A REVIEW ON LIFILEUCEL TUMOR-INFILTRATING LYMPHOCYTE THERAPY FOR ADVANCED MELANOMA

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ABSTRACT: *Advanced melanoma remains a major therapeutic challenge despite significant advances in immunotherapy, largely due to resistance to immune checkpoint inhibitors such as anti–programmed death-1 antibodies. Tumour-infiltrating lymphocyte therapy has emerged as a promising adoptive cell-based immunotherapeutic approach that utilizes a patient's own tumour-reactive immune cells to overcome treatment resistance. Lifileucel, also known as AMTAGVI, is the first FDA-approved autologous tumour-infiltrating lymphocyte therapy for patients with unresectable or metastatic melanoma who have progressed following PD-1 blockade and, when applicable, BRAF and MEK inhibitor therapy.*

This review summarizes the development, historical evolution, mechanism of action, pharmacodynamics, dosage and administration, safety profile, and clinical efficacy of lifileucel in advanced melanoma.

Key words: Lifileucel, Amtagvi, TIL therapy, Immunotherapy, Melanoma.

1. INTRODUCTION

Cutaneous melanoma is a skin tumour caused by the uncontrolled proliferation and transformation of melanocytes in the skin's stratum basale.[1] Melanoma is the fastest-growing solid tumour worldwide.[2] Melanoma is a skin tumour caused by the transformation and uncontrolled multiplication of melanocytes in the stratum basale of the skin.[3]There are five forms of cutaneous melanoma: superficial spreading, lentigo, acral lentiginous, Amelanotic and nodular.[4]Immunotherapy proposes that rather than treating tumour cells, one should boost a patient's immune system to more effectively attack the tumour. This method has transformed melanoma therapy, resulting in

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exceptional rates of long-term disease control and survival in patients with metastatic disease. However, numerous problems and opportunities exist to improve this treatment strategy.

One of the most clinically significant current difficulties is managing patients who develop resistance to anti-PD-1 medication. The addition of a multikinase inhibitor to pembrolizumab has been investigated as a strategy for overcoming anti-PD-(L)1 resistance. The phase II LEAP-004 trial assessed the combination of lenvatinib and pembrolizumab in patients with melanoma who had progressed on PD-1/L1 inhibitors. [5] Originating from decades of pioneering research at the National Cancer Institute and further developed by Iovance Biotherapeutics, lifileucel received accelerated regulatory approval in February 2024. The therapy involves surgical tumour resection, ex vivo expansion and selective enrichment of tumour-derived lymphocytes, lymphodepleting chemotherapy, and subsequent reinfusion of the expanded cells with interleukin-2 support.

Clinical evidence from pivotal phase II trials demonstrates durable objective responses and meaningful tumour regression in heavily pretreated, immune checkpoint inhibitor–refractory melanoma patients. Adverse events are generally manageable and are primarily associated with lymphodepletion and cytokine administration. Ongoing clinical investigations are evaluating lifileucel in earlier disease settings, special populations, and combination strategies to further improve efficacy and tolerability.[6]

Given the limitations of existing immunotherapeutic strategies and the emerging evidence supporting TIL-based approaches, this study aims to evaluate the role of TIL therapy, with particular emphasis on lifileucel, in the management of advanced melanoma. By reviewing current clinical evidence, mechanisms of action, and therapeutic outcomes, this study seeks to assess the potential of lifileucel to improve treatment responses and contribute to the evolving immunotherapy landscape in melanoma.

2. OVERVIEW

Lifileucel is the outcome of years of adoptive cell therapy and tumour immunology research.[7]

Dr. Steven Rosenberg conducted groundbreaking research on TIL therapy as a cancer treatment in the 1980s.[8] Lifileucel was made by Iovance Bio Therapeutics, which licensed and further developed the TIL therapy for commercial use.[9,10]It has undergone

extensive clinical testing, with a focus on patients with metastatic melanoma who have not responded to immune checkpoint inhibitors or other treatments (such as anti-PD-1/PD-L1 therapy).Lifileucel has the potential to dramatically enhance patient outcomes.[11]It is an autologous T cell immunotherapy that has been expedited for the treatment of adult patients with metastatic or unresectable melanoma who have previously had PD1 blocking antibody treatment and, if BRAF V600 positive, a BRAF inhibitor alone or in combination with a MEK inhibitor. The speedy approval was revealed on February 16, 2024.[12]

3. DEVELOPMENT OF AMTAGAVI

Over the last three decades, the NCI's Surgery Branch, led by surgeon-scientist Steven Rosenberg, has pioneered and refined tumour infiltrating lymphocyte (TIL) therapy. In the late 1980s, his research demonstrated that TILs derived from a patient's tumour and grown with interleukin 2 may decrease metastatic melanoma.[13] NCI investigators subsequently enhanced the method by optimising production and adding lymphodepleting chemotherapy, laying the groundwork for lifileucel's. To facilitate translation, NCI signed a Cooperative Research and Development Agreement with Lion Biotechnologies (later Iovance Biotherapeutics) in 2011, which allowed for protocol and process transfer for multicenter trials. The NIH issued an exclusive patent license in 2015, with subsequent revisions broadening rights and providing non-exclusive access in line with public health aims.[14] As the program progressed, NIH and Iovance revised the licensing structure to strike a compromise between broad public health access and ongoing innovation. In 2021, the parties signed an Amended and Restated NIH patent license, which included additional rights and broader non-exclusive access. In 2022, a Second Amended and Restated license adds additional exclusive and non-exclusive rights related to TIL engineering and potency. These NIH discoveries, patents, and collaborative procedures pave the way for the FDA's fast approval of Amtagvi on February 16, 2024.[15]

4. MECHANISM OF ACTION

AMTAGVI Tumor-Infiltrating Lymphocyte (TIL) therapy directs the patient's immune system to precisely attack cancer cells. Lymphocytes, or immune cells, are extracted from a tumour, cultivated in a lab, and then reintroduced into the patient's body as part of TIL therapy. TIL treatments aim to employ the patient's immune system to locate and eradicate melanoma cells.[16] The injected TILs are enhanced and selected with the specific goal of finding and destroying tumour cells. AMTAGVI combats melanoma cells by enhancing the immune system's innate response. Lymphocytes that aggressively seek out and destroy melanoma cells are frequently derived from tumours. These lymphocytes are predicted to recognise and target cancer cells more effectively after being enhanced and reinfused.[17] The reinfused tumor-infiltrating lymphocytes move through the bloodstream until they detect the tumour nearby, which is triggered by chemokines released by the tumour. The TIL then escapes the capillaries and moves to the tumour site. When TILs reach the tumour, they use their T cell receptors to detect tumor-antigen peptides presented by MHC molecules on the tumour cell surface. When tumor-infiltrating lymphocytes (TIL) recognise tumour antigens, they activate and create perforin, a protein that forms holes. The newly formed holes aid in the transfer of granzyme, a pro-apoptotic protease produced by activated tumor-infiltrating lymphocytes that causes lysis of the targeted cancer cell. The injected TIL causes tumour shrinkage through direct cell killing and may also induce cytokine-mediated tumour cell eradication.[18]

AMTAGVI (Lifileucel) works by returning enlarged tumor-infiltrating lymphocytes (TILs) into the patient, which then kill malignant cells. The presence of immunosuppressive cells such as regulatory T cells (Tregs) and the expression of immunological checkpoints like PD-L1 on tumour cells pose the most significant obstacle in the tumour microenvironment. Despite these hurdles, TILs used in AMTAGVI therapy frequently preserve functional activity because to selective enrichment during ex vivo expansion. This pathway promotes the proliferation of tumor-reactive $CD8^+$ T lymphocytes with high-avidity TCRs, which are often resistant to PD-1-mediated exhaustion. Furthermore, the lymphodepletion regimen delivered prior to TIL infusion lowers repressive immunological elements (e.g., Tregs and myeloid-derived suppressor cells), allowing infused TILs to grow and function in vivo. IL-2, when delivered after TIL reinfusion, serves a purpose other than to promote T cell proliferation.[18] It increases TIL survival, improves cytolytic function, and promotes

homeostatic proliferation of infused lymphocytes. However, IL-2 plays a dual role, potentially boosting Treg expansion, therefore clinical regimens optimise timing and dose to maximise effector T cell function while minimising immunosuppressive expansion.

Furthermore, recent research has shown that epigenetic reprogramming during TIL growth can restore exhaustion-associated chromatin states, allowing previously dysfunctional T cells to resume effector capabilities. This epigenetic remodelling promotes increased cytotoxicity, cytokine production, and long-term persistence following reinfusion. TILs can trigger secondary immune responses by producing pro-inflammatory cytokines (e.g., IFN-γ, TNF-α) that recruit and activate endogenous immune cells, potentially leading to tumour spread.[18]

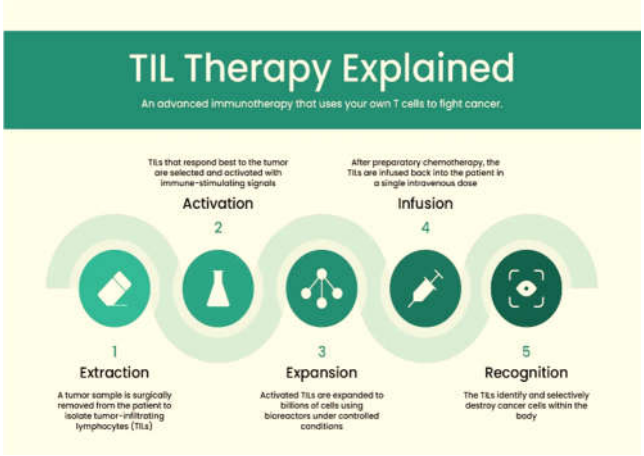

Fig.4.1 Workflow of TIL- based cancer Immunotherapy

5. PHARMACODYNAMICS

Lifileucel promotes an antitumor immune response. In a clinical trial for unresectable or metastatic melanoma, the objective response rate was 31.5%, and the median time to initial response to lifileucel was 1.5 months.[18]

6. DOSAGE AND ADMINISTRATION

AMTAGVI is provided as an infusion containing a suspension of tumor-derived T lymphocytes. The dosage is administered in one to four IV infusion bags suited to each patient, each in its own metal cassette for preservation. Each treatment contains around 7.5 × 10 9 to 72 × 10 viable cells. AMTAGVI should only be administered autologously. The patient's identification should match the patient IDs on the infusion bags and AMTAGVI cassettes. [19]Cryoshipped AMTAGVI is delivered to the treatment facility in the vapour state of liquid nitrogen. Every treatment facility must have liquid nitrogen kept in the vapour phase. The infusion bags and metal cassettes that make up the liquid nitrogen cryoshipper are labelled uniquely for each product and patient. Each of the one to four cryopreserved infusion bags is intended to hold one to four times the dose of AMTAGVI and includes its own protective metal cassette. If several bags are available, defrost and infuse one at a time.[20] Wait until the preceding bag has been safely and completely delivered before defrosting the next one. The product should not be frozen until ready for infusion. To defrost AMTAGVI, use a dry thaw method or a water bath at a temperature of 35-9 ◦ C to 39 ◦ C until the infusion bag is clear of visible ice or frozen materials.[18] The total time between the start of the warming and its finish should not exceed ten minutes. Schedule the infusion and thawing of AMTAGVI. Confirm the infusion time ahead of time, and adjust the thawing start time to ensure that AMTAGVI is ready for infusion when the patient arrives.[18] Begin the infusion as soon as one container of AMTAGVI is thawed, and finish within three hours at room temperature (18-25°C).Before initiating the lymphodepleting regimen, IL-2 (aldesleukin) and AMTAGVI should be readily available. Before delivering an AMTAGVI infusion, a chemotherapeutic regimen is used to deplete lymph nodes, as shown.[21] This contains two days of intravenous cyclophosphamide 60 mg/kg with Mesna, followed by five days of intravenous fludarabine 25 mg/m. Following fludarabine treatment, AMTAGVI is injected. Diphenhydramine, acetaminophen, or another H1-antihistamine should be administered to the patient 30 to 60 minutes before the AMTAGVI infusion. Systemic corticosteroids should not be given preventively since they may interfere with AMTAGVI function.[22]

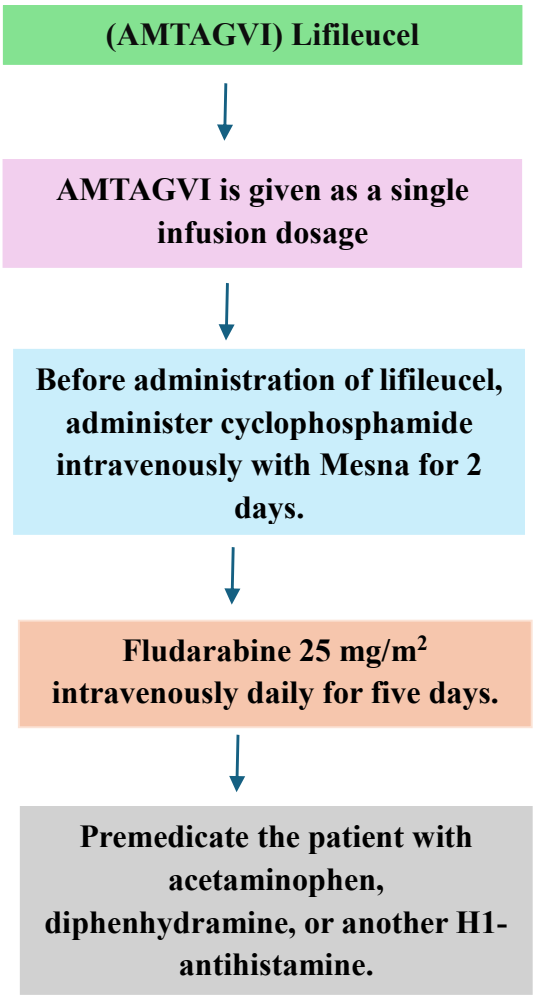

Fig.2 Administration protocol for Amtagvi

7. SIDE EFFECTS

The most common adverse reactions are chills, fever, fatigue, tachycardia (abnormally fast heart rate), diarrhoea, febrile neutropenia (fever associated with a low level of certain white blood cells), oedema (swelling caused by fluid buildup in body tissues), rash, hypotension, hair loss, infection, hypoxia (abnormally low oxygen levels in the body), and feeling short of breath.

Patients using lifileucel may experience sustained severe low blood count, severe infection, heart dysfunction, impaired respiratory or renal function, or fatal treatment-

related consequences. The prescribing material includes a boxed warning that describes these risks.[18]

8. CURRENT CLINICAL EVIDENCE ON LIFILEUCEL (LN-144)

Lifileucel (LN-144) is an autologous tumor-infiltrating lymphocyte (TIL) therapy being tested in clinical trials for advanced solid tumours, particularly melanoma.[23] In a pivotal phase II study (NCT02360579), Lifileucel demonstrated a high overall response rate, with tumour reduction observed in approximately 81% of patients with advanced melanoma who had progressed after immune checkpoint inhibitors and targeted therapies, indicating its potential role in ICI-refractory disease.[18]

Subsequent multicenter phase II trials (NCT03645928) revealed clinically significant and long-lasting responses in extensively pretreated solid tumour patients, including non-small cell lung cancer, with a tolerable safety profile.[18] A subgroup of patients experienced grade ≥3 haematologic toxicities, including anaemia and thrombocytopenia, which were associated with lymphodepleting treatment.[24]

Ongoing trials are looking at Lifileucel in early disease stages, particular populations, and combination tactics.[25] These include neoadjuvant and adjuvant use in melanoma, therapy of melanoma brain metastases, paediatric and young adult solid tumours, uveal melanoma, and certain sarcomas, as well as combinations with pembrolizumab and modified lymphodepletion regimens.[26]

Overall, existing data points to Lifileucel as a promising adoptive cell therapy for patients with advanced, treatment-refractory melanoma, with ongoing research targeted at improving its clinical use and broadening its therapeutic spectrum.[27,28]

9. CONCLUSION

Lifileucel TIL immunotherapy has demonstrated encouraging results in cancer treatment. Its ability to produce extended reactions while increasing overall survival highlights its potential as a substantial therapeutic alternative. Nonetheless, additional research is required to overcome constraints like as manufacturing challenges, potential side effects, and the need for biomarkers to predict patient responses.

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