CHAPTER 7

DNA copy number profiles of primary tumors as predictors of response to chemotherapy in advanced colorectal cancer

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Abstract

Background: Colorectal cancer (CRC) is biologically a heterogeneous disease, which may affect response to drug therapy. We investigated the correlation of genome wide DNA copy number profiles of primary tumors with response to systemic chemotherapy in advanced CRC.

Material and methods: DNA was isolated from formaldehyde-fixed paraffin embedded primary tumors of 32 patients with advanced CRC, which were selected based on either a good response (n=16) or a poor response (n=16) to first-line combination therapy with capecitabine and irinotecan. High resolution DNA copy number profiles were obtained by means of 30k oligonucleotide-based array comparative genomic hybridization (aCGH).

Results: Unsupervised hierarchical cluster analysis of the aCGH data revealed two clusters of 19 and 13 tumors, respectively, and cluster membership showed a significant correlation with response status ($P<0.03$). The non-responders had fewer chromosomal alterations compared to the responders, in particular less losses were found ($P<0.03$). Most prominent differences between the two groups were losses of regions 18p11.32-q11.2 ($P<0.02$) and 18q12.1-q23 ($P<0.03$), which were more frequently observed in responders.

Conclusion: Differences in DNA copy number profiles of primary CRCs are associated with response to systemic combination chemotherapy with capecitabine and irinotecan. Responders overall had more chromosomal alterations, especially loss of chromosome 18.

Introduction

Colorectal cancer (CRC) is the second leading cause of cancer death in the western world with nearly 204.000 deaths per year in Europe [1]. Clinical outcome mainly depends on the tumor stage at time of diagnosis. In patients with advanced disease the response to systemic therapy is a major determinant of prognosis.

Approximately 50% of all CRC patients develop distant metastases and will ultimately die from the disease. In patients with advanced CRC, 5-fluorouracil (5-FU) in combination with leucovorin (LV) has been the standard drug therapy for many years. During the past decade median overall survival times have further increased by the incorporation of new cytotoxic drugs like irinotecan and oxaliplatin. Furthermore, the oral fluoropyrimidine prodrug capecitabine has been proven a useful alternative to intravenous 5-FU [2]. Recently, incorporation of the anti-angiogenic drug bevacizumab has increased survival as well [3]. While overall these new drug regimens have resulted in increased response rates and prolonged median survival, treatment strategies are still based on a "one size fits all" approach to which only a subset of patients will respond. Therefore, predictive markers are needed to identify those patients who will maximally benefit from the available treatment options [4].

For predicting response to 5-FU, most research has been focused on enzymes involved in its mechanism of action like thymidylate synthase (TS) and dihydropyrimidine dehydrogenase (DPD), and thymidine phosphorylase (TP), which plays a role in the activation of capecitabine [5-9]. Recently, selection of advanced CRC patients with a low expression of TS and DPD, doubled response rate to 5-FU/LV compared to no selection. Yet, still 60% of the patients did not respond [9]. In addition, microsatellite instability status may be a predictor of 5-FU based therapy [10].

Response to DNA-topoisomerase I inhibitor irinotecan, has been associated with expression levels of the target Top-I [11,12] and of UDP-glucuronosyltransferase (UGT1A1), which metabolizes SN-38, the active metabolite of irinotecan [13-15].

In addition to these specific enzymes, other factors are likely to influence response to therapy. CRC is biologically a heterogeneous disease, and this biological diversity can be determined at the DNA level, mRNA level and protein level. CRC also shows heterogeneous phenotypes and differences in clinical behavior, including the risk of metastasis and the response to therapy. We are just starting to understand the interactions between this biological and clinical heterogeneity. A substantial part of the biological diversity in CRC occurs at the level of chromosomes giving rise to DNA copy number alterations [16]. Moreover, recent data show that DNA copy number profiles may be indicative for the response to therapy [17-20]. The aim of the present study was to test

whether genome wide DNA copy number profiles of primary tumors can predict the response to combination therapy with capecitabine and irinotecan in advanced CRC.

Patients and Methods

Patients

Thirty-nine patients with previously untreated advanced CRC, who received first-line combination chemotherapy with capecitabine and irinotecan were selected from the series of the CAIRO study, a randomized phase III study of sequential versus combination chemotherapy of the Dutch Colorectal Cancer Group (DCCG). Details on patient eligibility, study design and results have been published [21]. The study was approved by the Central Committee of Human-related Research and by the local ethics committees of all participating centers.

Chemotherapy

Patients received capecitabine (1000 mg/m^2) twice daily for 14 days plus irinotecan (250 mg/m^2) on day 1 at intervals of 3 weeks. Tumor response was assessed by CT scan using the RECIST [22] for evaluation of response every 9 weeks, at onset of clinical signs of progression and in case of premature discontinuation of study treatment. Partial response (PR) (at least a 30% decrease in the sum of the longest diameter) or complete response (CR) (the disappearance of all lesions) was confirmed by CT scan after 4 weeks and in case of stable disease (SD) (less than a 30% reduction and less than a 20% increase in the sum of the longest diameter of all measured lesions and the appearance of no new lesions), after a period of 6 weeks. Treatment was continued until disease progression or unacceptable toxicity, whichever came first.

For the present study, best observed response was used to classify patients into two groups. Of the 39 patients, nineteen achieved complete or partial response as best response (responder group) and 20 patients had progressive disease (non-responder group). Median progression-free survival (PFS) in first-line treatment for responders was 11.6 months (range 8.9-23.2), and for non-responders 2.1 months (range 1.5-4.1).

DNA isolation

DNA from primary tumors and matched normal tissue, resected prior to chemotherapy, was isolated from formaldehyde-fixed paraffin embedded tissue, using an extensively validated protocol [16]. Tumor tissue was microdissected from marked tumor rich areas on hematoxylin stained

sections, by scraping off tumor tissue with a surgical blade, as described before. Two out of 39 samples were excluded because of tumor cell content less than 80% [23].

Array Comparative Genomic Hybridization (aCGH)

High resolution DNA copy number profiles were obtained by means of oligonucleotide based aCGH as described by van den IJssel et al. [24]. Arrays contained 60mer oligonucleotides, representing 28.830 unique genetic locations designed by Compugen (San Jose, CA, USA).

Normal DNA of the same patient was hybridized as reference for every aCGH experiment to avoid contamination of somatic DNA copy number alterations in the tumor with copy number variations and segmental duplications present in germline DNA [25], since the current high resolution arrayCGH platforms are more sensitive to this bias when (pooled) reference DNA of other sources is used, than previously used classical CGH or bacterial artificial chromosome array platforms.

aCGH data analysis

Log2 ratios (tumor/normal reference signal) of each aCGH experiment were normalized by subtraction of the mode value of the log2 ratios of all the oligonucleotides on chromosome 1-22. The quality of the DNA isolated from formalin-fixed paraffin embedded tissue may vary, which can affect the signal quality of aCGH data. Therefore, a measure of variation, the Median Absolute Deviation (MAD) value was calculated as a quality measure of the final aCGH data. In the present study 32 out of 37 experiments had MAD-values ≤0.22 and were accepted for analysis. Mean MAD value of these 32 tumors was 0.16 (range 0.10-0.22), the 5 tumors excluded had considerably higher MAD values (mean of 0.29, range 0.25-0.32) [26].

CGHcall software [27] was used for the calling of gains, high-level amplifications and losses, and converting log2 ratios into ordinal data, i.e. '+1' for gains, '+2' for high-level amplifications, '-1' for losses, and '0' if no DNA copy number alterations were present.

We used the CGHregions algorithm to reduce our dataset to chromosomal regions of losses, gains and high-level amplifications, using a threshold of 0.01 [28].

Unsupervised hierarchical cluster analysis

To analyze the distribution of whole genome DNA copy number profiles in these advanced CRC samples, unsupervised hierarchical cluster analysis was performed using Weighted Clustering of Called aCGH data (WECCA) [29].

Clustering of aCGH data as discrete levels rather than continuous log2 ratios greatly improves the sensitivity and the specificity [30]. WECCA was applied to the region data, and weight was assigned to each region, thereby determining their relative influence on the clustering. To reflect differences in the regions' coverage of the genome, we chose a region's weight depending on the number of oligonucleotides (1 = 1-50 oligonucleotides; 2 = 51-150 oligonucleotides; 3 = 151-250 oligonucleotides, until 11 = 1051-1150 oligonucleotides).

Statistical analysis

To determine differences in clinicopathological features between responder and non-responder patients, the Mann Whitney U test for comparing means of continuous variables between the two groups, and the two-sided Fisher's exact test and the Chi-square test for testing significance of differences in distribution of categorical variables were used, respectively, where applicable. Survival analysis, applied on region data, was performed by Kaplan-Meier survival analysis with log-rank testing. SPSS version 15.0 statistical software package (SPSS Inc., Chicago, IL) was used.
To calculate differences in DNA copy numbers between tumors of responders and non-responders, we performed a Wilcoxon test with ties, the p-values of which were corrected for multiple testing using a permutation version of false discovery rate (FDR). To gain statistical power and allow easier interpretation of the results, calculations were performed using regions of gains and losses (defined as described above) [28], rather than the individual 30K oligonucleotides. P-values of less than 0.05 (after FDR correction where applicable) were considered statistically significant.

Results

Patients

Patient and tumor characteristics were well balanced between 16 responders and 16 non-responders. Responder patients had a significantly better progression free survival (PFS) and overall survival (OS), both $P<0.001$ (Table 1)

Table 1. Clinical and pathologic characteristics of patients in relation to their response status

	All eligible patients (n=32)	Responders (n=16)	Nonresponders (n=16)	P value
Age, year (Mean±SD)	60.5±10.2	60.9±9.3	60.1±11.4	0.79
Sex, n (%)				
Male	21 (66%)	10 (62.5%)	11 (69%)	1.00
Female	11 (34%)	6 (37.5%)	5 (31%)	
Site of primary tumor, n (%)				
Colon	16 (50%)	9 (56%)	7 (44%)	0.50
Rectosigmoïd	7 (22%)	4 (25%)	3 (19%)	
Rectum	9 (28%)	3 (19%)	6 (37%)	
Tumor grade, n (%)				
Well differentiated	1 (3%)	1 (6%)	0 (0%)	0.59
Moderately differentiated	16 (50%)	8 (50%)	8 (50%)	
Poorly differentiated	15 (47%)	7 (44%)	8 (50%)	
Predominant localization of metastases, n (%)				
Liver	26 (81%)	14 (87.5%)	12 (75%)	0.65
Extrahepatic	6 (19%)	2 (12.5%)	4 (25%)	
Performance status, n (%)				
0-1	31 (97%)	16 (100%)	15 (94%)	1.00
2	1 (3%)	0 (0%)	1 (6%)	
Serum LDH, n (%)				
Normal	23 (72%)	12 (75%)	11(69%)	1.00
Abnormal	9 (28%)	4 (25%)	5 (31%)	
MRR status, n (%)				
pMMR	31 (97%)	16 (100%)	15 (94%)	1.00
dMMR	1 (3%)	0 (0%)	1 (6%)	
Survival, months (Median)				
PFS	7	13	2	<0.001
OS	17	32	8	<0.001

LDH, lactate dehydrogenase; pMMR, proficient mismatch repair system; dMMR, deficient mismatch repair system; PFS, progression free survival; OS, overall survival

Unsupervised hierarchical cluster analysis divides tumors into two clusters

Unsupervised hierarchical cluster analysis of the aCGH data revealed two clusters of 19 and 13 tumors. Thirteen out of 19 tumors of cluster 1 consisted of non-responders, while 10 of 13 tumors of cluster 2 were responders (Figure 1). Cluster membership showed a significant correlation with response status ($P<0.03$).

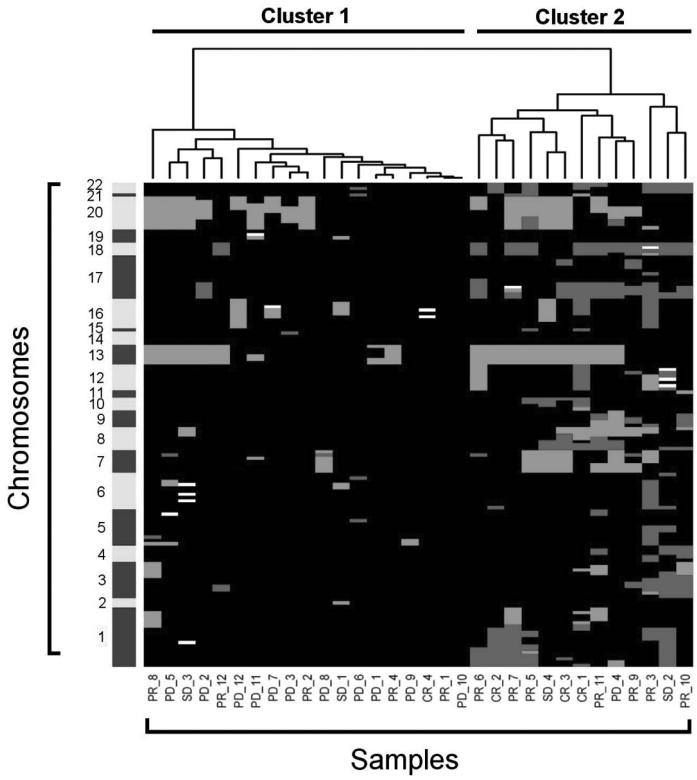

Figure 1. Unsupervised hierarchical cluster analysis of 32 advanced CRC samples, based on chromosomal gains and losses detected by aCGH. Rows represent chromosome 1 till 22 (different chromosomes are indicated by alternating blue and yellow colors), and every column represents a separate tumor. Green cells represent gains, red cells represent losses, white cells represent amplifications, and black cells indicate no abnormality. Two clusters emerged, cluster 1 containing mainly non-responders and cluster 2 responders.

Differences in DNA copy number profiles between responders and non-responders

The average number of chromosomal alterations per tumor was 6.3 (median 5.0, range 0-19), with a mean number of 3.2 gains (median 3.0, range 0-9) and 3.2 losses (median 1.5, range 0-12).

Less alterations were observed in the 16 non-responders ($P<0.2$) compared to the 16 responders, especially for losses ($P<0.03$). The median number of chromosomal alterations of the 16 responders was 7.0 (range 1-19), with a median number of 3.0 gains (range 1-8) and 3.5 losses (range 0-12). For the 16 non-responders the median chromosomal alterations was 4.0 (range 0-14), with a median

number of 2.5 gains (range 0-9) and 0 losses (range 0-12). The number of chromosomal alterations per response status (CR, PR, and PD) is presented in Table 2. Eight tumors (3 responders and 5 non-responders) had high-level amplifications, which were distributed over the whole genome (Table 3).

Table 2. Chromosomal alterations of the 32 tumors according to response status

Complete response (n=4)				
	Total	**Mean**	**Median**	**Range**
Total	33	8.3	6.0	2-19
Losses	19	4.8	3.5	0-12
Gains	14	3.5	3.0	1-7
Ampl.	2	0.5	0.0	0-2
Partial response (n=12)				
	Total	**Mean**	**Median**	**Range**
Total	93	7.8	7.0	1-18
Losses	53	4.4	3.5	0-12
Gains	40	3.3	3.0	1-8
Ampl.	3	0.3	0.0	0-2
Progressive disease (n=16)				
	Total	**Mean**	**Median**	**Range**
Total	77	4.8	4.0	0-14
Losses	29	1.8	0.0	0-12
Gains	48	3.0	2.5	0-9
Ampl.	10	0.6	0.0	0-5

Ampl., high-level amplifications

In figure 2 frequencies of gains and losses per oligonucleotide are plotted. Most frequently observed chromosomal alterations (i.e. >30%) in the 16 responders were loss of 1p36.33-36.23 (31%), 3p21.31 (31%), 17p13.3-p11.2 (50%), 18p (50%), 18q (56%) and 22q13.2-q13.33 (38%), and gain of 8q12.2-q24.13 (31%), 8q24.13-q24.3 (38%), 13q (63%), 20p13-p12.1 (31%), 20q11.21-q13.32 (50%) and 20q13.33 (44%). For the 16 non-responders the most frequent alterations were gain of 13q (median of 38%), 20q11.21-q13.32 (56%) and 20q13.33 (38%).

Table 3. High-level amplifications per tumor in three responder patients and five nonresponder patients

Samples	Regions	Amplicon size (Mb)
Responders		
CR-4	16p11.2	0.46
	16q12.1	0.61
PR-3	8q24.3	0.23
	18q21.1	0.61
PR-7	17p11.2	1.24
Nonresponders		
PD-5	5q35	0.12
PD-7	16q12.2-13	3.25
PD-11	19q13.1-13.2	3.10
PD-14	12p13.3	2.45
	12p13.3	0.13
PD-15	1p34	4.23
	6p21.3	0.23
	6p21.3	0.72
	6p21.1	2.59
	13q12	2.85

CR, complete response; PR, partial response; PD, progressive disease

The 16 non-responders had more gain of the regions 16p11.2-q12.1 and 16q12.2, while the 16 responders had more loss of the same regions (P=0.03). However, the FDR value of 0.31 for both regions on chromosome 16 indicates that the relevance of this finding should be interpreted with caution (Table 4). Loss of whole chromosome 18 was significantly more frequent in the 16 responders. Loss of the region 18p11.32-q11.2 was observed in 8 responders, whereas in non-responders a loss was found only once (P<0.02, FDR=0.06). Loss of 18q12.1-q23 was observed in 9 responders, while in the group of non-responders 2 losses of this region were found (P<0.03, FDR=0.06).

Given the fact that these patients were treated with DNA-topoisomerase I inhibitor irinotecan, the response status was checked against the called DNA copy number ratio for *TOP1* on chromosome 20q. Responders and non-responders did not show significant differences in frequency of DNA copy number changes of *TOP1*. Gain of *TOP1* was found in 8 responders and in 9 non-responders. Exact DNA copy number ratios for *UGT1A1*, an enzyme that metabolizes the active metabolite of irinotecan SN-38, were not available because no oligonucleotide for this gene was spotted on the array. DNA copy number changes of the oligonucleotides flanking the locus of *UGT1A1* on chromosome 2q37 were uncommon overall. Only one patient from the responder group showed a gain of this region.

Table 4. Chromosomal areas which are significantly different between responder and nonresponder patients.

Region	Size (Mb)	Responder (n)			Nonresponder (n)			Difference between responder and nonresponder	
		Gain	Ampl.	Loss	Gain	Ampl.	Loss	P value	FDR
16p11.2-16q12.1	19.1	0	0	2	4	0	0	0.032	0.31
16q12.2	0.4	0	0	2	3	1	0	0.032	0.31
18p11.32-18q11.2	22.5	1	0	8	0	0	1	**0.015**	**0.06**
18q12.1-23	52.2	0	0	9	0	0	2	**0.024**	**0.06**

Most significant regions, based on the criteria of P<0.05 and low FDR value, are indicated in bold. Significance of the regions 16p11.2-16q12.1and 16q12.2 was reached only when the data were dichotomized in 'gains' and 'losses'. For the regions 18p11.32-18q11.2 and 18q12.1-23, data were dichotomized in 'losses' and 'no losses'.
Amp, high-level amplification; FDR, false discovery rate.

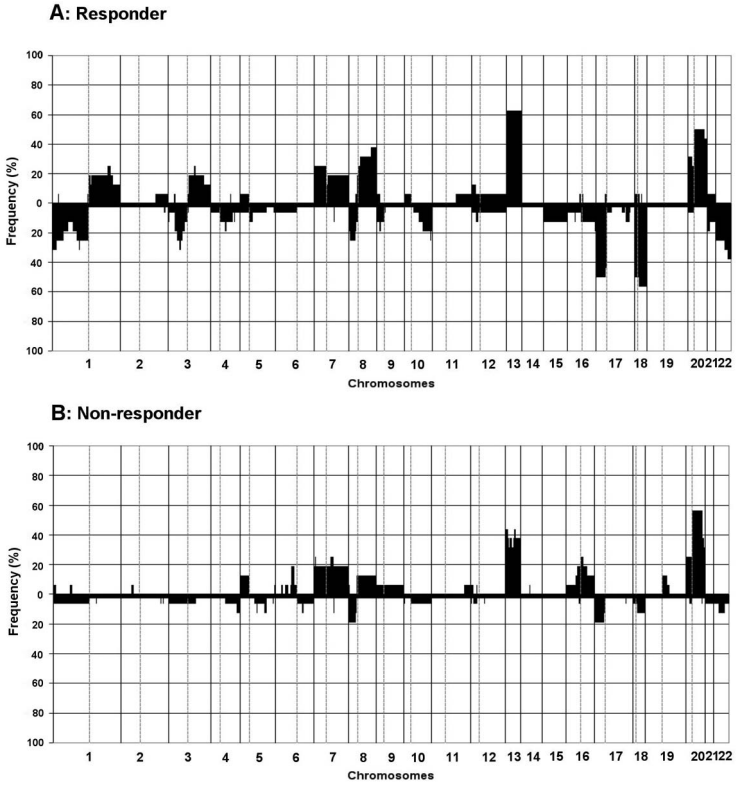

Figure 2. Frequency plot of DNA copy number gains and losses throughout the genome in **A**) 16 responders and **B**) 16 nonresponders. X-axis displays clones spotted on the array sorted by chromosome and basepare position. Y-axis displays frequency of tumors with gains (above zero) or losses (below zero). The boundaries of individual chromosomes are indicated by black vertical lines and location of centromeres are delineated by grey-dotted vertical lines.

Survival analysis

Patients who showed a good response to chemotherapy had a significantly better PFS and OS than patients that did not respond to treatment, both P<0.001 (Figure 3A en 3B). Comparing chromosomal alterations of all 32 CRCs with the overall survival status of the patients showed that tumors with loss of 1p36 or gain of 13q have a significantly better overall survival than tumors without these alterations. On chromosome 1p36 loss of four regions, 1p36.33-p36.32, 1p36.32-p36.23, 1p36.22-p36.21 and 1p36.13-p36.11 had a significantly better overall survival, respectively P < 0.05, P < 0.01, P < 0.05 and P < 0.05 (Figure 3C and 3D). The three regions with a significantly better overall survival distributed over whole chromosome 13q were 13q12.11, 13q13.1-q14.3 and 13q14.3-q34, with a P-value of P < 0.03, P = 0.02 and P < 0.03, respectively (Figure 3E and 3F). Table 5 shows the size of these regions and the number of oligonucleotides per region.

Table 5. Chromosomal alterations which give a better overall survival, when present in the tumor.

	Region	Size (Mb)	Oligonucleotides (n)	P value
Loss of 1p36	1p36.33-32	0.54	15	<0.05
	1p36.32-23	6.16	55	<0.01
	1p36.22-21	6.65	76	<0.05
	1p36.13-11	9.53	139	<0.05
Gain of 13q	13q12.11	3.91	27	<0.03
	13q13.1-14.3	21.81	176	0.02
	13q14.3-34	61.23	230	<0.03

Mb, Megabase

The most frequently observed alterations in the responders, loss of regions 18p11.32-q11.2 and 18q12.1-q23, had no significant impact on PFS (P<0.06 and P=0.11 respectively) or OS (P=0.11 and P=0.35 respectively).

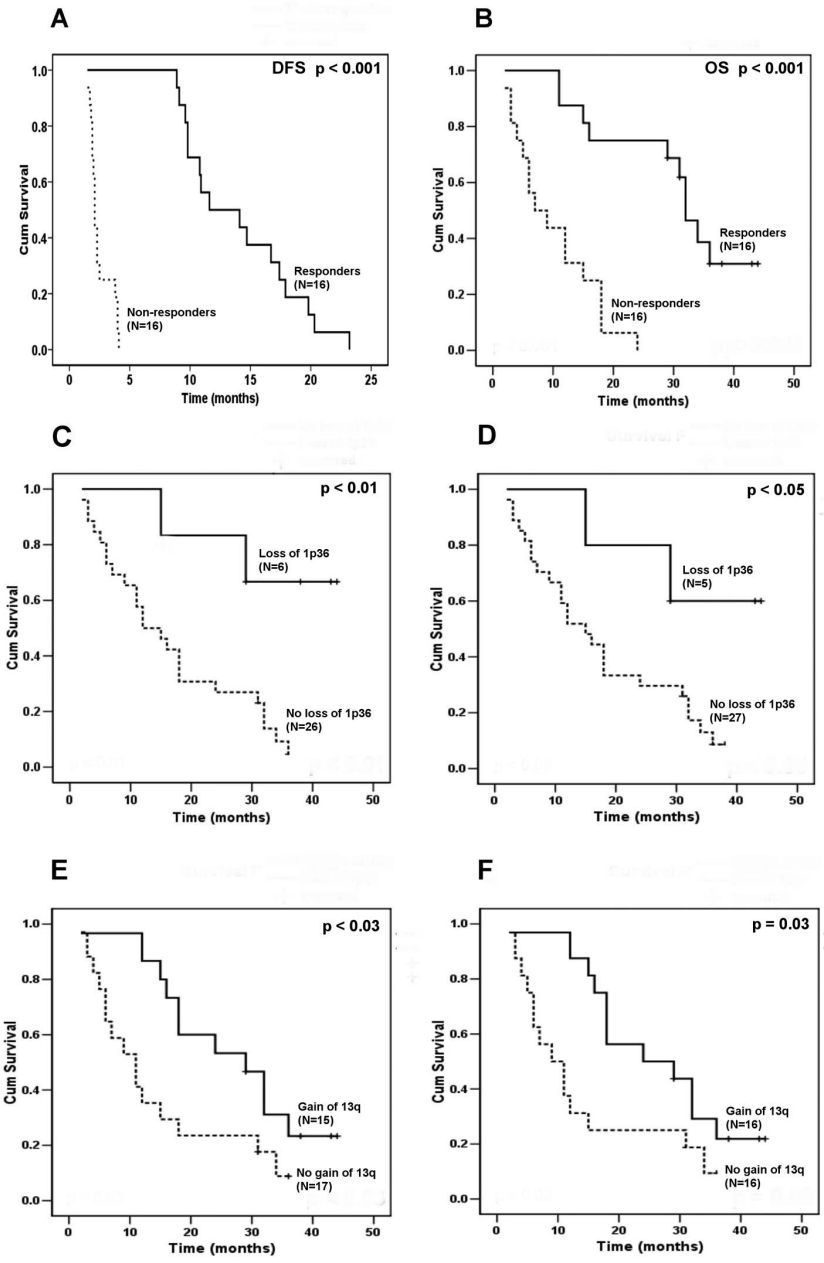

Figure 3. Kaplan-Meier **s**urvival analysis of 32 patients with advanced CRC. Patients with a good response to combination therapy with capecitabine and irinotecan had a significant better progression free survival (PFS) **A**) and overall survival (OS) than non-responders **B**). Cumulative survival curves **C** till **F** show the chromosomal alterations that gave a significantly better overall survival: loss of 1p36.23-p36.32 **C**), loss of 1p36.11-p36.13, 1p36.21-p36.22 and 1p36.32-p36.33 had the same survival curve **D**), gain of 13q13.1-q14.3 **E**), and also gain of 13q12.11 and 13q14.3-q34 had the same survival curve **F**).

Discussion

We demonstrate that genome wide DNA copy number profiles of primary tumors of advanced CRC patients significantly correlate with response to combination therapy with capecitabine and irinotecan. Unsupervised hierarchical cluster analysis of the tumors yielded two clusters, one of which contained mainly non-responders and the other cluster responders. Although, unsupervised hierarchical cluster analysis in a small dataset like the present harbors the risk of false positive findings, these results indicate that advanced CRC patients are a heterogeneous group at the level of genome wide DNA copy number status, and that these differences are relevant for the response to chemotherapy. A large scale validation study is presently ongoing. This will also allow working out in more detail which candidate genes at loci with discriminating DNA copy number alterations are causally involved in the response to drug therapy. A further point to be kept in mind is that the current study design does not allow to discriminate between DNA copy number alterations that provide an a priori better prognosis in patients with advanced CRC and DNA copy number alterations that reflect a better responsiveness of tumor cells to the drug therapy given. To this end, a control arm would be required that has not received any drug therapy at all, and the CAIRO study did not contain such a control arm.

Analysis of the specific DNA copy number alterations that differ between responders and non-responders may unravel the biology behind the response phenotype. In the present series we did not find any specific narrow DNA copy number alterations that could point to a single specific gene involved. However, the DNA copy number alterations that differ considerably between both response phenotypes involve large alterations, such as loss of chromosome 18, which was significantly more present in the responders. Furthermore, tumors with loss of 1p36 or gain of 13q are associated with a significantly better overall survival compared with tumors without these alterations. This suggests that if gene dosage effects influence response to drug therapy, this probably will be caused by altered expression of multiple genes rather than one or a few individual genes. There is ample evidence that low level chromosomal gains changes the expression of many genes at these altered loci [26,31]. This may complicate the attempts to unravel the mechanisms involved, but may yield novel biomarkers, which will help to explain the effects of the drugs. Chromosomal alterations have given more insight in the process of tumor progression, e.g. chromosomal gain of 13q, loss of 1p36 and 18q, and especially the tumor suppressor genes on 18q, *DCC* and *Smad4* (*DPC4*) have been widely studied in colorectal carcinogenesis. *DCC* at 18q21.2 is lost in 50% to 70% of CRCs and plays a role in apoptosis induction [32,33] and *Smad4* (*DPC4*) at 18q21 encodes intracellular transducers of the transforming growth factor-β (TGF-β) apoptosis pathway. In adjuvant 5-FU based chemotherapy, retention of heterozygosity of 18q and positive

expression of DCC are predictors of positive outcome [32,34-36]. This is in contrast to the role in treatment of advanced disease such as in the present study, where we show that loss of 18q is associated with the response phenotype to therapy. A possible explanation for this paradox is that the predictive effect of 18q loss in adjuvant setting is blurred by the prognostic effect of these alterations which is independent of adjuvant therapy.

TYMS the gene encoding TS, is located on 18p11.32 and low TS expression levels in metastatic CRC are associated with better response to 5-FU based therapy [5,8,9]. Our observations, that advanced CRC patients with loss of the region 18p11.32-q11.2, that harbors the *TYMS* gene, had a better response to combined therapy with capecitabine and irinotecan seems consistent with these results, because loss of *TYMS* may give low TS expression levels. Wang and colleagues describe that *TYMS* gene amplification is responsible for 5-FU resistance. Amplification of the *TYMS* gene was found in metastases of advanced CRC patients only after treatment with 5-FU and these patients had a significantly worse overall survival in comparison to patients without this amplification [18]. This suggests that tumors with loss of 18p11.32 could have a good response to capecitabine treatment because no *TYMS* gene amplification was possible.

Loss of 1p36 has been found to be associated with a lower percentage of stroma in CRC [37], while CRCs with a lower percentage of stroma have also been found to have a better prognosis [38]. However, loss of 1p36 has also been correlated with poor prognosis [39,40] and metastasis [41]. In our series of CRCs, loss of 1p36 was found in 19% of all primary tumors and the overall survival in patients with tumors showing a loss of four regions, 1p36.11-p36.13, 1p36.21-p36.22, 1p36.23-p36.32 and 1p36.32-p36.33, was significantly better compared to patients with tumors without these alterations. In particular, the frequencies of 1p36 deletion in responders and non-responders were 31% and 6%, respectively.

To our knowledge, a correlation between 13q gain and survival has not been presented before.

Del Rio and colleagues have found a gene expression signature of 14 genes in advanced CRC patients that may be of value in the prediction of the response to chemotherapy [42]. However, these data mainly concerned genes on other chromosomal regions than found in the present study. Their results were based on a relatively small sample size (n=21) and this gene expression signature has to be still validated and improved with a larger cohort of patients. Moreover, overexpression of the 14 genes found by Del Rio et al. could be due to other mechanisms than DNA copy number changes.

Although the aim of the present study rather was meant to provide proof of concept that differences in genome wide DNA copy number profiles are associated with response to systemic therapy in advanced CRC, it is tempting to obtain an impression of the diagnostic potential of such markers. Therefore, sensitivity, specificity and predictive value, based on the current data were

computed for loss of 18p11.32-q11.2, loss of 18q12.1-q23, and cluster membership, acknowledging the limitations of this exercise (Table 6). Loss of 18p11.32-q11.2 and 18q12.1-q23 as markers of response to combined capecitabine and irinotecan had sensitivities of 50% and 56%, respectively, and specificities of 94% and 88%, respectively. Positive predictive values for response were 89% and 82%, respectively, and negative predictive values were 65% and 67%, respectively. When cluster membership was taken as test, sensitivity and specificity for were 63% and 81%, respectively with positive and negative predictive values for response of 77% and 68%, respectively.

In conclusion, in this pilot study genome wide DNA copy number profiling of primary tumors of advanced CRC patients revealed genomic loci, of which the copy number status may serve as predictive markers of response to systemic chemotherapy. Tumors of patients with a good response to chemotherapy had an increased number of chromosomal alterations, in particular loss of regions 18p11.32-q11.2 and 18q12.1-q23. A large scale validation study, also using a higher resolution aCGH platform, is presently ongoing and will learn whether the findings of the present study can be of actual value in the selection of patients for chemotherapy.

Table 6. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for response to combined capecitabine and irinotecan therapy in a selected series of 32 advanced colorectal cancers, based on DNA copy number variables.

	Responders		Nonresponders					
	Present	**Absent**	**Present**	**Absent**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
Cluster 2 membership	10	6	3	13	0.63	0.81	0.77	0.68
Loss of 18p11.32-q11.2	8	8	1	15	0.5	0.94	0.89	0.65
Loss of 18q12.1-q23	9	7	2	14	0.56	0.88	0.82	0.67

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