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Review

A systematic review and meta-analysis of the relationship between chromosome 18q genotype, DCC status and colorectal cancer prognosis

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Abstract

Results from studies investigating the relationship between colorectal cancer survival and chromosome 18q allelic imbalance (AI)/loss of DCC expression (LOE) have been inconsistent. We have reviewed and pooled published studies to estimate the prognostic significance of chromosome 18q status more precisely. Data from 27 studies were eligible. Survival data were pooled using standard meta-analysis techniques. Considerable variation between assessment method, marker choice, and threshold for assigning AI/LOE was observed. Pooling data from a 2189 cases from 17 studies showed significantly worse overall survival in patients with AI/LOE (HR = 2.00, 95%CI: 1.49–2.69), maintained both in the adjuvant setting (HR = 1.69, 95%CI:1.13–2.54), and also by method (HR = 1.67, 95%CI: 1.19–2.36, genotyping microsatellites; HR = 3.00, 95%CI: 1.98–4.56, immunohistochemistry). There was however evidence of heterogeneity and publication bias. Cancers with chromosome 18q loss appear to have a poorer prognosis. Prospective studies using consistent methodology are needed to precisely quantify its effect and role in patients with stage II–III disease.

Keywords: Allelic imbalance; Chromosome 18q; Colorectal cancer; DCC; Meta-analysis

1. Introduction

Colorectal cancer (CRC) is one of the most common malignancies in developed countries [1]. In North America, it is the most common cause of cancer-related mortality in non-smoking men and in non-smoking women it is the second common cause of cancer [2]. Although prognosis has improved over the past three decades and a greater proportion of patients now present with potentially curable disease [3], the outlook for most patients still remains relatively poor.

Molecular analyses have shown that the natural history of all CRCs is not the same [4]. Whilst a small proportion of CRCs develop from the microsatellite-instability (MSI) (chromosome-stable) pathway, many develop from the chromosomal-instability (microsatellite-stable) pathway and are characterised by aneuploidy, allelic losses, amplifications and translocations [5]. Although CRC prognosis is stage and grade dependent, cancers with similar clinico-pathological features may show significant differences in outcome. This is likely in part to be due to underlying molecular heterogeneity. Identifying molecular markers of prognosis as an adjunct to traditional staging systems is clearly highly advantageous, and has not surprisingly been an area of active research in recent years [6].

One of the most promising markers studied to date, observed in up to 70% of CRCs, is chromosome 18q loss [7]. This has been evaluated by a number of methods, including loss of heterozygosity (LOH)/allelic imbalance

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(AI), and *DCC* gene expression. *DCC* maps to chromosome 18q21 and is a key gene involved in colorectal carcinogenesis [8] and the primary candidate for the biological effect of chromosome 18q AI [9,10]. Chromosomal loss at this region is thought to result in haploinsufficiency at *DCC* and therefore reduced protein expression.

A number of studies have investigated the relationship between chromosome 18q AI, DCC expression and prognosis in CRC. Although many have reported a poorer survival with these phenotypes, estimates of the prognostic value have differed considerably between studies, as have the methods used. To gain a better insight into the value of chromosome 18q AI and loss of DCC expression (LOE) as prognostic markers we have undertaken a systematic review of published studies and used standard meta-analysis techniques as per Cochrane [11] and QUOROM [12] guidelines to derive a more precise estimate of the prognostic significance of this phenotype.

2. Materials and methods

2.1. Eligibility criteria

Studies were eligible for pooling if survival was assessed in CRC patients stratified by chromosome 18q genotype or DCC expression status. The primary outcomes of interest were overall survival (OS) and disease-free survival (DFS). Only studies providing information on survival were included. Studies assessing the relationship between chromosome 18q markers and stage were not eligible. Care was taken to account for overlapping and duplicated datasets.

2.2. Identification of studies

Studies were identified using the PubMed electronic database (http://www.pubmed.com) until 26/09/2004. The search strategy included the keywords ''colon cancer'', ''rectal cancer'', ''colorectal cancer'', ''chromosome 18'', ''loss of heterozygosity'', ''LOH'', ''DCC'', ''allele'', and ''imbalance''. All studies matching eligibility criteria were retrieved and bibliographies checked for relevant publications. Review articles and bibliographies of other relevant studies identified were hand-searched to identify additional studies. Only published studies in peer-reviewed journals were included. Unpublished data were not sought. Data from abstracts and letters were not sought. Pharmaceutical industries and authors were not contacted.

2.3. Statistical analysis

The association between chromosome 18q marker, OS and DFS was derived as a weighted average of study-specific estimates of the hazard ratio (HR), using inverse variance weights [11]. The logHR and corresponding standard error were used as data points for the meta-analysis. In studies not quoting the HR or 95% confidence interval (CIs), where possible, these were calculated from data presented using a hierarchical series of steps as per Parmar and colleagues [13]. Data points were calculated from two of the following parameters: the HR point estimate, the logrank statistic or its *P*-value, the O–E statistic (difference between numbers observed and expected events) or its variance. If these were unavailable, the total number of events, the number of patients at risk in each group and the logrank statistic or P-values, were used to derive an estimate of the HR. Studies in which insufficient data was presented for data point extraction were included for systematic review but excluded from meta-analysis. This applied to 10 studies [14–23] and the stage II CRC data set from Watanabe and colleagues [24] (12 data sets in total) all of which were based on small sample sizes (median 56, range 24(18)–121(22)), with eight [15–19,22–24] reporting non-significant outcomes, and four [14,20,21] significant outcomes, all but one of which [20] favoured AI/DCC LOE as a marker of poor prognosis. The study by Barratt and colleagues [25] presented survival outcomes by AI at two microsatellite markers separately. Pooling was performed with data from each marker used separately to assess the impact of each on the pooled HR estimate. The marker resulting in the most conservative pooled HR was used for subsequent analyses.

Studies were searched for, identified, and eligibility confirmed independently, by both authors. Authors extracted the data independently of each other, and disagreements were resolved by discussion. Characteristics of the studies were extracted from published articles and summarised in a consistent manner to aid comparison. Allele imbalance and expression status was dichotomised using data and groupings provided in contributing studies. Markers used for genotyping were mapped in relation to *DCC* using the May 2004 (hg17) assembly of the human genome as implemented in the UCSC Genome Browser (http://genome.ucsc.edu/cgi-bin/hgGateway).

Significance values quoted in papers as less than the specified threshold were assumed to be at that threshold, resulting in conservative estimates of significance levels. Hazard ratios obtained after adjusting for other covariates were used when provided. Meta-analysis was performed using the random-effects model. This assumes that studies were a random sample of a hypothetical population of studies taking into account variability within and between studies [26]. Heterogeneity (p_{het}) was formally tested using Cochran's Q statistic [27]. Specific analyses considering confounding factors were not possible as raw data was not available.

Evidence of publication bias was examined by constructing forest plots of HRs [28]. Studies were plotted

in order of decreasing variance of the logHR. Horizontal lines represent 95% confidence intervals. Each box represents the HR point estimate and its area is proportional to the weight of the study. The diamond (and broken line) represents the overall summary estimate, with CIs given by its width. The unbroken vertical line is at the null value (HR = 1.0). Estimates from small studies that have less precision in estimating the underlying HR will therefore scatter widely. In the absence of publication bias the plot resembles a symmetrical inverted funnel. Publication bias was also formally assessed by the Egger [28] and Begg methods [29] and its effect assessed by the trim-and-fill method of Duval and Tweedie [30]. Statistical computations were undertaken using STATA version 7.0 (Stata Corporation, College Av. TX 77840, USA).

3. Results

3.1. Eligible studies

Thirty-eight potentially eligible studies were identified and retrieved [10,14–25,31–55] (Fig. 1). Studies by Watanabe and colleagues [24] and Choi and colleagues [14] presented survival data on patients with stage II and III CRC separately, and each of these were treated as separate data sets.

Six of the 38 retrieved studies were not relevant and therefore excluded: four did not assess survival [41,47,48,53], one did not assess chromosome 18q [51], and one did not assess AI [34]. Of the remaining 32 potentially eligible studies, five were ineligible – three did not assess survival by chromosome 18q AI alone [43,52,54], and two reported overlapping/duplicated datasets [32,45]. Specifically, the data set from Bardi and colleagues [32] overlapped with and was updated by Bardi and colleagues [21]; the latter being used as the primary reference. The study by Offerhaus and colleagues [45] overlapped with Kern and colleagues [40], with the latter used as the primary reference since this allowed accurate data point evaluation and a larger sample size. There were therefore 27 eligible studies (29 data sets) assessing survival by chromosome 18q AI/DCC expression eligible for systematic review and meta-analysis.

3.2. Study characteristics

Characteristics of the eligible studies are summarised in Table 1. Study design pertaining to time frame of molecular data analysis with respect to survival analysis

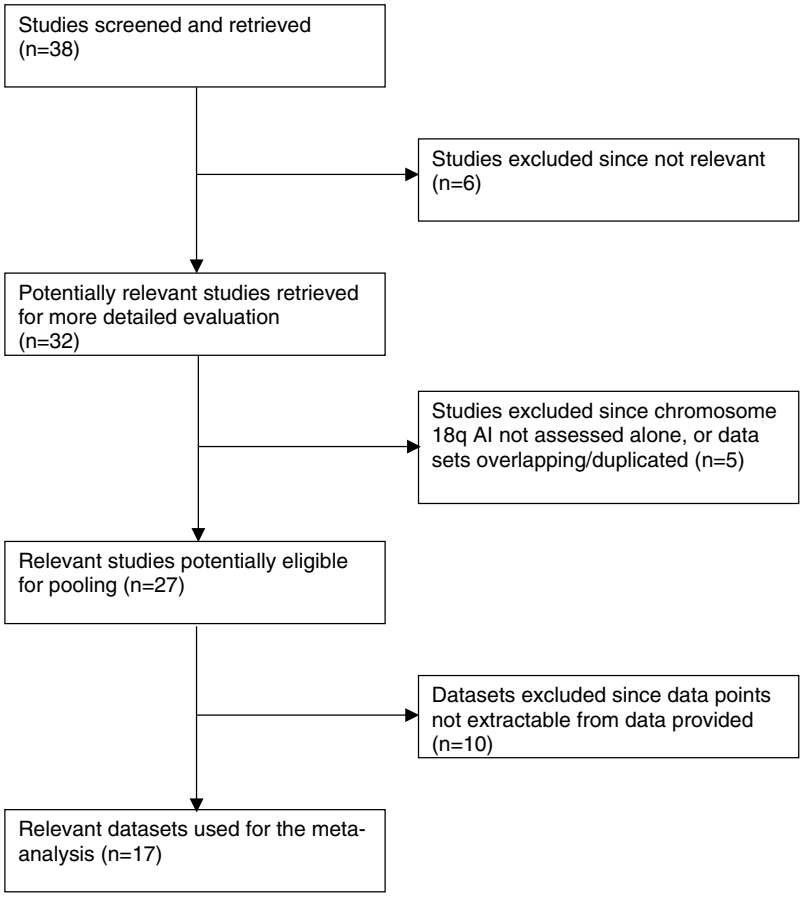

Fig. 1. Flow of studies as per QUORUM guidelines.

Table 1
Summary of studies of allele imbalance/DCC loss of expression and colorectal cancer prognosis

Study	Study details				Assessment of AI/DCC			
	Clinical trial	Stage	Site	Sample size[a]	Method	Lesion evaluated	No. AI/DCC LOE	%
Jen *et al.* [10]	No	II–III	Colorectal	145	Genotyping	Primary	90	67
Choi *et al.* [14]	No	II	Colorectal	53	Genotyping	Primary	39	74
Choi *et al.* [14]	No	III	Colorectal	63	Genotyping	Primary	54	86
Rooney *et al.* [15]	No	III	Colorectal	29	CGH	Primary	9	31
Lindforss *et al.* [16]	No	II–III	Colorectal	47	Genotyping	Primary	47	78
Cohn *et al.* [17]	Yes	I–IV	Colorectal	33	Southern analysis	Primary	8	24
Gerdes *et al.* [18]	No	I–IV	NR	24	Southern analysis	Primary	15	63
Laurent-Puig *et al.* [19]	No	I–IV	Colorectal	109	Southern analysis	NR	NR	NR
Knosel *et al.* [20]	No	I–IV	Colorectal	37	CGH	Primary, lymph-node, or metastasis	NR	NR
Bardi *et al.* [21]	No	I–IV	Colorectal	115	Karyotyping	Primary	29	25
Martinez-Lopez *et al.* [22]	No	I–III	Colorectal	121	Genotyping	Primary	54	45
Font *et al.* [23]	No	I–III	Colorectal	77	Genotyping	Primary	25	39
Watanabe *et al.* [24]	Yes	II	Colorectal	58	Genotyping	Primary	155	57
Watanabe *et al.* [24]	Yes	III	Colorectal	221	Genotyping	Primary	155	57
Barratt *et al.* [25]	Yes	II–III	Colonic	108	Genotyping	Primary	144	63
Carethers *et al.* [33]	No	II	Colorectal	70	Genotyping	Primary	30	43
Aschele *et al.* [31]	Yes	IV	Colorectal	42	Immunohistochemistry	Primary, lymph-node, or metastasis	23	55
De Angelis *et al.* [35]	No	I–IV	Colorectal	67	CGH	Primary	37	55
Diep *et al.* [36]	No	I–IV	Colorectal	184	Genotyping	Primary	129	70
Dix *et al.* [37]	No	II–III	Colorectal	55	Southern analysis	Primary	19	34
Halling *et al.* [38]	Yes	II–III	Colorectal	386	Genotyping	Primary	82	21
Jernvall *et al.* [39]	No	I–IV	Colorectal	195	Genotyping	Primary	101	52
Kern *et al.* [40]	No	I–III	Colorectal	48	Southern analysis	Primary	35	73
Lanza *et al.* [42]	No	II–III	Colonic	112	Genotyping	Primary	60	54
O'Connell *et al.* [44]	No	II–III	Colorectal	59	Southern analysis	Primary	20	34
Ogunbuyi *et al.* [46]	No	I–III	Colonic	126	Genotyping	Primary	67	53
Saito *et al.* [49]	No	I–IV	Colorectal	146	Immunohistochemistry	Primary	81	56
Shibata *et al.* [50]	No	II–III	Colorectal	132	Immunohistochemistry	Primary	66	50
Zauber *et al.* [55]	No	II	Colorectal	93	Genotyping	Primary	67	69

AI, allelic imbalance; CGH, comparative genomic hybridisation; LOE, loss of expression; NR, not reported.
[a] Assessed for survival.

was only reported in three of the 27 studies, two of which were performed prospectively [17,19], and one retrospectively [20]. In the remainder, design was not explicitly reported, and a retrospective design was assumed. Only seven of the eligible studies reporting molecular analyses were performed blinded to clinical data [24,25,31,33,40,46,50] and in the remainder (20/27), studies were either not blinded or status was not specifically reported, resulting in the potential for bias. Patient ascertainment between the eligible studies varied with only a minority (5/27, 19%) [17,24,25,31,38] based on tumours from unselected patients enrolled in clinical trials. The majority of studies assessed tumours from patients not involved in such studies and may have been open to selection bias.

Site of primary tumour was relatively consistent between the eligible studies, with most (23/27, 85%) investigating patients with either colonic or rectal cancers. Only three studies were site specific, assessing patients with colonic disease [25,42,46] and no studies investigated patients with only rectal disease (Table 1). Stage of disease differed between the contributing studies. Tumours from patients with localized or locally advanced CRC (stage I, II, III or II–III) represented the largest group (17/27, 63%) [10,14–16,22–25,33,37,38,40,42,44,46,50,55], with three of these involving patients treated in the context of clinical trials [24,25,38]. The majority of these stage I–III studies (13/17, 76%) assessed patients potentially suitable for adjuvant chemotherapy (stage II or III CRC) [10,14–16,24,25,33,37,38,42,44,50,55] and of these, only three investigated trial patients [24,25,38], with one investigating colonic disease [25], and two CRC [24,38]. Of the remaining seven datasets, all but one investigated patients with any CRC stage (stages I–IV) [17–21,35,36,39,49], and one was based on only stage IV patients [31] (Table 1).

3.3. Molecular methods

Five methods (immunohistochemistry, karyotyping, and assessment of AI by comparative genomic hybridisation (CGH), Southern analysis or genotyping microsatellites) were used to assess AI/DCC LOE prior to correlating with survival outcomes (Table 1). All but one of the 27 eligible studies [19] reported source of tumour tissue assessed, and of the remainder all but two [20,31] were based on analysis of primary resected tumour. These used either primary tumour, lymph node, or metastasis for immunohistochemistry [31] or CGH [20].

Three studies assessed DCC expression by immunohistochemistry [31,49,50]. All used different antibodies; two used different monoclonal antibodies [31,49], and one a polyclonal [50]. Expression was dichotomised on the basis of either presence or absence of staining in two studies [49,50] or a 10% threshold of immunoreactivity [31]. Whilst two studies assessed expression in primary tumour [49,50] and one on either primary tumour, associated lymph node, or metastasis [31], DCC LOE occurred at similar rates between the three studies (range 50(50)–56%(49), Table 1). Only one of the eligible studies investigated DCC expression and AI by microsatellite genotyping [49], with survival analysis based on DCC expression. In this study, reduced DCC expression was observed in all 16 samples with chromosome 18q AI but no further details about the relationship between AI and DCC expression were reported.

One study was based on karyotype of resected primary tumour, using standard cytogenetic methods [21]. Although the survival impact of a number of chromosomal aberrations were assessed, data was only presented for loss of whole chromosomes, and specific survival correlates for sub-bands was not reported.

Of the remaining 23 studies, all assessed chromosome 18q AI; six by Southern analysis [17–19,37,40,44], three by CGH [15,20,35], and 14 by genotyping microsatellite markers [10,14,16,22–25,33,36,38,39,42,46,55]. Most (18/23, 78%) reported microdissecting tissue to enrich for tumour, and 12 [10,14,15,17,20,22,23,25,38,40,44,46] of these reported the minimum threshold of tumour required for analysis (median 68%, range 50(15;25)–80(17)%).

The six studies based on Southern blot analysis [17–19,37,40,44] were all published prior to 1998, after which time genotyping microsatellites became more common. Only three of the studies reported the genomic regions that probes mapped to: chromosome 18q21 (17), 18q21.2 (19), and 18q22.2 (18). Frequency of chromosome 18q AI was reported in five of the six studies (median 34%, range 24(17)–73%(40)).

Of the three studies based on CGH [15,20,35] all but that by Knosel and colleagues [20] were based on analysis of primary tumour, with the latter based on analysis of either primary, associated lymph node, or metastasis. Thresholds used to assign AI varied between the studies. Two studies [15,35] used a 0.15 difference in fluorescence ratio (FR), whereas the study by Knosel [20], used a threshold resulting in a significance of >99% on *t*-testing. Rate of chromosome 18q AI was only reported in two of the three studies [15,35] (median 43%). Although the chromosome 18q regions tested for survival were not fully reported, regions correlating significantly with survival in two of the studies were reported: chromosome 18qcen-21 (35), and 18q11.2 (20).

The most common method used to assess AI, implemented in fourteen studies [10,14,16,22–25,33,36,38,39,42,46,55] was by genotyping microsatellite markers. All of these studies genotyped primary resected tumour. All but one [24] reported markers genotyped, and in this study the marker list was not available from the publisher's web-site. All markers reported in the 13 studies mapped to chromosome 18q. The median number of

markers assessed was three (range 2(16;25;36;42;55)–7(39)). All but one study [36] genotyped dinucleotide markers, and this was the only study to be based solely on analysis of non-dinucleotide (tetranucleotide) markers. The distribution and choice of markers varied between studies. Markers mapped to a region spanning 43.6 megabases (Mb) on chromosome 18q. The median spread of markers genotyped was 31.5 Mb (range 4.6(42)–43.6(14;38)), and in the majority of studies (9/13, 69%) markers mapped to a region that encompassed *DCC* [14,16,22,23,33,36,38,39,46] with a median span of 35.9 Mb (range 13.2(33)–43.6(14;38)). The remaining four studies [10,25,42,55] genotyped markers mapping telomeric to *DCC*, and in these the median distance of the nearest marker from *DCC* was 10.1 Mb (range 0.2(25)–17.5(42)), whilst the furthest was 22 Mb (range 17.5(25)–22.1(42)).

Most of the microsatellite-based studies (9/14, 64%) did not specify the number of markers displaying LOH required to define the tumour as having AI and in these AI is likely to have been defined on the basis of LOH observed in at least one of the markers tested. The marker threshold was, however, defined in the remaining five studies [14,25,33,39,46], and was either LOH in at least one marker [25,33,46], two or more markers [39], or five markers [14]. The reduction in intensity of heterozygous electrophoretograms observed required to define AI was reported in all but one [55] of the genotyping studies, and ranged from 33% (46) to 75% (36) (median 55%).

The presence of MSI as a potential confounder to the interpretation of AI due to the presence of new somatic alleles was acknowledged in most (8/14, 57%) of the genotyping studies, with five assigning these tumours as non-assessable for AI [22,25,38,39,46], and three defining them as having no AI [10,24,42]. However, five studies did not take account of MSI [14,16,23,33,55] and one [36] did not report the potential for this confounding.

The median frequency of chromosome 18q AI observed in the 14 microsatellite-based studies, was 56% (range 21% (38)–74 (14)%). When assessed by CGH, Southern analysis, or microsatellite genotyping, the median rate of chromosome 18q AI observed was 54%, with no significant difference between the three groups ($P = 0.41$). This rate of AI observed was similar to that of DCC LOE (median 55%).

3.4. Relationship between AI, DCC expression and survival

Seventeen datasets from 17 studies [10,24,25,31,33,35–40,42,44,46,49,50,55], presented survival data in a suitable format for data point extraction (Fig. 1). Datasets were based on patients treated from seven countries. All but two [44,46] of the studies assessed OS, and over half (12/17, 71%) provided estimates of either the HR associated with AI/DCC LOE and/or its associated CI (Table 2). These were calculated from information presented in the remainder.

Sample sizes assessed for survival ranged from 42 (31) to 386 (38) (median 112) patients. This resulted in OS data from a total of 2189 patients and DFS data from 683 patients available for pooling. Median follow-up was only presented by just over half (9/17, 53%) [25,31,33,37,38,40,42,50,55] of the studies (range 19 (37)–101 (38), median 72 months).

Table 2
Results of survival analyses by individual study

Study	Overall survival			Disease-free survival		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Jen *et al.* [10]	2.83^{U}	1.32–6.08	0.008	–	–	–
Watanabe *et al.* [24]*	2.04^{M}	1.3–3.2	0.002	–	–	–
Barratt *et al.* [25]**	0.86^{NR}	0.57–1.28	0.45	–	–	–
Aschele *et al.* [31]	2.16^{M}	1.04–4.49	0.04	–	–	–
Carethers *et al.* [33]	1.22^{M}	0.32–4.72	0.84	–	–	–
De Angelis *et al.* [35]	3.32^{U}	1.31–8.42	0.011	–	–	–
Diep *et al.* [36]	2.7^{U}	1.33–5.48	0.006	–	–	–
Dix *et al.* [37]	NR	NR	NR	–	–	–
Halling *et al.* [38]	1.2^{M}	0.81–1.76	0.37	1.25^{M}	0.81–1.91	0.32
Jernvall *et al.* [39]	NR	NR	NR	–	–	–
Kern *et al.* [40]	4.17^{M}	NR	0.025	–	–	–
Lanza *et al.* [42]	7.13^{M}	2.1–23.9	<0.001	5.76^{M}	2.2–15	<0.001
O'Connell *et al.* [44]	–	–	–	5.42^{U}	0.68–3.37	NR
Ogunbuyi *et al.* [46]	–	–	–	2^{M}	1.26–3.86	0.003
Saito *et al.* [49]	4.42^{M}	1.81–10.9	0.001	–	–	–
Shibata *et al.* [50]	3.155^{M}	1.7–5.852	<0.001	–	–	–
Zauber *et al.* [55]	0.91^{M}	NR	0.86	–	–	–

Hazard ratios (HRs) and associated 95% confidence intervals (CIs) are given as quoted unless stated otherwise. –, not performed; * cohort of stage III tumours; ** data associated with D18S61; CI, confidence interval; HR, hazard ratio; M, multivariate analysis; NR, not reported; U, univariate analysis.

Table 2 details results of survival analyses performed by each of the eligible studies, and Fig. 2 shows a plot of HR(s) and associated 95% CIs for OS from each study, stratified by method of chromosome 18q assessment. Of the 2189 patients with OS data 1206 had AI/DCC LOE and the pooled HR across all studies was 2.00 (95%CI: 1.49–2.69; $P_{het} < 0.0001$). Although there was evidence of publication bias ($P = 0.05$, Egger's test), tumours with AI/DCC LOE maintained a poorer OS (HR = 1.63, 95%CI: 1.21–2.20) after adjustment [30].

These results were obtained using survival data at the microsatellite marker D18S61 from Barratt and colleagues [25]. Substituting this with OS data at D18S851 had no effect on the pooled HR (HR = 2.04, 95%CI: 1.58–2.64; $P_{het} = 0.01$). Survival data associated with D18S61 was used as the primary data set for all further analyses in place of that at D18S851 since the study-specific HR from the former was more conservative (Table 2).

When stratified by assessment method, genotyping microsatellites and immunohistochemistry, both were associated with a significant poorer prognosis (HR = 1.67, 95%CI: 1.19–2.36 and HR = 3.00, 95%CI: 1.98–4.56, respectively). There was, however, significant evidence of heterogeneity within the sub-groups ($P = 0.013$). The impact of other methodologies could not be assessed due to sample sizes in studies based on alternate technologies.

Nine data sets provided OS information on 1322 patients with locally advanced (stage II or III) CRC [10,24,25,33,37,38,42,50,55], of which 713 had AI/DCC LOE. Pooling OS data from these studies confirmed a poorer prognosis (pooled HR = 1.69, 95%CI: 1.13–2.54;$P_{het} = 0.001$). The prognostic utility of this chromosome 18q phenotype for patients with stage IV was difficult to assess since although five studies presented survival data for cohorts that included stage IV disease [31,35,36,39,49], only one [31] investigated patients with stage IV disease exclusively. Pooling OS data from a total of 634 patients (at least 107 with stage IV CRC) across these five studies, of which 371 had AI/DCC LOE, the pooled HR for OS was 2.34 (95%CI: 1.64–3.34; $P_{het} = 0.24$).

Fig. 3 shows the relative chromosomal position of markers assessed in studies suitable for OS meta-analysis. To investigate the role of marker position with respect to *DCC*, OS data was pooled from eight studies [31,33,36–39,49,50] that assessed markers that either encompassed or assessed *DCC* directly. Based on 1210 patients of which 531 had AI/DCC LOE, the poor OS in tumours with AI/DCC LOE was maintained (HR = 1.95, 95%CI: 1.40–2.72; $P_{het} = 0.05$). This was also observed when pooling OS data from 458 patients from four studies [10,25,42,55] that reported markers not encompassing *DCC*, AI/DCC

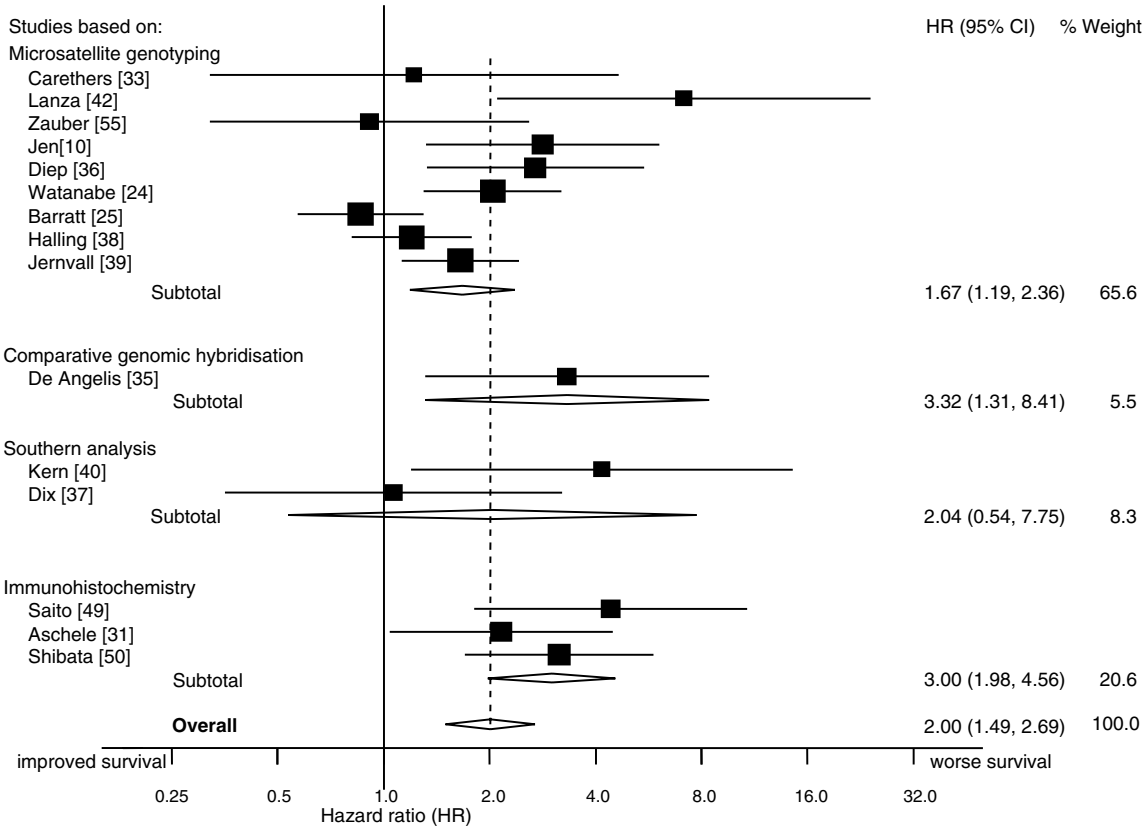

Fig. 2. Forest plots of hazard ratios (HRs) of overall survival (OS) from all eligible studies, stratified by method of assessment.

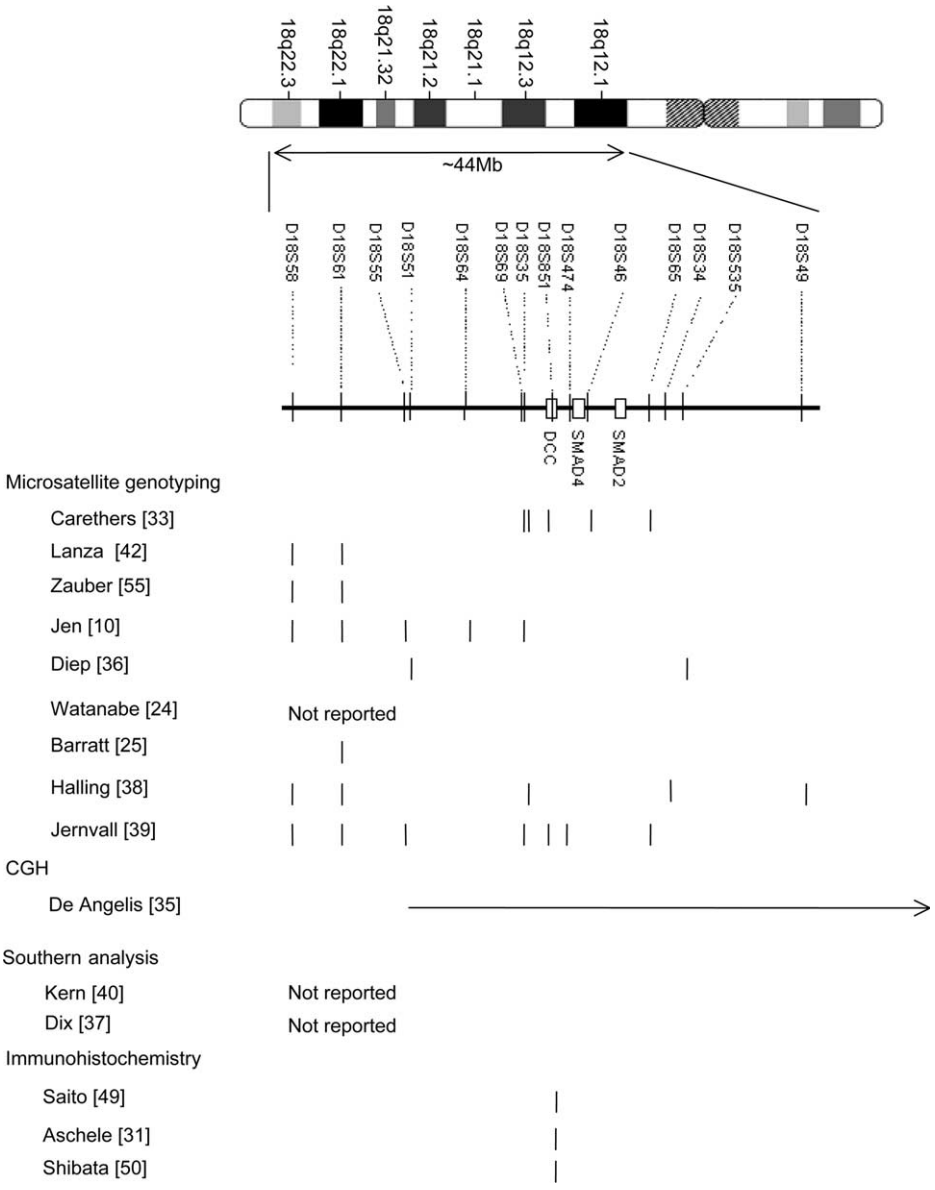

Fig. 3. Chromosome 18q schematic indicating position of markers assessed relative to major genes from studies assessing overall survival (OS). CGH, comparative genomic hybridisation.

(HR = 1.84, 95%CI: 0.74–4.58; $P_{het} = 0.001$), although this did not reach statistical significance.

All of the four studies that assessed DFS [38,42,44,46] presented the HR and its corresponding 95% CI (Table 2). All assessed chromosome 18q AI, three by genotyping microsatellite markers [38,42,46], and one by Southern analysis [44]. This provided DFS data on 683 patients of which 229 tumours had AI/DCC LOE. As seen for OS, a poorer DFS was observed for chromosome 18q AI (HR = 2.30, 95%CI: 1.21–4.39; $P_{het} = 0.02$). Again, this result should be interpreted with caution due to significant heterogeneity. Subset analysis of DFS data was not performed due to paucity of data.

4. Discussion

The molecular profile of CRCs is likely to be a determinant of clinical outcome. This is particularly important in locally advanced disease, where the identification of patients most likely to benefit from adjuvant chemotherapy is of direct clinical relevance. A proportion of patients with stage II and III disease can be cured by surgery alone and do not derive any benefit from adjuvant therapy [56]. The identification of robust molecular prognostic markers to supplement conventional pathological staging systems is therefore highly desirable. The notion that chromosome 18q AI/DCC LOE is a determinant of CRC prognosis is eminently

plausible given the differences between the biology of chromosomally stable and unstable cancers.

We sought to determine a better estimate of the prognostic impact of this phenotype by pooling data from published studies. A systematic review process was adopted in ascertaining studies, thereby avoiding selection bias on the basis of study quality. Although different methods were used to assess chromosome 18q status, estimates of the frequency of AI were similar across all methods including rate of DCC LOE. Seventeen studies provided suitable data for meta-analysis and using these, pooled estimates of outcome of CRCs supported the notion chromosome 18q AI/DCC LOE is predictive for poor survival.

The value of AI/DCC LOE in predicting poor OS was maintained in patients with stage II/III CRC, indicating its value in potentially stratifying these patients by need for or type of adjuvant therapy. Pooling data for patients with only stage II or only stage III CRC was not possible due to paucity of studies.

The poor prognosis associated with this chromosome 18q phenotype was maintained both in studies assessing *DCC* directly or genotyping encompassing microsatellites, and in those in which markers mapped to a genomic region excluding *DCC*, indicating that the poor prognosis observed may not be entirely due to *DCC*. However, this latter result should be interpreted with caution considering the small number of contributing studies, and heterogeneity observed.

Whilst CRCs with chromosome 18q AI/DCC LOE seem to be associated with a poorer prognosis, one caveat to this conclusion is that there was significant evidence for heterogeneity between studies. The impact of heterogeneity between studies on summary estimates of the relative hazard is difficult to assess. Given the differences in study groups (*e.g.*, stage, site of malignancy), some degree of heterogeneity may be expected. Some of the heterogeneity observed is also likely to reflect differences in methodology and criteria used to assign AI or expression. Although a number of methods were used to assess AI, there was little consistency in criteria both in terms of marker choice on chromosome 18q, and thresholds required to determine AI *per se*. In expression studies, antibodies varied as did expression thresholds. Methods utilising microsatellite genotyping gave no information on homozygous loci. Moreover, the functional interpretation of such genotypes relies on the assumption of a diploid genome, a state not typical for these tumours [57].

Whether by expression analysis or assessment of chromosome 18q AI, the optimal method of assessing this phenotype is unclear at present. Methods based on genotype have been widely published and allow assessment of a larger genomic region than immunohistochemistry. However, the widespread use of AI assessment in routine diagnostic laboratories has limited applicability, since these methods require specialist equipment, may require use of radioactivity, and can be difficult to interpret. Assessment of AI is therefore likely to be only available at central laboratories, and currently remains primarily a research tool. Although an immunohistochemical-based method of assessing DCC expression remains by far the most practical, both in terms of cost and labour intensiveness, antibodies to DCC have only recently become commercially available, and assignment of expression is currently semi-quantitative. Moreover, only few studies based on limited number of patients have used this method. For any further studies, the method used to assess AI/DCC is critical, and this report highlights the urgent need for standardization prior to incorporation into prospective clinical studies.

Publication bias is a major concern in all forms of meta-analysis, as studies reporting significant or positive findings are more likely to be published than those reporting non-significant results [28]. Indeed, it is not unusual for small-sized early studies to report a positive relationship or large effect that subsequent much larger studies fail to replicate. In the present study, although there was some evidence for publication bias, AI/DCC LOE still maintained prognostic utility after correction.

As with any systematic review further sources of bias may have impacted on these results. Another potential source of bias is whether all relevant studies have been identified. Whilst a small number of part-published studies may have been omitted, we believe that we have identified all key published analyses. Furthermore, we believe we have avoided bias in selecting studies by adopting rigid inclusion criteria, thereby avoiding selection bias on the basis of study quality.

Although reports were not quality scored for inclusion [58,59] it is clear that some studies were not optimally designed. A number of the larger studies show less of an effect of AI/DCC LOE on prognosis, and it is noteworthy that many of the published studies reviewed here have insufficient power to detect an association if the true HR is small. Notwithstanding the above, studies in this meta-analysis provide data on over 2000 CRC patients, of which approximately 1200 had chromosome 18q AI/DCC LOE and support the tenet that AI/DCC expression is a determinant of prognosis in CRC. This effect was observed in both studies genotyping microsatellites and assessing DCC expression, was not influenced by genomic position of marker, and maintained in the adjuvant setting. Based on pooled findings AI/DCC LOE is associated with $\sim 50\%$ or worse prognosis compared to tumours chromosomally stable or with normal DCC expression.

Although the biology of chromosomally unstable tumours is distinct from other CRCs, the precise mechanism of exactly how chromosome 18q AI/DCC LOE affords a poorer prognosis is not obvious. Moreover, it

remains unclear whether this prognosis is specific to chromosome 18q AI, or other genomic regions. Our overview of the data would suggest that the genomic region around and including *DCC* plays a major role in prognosis, consistent with recent data indicating that *DCC* acts as a conditional tumour suppressor [60]. The role of *DCC* in colorectal carcinogenesis may, however, be relatively minor, compared to the tumour suppressor *SMAD4*, which maps approximately 1Mbp telomeric to *DCC*. In this context, loss of DCC expression as defined by the contributing studies as either complete [49,50] or partial [90%, (31)] expression loss, may in fact be a measure of other phenomena, such as loss of SMAD4 expression through common genomic loss. Such a notion would be supported by the observation that studies genotyping markers not encompassing *DCC* still demonstrated poorer survival in tumours with AI. A role for *DCC* over and above that of *SMAD4* as the mechanism for poorer survival in cancers with chromosome 18q AI remains putative. It also remains unclear whether chromosome 18q AI specifically confers a deleterious outcome per se or is in fact merely a marker for a polyploid karyotype, or mismatch repair proficient cancers. Studies investigating tumours with non-18q chromosomal aberrations have also reported deleterious outcomes, particularly in CRCs with aberrations at more than one site [14,15,35,36,40].

Whatever the mechanism, our findings indicate that chromosome 18q AI/DCC status has the potential to define a group of patients who may benefit from adjuvant chemotherapy following potentially curative surgery. However, validation in the context of prospective clinical trials using consistent methodology is required before introducing assessment of this phenotype routinely into CRC patient management strategies.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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References

1. GLOBOCAN 2000: cancer incidence, mortality and prevalence worldwide, Version 1.0. Lyon: IARCPress; 2001.
2. American Cancer Society caner facts and figures 2004. 2004. Internet Communication.
3. Rougier P, Mitry E. Epidemiology, treatment and chemoprevention in colorectal cancer. *Ann Oncol* 2003, **14**(suppl. 2), ii3–5.
4. Ilyas M, Straub J, Tomlinson IP, *et al*. Genetic pathways in colorectal and other cancers. *Eur J Cancer* 1999, **35**(3), 335–351.
5. Cottrell S, Bicknell D, Kaklamanis L, *et al*. Molecular analysis of APC mutations in familial adenomatous polyposis and sporadic colon carcinomas. *Lancet* 1992, **340**(8820), 626–630.
6. Bendardaf R, Lamlum H, Pyrhonen S. Prognostic and predictive molecular markers in colorectal carcinoma. *Anticancer Res* 2004, **24**(4), 2519–2530.
7. Vogelstein B, Fearon ER, Hamilton SR, *et al*. Genetic alterations during colorectal-tumor development. *N Engl J Med* 1988, **319**(9), 525–532.
8. Fearon ER, Cho KR, Nigro JM, *et al*. Identification of a chromosome 18q gene that is altered in colorectal cancers. *Science* 1990, **247**(4938), 49–56.
9. Zetter BR. Adhesion molecules in tumor metastasis. *Semin Cancer Biol* 1993, **4**(4), 219–229.
10. Jen J, Kim H, Piantadosi S, *et al*. Allelic loss of chromosome 18q and prognosis in colorectal cancer. *N Engl J Med* 1994, **331**(4), 213–221.
11. Alderson P, Green S, Higgins JPT. *Cochrane Reviewers' Handbook 4.2.2. The Cochrane Library*. Chichester, John Wiley & Sons, Ltd, 2004.
12. Moher D, Cook DJ, Eastwood S, *et al*. Improving the quality of reports of meta-analyses of randomised controlled trials: the QUOROM statement. Quality of reporting of meta-analyses. *Lancet* 1999, **354**(9193), 1896–1900.
13. Parmar MK, Torri V, Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. *Stat Med* 1998, **17**(24), 2815–2834.
14. Choi SW, Lee KJ, Bae YA, *et al*. Genetic classification of colorectal cancer based on chromosomal loss and microsatellite instability predicts survival. *Clin Cancer Res* 2002, **8**(7), 2311–2322.
15. Rooney PH, Boonsong A, McKay JA, *et al*. Colorectal cancer genomics: evidence for multiple genotypes which influence survival. *Br J Cancer* 2001, **85**(10), 1492–1498.
16. Lindforss U, Fredholm H, Papadogiannakis N, *et al*. Allelic loss is heterogeneous throughout the tumor in colorectal carcinoma. *Cancer* 2000, **88**(12), 2661–2667.
17. Cohn KH, Ornstein DL, Wang F, *et al*. The significance of allelic deletions and aneuploidy in colorectal carcinoma. Results of a 5-year follow-up study. *Cancer* 1997, **79**(2), 233–244.
18. Gerdes H, Chen Q, Elahi AH, *et al*. Recurrent deletions involving chromosomes 1, 5, 17, and 18 in colorectal carcinoma: possible role in biological and clinical behavior of tumors. *Anticancer Res* 1995, **15**(1), 13–24.
19. Laurent-Puig P, Olschwang S, Delattre O, *et al*. Survival and acquired genetic alterations in colorectal cancer. *Gastroenterology* 1992, **102**(4 Pt 1), 1136–1141.
20. Knosel T, Schluns K, Stein U, *et al*. Genetic imbalances with impact on survival in colorectal cancer patients. *Histopathology* 2003, **43**(4), 323–331.
21. Bardi G, Fenger C, Johansson B, *et al*. Tumor karyotype predicts clinical outcome in colorectal cancer patients. *J Clin Oncol* 2004, **22**(13), 2623–2634.
22. Martinez-Lopez E, Abad A, Font A, *et al*. Allelic loss on chromosome 18q as a prognostic marker in stage II colorectal cancer. *Gastroenterology* 1998, **114**(6), 1180–1187.
23. Font A, Abad A, Monzo M, *et al*. Prognostic value of K-ras mutations and allelic imbalance on chromosome 18q in patients with resected colorectal cancer. *Dis Colon Rectum* 2001, **44**(4), 549–557.
24. Watanabe T, Wu TT, Catalano PJ, *et al*. Molecular predictors of survival after adjuvant chemotherapy for colon cancer. *N Engl J Med* 2001, **344**(16), 1196–1206.

25. Barratt PL, Seymour MT, Stenning SP, *et al*. DNA markers predicting benefit from adjuvant fluorouracil in patients with colon cancer: a molecular study. *Lancet* 2002, **360**(9343), 1381–1391.
26. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986, **7**(3), 177–188.
27. Cochran WG. The combination of estimates from different experiments. *Biometrics* 1954, **10**, 101–129.
28. Egger M, Davey SG, Schneider M, *et al*. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997, **315**(7109), 629–634.
29. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994, **50**(4), 1088–1101.
30. Duval S, Tweedie R. Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics* 2000, **56**(2), 455–463.
31. Aschele C, Debernardis D, Lonardi S, *et al*. Deleted in colon cancer protein expression in colorectal cancer metastases: a major predictor of survival in patients with unresectable metastatic disease receiving palliative fluorouracil-based chemotherapy. *J Clin Oncol* 2004, **22**(18), 3758–3765.
32. Bardi G, Johansson B, Pandis N, *et al*. Cytogenetic aberrations in colorectal adenocarcinomas and their correlation with clinicopathologic features. *Cancer* 1993, **71**(2), 306–314.
33. Carethers JM, Hawn MT, Greenson JK, *et al*. Prognostic significance of allelic lost at chromosome 18q21 for stage II colorectal cancer. *Gastroenterology* 1998, **114**(6), 1188–1195.
34. Curran B, Lenehan K, Mulcahy H, *et al*. Replication error phenotype, clinicopathological variables, and patient outcome in Dukes' B stage II (T3,N0,M0) colorectal cancer. *Gut* 2000, **46**(2), 200–204.
35. De Angelis PM, Stokke T, Beigi M, *et al*. Prognostic significance of recurrent chromosomal aberrations detected by comparative genomic hybridization in sporadic colorectal cancer. *Int J Colorectal Dis* 2001, **16**(1), 38–45.
36. Diep CB, Thorstensen L, Meling GI, *et al*. Genetic tumor markers with prognostic impact in Dukes' stages B and C colorectal cancer patients. *J Clin Oncol* 2003, **21**(5), 820–829.
37. Dix BR, Robbins P, Soong R, *et al*. The common molecular genetic alterations in Dukes' B and C colorectal carcinomas are not short-term prognostic indicators of survival. *Int J Cancer* 1994, **59**(6), 747–751.
38. Halling KC, French AJ, McDonnell SK, *et al*. Microsatellite instability and 8p allelic imbalance in stage B2 and C colorectal cancers. *J Natl Cancer Inst* 1999, **91**(15), 1295–1303.
39. Jernvall P, Makinen MJ, Karttunen TJ, *et al*. Microsatellite instability: impact on cancer progression in proximal and distal colorectal cancers. *Eur J Cancer* 1999, **35**(2), 197–201.
40. Kern SE, Fearon ER, Tersmette KW, *et al*. Clinical and pathological associations with allelic loss in colorectal carcinoma corrected. *JAMA* 1989, **261**(21), 3099–3103.
41. Kochhar R, Halling KC, McDonnell S, *et al*. Allelic imbalance and microsatellite instability in resected Duke's D colorectal cancer. *Diagn Mol Pathol* 1997, **6**(2), 78–84.
42. Lanza G, Matteuzzi M, Gafa R, *et al*. Chromosome 18q allelic loss and prognosis in stage II and III colon cancer. *Int J Cancer* 1998, **79**(4), 390–395.
43. Massa MJ, Iniesta P, Gonzalez-Quevedo R, *et al*. Differential prognosis of replication error phenotype and loss of heterozygosity in sporadic colorectal cancer. *Eur J Cancer* 1999, **35**(12), 1676–1682.
44. O'Connell MJ, Schaid DJ, Ganju V, *et al*. Current status of adjuvant chemotherapy for colorectal cancer. Can molecular markers play a role in predicting prognosis? *Cancer* 1992, **70**(6 Suppl), 1732–1739.
45. Offerhaus GJ, De Feyter EP, Cornelisse CJ, *et al*. The relationship of DNA aneuploidy to molecular genetic alterations in colorectal carcinoma. *Gastroenterology* 1992, **102**(5), 1612–1619.
46. Ogunbiyi OA, Goodfellow PJ, Herfarth K, *et al*. Confirmation that chromosome 18q allelic loss in colon cancer is a prognostic indicator. *J Clin Oncol* 1998, **16**(2), 427–433.
47. Ookawa K, Sakamoto M, Hirohashi S, *et al*. Concordant p53 and DCC alterations and allelic losses on chromosomes 13q and 14q associated with liver metastases of colorectal carcinoma. *Int J Cancer* 1993, **53**(3), 382–387.
48. Peng Z, Zhang F, Zhou C, *et al*. Genome-wide search for loss of heterozygosity in Chinese patients with sporadic colorectal cancer. *Int J Gastrointest Cancer* 2003, **34**(1), 39–48.
49. Saito M, Yamaguchi A, Goi T, *et al*. Expression of DCC protein in colorectal tumors and its relationship to tumor progression and metastasis. *Oncology* 1999, **56**(2), 134–141.
50. Shibata D, Reale MA, Lavin P, *et al*. The DCC protein and prognosis in colorectal cancer. *N Engl J Med* 1996, **335**(23), 1727–1732.
51. Slebos RJ, Baas IO, Clement M, *et al*. Clinical and pathological associations with p53 tumour-suppressor gene mutations and expression of p21WAF1/Cip1 in colorectal carcinoma. *Br J Cancer* 1996, **74**(2), 165–171.
52. Vogelstein B, Fearon ER, Kern SE, *et al*. Allelotype of colorectal carcinomas. *Science* 1989, **244**(4901), 207–211.
53. Young J, Buttenshaw R, Butterworth L, *et al*. Association of the SS genotype of the L-myc gene and loss of 18q sequences with a worse clinical prognosis in colorectal cancers. *Oncogene* 1994, **9**(4), 1053–1056.
54. Zhou W, Goodman SN, Galizia G, *et al*. Counting alleles to predict recurrence of early-stage colorectal cancers. *Lancet* 2002, **359**(9302), 219–225.
55. Zauber NP, Wang C, Lee PS, *et al*. Ki-ras gene mutations, LOH of the APC and DCC genes, and microsatellite instability in primary colorectal carcinoma are not associated with micrometastases in pericolonic lymph nodes or with patients' survival. *J Clin Pathol* 2004, **57**(9), 938–942.
56. Benson III AB, Schrag D, Somerfield MR, *et al*. American Society of Clinical Oncology recommendations on adjuvant chemotherapy for stage II colon cancer. *J Clin Oncol*. 2004, 22, 3408–3419.
57. Tomlinson IP, Lambros MB, Roylance RR. Loss of heterozygosity analysis: practically and conceptually flawed? *Gen Chromosom Cancer* 2002, **34**(4), 349–353.
58. Dickersin K, Berlin JA. Meta-analysis: state-of-the-science. *Epidemiol Rev* 1992, **14**, 154–176.
59. Sacks HS, Berrier J, Reitman D, *et al*. Meta-analyses of randomized controlled trials. *N Engl J Med* 1987, **316**(8), 450–455.
60. Mazelin L, Bernet A, Bonod-Bidaud C, *et al*. Netrin-1 controls colorectal tumorigenesis by regulating apoptosis. *Nature* 2004, **431**(7004), 80–84.