Chapter

Utilising Proteomics and Organoid Cultures for Predicting Treatment Response in Colorectal Cancer

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Abstract

Colorectal cancer (CRC) remains one of the most frequently diagnosed tumours worldwide. Despite advances in surgical intervention and therapeutics, development of chemoresistance remains a challenge to treating CRC. Predicting treatment response in CRC has strongly relied on genomics, transcriptomics and epigenomics, combined with different cancer staging and classification systems. Despite being beneficial, these omics technologies fail to provide any assessment at a protein level. Thus, having high-throughput tools that assess tumour response to therapy at a protein level will definitely complement the current approaches. In this regard, the field of proteomics holds promise to understand treatment response in tumours. Additionally, patient-derived tumour organoids are replacing the traditional cell lines and xenograft models as the preferred *in vitro* models for predicting clinical response due to being a better representative model of typical tumour characteristics *in vivo*. Combining proteomics and tumour organoids can provide more personalised and optimal treatments for CRC in the coming years. This chapter aims to provide an overview of the progress made in proteomic research and use of organoids for understanding CRC treatment response, together with discussing the strengths and limitations of these two approaches when linked together. This overview will then be used to propose future perspectives.

Keywords: colorectal cancer, proteomics, organoids, treatment response, prediction

1. Introduction

Despite the methodological advancements made in cancer detection and treatment administration, colorectal cancer (CRC) remains one of the most common types of gastrointestinal malignancies diagnosed worldwide [1]. Development of this tumour involves genetic, histological and morphological changes which arise within the crypt cells of the colon or rectum. Hyperproliferation of these cells gives rise to benign polyps which protrude the surface of the epithelial cells within the intestinal lumen. Progression of pre-cancerous polyps can take a few years or decades to become malignant polyps, referred to as adenocarcinomas. This phenomenon is associated with different forms of inherited, acquired and epigenetic mutations in different proto-oncogenes and tumour suppressor genes, which accrue in several mechanisms [2, 3].

To deal with CRC progression and metastasis, different staging and classification systems together with different modes of treatment have been established throughout the years [4, 5]. Despite the advancements made in therapeutic strategies, CRC mortality rate remains high, and development of chemoresistance due to different circumstances remains a major constraint to patients being treated [6–8].

Current research and preclinical treatment development is centred around the traditional tumour biology research models of xenografts and two-dimensional (2D) cell culturing. Unfortunately, cell lines in particularly, do not always present an integrative microenvironment of cells living within a tissue, cannot replicate tumour heterogeneity and at times cannot retain all genetic information. Additionally, for xenografts, genetics and growth environment tend to differ from those of patients, have a lower success rate, are more time consuming and costly [9]. All in all, measures to evaluate the standardisation of CRC therapy are not well established, thus the urge to develop new tumour models and to identify accurate and substantiated predictive markers is required, so that clinicians can appropriately select which chemotherapy to administer.

Throughout the last decade, various research teams have taken the initiative to predict treatment response through different high-throughput methodologies, some of which in the coming years could potentially accompany the current staging and classification systems used. Proteomics, which is the study of proteomes and their functions in cells and tissues, is one of the fields that has stood out the most, due to the promising opportunities it has presented when it comes to understanding treatment response in various tumours, including CRC [10–12]. Additionally, three-dimensional (3D) culturing is another high-throughput technique which has made rapid progress in the fields of drug discovery and screening. This form of culturing is an advanced system in which cells from both healthy or tumour tissues are cultured as spheres in a scaffold or non-scaffold-based system. In turn, this approach provides a better representation of an *in vivo* environment when compared to the traditional 2D monolayered cell culturing system [13–15]. This model permits the development of either spheroids (through cell lines using a scaffold or non-scaffold system) or organoids (through tissue samples using a scaffold system). The two models have similar and distinctive purposes, however the preparation, time, and tumour cell sources needed to establish the respective model differs [15]. Patient derived organoids (PDOs), have shown potential in different research fields, including high throughput drug screening analysis and to analyse the efficacy of different treatments [13, 16]. However, their use in predicting treatment response in relation to proteomics is still fairly novel, thus further research is still ongoing.

The purpose of this chapter is to first provide an overview of the current CRC staging and classification systems and their involvement in predicting treatment administration. Then, the chapter will address the involvement and progress of proteomics and PDOs, in predicting therapy response in CRC. Based on this, it will end by discussing the strengths and limitations of these two approaches when linked together, as well as propose potential future perspectives in this field.

2. Colorectal cancer (CRC)

Like many cancers, CRC development involves multiple different mutations and is linked to various risk factors. Most of the diagnosed patients display alterations in a number of proto-oncogenes and tumour suppressors which result in the

dysregulation of specific signalling pathways: mainly the Wingless-related integration site (WNT)/β-catenin pathway (mutations in the adenomatous polyposis coli (*APC*) gene), Rat Sarcoma Virus/Rapidly Accelerated Fibrosarcoma/Mitogen activated protein kinase/Extracellular signal regulated protein kinase (RAS-RAF-MEK-ERK) pathway (mutations in the *KRAS* gene), transforming growth factor-beta (TGF-β) pathway (mutations in the mothers against decapentaplegic homologue 2 and 4 (*SMAD2/4*) genes), p53 related pathways (mutations in the tumour protein 53 (*p53*) gene), phosphatidylinositol-3-kinase/Akt/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway (mutations in the *PIK3CA* gene) and DNA mismatch repair system (several gene mutations), among others [2, 17].

CRC development is also dependent on three different pathways: (1) microsatellite instability (MSI) pathway (2) chromosomal instability (CIN) pathway and (3) CpG island methylator pathway (CIMP) [2, 3, 18]. MSI-tumours are linked to mutations and inactivation of the DNA mismatch repair system which arise from gene errors due to DNA polymerase slippage, giving rise to uneven microsatellite lengths [2]. The genes typically affected in these pathways include MutL homologue 1 or 3 (MLH1 or MLH3), MutS homologue 2 or 6 (MSH2 or MSH6) and post-meiotic segregation 2 (PMS2). Furthermore, CIN tumours account for the bulk of the cases, and these arise due to mutation build up in the *TP3*, *APC* and *KRAS* genes, among others which occur less frequently [3, 18]. As for CIMP tumours, these exhibit a high degree of promoter hypermethylation on tumour suppressor genes, giving rise to transcriptional inactivation [2, 3].

Considering the known mutations and pathways affected, the CRC carcinogenesis genetic model proposed by Fearon and Vogelstein [19] is at present the accepted model for CRC progression. Since CRC is considered as a heterogeneous disease, patients present unique genetic and epigenetic modifications; hence, the therapy administered, mortality and heterogeneity differ between patients [20]. Current CRC therapy options are limited, thus treatment selection for each patient is dependent on the classification (extent) of the tumour as will be explained in the coming sections.

2.1 Staging and classifications

As introduced previously, CRC is a heterogeneous disease comprised of different subtypes, which can be distinguished by the clinical and/or molecular features presented. Due to the different mutations and pathways that have been defined for CRC development, biologically distinct groups having their respective characteristics have been proposed. The currently available technologies have enabled the generation of large-scale sequencing data for the identification of genetic and epigenetic CRC alterations. To understand and classify CRCs into different subtypes that can be used to predict treatment response, prognosis and cancer relapse risk, different molecular biomarkers have been utilised, including: (1) CRC developmental pathways (CIN, MSI, CIMP), (2) polymerase ε (POLE) mutations, (3) LINE-1 Hypomethylation, (4) RAS, BRAF, and PIK3CA mutations in the MAPK/PIK3 pathway, (5) mutations in the WNT/APC/CTNNB1/TGF-β pathway, (6) TP53 mutations and (7) immune biomarkers and the microbiome [20].

Different classifications have been established and proposed to categorise CRC diagnosis by molecular subtype [20–30]. These CRC molecular subtypes consider different biological features, alterations and clinical behaviour. However, the currently most accepted CRC classification is that proposed by Guinney et al. [30],

composed of four consensus molecular subtypes (CMS, CMS1-CMS4). These subtypes are based on different levels of immune infiltration, distinct mutations and altered somatic copy number alterations. In general, the CMS classification system is the most robust from the established classifications due to having a clear biological interpretability. Thus, it is expected to continue being used for future clinical stratification and subtype-based targeted interventions. All in all, despite the various classifications systems being useful for predicting treatment outcome in patients, such systems do not consider tumour heterogeneity which is typically the reason for therapy resistance.

Prior to the development of CRC classifications, categorisation of diagnosed CRC patients was based on clinical and pathological features, mainly the degree of differentiation, the stage of the tumour and the localisation of the tumour [21]. Various CRC staging systems were established by surgeons to categorise the four CRC stages (I–IV) for diagnosis and treatment. The preferred staging system is known as the American Joint Committee on Cancer (AJCC) tumour-node-metastasis (TNM) staging model, first implemented in 1977 [31]. Since then, this model has been continuously revised, with the latest being the eighth edition, released and implemented globally in 2018 [31]. Prior to this system, two other models were developed, the Dukes' staging system [32] and the Modified Astler-Coller (MAC) classification [33], implemented in 1932 and 1954, respectively. The limitation of these two models is that only tumour invasion depth and lymphatic metastasis is considered [32–35]. Thus, both have now been replaced with the TNM staging model, which is dependent on (1) tumour size and invasion (T), (2) regional lymph nodes involvement (N) and (3) metastasis (M) (**Table 1**) [31, 34]. Lastly, CRC histological grading is denoted as 'G' and this defines the state of cell differentiation when compared to a healthy cell (G1: well differentiated, G2: moderately differentiated, G3: poorly differentiated and G4: undifferentiated) [37].

Cell Type	**TNM Stages (AJCC-8)**				**Other Staging Methods**	
	Stage	**T**	**N**	**M**	**Dukes**	**MAC**
Healthy	0	Tis	N0	M0	—	—
Polyp	I	T1–T2	N0	M0	A	A–B1
Tumour	IIA–IIC	T3–T4b	N0	M0	B	B2–B3
Extended to Lymph Node	IIIA–IIIC	T1–T4b	N1c–N2b	M0	C	C1–C3
Metastasis	IVA-IVC	Any T	Any N	M1a–M1c	D	D

Roman numbers (I–IV) describe disease severity (least to most severe—I to IV). Stage 0 are carcinoma in situ, stage I cancers are small, less deeply invasive and have not reached the lymph nodes, stage II and III cancers refer to tumours which have increased in size and stage IV cancer refers to distant metastasis. Tis; tumour limited to mucosa, T1; tumour invaded submucosa, T2; tumour invaded muscalaris propria, T3; tumour invaded subserosa and beyond but not to other organs, T4; tumour invaded other organs (T4a: Invades visceral peritoneum, T4b: Invades or adheres to other organs or structures). N0; no regional lymph nodes (RNLs) metastasis, N1; metastasis to 1–3 RNLs (N1a: 1 RLN metastasis, N1b: 2–3 RLNs metastasis, N1c: metastasis into areas of fat near lymph nodes but not in the nodes), N2; metastasis to 4 or more RLNs (N2a: metastasis to 4–6 RLNs, N2b: metastasis to 7 or more RLNs). M0; no distant metastasis, M1; distant metastasis (M1a: metastasis to distant organ/site without peritoneal metastasis, M1b: metastasis to 2 or more organs/sites without peritoneal metastasis, M1c: metastasis to peritoneal surface with or without other organ/site metastases). Information summarised in this table was retrieved from: [4, 31, 34, 36].

Table 1.
A summarised classification for the different CRC staging systems.

2.2 Treatment administration

Different approaches are considered when treating CRC, starting with simple endoscopic polypectomy to remove any polyps which are benign or potentially malignant, to more sophisticated surgical interventions to eradicate non-metastatic primary tumours. Stage 0 to early-Stage II CRC are normally curative through surgery [4, 9], however nowadays some patients are inoperable due to bulky tumours. Thus, a range of therapy regimens are selected to shrink the metastatic lesion, which prolongs patient survival rates and reduces risk of metastatic spread due to microscopic tumour foci, distant from the primary tumour location [3, 9]. Nowadays, Stage I/II CRC patients can also receive neoadjuvant chemotherapy, while late III/IV stages CRC receive adjuvant treatment (**Table 2**), with the latter form of therapy at times also being administered to high risk stratified Stage II CRC patients [4, 5]. Cytotoxic agents [5, 8], administered as single agents or in combination, immunotherapy [38], targeted therapy [40] and sometimes radiotherapy [4], are the main treatment regimens for CRC (**Table 2**). Through these approaches, clinicians attempt to improve the response rate and overall survival of patients, especially those with metastatic CRC (mCRC). Despite the wide range of treatments available, it is estimated that around 90% of patients with late-stage CRC are resistant to the available frontline therapy [14]. Thus, combination therapy has been implemented to prevent the development of chemoresistance, to increase response rate and to reduce potential toxicity which arises when single cytotoxic agents are administered [5].

2.3 Current approaches for predicting treatment administration

Most registered CRC studies with targeted medicines in previous decades had no pre-planned biomarker analyses, apart from exploratory analysis, and did not stratify patients into biomarker-defined subgroups [29]. Significant advances being implemented have demonstrated slightly improved treatment predictions. Despite this, selecting which form of therapy to administer remains a complex process for each patient due to the lack of evidence for the CRC therapy existing, particularly chemotherapy [8]. Individual cancer patient therapy is presently dependent on clinical gene sequencing, however only 7% of the population benefits from personalised care established from next-generation sequencing (NGS) [9].

One of the initial advances arose from a retrospective correlative clinical trial analysis which focused on innate resistance to anti-EGFR treatment due to the KRAS mutations on exon 2. This biomarker stratification served as the first precision medicine CRC model ('one gene, one drug' paradigm), since patients harbouring KRAS mutations on exon 2 do not benefit from cetuximab and panitumumab [29, 40]. However, this concept had major limitations when it was employed to study potential predictive CRC markers [29]. Similar efficacy was obtained when administering BRAF inhibitors [41] or MEK inhibitors [42] to advanced CRC bearing specific *BRAF* or *KRAS* mutations, respectively. Other molecular biomarkers have shown to serve as predictive biomarkers in CRC, including miRNAs, Phosphatidylinositol 3-kinase catalytic subunit alpha (PI3KCA), VEGF and Human epidermal growth factor receptor 2 (HER2) [20, 43–45]. Furthermore, specific biomarkers for selected cytotoxic agents have served as biomarkers for predicating efficacy and toxicity of said agents [45, 46].

From the known classifications, CMS subtypes have shown to be of prognostic significance due to being suitable for the assessment of therapy responses and treatment choice [47–49]. For instance, Kwon et al. [50] used this classification to categories 101

Treatment	Class	Mechanism of action	Application
5-Fluorouracil (5FU) (cytotoxic agent)	Antimetabolite (pyrimidine analogue)	Inhibits thymidylate synthase (TS)	Alone or in combination for adjuvant or palliative care
Capecitabine (cytotoxic agent)	Antimetabolite (pyrimidine analogue)	Inhibits TS	Alone or in combination for adjuvant (Stage III) treatment
Irinotecan (cytotoxic agent)	Topoisomerase I (Topo I) inhibitor	Inhibits Topo I	Combined with FOLFOX, capecitabine or cetuximab for mCRC
Oxaliplatin (OXA) (cytotoxic agent)	Alkylating agent (platinum compound)	Inhibits DNA replication/ transcription	Combined with FOLFOX for adjuvant treatment and mCRC
Regorafenib (targeted therapy)	Kinase inhibitor	Tyrosine kinase inhibitor	Alone for mCRC
Cetuximab (targeted therapy)	Monoclonal antibody	EGFR inhibitor	Alone or combined with irinotecan or FOLFOX for mCRC
Bevacizumab (targeted therapy)	Monoclonal antibody	Vascular endothelial growth factor (VEGF) ligand inhibitor	Combined with FOLFIRI for mCRC
Panitumumab (targeted therapy)	Monoclonal antibody	EGFR inhibitor	Alone or combined with FOLFOX or FOLFIRI for mCRC
Pembrolizumab (immunotherapy)	Monoclonal antibody	Inhibits programmed cell death protein 1 (PD1)	mCRC
Nivolumab (immunotherapy)	Monoclonal antibody	Inhibits PD1	mCRC
Aflibercept (targeted therapy)	Recombinant fusion protein	VEGF-A and placental growth factor (PIGF) inhibitor	Alone or combined with FOLFIRI for mCRC
FOLFOX	Combination treatment (5-FU, leucovorin (LV) and OXA)		Adjuvant chemotherapy
FOLFIRI	Combination treatment (5-FU, LV and irinotecan)		Adjuvant chemotherapy
FOLFIRINOX	Combination treatment (5-FU, LV, irinotecan and OXA)		Adjuvant chemotherapy
XELOX	Combination treatment (OXA and capecitabine)		Adjuvant chemotherapy
Radiotherapy	At times combined with 5-FU or capecitabine		mCRC

Information summarised in this table was retrieved from: [4, 5, 8, 38, 39].

Table 2.
Current therapy used against colorectal cancer (CRC).

patients with stage III CRC which were treated with FOLFOX. However, despite the significant role shown by CMS subtypes in predicting treatment response throughout the last decade, this classification is not suitable for selecting patients for treatment with anti-VEGF or anti-EGFR agents [21].

The introduction of NGS with pre-screening approaches and clinical sample trials, together with the use of advanced preclinical models (organoids), are now being implemented to further characterise target agents in CRC [29]. This has helped in identifying and validating new predictive biomarkers, as well as gaining a better understanding of dynamic target inhibition so as to develop novel combination therapy which improves the overall patient outcome. Lastly, proteomics is another field which has slowly started to be implemented in treatment prediction throughout the last decade [10–12], however the advancements made will be discussed in the coming section.

3. Proteomics

Proteomics is generally defined as the comprehensive study of the proteins inside a cell, considering both their levels and distribution. Proteomes are dynamic and change in a spatial, temporal, or chemical manner, expanding the roles that the available complement of proteins can perform within a cell. One of the major aims of such investigations is to deduce the changes to biological pathways and cellular operations with the onset and progression of disease [51].

Similar to the expansion of genomic and transcriptomic information by inclusion of epigenetics (e.g. CpG promoter methylation), the acquisition of information from post-translational modifications (PTMs) can be considered as epiproteomics. The most common PTMs investigated are phosphorylation, acetylation and methylation, although proteins can undergo over 200 PTMs, which depend on cell type, cellular context, biological condition, and other parameters. Each PTM can alter protein properties, having some form of effect on protein function [51–54] and can also confer distinct biomarker properties to proteins [55]. Phosphorylation and acetylation are linked to protein activation, while methylation can alter the majority of the protein characteristics depending on the cellular conditions [54]. Throughout cancer development and the eventual therapy resistance, the aberrant signalling arising is not only due to an overall change in protein expression, but also due to changes in protein activity arising from the addition or removal of PTMs taking place on key proteins [52, 53, 56].

Proteomics incorporates numerous methods utilised for the measurement, large scale recognition, characterisation and analysis of proteins [53, 54]. With the continual development taking place in this field and its application in various diseases, including cancer, substantial improvement has been achieved in discovering clinically applicable biomarkers [57]. The main tool used for proteomics is mass spectrometry (MS), principally because it is sensitive, versatile, and can identify target proteins found in complex sample matrices. The approach most commonly used is known as bottom-up proteomics, also called "Shotgun Proteomics", in which the protein sample is enzymatically or chemically digested and then separated by liquid chromatography (LC) before being identified by tandem mass spectrometry (MS/MS), hence the name LC-MS/MS. On the contrary, the less popular, top-down approach analyses intact proteins, with the major advantage of the latter being the complete coverage of the protein sequence [58–60].

This omics approach permits the qualitative and quantitative profiling of several proteins within a sample. LC-MS/MS is the key approach to obtaining high-resolution spectra of mixed peptides, which in turn permit identification of sensitive and unique

biomarkers [57, 58]. For accurate quantification analysis and minimal discrepancies, both label-based and label-free quantification approaches have been developed, both of which have been used in clinical research [57, 60–62]. Through label-based approaches, the tagged protein can be compared to the control proteins tagged with isotope-free markers in a qualitative or quantitative manner [57, 58]. Different forms of labels having been developed, including SILAC (stable isotope labelling by amino acids in cell culture), Heavy methyl-SILAC (hmSILAC), Tandem Mass Tag (TMT), Isotope-Coded Affinity Tag (ICAT) and isobaric Tag for Relative and Absolute Quantitation (iTRAQ) [57–63]. These labelling approaches permit multiplexing of several samples under different experiment conditions within the same run and reduce the experimental biases and time needed for analysis [59, 61]. As for label-free approaches, "Targeted Proteomics" is preferred due to its high sensitivity, accuracy and reproducibility [58, 61]. This technique allows the focus on a subset of proteins of interest and is possible through Multiple Reaction Monitoring-Mass Spectrometry (MRM-MS), Selected Reaction Monitoring-Mass Spectrometry (SRM-MS), or Sequential Window Acquisition of all Theoretical fragment ion spectra (SWATH) [11, 57, 61]. Through the MS analysis performed, proteins can be quantified based on the intensity of the signals or spectral counts obtained for the peptides of interest. Apart from MS-based approaches, the amount of protein within a sample can also be semi-quantitatively or quantitatively analysed through antibody arrays or enzyme-linked immunosorbent assays (ELISA) [11, 55, 57].

Different studies have applied proteomics to CRC using most of the aforementioned approaches to investigate either cell lines or patient tissues samples [12, 55, 58, 64, 65]. However, utilisation of proteomics and CRC PDOs to understand and predict treatment response in a clinical settings has been extremely limited, thus this will be the main focus in the next sections.

3.1 Clinical proteomics

Current clinical cancer testing relies heavily on genomics to identify and classify patient tumours based on known mutations in key genes within the regulatory biochemical pathways important for a specific cancer type. This is due to the ease and accessibility of genetic techniques. However, such genetic biomarkers for diagnosis, prognosis and therapeutic effectiveness fall short of their aim as they do not take into consideration all the downstream changes that the products of such genes undergo, until they come to perform their cellular roles as proteins. Furthermore, genomics gives no information related to protein localisation, turnover, PTMs or functional activity, all of which can impinge on the effectiveness of therapeutics [56, 57].

The primary purpose of clinical proteomics is to analyse the proteome and its modifications in body fluids, cells and tissues so as to ascertain distinctive or signature biomarkers which can be utilised in a clinical setting, so as to promote personalised medicine [61, 63]. This interdisciplinary field highlights the efforts and research needed to further move forward. Clinical proteomics translates the biochemical data generated in the lab related to tumour changes undergone throughout the process of carcinogenesis up to metastasis and therapeutic evasion into patient-specific data, which provides a useful tool in improving decision-making to define the steps that can be taken to better treat a patient in a targeted manner. Clinical proteomics thus adds a critical layer of information to the available genomic data such that while the genomics provides the complement of mutations that give the tumour growth advantages, metastatic properties and resistance to therapy, the proteomics

provides an indication of any aberrant protein activity in the tumour, adding the functional consequences of the genomic data at the proteomic level [56].

Clinical proteomics can thus benefit patients with regards to cancer detection, treatment and management. As proteomic technologies improve and the potential of clinical proteomics grows, the applications and benefits for patients will improve. The application of serum proteomics could improve early cancer detection through non-invasive testing. The availability of reliable biomarkers for diagnosis and molecular classification at an early stage would increase the therapeutic options. The quantification of enzymatic activity using high-throughput array-based proteomics would allow more personalised therapeutic regimens targeting the most critically dysregulated pathways. Therapeutic efficacy and toxicity could then be assessed in real-time so as to adjust dosage or change treatment if resistance is detected [56].

3.2 Predictive biomarkers for clinical proteomics

In recent years, the search for protein biomarkers has become crucial. Biomarkers, as defined by the National Cancer Institute, are biological molecules found within the blood, other body fluids or tissues, which may be used as indicators for identifying signs of a normal/abnormal process, or of a pathological condition. Identifying biomarkers is of significant interest because these markers are suitable for: (1) evaluating clinical prognosis, (2) assessing and identifying risk of recurrence (diagnostic biomarkers), (3) following the development of disease or predicting relapse (prognostic biomarkers) and (4) determining and improving patients' response to therapy (predictive biomarkers) [11, 61]. Cancer biomarkers in the clinic are used to provide quantifiable information about the aberrant cellular processes arising in tumours and this information is critical for targeting the molecular mechanisms driving the cancer as well as determining the effectiveness of the therapeutic regimens administered to patients. While at a clinical level, diagnostic biomarkers assisting in histopathological tumour classification are the most commonly used, both prognostic and predictive biomarkers are needed for clinicians to determine a tumours level of malignancy and to exploit therapeutic sensitivities so as to provide more effective treatment regimens, respectively [66]. Through proteomics, one can examine several tumour proteins, thus hypothetically generating novel therapeutic targets and markers for CRC. Additionally, protein markers could be measured easily through routinely available body fluids, thus reducing the necessity for fresh or frozen tissue biopsies. Even though different research groups have shown that CRC leads to fluctuations in the blood proteome [67, 68], blood biomarkers specific to CRC have not been validated or approved for clinical uses.

As well reviewed by Chauvin and Boisvert [62] and Lee et al. [69], predictive biomarker discovery has proven to be quite a laborious process, with three stages being involved: (1) discovery/screening, (2) verification and (3) validation. The initial step is performed via shotgun proteomics, using small cohorts of patient tissue samples, whose proteins can be extracted and analysed through MS. The proteome is examined to monitor and identify any dysregulated proteins between different groups of patients (e.g. responsive vs. unresponsive). Different labelling techniques are applied to better quantify the proteins within samples. In the second stage, the proteins presenting the biggest changes between the different cohorts are selected for verification. Targeted proteomics is used here as it facilitates precise and accurate quantification of the selected proteins, across a slightly larger cohort. Thirdly, the validation stage involves the clinical assessment phase of the biomarkers which involves very large

cohorts to validate the sensitivity and specificity of the putative biomarkers. The main drawback of the latter stage is that very few studies have been reported with regard to protein biomarker identification for predictive therapy response through the use of proteomics and human samples, especially for CRC [61]. Lastly, the ideal biomarker selected should be sensitive and precise for the proteins of interest in a cost-effective assay, which is fast and robust against both inter-operator and inter-institutional variability. For a biomarker to be reliable it has to be validated through a regulated clinical study having a variety of patients, utilising thorough standards for each step, from sample collection to result analysis, all of which should be reproducible by different laboratories [56].

It has become apparent that no single biomarker exists for a particular cancer type due to the substantial heterogeneity existing within the proteome of patients, together with the processes involved in the development of the disease or therapy resistance. Moreover, most biomarker breakthroughs employ laborious searchers for one or a small range of dysregulated proteins in cancer samples, through which a panel of biomarkers can be selected for clinical analysis [56]. Different proteomic approaches have been utilised to identify new CRC biomarkers to elucidate not only molecular mechanisms, but to also predict treatment response. However, the latter has only been slightly investigated, especially from a clinical perspective and through the use of PDOs. Despite being far from pathophysiological tumour conditions, cell lines have been used mostly to model and reveal predictive biomarkers through proteomics, due to being inexpensive and easy to manipulate to generate resistant cultures. Most studies that used cell lines have made use of both gel-based and gel-free approaches, in order to compare the differential protein expression profiles in cell lines pre- and post-treatment administration [70–72]. Even though PTMs have not being given that much importance in their potential use as predictive biomarkers, some research groups have or are currently investigating their potential through the use of 2D cell lines [73–75], 3D spheroid cultures [73, 76, 77] or patient samples [78, 79], with the majority focusing on phosphorylated proteins. Use of spheroids for proteomic studies provides more valuable data about how therapy might affect an *in vivo* tumour when compared to 2D cultured cell lines [80].

The different CRC-related proteins discovered from proteomic-based studies indicate that these might be novel predictive biomarkers for CRC. Thus, further proving that proteomics is an absolute, highly reliable and translatable research tool for identification of novel biomarkers in cancers. However, further investigation on current putative biomarkers, together with others yet to be discovered can result in the development of a panel of markers which have adequate sensitivity and specificity for CRC in a clinical and therapeutic setting. Apart from total protein levels, more research efforts are being put into quantifying protein activity and the levels of key PTMs in an effort to provide patients with more suitable therapy regimens [56].

3.3 Limitations in clinical proteomics

Protein and peptide level identification through different MS-based approaches can recognise and quantify hundreds to thousands of proteins within a biological sample, however this only depends on the complexity and amount of the starting material [64]. Despite this, even from simplified cancer models such as cell lines, where protein yield is generally high, there is very limited amount of information present on most detected proteins, and their potential use as clinical biomarkers. In comparison, protein yield from clinical samples is much lower, due to the complexity

of the samples [64]. Even though studies reporting the detection and quantification of differentially expressed proteins in CRC through various approaches, a full understanding of the implications and functionality arising due to such dysregulations is required for a significant inference. Moreover, identifying the proteins of interest within a particular sample remains cumbersome at times. It is expected that the results derived from the different proteomic approaches will be combined with data collected from other omics approaches to further understand the significance of such dysregulation, as will be discussed in Section 5.

As for PTM-based research, identification and characterisation of PTMs is a challenging task, since these modifications are generally present in low (sub-stoichiometric) amounts and their existence is mostly transient, thus further making it difficult to analyse [64]. Sample preparation for PTM analysis through MS is laborious, requires a large amount of the starting material and contains several optimisation stages when compared to normal global proteome analysis. Additionally, we lack reliable tools and methods for studying PTMs and we lack enrichment techniques for specific PTMs, particularly those making use of antibodies. Commercially available antibodies that are capable of detecting and enriching PTMs are limited in availability, are of low quality and have low binding efficiency. Moreover, the production and application of antibodies is a long and costly process.

Most advancements made in order to (1) increase the number of modified protein or peptides identified and (2) to quantify the difference between modified and unmodified proteins or peptides have focused mostly on phosphorylation. Thus, it is expected that future advanced research will centre around other PTMs, particularly methylation, since this modification has been given the least importance when it comes to identification and quantification [59]. The implication and functional roles for most PTMs arising on proteins in CRC throughout cancer development and the eventual therapy resistance, remains unknown. Moreover, there are still several aspects of PTM biology that need to be defined such as their position, degree and the affector enzymes responsible for giving rise to the different PTMs.

Despite different labelling techniques currently available, the disadvantage of these approaches is the incorporation of a light or heavy amino acid to cells in culture in case of SILAC and hmSILAC [59], or the addition of chemically bonded mass labels to the peptides following preparation, as in the case of iTRAQ and TMT [58, 61], both of which complicate the sample preparation workflow. Consequently, this comes with additional disadvantages due to their high costs, and these techniques being scarcely or not used at all in shotgun proteomics on human samples since label-free quantification is preferred here. The problem with label-free approaches is that accuracy is much lower, the analysis system is quite complex since sophisticated software tools are needed, and multiplexing is not possible, when compared to the labelling approaches [57, 58]. Another limitation for SILAC and hmSILAC is that these two can only be applied to cell culturing samples, but not directly to patient tissue samples. Thus, for this reason, the better option would be to combine the generation of PDOs with these labelling approaches [59, 62]. Moreover, not all of these labelling techniques can be applied to all samples [11].

A common clinical limitation for cancer proteomics studies in general is the patient cohort size available, particularly when high resolution proteomics workflows are applied. Sample analysis for such workflows can take up to 24 h of instrument analysis time, thus limiting studies to either a handful of individual sample analysis or to pooled sample analysis [13]. This is slowly being overcome due to the development of multiplexed MS approaches and the decrease in instrument analysis time needed

due to ongoing development in instrument speed, thus permitting for larger scale clinical proteomic analyses in the near future. Another drawback is the long process of clinical approval needed for the discovery of new biomarkers through proteomic approaches. This is obviously expected, since as explained in Section 3.2, the validation phase demands a lot of further work, to ensure the biomarker selected provides reproducible data. This is not only a limitation in this field but research in general and it is one of the reasons why most putative and candidate biomarkers do not go beyond the proof-of-concept phase [60].

Considering the current knowledge gained through clinical proteomics, these limitations, as well as others well reviewed by Maes et al. [81], will not hinder the discovery and the growing panel of potential biomarkers suitable for the analysis of CRC development, progression and treatment response. Significant scientific and technical limitations are yet to be overcome in the process of identifying putative biomarkers through proteomics, however the constant advancement being made in this field are expected to decrease or eliminate the current bottlenecks.

4. Organoids

Development of 'mini-gut' organoids were first pioneered by Sato et al. [82]. These 3D models are self-organised multicellular structures, primarily derived from adult multipotent stem cells (ASCs-organ specific), human pluripotent stem cells (hPSCs-can differentiate into multiple cell types), embryonic stem cells (ESCs) or cancer stem cells (CSCs) [17]. Recent advancements have enabled the development of these CRC models through different approaches, particularly using patient tumour samples, which in turn provide a better representation of *in vivo* tumours [83]. Organoids are established by culturing cells extracted from tumour tissues in a supportive extracellular matrix (ECM), such as matrigel or basement membrane extract, with collagen IV, laminin and entactin also being major components [14, 84]. The ECM enables long-term proliferation and differentiation capacities; however, these two factors are also dependent on a cocktail of growth factors, small molecules and inhibitors which are supplemented to the culturing medium [14, 84, 85]. Based on the conditions provided, the typical SC niches found within the intestinal crypts are produced, which permit proliferation and differentiation of cells which self-organise into 3D structures. Over the years, organoids have shown to be better models for research in different fields when compared to cell lines and xenografts. Of note, organoids have been implemented to study CRC from different perspectives, such as: initiation, progression and invasion of CRC [84], genetic mutations [83], intratumoral heterogeneity and tumour evolution [86], and drug screening or development [9, 14, 16, 83–86].

4.1 Use in predicting treatment response

Drug screening through PDOs has not been limited to only cancer therapies but has been utilised to screen drugs for a range of diseases, thus further proving the usefulness of these models. It is expected that therapy screening through organoids will further help in predicting treatment response in patients, thus the value of PDOs in predicting the response of cytotoxic agents, targeted therapy and radiotherapy has also started to be investigated. For years, compounds displaying cytotoxic activity on cultured cancer cell lines resulted in being unsuccessful in the beginning stages of

clinical studies. This ineffectiveness is because of dissimilarity between genetically unstable immortal cell lines and patient tumours, and due to cell lines not representing the whole tumour. This has shown to not be the case with PDOs, since genetic and phenotypic characteristics are preserved over long-term culturing, the original features (heterogeneity) of the tumours they are derived from are recapitulated and cell-to-cell or cell-to-matrix interactions are maintained. Different research teams have demonstrated the benefit of using PDOs for drug screening in different settings, mainly; (1) drug innovation, (2) toxicity analysis and (3) precision medicine. Thus, PDOs are a unique system to test and predict drug effects within tumour tissues collected from a patient [17].

Recent reports which made use of intestinal organoids showed the adverse consequences of treatment [9, 86–90]. For instance, organoid cultures showed to be suitable for the detection of genotypes to drug association [86]. Through gene assessment, which revealed a number of altered genes, the authors designed a customised library to screen the sensitivity of a range of drugs, with the relationship between the two being detected through high throughput drug screening. For example, organoids harbouring *KRAS* mutations showed resistance to afatinib and cetuximab, while only two out of 10 *KRAS* wild-type organoid were insensitive to cetuximab [86]. In another study, therapy response of 23 CRCs in clinical trials was compared to that of PDOs. The group found 93% specificity, 100% sensitivity, 88% positive predictive value, and 100% negative predictive value in predicting response to targeted agents or chemotherapy in CRC patients [89]. Interestingly, PDOs have also been utilised to monitor the effect of radiotherapy, whereby PDOs are exposed to such treatment through an irradiator [91, 92]. It should be noted that there have also been times were patients who received PDOs informed therapy did not have any clinical benefit, as discussed in Ooft et al.'s [93] study. Considering all these studies, together with others also discussed in recent reviews by Furbo et al. [91] and Flood et al. [94], it is clearly evident that PDOs can be exploited for therapy analysis, to stimulate cancer behaviour *ex vivo* and incorporate molecular pathology in the verdict process of clinical trials.

4.2 Organoid limitations

Despite being among one of the most reliable models currently available to understand and predict treatment response, use of organoids also has its limitations.

The success rate of PDOs is not only affected by intrinsic experimental difficulties, including bacterial contamination and small tissue sample sizes, but it is also dependent on the culturing medium selected and the characteristics of the tumour (subtypes and mutations) [17]. Additionally, culturing of PDOs can at times be difficult, especially from patients having mucinous tumours, MSI tumours, poorly differentiated, and tumours bearing the BRAF gene mutation [95]. This suggests that patients having any of these characteristics are less prone to be contenders for *ex-vivo* drug testing under standard culturing conditions. No standardised culturing methodologies exist, and the culturing medium used can vary between one organoid and the next, thus experimental variation arises [94]. In addition to the culturing stages, preparation of these cultures is only possible when there is access to a hospital or 'tissue network' through which patient samples can be obtained, together with the required expertise needed to prepare and maintain organoids, which can be considered as additional limitations [86]. In fact, the success rates of organoid development, even with substantial experience, is estimated to be around 70% [83]. Additionally,

the lack of easy and reproducible readout approaches limits their use in high-throughput drug screening studies.

Intratumour heterogeneity is another problem which has to be considered, since at the start of culturing, PDOs present genetic stability and heterogeneity [94]. However, throughout the course of duration this cannot be predicted. During therapy, tumours change over time, thus PDOs established during one interval only represent that specific tumour at the time of culturing [17]. Furthermore, some organoids cannot be expanded for a long period of time, thus improvement in the cell culturing medium should be considered. Since a number of different inhibitors are generally also added throughout the culturing period, these might have a significant effect on signalling pathways and gene expression but could also alter drug sensitivity. Considering all these limitations, further effort is still needed to address these drawbacks, however specific organoids can still be effective models for monitoring and predicting tumour response to different treatments.

5. Advancements in predicting treatment response

To better understand the complex mechanisms and processes involved in CRC, research teams have started to move beyond single omics approaches and have started to integrate multi-omics approaches. This approach involves comprehensive and integrated analyses which are produced from different omics methods, such as proteomics, genomics, metabolomics, epigenomics, and transcriptomics. This multi-analysis can generate much larger datasets compared to only single analysis, thus providing more significant information on the pathophysiology of diseases. In turn, this further supports disease diagnosis, treatment administration and development. Moreover, the implementation of combining omics approaches will most likely have a bigger impact on translational studies, including tumour biology and cancer therapy [57]. As will be discussed in Section 5.2, despite multi-omics proving to be a powerful approach for molecular characterisation and discovery of novel biomarkers, this approach is impeded due to the lack of a standard workflow which can be applied to different cancer types [69]. As this field continues to advance and mature, it is highly likely that combining these different approaches will lay out records of all omics-based data as a whole, which will help provide more significant information at a molecular level for discovering novel predictive biomarkers.

The past and ongoing advances in omics tools have allowed systematic and extensive identification of molecular markers in CRC [58, 69]. Moreover, the involvement of PDOs in both proteomics, and other omics techniques, has slowly started to be implemented throughout the last few years. In relation to CRC, use of PDOs together with the different omics techniques has only been slightly investigated, as will be discussed in the coming sections. A look into the challenges currently being faced in multi-omics in relation to treatment prediction, together with potential future ideas to be considered in this field will also be discussed.

5.1 Combining proteomics and organoids for treatment response

With the recent advancements made in culturing PDOs for use in precision medicine, combining organoids and proteomics together would become valuable for quantifying protein expression changes, thus identifying novel signalling pathways, and suitable biomarkers for better understanding therapeutic response [62]. As of

yet, published data tackling the topic of 'PDOs and proteomics as tools for treatment prediction in CRC' has been very limited, as to our knowledge, only one study has been reported to date in relation to this matter. Schumacher et al. [96] made use of well-characterised CRC organoids and targeted proteomics to investigate the effect of tumour heterogeneity on the KRAS/MAPK-signalling pathway and the effects of treatment by inhibitors targeting EGFR and downstream effectors. Their data showed that heterogeneity presented variable response to EGFR inhibition. These findings could help in improving preclinical assessment of individual tumours by modelling heterogeneity in cultures, to better comprehend therapeutic failure in clinical situations and to improve therapy response prediction [96].

Despite only one study highlighting the potential of combining proteomics and PDOs for analysis of treatment response, this should further encourage other research groups to make use of such an approach in their research interest. This is because proteomic data will further facilitate the mechanistic understanding of differences observed in PDOs treated with various forms of therapy. As discussed in Section 3.2, proteomics together with cell lines have been used to investigate treatment response. However, it is time to replicate such analysis but through the use of PDOs to determine whether the same outcome can be reproduced or not, considering the differences between the two forms of culturing. Moreover, the data collected through PDOs should be of more significance since they provide a better representation of the atypical *in vivo* environment. Another benefit which comes with utilising organoids for treatment response through proteomic analysis is that non-cancerous organoids can also be established. This permits comparison between healthy and tumour proteomes, something which is not possible with either spheroid cultures, or 2D cell cultures [80]. However, it also provides information on whether the therapy being tested is harmful to healthy organoids as well.

Analysis can also be slightly hindered when combining PDOs and proteomics together. One of the main issues is the supporting medium in which the PDOs are generally cultured, that being Matrigel. As discussed in Section 4, since this matrix is composed of several growth factors which are needed to maintain the organoids in culture, this can hamper LC-MS/MS identification of peptides through ion suppression effects [97]. Furthermore, since the matrix is also composed of several proteins, the MS data collected contains a higher background of unwanted peptides within the sample, thus resulting in less identification of organoid proteins [98]. To eliminate such background, one would have to run a sample of matrigel on its own.

Apart from PDOs and proteomics being combined together to understand and predict CRC treatment response, these two approaches have previously been applied to study other biological characteristics, such as protein abundance, signalling pathway analysis, heterogeneity, PTMs, protein localisation and protein–protein interactions [62, 96, 99–101]. Overall, collection of proteomics data from CRC PDOs has been limited and has not been explored enough yet, thus this opens avenues for more novel development in the coming future, especially with respect to predicting treatment response.

5.2 Challenges and future prospects

Further understanding CRC progression, as well as identifying potential predictive biomarkers can refine therapy administration and patient care. The ongoing advancements being made through the different omics approaches will enable a more precise treatment prediction, especially if the use of PDOs is further implemented

in this field. Logically, when comparing the different omics approaches, particularly transcriptomics and proteomics, the latter is more suitable for novel therapy strategies since most protein-based biomarkers depend on the dysregulated protein signalling pathways and their respective PTMs. The proteome provides much more information on the functional state of the cells and tissues over a longer period of time. Proteome profiling of several dysregulated cell signalling cascades are anticipated to provide a better prediction on the behaviour of the disease when compared to single pathway investigations. Further implementing multi-omics studies will improve our understanding of not only treatment outcomes, but cancer related research as a whole. Ideally, different omics approaches should also start being implemented together when using CRC PDOs to understand and predict treatment response. Utilising more than one omics approach and PDOs to understand specific biological characteristics has slowly started being introduced, based on current published data [96, 100, 101].

Another way by which treatment response could be studied is through the use of array-based proteomic platforms, such as the use of peptide or protein arrays. Similar to MS approaches, this technique can provide multiplexing and sensitive analysis, however through the use of lower amounts of sample. Using minimal amounts of patient samples would be of significant benefit in a clinical setting. Additionally, such techniques can be advantageous in situations where MS analysis is not readily available, since these offer a cheaper yet reliable alternative. The use of protein and peptide arrays has shown promising results in disease biomarker discovery with different platforms [56, 69] being readily available for screening aberrant protein expression, including enzymes. In fact, such arrays have shown potential in monitoring treatment response by targeting specific PTMs and monitoring enzyme activity, with most of the currently published studies focusing on phosphorylation and kinase enzymes [102–104]. Most of these studies made use of either cell lines or patient tissues samples, however to our knowledge there have not been any published reports which made use of this technique to predict treatment in CRC through PTMs or enzyme activity. Moreover, the enzyme activity analysis of cell lysates collected from pre- and post-treated PDOs has not been reported, thus it could be a possible investigation in the coming future. Considering the positive results obtained it is expected that this same approach is to be applied to other PTMs and enzymes such as methylation and methyltransferase enzymes, which is something currently being investigated by our group.

Ideally, more focus is given to precision oncology or precision medicine, whose objective is to make use of molecular features and markers within an individual tumour to guide in therapy selection [63, 105]. This field focuses on selecting therapy based on genomic alterations, however the patient generally does not respond to the treatment selected based on genomics or responds throughout the early stages but then leads to relapse and resistance. By now, it has become evident that biological complexities which control drug response do not only depend on genomics data alone, but additional evidence is needed to fully unlock the potential of this field in predicting treatment response. As discussed in this chapter, proteomics-based data has been underutilised in this field, however the National Cancer Institute's Clinical Proteomic Tumour Analysis Consortium (CPTAC) have now started to combine proteomics data with information retrieved through transcriptomics profiling and genomics [70, 105, 106]. This is referred to proteogenomics, which provides functional contexts to explain and compare genomic and transcriptomic alterations in relation to proteomics data collected from MS, which in turn also improves the detection of proteins variants within a sample [59, 105, 107]. Moreover, the benefits arising

through this field are manifold, as well reviewed by Sheynkman et al. [107]. Despite these advantages, drawbacks are also inherently present, mainly because of false positives and false negatives, difficulty in detecting low abundance or novel peptides and the need for bioinformatics tools to analyse such large data sets [107]. Protein variants discovered through proteogenomics might be potential biomarkers for specific cancer types, which can assist in identifying therapeutic targets [59]. Incorporating proteogenomic analysis will open up new avenues for biological discoveries and it will most certainly lead to a vast range of opportunities for the identification of novel therapeutic targets. In the context of CRC, proteogenomics has been reported to have been utilised to characterise and subtype this tumour [108, 109] and to predicting treatment sensitivity [63, 106].

One of the main problems with applying multi-omics approaches to PDO-based investigations, is the need for a substantial amount of cellular material, which is not always possible due to minimal patient samples. Besides, the general challenge for researchers performing omics analyses for therapeutic application is the large data sets which arise from any of the omics approaches. Proper data mining tools are needed to analyse not only proteomics data but combined omics data as a whole, since this a challenge for everyone. As more data is collected from different (1) sample types, (2) time points, (3) drugs, (4) patients, and so forth, integrating all this data together will continue to be challenging and remains the limiting step when it comes to understanding biomarkers and their potential in predicting treatment response in patients. Thus, computational technologies (Bioinformatics) are strongly needed in order to combine proteomics data with that derived from other omics techniques. Such bioinformatic tools can be considered a major backbone in generating a biologically relevant output. The problem with these tools is that high false discovery rates are generally obtained, especially when PTMs are involved, since high specificity and sensitivity is difficult to achieve. Some research groups have opted to design in-house prediction tools to verify the data analysis collected through the use of positive data sets, however these tools generally treat any other datasets as negative tools, thus reducing the prediction accuracy [59]. Furthermore, real-time analysis of proteomics data is required in order to increase the clinical applicability of proteomics and improve patient outcome. Moreover, combining and integrating proteomic real-time analysis with other omics technologies will further improve the clinical application of advanced technologies and improve patient outcome. Combining multi-omics data is not an easy feat, but nevertheless the goals are: (1) to develop new and improve current bioinformatic tools to combine such data, and (2) to maintain and continually update the available open access resources, such as the Human Protein Atlas [110] and the Reactome Project [111].

There have been various reports which made use of proteomics or multi-omics to analyse the drug response relationship in CRC cell lines, and there is enough evidence which demonstrates the benefits and limitations of cell lines as models of primary diseases [70]. However, controversy persists since cell lines are not a good representative for primary tumours, thus more research teams should implement the use of PDOs for not only CRC therapy prediction, but cancer treatment prediction in general. Additionally, it is still unclear whether cell lines are representative of primary tumours at a proteomic level, and to what degree molecular programs and proteogenomic connections are sustained under *in vitro* conditions. The significance of proteomic data as a predictor of anti-cancer therapy response in contrast to transcriptomics and genomics has not been systematically studied [70].

It is worth mentioning that development of sensitive and powerful methods in the field of proteomics are constantly being pioneered so as to overcome the challenges faced when analysing lesser amounts of specific protein markers of interest. It is strongly believed that these advancements will continue to promote proteomic studies on predictive biomarkers in CRC. In turn, any future data collected can further support the current approaches for predicting treatment support.

6. Conclusions

Survival rate of patients with advanced CRC has significantly improved throughout the years due to the introduction of chemotherapeutics, targeted therapies, and the combination of multidisciplinary techniques. Even though CRC molecular subtypes and classifications have assisted in the selection of the proper therapy to improve the overall patient outcome, the downside is that tumour heterogeneity is not considered. Despite the drawbacks and limitations encountered with these subtypes and classifications, more advanced approaches have now started to be implemented to overcome such difficulties.

PDOs have shown to be a more reliable and suitable model to study CRC treatment response, when compared to the commonly used cell lines. However, given the small number of studies conducted and published, many issues remain unanswered. The accumulation of studies regarding the predictive potential of PDOs in personalised medicine will definitely determine their ultimate relevance in the near future.

The ongoing progress of proteomics has presented new insights to the therapeutic field. New technologies and different approaches which are being developed have offered a different alternative through which the search for predictive biomarkers in CRC can be achieved. With further advances in proteomic technologies and a greater push for their application in clinical proteomics, the prospective benefits for cancer patients will concomitantly increase. Proteomics, along with other omics approaches have ushered CRC PDOs research into a new era, generating loads of novel information, which is sometimes at a pace too fast for proper validation and evaluation. The development of computational technologies through which data from different omics approached can be combined, validated and analysed will hopefully further strengthen our understanding of CRC, which will in turn help in better predicting and selecting the right treatment to administer.

Acknowledgements

This research was financed by the Malta Council for Science & Technology, for and on behalf of the Foundation for Science and Technology, through the FUSION: R&I Technology Development Programme.

Conflict of interest

"The authors declare no conflict of interest."

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