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% [80–100], BRAF (n = 22) was 99.4% [80–100], and PIK3CA (n = 17) was 95% [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.

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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 tumorigene- sis, 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-β 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 arc sine 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 arc sine transformation was pre-

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)
[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 \((n = 11)\) [44,48,49,51,56,59,60,63,68,69,70,72,77,80–82,94,100]. Less commonly reported markers included: PTEN \((n = 10)\) [24,44,48,49,52,53,58,63,67,71,76,79,94], APC \((n = 10)\) [44,48,51–53,58,63,64,88], 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 \((n = 5)\) [45,48,51,52,54,64]. This also included single studies of mitochondrial microsatellite instability (mtMSI), CpG island methylator phenotype (CIMP), neuroendocrine differentiation, and microRNA (miRNA) [60,99–101].

### 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).
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%)

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%)

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%).

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 1: KRAS biomarker studies (n = 50).

| Study                | Year | N   | Analysis | Codons | Sites of metastasis | Concordance (%) |
|----------------------|------|-----|----------|--------|---------------------|-----------------|
| Moorcraft et al.     | 2017 | 15  | NGS      | –      | –                   | 92              |
| Fujiiyoshi 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 | –      | –                   | 81.9            |
| Nemec 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 | –                   | 78.6            |
| Crumley et al.       | 2016 | 16  | NGS      | –      | L + D             | 93.8            |
| Vignot et al.        | 2015 | 13  | NGS      | –      | –                   | 100             |
| Jesinghaus et al.    | 2015 | 24  | NGS      | –      | L + D             | 77.4            |
| Sisar-Ekinci et al.  | 2015 | 31  | Pyro     | –      | –                   | 88.1            |
| Lau et al.           | 2015 | 82  | Sanger   | 12, 13, 61 | D             | 79.7            |
| Lee et al.           | 2015 | 74  | Seq      | 12, 13 | –                   | 97              |
| Lim et al.           | 2015 | 34  | NGS + Sanger | –       | –                   | 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            |
| Palogiannis et al.   | 2014 | 31  | Seq      | 12, 13, 61 | D             | 90.3            |
| Brannon et al.       | 2014 | 69  | NGS      | –      | –                   | 76.3            |
| Lee et al.           | 2014 | 15  | NGS      | –      | –                   | 80              |
| Murata et al.        | 2013 | 26  | Pyro     | 12     | L + D             | 94              |
| Miglio et al.        | 2013 | 45  | Seq      | 12, 13 | –                   | 100             |
| Vakiani et al.       | 2012 | 84  | Sanger   | 12, 13, 22, 61, 117, 146 | L + D             | 97.6            |
| Ververmaat et al.    | 2012 | 21  | NGS + Sanger | 12, 13, 146 | Li             | 85.7            |
| Knijn et al.         | 2011 | 305 | Seq      | 12, 13 | –                   | 96.4            |
| Park et al.          | 2011 | 17  | Seq      | 12, 13 | –                   | 76              |
| Watanabe et al.      | 2011 | 43  | Seq      | 12, 13 | –                   | 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 | –                   | 97              |
| Perrone et al.       | 2009 | 12  | Seq      | 12, 13 | –                   | 80              |
| Cejas et al.         | 2009 | 110 | Seq      | 12, 13 | –                   | 94              |
| Garm-Spindler et al. | 2009 | 31  | qPCR     | 12, 13 | –                   | 93.5            |
| Loupakis et al.      | 2009 | 53  | Seq      | 12, 13 | –                   | 95              |
| Molinari et al.      | 2009 | 38  | Seq      | 12, 13 | –                   | 92              |
| Gattenlöhner et al.  | 2009 | 21  | AS-PCR   | 12, 13 | –                   | 95              |
| Etienne-Grimaldi et al. | 2008 | 48  | PCR-RFLP | 12, 13 | –                   | 100             |
| Santini et al.       | 2008 | 99  | Seq      | 12, 13 | –                   | 96              |
| Artale et al.        | 2008 | 48  | Seq      | 12, 13 | –                   | 94              |
| Gattenlöchner et al. | 2008 | 106 | Seq      | 12, 13 | –                   | 99              |
| Weber et al.         | 2007 | 38  | Seq      | 12, 13 | –                   | 94.7            |
| Oliveira et al.      | 2007 | 28  | –       | –      | –                   | 67.9            |
| Albanese et al.      | 2004 | 30  | PCR-SSCP | 12, 13 | –                   | 70              |
| Zaubier et al.       | 2003 | 42  | Seq      | 12, 13 | –                   | 70              |
| Tórtola et al.       | 2001 | 51  | SSCP + Seq | 12, 13 | –                   | 100             |
| Al-Mulla et al.      | 1998 | 58  | PCR ASO  | 12, 13 | –                   | 87              |
| Suchy et al.         | 1992 | 109 | PCR ASO  | 12      | –                   | 100             |
| Losi et al.          | 1992 | 35  | AS-PCR   | 12, 13 | –                   | 100             |
| Ouadji 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.
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-strand 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 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             |
| Nemecck 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            |
| Brannom 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             |
| Vemaraf 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.   | 2011 | 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            |
| Moliniari et al. | 2009 | 38 | PCR Seq      | 600    | L + D               | 100             |
| Gartenlöhner 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, mesentry, 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.

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            |
| Nemecck 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            |
| Brannom 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       | 545, 420, 542, 545, 546, 1043, 1047 | L + D      | 98.8            |
| Vemaraf 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, mesentry, 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/unspecific.
| Study               | Year  | Biomarker | LN  | Liver | Lung | PTM  | Other |
|---------------------|-------|-----------|-----|-------|------|------|-------|
| Kleist et al.       | 2017  | mtMSI     | 97.1| 97.4  | 100  | 98   | 85.7  |
| Fujiyoshi et al.    | 2017  | KRAS      | 97.1| 97.4  | 100  | 98   | 85.7  |
| Moorcraft et al.    | 2017  | KRAS      | 100 | 100   | 100  | 100  | 100   |
|                     |       | BRAF      | 98.8| 98.7  | 100  | 92   | 100   |
|                     |       | MSI/MSI   | 100 | 100   | -    | 92   | 100   |
| Moorcraft et al.    | 2017  | KRAS      | 100 | 93    | 100  |      |       |
|                     |       | NRAS      | 100 | 100   | 100  |      |       |
|                     |       | BRAF      | 100 | 100   | 100  |      |       |
|                     |       | PIK3CA    | 100 | 86.7  | 100  |      |       |
|                     |       | PTEN      | 100 | 100   | 100  |      |       |
|                     |       | TPS3      | 100 | 100   | 100  |      |       |
|                     |       | APC       | 100 | 100   | 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  |      |      |       |
|                     |       | 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   | 100  |      |       |
|                     |       | PIK3CA    | 100 | 100   | 100  |      |       |
|                     |       | TP53      | 84.6| 100   |      |      |       |
|                     |       | APC       | 91.7| 100   |      |      |       |
|                     |       | SMAD4     | 84.6| 100   |      |      |       |
| Lau et al.          | 2015  | KRAS      | 78.6| 100   | 66.7 |      |       |
| Giannini et al.     | 2014  | KRAS      | 78.6| 100   | 66.7 |      |       |
| 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   |      |      |       |
|                     |       | TPS3      | 100 | 100   |      |      |       |
|                     |       | APC       | 93.9| 100   |      |      |       |
| Lee et al.          | 2014  | KRAS      | 80  | 93    |      |      |       |
|                     |       | BRAF      | 93.3| 73.3  |      |      |       |
|                     |       | TP53      | 73.3| 93.3  |      |      |       |
| Atreya et al.       | 2013  | PTEN      | 98  | 98    |      |      |       |
| Murata et al.       | 2013  | KRAS      | 100 | 94    |      |      |       |
|                     |       | BRAF      | 100 | 100   |      |      |       |
|                     |       | PIK3CA    | 100 | 88    |      |      |       |
|                     |       | MSI       | 100 | 96    |      |      |       |
| Vermaat et al.      | 2012  | KRAS      | 85.7| 100   |      |      |       |
|                     |       | NRAS      | 100 | 100   |      |      |       |
|                     |       | BRAF      | 100 | 100   |      |      |       |
|                     |       | PIK3CA    | 100 | 100   |      |      |       |
| Knijn et al.        | 2011  | KRAS      | 80  | 96.4  |      |      |       |
| Watanabe et al.     | 2011  | KRAS      | 69  | 90    |      |      |       |
| 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 | 100   |      |      |       |
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 unreported 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
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...
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 pooled Discordance Proportion are the random effects pooled estimates to take into account heterogeneity.

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