FUTURE PERSPECTIVES OF CIRCULATING TUMOR DNA IN COLORECTAL CANCER

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SELECTED HIGHLIGHTS

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KEY MESSAGES

- Liquid biopsy using ctDNA analysis has the potential to revolutionise colorectal cancer management with roles in:
  - Cancer diagnosis
  - Detection of tumor recurrence or minimal residual disease and determining prognosis
  - Tracking resistance and tailoring therapies
- The key benefit is the ability to obtain a dynamic, real-time picture of the tumor genomic landscape in a given patient, providing the opportunity to tailor therapies throughout the disease course, from diagnosis to the development of resistance
- Application of this technology to detect minimal residual disease and to monitor the emergence of molecular resistance have a high clinical relevance
- Large prospective trials are needed, and it is essential to standardise the techniques used to analyse ctDNA,
LIQUID BIOSPY: RATIONALE

• Resistance to targeted therapy develops over time due to tumor heterogeneity, clonal evolution and selection\(^1\)

• Tumor biopsy is the current gold standard for both diagnosis and monitoring of resistance, but has significant drawbacks including:
  • Difficulties obtaining sufficient tumor material for analysis
  • The need for invasive serial biopsies
  • Sampling bias due to tumor heterogeneity\(^2\)\(^-\)\(^4\)

• “Liquid biopsy” is the common term for characterising the genetic profile of a tumor based on a blood sample

CTCs and cfDNA have been demonstrated to provide prognostic information\(^1\)

LIQUID BIOPSY: CIRCULATING TUMOR CELLS OR DNA?

• Unlike ctDNA, circulating tumor cells (CTCs) offer the ability to gain insight into the characteristics of cells responsible for metastasis.¹

• However, CTCs occur at very low levels in blood (1 in 10⁶ to 10⁷ nucleated blood cells), presenting a challenge for detection.²
  • Some patients test positive for ctDNA and negative for CTCs.³
  • CTC counts are lower in CRC than in other cancers – CTCs released from a tumor in the colon may become trapped in the liver before reaching the systemic circulation.⁴

• Using current techniques, the diagnostic performance of ctDNA is superior to that of CTCs in CRC.

LIQUID BIOPSY: REAL-TIME MONITORING IS FEASIBLE WITH ctDNA ANALYSIS

SCOPE OF THE REVIEW

• This review focuses on the potential clinical applications of liquid biopsy using circulating tumor DNA (ctDNA) in colorectal cancer (CRC), including its role:
  - Diagnosis and screening
  - Determining prognosis
  - Monitoring tumor burden
  - Monitoring response
  - Evaluating resistance during treatment
  - Detecting recurrence
TUMOR DNA FOR LIQUID BIOPSY: POTENTIAL SOURCES

• Cancer patients have higher plasma levels of cell-free DNA (cfDNA) than healthy individuals, although cfDNA elevation is not specific to cancer\(^1\)
• ctDNA is the fraction of cfDNA that carries tumor-specific alterations\(^2\)
  • Apoptosis is the major mechanism of ctDNA release\(^2,3\)
  • Plasma is the preferred source of ctDNA for liquid biopsy, as serum ctDNA is diluted by genomic DNA released from white blood cells\(^4,5\)
• Tumor-derived **exosomal DNA** is promising as a future alternative to ctDNA\(^6,7\) (tumor-derived genomic material from extracellular vesicles is more concentrated and better preserved as compared to ctDNA)
• Analysis of circulating free or exosomal **microRNA** (miRNA) is also under investigation (see next slide)\(^8\)

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miRNAs are released from cells into blood, which then circulate in various secreted extracellular vesicles, such as apoptotic bodies and exosomes\textsuperscript{1}

AGO, Argonaute

## DETECTING ctDNA: ADVANCES IN SEQUENCING TECHNIQUES

<table>
<thead>
<tr>
<th>Principle of detection</th>
<th>Techniques</th>
<th>Sensitivity</th>
<th>Application</th>
<th>Limitations</th>
<th>Advantages</th>
<th>Clinical use with ctDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digital PCR</td>
<td>BEAMing, Droplet-based digital PCR, Microfluidic digital PCR</td>
<td>0.01%-0.1%</td>
<td>SNV, known genomic rearrangements only</td>
<td>Specific equipment, cost</td>
<td>High sensitivity</td>
<td>Recurrence, prognosis, monitoring response and resistance</td>
</tr>
<tr>
<td>Targeted deep sequencing</td>
<td>Safe-SeqS, TAmSeq, Ion-AmpliSeq CAPP-Seq</td>
<td>0.01%-0.1%</td>
<td>SNV, CNV, rearrangements across targeted regions only</td>
<td>PCR sampling bias and sequencing errors</td>
<td>High sensitivity, cost decreasing</td>
<td>Diagnosis, screening, prognosis</td>
</tr>
<tr>
<td>Whole-genome sequencing</td>
<td>PARE, Whole-exome sequencing</td>
<td>1%</td>
<td>Genome-wide SNV, CNV, rearrangements</td>
<td>Expensive, sensitivity improvement ongoing</td>
<td>Genome-wide applications</td>
<td>Future: diagnosis, screening, tracking resistance</td>
</tr>
</tbody>
</table>

BEAMing, beads, emulsion, amplification, and magnetics; CAPP-Seq, cancer personalized profiling by deep sequencing; CNV, copy number variation; PARE, personalized analysis of rearranged ends; PCR, polymerase chain reaction; Safe-SeqS, safe-sequencing system; SNV, single nucleotide variation; TamSeq, tagged-amplicon deep sequencing.
SCREENING AND DIAGNOSIS: METHYLATED ctDNA

• ctDNA detection of individual mutations such as KRAS and BRAF V600E has insufficient sensitivity for population screening (unmutated tumors are undetectable)

• Analysing aberrant DNA methylation of specific gene promoter regions in ctDNA is an alternative approach

• Methylated SEPT9 has been evaluated in a number of CRC screening studies, with mixed results (sensitivity from 50 to 90% mostly dependant of tumor stage)

  • A SEPT9 methylation assay is approved in the US for screening of CRC (Epi proColon®; Epigenomics, Inc., Germantown, MD)

• A BCAT1 and IKZF1 methylation assay identified ~70% of CRC in recent studies

SCROLLING AND DIAGNOSIS: MULTIGENE PANELS

• Multigene methylation panels may improve sensitivity and specificity\textsuperscript{1-5}

• A multi-target, stool-based CRC screening test (\textit{Cologuard}\textsuperscript{®})\textsuperscript{6} is also approved in the US

• Nevertheless, further data and standardisation are needed before these approaches are ready for clinical practice

PROGNOSTIC VALUE OF ctDNA

- Many CRC patients receive potentially toxic and unnecessary adjuvant therapy due to an inability to identify which patients will experience disease recurrence after surgery

- Detection of ctDNA before surgery has been linked to a high risk of recurrence\(^4,7-12\) and shorter disease-free survival and overall survival.\(^{12-15}\)

- Detection of ctDNA after surgical resection is associated with minimal residual disease and a high recurrence rate (>90%) which can be detected before radiologic recurrence (up to 10 months before)\(^1,2-6,17\)

- Large trials are needed to confirm the prognostic value of ctDNA

## DETECTING RECURRENCE AFTER CURATIVE SURGERY USING ctDNA

<table>
<thead>
<tr>
<th>Reference</th>
<th>Gene(s)</th>
<th>Treatment</th>
<th>Patients (N)</th>
<th>Detection rate of recurrence in plasma, %*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diehl 2008</strong>&lt;sup&gt;1&lt;/sup&gt;</td>
<td><em>APC, TP53, KRAS, PI3K</em></td>
<td>Surgery ± chemotherapy (61%)</td>
<td>18</td>
<td>100% (15/15)</td>
</tr>
<tr>
<td><strong>Frattini 2008</strong>&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Total DNA, <em>KRAS, p16INK4a</em></td>
<td>Surgery ± chemotherapy</td>
<td>70</td>
<td>100% (18/18)</td>
</tr>
<tr>
<td><strong>Ryan 2003</strong>&lt;sup&gt;3&lt;/sup&gt;</td>
<td><em>KRAS</em></td>
<td>Surgery ± chemotherapy (53%)</td>
<td>94</td>
<td>91% (10/11)</td>
</tr>
<tr>
<td><strong>Tie 2016</strong>&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Personalised assay</td>
<td>Surgery ± chemotherapy</td>
<td>230</td>
<td>41% (11/27) in surgery-only group</td>
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</table>

* (n positive ctDNA / n recurrence)

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MOONITORING TUMOR BURDEN AND RESPONSE ON TREATMENT

• Serial plasma ctDNA measurements can track tumor burden dynamics over time, outperforming carcino-embryonic antigen (CEA)\textsuperscript{1}

• Liquid biopsy also has potential for monitoring treatment response (see next slide)\textsuperscript{2-5}

• Changes in ctDNA levels may predict treatment response early in the course of therapy, thereby creating a critical window of opportunity for intervention (i.e. treatment modification)

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# ctDNA AND TREATMENT OUTCOMES

<table>
<thead>
<tr>
<th>Reference</th>
<th>Gene(s)</th>
<th>Treatment</th>
<th>Patients (N)</th>
<th>Detection rate in plasma, %*</th>
<th>Predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lefebure 2010</strong>&lt;sup&gt;1&lt;/sup&gt;</td>
<td><em>KRAS</em>, <em>RASSF2A</em> methylation</td>
<td>Chemotherapy</td>
<td>31</td>
<td>52% (12/23)</td>
<td>Shorter PFS in patients with positive ctDNA (median 5 vs 14 months; <em>P</em>=0.004).</td>
</tr>
</tbody>
</table>
| **Spindler 2014**<sup>2</sup> | Total cfDNA and *KRAS*       | Cetuximab + irinotecan        | 73           | –                            | Higher response rate in patients with >50% decrease in total cfDNA at cycle 3 (40% vs 17%; *P*=0.04)<sup>†</sup>  
Poor disease control in patients with high levels of *KRAS*-mutant ctDNA (0% vs 42%; *P*=0.048)<sup>‡</sup> |
| **Spindler 2012**<sup>3</sup>  | Total cfDNA and *KRAS*       | Cetuximab + irinotecan        | 108          | 78% (32/41)                  | Low baseline cfDNA associated with higher disease control rate                  |

* (n plasma positive / n tumors positive); † Versus patients with <50% decrease in cfDNA; ‡ Disease control rate for patients with *KRAS*-mutant ctDNA in the highest quartile vs those with lower levels.
PFS, progression-free survival.

cfDNA LEVEL HAS A STRONG PROGNOSTIC VALUE IN CRC

Progression-free and overall survival for patients stratified by cfDNA low* (blue line) and high* (dashed line) as reported by Spindler et al.\textsuperscript{1}

*Threshold of 7,100 alleles/mL

TRACKING RESISTANCE AND TAILORING THERAPY

- During targeted therapy, serial monitoring of ctDNA may enable early detection of molecular changes that confer resistance, providing an opportunity for treatment modification.

- A cornerstone for ctDNA analysis in CRC has been the identification of emerging RAS mutations for acquired resistance to anti-EGFR therapy\(^1\)-\(^4\)
  - Numerous secondary KRAS mutations have been identified in serum ctDNA and may be detected 5-10 months before radiologic documentation of disease progression\(^1\),\(^3\),\(^4\)

- Additional mechanisms of secondary resistance detected in ctDNA during anti-EGFR therapy include up to 70 novel mutations in MAPK pathway genes,\(^5\) and MET amplification\(^6\)

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Detection of circulating KRAS mutant DNA in a patient with acquired resistance to cetuximab therapy as reported by Misale et al.¹
CONCLUSION

• Large, well-designed trials using standardized methodology are needed to:
  - Identify the most sensitive biomarkers for CRC screening
  - Compare relevance of ctDNA mutation vs tissue at diagnostic but also to track resistance.
  - Validate clinical relevance of ctDNA to evaluated tumor burden (i.e. change earlier chemotherapy regimen if ctDNA increase before radiologic progression is associated with an increased survival ?)
  - Compare the performance of ctDNA with CTCs and, in future, extracellular vesicles

• Transition of blood-based assays from bench to bedside is ongoing