

The Tumor Microenvironment and Clinical Implications of Radiotherapy and Immunotherapy for Liver Metastases from Non-Small Cell Lung Cancer (NSCLC)

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Abstract. Immunotherapy emergence has triggered a profound change in the approach to treating advanced non-small cell lung cancer (NSCLC). However, challenges arise when tumors metastasize to critical sites such as the brain, bones, and liver, impeding the efficacy of immunotherapeutic interventions. Particularly, liver metastases exhibit a considerably unfavorable prognosis in comparison to other metastatic sites, owing in part to the prevailing immunosuppressive microenvironment within the liver that hampers immune-based therapies. Hence, it is imperative to investigate novel strategies that can bolster immune responses and prolong the survival of individuals with liver metastases (LM). Radiotherapy (RT) stands out as a primary modality in cancer treatment. Preclinical studies have revealed its capability to reshape the tumor microenvironment (TME), shifting it from an immunosuppressive setting to one that promotes immune activity. This transformative mechanism holds the promise of amplifying the effectiveness of immune-based treatments. This article will discuss the mechanisms by which radiotherapy improves the immunosuppressive microenvironment in the liver, provide a comprehensive review of the research progress on the combined application of RT and immunotherapy for LM, and offer insights into the establishment of novel therapeutic strategies for patients with LM.

Keywords: liver metastasis (LM); immune checkpoint inhibitors (ICIs); tumor microenvironment (TME); Immunotherapy; Radiotherapy (RT); non-small cell lung cancer (NSCLC).

1. Introduction

According to estimates, lung cancer accounts for about two million new cases and one million deaths each year, making it one of the principal factors behind cancer-related deaths worldwide. There are two primary classifications of lung cancer: NSCLC and small cell lung cancer (SCLC), with NSCLC comprising about 85% of diagnosed cases [1]. The thorough investigation of ICIs has brought about a revolution in NSCLC treatment. This transformation has significantly improved the prognosis of lung cancer patients, whether used as standalone therapies or in combination with chemotherapy. However, within the population of metastatic NSCLC patients, immunotherapy is not effective when the metastatic site is the brain, liver, bone, etc. This may be due to factors such as the blood-brain barrier and special tumor microenvironment that limit the efficacy of immunotherapy. Among these, liver metastases exhibit a poorer prognosis and diminished immune response compared to other metastatic sites. Liver metastasis (LM) are detected in around 4% of NSCLC, and patients typically experience a median overall survival of about 4 months. Therefore, there is a need to explore effective strategies to overcome these obstacles, enhance immune benefits for such patients, and prolong their survival.

Fundamentally, tumor growth is determined by the inherent properties of tumor cells and their surrounding tumor microenvironment (TME). TME at different anatomical locations exhibits specificity, and these tissue-specific TME regulate tumor growth and progression, influencing the response to therapy. The liver, acting as an immune-privileged organ, typically shows immune-inhibitory conditions in its microenvironment. This might restrict immune reactions. Radiotherapy (RT), a fundamental cancer treatment, is extensively employed in anticancer therapies. Preclinical research indicates that radiotherapy does more than just eliminate tumor tissue; it also alters the tumor

microenvironment through numerous mechanisms. This transformation turns the TME from immune-suppressive to immune-stimulatory, thereby boosting immune responses [2].

This review will focus on the radiotherapy reshapes the immune-suppressive microenvironment in the liver, combined with the latest advancements in immunotherapy. It seeks to offer an in-depth examination of the research evidence for the combined use of radiotherapy and immunotherapy. The goal is to offer insights for clinical treatment strategies in patients with LM in NSCLC.

2. ICIs in the Therapeutic Approach to NSCLC

Currently, the indications for immunotherapy in NSCLC primarily focus on treatment of choice for patients with advanced NSCLC lacking driver gene mutations. According to the latest guidance, from National Comprehensive Cancer Network (NCCN), ICIs as a suitable treatment option for individuals with metastatic NSCLC [3].

The use of immunotherapy in advanced driver gene-negative NSCLC patients can lead to long-term survival. Diverse immunotherapy approaches have demonstrated survival benefits in several pivotal Phase III clinical trials for the initial treatment of advanced NSCLC.

Within first-line monotherapy immunotherapy investigations involving NSCLC, the latest follow-up survival outcomes from the KEYNOTE-001, KEYNOTE-024, and KEYNOTE-042 trials indicate 5-year overall survival rates of 29.6%, 31.9%, and 25%, sequentially. This demonstrates an almost twofold increase when compared to the chemotherapy group, providing substantial support for the consideration of pembrolizumab as a viable treatment avenue for patients with advanced NSCLC displaying PD-L1 positivity.

When exploring combination chemotherapy, regardless of PD-L1 status, the results of the KEYNOTE-021, KEYNOTE-189, and KEYNOTE-407 trials indicate that combination therapy leads to improved OS. In comparison to chemotherapy alone, In the case of pembrolizumab combined with chemotherapy, the median overall survival (mOS) was 34.5m vs 21.1m, 22.0m vs 10.6m, and 17.1m vs 11.6m, respectively. Additionally, the IMpower130 study demonstrates that the combination of atezolizumab and chemotherapy significantly improves outcomes (vs chemotherapy alone, mOS 18.6m vs 13.9m).

In the Checkmate-9LA and CheckMate-227 trials, the focus was on assessing the safety and effectiveness of combining two ICIs, Nivolumab and Ipilimumab, for the management of advanced NSCLC without mutations in the driver gene. The results indicate that, compared to the chemotherapy group, patients can benefit from the dual immune checkpoint inhibitor regimen.

Unfortunately, despite the prior research findings demonstrating the benefits of immunotherapy for advanced NSCLC patients, subgroup analyses within IMpower130 indicated no improvement in PFS and OS for patients with LM (mOS: 10 months vs. 8.8 months; mPFS: 4.2 months vs. 4.4 months). The 5-year results from CheckMate-017 and CheckMate-057 trials indicate that in NSCLC patients with LM, Nivolumab provides survival benefits compared to docetaxel treatment. However, patients without LM experience even greater benefits. In the Nivolumab group, the 3-year OS rate was 17% for the non-liver metastasis subgroup and 8% for the liver metastasis subgroup. Likewise, Yu et al. noted that the presence of baseline LM was connected to a decreased response to immunotherapy. Research indicates that the tumor immune-suppressive microenvironment is a significant factor leading to poor responses to immunotherapy. Further examination of the liver's microenvironment unveils that it often maintains an immune-suppressive state, potentially resulting in resistance to immunotherapy in the tumor microenvironment.

3. Microenvironment in Liver Metastases

The liver has a unique anatomical structure and dual blood supply and is responsible for various metabolic, detoxification, and immune functions in the human body. These functions result in the continuous exposure of the liver to a large number of antigens, including those from the gut

microbiota, food, drugs, toxins, and self-tissues. To prevent harmful immune reactions and clear potential pathogenic invasions, the liver needs to maintain an immune-tolerant environment. Nonetheless, it is this immune-tolerant environment that could potentially foster the creation of an immunosuppressive tumor environment in LM cases. This, in turn, assists the tumor in evading the immune response, hampers immune reactions against liver metastasis, and diminishes the efficacy of immune treatments.

This immunosuppressive characteristic is primarily due to the liver's rich cellular composition, including both non-immune and immune cells, many of which exert immunosuppressive functions within the tumor microenvironment.

Liver sinusoidal endothelial cells (LSECs): The majority of the non-parenchymal liver cell population consists of LSECs, which line the low-shear sinusoidal capillary channels of the liver. In the TME of LM, LSECs exert detrimental effects primarily due to local inflammation, which leads to heightened expression of LSEC adhesion receptors. It directly or indirectly promotes cancer cell adhesion, enhances cell migration and invasion capabilities, contributing to immune evasion by tumor cells. In contrast, LSECs play a role in antigen presentation by expressing MHC II molecules, presenting antigens to CD4⁺ T cells, inducing their differentiation into Tregs, and facilitating the formation of an immune-tolerant microenvironment in the liver.

Kupffer cells (KCs): Approximately 10% of the total liver cell population comprises KCs, which play a significant part in maintaining an immune-suppressive environment. KCs are located within the liver sinusoids. These mature cells have various surface receptors like scavenger receptors, TLRs, and NLRs. These receptors allow KCs to detect and engulf pathogens and dying cells. Additionally, Within the liver, KCs draw in other cells possessing pro-metastatic attributes. This collaborative effort contributes to the formation of a tolerant immune environment [4].

Regulatory T cells (Tregs): Within the context of immunosuppression, Tregs are considered the primary drivers of T cell dysfunction and have the capability to generate anti-inflammatory cytokines, such as TGF- β and IL-10, in order to sustain their immunosuppressive traits.

Myeloid-derived suppressor cells (MDSCs): In the liver, a significant number of MDSCs are present, and they are recruited from the bone marrow thanks to chemokines, which are secreted by LSECs and KCs. MDSCs have the capacity to produce various factors, including VEGF and CSF. They inhibit NKs activity and obstruct adaptive T cell responses by releasing arginase. Furthermore, MDSCs induce T cell apoptosis through the generation of ROS and CCL5, which serve to recruit Tregs, resulting in an amplified immune-suppressive microenvironment [5].

Tumor-associated macrophages (TAMs): As cancer progresses, KCs, the tissue-resident macrophages in the normal liver, undergo phenotypic changes when stimulated by pro-tumor factors. These changes transform them into TAMs. It has two types: M1 and M2 macrophages. M2-like TAMs are particularly crucial in promoting cancer development. They suppress anti-tumor immune responses, boost angiogenesis, and enhance tumor cell invasion, mobility, and intravasation. They also express various molecules, which induce T cell apoptosis or dysfunction, hampering cytotoxic anti-tumor immune reactions. Additionally, in studies on liver metastasis, it was found that FasL⁺CD11b⁺F4/80⁺ monocytes can trigger apoptosis in activated antigen-specific Fas⁺CD8⁺ T cells. This process depletes cytotoxic immune T cells from the systemic circulation, reducing responsiveness to immunotherapy. Because cancer cells grow uncontrollably and immune cells don't work properly, the tumor environment often lacks oxygen. It leads to CD8⁺ T cell death and reduces their ability to kill cancer cells [6]. Moreover, TAMs can team up with other immune cells that promote tumor growth, like Tregs and MDSCs, to maintain an immune-suppressed environment by releasing IL-10 and TGF- β . This hampers DCs, T cells, and NKs' activity [7].

Tumor-associated fibroblasts (CAFs): CAFs are a kind of critical component of the TME. They secrete various molecules such as growth factors, cytokines, and chemokines, which contribute to promoting tumor initiation, progression, and resistance to various therapeutic strategies. Recent research has uncovered a novel mechanism of immune suppression mediated by CAFs within TME. This mechanism involves the increased uptake of oxidized lipids by CD36⁺CAFs, potentially

triggered by elevated lipid oxidation within tumors. Consequently, it leads to heightened lipid peroxidation, activation of p38 kinase, and the upregulation of macrophage migration inhibitory factor in CD36⁺CAFs [8]. These findings provide fresh insights into potential clinical therapeutic strategies.

The liver's immune-suppressive microenvironment composed of these immune-inhibitory cell populations might play a part in the unsatisfactory immunotherapy outcomes in individuals with NSCLC liver metastases.

4. The impact of Radiotherapy on the TME

The immunosuppressive TME is a significant obstacle to successful cancer treatment. Radiation therapy, as a primary approach in cancer therapy, not only directly destroys cancer cells but also has the capacity to impact elements of TME, such as tumor vasculature and immune system cells. Radiation therapy offers the potential to remodel the immunosuppressive microenvironment associated with liver metastases, thereby bolstering the immune response in patients with liver metastasis.

RT can directly kill tumor cells, releasing tumor antigens. It induces immunogenic cell death, releasing radiation-related antigenic proteins (PAAPs) and tumor-associated antigens (TAAs), et al.. Additionally, it releases molecules that activate dendritic cells (DCs) and enhance their antigen presentation capabilities. Activated DCs subsequently migrate to lymph nodes and stimulate T lymphocytes. Therefore, elevating tumor antigens, stimulating dendritic cells (DCs), and improving the presentation of antigens to T cells, the immunogenicity of tumor cells can be enhanced.

Furthermore, radiation treatment stimulates the type I interferon (IFN) and cGAS-STING signaling pathways, resulting in the generation of pro-inflammatory proteins. Additionally, through releasing chemokines, it increases T cell migration to the tumor site and expands and activates CD8⁺ T cells in draining lymph nodes.

Simultaneously, radiation therapy upregulates VCAM-1 expression on endothelial cells, enabling T cell extravasation. It influences endothelial cell survival, rewires macrophages so they release NO, reduces VEGF and ANG2 secretion, inhibits new blood vessel formation, enhances T cell infiltration, and promotes vascular normalization, thus improving the hypoxic tumor microenvironment.

Additionally, RT induces the release of pro-inflammatory cytokines. These cytokines contribute to the upregulation of VCAM-1 on tumor endothelial cells, facilitating T lymphocyte infiltration. Furthermore, type I IFNs released stimulate DCs to present TAAs to T lymphocytes, thereby activating specific T cell responses within the irradiated area and lymph nodes. Consequently, radiation can alter the tumor's metabolic state and enhance immune responses.

Figure 1 [9] illustrates the impact of different RT doses on the components of TME. Therefore, the combination of radiotherapy and immunotherapy has brought new hope for "cold" tumors in terms of immune response.

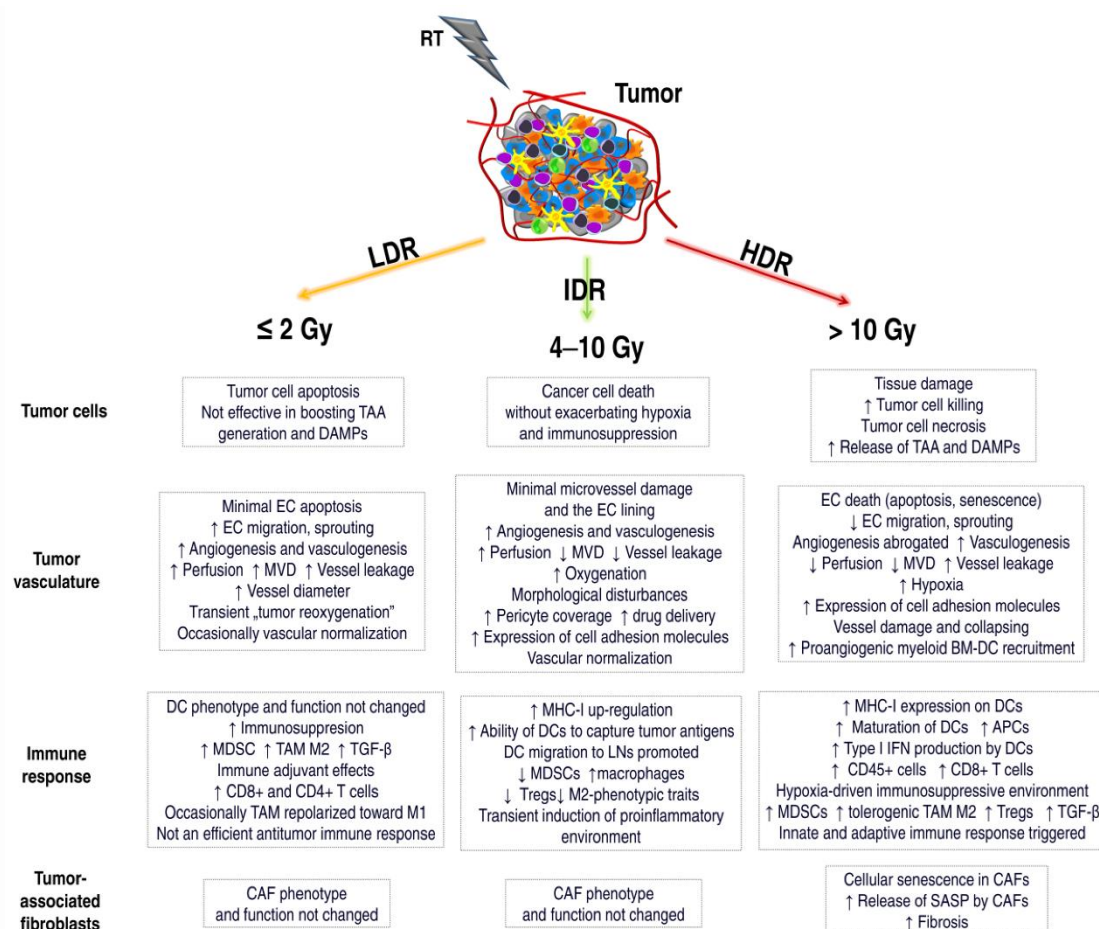


Fig.1 Radiation doses exert varying effects on cancer cells and the adjacent TME, influencing aspects such as tumor vascularization, immune system cells, and CAFs.

5. Clinical Application of RT Combined with ICIs in Liver Metastasis

A study published in Nature reveals that in mice without liver tumors, the number of T cells can return to normal after receiving immunotherapy. However, in mice with liver tumors, even after immunotherapy, there is no significant increase in T cell count. Through single-cell sequencing, researchers observed a significant increase in macrophages in mice with liver metastases, while the number of T cells decreased significantly. This indicates that liver metastasis leads to a significant proliferation of macrophages in the liver, and some macrophages (CD11b⁺F4/80⁺ type) recruit T cells from the entire body to the liver, inducing their death, thereby reducing the number and the role of T cells and diminishing the effectiveness of immunotherapy.

To address this issue, researchers found that liver-directed radiation therapy can reverse this adversity in the case of liver metastasis. By using radiation therapy to eliminate liver tumors, immune cells can return to normal, and after treatment with PD-1/PD-L1 drugs, they exhibit their original anticancer effects, causing tumors to shrink or even disappear.

A combination of radiation and immunotherapy with Ipilimumab + Nivolumab showed notable success in a patient with stage IV melanoma with numerous metastases to the liver, lungs, bones, and brain. The patient received liver LDRT (4×1.4Gy) in conjunction with immunotherapy. Two years later, there were no signs of disease, and the liver lesions reached complete remission (CR) [10].

The potential clinical promise of combining RT with ICIs for NSCLC is evident. Nevertheless, determining the optimal timing and sequence for administering this combination therapy necessitates further investigation. Currently, ongoing clinical studies are in progress, and we anticipate the release of follow-up data in due course.

6. Conclusion

Patients with LM from NSCLC constitute a unique subset within advanced lung cancer, marked by limited responses to immunotherapy. However, there is a dearth of clinical research specific to this subgroup, posing challenges in exploring suitable immunotherapeutic approaches through subgroup analysis in large-scale clinical studies. Thus, it is crucial to conduct a comprehensive review of preclinical research, synthesize the pathophysiological mechanisms underlying NSCLC liver metastasis, and investigate the functions of several immune cell types inside the liver's immunological milieu. These efforts are essential for developing innovative treatment strategies tailored to these patients' distinctive characteristics.

The TME significantly influences anti-tumor immune responses. The liver, with its intricate and unique immune-tolerant microenvironment, has complex associations with various immune cell populations. These cells may assume different functions at different tumor development stages, influenced by interactions with cytokines, chemokines, and other factors. Research into the liver immune microenvironment has advanced significantly in populations with liver metastases originating from hepatocellular carcinoma and colorectal cancer. However, investigations into the liver metastasis microenvironment in the context of lung cancer remain relatively limited. Our primary goal is to consolidate existing knowledge concerning immune cell roles and mechanisms, as revealed in preclinical studies, to inspire further research into treatment outcomes for lung cancer patients facing the challenge of LM.

Immunotherapy holds promise for extending the survival of patients with advanced lung cancer over extended periods. However, its effectiveness as a standalone treatment for NSCLC liver metastasis is suboptimal, necessitating exploration of potential synergistic therapies. This article comprehensively reviews clinical advancements in immune-based treatments, particularly their relevance to patients with liver metastases. Unfortunately, the current literature primarily comprises preclinical investigations and sporadic case reports. Therefore, prospective trials and large-scale randomized clinical studies are essential to definitively establish the efficacy of combined treatments.

In the future, it is imperative for the scientific community to explore the intricate mechanisms governing synergy and antagonism among different treatment regimens. This exploration aims to determine optimal timing and immunotherapeutic strategies, thereby refining protocols and improving the prognosis for this specific patient cohort, enabling them to fully benefit from immunotherapy.

In conclusion, to optimize the efficacy of immunotherapy in this distinct patient subset, a thorough understanding of the intricate immune microenvironment in NSCLC patients with liver metastasis, along with extensive clinical research, is essential.

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