Review

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Review

Interactions Between Circulating Tumor Cells and the Immune System in Colorectal Cancer: Friends or Foes?

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Simple Summary

Colorectal cancer often spreads to other organs through circulating tumor cells (CTC) that travel in the bloodstream. Normally, the immune system should recognize and destroy these cells. However, CTC can hide or protect themselves using several strategies. They can reduce their visible "identity tags" (MHC-I), display "do not kill me" signals such as PD-L1, or surround themselves with platelets and neutrophil traps to form a physical shield. CTC can cluster with immune cells, turning them into helpers rather than attackers. This review explains how CTC escape and manipulate the immune system in colorectal cancer. Understanding these mechanisms may help doctors predict which patients need stronger treatments. Moreover, studying CTC through a blood test (liquid biopsy) could guide personalized immunotherapy, such as immune checkpoint inhibitors or custom-made vaccines. Unraveling this battle between CTC and the immune system offers new hope for stopping metastasis and improving patient survival.

Abstract

Colorectal cancer (CRC) is a leading cause of cancer death worldwide, mainly due to metastasis. Circulating tumor cells (CTC) act as the biological "seeds" of dissemination, traveling through the bloodstream to colonize distant organs. However, the blood is a hostile environment where CTC must constantly face immune pressure. This review explores the bidirectional interactions between CTC and immune cells in CRC, asking whether CTC are merely vulnerable targets of immunosurveillance or can exploit the immune system for survival and metastasis. We dissect intrinsic and extrinsic immune evasion mechanisms, including MHC-I modulation, immune checkpoint expression (PD-L1, CD47, FasL), platelet cloaking, and neutrophil extracellular traps (NET). Furthermore, we examine how CTC form heterotypic clusters with monocytes, neutrophils, and lymphocytes, creating pro-metastatic niches and promoting phenotypic plasticity. The impact of CTC on systemic immunity, including reprogramming of NK cells, T lymphocytes, and myeloid-derived suppressor cells (MDSC), is discussed. Importantly, we highlight the emerging role of CTC as dynamic biomarkers for immunotherapy, focusing on the predictive value of PD-L1+ CTC and the potential of CTC-derived neoantigens for personalized vaccination. Despite progress, challenges remain in standardization, detection sensitivity, and clinical validation. Understanding the equilibrium between immune elimination and evasion by CTCs is crucial to develop novel interventions that interrupt the metastatic dialogue and improve outcomes for CRC patients.

Keywords: colorectal cancer; circulating tumor cells; immune evasion; immunotherapy; PD-L1; tumor microenvironment; metastasis; liquid biopsy; neutrophil extracellular traps; immune checkpoint

1. Introduction

Colorectal cancer (CRC) represents one of the most frequently diagnosed neoplasms and one of the leading causes of cancer death worldwide [1]. Despite advances in screening strategies, surgery, and systemic therapies, patient prognosis remains strictly linked to the appearance of distant metastases, particularly in the liver and lungs [2,3].

Metastasis is not a random event but a highly orchestrated biological process that includes the acquisition of invasive characteristics, degradation of the extracellular matrix, intravasation into the bloodstream or lymphatic system, survival in adverse hemodynamic conditions, and finally, extravasation and colonization of a new tissue microenvironment [3–5]. This pathway implies a profound phenotypic and molecular plasticity of tumor cells, which must adapt to selective pressures different from those present in the primary tumor [4,5].

In this context, circulating tumor cells (CTC) play a crucial role, configuring themselves as the biological "seed" of the metastasis according to the "seed and soil" model [6,7].

CTC represent an extremely rare and heterogeneous population, characterized by morphological, phenotypic, and functional variability [7–10]. They can present epithelial, mesenchymal, or hybrid traits, reflecting the dynamics of epithelial-to-mesenchymal transition (EMT) and its reversibility [11,12]. Beyond their prognostic and predictive relevance, CTC constitute a real-time model of tumor evolution, as they reflect the selective pressure exerted by therapies and the systemic microenvironment [13,14]. However, the blood compartment is not simply a passive means of transport: it is a hostile environment, characterized by mechanical stress, anoikis, and, above all, constant monitoring by the immune system [8,14–16].

The immune system indeed plays a central role in controlling tumor progression through the process of immunosurveillance, whereby effector cells such as cytotoxic T lymphocytes (CTL), Natural Killer (NK) cells, and macrophages recognize and eliminate transformed cells [17,18]. However, the interaction between tumor and immune system is not static but evolves over time according to the immunoediting paradigm, articulated in the phases of elimination, equilibrium, and escape [19,20].

In an initial phase, the most immunogenic tumor cells can be recognized and destroyed; subsequently, an equilibrium phase can establish itself in which immune pressure selects less immunogenic clones; finally, in the escape phase, subpopulations capable of evading immune recognition emerge through mechanisms such as down-regulation of tumor antigens, expression of immunosuppressive molecules, or induction of a tolerogenic microenvironment [19–21].

CTC are positioned exactly in this dynamic balance between elimination and evasion (Figure 1). During their time in the circulation, they are exposed to immune cells and soluble mediators, but they can also establish protective interactions, for example by associating with platelets or modulating the expression of immune checkpoint molecules [16,22]. Therefore, a biologically and clinically relevant question arises: do CTC simply represent vulnerable targets of systemic immune activity, or are they capable of exploiting the immune system, directly or indirectly, to increase their survival and metastatic potential? Understanding this dual nature of CTC could provide new interpretative keys to metastatic progression in CRC and open innovative perspectives for targeted therapeutic strategies.

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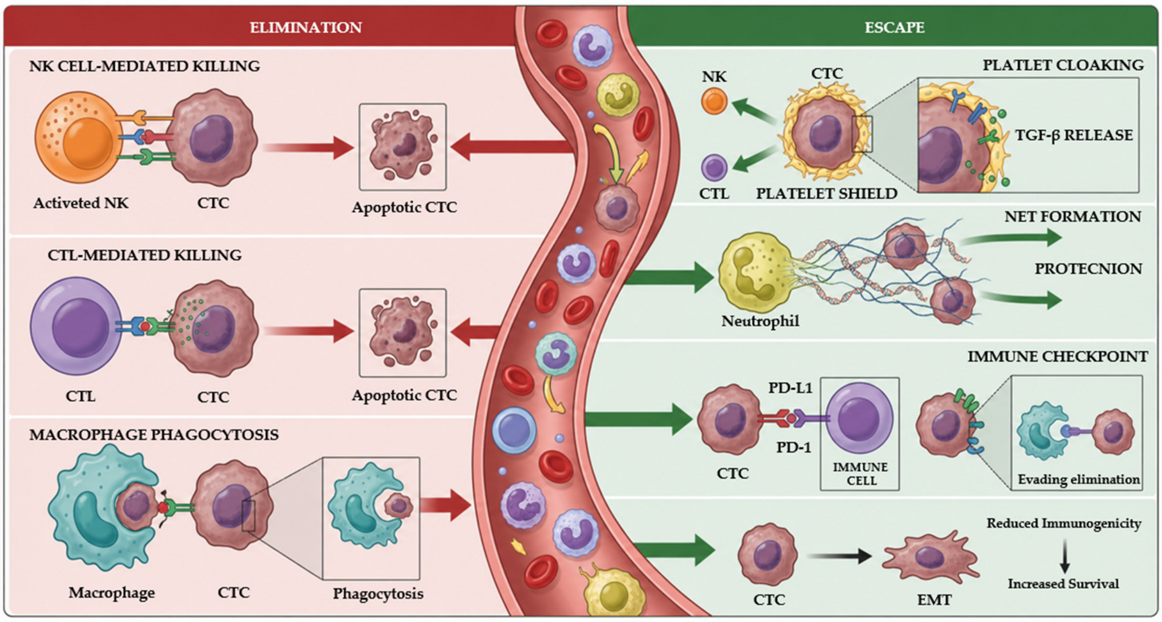

Figure 1. Journey of CTC in the bloodstream: interplay between immune elimination and evasion. The left panel illustrates the principal mechanisms involved in CTC elimination, including natural killer (NK) cell-mediated cytotoxicity, cytotoxic T lymphocyte (CTL)-mediated killing, and macrophage phagocytosis. The right panel depicts the main immune escape strategies adopted by CTC, such as platelet cloaking, neutrophil extracellular trap (NET) formation, immune checkpoint exploitation, and epithelial-to-mesenchymal transition (EMT), which collectively enhance survival, immune evasion, and metastatic dissemination.

2. Immune Escape Mechanisms of CTC in Colorectal Cancer

CTC represent an extremely heterogeneous and dynamic population, capable of surviving in the bloodstream despite constant surveillance by the immune system [16]. In CRC, several immune evasion mechanisms allow CTC to escape recognition and destruction by immune cells, thus favoring metastatic dissemination [23] (Figure 2). Among these, modulation of antigen presentation, expression of immune checkpoint molecules, and interaction with blood components such as platelets and neutrophils are particularly relevant [22,24–26] (Table 1).

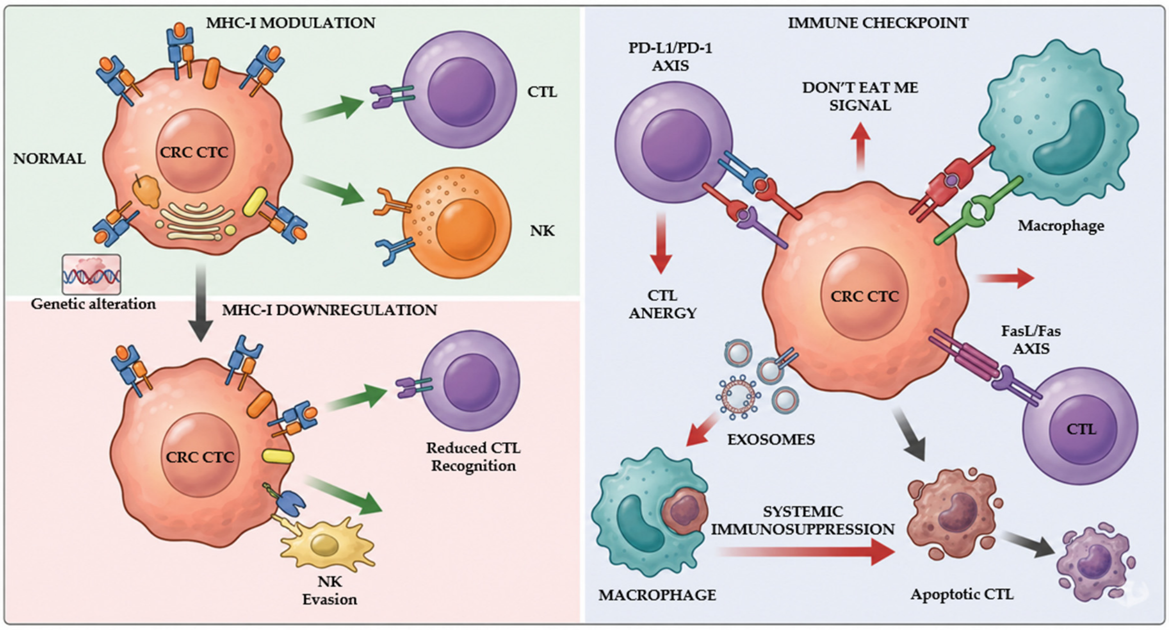

Figure 2. MHC-I modulation and immune checkpoint expression on CRC CTC. The left panel illustrates the impact of major histocompatibility complex class I (MHC-I) modulation on immune cell recognition. Under physiological conditions, preserved MHC-I antigen presentation machinery, including components such as TAP1 and LMP7, supports cytotoxic T lymphocyte (CTL) recognition and activation, while also influencing natural killer (NK) cell responses. Conversely, downregulation or alteration of MHC-I and associated antigen-processing machinery reduces CTL-mediated recognition and contributes to immune escape, with concurrent modulation of NK cell susceptibility. The right panel depicts key immune checkpoint and immune evasion pathways exploited by CRC CTC, including the PD-L1/PD-1 axis, CD47/SIRPα signaling, and FasL/Fas interactions, which collectively promote T-cell exhaustion or apoptosis, inhibit macrophage-mediated phagocytosis, and foster systemic immunosuppression, thereby enhancing CTC survival and metastatic potential.

Table 1. Key mechanisms of immune evasion of CTC in CRC.

Mechanism	**Key molecules**	**Effect on immune cell**	**Evidence in CRC**
Downregulation of antigen presentation machinery	MHC-I, TAP1/2, β2-microglobulin, LMP7	Reduced recognition by CD8+ CTL; partial loss prevents “missing self” recognition by NK cells	[27–31,33,35,36]
Upregulation of immune checkpoints	PD-L1	Inhibition of T cell cytotoxicity via PD-1; T cell exhaustion	[37,38,40–46]
“Don’t eat me” signal	CD47	Inhibition of macrophage phagocytosis via SIRPα; cis-masking of SLAMF7	[39,47–51]
Fas counterattack	FasL	Induction of apoptosis in Fas-expressing T lymphocytes	[52–54]
Alternative Immune checkpoint	PD-L2	Suppression of T cell function; expansion of Tregs; expressed on tumor exosomes	[55–57]
Platelet cloaking	Platelet MHC-I, TGF-β, CD155	Physical shield; transfer of MHC-I to CTC; suppression of NK cytotoxicity via CD155-TIGIT; induction of EMT	[58–65]
Neutrophil extracellular traps (NET)	NET-DNA, CEACAM1, histones	Physical barrier blocking CTL/NK contact; chemotaxis via CCDC25; induction of EMT	[66–75]

CEACAM1 (carcinoembryonic antigen-related cell adhesion molecule 1), CTL (cytotoxic T lymphocyte), EMT (epithelial-to-mesenchymal transition), FasL (Fas ligand), LMP7 (large multifunctional protease 7), MHC-I (major histocompatibility complex class I), NET (neutrophil extracellular trap), NK (natural killer), PD-1 (programmed cell death protein 1), PD-L1 (programmed death-ligand 1), SIRPα (signal regulatory protein alpha), SLAMF7 (signaling lymphocytic activation molecule family member 7), TAP1/2 (transporter associated with antigen processing 1/2), Treg (regulatory T cell).

2.1. Modulation of MHC Expression and the Antigen Presentation Machinery

One of the main mechanisms through which CTC evade the immune system is the reduction of expression of major histocompatibility complex class I (MHC-I) molecules, which are essential for presenting tumor antigens to CTL [27]. This phenomenon is often sustained by alterations in key components of the antigen processing and presentation machinery (APM), including TAP1/2, β2-microglobulin, and immunoproteasome subunits, whose loss or epigenetic repression reduces the peptide load presented on MHC-I and favors immune escape [28]. Ling et al. explored the possible molecular mechanisms involved in tumor immune evasion in CRC and found that TAP1 down-regulation correlates with immune evasion and unfavorable prognosis in CRC patients. Furthermore, to understand the possible regulatory mechanisms of TAP1 expression, the relationship with DNA methylation was studied, and it emerged that most of the CpG sites associated with TAP1 that were most involved clustered in 3 distinct regions along the gene: near the promoter region upstream of exon 1, in a region encompassing exons 2 and 3, and in a region encompassing exon 11. Focusing the analysis on a total of 9 CpG sites closest to the TAP1 promoter, a statistically significant difference was highlighted regarding the methylation status among these groups, but all CpG sites showed a higher level of methylation in the group of tumors with low TAP1 expression [29].

Furthermore, microenvironmental factors such as hypoxia can coordinately reduce MHC-I, TAP1/2, and LMP7, further limiting antigen presentation [30]. In the study by Sethumadhavan et al., it was demonstrated that oxygen tension in the TME modulates MHC-I-mediated antigen presentation. In particular, hypoxia induces a downregulation of MHC-I expression in HIF-dependent manner, as evidenced both in in vivo models and in hypoxic three-dimensional tumor cultures, but not in two-dimensional systems. Concurrently, the same study highlighted that hypoxia reduces the expression of key components of the antigen presentation machinery, including TAP1/2 and LMP7. Conversely, hyperoxia conditions promote the transcriptional upregulation of MHC and associated proteins (TAP2, LMP2/7) [30]. In the context of CRC and other solid tumors, intratumoral heterogeneity of MHC-I expression reflects a process of genetic and epigenetic immune editing, where clones with reduced APM/MHC-I function are selected under T-cell pressure and can dominate advanced and metastatic lesions [28,31]. The partial or reversible loss of MHC-I allows tumor cells to escape CTL surveillance while limiting NK cell activation, which recognize the complete absence of MHC-I as a “missing self” signal via inhibitory receptors such as KIR and NKG2A; for this reason, complete loss of MHC-I is rare in solid tumors [32]. This is consistent with the hypothesis that CTC present reduced or mosaic expression of MHC-I compared to the primary tumor, suggesting the selection of subclones more resistant to CTL surveillance during dissemination [33].

However, a complete loss of MHC-I renders cells highly susceptible to NK cell activity, which recognize the “missing self” phenotype through the absence of inhibitory MHC-I ligands for KIR/NKG2A receptors as demonstrated by Bern et al. [34]. For this reason, complete loss of MHC-I is relatively rare in solid tumors, while partial or reversible configurations are more frequent, often subclonal, allowing escape from CTL while limiting full NK activation [35,36]. It follows that CTC tend to maintain sufficient MHC-I levels to provide inhibitory signals to NKs and avoid immediate cytotoxicity, but not enough to allow effective antigen presentation and a robust CTL response, thus achieving a sophisticated form of adaptive immune escape [36].

2.2. Expression of Immune Checkpoints on CTC

CTC can actively contribute to immune suppression through the expression of immune checkpoint molecules, particularly Programmed Death-Ligand 1 (PD-L1) [37]. The interaction between PD-L1 expressed on CTC and the PD-1 receptor on T lymphocytes leads to inhibition of cytotoxic activity, promoting a state of T cell exhaustion [38]. In CRC, several pieces of evidence indicate that a subpopulation of CTC expresses PD-L1, often associated with more aggressive phenotypes and EMT characteristics [38].

The foundational 2014 study by Steinert et al. demonstrated for the first time, through gene expression analysis on manually isolated CTC from CRC patients, a marked upregulation of CD47 and transcriptional alterations compatible with a dormant state, suggesting that CTC adopt active immune escape mechanisms to survive in the circulation [39]. A retrospective study on 182 CRC patients showed that 42.2% of CTC-positive patients expressed PD-L1 on CTC, with association across all disease stages, including stable disease, suggesting a role as a dynamic biomarker of minimal residual disease (MRD) [40]. A broader analysis of 666 CRC patients confirmed that 74.6% of patients with CTC presented PD-L1 expression, with CTC clusters also found in 13% of cases [41]. Satelli et al. in 2016 isolated CTC with an EMT phenotype (surface vimentin positive) from metastatic CRC patients and demonstrated that nuclear expression of PD-L1 in CTC was significantly associated with reduced survival [42]. The association between PD-L1 and EMT in CRC was confirmed by Secinti et al.: PD-L1 expression in colorectal cancer cells correlated significantly with EMT status ($p < 0.001$), lymph node metastases, advanced stage, and reduced disease-free survival [43].

In vitro studies have demonstrated that FGFR2 induces PD-L1 expression in CRC cell lines (SW480 and NCI-H716) through the JAK/STAT3 pathway, with in vivo confirmation in murine xenograft models [44]. Furthermore, Wu et al. showed that CRC cell-derived exosomes containing miR-372-5p upregulate PD-L1 in macrophages through the PTEN/AKT/NF-κB pathway, suppressing the activity of CD3+CD8+ T lymphocytes in co-culture [45]. PD-L1 expression can be induced by inflammatory signals, such as IFN-γ, present in the tumor microenvironment and the bloodstream [46].

Beyond PD-L1, other immunoregulatory molecules such as PD-L2, CD47, and FasL can be expressed by CTCs, further contributing to their ability to evade the immune response. Regarding CD47, in 2012, Willingham demonstrated that CD47 is overexpressed in CRC tumor cells and that blocking CD47 with monoclonal antibodies restored macrophage phagocytosis in vitro and inhibited tumor growth in murine xenograft models with patient-derived tumor cells [47]. In 2023, Tang et al. discovered an additional SIRPα-independent mechanism: CD47 cis-masks the pro-phagocytic ligand SLAMF7 on the tumor surface, and blocking CD47 (but not SIRPα) restores phagocytosis [48]. In 2025, Miller et al. clarified the signaling cascade downstream of SIRPα, demonstrating how CD47 inhibits Vav phosphorylation in macrophages, blocking Rac activation and IgG-mediated phagocytosis [49]. Specifically in CRC, Arai et al., analyzing 14,287 CRC cases, demonstrated that high CD47 expression is associated with CMS1 and CMS4 subtypes and the activation of oncogenic pathways (MAPK, PI3K, TGF-β) [50]. Furthermore, Kang et al. demonstrated in vivo that dual blockade of CD47 and TNFR2 in murine CRC models produces synergistic antitumor effects, with reduction of Tregs and increased CD8+ activation [51].

Concerning FasL, as early as 1996, the “Fas counterattack” was demonstrated in CRC: the SW620 cell line expresses functional FasL and kills Jurkat T lymphocytes in a Fas-mediated manner in vitro, while the same tumor cells are resistant to Fas-mediated apoptosis [52]. Later, these results were extended by demonstrating that CRC cells release soluble FasL that induces dose-dependent apoptosis in Jurkat lymphocytes without cell-cell contact, and that serum levels of soluble FasL are significantly elevated in CRC patients [53]. Specifically regarding CTC, Papadaki et al. demonstrated Fas/FasL expression on CTC in metastatic breast cancer patients: FasL was expressed in 92.3% of CTC, and Fas/FasL co-expression was associated with significantly reduced PFS (9.5 vs 13.4 months; $p = 0.009$) [54].

Regarding PD-L2, it functions as an immune checkpoint independent of PD-L1 and is expressed in about 40-80% of CRCs [55]. In 2023, Lv et al. verified PD-L2 expression in tumor-associated macrophages (TAM) in CRC using single-cell RNA sequencing (scRNA-seq), multiplex immunofluorescence, and flow cytometry. PD-L2+ TAM showed a pro-tumoral M2 phenotype and increased the migratory, invasive, and proliferative capacity of colon cancer cells in transwell and colony formation assays [56]. In 2024, Zhu et al. conducted in vivo experiments on C57BL/6 mice with the MC38 cell line, demonstrating that targeting PD-L2 emerges as a complementary therapeutic strategy to PD-1/PD-L1 blockade. Although PD-L2 is inducible by IFN-γ like PD-L1, it shows a unique spatial distribution in the tumor microenvironment. Blockade of both checkpoints revealed a significant correlation with the infiltration of various immune cells, including multiple dendritic cell subtypes, implying enhanced antigen presentation [55]. Also, in 2024, Liu et al. demonstrated that PD-L2 is expressed mainly on tumor-derived exosomes (TDE-PD-L2) with surface localization. Under immunocompetent conditions, TDE-PD-L2 is sequestered by immune cells in a PD-1-dependent manner, systemically suppressing T cell function by increasing the proportion of Tregs and decreasing cytotoxic CD8+ T cells both in tumor-infiltrating lymphocytes and in the spleen [57].

In conclusion, the expression of these checkpoints on CTC not only favors survival in the circulation but also represents a potential dynamic biomarker for monitoring response to immunotherapies in CRC.

2.3. Extrinsic Protection Mechanisms: “Platelet Cloaking” and Neutrophil Extracellular Traps

In addition to intrinsic mechanisms, CTC exploit “extrinsic” protection strategies, interacting with blood components to create a physical and functional barrier against the immune system (Figure 3). One of the most studied mechanisms is “platelet cloaking,” the coating of CTC by platelets [58]. The study by Placke et al. demonstrated in vitro that tumor cells are rapidly coated by platelets forming heterotypic aggregates with a “pseudonormal” phenotype due to the transfer of platelet MHC class I onto the tumor cell surface, preventing missing self-recognition by NK cells. This transfer, in fact, suppresses NK cytotoxicity and IFN-γ production [58]. More recently, it has been confirmed that activated platelets aggregate and encapsulate CTC forming tumor microthrombi containing fibrin clots that act as protective barriers, interacting with NK cells, macrophages, neutrophils, and T cells to facilitate metastasis [59]. A 2011 study demonstrated both in vitro and in vivo that platelet TGF-β and direct platelet-CTC contact synergistically activate TGFβ/Smad and NF-κB pathways in tumor cells, inducing a transition to an invasive mesenchymal phenotype. Indeed, ablation of TGFβ1 expression induced exclusively in platelets protected mice from pulmonary metastasis formation in vivo [60]. Cluxton et al. described two other mechanisms by which platelets help tumor spread: (1) an immune decoy mechanism whereby platelets induce the release of soluble NKG2D ligands from tumor cells suppressing NK degranulation and IFN-γ production; (2) a TGF-β-mediated mechanism of suppressing the CD226/CD96-CD112/CD155 axis as an NK antitumor pathway [61]. Sun et al. identified by scRNA-seq and multiplex immunofluorescence that direct platelet adhesion to CTC upregulates the inhibitory checkpoint CD155 suppressing NK cytotoxicity exclusively through the CD155-TIGIT interaction; blocking TIGIT in vivo restored NK immunosurveillance and markedly reduced metastasis [62]. Specifically for CRC, Plantureux et al. demonstrated that platelets extravasate into the tumor microenvironment and interact with tumor cells in a cadherin-6-dependent manner, generating chimeric microparticles (iMP) that in the circulation induce EMT and promote metastasis, while locally they recruit macrophages with antitumor activity [63]. Furthermore, in 2023, colon CTC cell lines were used to study CTC-platelet cross-talk, demonstrating that conditioned medium from CTC induces platelet aggregation and activation, while co-culture with platelets increases the expression of genes involved in invasiveness (MMP2, MMP9, VEGFA) and maintains mesenchymal markers in CTC [64]. In vivo, it has also been demonstrated that podoplanin expressed on tumor cells in mice induces the release of TGF-β from platelets, thus promoting EMT and metastasis formation [65].

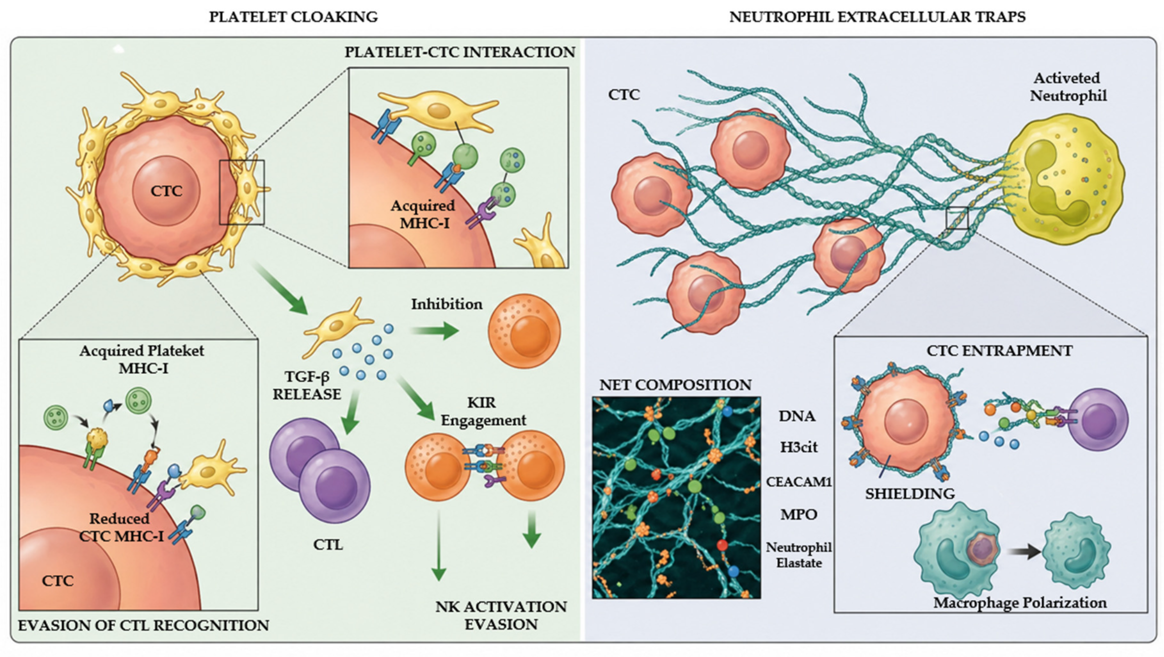

Figure 3. Extrinsic protection mechanisms: platelet cloaking and neutrophil extracellular traps. The left panel illustrates platelet-mediated cloaking as a key extrinsic immune evasion strategy adopted by CTC. Platelet adhesion to the CTC surface provides a physical shield that masks tumor-associated antigens, facilitates the transfer of platelet-derived MHC-I molecules, and promotes the release of immunomodulatory factors such as transforming growth factor-β (TGF-β). Collectively, these mechanisms impair cytotoxic T lymphocyte (CTL) recognition and inhibit natural killer (NK) cell activation, thereby enhancing immune escape and CTC survival. The right panel depicts the formation of neutrophil extracellular traps (NET), consisting of extracellular DNA fibers decorated with histones and granule-associated proteins, which entrap CTC within the circulation. NET-mediated sequestration provides a protective physical barrier against immune effector cells, facilitates interactions with immune and stromal components, and may promote macrophage polarization toward a pro-tumorigenic phenotype, ultimately favoring metastatic dissemination.

Concurrently, Neutrophil Extracellular Traps (NET) represent an additional mechanism supporting CTC survival. NET are filamentous structures composed of DNA and antimicrobial proteins released by activated neutrophils [66]. In the tumor context, NET can trap CTC in the circulation or in capillaries, facilitating endothelial adhesion and metastasis formation; protect CTC from attack by immune cells; promote a pro-inflammatory and pro-thrombotic state favorable to metastatic colonization [66]. In 2017, Najmeh et al. demonstrated, through an intra-abdominal sepsis mouse model (mimicking the post-operative inflammatory environment), that NET sequester CTC through β1-integrin-mediated interactions both in vitro and in vivo [67]. Yang et al. demonstrated that NET-DNA acts as a chemotactic factor attracting tumor cells. Briefly, the transmembrane receptor CCDC25 on tumor cells recognizes NET-DNA and activates the ILK-β-parvin pathway, increasing cell motility and thus metastatic capacity [68]. Furthermore, it has been shown that NET wrap and coat tumor cells, shielding them from CD8+ T lymphocyte and NK cytotoxicity by physically obstructing contact between immune cells and tumor targets [69].

In CRC, high levels of NET have been associated with unfavorable prognosis and increased risk of metastasis, suggesting a key role for these structures in disease progression. In 2020, Rayes et al. identified NET-associated CEACAM1 as an essential element for NET-CTC interaction in CRC [70]. Conversely, Stehr et al. detected citrullinated NET (H3cit+) in 44% of colon cancer tissues (37/85 patients), with a significant association with high histological grades and lymph node metastases. In vitro, purified NET induced filopodia formation, cell motility, and EMT in CRC cell lines, promoting the upregulation of vimentin, fibronectin, ZEB1, and Slug and inducing the downregulation of E-cadherin and EpCAM [71]. Lactate dehydrogenase A (LDHA) expression also appears to play a role in NET-promoted metastasis. Li et al. demonstrated, in fact, that inhibition of LDHA or NET formation effectively inhibited NET-induced liver metastasis in vivo, suggesting an active role of LDHA regulation through the PI3K/AKT pathway in inducing EMT in CRC cells [72]. In 2026, Pan et al. demonstrated how *Escherichia coli* recruits RIPK2 in neutrophils, promoting NET formation via p-MLKL, which in turn stabilize STAT3-dependent enhancer-promoter loops in CRC cells, reinforcing Tns1 transcription and facilitating liver metastasis [73]. Regarding the prognostic value of NET in CRC, a systematic meta-analysis on 5202 cancer patients confirmed that high NET levels are significantly associated with worse OS (HR 1.80; 95% CI 1.35-2.41) and DFS (HR 2.26; 95% CI 1.82-2.82), independent of the sample source (tissue or blood) [74]. However, in a study of 1,927 CRC patients across three independent cohorts, it emerged that intratumoral Cit-H3+ NET densities were not associated with survival, whereas a higher density of CD66b+ granulocytes was associated with longer CRC-specific survival (multivariate HR 0.53; 95% CI 0.38-0.73), suggesting that the prognostic role of NET in CRC may be more complex than initially hypothesized [75].

3. CTC Cellular Interactions in the Circulation

It is now clear that CTC do not travel in the bloodstream as isolated entities but interact dynamically with various components of the immune system and beyond [76]. These interactions represent a crucial step in the metastatic process, influencing both CTC survival and their ability to colonize distant sites [77]. In particular, the dialogue between CTC and immune cells can have a dual role: on one hand, it promotes the recognition and elimination of tumor cells; on the other, it can favor immune evasion mechanisms and support the formation of pro-metastatic niches [8,78]. Understanding the nature of these interactions is therefore essential to clarify the fate of CTCs in the circulation and their contribution to colorectal cancer progression.

3.1. CTC-PBMC Clusters: Cellular Crosstalk and Pro-Metastatic Niches

An emerging aspect in CTC biology is their ability to form clusters [79]. It has been widely demonstrated that CTC clusters have a metastatic potential 23-100 times higher than single CTC [80]. In a clinical study conducted on 103 CRC patients, Divella et al. noted that the presence of clustered CTC was significantly associated with elevated levels of TGF-β and CXCL1 and reduced overall survival. Taken together, these results showed that CTC clustering represents a negative prognostic factor [81].

CTC clusters can be divided into homotypic, composed solely of tumor cells, and heterotypic, arising from the aggregation of tumor cells with peripheral blood mononuclear cells (PBMC), including lymphocytes and monocytes [82] (Figure 4). These cellular aggregates are not simple passive associations but highly organized structures in which intense molecular crosstalk is established [83]. For example, within CTC-PBMC clusters, tumor cells can benefit from paracrine signals and direct cell-cell contacts that increase their survival in the circulation, protecting them from hemodynamic stress and immune attacks [84]. The study by Bobkov et al. demonstrated that hyaluronic acid (HA) mediates CTC clustering independently of adherent junctions and that HA acts as an anchoring platform to promote heterotypic cluster formation by recruiting immune cells and increasing CTC survival under hemodynamic stress [85].

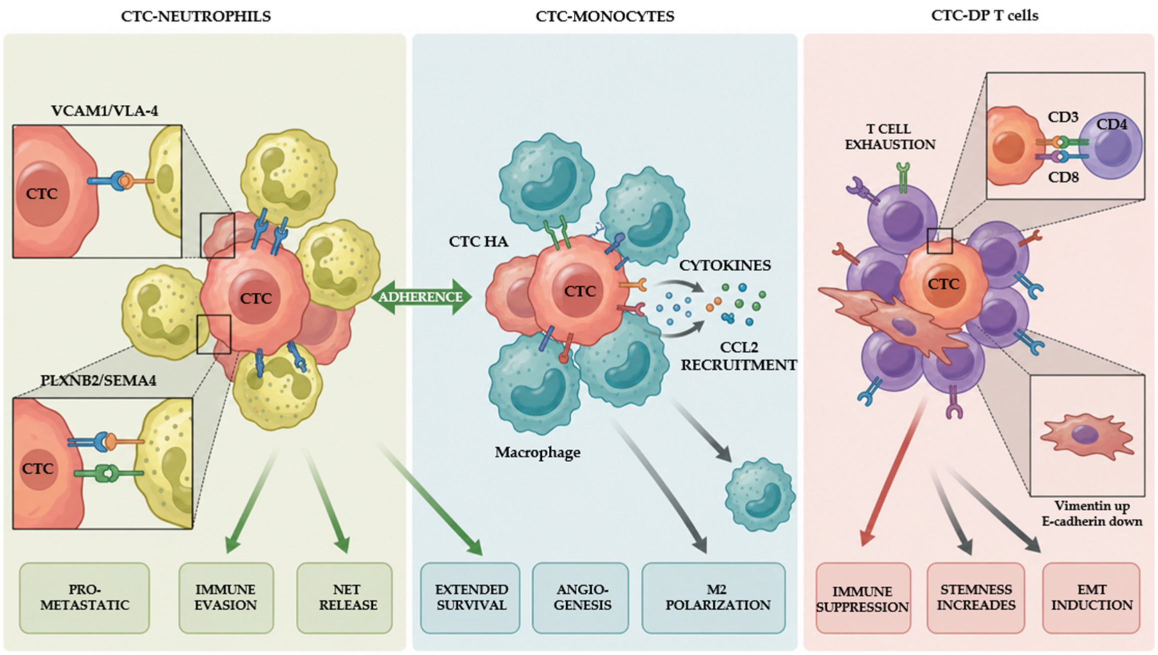

Figure 4. Heterotypic CTC clusters and their pro-metastatic role.

Moreover, PBMC associated with CTC can actively contribute to creating a favorable microenvironment for metastatic dissemination [82]. A study characterizing CTC-leukocyte clusters analyzed blood samples from 70 breast cancer patients and found that CTC-neutrophil clusters were the most frequent population with greater metastatic competence compared to single CTC [86]. Furthermore, an in vitro study highlighted that neutrophils form microtentacles (McTN) composed of detyrosinated/acetylated α-tubulin and vimentin, which facilitate heterotypic cluster formation [87]. According to Ju et al., CTC-neutrophil clusters help CTC survive in the hostile vascular environment by enhancing their metastatic capabilities. Conversely, Spiegel et al. demonstrated in murine models that CD11b+/Ly6G+ neutrophils promote metastasis by inhibiting NK cell function and facilitating extravasation through the secretion of IL-1β and metalloproteinases [88].

Scholten et al. not only noted that over 75% of CTC-positive blood samples from advanced breast cancer patients contained heterotypic CTC-white blood cell (WBC) clusters but also that a rare subpopulation of CD4+CD8+ double-positive T cells (DPT) was enriched 140-fold in CTC clusters compared to their frequency in WBC. Moreover, the interaction between CTC and DPT cells, mediated by the VCAM1/VLA-4 axis, conferred unique exhaustion and immunosuppression characteristics on tumor cells [89].

Monocytes and certain lymphocyte subpopulations can release cytokines, chemokines, and growth factors that promote the phenotypic plasticity of CTC, including EMT, thus increasing their invasiveness and extravasation capacity. Schuster et al., through computational ranking, identified the transmembrane protein Plexin-B2 (PLXNB2) as a key mediator of CTC-monocyte clusters. PLXNB2 was found to be enriched in CTC clusters compared to single CTC from advanced breast cancer patients. In vivo, loss of PLXNB2 reduced the formation of both homotypic and heterotypic clusters, reducing spontaneous metastasis, whereas the interaction of PLXNB2 with SEMA4C (on tumor cells) and SEMA4A (on monocytes) promoted homotypic and heterotypic clustering, respectively, guiding lung metastasis [90]. Amin et al. demonstrated through an in vitro study that tumor cell-monocyte crosstalk induces the early release of TNF-α and IL-6, thus promoting migration, invasion, colony formation, and EMT [91]. Specifically for CRC, a study conducted by Wei et al., both in vitro and in vivo, highlighted that CD163+ TAM induce EMT through the secretion of IL-6 and activation of the JAK2/STAT3/miR-506-3p/FoxQ1 axis, increasing the proportion of mesenchymal CTC, which in turn produce CCL2 that recruits macrophages, creating a positive feedback loop [92].

A further relevant element is the ability of CTC-PBMC clusters to act as pre-conditioned metastatic “seeds.” These aggregates can facilitate adhesion to the endothelium and subsequent engraftment in secondary tissues, acting as functional units capable of rapidly establishing a pro-metastatic niche. In this context, the crosstalk between CTC and immune cells not only favors survival in the circulation but also directly contributes to the efficiency of the metastatic process [93]. Vrynas et al. demonstrated through microfluidic models, which mimic human capillary bifurcations, how CTC interact with capillary beds, and it emerged that CTC release extracellular vesicles, termed shearosomes, capable of polarizing monocytes towards pro-tumoral M2 macrophages and altering endothelial barrier integrity, thus influencing the metastatic cascade [94] (Table 2).

Table 2. Heterotypic CTC clusters: composition and pro-metastatic mediators.

Cluster type	Key mediators / crosstalk	Functional effect	Model	Ref.
CTC-neutrophil	Microtentacles (detyrosinated tubulin, vimentin); IL-1β, MMPs	Enhanced survival, inhibition of NK cells, extravasation	CRC patient samples, murine models	[86–88]
CTC-monocyte / TAM-like	Plexin-B2 (PLXNB2) with SEMA4A; TNF-α, IL-6, TGF-β, IL-10; JAK2/STAT3/miR-506-3p/FoxQ1	EMT, mechanical fitness, clustering, extravasation, M2 polarisation	Breast cancer models, CRC in vitro/in vivo	[90–92,96,98–104]
CTC-DPT	VCAM1/VLA-4 axis	T cell exhaustion, immunosuppression	Advanced breast cancer	[89]
CTC-PBMC	Hyaluronic acid (HA); shearosomes (extracellular [86–88vesicles)	Clustering, protection from hemodynamic stress, M2 polarisation, endothelial barrier alteration	Microfluidic models, breast/CRC	[85,94]
CTC-macrophage	CCL2 loop, IL-6, JAK2/STAT3	Increase in mesenchymal CTCs, positive feedback recruitment	CRC in vitro/in vivo	[92]

CCL2 (C-C motif chemokine ligand 2), CTC (circulating tumor cell), DPT (double-positive T cell), EMT (epithelial-to-mesenchymal transition), FoxQ1 (forkhead box Q1), HA (hyaluronic acid), IL (interleukin), JAK2 (Janus kinase 2), M2 (alternatively activated macrophage polarization state), McTN (microtentacle), MMP (matrix metalloproteinase), NK (natural killer), PBMC (peripheral blood mononuclear cell), PLXNB2 (plexin B2), SEMA4A (semaphorin 4A), STAT3 (signal transducer and activator of transcription 3), TAM (tumor-associated macrophage), TGF-β (transforming growth factor beta), TNF-α (tumor necrosis factor alpha), VCAM1 (vascular cell adhesion protein 1), VLA-4 (very late antigen-4).

3.2. Macrophages and Myeloid-Derived Suppressor Cells

Among the immune populations involved in the interaction with CTC, macrophages and myeloid-derived suppressor cells (MDSC) play a central role in promoting tumor progression and immune evasion [95]. A study in prostate cancer showed that CTC isolated from patient blood are co-isolated with macrophage-like cells with TAM markers. Single-cell proteomic analysis and phenotyping revealed that macrophages promote epithelial-mesenchymal plasticity in tumor cells, conferring a “mechanical fitness” phenotype, characterized by softness and high adhesiveness, resulting in increased resistance to hemodynamic stress and facilitation of protective clustering [96].

Macrophages, particularly TAM-like, can interact with CTC both directly and indirectly [97]. In the circulation, monocytes can differentiate into cells with pro-tumoral characteristics, supporting CTC through the secretion of soluble factors that promote survival, migration, and invasiveness [98]. Molecules such as IL-10, TGF-β, and angiogenic factors contribute to creating an immunosuppressive and pro-metastatic environment. As early as 1995, it was demonstrated that TGF-β potentiates the ability of macrophages to produce IL-10 in both healthy and tumor-bearing mice [99]. Subsequently, Li et al., using a 3D microfluidic assay, highlighted that TNF-α and TGF-β1 secreted by macrophages synergistically increase the speed and persistence of tumor cell migration through the extracellular matrix, in an MMP-dependent manner [100]. A recent study in CRC demonstrated that TGF-β builds a double immune barrier that blocks the recruitment of memory CD8+ T cells and instructs TAM to express SPP1, promoting collagen decomposition and consequently the accumulation of TAM and fibroblasts, driving immunotherapy resistance in liver metastases [101]. Harney et al. demonstrated using two-photon intravital microscopy that TIE2hi macrophages in the tumor microenvironment of metastases cause local and transient vascular permeability via VEGFA, thus allowing tumor cell intravasation [102].

Furthermore, macrophages can facilitate CTC extravasation by modulating endothelial integrity and promoting tissue remodeling processes. The role of macrophages in tumor cell extravasation and pre-metastatic niche formation was clarified by Genna et al. through in vitro and in vivo studies, demonstrating, firstly, the formation of thin membranous connections between macrophages and tumor cells across the endothelial barrier and, secondly, that direct contact between macrophage and tumor cell is necessary to promote extravasation [103]. Häuselmann et al. also investigated the same aspect, demonstrating that monocytes recruited via endothelial E-selectin, induced by tumor CCL2, modulate endothelial VE-cadherin junctions, causing the opening of tight junctions and thus facilitating transendothelial migration of tumor cells [104].

MDSC represent another key component in the negative regulation of the anti-tumor immune response [105]. These cells are known for their potent immunosuppressive activity, exerted through various mechanisms, including the production of arginase-1, nitric oxide (NO), and reactive oxygen species (ROS), which inhibit the function of T cells and NK cells [106]. The interaction between CTC and MDSC can therefore significantly reduce immune pressure, allowing tumor cells to survive and disseminate [107].

Moreover, MDSC can directly contribute to the formation of pre-metastatic niches, favoring the recruitment of other immunosuppressive cells and the deposition of extracellular matrix components. This process creates a permissive environment for CTC engraftment in distant sites [108]. MDSC recruited to pre-metastatic lungs, for example, produce IL-1β, which increases endothelial E-selectin expression and promotes tumor cell arrest [109]. Recent evidence also suggests that CTC can actively modulate the recruitment and expansion of MDSC, establishing a positive feedback loop that amplifies systemic immunosuppression [110]. A study of 38 HER2-negative metastatic breast cancer patients noted a higher frequency of Tregs and CD14+CD15- MDSCs in patients with detectable CTC, suggesting that CTC may be under the control of the immune system and that different immune evasion mechanisms are involved during their biological evolution [111].

Overall, macrophages and MDSC not only protect CTC from immune surveillance but actively enhance their metastatic potential, thus representing promising therapeutic targets in metastatic CRC.

4. The Role of the Immune System in CTC Elimination: Still Effective?

The immune system represents the first line of defense against CTC, but its real effectiveness in controlling metastatic dissemination remains a matter of debate [112,113]. Preclinical evidence indicates that the vast majority of CTC are eliminated in the bloodstream; however, clinical data demonstrate that a proportion of CTC nonetheless manage to persist and give rise to metastases, suggesting that the immune system exerts a powerful but incomplete selective pressure [112,113].

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Among the components of innate immunity, NK cells play a crucial role in the elimination of CTC due to their ability to recognize cells with reduced MHC class I expression, a feature frequently observed in CRC cells [114]. NK induce direct cytotoxicity through the release of perforin and granzymes, in addition to secreting cytokines such as IFN-γ that amplify the immune response [114].

In vivo studies have provided direct evidence of the protective role of NK in CRC. In a murine xenograft model with HT29 colon carcinoma cells, mice lacking functional perforin in NK (pfp/rag2) developed lung metastases in 81% of cases (13/16) compared to 25% (5/20) of mice with functional NK (rag2), with an average number of metastases almost 4 times higher (789 vs 210); computational modeling estimated that perforin-dependent NK cytotoxicity eliminates about 80% of CTC and forces residual tumor cells into a dormant state for at least 30 days [115]. Similarly, NK depletion with anti-asialo GM1 antibody in C57BL/6 mice increased lung metastases 10-fold after intravenous tumor cell inoculation, and prolonged NK suppression dramatically accelerated the development of spontaneous lung metastases without affecting primary tumor growth [116]. In murine models of CRC liver metastasis (MC38), NK depletion significantly increased metastatic burden, and CXCR3+CD49a+ NK proved to be the subpopulation with the highest cytotoxic capacity; conditional deletion of Cxcr3 in NKp46+ cells compromised NK accumulation and function in metastases [117]. Furthermore, STING signaling in macrophages promotes NK antitumor function in CRC liver metastases through 4-1BBL/4-1BB co-stimulation; NK depletion in wild-type mice increased metastatic burden, while no significant effect was observed in myeloid STING-deficient mice [118]. In vitro studies on patient-derived CRC organoids (PDO) confirmed that most CMS2/CMS3 subtype PDO are susceptible to NK-mediated lysis, and that MHC-I deficiency and NKG2D ligand expression on organoids facilitate cytotoxicity; pharmacological targeting of HIF1A/EPAS1 or TGF-βR1, or the use of anti-CEACAM1 antibodies, further enhanced PDO killing [119]. Allogeneic NK derived from cord blood demonstrated effective antitumor activity in primary and metastatic CRC cell suspensions, inducing tumor cell lysis, conversion of monocytes into activated dendritic cells, and activation of CD8+ and CD4+ T cells with reduction of activated Tregs; combination with R848 induced a pro-inflammatory shift with increased IFN-γ, IL-2, and IL-12p70 [120] (Table 3).

Table 3. Summary of preclinical and clinical evidence on NK cell activity against CRC CTC.

Model system	Condition	Effect	Key findings	Ref
Murine xenograft	pfp/rag2 mice (perforin-deficient NK) vs rag2 (functional NK)	Lung metastases: 81% vs 25%; mean number 789 vs 210	Perforin-dependent NK cytotoxicity eliminates ~80% of CTC; forces dormancy for ≥ 30 days	[115]
C57BL/6 mice (intravenous CRC cells)	NK depletion with anti-asialo GM1	10-fold increase in lung metastases; accelerated spontaneous metastases	NK control both initial seeding and spontaneous dissemination	[116]
Murine CRC liver metastasis (MC38)	NK depletion; CXCR3 conditional deletion	Increased metastatic burden; CXCR3+CD49a+ NKs are key cytotoxic subset	CXCR3 required for NK accumulation and function in metastases	[117]
Murine CRC liver metastasis	STING signalling in macrophages; NK depletion	Increased metastatic burden in NK-depleted wild-type; no effect in myeloid STING-deficient	4-1BBL/4-1BB co-stimulation from macrophages promotes NK antitumor function	[118]
Patient-derived CRC organoids (PDOs)	CMS2/CMS3 PDO; HIF1A/EPAS1 or TGF-βR1 targeting; anti-CEACAM1	Enhanced NK-mediated lysis	MHC-I deficiency and NKG2D ligands facilitate killing; pharmacological targeting further potentiates lysis	[119]

Allogeneic cord blood NK + primary/metastatic CRC cells	NK alone or with R848	Tumor cell lysis; monocyte→DC conversion; CD8+/CD4+ T activation; Treg reduction	Combination with R848 increases IFN-γ, IL-2, IL-12p70	[120]

CEACAM1 (carcinoembryonic antigen-related cell adhesion molecule 1), CMS (consensus molecular subtype), CRC (colorectal cancer), CTC (circulating tumor cell), CXCR3 (C-X-C chemokine receptor type 3), DC (dendritic cell), EPAS1 (endothelial PAS domain protein 1), HIF1A (hypoxia-inducible factor 1-alpha), IFN-γ (interferon gamma), IL (interleukin), NK (natural killer), PDO (patient-derived organoid), pfp (perforin), rag2 (recombination activating gene 2), STING (stimulator of interferon genes), TGF-βR1 (transforming growth factor beta receptor 1), Treg (regulatory T cell).

5. Impact of CTC on Systemic Immunity in CRC

CTC are not simply passive targets of the immune system but actively act as modulators of systemic immunity in CRC, inducing profound alterations in peripheral immune populations and creating an environment favorable to metastatic dissemination [23,78,121] (Figure 5).

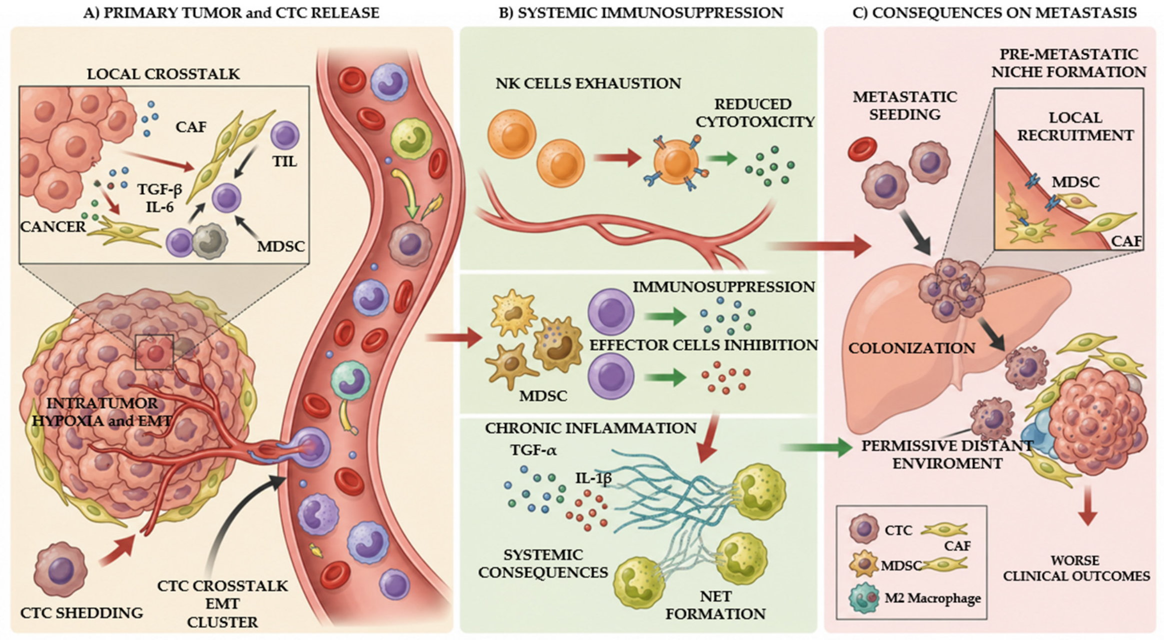

Figure 5. Impact of CTC on systemic immunity: from local crosstalk to peripheral immunosuppression.

In CRC patients, the presence of CTC is associated with quantitative and functional remodeling of circulating immune cells. A study of 200 CRC patients (stages II-IV) demonstrated that CTC-positive patients (> 3 CTC) present significantly lower levels of NK cells (16.8% vs 22.3%, $p \leq 0.05$) compared to CTC-negative patients, with a redistribution of NK subpopulations characterized by an increase in immunosuppressive NK cells (8.8% vs 5.0%, $p \leq 0.05$) and immature CD16dimCD56bright cells (13.1% vs 5.9%, $p \leq 0.05$) at the expense of cytotoxic CD16+CD56dim cells (82.5% vs 90.4%) [122]. These changes indicate a disorder in NK maturation and an inhibition of their antitumor properties [122]. The impact of CTC also extends to T lymphocytes. A study of 60 CRC patients revealed that in CTC-positive patients, the number of strong and moderate correlations between systemic immunological factors involving CD8+ is drastically reduced compared to CTC-negative patients (7 vs 19), while correlations with Tregs are increased (5 vs 3). In stages III and IV, a total disruption of correlations between systemic immune factors and proliferating tumor cells is observed, suggesting that the presence of CTC, rather than the tumor stage itself, is associated with an imbalance of systemic and local immune factors [123]. Furthermore, a study on 299 CRC patients demonstrated a significant inverse relationship between the intensity of tumor lymphocytic infiltration and the presence of CTC: CTC were 1.4 times more frequent in tumors with weak lymphocytic infiltration compared to those with moderate or strong infiltration (76.5% vs 56.3%, $p = 0.019$) [124].

CRC CTC exert their immunomodulatory action through direct and indirect mechanisms. Gene expression analysis on manually isolated CTC from CRC patients revealed a marked overexpression of CD47, an immune evasion mechanism that sends a "don't eat me" signal to macrophages, together with a significant downregulation of several metabolic pathways, suggesting a dormant state of viable CTC [39]. Recent studies have confirmed that 74.6% of CTC in CRC express PD-L1, allowing them to escape elimination by T lymphocytes and persist in a dormant state in the bloodstream [41]. This PD-L1 expression is present across all stages, even in early phases and in patients with progression-free disease status, suggesting that PD-L1+ CTC represent a dynamic biomarker of MRD [40].

CTC also induce systemic inflammation through the production of TLR2 and TLR4 ligands. A preclinical study demonstrated that CTC promote metastatic colonization of disseminated tumor cells by inducing systemic inflammation and neutrophil recruitment to pre-metastatic organs. Mechanistically, CTC-derived ligands for TLR2/4 induce the production of pro-inflammatory cytokines such as G-CSF and IL-6, which convert neutrophil function from tumor-suppressing to tumor-promoting. Moreover, CTC induce the production of endogenous ligands for TLR2/4 such as S100A8, S100A9, and SAA3, which amplify the stimulatory effect on pro-inflammatory cytokine expression [125]. In metastatic CRC, the presence of CTC clusters is significantly associated with elevated circulating levels of TGF-β and CXCL1 and reduced overall survival. A study of 103 metastatic CRC patients demonstrated that circulating cytokine levels are differentially associated with the two CTC populations (single vs clusters), with clusters representing a negative prognostic factor [81]. TGF-β, in particular, is one of the main immunosuppressive factors secreted by tumor cells, with elevated serum concentrations detected in CRC patients and associated with metastases [99]. A study on CRC also demonstrated that serum IL-17A levels correlate with the number of mesenteric CTC and with disease-free survival. IL-17A promotes tumor cell motility, matrix digestion, and angiogenesis, while GM-CSF stimulates CTC elimination by enhancing host immunity; ablation of IL-17A and administration of rGM-CSF effectively suppressed the increase in CTC and prevented metastasis in murine models [126].

Extracellular vesicles (EV) derived from CRC tumor cells represent a key mechanism through which CTC modulate systemic immunity. CRC EV are enriched in TGF-β1 and induce phenotypic alteration of T lymphocytes into Treg-like cells through activation of the TGF-β/Smad pathway and inactivation of the SAPK pathway. Treg-like cells induced by CRC EV have remarkable tumor growth-promoting activity in vitro and in vivo [127]. CRC EV also modulate TAM. A recent study demonstrated that TAM initially recognize CRC exosomes as foreign entities, triggering a pro-inflammatory response; however, over time, the content of these phagocytosed exosomes reprograms TAM into an anti-inflammatory and tumor-supportive phenotype, primarily through activation of the transcription factor NF-κB [128]. CRC EV containing miR-372-5p can be phagocytosed by both CRC cells and macrophages, regulating PD-L1 expression through the PTEN/AKT/NF-κB pathway and inducing an immunosuppressive microenvironment that promotes CRC development [45]. CRC tumor EV also suppress the CD28-CD80/86 co-stimulation pathway in tumor-infiltrating T lymphocytes and dendritic cells through miR-424 content, leading to resistance to immune checkpoint blockade. Tumor EV modified with miR-424 knockdown enhanced the T lymphocyte-mediated antitumor immune response in CRC models and increased the response to immune checkpoint blockade. Intravenous injections of modified tumor EV induced antigen-specific immune responses and enhanced the efficacy of immune checkpoint blockade in CRC models mimicking advanced-stage aggressive disease [129]. Recent studies have also demonstrated that EV derived from circulating tumor endothelial cells, enriched in mTOR, support G-CSF release and trigger phosphorylation of the downstream target of mTOR S6 (Ser235/236), ensuring tumor immunosuppression and metastatic growth through systemic and local expansion of immunosuppressive cells [130].

In vivo studies have confirmed that CTC promote metastatic colonization by inducing systemic inflammation and neutrophil recruitment to pre-metastatic organs, and that neutrophil depletion effectively abrogates the pro-metastatic effect of CTC [125].

A critical aspect of the impact of CTC on systemic immunity concerns the disruption of physiological correlations between immune components. In CTC-positive patients, pathological correlations are observed, such as a moderate direct correlation between the number of activated T lymphocytes and Ki-67+ tumor cells, while the number of correlations between intratumoral lymphocytes and tumor cells expressing proliferation and EMT markers is drastically reduced compared to CTC-negative patients (4 vs 10 in stage II, 1 vs 9 in stage III, 2 vs 9 in stage IV). This suggests that the presence of CTC destroys the functional architecture of the immune system, compromising both the local and systemic response [123].

In CRC, CTC are not simply transient tumor cells but active orchestrators of systemic immunosuppression. This ability to modulate systemic immunity represents a key mechanism through which CTC create a permissive environment for survival and metastatic colonization, and identifies new therapeutic targets to interrupt the metastatic cascade in CRC.

Schematic representation that summarizes the dynamic interplay between primary tumor–derived CTC and the immune system, highlighting the transition from local tumor microenvironment crosstalk to systemic peripheral immunosuppression. Tumor cell shedding and CTC release into the bloodstream promote immune remodeling through reduced natural killer (NK) cell activity, expansion of myeloid-derived suppressor cells (MDSC) and regulatory T cells (Treg), and chronic inflammatory signaling, including neutrophil extracellular trap (NET) formation. These systemic alterations establish a permissive pre-metastatic niche that supports metastatic seeding, colonization, and outgrowth at distant sites, ultimately contributing to increased metastatic burden and therapeutic resistance.

6. CTC as Biomarkers and Targets in CRC Immunotherapy

In recent years, CTC have gained increasing relevance not only as prognostic biomarkers but also as potential tools to guide immunotherapy strategies in CRC [123]. The possibility of analyzing CTC non-invasively through “liquid biopsy” allows obtaining dynamic and real-time information on the molecular and immunological status of the disease, partially overcoming the limitations of traditional tissue biopsies [131].

From an immunological perspective, CTC offer a unique opportunity to assess immune evasion mechanisms, identify therapeutic targets, and monitor treatment response [132]. In particular, the phenotypic and molecular characterization of CTC can provide indications on susceptibility to immunotherapy, contributing to patient stratification [133,134]. Moreover, CTC could themselves represent a therapeutic target, either through direct elimination approaches or through strategies aimed at interfering with the mechanisms that favor their survival and interaction with the immune system [76,78,135] (Figure 6).

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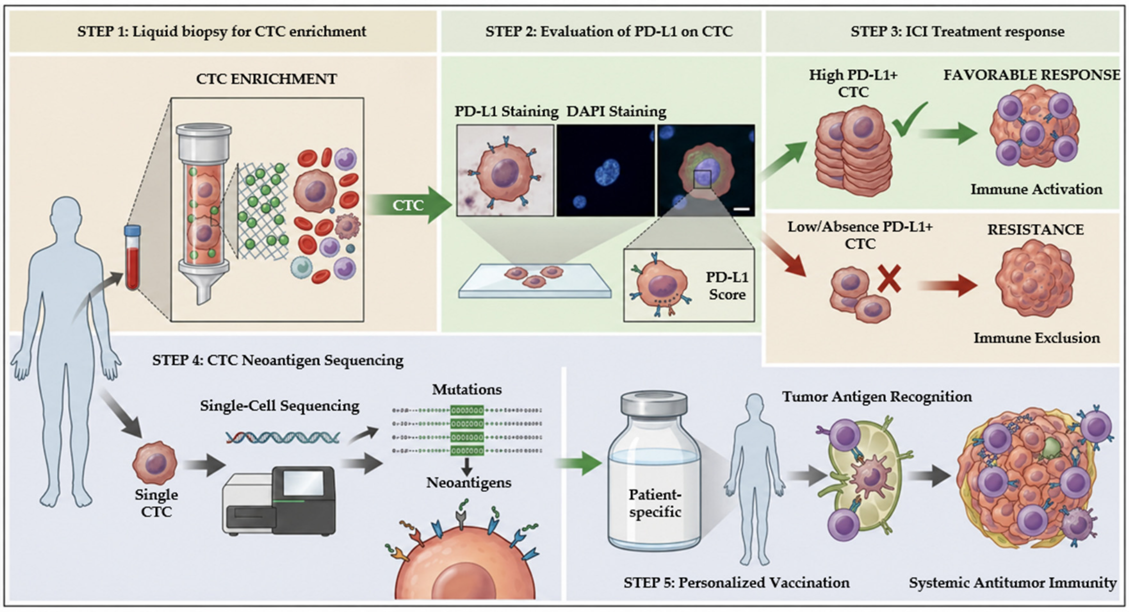

Figure 6. CTC as predictive biomarkers for immunotherapy and platforms for personalized vaccination.

However, despite the great potential, the clinical application of CTC in the immunotherapeutic field is still limited by several critical issues, including cellular heterogeneity, low frequency in peripheral blood, and lack of standardization in isolation and analysis methods. In this context, the integration of information derived from CTC with other emerging biomarkers could represent a promising strategy to improve patient selection and the efficacy of immunological therapies [136,137].

Schematic overview of circulating tumor cells (CTC) as predictive biomarkers for immunotherapy response and as a platform for personalized cancer vaccination. In the first track, liquid biopsy enables CTC enrichment, followed by PD-L1 characterization at the single-cell level to stratify patients undergoing immune checkpoint inhibitor (ICI) therapy. A high proportion of PD-L1–positive CTC is associated with favorable response and tumor regression, whereas low or absent PD-L1 expression correlates with resistance and disease progression. In the second track, isolated CTC are subjected to single-cell genomic profiling (WGS/WES) to identify somatic mutations and derive patient-specific neoantigens. These neoantigens are then exploited for the development of personalized cancer vaccines, ultimately promoting T-cell activation, tumor antigen recognition, and durable systemic antitumor immunity.

6.1. Predictive Value of PD-L1+ CTC in CRC Immunotherapy

PD-L1 expression on CTC represents one of the most studied biomarkers to predict response to immune checkpoint inhibitor-based immunotherapies. In a multicenter study of 155 patients with advanced solid tumors treated with anti-PD-1/PD-L1, Tan and colleagues demonstrated that patients with PD-L1-high CTC had a significantly higher disease control rate (75.68% vs 40.91%), longer median PFS (4.9 vs 2.2 months), and superior OS (16.1 vs 9.0 months) [133]. However, a meta-analysis by Ouyang et al. of 30 studies highlighted that, although pre-treatment PD-L1+ CTC tended to predict better PFS and OS for Immune Checkpoint Inhibitors (ICI) treatments, they were associated with unfavorable prognosis for non-ICI therapies [138]. Similarly, Kong et al. reported in a meta-analysis of 20 studies that baseline PD-L1 expression on CTC was associated with unfavorable prognosis in the overall analysis [139].

In CRC, where only a subset of patients (primarily those with microsatellite instability, MSI-high) significantly benefits from such treatments, the identification of reliable predictive markers is of fundamental importance [140]. In the CheckMate 142 study, Overman and colleagues

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demonstrated that in metastatic dMMR/MSI-H CRC, responses to nivolumab occurred independently of tissue PD-L1 expression, suggesting that dMMR/MSI-H status, rather than PD-L1, represents the main determinant of response [141].

PD-L1-positive CTC can reflect an active state of immune evasion, suggesting that the tumor uses the PD-1/PD-L1 axis to inhibit T cell response [142]. At the molecular level, in vitro and in vivo studies have demonstrated that oncogenic BRAF upregulates PD-L1 via MEK/c-JUN/YAP in CRC cells (SW480, NCI-H716), and that PD-L1 deletion reduces tumor growth in murine xenografts [143]. Furthermore, Feng and colleagues demonstrated that PD-L1 regulates chemotherapy sensitivity through BH3-only proteins BIM and BIK [143]. Similarly, Li et al. highlighted that FGFR2 promotes PD-L1 expression in CRC through the JAK/STAT3 pathway, inducing T cell apoptosis in co-culture and confirming these results in murine xenograft models [44].

Several studies have highlighted a correlation between the presence of PD-L1+ CTC and a higher probability of response to checkpoint inhibitors in certain tumor contexts. In a phase 1 study of 35 patients with advanced gastrointestinal tumors treated with anti-PD-1, Yue and colleagues reported that the disease control rate in patients with ≥ 20% PD-L1-high CTC was 64% vs 14% in others, and that baseline PD-L1-high CTC abundance predicted PFS [134]. Similar results were obtained in lung cancer: a prospective study on 53 patients demonstrated that patients with PD-L1+ CTC had Objective Response Rate (ORR) of 64.7% and Disease Control Rate (DCR) of 100% vs ORR 13.9% and DCR 83.3% in PD-L1-negative patients [144]. Furthermore, Wei et al. confirmed in a cohort of 52 patients that PD-L1+ CTC were associated with superior ORR (84.2% vs 36.8%), longer median PFS (16 vs 4 months), and superior OS [145].

However, in CRC, the results are still conflicting. In some cases, high PD-L1 expression on CTC has been associated with unfavorable prognosis and greater tumor aggressiveness, rather than with a better therapeutic response [42]. This observation is consistent with a meta-analysis by Wang et al. of 32 studies which highlighted that tissue PD-L1 expression was independently associated with unfavorable prognosis (OS: HR=1.93; Disease-free survival (DFS): HR=1.76) and correlated with lymph node metastases and poor differentiation [146]. Similarly, Jang et al. reported in a study of 169 stage III-IV CRC patients that PD-L1 SP263 was an independent indicator of shorter survival in multivariate analysis [147]. However, Alexander and colleagues concluded in a meta-analysis that PD-L1 on tumor cells in CRC has heterogeneous outcomes and does not meet the requirements of a prognostic marker, emphasizing the need for standardization of the evaluation methodology [148].

An additional element of complexity is represented by intra- and inter-patient heterogeneity of PD-L1 expression, as well as its variability over time and in response to treatments [149]. Wei et al. analyzed 422 primary CRC and demonstrated that the probability of inconsistent PD-L1 expression was 17.8% intra-tumorally, 31.4% between primary tumor and lymph node metastases, and 39.1% between primary tumor and distant metastases [150]. In the context of CTC, Janning and colleagues reported in non-small cell lung cancer (NSCLC) patients that 47% had only PD-L1-negative CTC, 47% had a mix of PD-L1+ and PD-L1- CTC, and only 7% had exclusively PD-L1+ CTC, with no correlation between PD-L1 on CTC and tissue PD-L1. Moreover, at disease progression, all patients showed an increase in PD-L1+ CTC, suggesting a dynamic mechanism of therapeutic escape [151]. This hypothesis is supported by a study by Nicolazzo et al. on 24 NSCLC patients treated with nivolumab, in which at 6 months, patients with PD-L1-negative CTC all had clinical benefit, while those with PD-L1+ CTC all had progressive disease [149]. The choice of antibody clone also influences the results: Gao et al. demonstrated in a CRC study with 3 PD-L1 antibody clones (22C3, SP263, E1L3N) moderate to good consistency among the clones, but with a “variegated and patchy” expression pattern confirming spatial heterogeneity [152].

Compared to tumor tissue, CTC could provide a more up-to-date picture of the immunological status of the disease, but standardization of cut-offs and detection methodologies remains an open challenge. A concordance study on 201 matched tissue/CTC pairs reported an overall concordance of 87.6%, with concordance of 85.1% and 87.6% for PD-L1 (clones 22C3 and 28-8 respectively) [153]. Page et al. analytically and clinically validated a CTC assay for PD-L1, demonstrating clinical sensitivity of 90% and specificity of 100%, supporting routine clinical use [154]. However, Nobin and colleagues highlighted in a study of 862 resected CRC that the prognostic value of PD-L1 differed significantly between antibody clones (73-10, SP263, 22C3), with positivity rates from 4% to 10% in tumor cells [155].

Overall, although PD-L1+ CTC represent a promising biomarker, their predictive value in CRC requires further validation in prospective clinical studies and on large, well-characterized cohorts. Preliminary data on 182 CRC patients showed that 42.2% of CTC-positive patients expressed PD-L1 on CTC, proposing this biomarker for highly individualized treatment plans, while a retrospective study on 666 CRC patients confirmed PD-L1 expression in 74.62% of patients with CTC, emphasizing the need for further clinical studies [41,42]. As concluded by Ouyang et al. in their meta-analysis, “the prognostic value of PD-L1+ CTC for ICI treatment needs validation by large-scale studies in the future” [138].

6.2. Neoantigen Profile of CTCs: Towards Personalized Vaccination?

Analysis of the mutational profile of CTC opens new perspectives in the field of personalized medicine, particularly for the identification of tumor neoantigens that could be exploited for therapeutic purposes [156]. Gao et al. developed Uni-C (Uniform Chromosome Conformation Capture), a method for profiling 3D chromatin architecture and genomic alterations at the single-cell level. Applying Uni-C to CTC from Patient-Derived Xenograft (PDX) mouse models of pancreatic cancer, integration of data from 7 CTC captured 88.7% of Single Nucleotide Polymorphisms (SNP) and INDEL and 75.0% of structural variants present in the tumor tissue, demonstrating that variants detected in CTC reflect the genomic characteristics of the tumor. Furthermore, in the spontaneous tumor model, the authors identified mutations in driver genes in CTC and predicted neoantigens, paving the way for early diagnosis and personalized therapeutic strategies [157].

Neoantigens, derived from tumor-specific somatic mutations, represent ideal targets for immunotherapy due to their high immunogenicity and limited expression in healthy tissues. Using WES analysis on 1,779 CRC samples, Chen et al. identified 1,550 recurrent mutations recognizable by multiple common Human Leukocyte Antigens (HLA) molecules, including KRAS G12D (8%), KRAS G12V (5.8%), and PIK3CA E545K (3.5%), proposing them as “public” neoantigens potentially usable as immunotherapeutic targets [158]. More recently, Yu et al. analyzed the immunogenicity of neoantigens in 5 CRC patients, demonstrating that 12.5% of tested neoantigen peptides showed high immunogenicity, correlated with high Tumor Mutational Burden (TMB) and distant metastasis. Furthermore, common neoantigens between primary and metastatic lesions showed low immunogenicity, suggesting selective immunological suppression [159]. In one of the largest studies on the neoantigen landscape in CRC, analysis of 7,053 CRC by NGS and immune epitope prediction identified 368,468 predicted neoantigens, of which 16,184 with high affinity. Among the recurrent high-affinity neoantigens, those derived from APC mutations were predominant in Microsatellite Stable (MSS) tumors (78%), whereas mutations in RNF43, ASXL1, and KMT2D prevailed in deficient Mismatch Repair/ Microsatellite Instability-High (MMRd/MSI-H) tumors [160].

CTC, being representative of the tumor component in circulation and potentially enriched for metastatic subclones, can provide valuable information on the repertoire of neoantigens relevant to disease progression. In a pioneering study, Heitzer et al. conducted the first comprehensive genomic profiling of CTC in stage IV CRC using array-CGH and NGS, isolating 37 intact CTC from 6 patients. Mutations in known driver genes (APC, KRAS, PIK3CA) found in the primary tumor and metastases were also present in the corresponding CTC. Using additional deep sequencing, most mutations initially found only in CTC were present at a subclonal level in primary tumors and metastases, demonstrating that CTC reflect the genomic complexity of the tumor [161]. Lyberopoulou et al. analyzed KRAS, BRAF, CD133, and PLS3 mutations in CTC from 52 CRC patients, finding a discordance between primary tumor and CTC of 5.77% for KRAS and 3.85% for BRAF, supporting the use of CTC as a non-invasive source for tumor molecular characterization [162]. More recently, Islam et al. employed the “PNA-LNA molecular switch” technique to detect KRAS G12V and BRAF

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V600E mutations in 71 primary tumors and 37 CTC samples from CRC patients, demonstrating that KRAS G12V mutations in CTC correlated significantly with lymph node metastases (p=0.002), pathological stage (p=0.005), and lymphovascular invasion (p=0.034) [163].

High-resolution sequencing techniques applied to CTC allow the identification of dynamic mutations and monitoring of clonal evolution in real time, offering an advantage over static biopsies [164]. In a 2021 study, Kozuka et al. applied scRNA-seq to 59 CTC isolated from 27 metastatic CRC patients, classifying them into four groups based on the expression of epithelial, EMT, and stemness genes. Patients in second or later lines with CTC expressing EMT genes showed significantly shorter PFS and OS, demonstrating the prognostic value of transcriptional profiling of CTC at the single-cell level [165]. Conversely, Cai et al. conducted single-cell exome sequencing of 150 cells isolated from primary tumor, liver metastasis, and lymph node metastasis of a stage IV colon cancer patient, revealing that liver and lymph node metastases originated from polyclonal CTC clusters, with greater genetic heterogeneity than the primary tumor [166].

In this context, neoantigen profiling from CTC could be used to develop personalized therapeutic vaccines or targeted adoptive immunotherapy strategies. In a phase 2 study published in Nature Medicine, Parkhurst et al. isolated personalized neoantigen-reactive T-cell receptors (TCR) from tumor-infiltrating lymphocytes of metastatic pMMR CRC patients, transduced autologous peripheral blood lymphocytes, and adoptively transferred these cells. Of 7 patients treated, 3 achieved objective responses per RECIST, including regressions of liver, lung, and lymph node metastases lasting 4-7 months. In one patient, about 20% of peripheral blood CD3+ lymphocytes expressed the transduced TCR more than 2 years after treatment [167]. In 2020, a personalized mRNA vaccine was developed by concatenating validated neoantigens, predicted neoepitopes, and driver gene mutations into a single mRNA construct to vaccinate patients with metastatic gastrointestinal cancer. The vaccine was safe and induced specific T cell responses against predicted neoepitopes not detectable before vaccination, including TCR targeting the KRAS G12D mutation [168]. Subsequently, Cho et al. developed a personalized mRNA vaccine platform based on the co-administration of MHC-I and MHC-II restricted neoantigens in CRC. In a murine CRC model, the vaccine demonstrated significant antitumor efficacy and reduction of post-surgical recurrence, with a synergistic effect when combined with immune checkpoint inhibitors [169]. Meanwhile, Chen et al. developed Neo-CRCVAS, a personalized neoantigen vaccine composed of 7 immunogenic peptides, combined with regorafenib (RegoNeo) for MSS CRC with liver metastases. In murine models, RegoNeo significantly improved tumor regression and survival, expanding a distinct population of Rgs2+ CD8+ T cells with strong cytotoxic activity, also validated in PDO [170].

However, several technical and biological challenges currently limit this application, including the difficulty of obtaining sufficient amounts of genetic material, clonal heterogeneity, and the need to validate the true immunogenicity of the identified neoantigens [159,171].

Despite these limitations, the integration of CTC analyses with multi-omic approaches and functional models could accelerate the development of personalized vaccination strategies, opening new perspectives for the treatment of CRC and other solid tumors [172]. For example, Vasudevan et al. developed a framework based on multi-omic analysis and artificial intelligence to identify tumor antigens and immune targets in CRC, identifying three key neoantigens (TTK, EZH2, KIF4A) as promising candidates for immunotherapy and categorizing patients into immune subtypes to optimize vaccine design [173].

7. Therapeutic Implications and Future Directions

Current evidence clearly indicates that CTC are not simply passive biomarkers of the disease but dynamic players involved in a complex network of interactions with the immune system [8]. Firstly, CTC play crucial roles in the metastatic cascade and tumor immune evasion, and their molecular characterization has opened new avenues in understanding the biology of metastasis and the response to therapies directed against metastatic cells [8]. It is now also known that CTC act both as biomarkers and active mediators of crosstalk with the immune microenvironment, influencing metastasis, immune evasion, and therapeutic resistance [121]. Furthermore, the controversial dialogue between CTC and the immune system has also been discussed in full detail, documenting both the antitumor activity and the immunosuppressive and pro-tumorigenic function mediated by NK cells, CD4 and CD8 T lymphocytes, Tregs, neutrophils, monocytes, macrophages, dendritic cells, and platelets [78].

In CRC, the fate of CTC in the circulation is the result of an unstable balance between immunosurveillance mechanisms and immune evasion strategies, in which the dialogue with innate and adaptive immune cells plays a determining role [77,112,123].

On one hand, effector components such as NK cells and CD8+ T lymphocytes contribute to the elimination of a significant proportion of CTC, exerting a selective pressure that favors the survival of the most aggressive and immune-evasive clones [174]. For example, Ruggeri et al. demonstrated that CRC patients present early and pronounced alterations of NK cells in peripheral blood, with a reduced frequency of total CD56+ cells and impaired cytotoxic and cytokine responses. Furthermore, plasma from CRC patients induced similar dysfunctions in healthy donor NK cells, suppressing mTORC1 signaling and effector activity through a TNF-α deficiency-mediated mechanism [175]. On the other hand, CTC are able to exploit and remodel the immune system to their advantage, through direct interactions with immune cells, the formation of heterotypic clusters, and the release of soluble factors and extracellular vesicles with immunomodulatory activity [76,82]. It has been demonstrated, in an in vitro and in vivo study, that small CRC-derived EV promote tumor immune evasion by upregulating PD-L1 expression in TAM through miR-21-5p and miR-200a, which synergistically regulate the PTEN/AKT and SOCS1/STAT1 pathways, resulting in decreased CD8+ T lymphocyte activity and increased tumor growth [176]. Regarding clusters, Wang et al. demonstrated in CRC that CTC-neutrophil clusters form at the vascular-immune interface of the primary tumor, guided by pericytes with high NNMT expression that activate the CXCL5/CXCR2 axis. Genetic and pharmacological inhibition of NNMT in pericytes eliminated CTC-neutrophil clusters and suppressed CRC liver metastases [177]. This dual role, already evident in the previous paragraphs, underscores how CTC are positioned at the center of a bidirectional network of signals that promotes metastatic dissemination.

The therapeutic implications of these processes are relevant. The integration of CTC into clinical pathways could improve patient stratification and therapy selection, particularly in the immunotherapeutic field [26,136]. The characterization of CTC in terms of expression of immunoregulatory molecules, such as PD-L1, or mutational and neoantigen profiles, paves the way for personalized medicine strategies [178]. In 2024, Dong et al. developed the NICHE microfluidic platform (Nanoplatform for Interrogating Living Cell Host-gene and Environment relationships) that integrates genetic and phenotypic profiling of single living CTC, enabling on-chip co-culture of CTC with immune cells and real-time quantification of phenotypic heterogeneities in response to ICI. The generated predictive index, validated on 80 NSCLC patients, demonstrated an AUC of 0.906, significantly higher than current clinical benchmarks for predicting immunotherapy response [179].

However, numerous challenges remain open. As highlighted by the international expert consensus, CellSearch® (Menarini Silicon Biosystems, Bologna, Italy) is currently the only platform with high-level evidence for clinical use, although emerging technologies are promising. Key challenges identified include improving detection sensitivity/specificity, standardizing workflows, generating robust data, and training clinicians [136]. The heterogeneity of CTC, their rarity in peripheral blood, and the lack of standardized methodologies still limit their full clinical applicability. Saini et al. conducted a comparative study of seven CTC enrichment methods across five technologies in lung cancer, demonstrating that recovery rates varied significantly between methods (from 18% to 70%) and that EpCAM-based methods showed a dramatic reduction in recovery with low EpCAM-expressing cells (from 70% to 1%), highlighting the need for phenotype-independent approaches [180]. Furthermore, the complexity of immune interactions, including the discrepancy between the peripheral compartment and the tumor microenvironment, requires integrated, multi-level approaches to be adequately understood [120].

In this context, future research directions should focus on the development of more sensitive and standardized technologies for CTC isolation and characterization, the integration of multi-omic data to more precisely define the immunological role of CTC, the clinical validation of CTC-derived biomarkers in prospective studies, and the exploration of new therapeutic strategies aimed at interrupting the crosstalk between CTC and the immune system. Ultimately, a better understanding of the interactions between CTC and the immune system could not only clarify the mechanisms underlying metastasis but also open new opportunities for early and more effective therapeutic interventions in colorectal cancer (Table 4).

Table 4. Ongoing challenges and future directions for CTC-based immunotherapies in CRC.

Challenge	Clinical impact	Potential solution	References
Heterogeneity of CTC	Underestimation of CTC; missed aggressive subclones	Phenotype-independent isolation methods (e.g., size-based, microfluidic); multi-marker panels	[7–12,76,180]
Low frequency of CTC in peripheral blood	Difficult to obtain sufficient material for molecular analysis	Pre-analytical enrichment; sensitive detection platforms (e.g., NICHE microfluidic)	[136,179]
Lack of standardization in isolation and analysis	Poor reproducibility across studies; no widely accepted cut-offs for PD-L1+ CTC	International consensus protocols; automated systems; validation in large prospective cohorts	[136,148,154,155]
Discrepancy between peripheral CTC and tumor microenvironment	CTCs may not fully reflect TME immune status	Integrated multi-omic approaches (scRNA-seq, proteomics) matched with tissue biopsies	[120,165,166]
Dynamic changes of PD-L1 expression on CTC (under therapy pressure)	Risk of false negative/positive for immunotherapy guidance	Serial liquid biopsies; real-time monitoring; use of multiple antibody clones	[149–152]
Validation of neoantigen prediction from CTC	Limited material for WES; low immunogenicity of common neoantigens	Single-cell sequencing; "public" neoantigen libraries (KRAS G12D, etc.); functional T cell assays	[157–163,171]
Clinical translation of CTC-based vaccination	Need for large trials; cost and logistics of personalized vaccines	mRNA vaccine platforms (personalised); neoantigen ranking by AI; combination with immune checkpoint inhibitors	[167–170,173]

AI (artificial intelligence), CRC (colorectal cancer), CTC (circulating tumor cell), mRNA (messenger RNA), PD-L1 (programmed death-ligand 1), scRNA-seq (single-cell RNA sequencing), WES (whole exome sequencing).

8. Conclusion

In conclusion, CTC in CRC embody a precarious balance between immune surveillance and evasion, a dualism that determines their metastatic fate. Their survival in the circulation is based on a multifaceted strategy: on one hand, they finely modulate MHC-I expression to evade cytotoxic T lymphocytes without fully alerting NK cells, while on the other, they exploit immune checkpoints such as PD-L1, CD47, and FasL to silence effector responses. Added to these intrinsic mechanisms are extrinsic protections such as platelet cloaking, which transfers MHC-I and activates pro-invasive TGF-β signals, and NET, which physically shield CTC and favor their endothelial adhesion. In the bloodstream, CTC do not travel in isolation but form heterotypic clusters with monocytes, neutrophils, and lymphocytes, fueling a crosstalk that increases resistance to mechanical stress and creates pro-metastatic niches, while macrophages and myeloid-derived suppressor cells amplify immunosuppression and facilitate extravasation. Concurrently, CTC themselves remodel systemic immunity: they alter peripheral lymphocyte subpopulations, promote a chronic inflammatory state, and release EV that convert immune cells into protumoral phenotypes, destabilizing the physiological correlations between local and systemic immunity. Although NK cells and CD8+ T lymphocytes eliminate a substantial proportion of CTC, selective pressure favors the emergence of increasingly resistant clones, often characterized by high PD-L1 expression. The dynamic analysis of PD-L1 on CTC, together with neoantigen profiling, is opening concrete perspectives for personalized immunotherapy, although cellular heterogeneity and lack of standardization still limit its clinical application. Overcoming these technological challenges and validating CTC-derived biomarkers in large prospective studies will allow these cells to be transformed from simple passive indicators into active therapeutic targets, with the aim of interrupting the immunosuppressive dialogue that sustains metastasis and improving treatment strategies in CRC.

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