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Modulation of Myeloid Cell Populations – An Alternative Approach to Cancer Immunotherapy

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Abstract. Lung cancer remains the leading cause of cancer-related mortality worldwide, accounting for 18% of all cancer-related deaths globally in 2020. The poor prognosis is largely due to the immunosuppressive tumor microenvironment (TME). Although immune checkpoint inhibitors can be used to treat a subset of lung cancer, limited efficacy in 'cold tumors' remains a major concern. Therefore, there is a need for effective immunotherapeutic strategies that remodel the TME. This study aimed to evaluate the potential of combining interleukin-37 (IL-37) with a CD40 agonist and to discover novel approaches to targeting myeloid-derived suppressor cells (MDSCs) and dendritic cells in lung cancer. We designed a preclinical study using murine lung cancer models to test whether IL-37 suppresses MDSCs recruitment and function while CD40 agonism enhances dendritic cell activation, antigen presentation, and $CD8^+$ T-cell priming. Additional work is required to understand the translational potential of this approach; however, this suggests that modulation of myeloid populations may represent a promising strategy to convert cold tumors into hot tumors and improve responsiveness to checkpoint blockade in lung cancer.

Keywords: Myeloid-derived suppressor cells (MDSCs), Tumor microenvironment (TME), Interleukin-37 (IL-37), CD40 agonist, Cold to hot tumor conversion

1. Introduction

1.1. Lung cancer overview

Lung cancer arises when lung cells undergo mutations that cause them to divide uncontrollably, forming tumors. These changes can be triggered by various factors, with smoking being the most significant risk factor. Other causes include exposure to radon gas, asbestos, certain occupational substances, and inherited genetic predispositions, such as germline TP53 mutations associated with Li-Fraumeni syndrome [1] or germline EGFR mutations predisposing individuals to familial lung cancer [2]. In 2024, lung cancer was the most commonly diagnosed cancer globally, with nearly 2.5 million new cases, and the leading cause of cancer-related deaths, with over 1.8 million deaths worldwide [3].

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1.2. Tumor Microenvironment (TME) and Myeloid-Derived Suppressor Cells (MDSCs)

1.2.1. TME

The tumor microenvironment (TME) comprises the tumor itself along with infiltrating immune cells, blood vessels, and signaling molecules. Far from merely supporting the immune system, the TME often works against it: tumors manipulate specific immune cells in this milieu to block immune attacks and shield the cancer. Among these cells, myeloid-derived suppressor cells (MDSCs) are particularly significant and exhibit potent immunosuppressive activity [4].

1.2.2. MDSCs

MDSCs are immature myeloid-lineage cells that, under normal circumstances, help regulate inflammation. In cancer, however, tumors co-opt MDSCs to shut down anti-tumor immune responses and foster an environment permissive to tumor growth. Elevated MDSCs levels correlate with poorer clinical outcomes and can hinder the effectiveness of immunotherapies such as checkpoint inhibitors [5].

These cells exhibit functional heterogeneity, partly shaped by changes in their energy metabolism —a field known as immunometabolism. Within the nutrient- and oxygen-deprived tumor environment, MDSCs rewire their metabolism (e.g., shifting from oxidative phosphorylation to glycolysis, mediated by pathways involving PI3K–AKT–mTOR and HIF-1α) to survive and maintain their suppressive function. Interfering with these metabolic adaptations may allow researchers to “reprogram” MDSCs into less harmful states [6].

1.2.3. Heterogeneity within the TME

Not all myeloid-related cells, including subsets of MDSCs, are uniformly immunosuppressive; some may support, rather than hinder, immune activity. Advances in high-resolution cellular profiling, particularly single-cell RNA sequencing (scRNA-seq) and mass cytometry (CyTOF), enable detailed analysis of immune cells within the TME. These techniques have uncovered significant diversity among myeloid cells, including distinctions among polymorphonuclear (PMN-) MDSCs subsets and classical neutrophils [7].

To move immunotherapy forward, an integrated understanding is needed, one that combines insights from MDSCs biology, immunometabolism, and single-cell analysis. This approach could facilitate the identification and targeting of the most suppressive immune-cell subsets in the TME, ultimately improving immunotherapy efficacy for a broader range of patients.

1.3. Comparison of previous immunotherapy approach to ours

1.3.1. Previous approach

In past years, many immunotherapeutic strategies including immune checkpoint inhibitors, cytokine therapies, and adoptive T-cell transfer have revolutionized oncology. However, the efficacy of these strategies in many solid tumors, such as lung cancer, still remains minor. This limitation is mainly due to the TME being dominated by MDSCs, which are key contributors to immune evasion, as they suppress T cell function and promote the secretion of inhibitory cytokines [8]. To explain, many solid tumors are “cold” in the first place, meaning that they initially lack the immune cell infiltration needed for these strategies to work. Previous strategies have often targeted either the suppression of

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MDSCs or the activation of DCs, but have rarely targeted both. For example focusing on drugs that reduce MDSCs suppressive functions, block their recruitment, or induce their differentiation into APCs, but does not present strategies explicitly designed to both suppress MDSCs and concurrently activate DCs [9].

1.3.2. IL-37 and CD40 agonist

Our proposal is to combine interleukin 37 (IL-37), a natural anti-inflammatory cytokine, with a CD40 agonist. IL-37 has been shown to reduce the number and suppressive activity of MDSCs, and to lower pro-tumor cytokine levels in the TME [10-12]. On the other hand, CD40 agonists are powerful activators of DCs, boosting the expression of co-stimulatory molecules and triggering IL-12 production — a signal that helps prime $CD8^+$ T cells to recognize and attack tumors [13-15]. IL-37 can strip away some of the immunosuppressive shield, while the CD40 agonist can enhance antigen presentation and T-cell activation, enabling the TME to regain its pro-inflammatory ability.

This dual modulation that we are proposing is anticipated to not only be effective in converting “cold” into “hot” tumors [16-17] but also make them far more responsive to checkpoint inhibitors such as anti-PD-1, to give the immune system its best chance at clearing lung tumors [16, 18].

In the following section we will summarize three primary research articles that our group selected as a foundation for our project on modulation of myeloid cell population – an alternative approach to cancer immunotherapy.

2. Reference article summary

2.1. Zalba et al. modulation of intratumoural myeloid cells, the hallmark of the anti-tumour efficacy induced by a triple combination: tumour-associated peptide, TLR-3 Ligand and α-PD-1, British journal of cancer

Zalba et al. applied immunotherapy combinations to dissect the kinetic of immune cells in non-inflamed TC-1/A9 tumor models. Using multiparametric flow cytometry and antibody depletion platforms, the authors characterized the intra-tumoral immune cell kinetics in the tumor microenvironment, identifying a biphasic immune response. Notably, they discovered an initial upsurge of proinflammatory myeloid cells that led to a further rise in effector CD8+ T lymphocytes at day 8. These subsets displayed an essential role in anti-tumor efficacy, as their depletion diminished the therapeutic effect, highlighting the need for a further understanding of critical immune cell populations and their kinetics over time. Additionally, they observed that the triple therapy induced an increase in proinflammatory monocytes and plasmacytoid dendritic cells, while decreasing immunosuppressive M2 macrophages and MDSCs, suggesting that an early remodeling of the tumor-associated myeloid cell compartment is crucial for anti-tumor efficacy. Through longitudinal analyses of the immune response, the study revealed the critical role of early modulation of the myeloid cell compartment and emphasized the importance of this modulation to allow subsequent infiltration of effector T lymphocytes. This research not only refines the understanding of immune dynamics in tumor microenvironment but also provide insight into how a cold tumor could become a hot tumor by an immunotherapeutic intervention.

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2.2. Mei et al. IL-37 dampens immunosuppressive functions of MDSCs via metabolic reprogramming in the tumor microenvironment, cell reports

The second study demonstrates that IL-37 can effectively inhibit tumor growth through its interaction with MDSCs within the tumor microenvironment. IL-37 alters the metabolic activity of MDSCs by promoting glycolysis and oxidative phosphorylation, enhancing ATP production. This metabolic reprogramming weakens the immunosuppressive function of MDSCs, which normally help tumors evade immune responses. In addition, IL-37 was shown to down-regulate key immunosuppressive genes such as Arg1 and Nos2, while also reducing the overall expansion of MDSCs. These changes correlated with increased survival rates in murine tumor models. The study suggests that IL-37 not only reshapes the tumor immune environment but may also contribute to the development of durable, long-term antitumor immunity. Overall, these findings present IL-37 as a compelling therapeutic target that holds promise for cancer immunotherapy, particularly when combined with existing approaches like immune checkpoint inhibitors to enhance treatment efficacy and broaden patient responsiveness.

2.3. Zhang, Lei et al. “single-cell analyses inform mechanisms of myeloid-targeted therapies in colon cancer.” cell

In this study, Zhang et al. utilize single-cell RNA sequencing (scRNA-seq) to explore the diversity of immune cells within the tumor microenvironment of colorectal cancer (CRC), specifically focusing on tumor-associated myeloid cells. They identify two key macrophage populations, $C1QC^{+}$ and $SPP1^{+}$, which differ in function, spatial distribution, and responsiveness to therapeutic intervention. It was further demonstrated that CSF1R inhibition, a common strategy to target myeloid cells, selectively affects certain macrophage subsets, leaving others largely untouched. In contrast, CD40 agonism reprograms resistant macrophage populations and enhances dendritic cell activation, improving T cell priming and promoting tumor regression. The study is validated through in vivo mouse models and further supports the notion that targeting the tumor myeloid compartment requires precision, as generalized myeloid depletion may be insufficient or counterproductive. Notably, the data presented suggest that evaluating specific immune cell signatures at the single-cell level can help guide rational combination therapies. This study not only clarifies the complexity of the myeloid landscape in CRC but also offers a path forward for developing more effective immunotherapies that are tailored to the immune architecture of individual tumors, potentially improving outcomes for patients with immunotherapy-resistant cancers.

3. Overall hypothesis

Combinational modulation of lung cancer cell models through IL-37 delivery and CD40 agonism reprograms the TME to convert immunologically "cold tumors" into "hot tumors". This will occur through a suppression of immunosuppressive MDSCs, activation of dendritic cells, and enhanced presentation of tumor-specific antigens, leading to the activation, expansion and memory formation of $CD8^{+}$ T cells. The approach will provide resistance to checkpoint blockade and promote anti-tumor immune efficacy in lung tumors [Figure 1].

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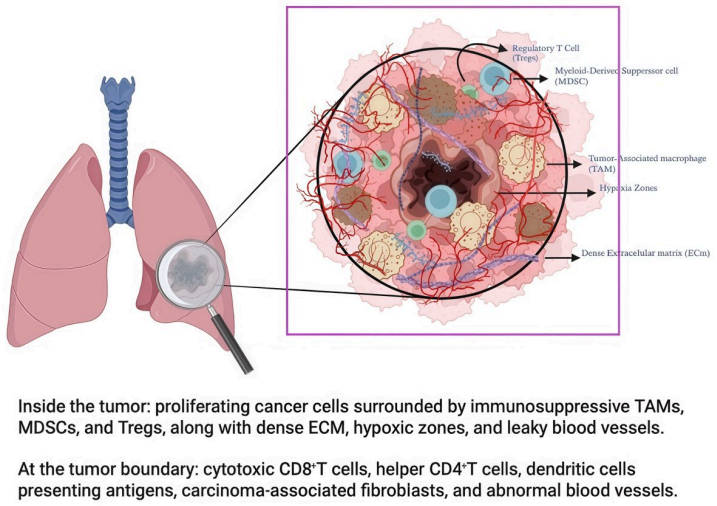

Figure 1. Visual depiction

4. Research aim

Our project proposes a combined immunotherapeutic approach designed to test whether combining IL-37 delivery with CD40 agonism could reprogram the pulmonary TME from immunologically "cold" to "hot" by MDSCs, mediated immunosuppression and enhancing dendritic cell (DC), driven $CD8^+$ T-cell immunity. We proposed to examine IL-37's ability to reduce MDSCs recruitment, suppressive function, and immunometabolic activity, while assessing CD40 agonist–mediated DC maturation and IL-12 production. Primary outcomes include changes in MDSCs and DC phenotypes (flow cytometry), $CD8^+$ T-cell priming, infiltration, and effector function, alongside tumor growth delay and survival.

5. Approach

We will test our treatment in mouse models of lung cancer with normal immune systems to study immune responses. Specifically, LLC1 and CMT167 lung cancer cells will be implanted into syngeneic C57BL/6 mice [19].

We will analyze immune cell populations in tumors using flow cytometry [20], focusing on MDSCs, activated dendritic cells (DCs), and $CD8^+$ T cells. Cytokine levels, including IL-12, IFN-γ, TNF-α, IL-10, and TGF-β [21], will be measured in blood and tumor tissue. We selected IL-12, IFN-γ, and TNF-α as key indicators of pro-inflammatory, anti-tumor immune activation, and IL-10 and TGF-β as representative immunosuppressive cytokines, enabling us to comprehensively evaluate the efficacy of our combinational treatment. Antigen presentation will be assessed using MHC-I tetramer staining to detect tumor-specific T cells. Tumor volume measurements occur at 72-hour intervals using digital calipers, with survival analysis continuing for 90 days post-treatment initiation. Anti-PD-1 [22] will be administered after primary treatment to assess checkpoint inhibitor response enhancement.

The experimental groups include:

- Untreated control
- IL-37 (Nold-Petry et al., 2015) alone
- CD40 agonist (Vonderheide, 2020) alone
- IL-37 plus CD40 agonist combination

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• Positive control (Poly(I:C) + anti-CD40)
• Negative controls (isotype antibodies) and injection controls (PBS/saline) [23]

IL-37 will be delivered systemically or intratumorally via recombinant protein or viral vector-mediated gene therapy. CD40 agonists will be administered intravenously to target antigen-presenting cells. The resulting immune reprogramming is expected to create an inflammatory, antigen-rich TME that promotes $CD8^+$ T cell infiltration and effector function. This environment should enhance tumor clearance and increase sensitivity to checkpoint inhibitors like anti-PD-1 [22], overcoming immunotherapy resistance in non-immunogenic lung tumors. The ultimate goal is to transform immune deserts into sites of durable anti-tumor immunity.

6. Anticipated results

6.1. Control group (no active treatment)

Tumors in the untreated control group are expected to remain immunologically "cold," with high MDSCs numbers, low activated DC and tumor-specific $CD8^+$ T cells frequencies, and more immune-suppressive cytokines including TGF-β, IL-6, and IL-10 [10, 11]. MHC-I tetramer staining will be weak in tumor-specific T cells, indicating poor antigen presentation. It is anticipated that tumors will grow rapidly, animals will exhibit brief survival, and tumors will respond poorly to anti-PD-1 therapy, in line with previous studies of untreated lung tumors in C57BL/6 mice [12].

6.2. IL-37 monotherapy

It is expected that mice treated IL-37 alone will exhibit a substantial reduction in the frequency of MDSCs in tumors when compared to controls [10], as well as lower serum and tumor tissue levels of IL-6, IL-10, and TGF-β [11, 12]. In comparison to controls, these alterations should allow for a slight rise in DC activation and tumor-specific $CD8^+$ T cells infiltration by partially easing immunological suppression. A minor improvement in antigen presentation is anticipated, along with a slight increase in MHC-I tetramer-positive cells. Without checkpoint inhibition, survival increases will be limited, and tumor growth rates will probably be slower than the control group but nonetheless progressing [11].

6.3. CD40 agonist monotherapy

We expect strong DC activation in the CD40 agonist–only group, along with increased production of IL-12 and co-stimulatory molecules such CD80 and CD86 [13, 14]. IFN-γ and TNF-α levels should rise in line with the mild stimulation of $CD8^+$ T cells. A change toward a more pro-inflammatory TME may cause a minor decrease in MDSCs levels compared to controls, but suppression won't be completely relieved. Although many cancers will still be partially resistant to anti-PD-1, the tumor growth reduction should be greater than in the IL-37 group, with a moderate extension of survival [14, 15].

6.4. IL-37 + CD40 agonist combination

The combination therapy group is expected to exhibit synergistic effects, including a significant increase in tumor-specific $CD8^+$ T cell infiltration and activation as seen by higher levels of IFN-γ and TNF-α [16, 17], a significant reduction in MDSCs and their suppressive cytokines (IL-6, IL-10, and TGF-β) [10, 11], and enhanced antigen presentation with significantly more MHC-I tetramer-

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positive T cells. Tumors in this group should shrink quicker, be more sensitive to anti-PD-1 therapy, and have significantly longer life periods than the control group and groups treated with single-agent therapies [16, 18].

6.5. Positive control (Poly(I:C) + anti-CD40)

This group is expected to demonstrate significant $CD8^+$ T cell priming, high IL-12 production, and maximal DC activation as part of a well-established immune-activating regimen [24, 25]. As a guide for the highest possible immune response, the immune activation profile could be similar to that of the combination therapy group.

6.6. Negative controls (inactive antibody / empty vector) & injection controls

We expect these groups to behave similarly to the untreated control, confirming that observed effects are due to active drug components rather than injection stress or non-specific antibody effects [10, 11].

7. Conclusion

We anticipate that IL-37 administration combined with a CD40 agonist can remodel the lung tumor microenvironment. IL-37 reduces the number and suppressive activity of MDSCs, while CD40 agonism activates DCs and improves $CD8^+$ T-cell priming. Together, these effects convert cold tumors into hot tumors, making them more responsive to checkpoint inhibitors.

When used alone, each treatment had limited benefits. IL-37 reduced MDSCs but only modestly improved T-cell activity. CD40 agonism activated dendritic cells but could not fully overcome MDSCs-driven suppression. The combination was stronger, producing more antigen presentation, greater $CD8^+$ T-cell infiltration, higher cytokine production, slower tumor growth, and better survival than either treatment alone.

These anticipated results would be consistent with earlier studies showing that IL-37 weakens MDSCs function and that CD40 agonists enhance dendritic cell activation. Our proposed work extends this by proving that targeting both cell types at once is more effective than targeting either alone.

The main implication is that focusing on a single immune pathway is not enough in lung cancer. A dual approach that reduces suppression while boosting activation provides a more complete immune response. Clinically, this strategy could improve checkpoint inhibitor therapy for patients with cold tumors.

In conclusion, combining IL-37 and CD40 agonism offers a clear path to overcoming resistance in lung cancer. Future studies should confirm these findings in more models and test safety and feasibility for patient treatment.

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