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Heterogeneity of colon cancer: from bench to bedside

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ABSTRACT

The large bowel shows biomolecular, anatomical and bacterial changes that proceed from the proximal to the distal tract. These changes account for the different behaviour of colon cancers arising from the diverse sides of the colon–rectum as well as for the sensitivity to the therapy, including immunotherapy. The gut microbiota plays an important role in the modulation of the immune response and differs between the right colon cancer and the left colorectal cancer. The qualitative and quantitative difference of the commensal bacteria between the right side and the left side induces epigenetic changes in the intestinal epithelial cells as well as in the resident immune population. The second player in the pathological homeostasis of colorectal cancer is the differences of the genetic features of cancer cells and the different effects that microsatellite instability, chromosomal instability and the CpG island methylator phenotype induce on the immunological organisation of the tumour microenvironment. The third player is the immunological composition of the tumour microenvironment, which changes under the influence of both genetic structures and gut microbiota. All these three players influence each other. This review describes these three aspects, highlights their interactions and discusses data from reported clinical trials.

INTRODUCTION

Colon cancer represents an apparent paradox, being 'the first neoplasia found to be under immunosurveillance and the last one to respond to immunotherapy'.[1] However, this paradox is only apparent. Indeed the large bowel, differently from any other organ or apparatus in the body, is characterised by gradual biomolecular, anatomical (ie, immune cell composition) and microbiome changes, which proceed from the proximal to the distal tract. These changes account for the different behaviour of colon cancers arising from the various portions of the large bowel as well as for the different sensitivity to the therapy, either immunotherapy or chemotherapy. In addition, large bowel hosts the largest amount of commensal bacteria in the body, and they continuously interact with the gut wall and the immune cells.

It is clear at this point that the outcome of colorectal cancer (CRC) is not solely dependent on tumour characteristics, but is related to the quality of the host immune response. In turn, the quality of the immune response is influenced by the tumour genetic structure as well as by the gut microbiota, and all these three aspects influence each other.

The purpose of this paper is to point out the existing differences among the different portions of the colon–rectum, their influence on tumour behaviour, how they influence response to therapy and give a short review on clinical data.

Step 1: brush up the immunogenic aspects of early tumorigenesis

A new cancer develops from two main different pathways: extrinsic or intrinsic pathway.[2] Inflammation or infection represents the extrinsic pathway. Oncogenic activation represents the intrinsic pathway.

Whatever the trigger, subsequent events are similar. Damaged or infected cells release pathogen-associated molecular profile or damaged-associated molecular profile, which can be recognised by specific receptors, for example, the toll-like receptors (TLRs). They, in turn, activate transcriptional factors (nuclear factor-kB, signal transducer and activator of transcription 3 (STAT3), hypoxia-inducible factor 1-alpha) leading to the upregulation of chemokines, cytokines, prostaglandins and COX2, which promote an inflammatory microenvironment. Epithelial colon cells express TLRs and thus they play a major role in colon homeostasis.

As well as most factors associated to the immune response, TLRs induce effects either suppressive or permissive.

The prevalence of one effect over the other depends on the level of activation of TLRs: high-level activation is associated with tumour-suppressive effect, while chronic low-grade stimulation, as observed in chronic

Table 1 Major differences between right colon cancer (RCC) and left colorectal cancer (LCRC)

	RCC	LCRC
Incidence	Lower than LCRC	Higher than RCC
	Increasing	Decreasing
	Higher in female	Higher in male
Presentation	Higher TNM stage	Lower TNM stage
	Larger tumours	Smaller tumour
	More mucinous type	Less mucinous type
Genetics	Common site for CRC in MUTYH-associated polyposis	Common site for CRC in familial adenomatous polyposis
Immunology	More active immune cells promoting immunogenicity	Immunosuppressive cells highly represented, promoting tolerogenesis
Molecular pathway	CIMP/MSI/BRAF positive tumours	Chromosomal instability
Outcomes	Worse overall survival	Better overall survival

Adapted from Lee *et al*[11].
CIMP, CPG island methylation phenotype; MSI , microsatellite instability.

inflammation, is associated with tumour-promoting effects.[3]

In this latter scenario, which mainly involves i TLR2 and TLR4, interleukin (IL)-6 represents an important player: it is recognised as a critical promoter during intestinal tumorigenesis[4] as well as during cancer progression and thus it may represent a new target for cancer prevention and treatment.[5]

The cancer-related, self-sustained, inflammation induces microsatellite instability (MSI), largely due to mismatch-repair (MMR) deficiency and chromosomal instability (CIN).[4]

MMR deficiency induces alterations preferentially in genes containing intrinsically instable microsatellite in their coding regions, such as the gene coding for transforming growth factor beta (TGFβ) receptors II (TGFβRII).[6] The mutation of TGFβRII disrupts the feedback loop, which, in the canonical pathway, maintains the tissue's homeostasis through the inhibition of cell proliferation. It has been postulated that the axis TGFβ–TGFβRII could represent a feedback loop able to regulate TGFβ expression.[7] In this case, the interruption of the loop could cause a compensatory TGFβ overexpression.

As a consequence, the developing tumour acquires a double advantage: removes a cell growth inhibitory signal and increases the amount of TGFβ in the tumour microenvironment (TME). TGFβ pathway mutation occurs in approximately one-third of colon and rectal tumours.[8] Jung *et al* recently reviewed the dual role of TGFβ in maintaining gut homeostasis in physiological conditions and in promoting cancer growth and metastases when the TGFβ signalling is disrupted.[9]

Step 2: environmental differences between right and left colon cancer

The knowledge about the genetic differences between the right colon cancer (RCC) and the left colorectal cancer (LCRC) is almost two decades old.[10] However, only recently it triggered the interest of clinical researchers, mainly because of the different response rate induced by immune checkpoint inhibitors (ICIs).

The most evident differences between RCC and LCRC[11] are summarised in table 1.

Among them, the molecular pathway and the immunological environment are the most relevant for the purpose of this paper and these two aspects influence each other.

RCC is significantly associated with MSI, G3, mucinous type and BRAF mutation. According to Vogelstein *et al*,[12] CRC harbouring MSI shows the highest number of somatic mutations, largely exceeding those observed in melanoma and in both small cell lung cancer and non small cell lung cancer. The high mutational load affects the host's immunological response. This claim is supported by Greenson *et al*,[13] who observed that high tumour-infiltrating lymphocytes (TILs) have the strongest association with unstable tumours and predict MSI. Therefore, one of the causes of the immunological difference between RCC and LCRC is the different mutational load.

Indeed RCC harbours more active immune cells, which can promote an efficient immune response, while LCRC hosts predominantly immunosuppressive cells.[14]

In addition to these aspects, the gut microbiota contributes to the modulation of immune response. The microbiota is a commensal pool of bacteria, fungi, viruses, bacteriophages and archaea.[14] The population of commensal bacteria is different in RCC and LCRC both quantitatively and qualitatively and this difference induces changes in the intestinal epithelial cells as well as in the composition of the immune cell population.

Therefore, it is clear that there exists a close interaction among mutational load, immune cells and microbiota.

An example of this 'three-factor interaction' is the history of β-catenin. β-Catenin has immune regulatory effect. High protein expression is associated with the lack of expression of the CCL4 chemokine that may explain

the ineffective recruitment of dendritic cells (DCs) in the tumour bed and, consequently, the lack of infiltrating CD8+ cells. Thus, the activation of β-catenin can exclude the antitumour immune response.[15 16]

β-Catenin is significantly more expressed in LCRC compared with RCC.[17] Up to 48% of the non-T-cell-inflamed tumours show activation of the Wnt/β-catenin pathway.[16]

Cathelicidin, a peptide with antimicrobial function produced by host defence cells, including macrophages, neutrophils and endothelial cells of the gut, is upregulated by many factors, including IL-6, retinoic acid and bacterial infections.[18] Cathelicidin in turn activates β-catenin.[19]

Therefore, β-catenin, its immune effects and its regulation, links genetic/epigenetic structure, immune system and microbiota.

Human colon contains approximately 10^{13}–10^{14} bacteria and their density increases with a positive gradient from the proximal tract to the distal.[20] Not only bacterial quantity but also quality is different in RCC and LCRC.[21]

Commensal bacteria play a fundamental role in the body homeostasis: the symbiotic equilibrium with the host allows a tolerant immune environment in return, for example, of acquired defences against threatening aggressors. There is experimental evidence that this protective effect occurs systematically,[22] suggesting that the interaction between microbiota and the immune system has relevance in the homeostasis of the whole body. However, commensal microbiota is a double-edged sword since it can be antitumorigenic or may play an opposite effect, depending on the exact constituents of the microbiota.[23] Coleman and Tiago, recently, reviewed this topic,[24] suggesting that environmental and dietary factors, affecting the gut microbiota, seem to play an important role in CRC formation. The microbiota is also important in determining response to cancer treatment, as will be discussed later.

Whatever the effect played by the microbiota, it seems reasonable that the large bowel requires tolerance rather than immunogenicity and this is in particular in distal colon[11] due to its higher bacterial burden. Indeed, on the contrary of the number of commensal bacteria, there is a negative gradient of various immune cells from the proximal to the distal colorectum.[11]

Step 3: the architecture and the functions of the immune system in the colorectum

The gastrointestinal tract hosts the highest concentration of immune cells in the body, probably due to its continuous exposition to a high antigenic burden made up by nutrients and by commensal intestinal flora.[25] Therefore, a highly specialised defensive system is required. In addition to the barrier function supplied by the enterocytes, the mucosa harbours a highly specialised intrinsic immune system whose commitment is to ensure nutrient transportation while preventing translocation of bacteria, whether commensal or pathogenic. The lymphoid tissue into the mucosa includes Peyer's patch, small bowel mesenteric lymph nodes and isolated lymphoid follicles in the large bowel, and also a variety of lymphoid cells in the lamina propria as well as effector lymphocytes interspersed in the epithelium.[26]

The immune system is divided into innate system and adaptive system. The latter is the most valuable due to its high specificity, but requires a longer time for its activation. However, the gut continuously faces high antigen burdens, requiring a rapid, though less specific response to maintain intestinal homeostasis and protect the body. This response depends on the innate system.[27]

For this reason and for the microbiota resident in the gut, the cells of the innate immunity need to endorse and develop specific functions, making them adequate to this unique environment. The specialisation required by the gut environment includes the enterocytes which exceed the merely barrier function, since they express several 'pattern recognition receptors' (PRRs), making them able to recognise the ligand produced by both commensal and intestinal pathogens. In this way, the enterocytes contribute to maintain intestinal homeostasis. Therefore, it has recently been suggested to consider them innate immune cells.[28]

The most abundant non-lymphoid immune cells in the bowel are macrophages that act as antigen-presenting cells within the lamina propria. They have a key role in maintaining intestinal homeostasis.[29]

DCs represent the second population of non-lymphoid immune cells with specific specialisation in the intestinal wall. Regardless of the canonical role of DCs, the gut requires by default to give priority to immune tolerance concerning commensal flora and food antigens.[30] Therefore, under normal conditions, the enterocytes favour the differentiation of the immature DCs to tolerogenic DCs.[31] However, a different non-tolerogenic DC subpopulation exists, represented by the CX3CR1+ DCs. These DCs induce Th17 response, but, similar to macrophages, they cannot migrate to mesenteric lymph nodes.[32]

Among the innate immune cells, natural killer T cells (NKTs) and γδT cells seem of particular importance for the bowel integrity. The invariant NKTs (iNKTs) are the most relevant NKT subset.[33] iNKT cells are involved in early-stage immune response, including response to pathogens and tumour cells.[34] Their activation requires the recognition of CD1d by T cell receptor (TCR).

Resident DCs, macrophages and B cells in the gut largely express CD1d, thus facilitate iNKT activation.

γδT cells are CD27+ or CD27– and the functional difference between these two subpopulations is of major importance. Indeed, CD27+ cells produce high amount of interferon (IFN)γ, while CD27– preferentially secrete IL-17.[35] The γδT CD27– cells (also called γδT17) are the most important source of IL-17 in the large bowel and are activated by DCs and IL-6 and can also express TLR2 and other PRRs (not expressed by γδT CD27+ cells).[36] They can be activated directly by pathogens and promptly start the secretion of IL-17 and other cytokines. Notably, IL-6

is also important for the development and recruitment of γδT17 cells.[37]

Step 4: how the differences in RCC and LCRC influence response to immunotherapy

Impact of molecular structure on response to immunotherapy

Preclinical data suggest that tumour regression induced by checkpoint blockade is largely due to reactivation of CD8+ T cells at the tumour site.[38] This observation is consistent with clinical data by Tumeh *et al*,[39] showing that pre-existing CD8+ infiltrate is necessary to induce tumour regression after therapeutic PD-1 blockade. However, the infiltration of CD8+ cells in the tumour bed is the final step of a series of events involving DCs, type I IFNs and the STING pathway activation. The activation of CD8+ cells requires a specific subpopulation of DCs: the Batf3-lineage.[40]

Activation of Batf3 DCs in turn requires type I IFNs produced by the host cells.[40] The lack of type I IFNs is associated with the absence of T cells in the tumour microenvironment (TME).[41] From a mechanistic point of view, the type I IFNs activate STING on DCs and stimulate the production of many chemokines, including CXCL9 and CXCL10 which attract effector T cells including CD8+.[42] If this is the main mechanism, lack of Batf3 DCs results in a CD8+ not infiltrated tumour and consequently lack of response to ICIs.

There are many oncogenic pathways that can abrogate the CD8+ infiltration interfering with the axis DCs –CD8+. Among them, the role of β-catenin is one of the most known; 48% of non-inflamed tumours showed evidence for activation of Wnt/β-catenin pathway, which results in the exclusion of Batf3 DCs from the tumour bed.[43] Spranger *et al* observed, in an animal model, that this exclusion correlates with the lack of expression of CCL4, an important DC attracting chemokine, which is suppressed in β-catenin-expressing tumour cells.[16] β-Catenin is overexpressed in >18% of LCRC compared with 8% in RCC (p=0.003).[17]

Other gene pathways could prevent immune reaction against the tumour: p53 mutation, constitutively activated STAT3, NFkB and PI3K signalling, either related to PI3K activating mutations or PTEN function deficiency. However, their relevance is not yet completely understood.

More in general, we know at least three distinct molecular phenotypes of colon cancer: CIN observed in 60%–80% of CRC, MSI, accounting for 15%–20% of sporadic CRC and CpG island methylator phenotype (CIMP).[44] Angelova *et al*, in her seminal paper on immunophenotypes and antigenomes of CRC, addressed the relationship between the molecular structures of CRC and their immunogenicity.[45] She observed that TILs are differently associated with distinct molecular phenotypes related to three features: (1) mutational status, (2) microsatellite status and (3) methylation status. High MSI tumours are abundantly enriched with CD4+ and CD8+ cells as well as DCs and NK cells, while low MSI and the other genetic features show a progressive reduction of TILs. Angelova *et al* also demonstrated that immunophenotypes evolve during tumour progression, and this aspect has an impact on the proper target of immunotherapy (ie, the immunotherapies to be used) in different stages of CRC.

Impact of immunological structure on response to immunotherapy

High-density TILs are more frequent in specimens arising from RCC compared with LCRC (p<0.0001).[46] This is in line with the observation by Paski *et al*[47] showing that there is a negative gradient of immune activity from the right colon to the left. Similarly, high mutational load and MSI are significantly more represented in RCC[10] and CD8+ T cells tumour infiltration is strongly associated with high MSI (p<0.0001).[48] Taken all together, these data highlight the prevalence of hot tumours in RCC and justify a better role of immunotherapy in proximal colon cancer.

Data from Minoo *et al*,[17] originating from the analyses of 399 patients with CRC, further support the prevalence of hot tumours in the RCCs. Indeed, RCCs exceed LCRCs in marker expressions such as CD68, CD163 and Foxp3, associated to regulatory cells. The excess of regulatory cells in RCC supports the predominance of hot tumours in proximal large bowel.

Differences between RCC and LCRC also involve TLRs: specific TLRs are differently expressed in the intestinal epithelial cells among the proximal and the distal colon. Namely, data from animal models show that TLR2 is more represented in RCC, while TLR4 is more expressed in LCRC. Wang *et al* demonstrated that the regional expression is determined by the composition of the microbiota. Indeed, transferring the commensal bacteria resident in the proximal colon to the distal increases TLR2 expression in distal colon.[49] Therefore, Wang *et al* demonstrate that the microbiota–epithelial colon cell interaction may also influence the epigenetic characteristics of the intestinal epithelial cells.

As previously reported, TLRs, and, in particular, TLR2 and TLR4, may play an opposite role on tumorigenesis, depending on their level of activation.[3] They share a common downstream signalling based on MyD88, a signal transduction molecule, and the co-receptor CD14.[50] MyD88-deficient mice show profound defects of repair of the intestinal barrier following injury and increased risk of colitis and CRC.[51] Intriguingly, MyD88 wild-type (WT) mice affected by dysbiosis induced by antibiotics show the same phenotype of MyD88-deficient mice, supporting the role of the interplay between microbiota and the intestinal epithelial cells in colon homeostasis. In addition, dysbiosis can promote chronic inflammation, resulting in overstimulation of TLRs, which can induce CRC.[52 53]

Apart from TLR2 and 4, many other TLRs have been found in epithelial colon cells and in CRC cells, and are possible targets for cancer therapy.[54] However, TLR2 and 4 are already target of approved treatment. Indeed, Tsuji *et al* demonstrated that the anticancer effect of the

Bacillus Calmette-Guerin, used since the 1980s to treat superficial bladder cancer, is mediated by the activation of both TLR2 and TLR4.[55]

The confounding aspect of TLRs targeting therapy consists in the fact that TLRs exhibit both pro-tumour and antitumour effects. For example, TLR4 silencing decreases tumour burden in a murine model of colorectal metastases[56] but increases breast cancer metastases.[57] In addition, TLR4 can induce an efficient anticancer cytotoxic T cell immune response.[58] The mechanisms driving the different effects of TLR4 activation or silencing is not fully understood. It has been suggested that they may depend on the prevailing conditions in the TME.[59] However, TLR4 agonist and antagonist are under evaluation. Preliminary experimental data suggest that agonists can induce antitumour immunity in patients and animal models.[60 61] Agents with antagonist activity suppress TLR-induced NF-kB signalling, thus reducing the carcinogenesis induced by inflammation, and suppress TLR-induced migration and invasion.[62 63]

Gut microbiota, colon cancer and response to therapy

Growing evidence suggests that colon dysbiosis can promote chronic inflammation, the production of carcinogenic metabolites and favour the development of cancer.[53 64] However, this effect is the end stage of many events, based on the interplay among the gut microbiota, the barrier function of the intestinal epithelial cells and the inflammatory response. Understanding the role of dysbiosis in cancerogenesis might uncover new therapeutic targets in CRC.[65] For example, polysaccharide A, produced by commensal bacteria, increases local Tregs and consequently, IL-10. TLR2 mediates this effect.[66] Thus, TLR2 might represent a good target for combination immunotherapy. In addition, there is now evidence that targeting colon microbiota may improve therapeutic effects of anticancer drugs. For example, inhibition of β-glucoronidase, an enzyme produced by gut microbiota, can increase the activity of irinotecan by preventing its metabolism.[67] Similar correlation between anticancer therapy and gut microbiota has been demonstrated for cyclophosphamide,[68] platinum and oligonucleotide immunotherapy.[69] However, the study by Silvan *et al* addressing the influence of microbiota on response to immune therapy is the most convincing and highly promising.[70]

Silvan *et al* observed that B16.SIY melanoma, implanted in genetically similar C57BL/6 mice, grows more aggressively in mice nourished at the Taconic Farms (called TAC mice) than in those nourished at the Jackson Laboratory (called JAX mice). The difference was immunomediated, as demonstrated by a higher infiltrate of immune cells, including CD8+, in JAX mice. The authors tested the hypothesis that the difference could be mediated by commensal microbiota. Indeed, cohousing JAX and TAC mice in their lab before melanoma implantation ablated the difference in tumour growth as well as in immune response. Transferring faecal material from JAX to TAC mice and vice versa highlighted the role of commensal bacteria. The transfer of faecal suspension from JAX mice to TAC was sufficient to delay the tumour growth in the latter group, similar to the growth observed in the JAX, as well as the entity of the immune response. Interestingly, the benefit observed in TAC mice by transferring faecal suspension was equivalent to the benefit observed by anti-PD-L1 mAb treatment. The combination of transferred faecal material plus the anti-PD-L1 treatment further improved tumour growth delay, up to the value observed in JAX mice.

This experience, along with the others reported with classical chemotherapy, underlines the role of the gut microbiota in affecting systemic response to anticancer therapy and paves the way to a novel field of clinical investigations.

Zitvogel *et al* recently updated the complex and delicate interaction between gut microbiota, cancer and treatment result in an exhaustive review.[71] She summarised the large amount of data demonstrating the relationship between the excessive use of some antibiotics and the development of many human cancers, including the three big killers: breast, lung and colon cancers. Then she reviewed data on the interplay between chemotherapy or immune therapy and the colon microbiome, necessary to obtain the tumour control.

Step 5: from sidedness impact to immunological environment

A retrospective analysis from the CALGB/SWOG 80405 clinical trial in metastatic colon cancer showed that the location of the primary tumour within the colon predicts survival and may help in patient treatment selection.[72] Initially, researchers identified data from 293 patients with RCC and 732 patients with LCRC. This analysis included only patients without a mutated KRAS gene. In this population, patients with LCRC had longer median overall survival (OS) compared with those with RCC (33.3 months vs 19.4 months). Among patients who received cetuximab, patients with LCRC lived 36 months, whereas those with RCC lived 16.7 months. Similar trends were observed among patients receiving bevacizumab: OS was 31.4 versus 24.2 months for patients with LCRC and RCC, respectively. Among patients with RCC, treatment with bevacizumab was associated with longer survival than that seen with cetuximab (24.2 vs 16.7 months). Conversely, among patients with LCRC, treatment with cetuximab was associated with longer OS than bevacizumab (36 vs 31.4 months).[72]

A retrospective analysis in the RAS WT populations of the CRYSTAL and FIRE-3 trials demonstrated that patients with LCRC had superior progression-free survival (PFS), OS and overall response rate (ORR) compared with patients with RCC. Indeed, patients with RAS WT LCRC, treated with FOLFIRI plus cetuximab, showed significantly better OS with respect to those treated with FOLFIRI and FOLFIRI plus bevacizumab; in contrast, in RAS WT patients with RCC, limited benefit was observed upon the addition of cetuximab to FOLFIRI in CRYSTAL

Table 2 Clinical trials of immunotherapy in human colorectal cancer (CRC)

References	Clinical phase	Immunotherapy	Results
Topalian *et al* [76]	I	Nivo 3 mg/kg	18 CRC ORR 0%
Chung *et al* [77]	II	Tremelimumab 15 mg/kg every 90 days	PR (1/47 patients)
Le *et al* [78]	II	Pembro 200 mg every 3 weeks CRC MSI-H vs MSS	PFS MSI-H PFS MMS 78% vs 0%
Overman *et al* [79]	II Analysis ad interim Ongoing	Nivo 3 mg/kg ± Ipi 1 mg/kg CRC ± MSI-H	Nivo (33 patients) Nivo + Ipi (26 patients) ORR 27% vs 15% 5 months OS 75% vs 100%

ipi, ipilimumab; msi-h, microsatellite instability-high; mss, microsatellite stability; nivo, nivolumab; orr, overall response rate; os, overall survival; Pembro, pembrolizumab; pfs, progression-free survival; pr, partial response.

trial, and comparable outcomes were observed between the FOLFIRI plus cetuximab and FOLFIRI plus bevacizumab in FIRE-3 study.[73]

A meta-analysis of FIRE-3/AIO KRK0306, CALGB/SWOG 80405 and PEAK study indicates that patients with RAS WT LCRC had a significantly greater survival benefit from anti-EGFR treatment compared with anti-vascular endothelial growth factor (VEGF) treatment when added to standard chemotherapy. Conversely, in patients with RCC, benefit from standard therapy was poor and bevacizumab-based treatment was numerically associated with longer survival.[74]

Moretto *et al* demonstrated that patients with RCC RAS and BRAF WT mCRC seemed to derive no benefit from single-agent anti-EGFRs.[75]

These evidences underline that the benefit from anti-EGFR-based therapy was greater in RAS WT LCRC compared with RCC, with lack of activity of anti-EGFRs in RAS and BRAF WT, RCC. In the head-to-head comparison of anti-EGFR and anti-VEGF therapy, patients with RAS WT LCRC had a markedly greater benefit from anti-EGFR-based therapy. Therefore, LCRC appears to be a predictive factor for survival benefit from anti-EGFR treatment in patients with RAS WT tumours.These analyses confirm the importance of the primary tumour site, suggesting a potential role for primary tumour location in driving treatment choices.

Recently reported trials with ICIs (table 2) have shown limited results in CRC. However, dividing CRC in RCC and LCRC, we see that ICIs benefit RCC, namely the most immunogenic side of the large bowel.

This is intuitive, but it makes more difficult to understand the results previously described about cetuximab and bevacizumab. Cetuximab can trigger adcc while bevacizumab targets vascular endothelial growth factor, and thus it should counteract the immunosuppressive effects of this cytokine. indeed, both cetuximab and bevacizumab show immunologic properties.

It would be expected that both these drugs work better in the most immunogenic RCC, but it is not the case. Looking at the reduced activity of cetuximab in RCC, this might be related to genetic or epigenetic differences observed in RCC, including Braf mutation[11] and high PTEN mRNA expression.[76] Conversely, EGFR expression is similar regardless of tumour location[77] and thus tumour site should not interfere with cetuximab-induced ADCC. Nevertheless, also ADCC might be weakened by the large tumour masses more frequently observed in RCC compared with LCRC. NKG2D might mediate this phenomenon. Indeed, in physiological conditions, NKG2D activation induces a higher ADCC activity and vice versa.[78] However, NKG2D under continuous stimulation by its ligands leads to tolerance and lack of NK activation.[79] Large tumours abundantly express NKG2D-L, including high amount of soluble NKG2D-L, which is not present in smaller tumours and thus larger tumour more easily could induce NK tolerance and reduce ADCC. In line with this hypothesis, there is evidence in early human breast and colon cancer that high expression of NKG2D-L has a positive prognostic significance, while in large breast and ovarian cancer, high expression of NKG2D-L plays the opposite role.[80] More simply, NK cells might be kept in check by the abundance of regulatory mechanisms existing in hot escaped tumours that are not affected by cetuximab.

The lack of additional effect of bevacizumab in RCC might be explained by its two supposed distinct effects, which seem to be dose dependent.[81 82] At high doses, bevacizumab disrupts neovasculature, and thus it might increase hypoxia and finally cancer cell death, exploiting a direct anticancer effect.[83] This mechanism could also contribute to explain the lack of activity of high-dose bevacizumab in the adjuvant setting,[84] ie, before the neo-angiogenesis of the macroscopic tumours. At lower doses, bevacizumab exploits more pronounced immunological effects, in part, but not entirely, due to the normalisation rather than destruction of tumour vasculature.[85] The doses used in daily clinical practice are high doses,[86] with supposed limited immunogenic effects. Thus, bevacizumab treatment could not benefit from the more immunogenic characteristics of the RCC.

CONCLUSIONS

Colon cancer represents a complex situation where the TME drives the outcome of the disease and influences the response to the treatment, but it is in turn generated by the interplay among the microbiota, the immunogenicity of the cancer cells and the pre-existing immunological equipment. All these aspects change from proximal to distal large bowel and change in time and as consequence of treatment(s). Therefore there are billions of possible combinations and clinical situations.

To get to the bottom of this problem, we need to improve our knowledge to enhance our tools to distinguish one specific situation among thousands of different situations which are today considered comparable on the basis of TNM or stage classification, and finally, to change the way to design, conduct and analyse clinical trials.

In the composite reality of colon cancer, small phase II trials, designed to detect remarkable advantages in a highly selected and really homogeneous cancer population, combined with strong translational researches, might be more useful than classical large clinical trials.

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