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The Potential of Combination Therapies and Patient Stratification to Improve CCR2 Inhibition Therapeutics

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Chemokines and their receptors are the communication mechanism used by cells of the immune system, allowing them to identify and eliminate pathogens and cancerous cells. However, it is becoming clear that chemokines and their receptors are also playing a role in tumor progression and metastasis [1,2]. An example of such coopting is the CCL2-CCR2 axis. The chemokine CCL2/MCP-1 (monocyte chemoattractant protein-1) is known to bind the CCR2 receptor on monocytes [3] and attract them to areas of need. What is now clear is that CCR2 levels are high in tumors of a number of cancer types. For example, in bladder cancer, 12 different patient datasets [4] all show that CCL2 expression is higher in the more advanced, muscle invasive disease than the non-muscle invasive disease [5]. In line with this, high CCL2 expression correlates with a worse overall survival in bladder cancer [5]. CCL2 and CCR2 are also associated with disease progression in many other cancer types including breast, ovarian, lung and colon [6,7].

In general, the elevated levels of CCL2 and CCR2 in tumors is the result of increased recruitment of monocytes into those tumors, most often the immunosuppressive M2 class of macrophages and myeloid derived suppressor cells (MDSCs), which limit the ability of the immune system to destroy the cancer cells [7,8]. The finding that increased levels of CCL2 and CCR2 correlate with increased pathology in cancers makes the targeting of the cell surface protein CCR2 a potential therapeutic target with small molecules or therapeutic antibodies [9]. While clinical trials to date have observed little to no responses in patients, the drugs are well tolerated, suggesting these drugs could be combined with those having a synergistic mechanism of action.

A recently published study examined this concept after identifying molecular CCR2 depletion as a potential avenue for enhancing the effectiveness of immune checkpoint inhibitors (ICI) in animal models. This study employed a functional genomic screen to identify genes whose inhibition led to synthetic lethality with ICIs in several mouse tumor models [5]. This work identified that inhibition in mouse models of two different cell surface receptors, either DDR2 [10] or CCR2, led to enhanced tumor response when mice were also treated with the ICI anti-PD-1. The CCR2 inhibition finding is consistent with the observation that CCR2 inhibition promotes a recovery in immunity to tumors [11]. Thus, the combination of CCR2 inhibition and ICI is an excellent combinatorial therapeutic approach to test in the clinic. In fact, a clinical trial is currently recruiting patients to investigate the combinatorial therapy of the CCR2 inhibitor BMS-813160 with Nivolumab, an ICI targeting the PD-1 molecule on immune cells. This phase II trial (NCT04123379) is focusing on patients with either non-small cell lung cancer (NSCLC) or hepatocellular carcinoma (HCC).

Another approach to improving the efficacy of CCR2 inhibition as monotherapy, or in combination with other therapeutics, would be to investigate the effect that patient stratification has on efficacy. This could be achieved by either stratification of patients by CCR2 levels in their tumors or CCL2 in their blood. To date, this stratification has not been employed. However, quantification of CCR2 protein levels in tumors has to date been approached in several ways and may be ready for clinical utility. One

promising technique is tumor transcriptional profiling on formalin fixed, paraffin embedded tissue blocks. This allows for gene expression signatures to be generated for each patient and this information compared to known signatures for certain therapeutics and prognostications [12]. For example, CCR2 gene expression levels can be determined. Additionally, the ratio of immune cells such as M1 macrophages (anti-tumor) to M2 macrophages (pro-tumor), can be scored [13,14] and even estimated computationally though various algorithms such as CIBERSORT [5,10,16]. Using this scoring, retrospective analysis has demonstrated that the M1-M2 scoring ratio has a typical hazard ratio below 1 for PFS and OS [17], suggesting this may be a good prognostic marker. However, this technique does have a limitation in that an invasive procedure is required to obtain the material prior to any therapy that would be guided by the results. This also means that other approaches are needed to monitor the patient's disease state in real-time. One non-invasive technique that would allow for real-time monitoring is assessing CCL2 plasma levels in patients [18]. While the procedure involves only a simple blood draw and follow-on ELISA assay, further study is needed to correlate the findings with cancer progression and outcome. While the above approaches are promising, they share a limitation in that they capture data on the tumor *in toto*, averaging signals from the different cell types as the tumor is assessed as a homogeneous mixture. Alternative methods can improve upon this aspect by assessing regions, or even cells, individually. Immunohistochemistry (IHC) has been used to score CCR2 levels in tissue microarrays (https://www.proteinatlas.org/ENSG00000121807-CCR2/pathology) but much of the work to date has centered on a potential role for CCR2 in central nervous system disorders [19]. IHC, when fully developed for a particular cell marker, has the benefit of providing clinicians with geographical information within the tumor and comparing CCR2 tumoral localization with those of other cell defining markers, referred to as multiplex IHC [15,20]. This additional information is particularly useful for immune cell markers, providing both biological insights as well as helping with prognostic and predictive determinations for patients. Flow cytometry, or even more advanced technologies such as imaging mass cytometry [10], is also strongly suited to investigating each cell individually and quantifying surface markers such as CCR2. Fresh tumor samples or tumor aspirates are enzymatically treated to yield a single cell suspension which is then probed with several cell markers, such as CCR2. Although similar to IHC, this technique has not been developed clinically for CCR2 evaluation in cancer as it has been for other diseases [21,22]. A newer technology, spurred by the advent of next generation sequencing, is single cell sequencing (Gouin and Theodorescu, in revision). Single cell suspensions are prepped from fresh tumor sample or aspirates and then placed into devices which isolate cells individually. Once isolated the nucleic acid from each cell is labeled with a unique barcode, thereby allowing for the pooling of the nucleic acid material for sequencing and a subsequent ascription of the sequencing data to an individual cell through demultiplexing. Thus, in addition to gathering expression or mutation data from each cell, the cell type (e.g., tumor, tumor associated fibroblast, adaptive or innate immune cell) can be identified using well known gene expression signatures. This allows, for example, the determination of CCR2 levels in the tumor but also determines which cells have the highest expression of CCR2 [23]. As with imaging mass cytometry, this approach can illustrate the tumor heterogeneity (e.g., immune cell infiltration) better than more classical techniques such as IHC, even in multiplexed form. However, it does have limitations in that no geography can be associated with any of the cell info, thereby making it unclear if high levels of CD8+ lymphocytes are within the tumor or on the periphery. This limitation has recently been resolved by a number of technologies that carry out numerous spatial transcriptomics or proteomics of a tumor, allowing visualization of gene or protein expression within the context of tumor architecture. These vanguard technologies are ideal for studying tumor-host interactions and also discovering novel biomarker panels to guide monotherapy or combinatory therapy in patients.

Therapeutic targeting of the CCR2-CCL2 axis appears to have therapeutic potential. Most notably, CCR2 inhibition exhibits substantial promise in increasing the rate of durable response in cancer patients also being treated with ICIs. Additional research is needed to identify the most accurate and practical method for determining the extent to which the CCR2-CCL2 axis is functioning for each patient and aligning that patient with the therapies that are most likely to provide them with a durable benefit.

Declaration

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