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Clinical Challenges of Consensus Molecular Subtype CMS4 Colon Cancer in the Era of Precision Medicine

Sophie Mouillet-Richard¹, Antoine Cazelles¹, Marine Sroussi¹, Claire Gallois^{1,2}, Julien Taieb^{1,2}, and Pierre Laurent-Puig^{1,3}

ABSTRACT

Over the past decade, our understanding of the diversity of colorectal cancer has expanded significantly, raising hopes of tailoring treatments more precisely for individual patients. A key achievement in this direction was the establishment of the consensus molecular classification, particularly identifying the challenging consensus molecular subtype (CMS) CMS4 associated with poor prognosis. Because of its aggressive nature, extensive research is dedicated to the CMS4 subgroup. Recent years have unveiled molecular and microenvironmental features at the tissue level specific to CMS4 colorectal cancer. This has paved the way for mechanistic studies and the development of preclinical models. Simultaneously, efforts have been made to easily identify patients

with CMS4 colorectal cancer. Reassessing clinical trial results through the CMS classification lens has improved our understanding of the therapeutic challenges linked to this subtype. Exploration of the biology of CMS4 colorectal cancer is yielding potential biomarkers and novel treatment approaches. This overview aims to provide insights into the clinico-biological characteristics of the CMS4 subgroup, the molecular pathways driving this subtype, and available diagnostic options. We also emphasize the therapeutic challenges associated with this subtype, offering potential explanations. Finally, we summarize the current tailored treatments for CMS4 colorectal cancer emerging from fundamental and preclinical studies.

Introduction

The definition of consensus molecular subtypes (CMS) in the landmark study by Guinney and colleagues in 2015, based on bulk transcriptomic profiling, has ushered a new era in which colorectal cancer can be viewed as a collection of distinct entities, with specific histopathologic, genomic, molecular, and clinical features (1). Since then, the CMS classification has become an essential reference for describing the diversity of colorectal cancer. This notably ensues from more dedicated in-depth studies demonstrating that this classification is also relevant in terms of tumor microenvironment (TME; ref. 2), miRNAs (3), or epigenomic landscape (4). The various characteristics of each subtype are summarized in **Table 1** and we refer the reader to several outstanding reviews for more details (5, 6). Briefly, the CMS1 group (14% of patients), referred to as “immune,” is enriched in patients with microsatellite instability (MSI; ref. 1). The “canonical” CMS2 (37% of patients) and “metabolic” CMS3 (13% of patients) subgroups are both characterized by an epithelial type and a good

prognosis (1). The so-called “mesenchymal” CMS4 subgroup (23% of patients) is that of worse overall survival (OS) and relapse-free survival (1), and, for this reason, the focus of the current review. Until now, the data on CMS classes were mostly gained on United States/European populations and the potential impact of race or ethnicity on CMS distribution remains to be evaluated.

Because the original classification algorithms were developed for bulk tissue, including both tumor cell themselves but also their surrounding TME (1), they turned out to be unsuitable for preclinical models such as cell lines for instance, in particular when attempting to identify CMS4-like profiles. This ensues from the fact that the original CMS4 label is notably based on a number of stromal rather than tumor-expressed genes. Classification algorithms have thus evolved to allow applicability to preclinical samples, including patient-derived organoids or cell lines (7, 8) and genetically-modified mouse models (9), or patient metastases (10). The same observation was the basis for designing the so-called colorectal cancer intrinsic subtype (CRIS) classification (11), where CMS4 tumor samples majorly split into the CRIS-B and CRIS-D subtypes (12). More recently, our team sought to describe colon tumors as weighted combinations of different CMS subsets instead of ascribing a unique CMS class defined as the most probable according to the original classifier (13). We discovered that colon cancer frequently belongs to multiple CMS and that this new level of intratumor heterogeneity is associated with a dismal prognosis. In addition, our study highlighted four combinations with most notable poor outcomes, including three with a CMS4 component. With the advent of new technologies, the CMS classification has been refined to adapt to single-cell data, leading to the definition of so-called intrinsic CMS that, combined with a quantitative assessment of the stroma, can describe colorectal cancer heterogeneity at a single-cell level (14). Over the past years, many studies have integrated the consensus classification as a reference framework to consider the diversity of colorectal cancer, most of them focusing on all CMS irrespective of their prognosis. Given its dismal prognosis, the current review aims to provide an overview of the current knowledge of CMS4 clinico-biological characteristics, its driving molecular pathways, as

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Table 1. Key characteristics of CMS subtypes.

	CMS1 “Immune”	CMS2 “Canonical”	CMS3 “Metabolic”	CMS4 “Mesenchymal”
Genetic features	MSI CIMP high	CIN high	CIMP low CIN low	CIN high
Mutations	<i>BRAF</i> ^{V600E}		<i>KRAS</i>	
Molecular pathways	JAK-STAT	WNT SRC MYC	Metabolic deregulation	EMT TGFβ Angiogenesis
TME	Immune infiltration	Immune desert	Immune desert	Fibroblastic infiltration Immunosuppression
Prognosis Stage I-III	Good	Good	Good	Poor
Prognosis Stage IV	Poor	Good	Intermediate	Intermediate

well as the diagnostic and therapeutic challenges pertaining to this subtype.

The Hallmarks of CMS4 Colorectal Cancer

Clinical hallmarks

CMS4 colon cancers stand out from other subtypes by their aggressiveness and poor prognosis. This distinctive feature that emerged from the seminal CMS classification paper (1) has remained unchallenged onward. While the CMS4 group globally accounts for 20%–25% of colorectal cancer cases (1), the prevalence significantly varies according to a number of variables and in particular disease stage (15). Indeed, the proportion of CMS4 cases was shown to gradually increase with stage, ranging from 10% in stage 1 to 38.5% in stage 4 patients (16). A consensus that emerged from several studies is that the CMS4 subtype is barely if not detected in adenomas and arises at the carcinoma stage (17, 18), notably after transition from an indolent CMS3 subtype. This finding relies on the comparison of matched precursor and carcinoma regions (19). Thus, beyond the more accepted model according to which the CMS4 profile is associated with sessile serrated lesions (SSL) impregnated by a TGFβ-rich environment (20), CMS4 colorectal cancer may alternatively originate from CMS3 tubular adenomas (TA; **Fig. 1**). Furthermore, a recent study has suggested that an obesity-associated TME could reprogram CMS2 tumors into a CMS4 subtype (21). This raises the question of the risk factors potentially involved in the emergence of CMS4 colorectal cancer. Until now, this issue has received little attention, and should feature among the priorities for future research. The CMS4 profile may also come up upon metastasis in various tissues (10, 22, 23), and was shown to be present in the vast majority of peritoneal metastases (24). Finally, the profile of inflammatory bowel disease-related colorectal cancer mainly falls in the CMS4 group (25). In terms of prognosis, except for survival after relapse that is worst in CMS1 cases, CMS4 colorectal cancer is consistently associated with a dismal outcome (1, 26), including at early stage (27). This also holds for mixed CMS tumors with a CMS4 component (13).

Histomorphologic hallmarks

In line with their enrichment in an epithelial-to-mesenchymal transition (EMT) signature (1), CMS4 tumors exhibit a mesenchymal morphology, and are characterized by an abundant expression of the

master EMT transcription factor ZEB1 easily detectable by IHC (28). A second outstanding and undisputed hallmark of CMS4 tumors is their stromal enrichment, which emerged both from transcriptomic signature (2) and histologic analyses (29). From a spatial point of view, it is worth noting that multisampling analyses have revealed an enrichment in the CMS4 subtype at the invasive margin as compared with the tumor core (11). In line with this, several studies have documented an association between the CMS4 subtype and tumor budding, defined as a single tumor cell or tumor cluster of up to four cells at the invasive margin (29–31), a trait that is associated with poor prognosis (32). Altogether, several works based on paired tumor regions (11, 19, 30) support the notion that the CMS4 tumor state arises from the progression of a less aggressive state, rather than originating from a distinct carcinogenesis pathway, perhaps with the exception of inflammation-induced colorectal cancer (25). In the future, spatial omics technologies may help gain further insight into this issue.

Biological hallmarks

In contrast to the enrichment of *BRAF* and *KRAS* mutation in the CMS1 and CMS3 subtypes, respectively, no genomic feature is specific to CMS4 tumors (1). One of the most scrutinized pathways originally found to be enriched in CMS4 colorectal cancer is the TGFβ pathway (1). The incrimination of TGFβ in the progression to a stromal-enriched colon cancer of poor prognosis actually preceded the establishment of the CMS classification (33). Follow-up studies have documented that TGFβ can direct human organoid cultures to a CMS4 profile (20) and that it fosters the emergence of an immunosuppressive contexture, leading to the proposition that combined TGFβ targeting and immune checkpoint blockade may represent an attractive therapeutic strategy for CMS4 colorectal cancer (34). While previous studies have shown that TGFβ, produced by the tumor, exerts a paracrine action on cancer-associated fibroblasts (CAF), rather than acting in an autocrine manner (33), the picture is perhaps not so clear. Indeed, we reported that CMS4-like colon cancer cells themselves respond to TGFβ, and notably control the expression of a number of CMS4-specific genes through this pathway (35). The initial view that CMS4 tumor cells cannot respond to TGFβ was based upon the observations that colorectal cancer often presents with mutational inactivation of this pathway, most notably in the *SMAD4* and *TGFBR2* genes (36). However, beyond the fact that such genetic alterations are not overrepresented in the CMS4 subgroup (1), it is now apparent that the induction of some TGFβ target genes does not require

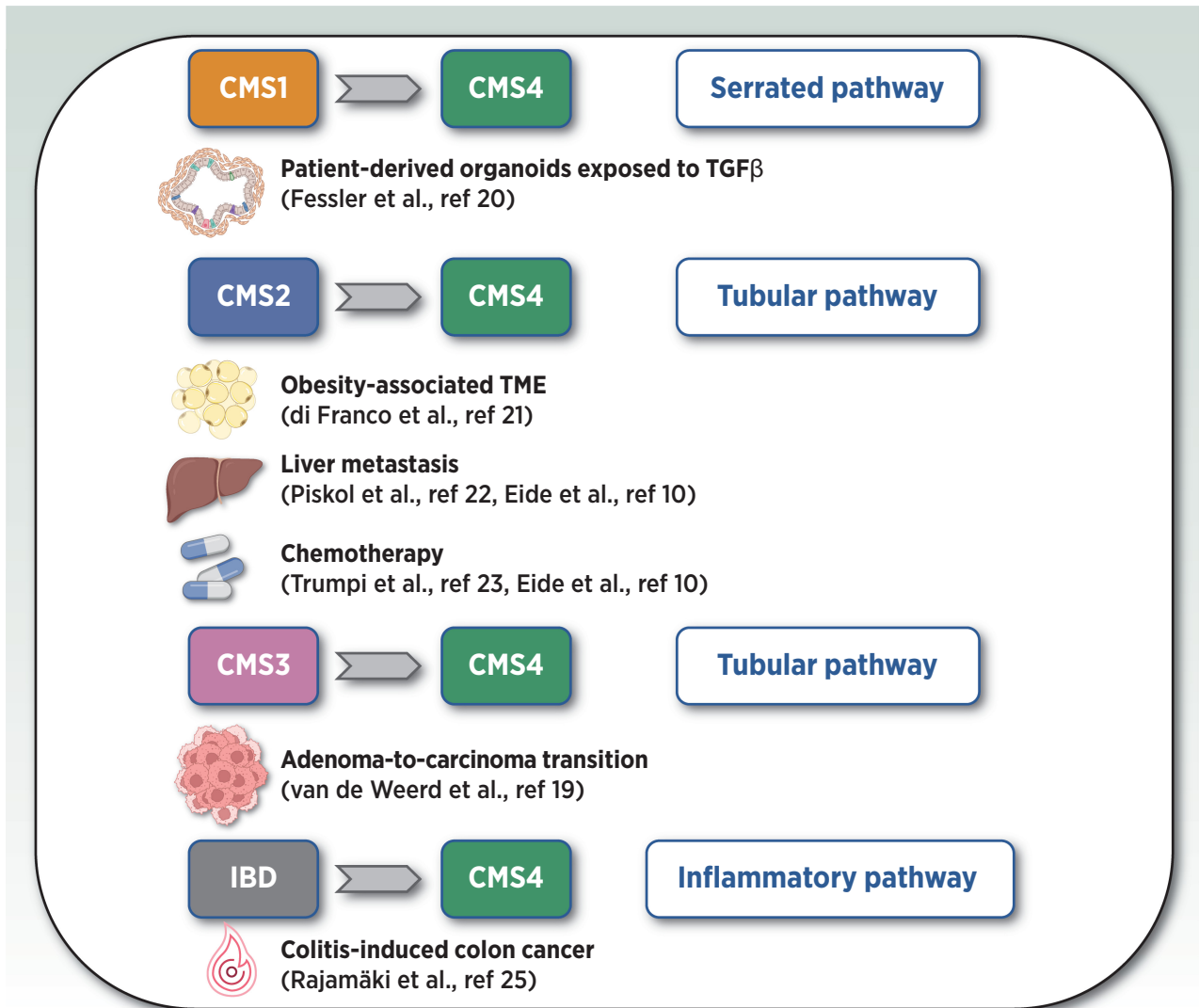


Figure 1.

Proposed pathways involved in the emergence of the CMS4 subtype. CMS4 colorectal cancer can arise along the serrated pathway within a TGF β -rich environment. Along the tubular pathway, the reprogramming from a CMS2 to a CMS4 subtype may take place in the context of obesity, metastasis, or after exposure to chemotherapy. Alternatively, the switching from an indolent CMS3 to an aggressive CMS4 subtype may occur along the adenoma-to-carcinoma transition. Finally, CMS4 colorectal cancer may arise on an inflammatory bowel disease (IBD) context.

SMAD4 (37). On this basis, it can be surmised that TGF β is central in the tumor-intrinsic and -extrinsic traits of CMS4 colorectal cancer. Several critical inquiries persist, especially regarding the processes that facilitate the early stages of TGF β activation, such as the liberation of TGF β from its latent-binding complex (37). We recently brought some insight into this issue by showing that the concentration of soluble TGF β in the supernatant of colon cancer cells is partly controlled by the cellular prion protein PrP^C (35). Indeed, we introduced PrP^C, a plasma membrane-tethered protein endowed with the capacity to participate in signal transduction events (38), as a key protagonist of CMS4 colon cancer, controlling the expression of many CMS4-specific genes in colon cancer cells through diverse signaling intermediates (35, 39). These include YAP and TAZ, the main effectors of the Hippo pathway, whose expression was shown to be significantly increased in CMS4 tumors in reverse phase protein arrays (1). Acti-

vation of YAP and/or TAZ appears to represent a cardinal trait of CMS4 colorectal cancer, similar to that of TGF β (40). On the basis of our first set of observations (35) and the multiple examples of cross-talk between the two pathways (41), we may postulate that the TGF β and the YAP/TAZ signaling axes cooperate to induce and/or maintain CMS4 features. Another effector is the integrin linked kinase (ILK), which we found to be overexpressed in CMS4 colon cancer and to act as a relay downstream from PrP^C to activate YAP/TAZ signaling (39). Among other biological hallmarks of this subtype shown to contribute to CMS4 traits feature elevated AKT3 (42) and KIT (43), reduced mitochondrial respiratory complex I activity (44)—possibly resulting from KIT overexpression (43)—or modified alternative splicing landscape (45). Relevant signatures further include β 3 integrin activation (1), which could in turn foster ILK activation (46), hypoxia, and DNA damage (47), potentially accounting for elevated PrP^C

expression (reviewed in ref. 48). Finally, some clues may be gained by examining genetically engineered mouse models that phenocopy CMS4 colorectal cancer, such as those based on Notch activation (9, 49), reduced atypical protein kinases C and consecutive elevated hyaluronic acid (50), cyclin A2 deletion (51) or ZEB2 overexpression (52). Noteworthy, ZEB2 activation was also depicted as a key downstream event in the reprogramming of CMS2 cancer cells into a CMS4 subtype in response to visceral adipose-derived factors, through a STAT3-miR200 axis (21).

Although some connections have already been established between various protagonists (see Fig. 2), still much work is needed to reach an integrated picture of the signaling circuitries operating in CMS4 cancer cells. We may also suspect positive regulatory loops to operate and foster the stability of the CMS4 state once unleashed upon a triggering signal, for example secreted by surrounding CAFs (53) or inflammation.

Toward the Definition of the CMS4 Ecosystem

According to the single-cell study by Joanito and colleagues (14), cancer cells from CMS4 colorectal cancer reside in either a fibrotic intrinsic CMS2 (iCMS2) or iCMS3 state according to the type of lesion: TA-type CMS4 correspond to the iCMS2-F class (F standing for fibrotic), while SSL-type CMS4 correspond to the iCMS3-F class. This dichotomy actually aligns well with the various reports altogether supporting a dual origin of CMS4 colorectal cancer (see above). Whatever their intrinsic subtype correspondence, the enrichment of CMS4 tumors in CAFs is a shared outstanding characteristic. Several cancer cell-produced signaling molecules, including TGF β and PDGF may contribute to CAFs activation (54). In turn, CAFs may also produce a variety of factors, such as TGF β , PDGF, IL11, HGF, and prostaglandins as well as extracellular matrix (ECM) molecules (55),

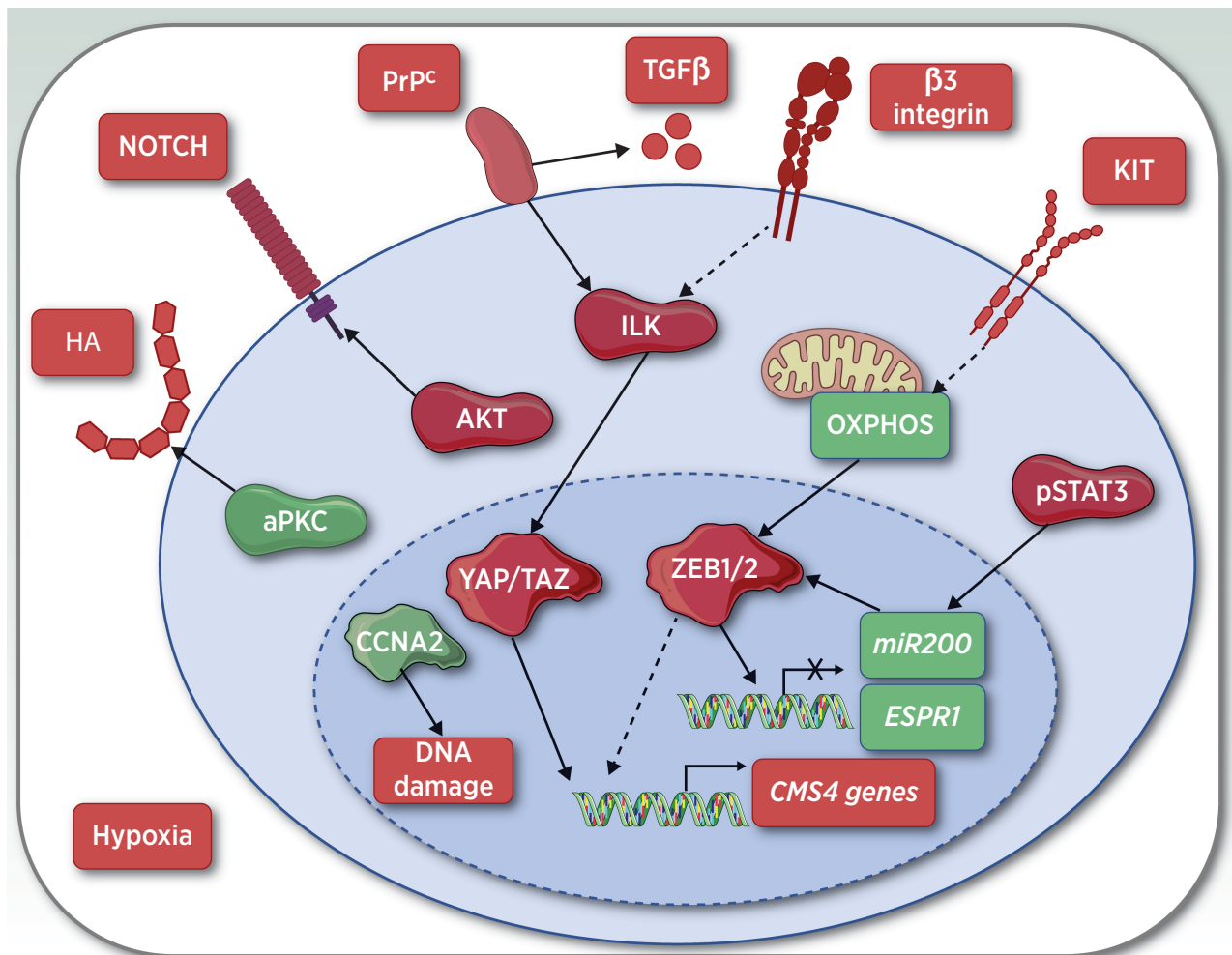


Figure 2.

Simplified model summarizing the biological hallmarks of CMS4 cancer cells and their potential interplays. Overexpressed/overactivated genes, proteins, or pathways are highlighted in red, and underexpressed/underactivated genes, proteins, or pathways are highlighted in green. Established links are indicated with a plain line: reduced atypical PKC expression and associated increased hyaluronic acid (HA) synthesis (50), AKT-dependent activation of NOTCH (49), PrP^c-dependent control on soluble TGF β levels, ILK and YAP/TAZ activation (35, 39), reduced oxidative phosphorylation and increased ZEB1 levels (44), ZEB1-dependent negative regulation of the ESPR1 splicing regulator expression (45), phosphorylation-dependent STAT3 activation and downstream silencing of miR200, itself promoting enhanced expression of ZEB2 (21), decreased levels of cyclin A2 (CCNA2), fostering DNA damage (51). Dashed lines represent potential links: β 3 integrin-dependent activation of ILK (46) and KIT-dependent reduction of oxidative phosphorylation (43).

possibly sustaining YAP/TAZ activation in cancer cells (40). Although the intricate interplay between cancer cells and CAFs in CMS4 colorectal cancer is far from completely understood, studies based on coculture experiments have unambiguously demonstrated that CAFs reinforce the CMS4 traits of cancer cells (53). Regarding the immunotolerant CMS4 TME, we posit an involvement of TGF β (56) and Kynurenine (57), both produced by cancer cells under the control of PrP^C (35, 39) and it is likely that the immunosuppressive trait of CMS4 is reinforced by CAFs (55). Finally, both cancer cells and CAF-derived factors, including TGF β and PDGF, are expected to promote endothelial cell infiltration and angiogenesis, again hallmarks of CMS4 (2). The TME archetype of CMS4 colorectal cancer is thus being progressively delineated, and spatial omics analyses should allow to refine the architectural organization of the different cell contingents soon. Such spatial approaches will certainly be key to grasping the architectural complexity of mixed CMS4 tumors.

Diagnosis of CMS4 Colorectal Cancer

The CMS classification was built on full transcriptome profiling, which is not yet compatible with the day-to-day management of patients with colorectal cancer in the clinics. To overcome this limitation, several diagnostic strategies have been developed (Fig. 3). At a molecular level, the group of O. Kranenburg proposed a qRT-PCR-based test based on the expression of several effectors of the PDGF pathway as well as the KIT receptor, with a high degree of robustness (58). A number of teams, including ours, have further developed NanoString-based classifiers (13, 22, 59). Other strategies

rely on histopathology, such as IHC, CMS4 being defined as pMMR, CDX2-negative and FRMD6, HTR2B and ZEB1-positive tumors (28). In addition, artificial intelligence is now opening up new avenues for colorectal cancer classification, with a high degree of robustness (29). In terms of minimally invasive methods, the detection of FAP (fibroblast activated protein) through PET imaging as a surrogate of CMS4 colorectal cancer appears extremely promising (60). An ideal scenario would be the possibility to exploit liquid biopsies, with obvious advantages in terms of ease of collection and repeated sampling, as well as analytes that can be monitored. One candidate circulating biomarker is PrP^C, which we found to be increased in CMS4 tumors and elevated in plasma derived from patients with metastatic colorectal cancer (35). However, the CMS classification was not available for the latter patients (35) and the question therefore remains as to whether circulating PrP^C levels can discriminate CMS4 patients from others. We may expect the current development of high-plex omic profiling technologies to yield new biomarkers of clinical utility in the coming years.

The Therapeutic Challenges of CMS4 Colon Cancer

The clinical value of the CMS classification has been the topic of intense investigation and recently reviewed by Ten Hoorn and colleagues (26). It is now quite well established that patients with stage II and III CMS4 colorectal cancer benefit less from adjuvant chemotherapy than other subtypes and that, in the metastatic setting, irinotecan-based regimens have superior efficacy compared with those with an

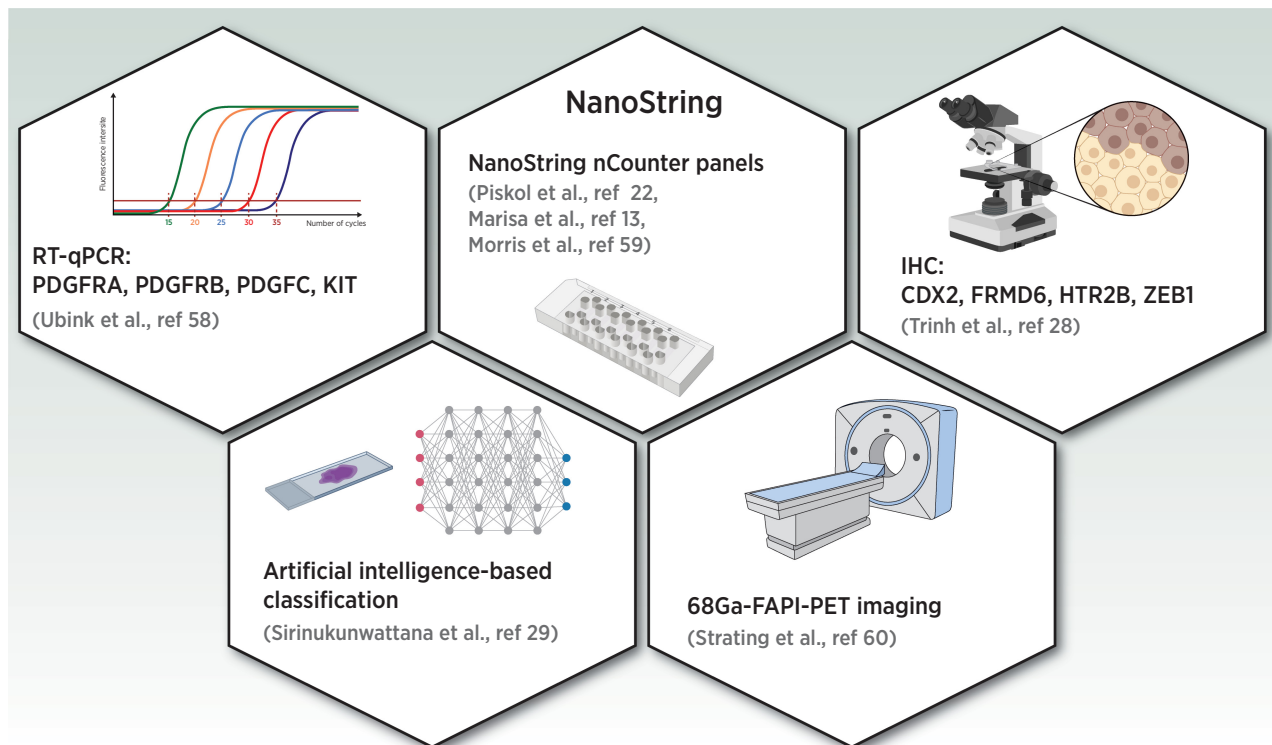


Figure 3.

Summary of the various diagnostic strategies to detect CMS4 colorectal cancer: qRT-PCR-based testing of *PDGFRA*, *PDGFRB*, *PDGFC*, and *KIT* (58); NanoString nCounter panels (13, 22, 59); IHC staining of CDX2, FRMD6, HTR2B, and ZEB1 (28); artificial intelligence-based classification (29); or FAPI-PET imaging (ref. 60; Adapted from an image created with BioRender.com).

Table 2. Summary of the studies having shown potential treatment efficacy in CMS4 colorectal cancer.

Target	Drug/strategy	Evidence	Reference
<i>Clinical trials</i>			
PDGF pathway	Imatinib	Suppression of mesenchymal genes in posttreatment samples ($n = 5$ patients with primary CMS4 colorectal cancer who consented to treatment prior to surgery)	(63)
Combined STAT3 and PD-1	Napabucasin + pembrolizumab	Immune-related objective response rate in 33% of patients (2 of 6 CMS4 patients with metastatic colorectal cancer)	(64)
Receptor tyrosine kinases	Nintedanib	Increased progression-free survival (PFS) from 1.3 to 2.2 months ($n = 60$ nintedanib-treated and $n = 59$ placebo CMS4 patients with metastatic colorectal cancer)	(65)
Kinases	Regorafenib	Increased PFS with regorafenib vs. placebo ($n = 26$ CMS4 patients with metastatic colorectal cancer)	(66)
<i>In silico prediction</i>			
Combined MEK and SRC		Gene expression signature of sensitivity	(69)
<i>Preclinical models</i>			
HSP90	Luminespib + 5-FU	Reduced tumor growth	(7)
PAK2	PAK2 shRNA	Reduced intraperitoneal metastases	(67)
AKT3	MK2206	Reduced cell growth	(42)
PrP ^C	Neutralizing antibodies	Reduced expression of CMS4 genes and soluble TGF β levels	(68)

oxaliplatin backbone (26). In addition, combining bevacizumab to FOLFIRI [folinic acid (FA), fluorouracil (5-FU) and irinotecan]—but not to capecitabine—is associated with better outcomes as compared with monotherapy, and the FOLFIRI + cetuximab combination (for RAS wild-type patients) is superior to the FOLFIRI + bevacizumab combination (26). Recently, results from the PanaMa trial showed an OS benefit from the addition of panitumumab to 5-FU and FA maintenance in CMS4 patients (61). The molecular mechanisms behind the resistance of CMS4 colorectal cancer to 5-FU and oxaliplatin-based regimens are not fully understood. Nonetheless, some explanation may be found in the intrinsic properties and the stromal enrichment of CMS4 colorectal cancer. Regarding signals, the strong TGF β impregnation of CMS4 colorectal cancer is likely to build an immunotolerant environment, as reviewed recently (56). Indeed, combining TGF β targeting with immune checkpoint blockade has demonstrated efficacy in *in vivo* CMS4 colorectal cancer models (34). However, following these preclinical results, dual TGF β and PD-L1 inhibition with bintrafust alfa was not effective in patients with liver metastasis (62). In contrast, positive responses have been reported for CMS4 patients treated with (i) imatinib—targeting the PDGF pathway—before surgery (ImPACCT trial), however in a very limited number of patients (63), (ii) a combination of napabucasin (a STAT3 inhibitor) and pembrolizumab (EPOC1503/SCOOP trial; ref. 64), (iii) the triple tyrosine kinase inhibitor nintedanib (LUME-Colon1 trial; ref. 65), or (iv) the multikinase inhibitor regorafenib (CORRECT trial; ref. 66; **Table 2**).

Other candidate targets have emerged from preclinical studies, including HSP90, combined with 5-FU (7), PAK2 (67) or AKT3 (42), or PrP^C, whose neutralization could have the combined advantage of reducing both TGF β and PDGF signaling (68). From gene expression signatures studies, KRAS-mutated CMS4 colorectal cancer was further predicted to respond to combined MEK and SRC inhibition (69). In terms of TME contexture, the prevalent stromal contingent is likely to contribute to resistance mechanisms, for instance through ECM deposition and cancer cells insulation (54), co-migration with cancer cells to metastatic sites (70), chemotherapy-induced secretome fostering metastasis, angiogenesis, immune evasion and/or drug

resistance (71), or even drug retention, as demonstrated in the case of oxaliplatin (72). A very recent study reported that coculture with CAFs promotes an increased resistance to SN-38 (irinotecan active metabolite) and gefitinib of CMS4 compared with CMS2 patient-derived tumor organoids (53). Thus, the stroma-rich hallmark of CMS4 colorectal cancer may represent a promising therapeutic vulnerability, as suggested by Dunne and colleagues (73). Finally, one challenging issue arising from intratumor heterogeneity pertains to the notable dismal prognosis of CMS4-associated combinations (13). Mechanistically, we may surmise that the exposure of CMS1 cancer cells to 5-FU may promote the release of metabolites acting on CMS4 cells to enhance their migratory and invasive capacities as well as their own resistance, according to our recently published work (74). Conversely, factors secreted by CMS4 cells or their surrounding CAFs, such as TGF β , may possibly rewire the TME contexture of CMS1 colon cancer to yield an immunosuppressive archetype.

Concluding Remarks

In recent years, our understanding of colorectal cancer diversity has grown to include the CMS taxonomy. It is now clear that the CMS4 subtype requires special attention for both diagnosis and treatment. Looking ahead, improvements in diagnosing CMS4 and identifying theranostic biomarkers, as demonstrated in successful examples (75), are expected to enhance patient stratification. This will enable the integration of CMS profiles into the clinical decision-making process. In addition, our knowledge of the biology of the CMS4 subgroup is expanding rapidly, opening up new possibilities for treatment. Beyond focusing on the tumor cells themselves, manipulating the TME could be a promising avenue for exploration. With the availability of advanced analytic tools and preclinical models, we can confidently anticipate overcoming the challenges that lie ahead.

Authors' Disclosures

C. Gallois reports consulting and/or advisory board participation for Servier, Sanofi, Merck, Pierre Fabre, and MSD and has received support for travel to meetings from Pierre Fabre. J. Taieb reports personal fees from Amgen, Astellas, AstraZeneca, BMS, Merck KGaA, MSD, Novartis, Ono Pharmaceuticals, Pierre Fabre, Roche

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