Review

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What are the main findings?

- The article explores the use of autologous activated lymphocytes with their ability to elicit robust anti-tumor immune responses as a promising treatment strategy for non-small cell lung cancer.
- The application of autologous lymphocyte therapy in lung cancer treatment in nearly 2,000 patients, with special focusing on the methods of procurement, quality control, and infusion protocols, was referred and thoroughly discussed.
- The article emphasizes the benefits of combining activated lymphocyte therapy with existing treatments like checkpoint inhibitors and chemotherapy to improve efficacy and reduce resistance.

What is the implication of the main finding?

- A wide variety of cell types like NK cells, cytotoxic T lymphocytes, LAK cells, TILs, and activated macrophages, contribute to immune responses that assist in eliminating cancer cells.
- The potential research gaps are identified and a wider adoption of immune cell therapy as a component of combination strategies for the treatment of lung cancer is proposed.
- The article highlights ongoing research into the tumor microenvironment and its role in immune evasion, suggesting that targeting these mechanisms could boost treatment success, and discusses the significance of clinical trials in validating the effectiveness of activated lymphocyte therapies and the importance of real-world evidence in guiding clinical practice.

Abstract: This review article explores the significance and prospects of using diverse T-cell variants in the context of combined therapy for lung cancer treatment. Recently, there has been an increase in research focused on understanding the critical role of tumor-specific T-lymphocytes and the potential benefits of autologous T-cell-based treatments for individuals with lung cancer. One promising approach involves intravenous administration of ex vivo-activated autologous lymphocytes to improve the immune status of patients with cancer. Investigations are also exploring the factors that influence the success of T-cell therapy and the methods used to stimulate them. Achieving a comprehensive understanding of the characteristics of activated lymphocytes and deciphering the mechanisms underlying their activation of innate anti-tumor immunity will pave the way for numerous clinical trials and the development of innovative strategies for cancer therapy.

Keywords: lymphocytes; lung cancer; tomotherapy; activation; tumor; immunotherapy; immunosuppression

1. Introduction

Small cell lung cancer (SCLC) is a special type of lung cancer, with rapid progression and metastasis to various organs, mainly through the lymphatic pathways. First, it spreads to the nearest bronchopulmonary lymph nodes and then to distant lymph nodes in the mediastinum. Lung cancer is the leading cause of cancer morbidity and mortality worldwide, accounting for about 2 million new cases and 1.8 million deaths annually. This shows the seriousness of the disease and its poor prognosis [1].

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The two main subtypes of lung cancer are small cell lung cancer and non-small cell lung cancer (NSCLC). NSCLC is the most common, comprising approximately 85% of all lung cancer cases. It can be categorized into three main types: adenocarcinoma, large cell carcinoma, and squamous cell carcinoma. Squamous cell carcinoma is the most widespread, accounting for 25-30% of all cases of lung cancer, and originates from squamous cells lining the airways and bronchi of the lungs. Smoking has a direct link to the development of squamous lung cancer. Identifying the subtype of lung cancer is important for designing effective treatment plans and prolonging patient survival. Currently, finding the right strategy for treating lung cancer remains a significant challenge that requires ongoing efforts in prevention, early detection, and development of innovative therapies [2].

Lung cancer, a formidable disease, often presents a significant challenge due to its late diagnosis. While early detection and surgical intervention offer the most effective treatment options, a sobering reality emerges. The majority of lung cancer cases are diagnosed at advanced stages, leaving many patients with limited treatment options. Only about a third of patients are diagnosed with stage I or II lung cancer, making them eligible for potentially curative surgery. Another third receive chemotherapy and/or radiation therapy, aiming to shrink the tumor and prolong survival. The remaining third are unfortunately diagnosed with advanced-stage disease, receiving palliative care to manage symptoms and improve quality of life. Additionally, even after complete surgical resection, more than half of patients experience recurrence, often in distant organs, highlighting the aggressive nature of lung cancer and its ability to evade treatment. The biological characteristics of lung cancer play a pivotal role in its progression and resistance to treatment. The presence of heterogeneous cell populations within primary lung tumors, known as intratumor heterogeneity, poses a significant challenge for treatment. Different cell types within the tumor can develop resistance to specific therapies, leading to treatment failure. This intricate interplay between tumor cells and their surrounding microenvironment fuels the progression of the disease. Cancer cells evade immune surveillance and promote angiogenesis, leading to metastasis and recurrence.

Addressing this complex problem requires a multi-faceted approach, including early detection through screening, personalized treatments tailored to the molecular characteristics of each tumor, and ongoing research to develop novel treatment strategies. Further research is crucial to understanding the underlying mechanisms of tumor heterogeneity, metastasis, and treatment resistance [3]. This knowledge may facilitate the development of combination therapy, including immunotherapy, to effectively treat this complex disease and improve quality and duration of life for people with lung cancer [4]. Although immunotherapy has the potential to significantly improve survival for patients with advanced non-small cell lung cancer (NSCLC), patients often do not respond well to these therapies at the beginning, and resistance develops early in treatment or over time [5]. Immune checkpoint inhibitors (ICIs), such as PD-1/PD-L1 compounds, have shown remarkable results in treating solid tumors [6], but their benefits are limited to a small number of patients [7]. To overcome this, researchers are investigating combining immunotherapy with radiation therapy [8]. This combination strategy aims to enhance the immune system's ability to fight cancer, potentially resulting in more effective and durable treatment prospects [9]. There are many approaches to treating cancer with drug therapy, including the use of durvalumab. The results of the PACIFIC trial show significant and long-term benefits for patients with stage III NSCLC who received durvabulum after chemoradiation, with significant overall survival and prolonged progression-free survival (PFS). These findings lay the groundwork for future studies aimed at identifying the most effective combinations of therapies [10,11].

Stereotactic body radiation therapy (SBRT), also known as stereotactical ablative radiotherapy (SABR), is becoming increasingly popular due to its ability to deliver highly focused high doses of radiation with fewer treatments [12]. This method has shown excellent local tumor control and manageable side effects, and high-dose radiation like SBRT stimulates the immune system more effectively than traditional radiation therapy [13].

Numerous studies have shown that low-dose radiation therapy, defined as less than 2 Gy per fraction, can alter the tumor's microenvironment effectively. This change transforms the stroma from immunosuppressing to immunostimulating, enhancing the efficacy of immunotherapy [14].

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Interestingly, high- and low-doses appear to complement each other when combined, suggesting potential synergistic benefits [15].

The combined use of immunotherapy and radiotherapy triggers a chain reaction that leads to tumor destruction. This process involves:

1) Tumor cell death: Radiation causes tumor cells to die through apoptosis (programmed cell death).

2) Tumor vasculature normalization: Radiation helps to normalize the abnormal blood vessels within the tumor, improving the oxygen supply and facilitating immune cell access.

3) Immune activation: The dying tumor cells release apoptotic bodies, danger signals, and tumor-associated antigens (TAA), as well as inflammatory cytokines. These signals activate dendritic cells and other antigen presenting cells (APC).

4) T cell stimulation: Activated DCs and APCs migrate to lymph nodes and present TAAs, stimulating the production of specific T-cells.

5) Tumor attack: These activated T cells target both the primary tumor in the radiation field and distant tumors [16].

Immunotherapy further enhances this process by activating the immune microenvironment. This combined approach creates a more effective immune response to cancer.

2. The Vital Role of T-Cells in the Treatment of Lung Cancer

As is known, the immune system's response to tumor cells is important, but it does not guarantee an adequate and effective antitumor response. Today, understanding the very nature of the complex cascade of interactions between the immune system and the tumor itself is an important issue in clinical research in the field of oncology. Following the approval of Nivolumab (2018) and Pembrolizumab (2019) for treatment of NSCLC patients, clinical results have been obtained indicating significant improvement in many patient conditions, although some patients did not respond positively to this treatment [17].

To improve existing immune cell therapies and develop new treatment methods for patients with oncological pathology, it is crucial to understand how immune responses occur and are formed. Once this is understood, we can apply comprehensive treatments to cancer patients [18].

Due to the ability of T-lymphocytes to destroy cells based on specific antigens, the immune system has become considered as an opportunity to use it in the fight against cancer. The acquired knowledge of the molecular and cellular processes of blood lymphocytes contributed to the emergence of new treatment directions in the field of oncology and the development of cellular vaccines based on the blocking of control points.

There is a high need for effective and low-toxic methods for treating lung cancer that require the development of novel methods using cell therapy.

The role of CD8+ T-cells in the immune response is currently well understood, while the function of CD4+ helper T-cells in this context is less well known. However, more and more evidence suggests that CD4+ positive T-cells still play a key role in the treatment of lung cancer, while a higher number of tumor-infiltrating CD4+ T-cells correlates with improved patient survival [18]. At the same time, positive CD4 cells are essential for the "presentation" of DCs by transmitting specific signals to CD8 cells, which allows them to recognize tumor cells and attack them. It is this process that allows the cross-priming of positive CD8+ T- cells, thereby increasing the ability to recognize tumor cells, attack them, and most importantly, develop long-term memory of positive CD8+ T-cells [19]. Recent studies have shown that these positive cells also play an important role in recognizing mutated tumor antigens and leading to mutations in NSCLC tumors.. This confirms that positive CD4+ T-lymphocytes play an important role in activating the immune response to tumor cells and can be considered a new direction for the development and use of immunotherapeutic cell products in the treatment of tumors [20].

Previous studies have been based on the ability of positive CD8+ T-cells to recognize tumor cells through MHC class 1 receptors and directly destroy them. However, as a result of numerous studies in this field, it has become known that the effectiveness of positive CD8+ T-lymphocytes largely

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depends on CD4+ T-cells. These interactions involve APC and are heavily dependent on interleukin-2 (IL-2) signaling.

Positive CD4+ T-lymphocytes play an important role in increasing the activity of CD8+ T-cells in the following ways:

1) Enhance effector functions: positive CD4+ T-lymphocytes help CD8+ T-cells become more effective at killing tumor cells [21].

2) Stimulate proliferation: it is positive CD4+ T-lymphocytes that stimulate the growth and reproduction of CD8+ T-cells.

3) Attracting to the location of tumor tissue: positive CD4+ T-lymphocytes help to attract CD8+ T cells to the tumor, thereby increasing the concentration of antitumor immune cells [22]. This demonstrates the importance of positive CD4 + T - blood lymphocytes in maximizing the effectiveness of CD8 +T-cell-based therapy and highlights the significance of interaction between these two types of T cells for developing the most optimal treatment strategies [23].

Currently, adoptive T-cell therapy (ACT) is used to multiply and inject natural T- lymphocytes that react with tumor tissue ex vivo in patients with lymphodispersion to destroy tumor cells by enhancing the T-cell link of the immune response. Adoptive T-cell therapy is divided into (i) isolation of natural tumor-reactive T-cells from existing tumor tissues or blood and (ii) genetic modification of T-cells to give them specific recognition from tumor cells [24].

While adaptive transfer of tumor-infiltrating lymphocytes (TILs) has shown promising preclinical results, clinical trials have yielded uneven results, with rare exceptions [25]. However, a recent study conducted by the National Cancer Institute suggests a potential solution: preparing a patient for chemotherapy beforehand before starting TIL therapy, which can significantly increase the efficacy of the treatment and lead to improved patient outcomes [26].

Since T-lymphocytes play a crucial role in the development and progression of tumors, this makes them the most important targets for the treatment of oncological processes. T-cell-based immunotherapy is a promising way to develop additional approaches to the traditional treatment of lung cancer. Further research is needed to assess the full potential of immune cell therapy methods and improve clinical outcomes for cancer treatment [27].

Positive CD4+ T-lymphocytes are important components of the adaptive immune system, working alongside with positive CD8+ cytotoxic T-lymphocytes. The ability of CD4+ T-lymphocytes to differentiate into various specialized subtypes, each of which plays a crucial role in supporting other immune cells, leads to the strengthening of antitumor immunity in several ways:

- Enhance positive CD8+ CTL and antibody response.

- Secreting effector cytokines such as interferon-γ (IFNy) and tumor necrosis factor-α (TNFa), which activate, direct and regulate the immune response.

- Direct cytotoxicity when CD4+ T-lymphocytes destroy tumor cells, which makes them attractive targets for the development of immuno-therapy methods [27,28]. Attempts to use positive CD4+ T-cells against tumors included vaccination with epitopes with peptides designed to stimulate the production of specialized CD4+ T-lymphocytes. These peptides were often derived from highly immunogenic tumor-associated antigens, such as representatives of the testicular cancer antigen family, such as NY-ESO1 [28].

Many studies have focused on measuring the increased production of tumor-specific T-cell cytokines, in particular IFNy, as one of the markers of enhanced CD4+ antitumor reactions. This approach was aimed at understanding and measuring the effectiveness of cellular vaccination in stimulating positive CD4+ T-cells to fight tumors [29].

3. Expanded Activated Autologous Lymphocyte Therapy

Expanded activated autologous lymphocyte (EAAL) therapy is a form of adoptive cell therapy that harnesses the patient's own immune components, specifically cytotoxic T cells and NK cells, to fight cancer. The study involved 19 patients with metastatic tumors who received EAAL treatment. Within two to four weeks of the treatment, the concentration of CD3+, CD8+, and CD56+ cells in the blood increased significantly compared to pre-treatment levels, suggesting that the EAAL effectively

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mobilized these immune cells to enhance the body's anti-cancer response. After EAAL infusion, there was also an increase in the number of IFN-producing cells, a critical cytokine for tumor immunity. This increase was particularly pronounced in both CD3+IFN+ lymphocytes (p=0.006) and CD3-IFN+ cells (p= 0.015), indicating that EAAL therapy enhances the capacity of T-cells and NK cells to produce this essential immune-regulating agent.

Furthermore, there was a notable increase in the proportion of IFN-producing cells within the CD3+, CD8+ and CD3 cohorts following infusion. This suggests that EAA treatment effectively boosted lymphocyte counts in peripheral blood, increasing the population capable of targeting and eliminating tumor cells [30].

This treatment regimen involved administering patients with their own mononuclear leukocytes. These are a specific type of white blood cells that have been treated with sodium periodate (IO4-) and then cultivated in a medium enriched with human recombinant IL-2. Subsequently, patients underwent regular low-dose IL-2 infusions every five days for two cycles, each lasting three weeks. This intervention resulted in various beneficial changes in the immune system, including an increase in markers associated with natural killer (NK) cells. A significant finding was the rise in the number of peripheral blood mononuclear cells expressing the surface marker Leu 11, indicating an enhancement in functional capabilities of NK cells.

Enhanced NK and Antibody-Dependent Cytotoxicity: The ability of NK cells to directly destroy target cells (NK-mediated cytotoxicity) and collaborate with antibodies (antibody-dependent cell-mediated cytotoxic) has been enhanced. A slight increase in spontaneous killing was noted, indicating a potential general activation of the immune system and an increased ability of immune cells to engage non-NK target cells.

These findings suggest that this treatment approach may strengthen the overall immune response, particularly with regard to NK cells. This allows for more efficient targeting and elimination of cancer cells. Notably, six patients experienced a reduction in their metastatic tumors by over 50%, affecting the lungs, liver, bones or soft tissues. It is important to note that none of the patients exhibited significant fluid retention or required extensive medical attention. Of the five patients who responded, four experienced relapse after an average of 5.2 months (with a standard deviation of one month) [31].

Although conventional treatments such as surgery, chemotherapy, and radiation are often used in cancer care, they can be limited in their effectiveness, particularly in advanced stages of the disease. Adoptive immunotherapy, on the other hand, which uses the patient's own immune cells activated with IL-2, has shown promising results for more advanced cancer cases.

A study conducted by Xie et al. (2019) investigated the safety and effectiveness of an immunotherapy approach using highly activated natural killer (NK) cells in 13 people with late-stage lung cancer. The study involved harvesting NK cells from the patients' blood, cultivating them for 12 days, and then reintroducing them into the patients' bodies through intravenous infusion.

To assess the impact of the treatment, the researchers evaluated various parameters both before and after the final infusion. This allowed them to monitor the patients' response to the therapy and determine its effectiveness. The study examined various immune cell types, levels of immune signaling proteins, carcinoembryonic antigen (CEA), thymidine kinase 1. The research focused on cytokine-induced killer (CIK) cells, which are developed from blood and culture with recombinant IL-2. The CD3+CD56+ subset is considered as more effective against tumors due to its enhanced ability to target and destroy cancer cells. The presence of CD56 on these cells, which is a marker that may be related to cancer cell interactions, highlights the significance of these findings [31,32].

After a three-month follow-up, about 85% patients maintained their stable condition, indicating that their cancer had not progressed. Conversely, about 15% patients showed signs of disease progression. The level of IFN increased significantly after treatment, while the level of carcinoembryonic antigen (CEA) decreased, which may indicate a reduction in cancer activity. Overall, the immune function of patients undergoing NK cell therapy remained stable [32]. The findings indicated that CIK cell therapy can enhance the effectiveness of conventional chemotherapy. Specifically, this treatment not only significantly improved immune function but also increased the

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total count of effector cells, which are responsible for directly attacking cancer cells, all without causing any major adverse effects [34].

A phase II clinical trial conducted by Li et al. showed the potential of CIK immunotherapy to augment the efficacy of standard chemotherapy in individuals diagnosed with NSCLC [35]. Their investigation has revealed important connections between CIK immunotherapy and several elements that might influence its effectiveness. Research by Chen et al. showed that the expression levels of MHC class I-related chain A (MICA) in patients with gastric cancer correlated with their responses to CIK treatment. Patients with higher MICA levels were more likely to experience positive outcomes from this therapy. A phase II/III study demonstrated that the combination of radiofrequency ablation and CIK therapy was both safe and effective for individuals suffering from colorectal liver metastases (CRLM). Several clinical studies have explored the effectiveness of CIK and DC-CIK cell therapies in lung cancer. Overall the findings consistently support the effectiveness of these treatments for particular form of cancer [35,36].

These outcomes highlight the potential of CIK immunotherapy as a promising alternative for treating cancer, particularly lung cancer. Further research is needed to thoroughly understand the mechanisms behind its effectiveness and evaluate its role in personalized medicine strategies [37,38]. In a study by Guo-Qing Zhang and his team (2015), the successful use of EAAL for the treatment of small cell lung cancer was described. This research comprised 32 patients diagnosed with SCLC, categorized into two separate groups: EAAL and a control group. The procedure for obtaining EAALs included several key steps. Blood was collected in the volume ranging from 20 mL to 100 mL with peripheral blood mononuclear cells (PBMC) isolated using Ficoll-hypaque gravity centrifugation technique. The isolated PBMCs cultured fourteen days in specific medium (IMSF-10) with cytokine IL-2 that facilitates proliferation and activation of immune cells. This method resulted in an increased population of EAAL. Before administering the EAALs to patients, distinct types and characteristics were identified through characterization [39]. Following the cultivation and in vitro expansion, a substantial increase ($p<0.01$) was noted in the percentages of CD3+, CD3+CD8+, CD45RO+, CD28+, CD29+, CD8+CD28+, and CD3+CD16+/CD56+ cells. In contrast, there was a significant reduction in the percentages of CD19+, CD3+CD4+, CD45RA+, CD4+CD25+, CD4+CD29+, and CD3-CD16+/CD56+ (NK cells). After the in vitro culture and expansion, notable rises ($p<0.05$) were observed in the percentages of CD3+ (T-cells), CD3+CD8+ (cytotoxic T-cells), CD45RO+ (memory T-cells), CD28+ (co-stimulatory receptor T-cells), CD29+ (integrin for cell adhesion), CD8+CD28+ (co-stimulatory receptor cytotoxic T-cells), and CD3+CD16+/CD56+ (T-cells showing NK cell markers). The Kaplan-Meier analysis of survival demonstrated that the median survival time was extended in EAAL-treated patients compared to controls, although the difference was not statistically significant. However, 1-, 2- and 3-year survival rates were significantly higher in the EAAL group than in controls ($p>0.05$), suggesting that EAAL may contribute to enhanced survival in SCLC patients. Additional analysis using multivariate Cox regression revealed that the number of chemotherapy sessions and the use of EAAL immunotherapy are both independent factors predicting survival in patients with SCLC. This implies that both factors have a significant effect on survival, even when accounting for other variables. The study demonstrated the effectiveness and safety of in vitro expansion and induction of EAALs. These findings strongly suggest that adoptive immunotherapy using EAALs can significantly prolong overall survival in SCLC patients [38,39]. A larger investigation with increased sample size is necessary to conclusively verify these findings [38].

The principal challenges in the clinical use of antigen-specific T-cells include expanding these cells to obtain a sufficient quantity, while minimizing the presence of regulatory T cells, preventing a reduction in their function, and determining their role and position in combined tumor immunotherapy. Moreover, there is a need to establish the most efficient protocols for their deployment. To encourage effective proliferation of antigen specific T cells, various researchers use different cytokines and their combinations.

Klebanoff C.A. et al. demonstrated that in addition to the traditionally employed IL-2 for activating adoptively transferred T lymphocytes, IL-21 and «homeostatic cytokines» like IL-7 and IL-15 should also be used because of their ability to encourage the maturation of memory T-cells [40].

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Sennikov S.V. et al. explored the activity of antigen-specific T-cells (both cytotoxic T lymphocytes (CTL) and helper T-cells (Th) and discovered that commonly used modulators designed to boost cytotoxic anti-tumor responses, such as pro-inflammatory cytokines (IL-12 and IL-18), IDO inhibitors (L-methyl-1-tryptophan), and COX-2 inhibitors (celecoxib), did not significantly influence the cytotoxic activity of these cells.

Nonetheless, the analysis of cytotoxic factors (Fas, TRAIL, perforin, granzyme B) and cytokine secretion showed a significant rise in TRAIL-positive CD8+ lymphocytes and cells producing interferon-γ (in non-small cell lung cancer). Simultaneously, there was no stimulation of IL-4 and IL-10 production. These observations suggest a dominant activation of the Th1 immune cellular response, implying that these modulators may be more effective in steering the immune response towards a Th1-dominant profile, which is generally perceived as favorable for anti-tumor immunity [41].

The same study identified that epitope-specific lymphocytes isolated via magnetic separation and cultured with a cytokine mixture exhibited notably higher cytotoxic activity against tumor cells compared to activated mononuclear cell cultures and DCs. The research used mouse splenocyte, specifically CD8+ T-cell, which were programmed to recognize OVA257-264 epitopes presented by MHC-I. The researchers successfully developed a rapid ex vivo expansion method followed by cryopreservation to obtain and store antigen-specific T cells. This approach maintains cell viability and cytokine production, as well as the capacity of T cells to differentiate, migrate, and infiltrate tumors, leading to tumor regression [42,43]. Cryopreservation does not notably impair the key properties of cytotoxic T lymphocytes (CTLs), despite some studies suggesting otherwise (Galeano Niño et al., 2014) [42]. This findings supports the use of cryopreservation as a viable technique.

Most studies using T-cell activation show that CD8+ T-cells actively multiply and become the predominant cell type (on average >60%) during the culture process, demonstrating increased NK cell activity and reduced numbers of regulatory T-cells that inhibit tumor immunity. The infused activated CD8+ T-cells can help enhance the suppressive and cytotoxic actions of the patient's immune system through repeated infusions. This indicates that repeated infusions can build and strengthen the immune response. Additionally, the infused adoptive T-cells, including central memory CD8+ T-cells, can persist and accumulate in the body over time. This results in an increase in the total quantity of CD8+ T-cells and a reversal of the CD4:CD8 ratio in peripheral blood. This occurrence has also been observed in other clinical studies, indicating that adoptive T-cell therapy can have lasting effects on the immune system [44].

Research has shown that T-cells isolated from patients in the early stages of the disease often exhibit a stronger anti-tumor response compared to those isolated after successful treatment [44]. This suggests that the tumor microenvironment and the advancement of the disease can impact the effectiveness of T-cells. Possible explanations for this phenomenon include:

-T-cell exhaustion or anergy: T-cells can become exhausted or unresponsive due to prolonged exposure to tumor antigens.

-Influence of the tumor microenvironment: The tumor microenvironment can suppress T-cell function, particularly through the presence of regulatory T-cells (Tregs).

These findings could explain the limited success of lymphokine-activated killer (LAK) and tumor-infiltrating lymphocyte (TIL) therapy, as higher levels of Tregs have been observed during cultivation and in TILs. However, the role of Tregs in these therapies needs further exploration [45].

In the work of Chikilev I.O. and co-authorsLAK cells remain at the baseline level, despite the increase in the content of T-reg during long-term cultivation ex vivo. Whereas in other works a decrease in the functional activity of T-lymphocytes was demonstrated against the background of cultivation with IL-2, since IL-2 accelerates the aging process and the formation of the T-regulatory immunophenotype [46]. Some authors propose a method of immunomagnetic separation, which promotes the acceleration of tumor regression, due to the elimination or selective inhibition of T-reg [47]. At the same time, in addition to the T-reg factor, other factors also take place when introducing them into the body, in reducing the activity of lymphocytes activated ex vivo. One of them is cytokine-induced killer cells (CIK), obtained by culturing in vitro peripheral blood mononuclear cells

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(PBMC) with specific cytokines. As a result, a wide range of cells appears, most of which express CD3 and CD56 markers, the so-called natural killer T-cells (NKT). These NKT-cells have a unique ability aimed at cell proliferation and cytolysis, thereby bypassing the major histocompatibility complex (MHC), they can recognize and kill target cells [48].

In the work of Jinying Zhang et al., the use of a combination of chemotherapy with adoptive immunotherapy with autologous CIK cells (the CIK treatment group) in a group of patients with lung cancer (n=60) was demonstrated, while the second control group (n=60) received only chemotherapy. The primary objective was to evaluate progression-free survival and overall survival in both groups. In the in vitro incubation study, after 14 days, CIK was significantly higher in the study group by the percentage of CD3+, CD3+CD8+, CD3+CD56+ and CD3-CD56+ cells ($P < 0.05$), demonstrating the high potential of this immune cell population. Moreover, these data were correlated with clinical symptoms in 60 patients in the CIK treatment group towards improvement. No adverse events or serious toxicities were observed during the study when using autologous CIK cells in combination with chemotherapy.

The group where patients received a combination of CIK and chemotherapy demonstrated significantly better survival rates compared with the control group. According to the study results, the median progression-free survival at 3 years and 5 years was 44.7% and 26.8% in the CIK group compared with the control group and was significantly higher, and the median overall survival at 3 years and 5 years was 74% and 62% in the CIK group, which was also higher than in the control group ($P < 0.014$). These results indicate that the use of autologous CIK cells in combination with chemotherapy can improve the response to treatment, improve progression-free survival and overall survival in patients with lung cancer [49].

Cellular immunotherapy for CIK typically uses similar methods to extract these cells. Autologous CIK cells are obtained from peripheral blood by activating and expanding the patient's own PBMCs ex vivo and then reintroducing them back into the patient. CIK cells, often referred to as NKT cells, can be greatly amplified, 200- to 1000-fold, within 14-21 days of culture. This growth is achieved by initially stimulating the cells with CD3 antibodies and various combinations of cytokines. Ex vivo expanded CIK cells are characterized by the expression of CD3 and CD56 markers. They exhibit potent cytotoxic activity against various tumor cell lines and animal models bearing the tumor antigen [50]. There are several clinical studies shown that combining CIK immunotherapy with chemotherapy offers potential benefits over chemotherapy alone in patients with advanced NSCLC [51]. Immunotherapy represents a promising avenue for cancer treatment by selectively targeting and killing cancer cells while leaving healthy cells and tissues unharmed. Recent advances in clinical trials have fueled enthusiasm for combining adoptive cellular immunotherapy with traditional treatments to achieve potent, effective, and long-lasting clinical responses [51].

In addition, many studies have described the effect of immune cell therapy on reducing the resistance of patients' tumor cells to chemotherapy. The combination of chemoimmunotherapy has the potential to mitigate the side effects of traditional chemotherapy, improve the patient's quality of life, and prolong the survival of patients with advanced NSCLC.

In a study by Runbo Zhong et al., fourteen patients received a combination of norelbine-platinum chemotherapy followed by vaccination with autologous CEA peptide-treated DC and CIK cells. These vaccines were administered every 30 days for four cycles. The study assessed the side effects of therapy, time to disease progression, and overall survival in both the chemoimmunotherapy group and the control group that received chemotherapy alone.

The study showed that chemoimmunotherapy was well tolerated with mild side effects. Allergic reactions (rash, itching) were more common in the chemoimmunotherapy group (64.2%) compared to the chemotherapy group (7.1%), but this difference was statistically significant ($p = 0.004$). Fever without infection was more common in the chemoimmunotherapy group compared to the chemotherapy group (71.4% vs. 21.4%, $p = 0.02$). Grade 3/4 fatigue was less common in patients receiving chemoimmunotherapy (7.1%) compared to the chemotherapy group (57.1%), $p = 0.01$ [52].

Researchers from the First Affiliated Hospital of Zhengzhou University, led by Jianming Huang and colleagues, conducted a study to investigate the use of cytokine-induced killer (CIK) cells in

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patients with advanced small cell lung cancer. Study participants were randomly divided into two groups: a combination treatment group that received chemotherapy plus CIK cell transfusion, and a control group that received chemotherapy alone. Short-term outcomes, overall survival, progression-free survival (PFS), and treatment-related adverse events were analyzed retrospectively. The results showed a significantly higher overall response rate in the combination treatment group (40.9%) compared to the control group (9.1%) ($p = 0.0339$). This indicates that the addition of CIK cell therapy to chemotherapy significantly improved the treatment response in patients with advanced small cell lung cancer. The study also showed that progression-free survival was significantly longer in the combination treatment group (8 months) compared to the control group (4 months) ($p = 0.005$). Importantly, no serious adverse events were observed after CIK cell infusion. These results strongly suggest that the combination of chemotherapy with CIK cell immunotherapy may be a safe and effective treatment option for patients with advanced small cell lung cancer (SCLC) [53].

In a phase II clinical study led by Li R. et al., the use of autologous immunotherapy with CIK cells was found to enhance the effectiveness of standard chemotherapy in patients suffering from advanced NSCLC [53]. The stimulation of CIK cells by DCs significantly increased the antitumor effects, and the integration of chemotherapy with CIK/DC treatment led to improved clinical results for individuals with advanced NSCLC [54]. CIK cells are produced from peripheral lymphocytes using a combination of cytokines, which includes CD3 monoclonal antibodies, IL-2, and IFN-γ [55]. Moreover, pairing CIK cells with endostatin or DC-based cancer vaccines might demonstrate a synergistic benefit in enhancing clinical outcomes. In recent years, CIK cells have been widely employed as an immunotherapeutic approach for various malignancies due to their strong expansion and cytotoxic functions, especially when activated by DCs [56].

4. Adoptive Immunotherapy of Cancer Using Tumor-Infiltrating Lymphocytes

In 1987, Kradin and colleagues conducted a novel research effort, introducing adoptive immunotherapy using T-lymphocytes that were cultured for extended periods of time and depended on IL-2. These T-cells were administered to individuals suffering from metastatic lung adenocarcinoma, having been extracted from cancerous tissue explants and cultivated in a medium enriched with recombinant IL-2. The T lymphocytes displayed activation markers and exhibited the capacity to target and eliminate a wide range of tumors. Upon intravenous injection, indium-tagged T-cell blasts mainly concentrated in the lungs, liver, and spleen. Although external imaging revealed a low count of infused lymphocytes at tumor locations, five out of seven patients noted a decrease in their cancer. However, none of the patients experienced a reduction exceeding 50% of their overall tumor volume. Furthermore, three patients showed increased delayed hypersensitivity reactions to protein antigens after therapy. This suggests that tumor-derived T-cells, when cultured for a long time, can be safely injected into human patients and potentially enhance immune responses, helping to reduce tumors in vivo [57].

The ex vivo expansion and reinfusion of tumor-infiltrating leukocytes (TILs) have been effectively implemented for treating various cancers, usually involving IL-2 for TIL expansion and subsequent support of these cells after reinfusion. Despite this, the use of IL-2 poses challenges due to its inclination to preferentially amplify regulatory T-cells and myeloid cells, along with its systemic side effects [58]. Furthermore, a study evaluated the efficacy and safety of lifileucel (LN-145), a cell therapy based on autologous tumor-infiltrating lymphocytes, for patients with metastatic non-small cell lung cancer (mNSCLC) who had shown progression after prior immunotherapy. The cell product utilized in this research was derived from tumor tissues, mainly from lung specimens across various anatomical regions. The treatment showed efficacy against tumors that are often resistant to immunotherapy, including those lacking PD-L1 expression, with low mutational burdens, and harboring the STK11 mutation. Adverse effects were mostly predictable, two patients succumbed to treatment-related complications: cardiac failure and multi-organ failure. Nevertheless, lifileucel exhibits promise as a viable therapy for patients with mNSCLC who have not responded to prior treatments [59].

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The presence of tumor-infiltrating lymphocytes (TILs) has been found to correlate positively with lung cancer prognosis, suggesting that lung cancer is an ideal candidate for adoptive TIL therapy [60]. This approach entails the extraction and expansion of tumor-specific lymphocyte populations in vitro. Recent studies illustrate the potential of TIL-based therapies. A 2021 phase 1 trial evaluated the safety and initial efficacy of TIL therapy combined with nivolumab in late-stage NSCLC patients who had previously undergone nivolumab monotherapy. The results showed that most participants experienced confirmed clinical responses.

Patients received lymphodepletion therapy using cyclophosphamide and fludarabine, followed by TIL adoptive cell transfer (ACT) alongside IL-2, and subsequently underwent maintenance therapy with nivolumab. Results demonstrated that a significant number of patients had confirmed clinical responses. TILs showed longer engraftment when compared to those infused into untreated patients, and the level of engraftment correlated with clinical outcomes. It was notably evident that concurrent host immunosuppression plays a vital role; only 34% of patients with melanoma who had TIL infusion and high-dose IL-2 without prior lymphodepleting chemotherapy achieved objective clinical responses in earlier trials, prior to implementing host lymphodepletion. Many of these responses were temporary, with a lack of sustained persistence of the transferred cells noted in those studies [61].

5. Lymphokine-Activated Killer Cells

Regarding lymphokine-activated killer (LAK) cells, which can non-specifically target both autologous and allogeneic tumor cells, early attempts showed promising results in treating advanced cancers. A study involving 121 patients with malignant effusions due to advanced lung cancer indicated that the combination of autologous LAK cells and recombinant IL-2 resolved effusions in 58.6% of cases and significantly reduced them in 36.2%. Nevertheless, high IL-2 doses pose challenges due to serious side effects such as capillary leak syndrome, which can complicate LAK cell therapy in clinical practice [62].

A clinical trial involving 105 patients who underwent surgery for incurable primary lung cancer randomly assigned patients to two groups. The group receiving a combination of recombinant rIL-2 and LAK cells, in addition to radiation or chemotherapy, exhibited improved 7-year survival compared to the control group receiving radiation or chemotherapy alone [63]. Similar results were observed in a randomized phase III study [64].

Despite these promising findings, the use of LAK cells in clinical settings faces a significant hurdle: the high doses of IL-2 often lead to serious side effects such as capillary leak syndrome. This syndrome can cause hypotension, oliguria (reduced urine output), pulmonary edema, and dyspnea (difficulty breathing), making it a major obstacle in the development of LAK cells for clinical use.

The LAK cells exhibit remarkable cytotoxicity against cancerous cells in vitro, yet clinical trials have yielded disappointing results, with only a handful of exceptions. The key point of for their inability to exhibit cytolytic activity is their lack of specificity, which prevents LAK cells from selectively accumulating in tumor tissue.

Kimura et al. (1997) demonstrated effectiveness of adjuvant LAK cells/IL-2 adoptive immunotherapy in combination with chemoradiotherapy for 174 patients with stages I–IV NSCLC [64]. This prospective, randomized, phase III clinical study showed a significant improvement in patient survival among those receiving adjuvant IL-2 LAK immunotherapy. The five- and nine-year overall survival rates were 54% and 52% respectively, compared to 33% and 24% in the control group. The subgroup analysis revealed statistically significant differences in the five-year survival rates, favoring immunotherapy among patients who underwent curative resection. Specifically, the survival rates were 66% for those who received curative resection and 41% for those who did not. Additionally, there were differences in survival rates among patients with different types of cancer: 48% for patients with adenocarcinoma and 23% without; and 62% for patients with squamous cell carcinoma and 35% without [65,66].

Zhang et al. (2014) assessed the efficacy of adoptive transfer of NK and NKT mixed effector cells in patients with NSCLC [66]. NKT mixed effector cells were generated by expanding PBMCs ex vivo

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and then phenotypically characterized. The analysis revealed 1.7-times longer overall survival as an outcome measure compared to a control group (31.1 months vs. 18.1 months, $p = 0.008$), indicating a 43.8% reduction in the risk of death. The same significant trend was seen in two-year survival rate in the immunotherapy group (62.95% vs. 35.44%, $p < 0.05$). Different independent prognostic factors for patients with NSCLC was revealed in this study. They include clinical stage, gender, the use of tyrosine kinase inhibitors, number of chemotherapy cycles, and as can be predicted, application of immunotherapy which highlights the promising potential of NKT immunotherapy as a treatment option for patients with NSCLC [66,67].

In conclusion, based on a review of the literature, it can be stated that autologous lymphocytes appear to be the most promising for use in adoptive immunotherapy for lung cancer. Hideki Kimura's experiments showed that freshly isolated lymphocytes did not display cytotoxicity towards autologous tumour cells over time. However, when cultured in the presence of T-cell growth factor, IL-2, the lymphocytes became cytotoxic towards autologous tumour cells starting on the third day of culture, with peak cytotoxicity occurring on the seventh day. A comparative study of the effects of IL-2 and phytohaemagglutinin (PHA) revealed that while lymphocytes exposed to PHA exhibited significant cytotoxicity, those exposed to IL-2 demonstrated significantly higher activity [67].

A comprehensive review of clinical trials examining the use of autologous lymphocytes in cancer treatment reveals a consistent trend towards improved overall patient survival among those receiving this approach. In particular, a study conducted by Guo-Qing Zhang et al. highlights the significance of both the number of chemotherapy cycles administered and the implementation of EAAL immunotherapy as independent factors contributing to longer survival rates among patients with SCLC. Through the application of Cox multivariate regression analysis, this study identified a hazard ratio (HR) value of 2.801 for the number of chemotherapy cycles (95% confidence interval: 1.157–6.783), indicating that patients undergoing more than six cycles of chemotherapy exhibited significantly higher survival rates compared to those receiving six or fewer cycles. Similarly, an HR value of 3.278 was observed for EAAL immunotherapy (95% CI: 1.415–7.592), indicating a higher likelihood of prolonged survival for patients who received EAAL treatment compared to those in the control group. These findings underscore the promising prospects of autologous lymphocyte infusion immunotherapy, in enhancing survival outcomes for patients with SCLC. The results of the subgroup analysis revealed that the overall survival (OS) of patients in the female subgroup and those receiving chemotherapy for ≤6 cycles could be significantly prolonged after EAAL cellular immunotherapy ($p < 0.05$). Additionally, the OS of other subgroups also showed an improvement after EAAL, although it was not statistically significant ($p > 0.05$), as reported in the study [68].

6. Discussion

The treatment of NSCLC poses significant challenges due to the complexity of the disease and its heterogeneous nature. In light of the dismal five-year survival rate for lung cancer of 15–16%, there is an urgent need for innovative treatments. Cancer remains a leading cause of mortality and a major global public health concern, projected to continue as a significant contributor to morbidity and mortality in the decades to come. Bray et al. have estimated that the number of new cancer cases worldwide will rise to 22.2 million by the year 2030 [68]. Recent advances in immunotherapy have highlighted the potential of harnessing the body's own immune system to combat cancer. Adoptive immunotherapy, whether used as an adjunct or a standalone treatment, holds significant promise for addressing a wide range of malignant tumors [68,69]. This article explores the prospects of combined treatment strategies utilizing autologous activated lymphocytes, a promising strategy aimed at enhancing therapeutic efficacy and improving clinical outcomes.

Autologous activated lymphocytes are characterized by their ability to elicit robust anti-tumor immune responses. By isolating and subsequently activating a patient's own lymphocytes, it is possible to augment their capacity to recognize and target neoplastic cells. This personalized approach mitigates the risk of immune rejection while allowing for tailored interventions that are attuned to the unique tumor microenvironment of individual patients.

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Preliminary clinical studies have reported encouraging findings regarding response rates and overall survival in patients undergoing combined treatment regimens. Nevertheless, challenges persist, particularly with regard to the variability in patient responses and the need for optimized methodologies for the activation and expansion of lymphocytes. Additionally, the timing and sequencing of these therapies warrant further investigation to maximize therapeutic benefits.

The effector cells activated by cytokines are considered as an ideal candidate for cancer immunotherapy, including LAK cells, activated NK cells, DCs, activated lymphocytes, TILs, and CIKs, exhibit antitumor activity in various contexts. However, most methods for ex vivo expansion have proven challenging, and data supporting prolonged survival remain scarce. The article summarizes the factors that influence cell-based immunotherapies and outlines the strategies employed. Analysis of the clinical benefits of autologous lymphocyte treatment has revealed that T-cells through cell-to-cell interactions and cytokine activity influence on the initiation, progression, and metastasis of lung cancer, enhancing the overall survival rate of patients.

Lymphocyte apheresis, while a promising technique, faces several challenges. Patients undergoing apheresis are often in a weakened state, and their T-cells, being mature and differentiated, may exhibit reduced growth rates. This poses a significant hurdle for ex vivo expansion protocols, as these mature cells may not respond well to the expansion process. Additionally, damaged or weakened T-cells contribute to a lower quality cell population compared to healthy cells. In addition, tumor cells have multiple ways to evade the immune system. The tumor is heterogeneous and variable during its development. The interactions between cancer cells and immune cells not only create an immunosuppressive microenvironment around the tumor but also create a systemic effect, which reduces the effectiveness of immunotherapy [70,71].

Transfusing an adequate number of lymphocytes that can identify and eliminate tumor cells provides a strong foundation for effective adoptive cell therapy [72]. Earlier research conducted by the authors demonstrated that T-cells from tumor-free hosts can enhance antitumor immunity and alter the harmful balance between tumor cells and the host. Recently, cell therapy based on various types of a cancer patient's own cells has become an important additional treatment option after surgery, chemotherapy and radiotherapy However, the high effect of immunotherapy is achieved through its complex use with generally accepted treatment methods [73]. Nonetheless, utilizing multiple immune cell types has encountered considerable challenges, such as low efficiency and issues with cell expansion. One of the advantages of using autologous activated lymphocytes is that it eliminates ethical issues, since the effector cells are obtained by the usual method of collecting blood from the cubital vein or by apheresis technology [74].

Immunotherapy based on activated autologous lymphocytes has advanced significantly in the last 15 years, driven by studies involving various types of interleukins, LAK cells, tumor-infiltrating lymphocytes (TILs), and mixed lymphocyte-tumor culture (MLTC)-sensitized cytotoxic T lymphocytes in clinical trials. Although the overall tumor shrinkage response rate has been relatively modest at 9%, locoregional administration of TILs into malignant effusions has proven highly effective, achieving 77% shrinkage or elimination of these effusions, even in severely ill patients. This has resulted in improved quality of life for these individuals [75].

A wide variety of cell types, such as NK cells, cytotoxic T lymphocytes, LAK cells, TILs, and activated macrophages, contribute to immune responses that assist in eliminating cancer cells. These cells interact with each other, and their functions are modulated by various cytokines. Further research into the preparation of these cells and their clinical implications could lead to significant advancements in immunotherapy for various types of cancer.

This review summarizes the application of autologous lymphocyte therapy in lung cancer treatment, concentrating on the methods of procurement, quality control, and infusion protocols used in nearly 2,000 patients. We identify potential research gaps and ultimately promote the wider adoption of immune cell therapy as a component of combination strategies for the treatment of lung cancer.

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7. Future Perspectives

The landscape of NSCLC treatment is evolving, particularly with the integration of immunotherapies and personalized medicine. The combined treatment utilizing autologous activated lymphocytes holds significant promise for improving outcomes in NSCLC patients.

Future studies may explore the synergistic effects of combining autologous activated lymphocytes with existing therapies, such as checkpoint inhibitors, targeted therapies, and traditional chemotherapy. By leveraging multiple mechanisms of action, these combination approaches could lead to improved tumor responses and extended survival rates. As more clinical trials are initiated to test various combinations and techniques involving autologous activated lymphocytes, accumulating real-world evidence will provide insights into the practicality and effectiveness of these treatments. Collaborative efforts among research institutions, pharmaceutical companies, and healthcare providers will be essential to validate findings and inform clinical practice.

Advancements in genomic profiling and biomarker identification will facilitate the customization of treatment regimens with personalized treatment protocols. By selecting patients based on specific tumor characteristics and immune profiles, clinicians can optimize the use of activated lymphocytes, potentially leading to more effective and less toxic therapies.

Ongoing innovations in cellular engineering, such as CRISPR and CAR-T cell technologies, may enhance the effectiveness of autologous lymphocyte treatments. By genetically modifying T-cells to better recognize and attack NSCLC cells, researchers could significantly improve patient outcomes.

Research into the tumor microenvironment will continue to be crucial. By understanding how NSCLC cells evade immune detection and how the microenvironment suppresses immune responses, future therapies can be designed to counteract these mechanisms, thereby boosting the efficacy of activated lymphocytes.

As the field progresses, navigating the regulatory landscape will be critical for the approval of new therapies. Moreover, cost-effectiveness analyses will help determine the feasibility of widespread adoption of these advanced treatments in clinical settings. Future developments will likely emphasize the importance of patient involvement in treatment decision-making. Educational initiatives aimed at informing patients about the benefits and risks of using autologous activated lymphocytes will empower them to participate actively in their care.

Overall, the combined treatment of NSCLC using autologous activated lymphocytes represents a promising frontier in oncology. As research progresses and technology advances, the potential to enhance treatment efficacy, personalize patient care, and improve outcomes will drive the development of this innovative therapeutic approach. Collaborative efforts across disciplines will be essential to realize the full potential of these strategies in the fight against lung cancer.

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