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Research paper

Biomarker concordance between primary colorectal cancer and its metastases

D.S. Bhullar [1], J. Barriuso [1], S. Mullamitha, M.P. Saunders, S.T. O'Dwyer, O. Aziz *

Colorectal & Peritoneal Oncology Centre, The Christie NHS Foundation Trust, Manchester, UK
Division of Cancer Sciences, School of Medical Science, Faculty of Biology, Medicine and Health, University of Manchester, UK

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ABSTRACT

Background: The use of biomarkers to target anti-EGFR treatments for metastatic colorectal cancer (CRC) is well-established, requiring molecular analysis of primary or metastatic biopsies. We aim to review concordance between primary CRC and its metastatic sites.
Methods: A systematic review and meta-analysis of all published studies (1991–2018) reporting on biomarker concordance between primary CRC and its metastatic site(s) was undertaken according to PRISMA guidelines using several medical databases. Studies without matched samples or using peripheral blood for biomarker analysis were excluded.
Findings: 61 studies including 3565 patient samples were included. Median biomarker concordance for KRAS ($n = 50$) was 93.7% [67–100], NRAS ($n = 11$) was 100% [90–100], BRAF ($n = 22$) was 99.4% [80–100], and PIK3CA ($n = 17$) was 93% [42–100]. Meta-analytic pooled discordance was 8% for KRAS (95% CI = 5–10%), 8% for BRAF (95% CI = 5–10%), 7% for PIK3CA (95% CI = 2–13%), and 28% overall (95% CI = 14–44%). The liver was the most commonly biopsied metastatic site ($n = 2276$), followed by lung ($n = 438$), lymph nodes ($n = 1123$), and peritoneum ($n = 132$). Median absolute concordance in multiple biomarkers was 81% (5–95%).
Interpretation: Metastatic CRC demonstrates high concordance across multiple biomarkers, suggesting that molecular testing of either the primary or liver and lung metastasis is adequate. More research on colorectal peritoneal metastases is required.

1. Introduction

Approximately 1.4 million patients per annum worldwide are diagnosed with colorectal cancer (CRC). Metastatic (stage IV) disease at presentation results in a 5-year overall survival (OS) of 14% [1,2], whilst 50% of patients undergoing surgery with curative intent develop metastatic disease within 5 years [3]. The most commonly reported sites include lymph nodes (35–40%) [4], liver (50–60%) [5,6], lung (10–30%) [7], and peritoneum (5–20%) [8–10].

Advances in biological therapies such as anti-epidermal growth factor receptor (EGFR) antibodies (cetuximab and panitumumab) have resulted in biomarkers being used to target metastatic CRC (mCRC) [11]. KRAS is a key proto-oncogene downstream of EGFR and is activated in up to 50% of sporadic mCRC patients, with 95% of activations occurring in codons 12/13 of exon 2 [12]. Importantly, KRAS exon 2–4 mutations (involving codons 12, 13, 161, 117 & 146) demonstrate a significantly lower response to cetuximab and panitumumab [13,14]. Some studies have also indicated that KRAS mutations may confer resistance to bevacizumab [15,16]. The extended RAS family of oncogenes includes NRAS, with exon 2–4 mutations occurring in 3–5% of CRC's and similarly resulting in a lower response [17,18]. BRAF, a RAF gene kinase and immediate downstream effector of KRAS, shows mutations in nearly 10% of colorectal adenocarcinomas and is also a strong negative prognostic marker, predicting resistance to both cytotoxic and anti-EGFR therapy [19–21].

Mutations in genes other than those constituting the RAS/RAF pathway include PIK3CA and PTEN [22]. Approximately 15–20% of patients with mCRC, have mutations in exon 20 of PIK3CA and demonstrate resistance to cetuximab even in the presence of KRAS wild type [23]. Loss of expression of PTEN, a natural inhibitor of PI3K-initiated signalling, has itself also been associated with unresponsiveness to cetuximab and reduced OS [24,25]. Beyond the factors associated with EGFR signalling pathways, a number of other genes are significantly mutated in CRC, including APC (51–81%), TP53 (20–60%), and SMAD4 (10–20%) [12]. APC is the most prevalent gene in the establishment of sporadic colorectal malignancy [26]. Similar to APC, the TP53 gene is heavily

* Corresponding author at: Consultant Colorectal Surgeon, Colorectal and Peritoneal Oncology Centre, The Christie NHS Foundation Trust, Wilmslow Road, Manchester M20 4BX, United Kingdom.
E-mail address: omer.aziz@christie.nhs.uk (O. Aziz).
[1] Signifies co-first authors.

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Research in context

Evidence before this study

Genetic mutations in key biomarkers are known to predict outcomes and response to treatment in metastatic colorectal cancer. Concordance in these biomarkers between the primary and metastatic sites is an important factor to consider with both diagnostic and therapeutic implications. We systematically reviewed all published comparative studies reporting on biomarker concordance between primary and metastatic sites. These studies were identified using PubMed, MEDLINE, Ovid, Embase, Cochrane, and Google Scholar databases. Biomarkers that were studied included KRAS, BRAF, NRAS, PIK3CA and PTEN, among others.

Added value of this study

This study provides a comprehensive review of the concordance rates of genetic biomarker mutations between primary and metastatic sites in colorectal cancer. This allows us to quantify the predictive value of a metastatic site biopsy in determining the biomarker mutation status of the primary colorectal cancer. It also presents what is currently known about biomarker concordance by metastatic site.

Implications of all the available evidence

This study demonstrates a high genetic concordance rate between primary colorectal cancers and their liver/lung metastases. Despite peritoneal metastases being the third most common site, little remains known about their concordance with the primary colorectal cancer. There is currently no evidence that multiple metastatic site biopsies will provide benefit, with single sites sufficient for diagnosis and biomarker profiling provided adequate samples can be taken. This has important implications for reducing the time to diagnosis, commencement of treatment, and cost.

involved in the malignant transformation of CRC, with mutation in TP53 resulting in a non-functional p53 protein and reduced OS [27–30]. Unlike APC and TP53, which usually occur in early colorectal tumorigenesis, inactivation of SMAD4 is associated with late-stage or metastatic disease [31]. SMAD4, also known as DPC4, encodes a tumour suppressor that regulates transcriptional activity downstream of TGF-beta receptor signalling. Expression of SMAD4 is an important prognostic factor in CRC, with patients who retain higher levels of SMAD4 within their tumours having higher OS than those with low or absent expression [32,33].

This study aims to systematically review the literature and undertake meta-analysis where appropriate in order to determine the concordance between primary CRC and its metastatic site, with regards to the above-mentioned biomarkers and their combinations. It also aims to determine the variation in concordance by metastatic site, and the 'absolute concordance' in multiple biomarkers for mCRC. This is important for two reasons: First, it has implications for the understanding of how tumours evolve and differ between the primary and metastatic site. Studies demonstrating the dynamic changes in circulating DNA of mCRC patients with the clonal evolution and resistance to anti-EGFR treatments with time have suggested that the CRC genome adapts to drug schedules, providing a molecular explanation for changes in efficacy with re-challenge anti-EGFR therapies [34]. Second it also has implications for personalized treatment strategies used for patients based on single site biopsies [35,36]. This study tries to shed light in respect to the heterogeneity (studied as mutational discordance) between the primary and metastatic sites in light of evidence that significant intra-tumour heterogeneity exists between different points on the same primary CRC specimen [37]. There are cases where the primary tumour cannot be accessed, in which case knowledge on concordance between the metastatic sites (liver, lung, lymph node or peritoneum) is important.

2. Methods

This systematic review was undertaken in accordance with the PRISMA guidelines [38]. A literature search was undertaken by two independent reviewers (DB and OA) of all published studies using PubMed, MEDLINE, Ovid, Embase, Cochrane, and Google Scholar databases using the following MeSH terms: "colorectal neoplasm", "peritoneal neoplasm" and "mutation", plus additional search terms including: "primary colorectal cancer", "metastasis", "biomarker", and "concordance". Further references were identified manually using the bibliographies of relevant papers and review articles. Equal consideration was given to fully published studies and those available in only abstract form.

2.1. Study selection

Studies were included provided that patients had a confirmed diagnosis of metastatic colorectal adenocarcinoma, and mutational biomarker analysis on biopsies both from the colorectal primary and at least one site of metastasis. Studies were excluded if the primary and metastatic tumour samples were unmatched, concordance was reported in relation to peripheral blood samples instead of solid tumour, or there was insufficient data available to provide a value for concordance. The method and extent of mutational analysis was not a criterion for exclusion. In this study the biomarker concordance between primary tumour and metastasis was defined in terms of both mutant and wild-type pairs, and not limited to the mutant-only population.

2.2. Meta-analysis

The proportion of changes (discordance proportion) with exact 95% confidence intervals (CIs) was calculated for each study. The Freeman–Tukey double arcsine transformation was the chosen approach for the calculation of pooled estimates and corresponding 95% CIs [39,40]. If a study had a sample size below 10, the arcsine transformation was preferred. Random effects pooled estimates were calculated in order to take into account heterogeneity between estimates [41]. Statistical heterogeneity among studies was evaluated using the chi-square test statistic and was measured using the I^2 statistic, which is the proportion of total variation contributed by between-study variance tau-squared (τ^2) [42]. Publication bias was evaluated using funnel plots and the asymmetry test developed by Egger et al. [43]

All analyses were carried out with R software (http://cran.r-project.org/) with packages 'meta' and 'metafor'. All the reported *P* values were two sided.

3. Results

Literature search identified 1498 studies reporting on concordance in mCRC between 1991 and 2018. Of these, 61 articles including 3565 patients matched the selection criteria and were deemed suitable for qualitative synthesis as outlined in Fig. 1.

3.1. Concordance between primary CRC and its metastatic site

Concordance in individual biomarker status in patients with mCRC was reported in a range of oncogenes and tumour suppressor genes. The median reported concordance was 93.7% (range 67–100) for KRAS ($n = 50$) [24,44–93], 99.4% (range 80–100) for BRAF ($n = 22$)

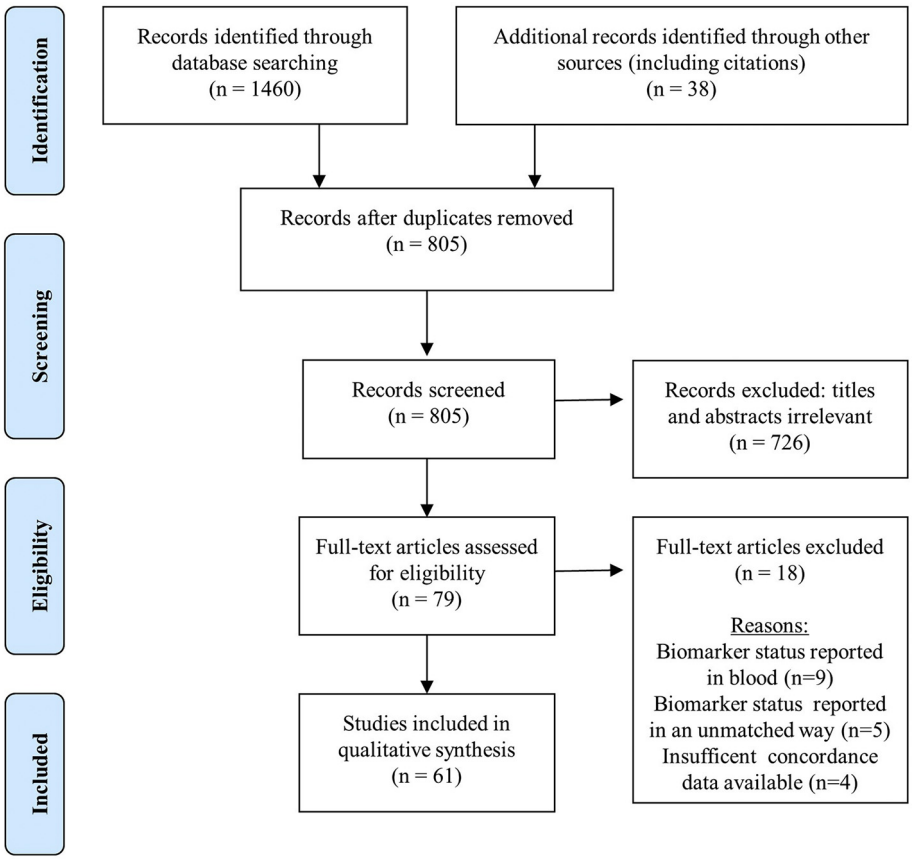

Fig. 1. PRISMA flow diagram of the literature search.

[44,45,48,49,54,60,61,63–65,67–69,71,73–76,79,80,83,86], 93% (range 42–100) for PIK3CA (n = 17) [44,48–51,53,54,58–60,63,65,67–69,73,76], 92.9% (range 73–100) for TP53 (n = 12) [44,48,51–53,58–60,63,64,68,87], and 100% (range 90–100) for NRAS (n = 11) [44,48,49,51,56,59,60,63,68,69,93]. Less commonly reported markers included: PTEN (n = 10) [24,44,48,53,63,67,71,76,79,94], APC (n = 10) [44,48,51–53,58,59,63,64,88], SMAD4 (n = 6) [51,53,54,58,63,64], and EGFR (n = 5) [24,71,79,95,96]. Additional data was also available on the concordance of MSI status and MMR genes (n = 5) [45,48,65,97,98]. Tables 1, 2, and 3 summarize the concordance rates for KRAS, BRAF, and PIK3CA, the three most commonly studied biomarkers.

Other CRC biomarkers infrequently reported in the literature included AKT (n = 4, range 68–90%) [24,48,49,52], CTNNB1 (n = 4, range 95–100%) [44,48,52,54], MET (n = 3, range 75–100%) [48,52,67], and FBXW7 (n = 3, range 83–100%) [44,48,52]. Only two or fewer studies accounted for mutational concordance in the following genes: STK11, KIT, FGFR3, GNAQ, NOTCH, ATM, ARID1A, and FAT4, in addition to various others [44,48,51,52,54,64]. This also included singular studies of mitochondrial microsatellite instability (mtMSI), CpG island methylator phenotype (CIMP), neuroendocrine differentiation, and microRNA (miRNA) [60,99–101].

3.2. Concordance depending on metastatic site

The liver was the most commonly included metastatic site within studies (n = 53 studies including 2276 patients), followed by the: lung (n = 37 studies including 438 patients), lymph nodes (n = 30 studies including 1123 patients), and peritoneum (n = 17 studies, including 132 patients). 27 studies analysed biomarker concordance separately within these metastatic sites as displayed in Table 4, thus allowing comparison between metastatic location and the possible impact on concordance. Of these studies, 21 gave concordance data specific to liver metastases [44–46,49–51,53,56,61,63–65,69,70,72,77,80–82,94,100], 11 for lung metastases [38,42,49,59,60,65,68,84–87] and 12 for lymph nodes [46,47,49,52,57,60,61,67,68,75,84,87]. Only two studies provided separate concordance for peritoneal metastases and likewise only single studies for ovarian and bone metastases were available, with most studies grouping these with other metastatic sites [45,53,63,89].

3.3. Absolute concordance

15 studies compared the overall molecular profiles of the matched primary and metastatic sites as shown in Table 5 [44,45,51,52,54,59,60,63,65,69,76,80,83,93,102]. An additional 2 studies reported on somatic variance between matched tumours [103,104]. Studies identified that the greater the number of genes included within the analysis, the lower the rate of absolute concordance. For example, a combination of KRAS and BRAF was concordant in 44 out of 48 of cases (91.7%) but sequencing of >1000 genes in a separate cohort saw concordance fall to only 1 in 19 cases (5.3%) [45,72].

3.4. Meta-analysis of the discordance

The discordance rate was assessed in 3066 patients for KRAS, 1312 patients for BRAF, 727 patients for PIK3CA and 626 patients with overall molecular profiles. There was no evidence for publication bias for PIK3CA and the overall molecular profiles (labelled as “ALL” in the Supplementary Fig. 1 (Egger's test: p = .76 and p = .08 respectively). However, publication bias was found in KRAS and BRAF studies (Egger's test: p = .01 and p = .01 respectively).

Table 1
KRAS biomarker studies ($n = 50$).

Study	Year	*N*	Analysis	Codons	Sites of metastasis	Concordance (%)
Moorcraft et al.	2017	15	NGS	–	Lu	92
Fujiyoshi et al.	2017	457	NGS	12, 13, 61	L + D	96.9
Petaccia de Macedo et al.	2017	97	Pyro	12, 13, 61	L + D	97.9
Pang et al.	2017	72	ARMS PCR	–	L + D	81.9
Nemecek et al.	2016	12	NGS	12, 13, 22, 61, 117, 146	L + D	75
Li et al.	2016	58	qRT-PCR + NGS	12, 13	D	81
He et al.	2016	59	PCR	12, 13, 61, 117	D	76.3
Kovaleva et al.	2016	14	NGS	12, 13	D	78.6
Crumley et al.	2016	16	NGS	–	L + D	93.8
Vignot et al.	2015	13	NGS	–	D	100
Jesinghaus et al.	2015	24	NGS	–	L + D	100
Siyar-Ekinci et al.	2015	31	Pyro	–	D	77.4
Lau et al.	2015	82	Sanger	12, 13, 61	D	88.1
Lee et al.	2015	74	Seq	12, 13	L + D	79.7
Lim et al.	2015	34	NGS + Sanger	–	Li	97
Kim et al.	2015	19	NGS	12, 13, 61	L + D	100
Kleist et al.	2014	151	Seq	12, 13, 61	L + D	86.8
Giannini et al.	2014	17	PCR + Pyro	12, 13	L + D	82.4
Paliogiannis et al.	2014	31	Seq	12, 13, 61	D	90.3
Brannon et al.	2014	69	NGS	–	D	100
Lee et al.	2014	15	NGS	–	Li	80
Murata et al.	2013	26	Pyro	12	L + D	94
Miglio et al.	2013	45	Seq	12, 13	L + D	100
Vakiani et al.	2012	84	Sanger	12, 13, 22, 61, 117, 146	L + D	97.6
Vermaat et al.	2012	21	NGS + Sanger	12, 13, 61, 146	Li	85.7
Knijn et al.	2011	305	Seq	12, 13	Li	96.4
Park et al.	2011	17	Seq	12,13	L + D	76
Watanabe et al.	2011	43	Seq	12, 13	D	88.4
Baldus et al.	2010	75	Pyro	–	L + D	74.7
Italiano et al.	2010	64	Seq	–	–	94.9
Mariani et al.	2010	38	Seq	12, 13	D	97
Perrone et al.	2009	12	Seq	12, 13	D	80
Cejas et al.	2009	110	Seq	12, 13	D	94
Garm-Spindler et al.	2009	31	qPCR	12, 13	D	93.5
Loupakis et al.	2009	53	Seq	12, 13	D	95
Molinari et al.	2009	38	Seq	12, 13	L + D	92
Gattenlöhner et al.	2009	21	AS-PCR	12, 13	L + D	95
Etienne-Grimaldi et al.	2008	48	PCR-RFLP	12, 13	Li	100
Santini et al.	2008	99	Seq	12, 13	D	96
Artale et al.	2008	48	Seq	12, 13	D	94
Gattenlohner et al.	2008	106	Seq	12, 13	L + D	99
Weber et al.	2007	38	Seq	12, 13	Li	94.7
Oliveira et al.	2007	28	–	–	L	67.9
Albanese et al.	2004	30	PCR-SSCP	12, 13	Li	70
Zauber et al.	2003	42	Seq	12, 13	L + D	100
Tórtola et al.	2001	51	SSCP + Seq	12, 13	BM	70
Al-Mulla et al.	1998	58	PCR ASO	12, 13	L + D	87
Suchy et al.	1992	109	PCR ASO	12	–	100
Losi et al.	1992	35	AS-PCR	12, 13	L + D	100
Oudejans et al.	1991	31	Seq	12, 13, 61	D	93.5

Table key [1–3]:
N = no. of patients with matched samples
L = local metastasis e.g. loco-regional lymph nodes
D = distant metastasis e.g. liver, lung, peritoneum, omentum, mesentery, brain, bone, ovary, uterus, vagina, small intestine, adrenal gland, pancreas
Li = Liver only
Lu = Lung only
BM = Bone marrow only
Abbreviations: ARMS, amplification-refractory mutation system analysis; ASO, allele-specific oligonucleotide hybridisation; AS-PCR; allele-specific polymerase chain reaction; NGS, next generation sequencing; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; Pyro, pyrosequencing; qPCR; quantitative PCR; qRT, quantitative reverse transcription; Sanger, sanger sequencing; Seq, sequencing; SSCP, single-stranded conformation polymorphism; —, information unavailable/unspecified.

(i) KRAS - Fig. 2 shows the discordance proportions reported for KRAS in each study included in the analysis. The heterogeneity between proportions ranged from 0% to 29% ($I^2 = 91\%$, $\tau^2 = 0.012$, $p < .0001$). The meta-analytic pooled discordance proportion was 8% (95% CI: 5–10%).
(ii) BRAF - Fig. 3 shows the discordance proportions reported for BRAF in each study included in the analysis. The heterogeneity between proportions ranged from 0% to 20% ($I^2 = 59\%$, $\tau^2 = 0.007$, $p < .0003$). The meta-analytic pooled discordance proportion was 8% (95% CI: 5–10%).
(iii) PIK3CA - Fig. 4 shows the discordance proportions reported for PIK3CA in each study included in the analysis. The heterogeneity between proportions ranged from 0% to 58% ($I^2 = 86\%$, $\tau^2 = 0.04$, $p < .0001$). The meta-analytic pooled discordance proportion was 7% (95% CI: 2–13%).
(iv) Overall molecular profiles – Fig. 5 shows the discordance proportions reported considering the overall molecular profiles. PIK3CA in for all studies included in the analysis. The heterogeneity between proportions ranged from 5% to 95% ($I^2 = 93\%$, $\tau^2 = 0.1$, $p < .0001$). The meta-analytic pooled discordance proportion was 28% (95% CI: 14–44%).

Table 2
BRAF biomarker studies ($n = 22$).

Study	Year	*N*	Analysis	Codons	Sites of metastasis	Concordance (%)
Moorcraft et al.	2017	15	NGS	–	Lu	100
Fujiyoshi et al.	2017	457	PCR-RFLP	600	L + D	100
Nemecek et al.	2016	12	NGS	600	L + D	92
Li et al.	2016	10	NGS	–	D	80
Jesinghaus et al.	2015	24	NGS	–	D	100
Lee et al.	2014	15	NGS	–	Li	93.3
Kleist et al.	2014	151	PCR Seq	–	L + D	98.7
Giannini et al.	2014	17	PCR + Pyro	600	L + D	94.1
Brannon et al.	2014	69	NGS	–	D	100
Murata et al.	2013	26	Pyro	600	L + D	100
Voutsina et al.	2013	83	Sanger + ARMS AS-PCR	600	D	100
Vermaat et al.	2012	21	NGS + Sanger	600	Li	100
Vakiani et al.	2012	84	Sanger	600	L + D	100
Park et al.	2011	20	Seq	600	L + D	90
Mariani et al.	2010	38	Seq	600	D	100
Italiano et al.	2010	48	PCR Seq	–	–	97.9
Baldus et al.	2010	75	Pyro	600	L + D	97.4
Perrone et al.	2009	12	PCR Seq	600	D	90.1
Molinari et al.	2009	38	PCR Seq	600	L + D	100
Gattenlöhner et al.	2009	21	AS-PCR	600	D	100
Artale et al.	2008	48	Seq	600	D	98
Oliveira et al.	2007	28	–	600 + 601	L	89.3

Table key [1–3]:
N = no. of patients with matched samples
L = local metastasis e.g. loco-regional lymph nodes
D = distant metastasis e.g. liver, lung, peritoneum, omentum, mesentery, brain, bone, ovary, uterus, vagina, small intestine, adrenal gland, pancreas
Li = Liver only
Lu = Lung only
BM = Bone marrow only
Abbreviations: ARMS, amplification-refractory mutation system analysis; AS-PCR; allele-specific polymerase chain reaction; NGS, next generation sequencing; PCR, polymerase chain reaction; Pyro, pyrosequencing; Sanger, sanger sequencing; Seq, sequencing; SSCP, single-stranded conformation polymorphism; −, information unavailable/unspecified.

4. Discussion

A total of 9 studies reported absolute concordance in KRAS point mutations within codons 12 and 13, despite apparent differences in sequencing methodologies, suggesting the stability in KRAS status between the primary and metastatic sites [53,54,59,63,66,81,88,91,92]. Of note, Tórtola et al. did identify some discordance when using single-stand conformational polymorphism (SSCP) analysis of bone micrometastases [89]. In this study, 17 mutations were found among the primary tumours compared to only 7 in corresponding metastases, and whereas the majority (88%) of mutated KRAS in primary CRC's were located in codon 12, in the bone marrow these mutations were

Table 3
PIK3CA biomarker studies ($n = 17$).

Study	Year	*N*	Analysis	Codons	Site of metastases	Concordance (%)
Moorcraft et al.	2017	15	NGS	–	Lu	86.7
Li et al.	2016	10	NGS	–	D	100
He et al.	2016	59	PCR Seq	542, 545, 1047	D	42.4
Kovaleva et al.	2016	14	NGS	542, 545, 1047	D	92.9
Nemecek et al.	2016	12	NGS	542, 545, 1047	L + D	83
Vignot et al.	2015	13	NGS	–	D	100
Jesinghaus et al.	2015	24	NGS	–	D	100
Lim et al.	2015	34	NGS + Sanger	–	Li	100
Kim et al.	2015	19	NGS	542, 545, 1047	L + D	100
Kleist et al.	2014	151	PCR Seq	542, 545, 1047	L + D	97.4
Brannon et al.	2014	69	NGS	–	D	94.2
Murata et al.	2013	32	Pyro	542, 545, 1047	L + D	88
Voutsina et al.	2013	83	Sanger + ARMS AS-PCR	525, 545, 1047	D	93
Vakiani et al.	2012	84	Sanger	345, 420, 542, 545, 546, 1043, 1047	L + D	98.8
Vermaat et al.	2012	21	NGS + Sanger	542, 545, 1047	Li	85.7
Baldus et al.	2010	75	Pyro	–	L + D	89.3
Perrone et al.	2009	12	PCR Seq	542, 545, 1047	D	90.1

Table key [1–3]:
N = no. of patients with matched samples
L = local metastasis e.g. loco-regional lymph nodes
D = distant metastasis e.g. liver, lung, peritoneum, omentum, mesentery, brain, bone, ovary, uterus, vagina, small intestine, adrenal gland, pancreas
Li = Liver only
Lu = Lung only
BM = Bone marrow only
Abbreviations: ARMS, amplification-refractory mutation system analysis; AS-PCR; allele-specific polymerase chain reaction; NGS, next generation sequencing; PCR, polymerase chain reaction; Pyro, pyrosequencing; Sanger, sanger sequencing; Seq, sequencing; −, information unavailable/unspecified

Table 4
Concordance studies by metastatic type ($n = 27$).

Study	Year	Biomarker	Biomarker concordance according to metastatic site (%)				
			LN	Liver	Lung	PTM	Other
Kleist et al.	2017	mtMSI		25	48		
Fujiyoshi et al.	2017	KRAS	97.1	97.4	100	98	85.7
		BRAF	100	100	100	100	100
		MSI/MSS	98.8	100	-	92	100
Moorcraft et al.	2017	KRAS		100	93		100
		NRAS		100	100		100
		BRAF		100	100		100
		PIK3CA		100	86.7		100
							100
		PTEN		100	100		100
		TP53		100	100 80		100
		APC		100			
Petaccia et al.	2017	KRAS	97.8	98.9	100		
Crumley et al.	2016	KRAS	92.3		100		
		TP53	92.3		100		
		APC	84.6		100		
He et al.	2016	KRAS		75.8	66.7	92.9	
		PIK3CA		39.4	44.4	47.1	
Kovaleva et al.	2016	KRAS		85.7	85.7		
		NRAS		100	100		
		PIK3CA		92.9	92.9		
		TP53		92.9	92.9		
					78.6		
		APC		71.4	100		
		SMAD4		92.9			
Li et al.	2016	KRAS	100	80	80		
		NRAS	100	100	50		
		BRAF	0	80	100		
		PIK3CA	100	100	100		
Vignot et al.	2015	KRAS		100		100	
		PIK3CA		100		100	
		PTEN		100		100	
		TP53		84.6		100	
		APC		91.7		100	
		SMAD4		84.6		100	
Lau et al.	2015	RAS		100	66.7		
Giannini et al.	2014	KRAS	78.6	100			
		BRAF	92.9	100			
Kleist et al.	2014	KRAS	88.1	83.3			
		NRAS	99.1	97.6			
		BRAF	99.1	100			
		PIK3CA	96.3	100			
		TP53	95.4	100			
Brannon et al.	2014	KRAS		100			100
		NRAS		100			100
		BRAF		100			100
		PIK3CA		98.5			100
		PTEN		100			100
		TP53		100			100
		APC		93.9			100
Lee et al.	2014	KRAS		80			
		BRAF		93.3			
		TP53		73.3			
		APC		53.3			
		SMAD4		93.3			
Atreya et al.	2013	PTEN		98			
Murata et al.	2013	KRAS	100	94			
		BRAF	100	100			
		PIK3CA	100	88			
		MSI	100	96			
Vermaat et al.	2012	KRAS		85.7			
		NRAS		100			
		BRAF		100			
		PIK3CA		100			
Knijn et al.	2011	KRAS	80	96.4			
Watanabe et al.	2011	KRAS		100	100		
Baldus et al.	2010	KRAS	69	90			
		BRAF	96	100			
		PIK3CA	87	95			
Cejas et al.	2009	KRAS		94.6	88.2		
Molinari et al.	2009	KRAS	100	92			
		BRAF	100	100			
		PTEN	87	89			
Etienne-Grimaldi et al.	2008	KRAS		100			

Table 4 (*continued*)

Study	Year	Biomarker	Biomarker concordance according to metastatic site (%)				
			LN	Liver	Lung	PTM	Other
Gattenlohner et al.	2008	KRAS	98.6	99	100	96	
Santini et al.	2008	KRAS		96		80	
Oliveira et al.	2007	KRAS	67.9				
		BRAF	89.3				
Tórtola et al.	2001	KRAS					70

Abbreviations: LN, lymph nodes; PTM, peritoneum. 'Other' – includes bone, ovary, pancreas etc.

mainly localised to codon 13 (71%) [89]. There are however studies that identified discordance rates in KRAS as high as 30% [86,87]. In the case of Baldus et al., the lower reported concordance was suggested to be attributable to intra-tumour heterogeneity; a phenomenon strongly evidenced in colorectal tumours as well as numerous other solid malignancies [37,73,105,106]. In a comparable study by He et al., a combination of low sensitivity testing and unrepeated mutation analysis of the tissues could have led to false-negatives [50,73]. This is effectively illustrated in the study by Vakiani and colleagues where concordance rates increased by 5% when more sensitive analysis methods were used [68]. KRAS status can therefore be determined from the primary tumour or the metastatic site as long as the sample is sufficient and sequencing technique adequate.

Although significantly fewer studies have evaluated NRAS status compared to KRAS in matched tumours, the available data suggests concordance rates are also high (median of 100%, range 90–100%) regardless of sequencing method [56,60,63]. BRAF notably demonstrated 100% mutation concordance in half of the identified studies, despite similar variances in the type of metastasis sampled and sequencing method [48,54,65,68]. Expanded analysis of BRAF mutations to include exons 11 and 15 did result in a slightly lower concordance (median of 99.4%, range 80–100%), with discordance limited to V600E region of exon 15 [76]. It is important to note that all of the studies reporting lower rates of BRAF concordance demonstrated a small sample size of paired specimens which may have impacted their results. This is aligned to the publication bias detected in this particular subset where usually smaller studies are published only with positive findings. Altogether 7 BRAF studies had sample sizes that were considered small, defined as less than or equal to 20 matched patient samples [49,71,76].

The results for PIK3CA in some cases show a much more discordant relationship between the primary tumour and the metastasis, with a median concordance of 93% (42–100%) [50]. While the aforementioned report is unhindered by its adequate sample size (>50 matched pairs), a combination of the low sensitivity method of sequencing within this study and the unusually high proportion of patients with detected PIK3CA mutation may somewhat explain its contradiction to the rest of the literature [50]. Thus, based on the consistent results of other studies, the concordance rate for PIK3CA is relatively high overall within the average CRC population [60,63,73]. Importantly, this study identified that some of the tumour suppressor genes (PTEN, TP53, APC and SMAD4) can show a more variable and discordant relationship between the primary tumour and the metastasis [59]. Mutation within these tumour suppressors, such as TP53, is at times more common in the metastasis than the primary [68]. However, cumulative data indicate that the PTEN and APC genes are among those demonstrating the greatest variation, revealing less consistency for higher rates of concordance [24,51,52,63,64,67,94]. We also found that although primary tumours and their metastases displayed a variable concordance with regards to MSI and EGFR, the number of studies reporting on these biomarkers was small making our findings difficult to interpret [24,45,95,97]. Nonetheless, EGFR does show notable evidence of a predisposition toward discordant expression [95,96].

This study was also able to determine biomarker concordance by metastatic site, with hepatic metastases most commonly studied and displaying a remarkably concordant relationship with the primary tumour [63,69]. The liver is a site that can be relatively easily biopsied with sufficient sample sizes (core biopsies). In contrast, pulmonary metastases are more challenging to sample and therefore not surprisingly were sampled less frequently than the liver. Much of the variation in concordance with lung metastases may be explained by this. It is however a valid site for biopsy and determination of RAS status. Peritoneal samples were extremely infrequently sampled and would also be

Table 5
Absolute concordance in all biomarkers ($n = 15$).

Study	Year	*N*	Sequencing	Biomarkers included	Sites of metastases	Absolute (%) concordance
Fujiyoshi et al.	2017	257	NGS	KRAS & BRAF	Li, Lu, LN, PTM	94.6
Moorcraft et al.	2017	15	NGS	KRAS, NRAS, BRAF, PIK3CA, PTEN, TP53, APC…	Lu	73
Kovaleva et al.	2016	14	NGS	KRAS, NRAS, PIK3CA, TP53, APC…	Li, Lu	57.1
Crumley et al.	2016	16	NGS	KRAS, TP53 & APC	LN, Li, Lu	81
Jesinghaus et al.	2015	24	NGS - 12 genes	KRAS, NRAS, BRAF, PIK3CA, PTEN, TP53, APC…	LN, Li, Lu, Br	84.4
Kim et al.	2015	19	NGS - 1321 genes	APC, KRAS, TP53, BRAF, PIK3CA…	LN, Li, Lu, Ov	5
Kleist et al.	2014	42	DNA PCR	KRAS, NRAS, BRAF, PIK3CA & TP53	Li, Abw, Ut, Ov, Va, PTM, OTM, SI	81
Brannon et al.	2014	69	NGS - 230 genes	KRAS, NRAS, BRAF…	Li, Lu, Ov	31.9
Murata et al.	2013	32	Pyro	KRAS, BRAF, PIK3CA & MSI	LN, Li	84
Vermaat et al.	2012	21	NGS + Sanger	KRAS, NRAS, BRAF, PIK3CA & EGFR	Li	85.7
Balschun et al.	2011	5	Sanger + Pyro	KRAS, NRAS, BRAF, PIK3CA & MSI	Li	20
Perrone et al.	2009	12	PCR	KRAS, BRAF, PI3KCA & PTEN	Li, Lu, Ut	58.3
Gattenlöhner et al.	2009	21	DNA PCR + AS-PCR	KRAS & BRAF	LN, Li, Lu, PTN, Mes, SI, ST	95
Artale et al.	2008	48	DNA PCR	KRAS & BRAF	Li, Lu, Ov, LN, Adr, Pan, OTM	92
Oudejans et al.	1991	31	DNA PCR	RAS	Li, Lu	87.1

Abbreviations: Abw, abdominal wall; Adr, adrenal gland; AS-PCR; allele-specific polymerase chain reaction; Br, brain; DNA, deoxyribonucleic acid; Li, liver; LN, lymph node; Lu, lung; Mes, mesocolon; NGS, next generation sequencing; OTM, omentum; Ov, ovary; PCR, polymerase chain reaction; PTM, peritoneum; Pyro, pyrosequencing; Sanger, sanger sequencing; SI, small intestine; ST, soft tissue; Ut, uterus; Va, vagina. (…) = additional CRC-related genes but not listed.

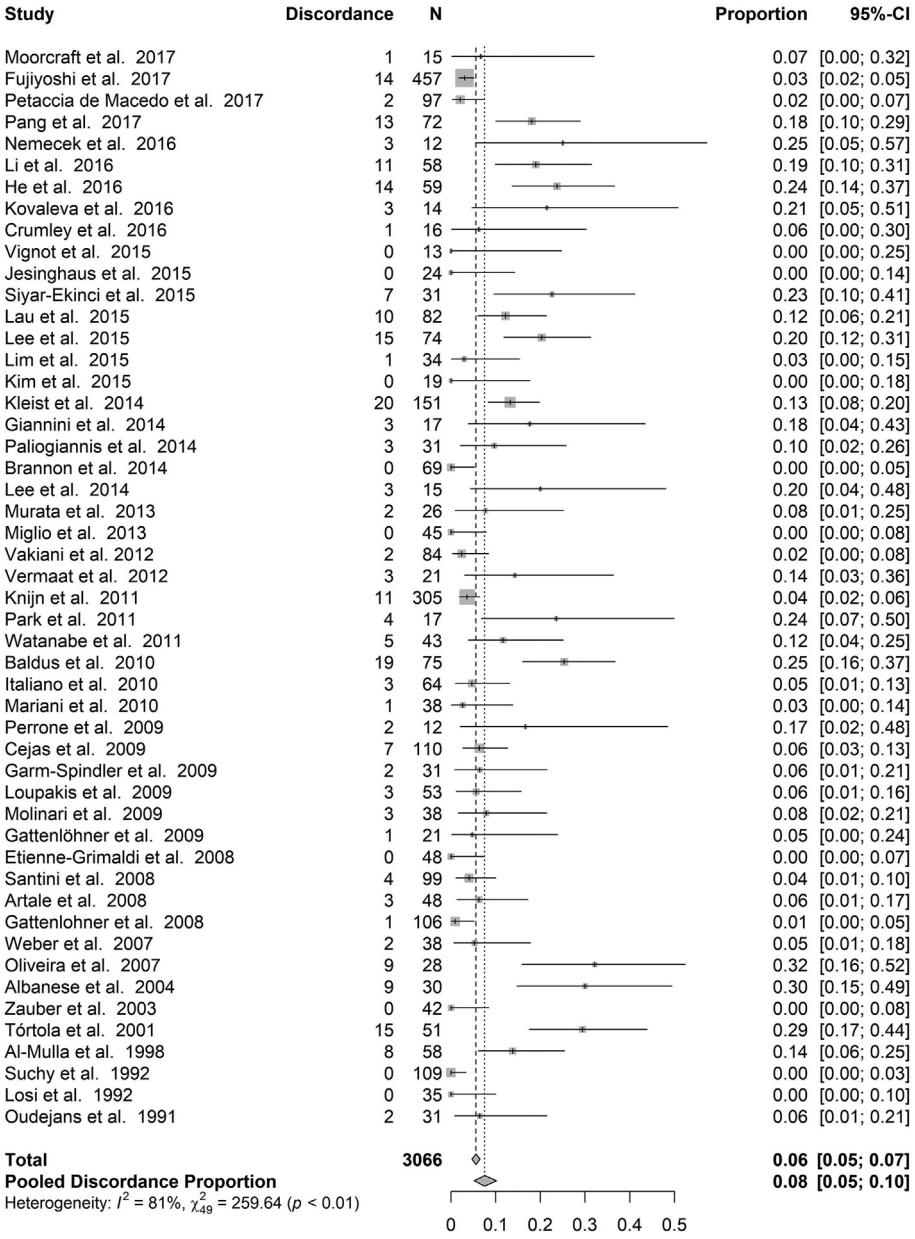

Fig. 2. Forest plot for proportion of discordance of KRAS. The estimate proportion and 95% CI interval at the level of the total number of cases reflects the calculation under a fixed effect model. The estimate proportion and 95% CI interval at the level of the Pooled Discordance Proportion are the random effects pooled estimates to take into account heterogeneity.

potentially more difficult to obtain sufficient tissue from due to their location and size. It is therefore difficult to comment on their relationship with the primary. Only two studies reported on biomarker concordance specifically comparing peritoneal metastases to the primary tumour [45,53]. This suggests the need to investigate this area, particularly as peritoneal metastases have a significantly worse median OS (16.3 months) compared to liver (19.1 months) and lung (24.6 months) metastases [107].

It is not surprising that we found that absolute concordance in more than one biomarker fell as the number of biomarkers was increased. For example limiting analysis to KRAS and BRAF demonstrated a 92–95% concordance [45,80,83], falling to 87% with extended RAS mutation analysis (including KRAS, NRAS and HRAS) [93]. Cohorts tested for KRAS and BRAF as well as additional alterations in PIK3CA/PTEN/TP53/APC demonstrate a more variable absolute concordance ranging from 57 to 84% [44,51,54]. Finally studies comparing 12, 230 and 1321 gene sequencing have demonstrated how absolute concordance falls as more genes are included within the analysis to only 5% [59,63,108]. This suggests that the metastases are distinctly different in their genetic composition to the primary tumour [64]. In any case poor absolute concordance may provide some explanation as to the complexities of colorectal tumours and their resistance to cytotoxic and biological therapies [109]. Furthermore, as metastases generally possess greater mutational load than primary tumours as well as less intra-tumour heterogeneity, basing clinical decision-making on the profile of metastases in preference to generalising the data obtained from primary tumours may be beneficial [64,67].

It is important to note a number of limitations of this study. First it should be noted that the studies included were retrospective and varied in their sample size. This was reflected in the meta-analysis showing a clear publication bias for the KRAS and BRAF papers. Second, there was heterogeneity in sample collection and processing techniques as well as sequencing techniques used which have evolved over time, making absolute comparisons difficult. This would for example explain

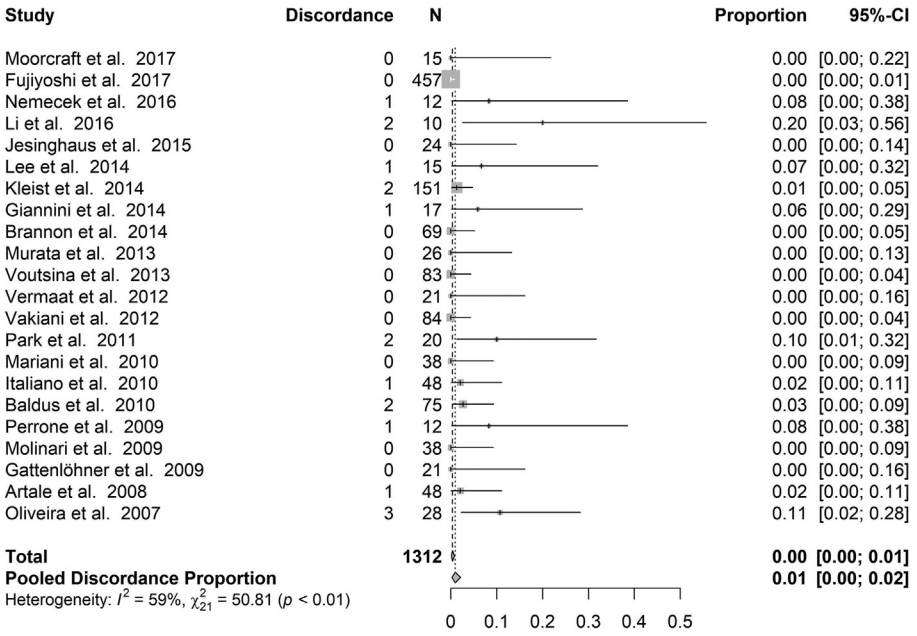

Fig. 3. Forest plot for proportion of discordance of BRAF. The estimate proportion and 95% CI interval at the level of the total number of cases reflects the calculation under a fixed effect model. The estimate proportion and 95% CI interval at the level of the Pooled Discordance Proportion are the random effects pooled estimates to take into account heterogeneity.

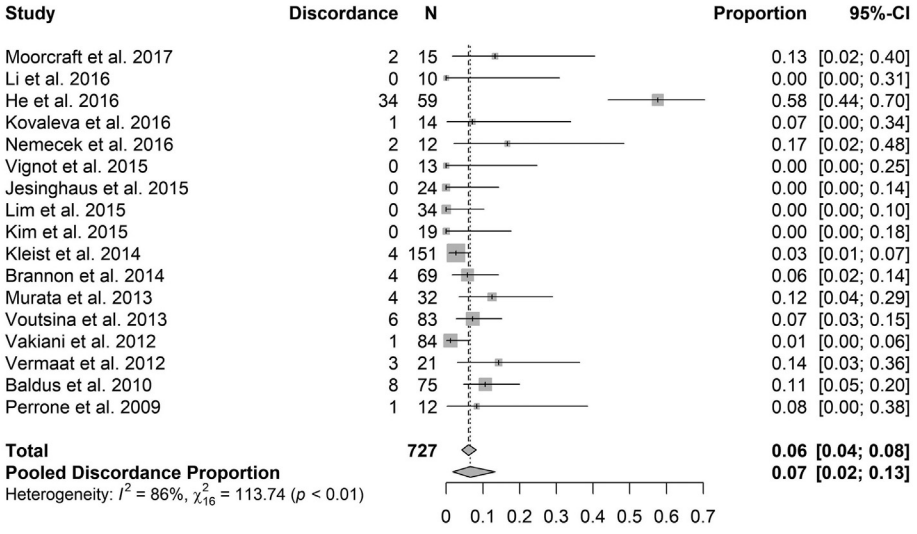

Fig. 4. Forest plot for proportion of discordance of PIK3CA. The estimate proportion and 95% CI interval at the level of the total number of cases reflects the calculation under a fixed effect model. The estimate proportion and 95% CI interval at the level of the Pooled Discordance Proportion are the random effects pooled estimates to take into account heterogeneity.

the higher concordance for newer biomarkers such as NRAS. Third, the patients varied in the systemic treatments they received, and the impact of this on the metastasis (tumour regression after chemo- or radiotherapy) may have affected the sequencing result through adequacy of the sample. Fourth, it is important to note that any change in concordance may represent the evolution of the tumour as it metastasises and therefore the time between the primary tumour and metastasis sampling is an important factor to consider which could not be accounted for. Finally inter-tumour heterogeneity means that sufficient samples may not have been possible to obtain in many of the studies to determine biomarker status especially at the metastatic sites.

Despite the above limitations, we feel that this study has demonstrated high biomarker concordance rate between primary tumours and the metastases with challenges in getting adequate sample size from metastatic sites likely to account for most discordance. This has important practical implications as it suggests there is little evidence for separate biopsy of the primary and metastatic sites. Whether the evolving use of liquid biopsy through circulating tumour DNA (ctDNA) analysis adds anything further to a patient's treatment options remains to be seen. We also feel that little information is currently available on peritoneal metastases and this is an area for further research.

5. Conclusion

mCRC demonstrates remarkably high concordance across a number of individual biomarkers, suggesting that molecular testing of either the primary or liver and lung metastasis is adequate for determining biomarker status to personalize treatment. More research is required to determine concordance in colorectal cancer peritoneal metastases which may explain why these tumours have a significantly worse prognosis compared to other sites. Clonal selection and tumour evolution add

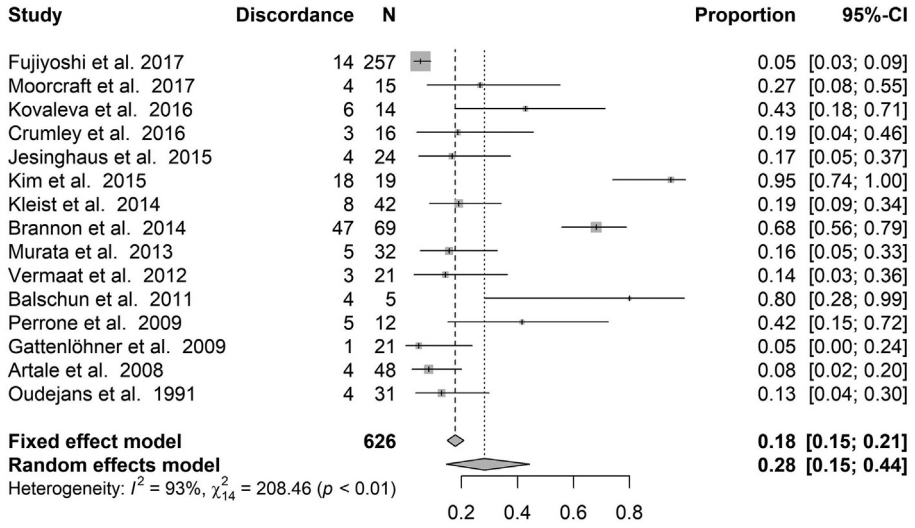

Fig. 5. Forest plot for proportion of discordance of studies considering the overall molecular profiles. The estimate proportion and 95% CI interval at the level of the total number of cases reflects the calculation under a fixed effect model. The estimate proportion and 95% CI interval at the level of the Pooled Discordance Proportion are the random effects pooled estimates to take into account heterogeneity.

further inherent complexities that may be addressed through new sampling strategies and novel technologies in development.

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Authors' contributions

Dilraj S Bhullar - Literature search, figures, study design, data collection, data analysis, data interpretation, writing.

Omer Aziz - Literature search, figures, study design, data collection, data analysis, data interpretation, writing.

Sarah T O'Dwyer - Study design, data analysis, data interpretation, writing.

Jorge Barriuso - Figures, study design, data analysis, data interpretation, writing.

Saifee Mullamitha - Data interpretation, writing.

Mark P Saunders - Data interpretation, writing.

Conflict of interest

The authors have declared no conflicts of interest.

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