Power System Protection and Control
ISSN:1674-3415

MECHANISMS DRIVING IMMUNOTHERAPY RESISTANCE IN COLORECTAL CANCER LIVER METASTASES

Dr. Latha Kiran Krishna Rajendran

General Practitioner, Elova Hospitals
27, 5th Cross, Lalbagh Main Rd, Sudhama Nagar, Bengaluru, Karnataka 560027
meetlathakiran@gmail.com

Recevied:22 November 2023 **Revised**: 27 December 2024 **Accepted**: 23 January 2024

ABSTRACT

Colorectal cancer liver metastases (CRLM) represent one of the most immunologically hostile environments encountered in clinical oncology which prevents most patients from benefiting from immune checkpoint inhibitors (ICIs). While microsatellite instability-high (MSI-H) tumors respond favorably to anti-PD-1 therapy the predominant microsatellite stable (MSS) subtype which accounts for 80-85% of cases shows almost complete resistance to immunotherapy in liver metastatic cases. The review presents the existing research evidence about ICI resistance mechanisms in CRLM which include hepatic immune tolerance mechanisms and tumor-specific molecular changes and immune cell dysfunction and stromal changes and metabolic changes and systemic immune system loss. The main resistance factors stem from the immunosuppressive activities of Kupffer cells and hepatic stellate cells and liver sinusoidal endothelial cells which create a barrier that prevents T-cells from entering the tumor through WNT/β-catenin pathways and TGF-β which inhibits cytotoxic immune responses and the tumor environment which leads to higher regulatory T cell and myeloid-derived suppressor cell expansion and the metastatic site which causes PD-L1 to be incorrectly increased. The article examines how current approaches which target these mechanisms through LAG-3 inhibition and VEGF/VEGFR co-blockade and TGF-β pathway antagonism and locoregional liver-directed therapies will be implemented in clinical practice. The design of effective combination treatment plans for transforming immunologically cold liver metastases into treatable lesions requires an understanding of resistance mechanisms.

Keywords: *Colorectal Cancer Liver Metastases (CRLM), immune checkpoint inhibitors, immunotherapy resistance, tumor microenvironment, TGF-β, WNT/β-catenin, Kupffer cells, regulatory T cells, microsatellite stable, hepatic immune tolerance*

INTRODUCTION

Colorectal cancer (CRC) stands as the third most frequently diagnosed cancer and the second most common cause of cancer deaths worldwide which results in 1.9 million new cases and 935000 annual fatalities. The liver functions as the main location where CRC spreads to other areas of the body because 50 percent of patients will develop colorectal cancer liver metastases (CRLM) throughout their illness and liver cancer spread represents the main factor which affects their chances of surviving long term. The five-year survival rate for patients with unresectable CRLM remains below 15% despite medical progress in chemotherapy and targeted treatments and surgical procedures.

ICI therapy has revolutionized cancer treatment during the past ten years because it delivers long-lasting results for various solid tumors which include melanoma and lung cancer and renal cell carcinoma and microsatellite instability-high colorectal cancer. Pembrolizumab and nivolumab achieved approval for first-line and refractory MSI-H/dMMR metastatic CRC based on remarkable response rates and prolonged progression-free survival. ICI therapy offers no significant advantages to the most common CRC patient group which consists of microsatellite stable (MSS) tumor patients because multiple prospective trials show an overall response rate of 0%.

DOI: 10.46121/pspc.52.1.5

Active liver metastases lead to worse treatment outcomes with immune checkpoint inhibitors for MSI-H patients than for patients who have only extrahepatic disease. Clinical data from 16 prospective trials which studied 1713 patients show that hepatic metastasis reduces the systemic objective response rate (ORR) for all patients regardless of their microsatellite status. The phenomenon occurs because the liver functions as an organ which blocks immune reactions to dietary substances and gut bacteria that enter through the portal system. The processes which enable CRLM to avoid immune destruction require examination to create successful treatment approaches. The study delivers an extensive examination of present evidence regarding resistance mechanisms that function at tumor-intrinsic and microenvironmental and cellular and metabolic and systemic levels in CRLM together with new methods that researchers use to defeat these obstacles.

THE LIVER AS AN IMMUNOLOGICALLY TOLEROGENIC ORGAN

2.1 Hepatic Immune Architecture and Portal Tolerance

The liver has developed its distinct immune system because it receives blood from two separate sources and it evolved to act as the body's primary defense system against the portal vein blood that contains gut-derived substances. The system needs to operate at a basic level of immune system suppression which researchers call "portal vein tolerance" that special resident cells including Kupffer cells and liver sinusoidal endothelial cells and hepatic stellate cells maintain.

LSECs show low co-stimulatory molecule expression under normal body conditions while their immune system control leads to Th1 response suppression and Th2 response enhancement. The cells release nitric oxide (NO) and interferon-gamma (IFN-γ) which cause apoptosis in tumor cells that enter through the sinusoids but during cancer spread they help tumor cells stick to blood vessels and leave the bloodstream because adhesion molecules.

The liver's antigen-presenting cells establish immune system control by removing T cells that respond to antigens while they activate regulatory T cells through TLR4 signaling pathways which create immune system non-responsiveness to portal antigens and extend this effect to metastatic tumor cells.

2.2 Kupffer Cells: A Dual-Edged Resident Macrophage

Kupffer cells (KCs) function as liver macrophages that represent 80 to 90 percent of all tissue macrophages which exist within the human body. The initial phase of metastatic seeding sees KCs kill tumors through their ability to consume cancer cells and create reactive oxygen species and produce TNF-α. KCs which established themselves within metastatic lesions now experience a functional shift toward developing immunosuppressive capabilities. The tolerant state of KCs enables them to produce interleukin-10 (IL-10), which leads to the creation of FOXP3+ regulatory T cells and increases their PD-L1 surface expression, resulting in decreased cytotoxic T lymphocyte (CTL) activity within the hepatic microenvironment. KC-derived IL-6 and matrix metalloproteinases (MMPs) and vascular endothelial growth factor (VEGF) create a pathway that enables cancer cells to invade and blood vessels to grow and cells to multiply. The process results in KCs switching from their active immune monitoring mode to their new role as active agents who assist with cancer development.

The protein Fibrinogen-like protein 1 (FGL1) functions as a significant LAG-3 ligand which both cancer cells and native liver cells release and which tumor-associated macrophages (TAMs) stabilize through their OTUD1 production.

2.3 Hepatic Stellate Cells and Cancer-Associated Fibroblasts

Hepatic stellate cells (HSCs) transform into myofibroblast-like cancer-associated fibroblasts (CAFs) which create dense extracellular matrix (ECM) structures while producing TGF-β and stromal cell-derived factor 1α (SDF-1α/CXCL12) and VEGF upon their activation through tumor-derived signals and inflammatory cytokines. The desmoplastic stromal reaction establishes a dual barrier system which prevents effector T cells from entering tumor nests and mimics the immune-excluded phenotype that occurs in various solid tumors. CAF-

DOI: 10.46121/pspc.52.1.5

secreted CXCL12 keeps T cells inside the stroma which prevents their movement through intraepithelial spaces and this process directly reduces cytotoxic lymphocytes' ability to destroy tumor cells.

TUMOR-INTRINSIC MECHANISMS OF IMMUNE EVASION

3.1 Low Neoantigen Burden and Impaired Antigen Presentation

The immunotherapeutic effectiveness of MSI-H tumors emerges from their high tumor mutational burden (TMB) which leads to increased neoantigen production that enables T-cells to react to multiple foreign peptides. The majority of CRLM cases are found in MSS tumors which exhibit both APC and KRAS mutations together with chromosomal instability, yet their somatic mutation rate remains considerably lower. The low number of immunogenic neoantigens results in reduced T-cell activation against tumor-specific antigens which leads to a "cold" immune phenotype that shows low density of tumor-infiltrating lymphocytes (TILs). The neoantigen shortage is worsened by MSS CRC which commonly shows genetic changes that affect the ability of cells to process and show antigens. MHC class I surface expression loss stops CD8+ T cells from detecting tumor-derived peptides which results in functional tumor invisibility despite T cell presence in the tumor area. Interferon-gamma signaling pathway changes disrupt antigen presentation by affecting JAK1/JAK2 and subsequent STAT1 activation.

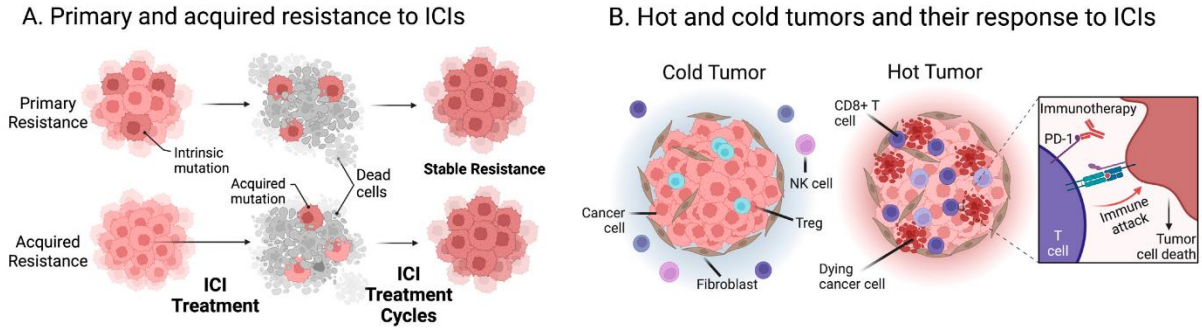

Figure 1.1 - Mechanisms of Primary and Acquired Resistance to Immune Checkpoint Inhibitors (ICIs) and the Immunological Landscape of Hot vs. Cold Tumors

3.2 WNT/β-Catenin Signaling and T-Cell Exclusion

The WNT/β-catenin pathway activation functions as the primary mechanism which scientists understand the best to create immune exclusion in CRC. The APC mutation which occurs in more than 70% of MSS CRC cases activates WNT/β-catenin signaling permanently because this genetic change creates a constant activation. The expression of nuclear β-catenin (CTNNB1) shows an inverse relationship to TIL density because it affects CD8+ and CD45RO+ and CD3+ cell counts at all mutation levels while "non-inflamed" CRC tumors show active WNT pathway signaling at higher rates. WNT/β-catenin activation creates a mechanism which prevents CD103+ dendritic cells from producing CCL3 thus stopping CD8+ T-cell recruitment. WNT11 functions as a non-canonical WNT ligand which liver metastases overexpress to reduce CXCL10 and CCL4 production in tumor cells through CAMKII-mediated β-catenin/AFF3 downregulation which disables T-cell chemotaxis. The WNT11-overexpressing tumor cells drive M2 macrophage polarization through their IL17D induction via the CAMKII/NF-κB pathway thus establishing two processes which block CD8+ T-cells from entering and functioning. The excess activity of β-catenin leads to increased PD-L1 production which results in the formation of additional Treg cells thus strengthening the immunologically cold phenotype.

DOI: 10.46121/pspc.52.1.5

Power System Protection and Control
ISSN:1674-3415

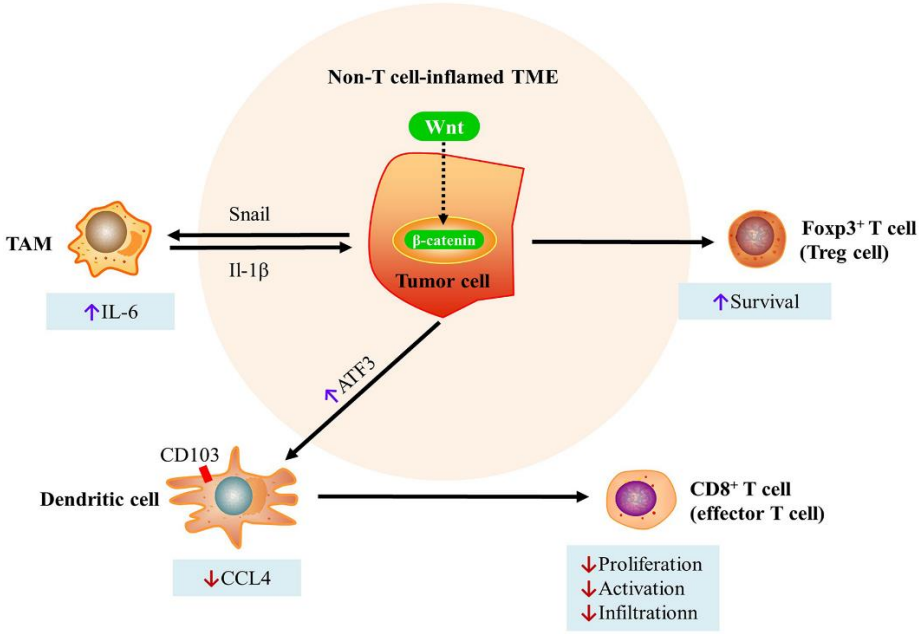

Figure 1.2-Wnt/β-Catenin–Mediated Immune Exclusion in Non–T Cell-Inflamed Tumor Microenvironment (TME)

3.3 MAPK Pathway Activation

The cancer-causing KRAS and BRAF mutations which create CRC tumors activate the MAPK pathway through MEK and ERK components which then enable cancer cells to grow while blocking their immune defense mechanisms. The activation of MAPK pathways leads to a decrease in IFN-γ-dependent antigen presentation together with less T-cell infiltration and the establishment of an immunosuppressive cytokine environment. The KRAS signaling pathway leads to VEGF upregulation which causes dendritic cells to lose their ability to differentiate while T-cells undergo exhaustion and regulatory T-cells and myeloid-derived suppressor cells enter the tumor microenvironment, thus creating an environment that prevents immune system access to the hepatic metastatic area.

THE IMMUNOSUPPRESSIVE CELLULAR MILIEU OF CRLM

4.1 CD8+ T-Cell Depletion and Systemic Immune Sequestration

The main clinical observation which has the greatest clinical significance among CRLM symptoms shows that patients experience a total loss of effector CD8 T cells throughout their body which occurs because the disease extends beyond their liver metastases and the body loses cytotoxic lymphocytes in their primary tumors and all other areas of their disease. Preclinical work in orthotopic mouse models demonstrated that liver metastases eliminate activated CD8+ T cells from the systemic circulation: within the liver, activated antigen-specific Fas+CD8+ T cells undergo apoptosis following interaction with FasL+CD11b+F4/80+ monocyte-derived macrophages, which creates an immunologically inert microenvironment that enables tumors to grow while simultaneously blocking immune defense at distant locations of the disease. The systemic exhaustion of CD8 T cells throughout the body leads to worse treatment outcomes for active CRLM patients who receive immune checkpoint inhibitors because their extrahepatic lesions show lower treatment responses.

4.2 Regulatory T Cells and TIM-3 Upregulation

CRLM microenvironment shows significant growth of FOXP3+ regulatory T cells which operate as primary defenders of antitumor defense mechanisms. The Tregs in CRLM establish the main pathway for IL-10 production which causes increased PD-L1 levels on monocytes that result in decreased CD8+ T-cell movement

DOI: 10.46121/pspc.52.1.5

and power. The TGF-β which Tregs produce stops CD4+ and CD8+ effector T-cells from functioning while it reduces their ability to create IFN-γ and IL-2 and it prevents natural killer (NK) cells from destroying targets. The liver TME shows increased T-cell immunoglobulin mucin-3 (TIM-3) expression on Treg cells because complex cellular interactions within this environment drive this process. The TIM-3/Treg mechanism creates an immunosuppressive system which maintains itself through regulatory cell activation that controls macrophage-driven immune suppression which leads to Treg expansion.

4.3 Myeloid-Derived Suppressor Cells

Myeloid-derived suppressor cells (MDSCs) accumulate excessively in CRLM which creates a significant barrier that prevents successful antitumor immune responses. The CRLM tumor microenvironment contains monocytic MDSCs at high levels which use various methods to block T-cell activity by depleting arginine through arginase-1, producing reactive oxygen species, and releasing immunosuppressive cytokines such as IL-10 and TGF-β, and causing direct T-cell death. The preclinical study showed that MDSCs reduced CD8+ T-cell levels of CTLA-4, PD-1, ICOS, and Ki67 in the liver while simultaneously increasing CTLA-4, ICOS, and PD-L1 levels on Treg cells which resulted in reduced effector immunity and enhanced suppressor functions.

The N2 phenotype of tumor-associated neutrophils (TANs) which TGF-β induces, produces neutrophil extracellular traps (NETs) that protect tumor cells from immune system attacks while they activate the SDF-1α/CXCR4 pathway and epithelial-mesenchymal transition (EMT) process, which strengthens the CRLM immunosuppressive network.

4.4 Tumor-Associated Macrophages and M2 Polarization

MSS CRC tumors display tumor-associated macrophages as their main immune cell type which links their increased presence to unfavorable clinical outcomes based on most studies. Macrophages establish an M2 anti-inflammatory phenotype in CRLM because they produce high levels of IL-10 and TGF-β while secreting VEGF and reducing their ability to present antigens. The process of M2 polarization creates direct obstacles for adaptive immunity because it produces an "immune exclusion" effect which stops T cells from entering tumor nests. High TAM density predicts poor outcomes in CRC patients treated with ICI-based combinations, as demonstrated in a phase II trial evaluating regorafenib plus avelumab, where high TAM infiltration correlated with inferior clinical benefit.

TGF-β SIGNALING: A CENTRAL DRIVER OF HEPATIC IMMUNE SUPPRESSION

The TGF-β pathway controls all aspects of immunosuppression in CRLM because it uses multiple pathways to eliminate all forms of antitumor defense. Tumor cells and CAFs and Tregs and macrophages release TGF-β in large quantities within the hepatic metastatic niche which leads to strong suppression of CD4+ T-cell and CD8+ T-cell activation and proliferation and NK cell cytotoxicity reduction and naive T cell differentiation into immunosuppressive FOXP3+ Tregs. The system induces TAMs to develop M2 phenotype characteristics while it generates N2-TAN development which establishes an immunosuppressive system that strengthens itself.

The CRLM condition shows intensified TGF-β signaling which causes desmoplastic stromal changes that create a physical barrier which prevents T cells from reaching tumor cells. Preclinical models have consistently demonstrated that inhibition of the TGF-β pathway with small-molecule inhibitors reduces liver metastatic burden and attenuates immune evasion. The presence of aberrant APC/β-catenin mutations in MSS CRC leads to increased TGF-β pathway activation which establishes a connection between genetic changes in tumors and the resulting immunosuppression in their microenvironment. TGF-β emerges as a key therapeutic target for CRLM treatment according to the results of this research.

PD-L1 UPREGULATION AND CHECKPOINT PATHWAY EXPLOITATION

The PD-L1 expression levels in CRLM show substantial increase when compared to their primary tumors which demonstrates that metastatic tumors more effectively stop CD8+ T cell infiltration. The elevated PD-L1 levels present in the hepatic metastatic niche lead to T cell exhaustion through continuous PD-1/PD-L1

DOI: 10.46121/pspc.52.1.5

interaction which decreases lymphocyte functions. The high PD-L1 levels found in CRLM result from both tumor cells and stromal cells which include macrophages CAFs and LSECs.

The oncogenic pathways all lead to PD-L1 upregulation because WNT/β-catenin and PI3K/AKT and MEK/ERK and HGF/MET pathways create multiple ways for tumor cells to use immune checkpoints which makes anti-PD-1/PD-L1 treatment fail to restore T-cell activity. Tregs release high IL-10 levels in the CRLM microenvironment which leads to PD-L1 upregulation on monocytes that creates additional immune suppression which goes beyond the tumor cell PD-L1 expression.

The co-expression of multiple co-inhibitory receptors (PD-1 TIM-3 LAG-3 TIGIT) on tumor-infiltrating T cells in CRLM represents advanced T-cell exhaustion that single checkpoint blockade cannot adequately reverse. LAG-3 which binds MHC II and FGL1 the latter highly expressed in liver tissue has become a clinically important resistance mechanism and therapeutic target.

METABOLIC REPROGRAMMING OF THE TUMOR IMMUNE MICROENVIRONMENT

The metabolic environment of colorectal liver metastases creates an extreme challenge which prevents the immune system from mounting successful attacks against cancer. Tumor cells use aerobic glycolysis through the Warburg effect to produce large amounts of lactate which causes TME acidification. This process results in direct hindrance of NK cell and CD8+ T-cell functions while it drives macrophages to adopt an immunosuppressive M2 phenotype. Researchers are developing new methods to combat cancer through the treatment of lactate dehydrogenase (LDH) and MCT1/4 monocarboxylate transporters that exist in the CRLM microenvironment. Amino acids create another pathway through which organisms develop resistance to treatment. The enzyme indoleamine 2,3-dioxygenase (IDO) depletes tryptophan to produce kynurenine which acts as a strong suppressor of T-cell growth and an inducer of Treg development. Arginase-1 which MDSCs and M2 macrophages release into CRLM tissue depletes arginine which T-cells need to activate their receptor signals and grow. The metabolic changes lead to a space which lacks nutrients and contains high levels of acid and immune system blockers, making it impossible for immune cells to work properly there.

STRATEGIES TO OVERCOME IMMUNOTHERAPY RESISTANCE IN CRLM

8.1 Combination Immune Checkpoint Blockade

Researchers have investigated combination treatment methods that target different checkpoint pathways because CRLM uses multiple co-inhibitory pathways. The dual CTLA-4/PD-L1 block demonstrated preclinical efficacy in orthotopic mouse models because it reduced colon cancer development while it blocked liver metastasis and it increased T-cell infiltration into tumors. The clinical trials testing dual checkpoint blockade in unselected MSS CRC patients have produced unsatisfactory results because they showed only minimal objective responses. The novel bispecific and Fc-enhanced antibodies which include botensilimab with balstilimab have demonstrated potential to overcome the drawbacks of standard ICI combination treatments for refractory MSS mCRC patients.

8.2 LAG-3 and FGL1-Targeted Therapy

The detection of FGL1 as the primary LAG-3 receptor present in liver tissue creates strong justification for using LAG-3 inhibitors during treatment of CRLM. The experimental CRLM model studies show that new treatments which use anti-LAG-3 together with anti-PD-1 improve immunotherapy results by breaking the FGL1/LAG-3 connection which restores T-cell activity. The clinical testing of this combination method has begun because it stands as one of the strongest scientifically supported methods to increase ICI treatment success in patients with liver metastatic disease.

8.3 Anti-VEGF/VEGFR Combinations

The agents that target VEGF create normal blood vessel development in CRLM tumors which leads to better T-cell movement into the tumor area and the agents also treat the immunosuppressive effects of VEGF which include disrupted DC development and T-cell fatigue and Treg/MDSC recruitment. The combination of anti-

DOI: 10.46121/pspc.52.1.5

PD-1 therapy with anti-VEGF/VEGFR agents has demonstrated practical potential in laboratory studies and currently exists as a subject of research in active clinical investigations.

8.4 TGF-β Pathway Inhibition

Multiple TGF-β receptor kinase inhibitors and anti-TGF-β antibodies are currently undergoing clinical testing for the treatment of CRLM. Bifunctional molecules that simultaneously block TGF-β and PD-L1 (bintrafusp alfa and related constructs) use their mechanism to disrupt the primary immunosuppressive pathway which operates in the hepatic TME. The studies prove that TGF-β inhibition stops effector T cell exclusion from stromal-dense CRLM microenvironments while it brings back T cell operational capacity.

8.5 Locoregional Liver-Directed Therapies Combined with Immunotherapy

The combination of locoregional ablative therapies which include stereotactic body radiotherapy and hepatic arterial infusion and transcatheter arterial chemoembolization with systemic ICI therapy creates an effective medical method to break down the immune defenses of the liver. The process of liver metastasis radiation treatment results in the destruction of cancer cells through immunogenic cell death while it reduces MDSC cell numbers and it improves the ability of CD8+ T-cells to enter the liver thus making the cold immunological space more suitable for checkpoint blockade therapy. Research studies have shown that the combination of radiotherapy and immunotherapy results in changes to T-cell receptor patterns and leads to M2 macrophage transformation back to their original state yet the approach still needs to be validated through clinical trials.

BIOMARKERS AND FUTURE DIRECTIONS

Clinical management of CRLM requires development of specific biomarkers which can assess immunotherapy response beyond existing microsatellite testing methods. The density of tumor-infiltrating lymphocytes which includes the presence of cytotoxic CD8+ T-cells functions as a better prognostic biomarker than tumor mutational burden (TMB) for evaluating MSS CRC patients who participated in ICI combination trials. Researchers discovered that serum WNT11 expression serves as a potential biomarker which doctors can use to monitor CRLM patients who undergo immunotherapy treatment. Scientists used machine learning-based bioinformatics to analyze single-cell transcriptomic data from the liver tumor microenvironment which identified new molecular targets and patient populations who would benefit from specific treatment combinations. Future research priorities include conducting longitudinal studies which use serial biopsies and circulating tumor DNA (ctDNA) profiling to track resistance mechanism development and creating biomarker-driven adaptive clinical trial platforms that use translational endpoints and establishing standardized immunophenotyping protocols for cross-trial comparisons. The IL-33/ST2 axis and NET formation inhibitors which include PAD4 inhibitors and PORCN inhibitors that target WNT secretion need additional evaluation through early-phase clinical trials.

CONCLUSION

The resistance of immunotherapy to CRLM develops through the interaction of both internal tumor factors and external biological processes which function at multiple levels of biological existence. The hepatic tolerogenic immune environment functions as the fundamental system which enables tumor-derived WNT/β-catenin activation and TGF-β-mediated immune suppression and Treg and MDSC expansion and metabolic reprogramming and advanced T-cell exhaustion to make immune checkpoint inhibition fail. The liver metastases cause complete CD8+ T cell system depletion which creates immunosuppression that affects all disease sites beyond the liver area.

Progression toward this resistance must happen through combined strategies which medical science has developed to target five different suppressive mechanisms that include tumor-based immune defense elimination and myeloid cell transformation and metabolic system restoration and blood vessel system changes and connective tissue protection. The combination therapies need to be tested through biomarker-based clinical trials which include both clinical study and translational research to determine their effectiveness for specific patient groups while translating preclinical research into sustained clinical success. The growing understanding

DOI: 10.46121/pspc.52.1.5

of CRLM resistance molecular structure enables researchers to transform liver metastases which lack immune activity into responsive treatment sites which will lead to better survival outcomes for this difficult-to-treat disease.

REFERENCES

1. Ilerhunmwuwa NP, Sahin IH, Saeed A. Immunotherapy resistance in colorectal cancer with liver metastases: challenges and therapeutic advances. *Chin Clin Oncol.* 2024;14(1):7. doi:10.21037/cco-24-93.
2. Sunagua Aruquipa MP, Donadio MS, Peixoto RD. Liver metastasis and resistance to immunotherapy in microsatellite stable colorectal cancer: a literature review. *ecancer.* 2024;18:1771. doi:10.3332/ecancer.2024.1771.
3. Liu Y, Zhang Q, Xing B, et al. Immune phenotypic linkage between colorectal cancer and liver metastasis. *Cancer Cell.* 2022;40:424–437.
4. Yu J, Green MD, Li S, et al. Liver metastasis restrains immunotherapy efficacy via macrophage-mediated T cell elimination. *Nat Med.* 2021;27:152–164.
5. Li J, et al. FGL1 secretion by hepatic cells stabilized by OTUD1 activates LAG-3 on T cells in CRLM. *J Hepatol.* 2023.
6. Tauriello DVF, Palomo-Ponce S, Stork D, et al. TGFβ drives immune evasion in genetically reconstituted colon cancer metastasis. *Nature.* 2018;554:538–543.
7. Bagby SM, Hartman SJ, Navarro NM, et al. Sensitizing microsatellite stable colorectal cancer to immune checkpoint therapy utilizing Wnt pathway inhibition. *Cancer Res.* 2020;80(16_Suppl):6647.
8. Gu J, Zhou J, Chen Q, et al. WNT11 promotes immune evasion and resistance to anti-PD-1 therapy in liver metastasis. *Nat Commun.* 2024. doi:10.1038/s41467-025-56714-z.
9. Chen EX, Loree JM, Titmuss E, et al. Liver metastases and immune checkpoint inhibitor efficacy in patients with refractory metastatic colorectal cancer: a secondary analysis of a randomized clinical trial. *JAMA Netw Open.* 2023;6(12):e2346094.
10. Overman MJ, Gelsomino F, Aglietta M, et al. Nivolumab plus relatlimab in patients with MSI-H/dMMR metastatic colorectal cancer: the phase II CheckMate 142 study. *J Immunother Cancer.* 2024;12(5):e008689.
11. Bullock AJ, Schlechter BL, Fakih MG, et al. Botensilimab plus balstilimab in relapsed/refractory microsatellite stable metastatic colorectal cancer: a phase 1 trial. *Ann Oncol.* 2024.
12. Zhang B, Halder SK, Zhang S, et al. Targeting transforming growth factor-β signaling in liver metastasis of colon cancer. *Cancer Lett.* 2009;277:114–120.
13. Kamal Y, Schmit SL, Frost HR, et al. The tumor microenvironment of colorectal cancer metastases: opportunities in cancer immunotherapy. *Immunotherapy.* 2020;12:1083–1100.
14. Wu Z, Zhang Y, Cheng Y, et al. PD-1 blockade plus COX inhibitors in dMMR metastatic colorectal cancer: the PCOX trial. *Med.* 2024;5:998–1015.

DOI: 10.46121/pspc.52.1.5

15. Ashekyan O, Shahbazyan N, Bareghamyan Y, et al. Transcriptomic maps of colorectal liver metastasis: machine learning of gene activation patterns for precision medicine. *Cancers (Basel).* 2023;15:3835.
16. Vaswani A, Shazeer N, Parmar N, et al. Attention is all you need. *Adv Neural Inf Process Syst.* 2017;30. [Cited for ML methodology in bioinformatics analyses of CRLM data.]
17. Santagata S, Rea G, Castaldo D, et al. Hepatocellular carcinoma tumor microenvironment is more suppressive than colorectal cancer liver metastasis tumor microenvironment. *Hepatology Int.* 2023. doi:10.1007/s12072-023-10537-6.
18. Fukuoka S, Hara H, Takahashi N, et al. Regorafenib plus nivolumab in patients with advanced gastric or colorectal cancer: REGONIVO trial. *J Clin Oncol.* 2020;38(18):2053–2061.

DOI: 10.46121/pspc.52.1.5