Clinical Relevance of Circulating Tumor Cells in Immunology

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Circulating tumor cells (CTCs) are a heterogeneous population of tumor cells that have shed from a tumor into the lymphatics and vasculature, ultimately disseminating into blood circulation. Immune modulation is a hallmark of cancer. Cancer–immune interaction shapes the course of disease progression at every step of tumorigenesis, including metastasis, of which circulating tumor cells (CTCs) are regarded as an indicator.

Circulating Tumor Cells | immune | Therapeutic Target

1. Circulating Tumor Cells (CTCs) as a Therapeutic Target

Although there is substantial evidence that the immune system plays a complex role in influencing the genesis, survival and successful seeding of Circulating tumor cells (CTCs), surprisingly little is available on the therapeutic targeting of CTC–immune cell interactions. Although there is ample evidence that successful chemo/radio/immunotherapeutic modalities, targeting the primary tumor, also promote the elimination of CTCs [1][2][3][4], the studies outlined below are those that aim specifically at CTCs as a target of therapy.

An important avenue of CTC elimination involves their phagocytosis by macrophages. However, in most solid malignancies, macrophages often present with a tumor-promoting phenotype with impaired phagocytic functions (i.e., TAMs [5]). There have been a few approaches reported to promote macrophage-dependent elimination of tumor cells, primarily via monoclonal antibody therapies. Gul et al. reported that in a murine model of melanoma, during transit through the liver, CTCs are susceptible to phagocytosis by Kupffer cells (liver macrophages) [6][7]. They reported that CTC opsonization, following monoclonal antibody therapy, using antibodies against cancer-specific antigens stimulates Kupffer cells to eliminate melanoma CTCs, via antibody-dependent phagocytosis [6]. Their report of Kupffer-dependent CTC clearance is backed by a similar report from van der Bij et al., using a murine colorectal cancer model [8]. The “do not eat me” signal, CD47, has also been targeted via monoclonal antibodies (mAbs) to reverse CTC immune evasion. Lian et al. reported that the simultaneous blockage of PD-L1 and CD47 in a murine breast cancer model reduces metastasis more effectively compared to single therapy via CTC inhibition [9][10]. Although they did not mechanistically investigate the cause of CTC reduction, other groups have shown that CD47 blockage promotes macrophage-dependent phagocytosis [11][12].

Another approach at CTC elimination utilizes the close association between CTCs and platelets during circulatory transit. Li et al. engineered platelets that overexpress membrane-bound TRAIL to promote CTC apoptosis [13]. They demonstrated that in murine models for breast and prostate cancers, recruitment of TRAIL-overexpressing platelet

to CTC clusters (as part of the CTC “cloaking” strategy) resulted in reduced metastasis and CTC viability [13]. A more recent study by Ortiz-Otero et al., using TRAIL-overexpressing platelets, also reported similar results with primary-tumor-derived CTCs [14]. Another reported approach using TRAIL, involves generating TRAIL and E-selectin-containing liposomes [15]. These liposomes bind circulating leukocytes via their E-selectin (effectively coating these leukocytes with the accompanying TRAIL molecules with monocytes, neutrophils and NK-cells, showing the highest level of coating). These cells are then primed to promote TRAIL-mediated CTC apoptosis and were shown to successfully reduce lung metastasis in a murine colorectal cancer model [15]. Recent research has also suggested that EpCAM can be utilized as a target antigen for CAR-T cell therapy, potentially selectively eliminating CTCs. However, their reported efficacy seems to also be accompanied by an unfavorable toxicity profile [16].

Immunotherapeutic modalities are often prescribed as second or subsequent-line drugs, usually prescribed for late-stage disease, when CTCs are usually also at their peak abundance [17]. Overall, larger studies are required to derive stronger conclusions on the utility of CTCs to predict ICI efficacy and to refine patient stratification. For a more in-depth review of CTC in immunotherapeutics, Leone et al. and Schuster et al. also summarized the clinical evidence [18][19].

2. CTCs as Diagnostic and Therapeutic Biomarkers

In contrast to the scarcity of evidence found for CTC-targeted therapies, a much more well-researched area of CTC biology concerns their utility as biomarkers (a representative surrogate for primary and metastatic tumors, used as clinical guides for patient stratification, prognostic determination, or therapeutic evaluation [20]). Often grouped together with circulating tumor DNA (ctDNA), as cancer-specific biomarkers obtained from the blood, liquid biopsies are much easier and less invasive to obtain compared to tumor biopsies [21]. The value of CTC liquid biopsies in cancer prognostics has been well documented for many solid tumors in the clinical setting. The $EpCAM^+$ CTC-based CellSearch test is currently the only FDA-approved clinical platform for use in prognostics and patient stratification in metastatic breast, prostate and colorectal cancer [22]; however, this have also have been used to research other malignancies [23].

Although primarily associated with metastasis and late-stage malignancies, CTCs have also been demonstrated to be present in the early stages of many cancers [24], as even stage I epithelial tumors have been shown to release CTCs [25][26]. This has driven research into the utility of CTCs as a highly specific early diagnostic tool. In patients with risk factors to the development of malignancies, CTCs may be released in small quantities by tumors which are not macroscopically detectable [27]. This approach allows for these clinically asymptomatic tumors to be detected early. Its role in screening has been demonstrated for early detection of lung cancer in patients with COPD [28], as well as for early or asymptomatic HCC [29], prostate cancer and breast cancer [30].

With the use of scRNA-seq becoming increasingly common, deeper investigations into the transcriptomic diversity of CTCs have been some of the more recent highlights in the field. Using metastatic HCC samples, D’avola et al. used scRNA-seq to identify distinct expression profiles in different HCC CTC populations, showing that some CTCs

show upregulation of angiogenesis-related genes, and in others, KRAS and G2M checkpoint genes [31]. In metastatic breast cancer, De Luca et al. showed that not only do CTCs exhibit significant interpatient heterogeneity, single CTCs may possess unique mutations which will be lost in bulk analysis [32]. In the same paper, they also reported that the mutational landscape of CTCs may be completely altered following treatment [32]. A recent publication by Sun et al. described a high degree of spatial heterogeneity of the CTC transcriptional landscape in HCC via scRNA-seq [33]. They identified a large number of transcriptomic differences between CTCs drawn from liver efferent (representing primary tumor heterogeneity) and efferent (representing CTC adaptation in circulation) vessels. They also identified CCL5 as being increasingly upregulated, the longer CTCs circulate as an immune evasion mechanism, by recruiting Tregs in circulation [33]. Single-cell analysis also allows for better discrimination of CTC subpopulations, classifying them based on their transcriptional signatures or drug resistance. For example, in metastatic pancreatic cancer, Ting et al. was able to segregate CTCs into three transcriptionally distinct groups (classical CTCs, platelet-adherent CTCs and a subset, exhibiting high proliferation [34]). Aberrant signaling pathways in small populations of CTCs may also contribute to partial drug resistance. Miyamoto et al. reported that increased noncanonical Wnt signaling, in a population of pancreatic cancer CTCs, allowed their survival against antiandrogenic agents, which potentially allows for relapse or treatment failure [35].

A more recent development in the field is research into the predictive and evaluative value of CTCs in immunotherapeutic regimens. The CTCs hold substantial promise as immunotherapeutic biomarkers due to their progressive enrichment during disease progression. Immunotherapeutic modalities are often administered as second or subsequent-line drugs, usually prescribed for late-stage disease, when CTCs are at their peak abundance [17]. As mentioned previously, PD-L1 has been discovered on CTCs, and this has driven interest in their role during immune checkpoint inhibitor (ICI) therapy. Although most of the research in the field have been focused on ICI therapies, some recent work on their utility in adoptive cell transfer and DC immunotherapy will also be highlighted.

Immune checkpoint inhibitor therapy has been the most successful immunotherapeutic modality to date, with anti-CTLA-4 and anti-PD-1/PD-L1 mAbs already in clinical use [36]. Additionally, CTC quantification and qualitative analysis in patients receiving ICIs suggests that their analysis pre- and post-treatment may be prognostically useful in predicting overall survival and treatment response. In malignant melanoma, two separate groups used a combination of chemotherapeutic and ICI agents to investigate the prognostic utility of CTCs and concluded that changes to CTC counts post-treatment is a good marker of prognosis [37][38]. More detailed qualitative analysis of melanoma CTCs showed that the presence of PD-L1$^+$ CTCs pre-treatment predicts sensitivity to anti-PD-1 ICIs [39] [40]. Conversely, expression of Catenin Beta 1 on CTCs is a predictor of resistance to ICI therapy [41]. More in-depth analysis of melanoma CTCs using RNA signatures have also been recently reported [42].

In lung cancer, the presence of PD-L1 on NSCLC CTCs was reported as a predictor of lower overall survival (OS) [43], although a different group did not find a statistically significant result [44]. Another group reported poorer prognoses of patients with PD-L1$^+$ CTCs and that persistence of PD-L1$^+$ CTCs after nivolumab therapy is associated with worse overall survival [45]. Further studies by Guibert et al. and Dhar et al. showed that higher PD-L1$^+$ CTC count pre-treatment is associated with worse progression-free survival (PFS) and that CTCs express

higher levels of PD-L1 compared to primary tumors [46][47]. Although ICIs have not been extensively studied for breast carcinomas, Mazel et al. demonstrated the presence of PD-L1 on 68% of breast cancer CTCs [48]. Expanding on this, Schott et al. found that PD-L1 positive CTCs are present on both earlier and metastatic disease stages [49] and reported that one patient showed a reduction in PD-L1$^+$ CTCs, with successful nivolumab and ipilimumab treatment.

Other malignancies with CTCs being studied as biomarkers for immunotherapy include prostate, bladder [50], head and neck squamous cell carcinoma (HNSCC) and HCC. In metastatic prostate cancer, with CTCs expressing AR-V7, Boudadi et al. reported that nivolumab and ipilimumab are efficacious only when tumors also show DNA-repair deficiencies [51]. In HNSCC, the presence of PD-L1$^+$ CTCs in HNSCC pre-treatment was associated with poorer prognoses [44] and lower PFS and OS post-chemoradiotherapy [52]. In HCC, the presence of PD-L1$^+$ CTCs is a predictor of worse OS but predicts a positive response to nivolumab treatment [53].

Studies addressing the role of CTCs in DC vaccination and ACT therapies are much less prevalent than for ICIs, with only the DC vaccine Sipuleucel-T in clinical use for prostate cancer [54]. Rekoske et al. found that PD-L1 expression on CTCs increased after administration of Sipuleucel-T and is associated with sustained T-cell responses and longer PFS [55]. In autogeneic NK-cell adoptive cell transfer for breast cancer and NSCLC, CTC quantity is negatively correlated with therapeutic efficacy [56][57].

Although momentum has been growing in efforts to bring relevance to CTCs, as both targets and biomarkers of cancer therapy, much work remains. Most studies targeting CTCs via immunotherapies are still at the preclinical stage and clinical biomarker studies often involve small study populations. Deeper investigations are still required to fully translate the mechanistic findings of CTC immunology onto the bedside.

3. Future Avenues of CTC Research

Efforts to understand CTC complexity have recently been supported by the increasing accessibility of single-cell transcriptomic analysis. In addition to the high spatiotemporal heterogeneity seen in primary lesions, CTCs may undergo various degrees of EMT, resulting in an even more varied transcriptome [58]. Further, CTC clusters have been shown to be composed of CTCs at different stages of EMT, where individual CTCs possess specialized roles in causing successful metastasis [58][59]. Appreciating the diversity of CTCs may illuminate more complex immune–CTC interactions, although research on the topic is very limited at the time of writing. Notably, Sun et al. demonstrated the spatial heterogeneity of CTCs by investigating transcriptomic differences between HCC CTCs drawn from liver efferent and afferent vessels (reflecting newly-released and circulation-adapted CTCs, respectively [33]). They identified CCL5 production as a CTC immune evasion strategy by recruiting Tregs in circulation, with levels of CCL5 increasing, the longer CTCs are in circulation [33]. Single-cell analyses also allow for better discrimination of CTC subpopulations, classifying them based on their transcriptional signatures [34] or drug resistance [35]. Identifying the immunological significance of these subpopulations may be an interesting avenue of research.

With the recent advent of personalized neoantigen cancer vaccines [60], CTCs may find utility for neoantigen screening. Neoantigen vaccines have been shown to be successful modalities for multiple cancer types, with very favorable toxicity profiles, due to their high specificity [61][62][63]. Although these studies relied on the sequencing of primary tumor biopsies, performing neoantigen screening via liquid biopsies may become increasingly feasible. A recent study in NSCLC, by Jia et al., showed that neoantigens identified from primary tumor biopsies were also detectable on ctDNAs. They then tracked the success of ICI therapy via fluctuations in neoantigen ctDNA abundance [64]. This suggests that neoantigen sequences can be tracked from liquid biopsies. However, as of the time of writing, there are still no reports on neoantigen detection using CTCs. A large caveat to this approach, however, is that there may be varying levels of similarity between the transcriptomic profile seen in CTCs and primary or metastatic lesions [58][65][66]. As such, extrapolating any neoantigen findings in CTCs to primary or metastatic lesions requires much scrutiny to establish their clinical significance.

Another field that is rapidly developing is the use of machine learning methods for CTC analysis, which provides a fully automated and robust platform for CTC enumeration. These methods can identify and characterize CTCs in heterogeneous liquid biopsies in a reproducible and accurate manner [67][68]. The CTC counts were obtained by combining autoencoding convolutional neural networks (CNN) with advanced visualization techniques to predict overall survival in metastatic breast cancer patients [69]. The CNN have also been employed to detect and classify rare CTC cells from metastatic renal cell carcinoma patients [70]. Likewise, deep learning radiomics was employed for CTC counts to predict disease recurrence in early-stage non-small cell lung cancer patients, treated with stereotactic body radiation therapy [71].

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