339323	7.06E-01	22	58	ENTPD6	PYGB	ABHD12	VSX1	ACSS1	LOC284798
Amplification	chr20	20q13.33	60541887	61594432	0.060469	22	58	**SS18L1**	hsa-mir-133a-2	hsa-mir-3195	COL9A3	LAMA5	NTSR1
Deletion Peak	chr1	1p36.22	11340473	11721692	5.43E-17	22	58	FBXO2	PTCHD2				
Deletion Peak	chr1	1p35.1	33911648	35165737	1.62E-04	22	58	hsa-mir-552	ZSCAN20	C1orf94	CSMD2	HMGB4	LOC402779
Deletion Peak	chr1	1p32.2	57277657	59041576	0.026128	22	58	C8A	C8B	DAB1	OMA1		
Deletion Peak	chr1	1q21.2	147826659	149815535	1.67E-09	22	58	FCGR1A	HIST2H2AA3	HIST2H4A	NBPF14	FAM91A2	HIST2H3C
Amplification	chr8	8q12.2	61419729	61922458	1.38E-05	21	55	**CHD7**	RAB2A				
Amplification	chr8	8q24.3	145008707	145069677	7.37E-04	21	55	hsa-mir-661	GRINA	PLEC	PARP10	MIR661	
Amplification	chr12	12p13.31	6288835	6635273	1.05E-07	21	55	CD9	CD27	LTBR	SCNN1A	VAMP1	TNFRSF1A
Amplification	chr12	12p12.3	15786388	15956939	1.93E-02	21	55	EPS8					
Amplification	chr12	12q23.3	106903322	107159404	3.64E-06	21	55	RFX4	POLR3B				
Amplification	chr13	13q22.1	73539854	73986627	6.40E-15	21	55	**KLF5**	PIBF1				
Amplification	chr13	13q31.1	79623692	81759787	0.0038951	21	55	SPRY2	NDFIP2	RBM26	RBM26-AS1		
Amplification	chr19	19q13.11	33524070	33550588	2.78E-08	21	55	RHPN2					
Deletion Peak	chr1	1p36.32	2583273	3046462	1.51E-01	21	55	hsa-mir-4251	ACTRT2	FLJ42875	MIR4251		
Deletion Peak	chr1	1p33	49127187	49269570	0.00014111	21	55	BEND5					
Amplification	chr8	8q24.13	126172341	126409667	6.25E-08	20	53	NSMCE2					
Amplification	chr12	12p11.22	28285004	29809540	0.029524	20	53	**OVCH1**	ERGIC2	CCDC91	FAR2	TMTC1	
Amplification	chr12	12q14.1	59215534	59511652	0.023305	20	53	**LRIG3**					
Amplification	chr12	12q21.33	89702070	89938589	4.17E-02	20	53	DUSP6	GALNT4	POC1B	POC1B-GALNT4		
Amplification	chr13	13q34	110188242	110447636	8.46E-10	20	53	IRS2					
Amplification	chr20	20p12.2	10562659	11565455	0.0023524	20	53	**PLCB4**					
Deletion Peak	chr1	1p36.11	27714703	27993259	0.0001256	20	53	FGR	GPR3	WASF2	AHDC1		
Deletion Peak	chr1	1p31.3	63322562	63861012	0.04931	20	53	FOXD3	LINC00466				
Deletion Peak	chr1	1q21.1	121302698	144851598	6.68E-13	20	53	**PPIAL4G**	hsa-mir-3118-3	hsa-mir-3118-2	hsa-mir-3118-1	PPIAL4A	LOC375010

Deletion Peak	chr15	15q25.2	83732906	84327770	1.63E-05	20	53	BNC1	SH3GL3	HDGFRP3	TM6SF1	MIR4515	
Amplification	chr3	3q26.2	168837737	169096899	1.86E-03	19	50	**MECOM**					
Amplification	chr7	7q21.2	92224326	92489812	0.00061722	19	50	**CDK6**					
Amplification	chr7	7q36.1	149933601	150522564	0.024645	19	50	RARRES2	GIMAP2	TMEM176B	REPIN1	GIMAP4	GIMAP5
Amplification	chr12	12p13.33	1780566	1928288	1.48E-03	19	50	ADIPOR2	CACNA2D4				
Amplification	chr12	12p12.1	22647857	22853304	0.12792	19	50	KIAA0528	ETNK1				
Amplification	chr12	12q21.2	76064346	76454447	0.018059	19	50	NAP1L1	PHLDA1				
Amplification	chr13	13q12.12	25035608	25338131	0.0025603	19	50	PARP4	ATP12A	TPTE2P6			
Amplification	chr13	13q33.2	106659101	107272113	4.56E-06	19	50	EFNB2	ARGLU1	LINC00460			
Amplification	chr20	20p12.1	17504102	18005112	9.48E-01	19	50	BFSP1	RRBP1	DSTN	SNX5	OVOL2	C20orf72
Amplification	chr20	20q11.21	30755699	30983019	0.11002	19	50	**ASXL1**	hsa-mir-1825	PLAGL2	KIF3B	POFUT1	TSPY26P
Deletion Peak	chr1	1p36.23	6829583	7855813	7.70E-02	19	50	**CAMTA1**	VAMP3				
Deletion Peak	chr1	1p34.3	38554331	39332792	8.21E-04	19	50	RRAGC	LOC339442	MIR3659			
Deletion Peak	chr1	1p22.1	91990919	92419268	0.041298	19	50	TGFBR3	HSP90B3P				
Deletion Peak	chr1	1p21.3	95709940	98681347	0.0055657	19	50	**DPYD**	hsa-mir-137	PTBP2	MIR137HG	MIR137	FLJ31662
Deletion Peak	chr1	1p13.2	112239322	112945981	2.51E-07	19	50	KCND3	DDX20	FAM212B	LOC100506343		

SUPPLEMENTATY TABLE 9. Most frequent alterations in Polyclonal Pancolonic Synchronous Colorectal cancers.

Change	Chr	Cytoband	Start	End	q values	cases	%					
Amplification	chr8	8q24.3	142073847	142429472	1,56E-05	21	75	GPR20	DENND3	SLC45A4	LOC731779		
Amplification	chr8	8q24.13	126172341	126409667	6,25E-08	20	71	NSMCE2					
Amplification	chr8	8q24.22	133713111	134615749	1,98E-05	20	71	**NDRG1**	ST3GAL1	SLA	TG	WISP1	PHF20L1
Amplification	chr8	8q24.3	145008707	145069677	7,37E-04	20	71	hsa-mir-661	GRINA	PLEC	PARP10	MIR661	
Amplification	chr7	7p11.2	55189547	55202597	1,46E-33	19	68	**EGFR**					
Amplification	chr8	8q12.2	61419729	61922458	1,38E-05	19	68	**CHD7**	RAB2A	LOC100130298			
Amplification	chr8	8q23.1	109991638	111117737	7,78E-02	19	68	**PKHD1L1**	TRHR	EBAG9	KCNV1	SYBU	ENY2
Amplification	chr8	8q24.21	128999641	130128117	3,72E-05	19	68	hsa-mir-1208	hsa-mir-1207	PVT1	MIR1206	MIR1207	MIR1208
Amplification	chr20	20q13.31	56044263	56240496	4,27E-10	19	68	PCK1	PMEPA1	ZBP1	CTCFL		
Amplification	chr8	8q24.12	120928956	121937244	0.008956	18	64	SNTB1	COL14A1	MTBP	MRPL13	DEPTOR	
Amplification	chr20	20q12	39650599	39727694	1,14E-06	18	64	**TOP1**					
Amplification	chr20	20q13.12	45854488	46278181	0.0019868	18	64	NCOA3	ZMYND8	LOC100131496			
Amplification	chr20	20q13.13	48792592	49133279	1,31E-03	18	64	**PTPN1**	CEBPB	LOC284751			
Amplification	chr20	20q13.2	52186785	52304107	7,71E-23	18	64	ZNF217					
Amplification	chr20	20q13.33	60541887	61594432	0.060469	18	64	**SS18L1**	hsa-mir-133a-2	hsa-mir-3195	COL9A3	LAMA5	NTSR1
Amplification	chr8	8q22.2	101174187	101582666	0.0025603	17	61	SPAG1	RNF19A	ANKRD46	MIR4471		
Amplification	chr8	8q24.21	128150653	128561430	2,68E-02	17	61	POU5F1B	LOC727677				
Amplification	chr12	12p13.2	11870769	12039752	4,12E-06	17	61	**ETV6**					
Amplification	chr12	12p13.1	12845688	13104999	8,72E-07	17	61	**CDKN1B**	hsa-mir-614	hsa-mir-613	GPR19	GPRC5A	DDX47
Amplification	chr13	13q34	110188242	110447636	8,46E-10	17	61	IRS2					
Amplification	chr20	20q13.12	42924922	43123027	7,82E-01	17	61	HNF4A	TTPAL	FITM2	R3HDML	MIR3646	
Amplification	chr7	7p15.2	27168014	27250376	2,80E-11	16	57	**HOXA7**	**HOXA9**	**HOXA4**	**HOXA11**	**HOXA13**	hsa-mir-196b
Amplification	chr7	7p12.1	50571997	51393547	0.0025603	16	57	ALG10B	CPNE8				
Amplification	chr7	7q36.3	158271643	158660922	1,10E-08	16	57	hsa-mir-595	PTPRN2	NCAPG2	WDR60	ESYT2	MIR595

Amplification	chr13	13q14.11	41199532	41235807	7,50E-06	16	57	**FOXO1**					
Amplification	chr20	20p11.23	19665256	20032997	2,89E-04	16	57	NAA20	CRNKL1	RIN2	SLC24A3		
Amplification	chr20	20p11.21	22276300	22757959	1,05E-03	16	57	FOXA2	LINC00261	LOC284788			
Amplification	chr20	20p11.21	24994290	25339323	7,06E-01	16	57	ENTPD6	PYGB	ABHD12	VSX1	ACSS1	LOC284798
Amplification	chr20	20q11.21	30755699	30983019	0.11002	16	57	**ASXL1**	hsa-mir-1825	PLAGL2	KIF3B	POFUT1	TSPY26P
Amplification	chr3	3q26.31	171461612	171898475	0.0014993	15	54	PLD1	FNDC3B	TMEM212			
Amplification	chr7	7p21.1	16687543	17225606	2,27E-05	15	54	AGR2	TSPAN13	BZW2	AGR3		
Amplification	chr7	7q31.2	116409778	116458045	2,80E-11	15	54	CFTR	CTTNBP2	ASZ1			
Amplification	chr8	8q21.13	80909230	81115847	0.029524	15	54	TPD52	MRPS28				
Amplification	chr12	12p12.3	15786388	15956939	1,93E-02	15	54	EPS8					
Amplification	chr13	13q22.1	73539854	73986627	6,40E-15	15	54	**KLF5**	PIBF1				
Amplification	chr13	13q32.3	99818725	100051333	0.023305	15	54	**GPR183**	hsa-mir-623	GPR18	UBAC2	MIR623	FKSG29
Deletion Peak	chr1	1p35.3	29533081	31184217	8,35E-11	15	54	PTPRU					
Deletion Peak	chr17	17p11.2	21447016	25637342	1,05E-01	15	54	FLJ36000	MTRNR2L1	MIR4522			
Deletion Peak	chr18	18p11.32	809912	2548100	8,36E-10	15	54	ADCYAP1	LINC00470				
Amplification	chr7	7p15.3	24802000	26247113	0.019353	14	50	**HNRNPA2B1**	**NFE2L3**	hsa-mir-148a	CBX3	OSBPL3	CYCS
Amplification	chr7	7p14.3	29273987	29663844	0.033166	14	50	CHN2	PRR15				
Amplification	chr7	7p12.3	46406135	48149509	0.026086	14	50	HUS1	UPP1	TNS3	C7orf69	LINC00525	C7orf57
Amplification	chr8	8q11.23	53168383	53361210	1,97E-01	14	50	ST18					
Amplification	chr8	8q13.3	71088549	71278585	0.010927	14	50	**NCOA2**					
Amplification	chr12	12p13.31	6288835	6635273	1,05E-07	14	50	CD9	CD27	LTBR	SCNN1A	VAMP1	TNFRSF1A
Amplification	chr13	13q32.1	97841269	98151553	0.00031826	14	50	RAP2A	MBNL2				
Amplification	chr13	13q34	112666365	115169878	0.00061722	14	50	**TFDP1**	ATP4B	F7	F10	GAS6	LAMP1
Amplification	chr21	21q22.3	42811396	43938672	5,60E-12	14	50	**TMPRSS2**	MX1	TFF1	TFF2	TFF3	ABCG1
Deletion Peak	chr17	17p13.1	7529764	7571856	0.00012473	14	50	ATP1B2	SHBG				
Deletion Peak	chr18	18q21.32	56719437	57568692	0.0012857	14	50	GRP	LMAN1	RAX	SEC11C	CCBE1	CPLX4

SUPPLEMENTARY TABLE 10 Differential alterations between Segmentary and Pancolonic Polyclonal Synchronous Colorectal Cancer

					PM (n=38)		PP (n=28)							
change	**chr**	**Cytoband**	**start**	**End**	**Cases**	**%**	**Cases**	**%**						
Amplification	chr7	7q36.2	154664675	155798939	26	**68**	12	43	**DPP6**	EN2	HTR5A	INSIG1	SHH	PAXIP1
Deletion	chr1	1p36.23	8066915	8926867	23	**60**	7	25	**ERRFI1**	RERE	SLC45A1			
Deletion	chr1	1p32.2	57277657	59041576	22	**58**	7	25	C8A	C8B	DAB1	OMA1		
Deletion	chr1	1p36.11	27714703	27993259	20	**53**	7	25	FGR	GPR3	WASF2	AHDC1		
Deletion	chr1	1p31.3	63322562	63861012	20	**53**	6	21	FOXD3	LINC00466				
Deletion	chr1	1p36.23	6829583	7855813	19	**50**	7	25	**CAMTA1**	VAMP3				
Deletion	chr1	1p22.1	91990919	92419268	19	**50**	5	18	TGFBR3	HSP90B3P				
Deletion	chr1	1p21.3	95709940	98681347	19	**50**	7	25	**DPYD**	hsa-mir-137	PTBP2	MIR137HG	MIR137	FLJ31662
Amplification	chr14	14q11.2	1	20400579	17	45	6	21	OR4K5	OR11H2	OR4N2	OR4K2	POTEG	OR11H12
Deletion	chr10	10q11.22	46163328	47666436	14	37	4	14	PPYR1	GPRIN2	PTPN20B	SYT15	AGAP4	FAM21C
Deletion	chr11	11p15.5	1	295342	13	34	3	11	PSMD13	SIRT3	BET1L	RIC8A	ODF3	SCGB1C1
Deletion	chr12	12p13.1	12776974	12919324	13	34	3	11	**CDKN1B**	hsa-mir-613	GPR19	MIR613		
Deletion	chr13	13q14.11	39442549	41322731	10	26	2	7	**FOXO1**	**LHFP**	**TTL**	hsa-mir -320d-1	hsa-mir-4305	COG6
Amplification	chr5	5p15.2	14106137	15029265	5	13	11	**39**	TRIO	FAM105A	ANKH	FAM105B		MIR4637
Deletion	chr3	3p25.3	10302173	11199556	4	11	9	**32**	**SLC6A11**	hsa-mir-885	ATP2B2	SEC13	SLC6A1	GHRL
Amplification	chr5	5q23.2	127140487	127314580	4	11	9	**32**	[FLJ33630]					
Amplification	chr10	10p15.3	1	476001	4	11	11	**39**	**DIP2C**	ZMYND11	TUBB8			
Amplification	chr1	1p36.21	15455193	15779112	1	3	5	18	CTRC	TMEM51	EFHD2	FHAD1	C1orf126	