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Background

In recent years there has been an increasing focus on gene expression profiling to provide additional criteria for tumour sub-classification and improve prognostication, with the ultimate goal of individualising patient therapy. Numerous abnormalities in gene expression have been reported, the significance of which needs to be evaluated in well-designed studies of large clinical populations.

See Molecular pathology and biomarkers – implications for systemic chemotherapy.

Sampling and specimen handling considerations

The procurement of adequate tissue to determine the status of predictive and or prognostic biomarkers has become necessary to guide important treatment decisions.

The primary pathologist plays a central role in reviewing all available tissue samples and selecting the most appropriate tissue suitable for biomarker analysis. If there is inadequate quantity of neoplastic cells for analysis, false-negative results may occur due to dilution of mutant alleles. This is particularly relevant to RAS mutation analysis.[1][2] Most molecular testing can now be performed on archival paraffin embedded tissue, and this may be required several years after resection of the primary tumour. It is recommended that a suitable tissue block be designated for this purpose, which contains a high proportion of cancer (preferably >70%).[3]

Practice point

A suitable tissue block with a high proportion of tumour tissue (preferably over 70%) should be designated for the purpose of further molecular testing if required.

Systematic review evidence

In patients diagnosed with colorectal cancer who have undergone surgical resection or biopsy of the primary colorectal tumour, which molecular marker (BRAF/KRAS/NRAS/DNA mismatch repair /microsatellite instability) best predicts response to surgery, or adjuvant therapy or radiotherapy (disease-free survival, overall survival, disease-specific mortality, overall mortality, or relapse incidence)? (PTH1)

A total of 39 level II studies[4][5][6][7][8][9][10][11][12][13][14][15][16][17][18][19][20][21][22][23][24][25][26][27][28][29][30][31][32][33][34][35][36][37][38][39][40][41][42] and 66 level III-3 studies[43][44][45][46][47][48][49][50][51][52][53][54][55][56][57][58][59][60][61][62][63][64][65][66][67][68][69][70][71][72][73][74][75][76][77][78][79][80][81][82][83][84][85][86][87][88][89][90][91][92][93][94][95][96][97][98][99][100][101][102][103][104][105][106][107][108] were identified that evaluated the prognostic value of microsatellite stability status, DNA mismatch repair function, KRAS or BRAF mutation status for various outcomes related to patient response to treatment. All studies were at high risk of bias except 6 which were at medium risk of bias.[17][24][48][72][77][107]
The search strategy, inclusion and exclusion criteria, and quality assessment are described in detail in the Technical report.

Overall survival

KRAS mutation status

A total of 35 studies[6][12][15][19][23][24][28][29][30][39][42][45][46][49][55][56][58][62][64][65][74][77][79][80][81][85][86][91][95][97][98][99][102][106][108] reported the outcome of overall survival with respect to KRAS mutation status (any mutation versus wild type). All stages of colorectal cancer were included, as well as patients with metastatic disease.

Most studies reported a trend towards increased survival in those without KRAS mutations (wild-type KRAS), with half of the studies reporting at statistically significant difference.

No trends in overall survival and KRAS mutation status were reported against the clinical stage of colorectal cancer.

Thirteen studies[6][10][12][15][42][45][52][56][79][80][97][99][106] reported overall survival with respect to KRAS mutation status (any mutation versus wild type) in those who had anti-epidermal growth factor receptor (EGFR) treatment (cetuximab or panitumumab). Most studies reported a trend towards increased survival in those without KRAS mutations (wild-type KRAS), with nine of the studies reporting at statistically significant difference.

Nine studies[6][15][28][36][70][77][100][106][108] reported overall survival as an outcome with respect to BRAF mutation status (any mutation versus wild-type) in those treated with the combination of leucovorin calcium (folinic acid), 5-fluorouracil (SFU) and oxaliplatin (FOLFOX). All but one study reported no statistically significant difference.

BRAF mutation status

A total of 25 studies[4][14][15][18][24][29][30][42][43][52][53][55][58][60][62][77][79][90][95][96][97][99][102][103] reported overall survival as an outcome with respect to BRAF mutation status. The majority of studies report better survival in those with wild-type BRAF tumour gene, and this was statistically significantly different in all but six studies.[4][14][55][58][62][103]

Six studies[18][42][52][60][79][97] reported overall survival as an outcome with respect to BRAF mutation status in those who had anti-EGFR treatment (cetuximab or panitumumab). All studies report better survival in those with wild-type BRAF tumour gene, and this was statistically significantly different in all six studies.

Five studies[4][15][18][43][77] reported overall survival as outcome with respect to BRAF mutation status (any mutation versus wild type) in those who had FOLFOX. All studies report a trend towards increased survival in those without BRAF mutations (wild-type BRAF), with all but one study[4] reporting a statistically significant difference.
Microsatellite stability status

A total of 20 studies\(^{13}[19][20][24][30][34][35][41][48][61][62][63][71][75][86][89][90][92][96][104]\) reported overall survival as an outcome with respect to microsatellite stability status. There was a slight trend towards better overall survival in those with microsatellite instability, with only nine studies\(^{20}[30][34][41][61][75][86][104]\) reporting a statistical significant difference.

Eighteen studies\(^{4}[5][14][15][17][21][31][32][34][35][53][61][67][68][69][76][93][107]\) reported overall survival as an outcome with respect to DNA mismatch repair function (proficient verse deficient, and vice versa). There was no reported consistent trends of significant between studies.

DNA mismatch repair status

Five studies\(^{4}[15][67][69][107]\) reported overall survival as an outcome with respect to DNA mismatch repair function (proficient verse deficient, and vice versa) in those who had treatment. There were no consistently reported trends across the studies.

Progression-free survival

KRAS mutation status

A total of 21 studies\(^{7}[10][12][18][28][29][36][39][42][45][52][56][57][60][70][78][97][100][102][106][108]\) reported progression-free survival as an outcome with respect to KRAS mutation status. All studies reported a trend towards longer progression-free survival in those without primary tumour KRAS mutation, but fewer than 50% of studies reported a statically significant difference.

A total of 10 studies\(^{7}[10][39][45][52][56][57][60][97][106]\) reported progression-free survival as outcome with respect to KRAS mutation status (any mutation versus wild type) in those who had anti-EGFR treatment (cetuximab or panitumumab). Most studies reported a trend towards longer progression-free survival in those without KRAS mutations (wild-type KRAS), with six of the studies\(^{7}[39][52][56][57][97]\) reporting at statistically significant difference.

Six studies\(^{7}[18][36][70][100][108]\) reported progression-free survival as outcome in respect to KRAS mutation status (any mutation versus wild type) in those who had FOLFOX treatment. There were no consistently reported trends across the studies.

BRAF mutation status

Ten studies\(^{18}[26][29][42][52][57][60][96][97][102]\) reported progression-free survival as an outcome with respect to BRAF mutation status. All studies consistently reported longer progression free survival in those without BRAF mutation, and all but one study reported a statistically significant difference. All clinical grades of colorectal cancer were reported across these nine studies\(^{18}[26][42][52][57][60][96][97][102]\).
A total of seven studies\cite{18,26,42,52,57,60,97} reported progression free survival as an outcome with respect to BRAF mutation status in those who had anti-EGFR treatment (cetuximab or panitumumab). All studies reported longer progression-free survival in those with wild-type BRAF tumour gene, and this was statistically significantly different in six studies.\cite{18,26,42,52,57,97}

**Microsatellite stability status and DNA mismatch repair status**

Five studies\cite{21,66,68,69,96} reported progression-free survival as an outcome with respect to either microsatellite stability status or mismatch repair function status. No significant trends or differences were reported.

**Disease-free survival**

**KRAS mutation status**

Twelve studies\cite{19,23,24,25,40,46,49,51,55,62,73,77} reported disease-free survival as an outcome with respect to KRAS mutation status. Most studies consistently reported a trend towards longer disease free survival in those without KRAS mutations (wild-type KRAS). This difference was statistically significantly in only 5 of these studies.\cite{19,25,49,51,73}

Four studies\cite{25,51,73,77} reported disease-free survival as an outcome with respect to KRAS mutation status in those who had FOLFOX treatment. All studies consistently reported a trend towards longer disease-free survival in those without KRAS mutations (wild-type KRAS), but only two studies reported a statistically significantly difference.\cite{25,73}

**BRAF mutation status**

Ten studies\cite{14,19,24,53,55,62,74,77,82,90} reported disease free survival as an outcome with respect to BRAF mutation status. All studies consistently reported a trend towards longer disease free survival in those without BRAF mutations (wild-type BRAF). This difference was statistically significantly in five studies.\cite{53,55,77,82,90}

**Microsatellite stability status**

Seventeen studies\cite{13,19,22,24,34,35,40,41,54,59,62,71,75,82,89,90,105} reported disease-free survival as an outcome with respect to microsatellite stability status. Reported results were inconsistent across studies.

**DNA mismatch repair status**

Twelve studies\cite{5,14,17,31,32,34,35,53,59,67,76,107} reported disease free survival as an outcome with respect to mismatch repair function. Most studies consistently reported a trend towards longer disease free survival in those with deficient mismatch repair function. This difference was statistically significant in eight studies.\cite{5,14,17,31,32,35,53,107}
Objective response rate

RAS mutation status

Five studies[^10][^12][^27][^70][^79] reported objective response rate as an outcome with respect to KRAS or RAS (KRAS or NRAS) mutation status.

All studies consistently reported a trend towards greater response rate in those with wild-type KRAS tumours. This was statistically significant in three[^12][^27][^79] of the five studies.

BRAF mutation status

One study[^79] reported objective response rate as an outcome with respect to tumour BRAF mutation status. This single study reported a significantly greater objective response rate in those with tumour BRAF mutations.

DNA mismatch repair status

Three studies[^21][^68][^69] reported objective response rate as an outcome with respect to mismatch repair function. No significant trends or differences were reported.

Other outcomes

A number of other outcomes relating to treatment response were reported. These outcomes included pathological complete response, overall mortality, disease control rate, disease-specific survival, time to progression, disease recurrence, recurrence free survival, recurrence-free interval, distant metastases, clinical response, risk of recurrence, and time to recurrence. All these outcomes were reported in a single or very few studies, with few or no reported significant trends.

Evidence summary and recommendations

<table>
<thead>
<tr>
<th>Evidence summary</th>
<th>Level</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current evidence remains controversial as to the use of presently available molecular markers to predict prognosis and identify those patients who may benefit most from conventional adjuvant postoperative chemotherapy. There is emerging evidence to support the use of markers to inform specific targeted therapy.</td>
<td>II, III-2</td>
<td>[4][5][6][7][10][12][13][14][15][17][18][19][20][21][22][23][24][25][26][27][28][29][30][31][32][34][35][36][39][40][41][42][43][45]</td>
</tr>
</tbody>
</table>
### Evidence summary

| RAS | II, III-2 | [6], [12], [15], [19], [23], [24], [28], [30], [29], [39], [42], [45], [46], [49], [55], [56], [58], [62], [65], [64], [74], [77], [79], [80], [81], [82], [85], [86], [89], [90], [91], [92], [93], [95], [96], [97], [98], [99], [102], [106], [108], [7], [10], [18], [25], [27], [36], [40], [51], [52], [57], [60], [70], [73], [78], [100] |
| BRAF | II, III-2 | [4], [14], [15], [18], [19], [24], [26], [29], [30], [42], [43], [52], [53], [55], [57], [58], [60], [62], [74], [77], [79], [82], [90], [95], [96], [97], [99], [102], [103] |

**RAS**

There is consistent evidence that KRAS mutations are predictive of decreased overall survival (all stages of diseases including metastatic disease), decreased progression free survival (all stages of diseases including metastatic disease), and poorer objective response rate.

There is moderate consistent evidence that KRAS mutation predicts decreased disease free survival (stages I–IV) and decreased recurrence free survival (stages I–IV).

There is moderate evidence that, among patients who received anti-EGFR treatment, those with RAS (KRAS or NRAS) mutated tumours had decreased overall survival and progression-free survival compared to anti-EGFR treated patients with wild-type RAS tumours.

**BRAF**

There is consistent evidence that BRAF gene mutation is predictive for both decreased overall survival (all stages of diseases including metastatic disease) and progression free survival (all stages of diseases including metastatic disease).

There is moderate consistent evidence that BRAF mutation is predictive for decreased disease free survival (stages I–IV) and recurrence free survival (stages I–IV).
<table>
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<th>Evidence summary</th>
<th>Level</th>
<th>References</th>
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<tr>
<td>There is moderate evidence that, among patients who received anti-EGFR treatment, those with BRAF mutated tumours had decreased overall survival and progression-free survival than those with wild-type BRAF tumours.</td>
<td></td>
<td>[4], [5], [13], [14], [15], [17], [19], [20], [21], [22], [24], [30], [31], [32], [34], [35], [40], [41], [48], [53], [54], [59], [61], [62], [63], [66], [67], [68], [69], [71], [75], [76], [82], [86], [89], [90], [92], [93], [96], [104], [105], [107]</td>
</tr>
<tr>
<td>There is moderate evidence that, among patients who received FOLFOX treatment, those with BRAF mutated tumours had decreased overall survival than those with wild-type BRAF tumours.</td>
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<tr>
<td><strong>Microsatellite Instability</strong></td>
<td>II, III-2</td>
<td>[4], [5], [13], [14], [15], [17], [19], [20], [21], [22], [24], [30], [31], [32], [34], [35], [40], [41], [48], [53], [54], [59], [61], [62], [63], [66], [67], [68], [69], [71], [75], [76], [82], [86], [89], [90], [92], [93], [96], [104], [105], [107]</td>
</tr>
<tr>
<td>There is consistent evidence that tumour microsatellite instability predicts longer time to disease recurrence (stages I-IV), increased recurrence free survival (stages II-III), and a longer recurrence free interval (stages II-III).</td>
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<tr>
<td>There is inconsistent evidence that tumour microsatellite instability predicts increase overall survival (stages I-IV).</td>
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<tr>
<td>Microsatellite stability status was not shown to predict progression-free survival or disease-free survival.</td>
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<tr>
<td><strong>Mismatch repair</strong></td>
<td>II, III-2</td>
<td>[4], [5], [14], [15], [17], [21], [31], [32], [34], [35], [53], [59], [66], [67], [68], [69], [76], [96], [107]</td>
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<tr>
<td>There is consistent evidence that tumour mismatch repair deficiency predicts increased disease free survival (stage II-III) and decreased risk of recurrence (stages I-IV).</td>
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<tr>
<td>There is no consistent evidence that mismatch repair status predicts patient overall survival, progression free survival, or objective response rate.</td>
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</table>
Evidence-based recommendation

RAS mutation studies should be performed on patients with advanced (metastatic) colorectal cancer in whom anti-EGFR treatment is being considered. Cetuximab and panitumumab should only be considered for the treatment of patients with RAS wild-type metastatic colorectal cancer.

Grade: D

Evidence-based recommendation

There is emerging evidence suggesting that BRAF mutation may be associated with poor response to anti-EGFR treatment, and that BRAF mutation studies should therefore be performed on patients with advanced (metastatic) colorectal cancer.

Grade: D

Health system implications of these recommendations

Clinical practice

Implementation of the recommendation would not change the way that care is currently organised.

Resourcing

No additional resourcing will be necessary to implement the recommendation.

Barriers to implementation

No barriers to the implementation of this recommendation are envisaged.

Discussion

Unresolved issues

The prognostic value of molecular markers is yet to be defined to a degree that can be used in routine pathological analysis.
Studies currently underway

Clinical trials are currently underway to test targeted therapies in BRAF-mutated metastatic colorectal cancer, akin to the development of therapies for BRAF-mutated metastatic melanoma. Early results are promising but have generally been less favourable than the melanoma trials.[109][110][111][112]

It is not known if there are other studies underway in this field.

Future research priorities

It is suggested that further studies are done to more precisely define the prognostic value of these molecular markers.

References


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Appendices