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Review

How Does CAR-T Therapy's Efficacy Translate to Clinical Value for Hong Kong Patients with Refractory Hematological Malignancies, and How Can Optimized Manufacturing Help Close the Current Access Gap?

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Abstract: This comprehensive review examines the transformative potential of Chimeric Antigen Receptor (CAR)-T cell therapy in treating refractory hematological malignancies, with specific focus on its clinical implementation in Hong Kong SAR, China. CAR-T therapy represents a revolutionary advancement in blood cancer treatment, offering a potentially curative single-treatment option for patients who have exhausted standard therapeutic approaches. Global data demonstrates remarkable success, with documented durable remissions among over 40,000 treated patients worldwide. However, the translation of these benefits to Hong Kong's healthcare landscape faces significant challenges. The study analyzes critical manufacturing bottlenecks, extended wait times, and limited treatment slot availability that currently restrict patient access in Hong Kong SAR, China. Our research highlights promising technological improvements, specifically an EF1α-driven CD19 CAR construct achieving 59.9% transduction efficiency, exceeding typical clinical benchmarks of 30-70%. Through detailed statistical modeling, we demonstrate the potential for a seven-day reduction in manufacturing timeline, resulting in a 42% increase in treatment throughput. This optimization could significantly reduce patient attrition and potentially save 30-40 additional lives annually in Hong Kong SAR, China. The analysis encompasses manufacturing optimization strategies, regulatory considerations, and healthcare system integration requirements to enhance CAR-T therapy accessibility while maintaining therapeutic efficacy.

Keywords: car-t immunotherapy; hematological malignancies; cell therapy manufacturing; healthcare accessibility; clinical translation; treatment optimization; Hong Kong healthcare

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1. Background

1.1. Global and Hong Kong SAR, China, Burden of Hematological Malignancies

Globally, there are approximately 20 million new cancer cases and 9.7 million related deaths estimated for 2022, with projections of 18.5 million deaths by 2050 due to aging populations and improved diagnostics. This trend is evident in Hong Kong SAR, China, where cancer is the leading cause of mortality, accounting for one in every three deaths [1]. Within this significant burden, hematological malignancies account for about 1,000 new cases annually. In 2023 alone, the registered incidence reached 2,782, resulting in 966 deaths [2, 3] (As shown in Table 1).

Table 1. Number of Registered Deaths due to Blood Cancer in Hong Kong SAR, China 2023.

Disease Group	Male	Female	Total
Hodgkin lymphoma	4	5	9
Non-Hodgkin lymphoma	253	213	466

Multiple myeloma and malignant plasma cell neoplasms	92	54	146
Leukemia	185	160	345
Total	534	432	966

Compared to other types of cancer, hematological malignancies can affect individuals across the lifespan, from children with acute lymphoblastic leukemia to the elderly with diffuse large B-cell lymphoma [4]. This type of cancer is often systemic, spreading throughout the body from the onset, as the cancer cells originate in the bone marrow and the lymphatic system, which circulates throughout the body [5].

Common subtypes such as diffuse large B-cell lymphoma have a 30-40% likelihood of becoming resistant to standard therapies like chemotherapy and stem cell transplants [6]. For patients with certain types of Hodgkin and non-Hodgkin lymphoma, multiple myeloma, and leukemia, if conventional treatments have failed or if they have relapsed after at least two other treatments, it often leaves them with few options [3]. While advances in chemotherapy have significantly increased the survival rate for children with acute lymphoblastic leukemia to 80%, there were no effective treatments for those whose cancers returned or relapsed for many years. There remains a significant unmet need for effective treatments in relapsed or refractory cases across age groups.

1.2. CAR-T therapy as a potential solution

Chimeric Antigen Receptor (CAR) T-cell therapy has emerged as a transformative approach for refractory hematological malignancies. It offers a potential cure where none existed before [7]. This form of immunotherapy uses the patient's immune system to find and destroy cancer cells [8]. It alters the genes in a person's T cells to allow them to recognize and attack cancer cells. T cells are taken from the blood of patients or healthy donors and genetically modified to have a receptor called CAR on their outer surface. CAR allows T cells to attach to specific cancer cell antigens. This therapy is recommended when a cancer does not respond to other treatments, such as bone marrow transplantation, or the cancer has returned within 12 months after treatment, or after several treatments that do not succeed [4].

- **Chimeric:** The combination of different antibody or protein parts to construct a hybrid receptor [9, 10].
- **Antigen**: A specific protein marker that triggers immune responses to produce antibodies complementary in shape to it [11].
- **Receptor**: A biological recognition molecule inside or on the surface of a cell that binds to a specific substance and causes a specific effect in the cell [3, 12].
- **T-cells**: A type of lymphocyte involved in the immune response by fighting off pathogens and protecting organisms from disease [13].

The clinical application of CAR T-cell therapy has progressed rapidly since its initial approval [9]. In 2017, the first CAR T-cell therapy was approved in the USA for certain cases of Acute Lymphoblastic Leukemia (ALL) in children. Since then, other types of CAR-T cell therapies have been approved for various blood cancers, including multiple myeloma. Currently, there are 34,000 patients worldwide who have received commercially available CAR T-cell immunotherapies [2, 14].

Reports reveal CAR-T cell therapy has led to rapid improvement in some of the sickest patients, those who have tried multiple treatments without success [9, 15]. Physicians report remarkable outcomes, with tumors disappearing over weeks or even just a few days, and individuals who were nearing their last moments beginning to regain their lives [16]. One patient almost died during the therapy, and two weeks later was leukemia-free and engaging in activities like snowboarding [5, 17]. It was an extraordinary recovery.

As shown in Figure 1, findings demonstrate that patients treated with Yescarta, a type of CAR T-cell therapy used to treat two different types of non-Hodgkin lymphoma, large B-cell lymphoma (LBCL) and follicular lymphoma (FL), had an overall survival rate of 42.6%, which is notably high for patients with refractory large B-cell lymphoma [18].

Among the treated patients alive at five years, 92% have received no additional treatment since their one-time infusion of Yescarta [19]. Based on analyses of the five-year follow-up data, the overall survival rate was 42.6%, a significant figure for patients with refractory large B-cell lymphoma.

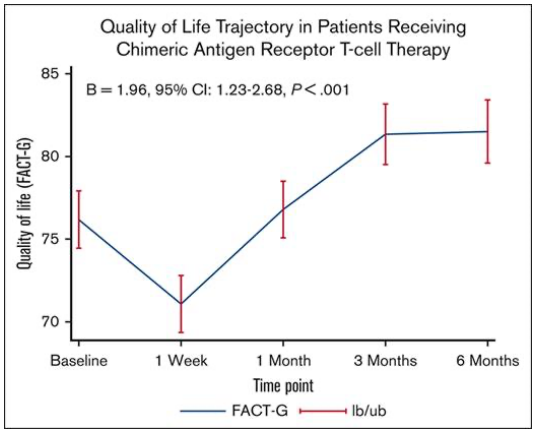

Figure 1. Quality of Life Trajectory in Patients Receiving CAR-T Therapy, as measured by FACT-G.

Evidence indicates that patients receiving CAR T-cell therapy demonstrate improved quality of life six months post-treatment, as measured by FACT-G, a specific questionnaire designed to measure the physical, social, emotional, and functional well-being of cancer patients [14]. Despite an initial decline in quality of life due to CAR-T therapy-related symptoms, such as fevers and fatigue, statistics have shown a significant increase to a median of 83.6, indicating a high quality of life by the six-month post-infusion mark [15].

1.3. Why Hong Kong SAR, China?

Existing treatments for refractory hematological malignancies face inefficiencies on several fronts [20, 21]. First, long waiting times arise due to the high demand created by heavily subsidized care. Secondly, the quality of life diminishes as patients endure cycles of hospitalizations, infections, and treatment-related side effects, even when transient responses are achieved. Thirdly, the affordability of treatment poses challenges in Hong Kong's mixed public-private healthcare system, where long-term drug costs and hospital stays can surpass the one-time expense of advanced cellular therapies. Finally, and most importantly for this study, access to innovative treatments such as CAR-T therapy is limited by manufacturing capacity and logistics.

Among these issues, manufacturing is undoubtedly the most critical gap, as it directly affects who can receive potentially curative treatment and when. CAR-T therapy requires an ex-vivo manufacturing process that can take several weeks to months between leukapheresis and infusion. In regions with mature CAR-T infrastructure, standardized and high-throughput manufacturing, along with a broader hospital network offering CAR-T cell therapy, help mitigate this attrition. Conversely, in Hong Kong SAR, China, among the 57 hospitals, only one offers CAR-T cell therapy, specifically for multiple myeloma, and aims to treat only five to ten patients annually, meeting only a small fraction of the local need [22]. In this context, optimizing and standardizing CAR-T manufacturing is not merely a refinement but a life-saving advancement.

Hong Kong SAR, China represents an urgent yet understudied setting for advancing CAR-T therapy implementation, particularly when contrasted with global leaders in CAR-T therapy. While other regions have treated tens of thousands of patients through scaled manufacturing networks, Hong Kong's curative success remains limited, with a relatively small number of patients treated over several years [12, 23]. This highlights Hong Kong's lag in translating CAR-T's proven efficacy into meaningful clinical access, as

only a small number are cured locally compared to thousands globally, and only one of the approved CAR-T products is used in Hong Kong SAR, China.

1.4. Research question and aims

Against this backdrop, the central question of this paper is: *How does CAR-T Therapy's Efficacy Translate to Clinical Value for Hong Kong SAR, China Patients with Refractory Hematological Malignancies, and How Can Optimized Manufacturing Help Close the Current Access Gap?* It is hypothesized that CAR-T therapy could offer superior clinical value compared to traditional salvage treatments in Hong Kong SAR, China, but the real-world benefits are currently undermined by manufacturing delays and limited capacity [9, 24].

To investigate the research question, the paper employs a multifaceted approach using a combination of primary experimental data and secondary research analyses [24, 25]. A primary in vitro experiment will be conducted to assess the transduction efficiency of the second-generation EF1a-driven CD19-targeted CAR construct, quantified by flow cytometry on transduced primary human 293T cells following lentiviral delivery [26]. The purpose is to generate original firsthand data to validate the therapy's preclinical efficacy in a lab-controlled setting and inform its translational value in the Hong Kong SAR, China context [27]. Additionally, a comprehensive literature review will incorporate worldwide data from authoritative sources, including clinical applications of CAR-T therapy and scientific reviews on the therapy's current stage of progress and limitations. Local case studies in Hong Kong SAR, China will be included to provide specific insights into accessibility and quality of life improvement, supplemented by financial analyses comparing one-time CAR-T expenses against chronic standard care.

1.5. Purpose and Significance

This research has significant social implications. Many patients with high-risk refractory blood cancers die before they can receive CAR T-cell therapy [9, 28]. The time-intensive ex vivo processing and quality control delay the manufacturing duration, which can take from three months to over a year. During this period, the rapidly progressing disease can lead to death, even though the CAR-T therapy is still pending [29, 30]. In this context, optimizing the transduction efficiency and expression strength of CAR constructs is not merely a technical refinement but a potentially life-saving advancement.

Research indicates that only about 40% of heavily pretreated relapsed or refractory multiple myeloma patients on the waiting list received the therapy within a year, with a quarter of patients dying while waiting [31]. The main issue is the lack of available slots due to limited production of the treatment. Furthermore, reducing the waiting time by two months can increase treatment efficacy by 14%, alongside a 3.3% increase in survival gains per treated patient [32]. These findings underscore the critical impact of timely access and highlight the urgent need to shorten the manufacturing timeline, which acts as the primary bottleneck for patient access, causing prolonged waits and restricted availability.

This paper has the potential to impact Hong Kong SAR, China's healthcare system by promoting broader access to CAR T-cell therapy [17, 33]. By presenting a solution that directly links laboratory optimization of CAR-T design with a shorter ex vivo manufacturing period, it could enable more hospitals to offer the therapy or increase the available slots, making this life-saving therapy more accessible to eligible patients in Hong Kong SAR, China [5, 34].

2. CAR-T cell Therapy

2.1. Mechanism of CAR-T cell Therapy

As depicted in the figure, a CAR T-cell possesses a distinct structure comprising four primary domains. The antigen recognition domain, situated within the extracellular domain, consists of laboratory-engineered antibodies. This domain interacts with potential targets, directing the CAR T-cell towards any tumor cell expressing a

corresponding protein. The hinge region, also referred to as the spacer, maintains a balance of rigidity and flexibility during the binding of the CAR to the target cell. The transmembrane domain, characterized by a hydrophobic alpha helix, stabilizes the entire CAR structure and secures it within the plasma membrane. Lastly, the intracellular signaling domain transmits signals to initiate a cascade of internal responses within the T-cells, promoting further multiplication in the body when the antigen-binding domain engages with the target cell [22, 35] (As shown in Figure 2).

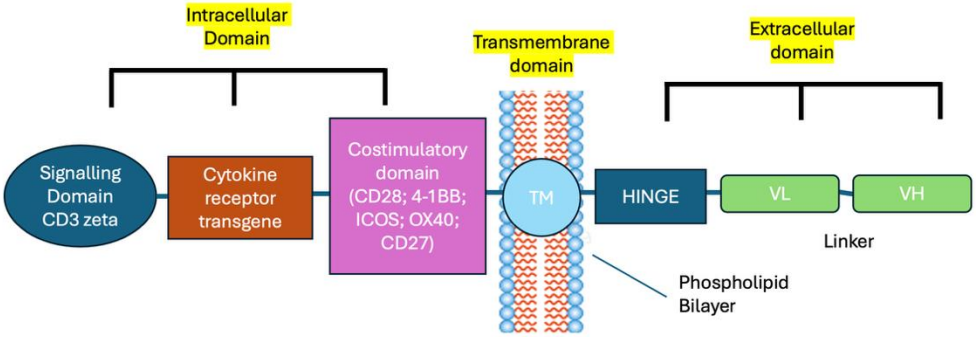

Figure 2. Structure of CAR T Cell.

2.2. How CAR T-Cell Therapy Works

The entire process of ex vivo CAR-T therapy takes approximately 3-5 weeks. Blood is initially harvested from the patient through leukapheresis, involving the insertion of a needle and a thin tube (cannula) into a vein in both arms. Blood is drawn from one arm and processed through an apheresis machine, which separates the T cells from the rest of the blood [36]. The remaining blood is then returned to the other arm, with the entire procedure lasting about 5 hours. The isolated T cells are sent to the treatment manufacturer's laboratories, where they are genetically engineered to produce synthetic proteins called CARs on the surface of the T cell. Each CAR spans the cell membrane, featuring both internal and external components [37]. The external component enhances the receptor's ability to recognize and bind to its target antigen on tumor cells, while the internal component contains signaling domains. Once the receptor binds to an antigen on the tumor cell, these stimulatory domains signal the T cell to proliferate until there are billions of them.

These cells are then reintroduced to the patient as a single infusion through the patient's central line, PICC line, or cannula [29, 38]. Medication is administered prior to the infusion to prevent any allergic reactions or fevers [39].

Following the CAR-T infusion, patients often require hospitalization for 2-3 weeks to monitor for any potential life-threatening toxicities [34]. These include cytokine release syndrome (CRS), an inflammatory response caused by an excessive release of cytokines into the bloodstream, which can damage organs and tissues, and immune effector cell-associated neurotoxicity syndrome (ICANS), a condition affecting the central nervous system that can occur after certain types of immune therapy treatments [40].

3. In Vitro Experiment

From plasmid design to functional detection of CAR expression, this experimental workflow details the testing of a second-generation CD19-targeting CAR-T cell product's transduction efficiency, as measured by flow cytometry [17]. This design closely mirrors clinically approved CD19 CARs that are already used in patients with challenging B-cell malignancies [16].

Optimized protocols that maximize transduction efficiency can significantly reduce the culture time necessary to achieve the target goal dose of approximately 50-100 million CAR+ T cells per dose [9, 10]. This directly shortens the patient wait time and

manufacturing window, potentially saving many lives [9]. This transduction efficiency experiment provides direct mechanistic evidence that a well-designed CAR construct yields a high CAR-positive percentage, addressing part of our research question: "How can optimized manufacturing help close the current access gap in Hong Kong SAR, China?" Robust CAR+ content supports stronger antigen-driven persistence in vivo, which is crucial for translating to clinical benefits in Hong Kong SAR, China.

3.1. Plasmid Design

The plasmid was specifically designed with components to optimize transduction efficiency [8]. The second-generation lentivirus backbone was chosen and provided by the lab, and the CAR insert was custom synthesized using SnapGene and cloned into the backbone by a biotechnology company, as shown in the circular plasmid diagram and the backbone of the lentivirus (As shown in Figure 3) (As shown in Figure 4).

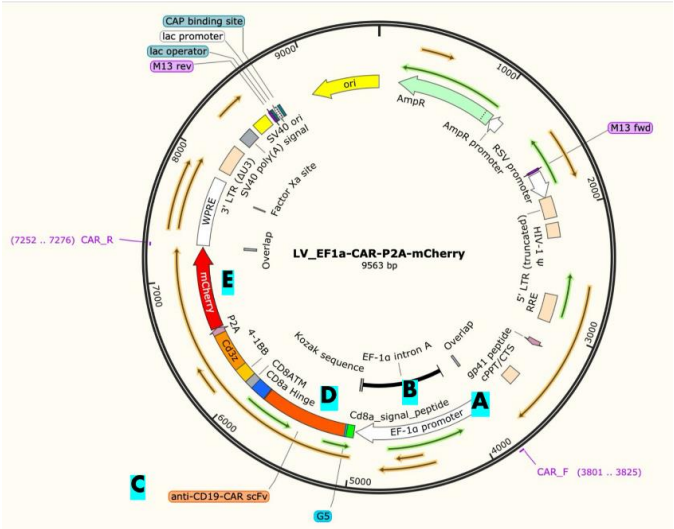

Figure 3: Circular Plasmid Diagram of LV_EF1a-CAR-P2A-mCherry

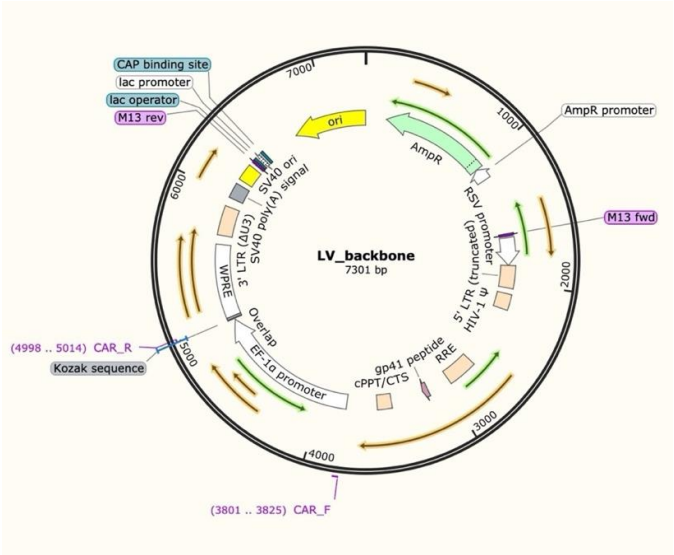

Figure 4: Lentivirus Backbone

The CAR architecture (5' to 3') included:

- EF1a promoter:
- CD8a signal peptide
- FMC63 scFv

- CD8a hinge + CD8a transmembrane domain
- 4-1BB (CD137) co-stimulatory domain: An intracellular signaling module
- CD3ζ signaling domain: Containing three intracellular activation domains for primary activation [5]. It is present in all clinically used CAR-T cells to deliver activation signals [9, 18].
- P2A self-cleaving peptide followed by mCherry

Browsing PubMed, all clinical trials up until December 1st, 2023, 86 out of the 105 clinical trials focused on hematological cancers, with over 3,312 patients, with around 90% of T-cell products using mouse-derived scFvs, second-generation CAR structure with 4-1BB or CD28 costimulatory domains [26].

EF1a promoter (labeled as "A" in the circular plasmid diagram): A gene promoter is a specific DNA sequence that is located upstream of its target genes; it acts as the binding site for RNA polymerase, which determines when and what gene is transcribed into mRNA. It helps control the timing, duration, and level of CAR expression [5]. Literature consistently shows EF1a provides long-term gene expression in T cells, and it is also one of the most widely used promoters for approved CAR-T cell therapies [3]. Thus, for this experiment, the EF1a promoter was selected to drive the expression of the CAR because it is superior to alternatives like CMV, which may have weaker expression in primary T cells; SFFV, which has the potential for silencing, which is the prevention of gene expression in primary T cells so that the corresponding protein is not produced; and PGK, which may lead to reduced transduction and CAR expression.

More detailed comparison could be seen in the table of different promoters used in CAR-T therapy [19] (As shown in Table 3).

Table 3. Different Promoters Used in CAR-T therapy.

Promoters	Expression strength in primary T cells	Silencing/long-term stability	Safety & Clinical familiarity in CAR T
EF1a	High and stable CAR expression	Active and resistant to silencing	Widely used in clinical-grade lentiviral CAR vectors with controllable CAR density
CMV	Weaker expression strength compared to EF1a	Well known to undergo silencing over week in vivo and during differentiation	Historically common but disfavoured for silencing
SFFV (Spleen Focus Forming Virus)	Very strong, often resulting in high percentage of CAR expression	Can be subject to silencing over time or be toxic in some cell line	Associated with higher genotoxic potential and rarely used in current clinical trials
PGK	Weaker expression strength compared to EF1a	Show stable activity for up to 60 days in certain stable cell lines (Jurkat) but may also be	Less common for first-line clinical CAR projects

susceptible to silencing
in primary T cells

CD8a signal peptide (labeled as "B" in the circular plasmid diagram): A signal peptide is a short amino acid sequence at the N-terminus (beginning) of the CAR construct [19, 33]. It directly guides the newly made CAR protein to the endoplasmic reticulum for processing, and ensures the CAR is inserted into the T-cell membrane to be displayed on the surface, rather than being kept inside the cytoplasm. The human CD8a chain signal peptide was chosen for the experiment, as it is the most used signal peptide for FDA-approved CAR-T products.

FMC63 scFv (labeled as "C" in the circular plasmid diagram): An antigen-binding domain (ABD) is the specific region on an engineered CAR receptor that recognizes and binds to CD19, a B-cell marker. The affinity of CAR for the target antigen (mainly CD19) is one of the key factors influencing its function, as it determines the sensitivity of the CAR-T cells [6]. FMC63 scFv is derived from an original FMC63 mouse antibody that targets the CD19 protein on B-cells, a key target for treating leukemia and lymphoma. It was selected as the ABD for this experiment because it is the most clinically validated for CD19 targeting. As shown in the figure, four out of the six FDA-approved CD19-targeted CAR T-cell therapies use the mouse-derived FMC63 scFv for antigen recognition (As shown in Figure 5).

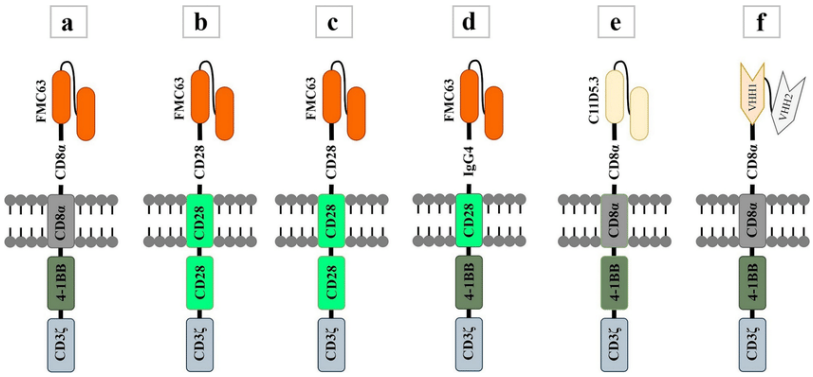

Figure 5: Structural components of six CAR-T products that have received FDA approval.

A=Tisagenlecleucel, B=Axicabtagene ciloleucel, C=Brexucabtagene autoleucel, D=Lisocabtagene maraleucel, E=Idecabtagene vicleucel, and F=Ciltacabtagene autoleucel [13].

CD8a hinge (labeled as "D" in the circular plasmid diagram): Hinge refers to a flexible protein linker region that allows the CAR to bind multiple targets at various distances, which is critical in determining the CAR's ability to bind to target cells and induce an effective T-cell response [22]. The CD8a hinge provides a flexible space between scFv and the membrane, improving the ability of the CAR to reach CD19 on the target cell surface and to form an effective immune synapse [15].

CD8a transmembrane domain (labeled as "D" in the circular plasmid diagram): A CD8a transmembrane domain is a hydrophobic alpha helix that anchors the entire CAR to the T-cell's surface membrane, acting as a link between the extracellular region and the intracellular signaling domains of the cell and a key factor that influences CAR expression and stability [40].

P2A self-cleaving peptide followed by mCherry (labeled as "E" in the circular plasmid diagram): The P2A sequence is a self-cleaving peptide that enables the co-expression of CAR and mCherry; it induces ribosomal skipping during translation, so the single mRNA transcript is translated into two separate proteins (CAR and mCherry). The mechanism achieves near 1:1 co-expression, in which both proteins remain structurally and functionally independent, with CAR binding to antigens and signaling normally, while

mCherry provides a red fluorescent signal that serves as a marker for the visualization of CAR expression rate [40].

3.2. Plasmid & Lentivirus Preparation

3.2.1. Amplification of plasmid

The lentiviral transfer plasmid LV_EF1a-CAR-P2A-mCherry was amplified on a large scale using the maxiprep protocol, which isolates and purifies a large quantity of pure plasmid DNA from bacterial cultures on a large scale [2].

3.2.2. Plasmid Transformation

Plasmid transformation is the process of introducing our circular plasmid (containing CAR gene) into the Stbl3 cell (the host), which is a special type of E. coli bacteria engineered for stable cloning [39]. The purpose is for large-scale production of the plasmid DNA [39].

1) Mix on ice: Put Stbl3 cells and the plasmid in an ice bucket to keep them stable. Then, add small amounts of our purified plasmid DNA to the cells, and mix gently [28, 32]. Immediately after adding, put the tube back into the ice bucket. Incubate on ice for 15 minutes, which lets the plasmid DNA bind to the outside of the bacterial cell membrane without the cells dying [26].
2) Heat shock: Move the tube to a 42°C water bath for 45 seconds [11, 34]. This sudden heat "shocks" the cell, which creates temporary pores in the plasma membrane and reduces the membrane potential, which makes the inside less negatively charged, so the plasmid DNA can enter the cytoplasm through these pores [24, 39].
3) Back on ice: Immediately put the tube back on ice for a few minutes [40]. This seals the pores quickly, trapping the plasmid inside the cell before it can leak out.
4) Recovery: Add nutrient-rich liquid broth SOC (without antibiotic) and incubate at 37°C with shaking overnight [33]. This lets the bacteria recover from the heat shock and begin replicating the plasmid.
5) Plate the transformation onto one/two 10 cm LB agar plates with ampicillin at 100 μg/mL [17].

3.2.3. Colony Selection & Expansion

1) A single and well-isolated colony was picked using a 200 μL pipette tip and inoculated into a 5 ml LB medium containing ampicillin [16].
2) Incubate in shaker at 37°C for 8 hours while shaking
3) The starter culture was diluted into selective LB medium and grown overnight (15 hours) at 37°C with shaking to obtain a dense bacterial culture [33].

3.2.4. Maxi-prep

The Qiagen plasmid plus maxiprep kit was used to purify the plasmid [3].

1) Bacterial cells were harvested by centrifugation at 6,000 x g for 15 minutes at 4°C and the supernatant was discarded
2) Plasmid DNA was extracted from the cell pellet, and it was resuspended in 8 ml of Buffer P1 (containing RNase A)
3) 8 ml Buffer P2 (an alkaline lysis buffer) was added to the pellet and gently mixed by inverting to denature chromosomal DNA, disrupt the cell membrane, and release plasmid DNA into solution. The lysate was then incubated at 25°C for 3 minutes [3].
4) 8 ml of pre-chilled Buffer P3 was added to renature the plasmid DNA; mixing was done by inverting 6 times [27]. This is to make all the components inside the bacterial cell clump together into a thick white precipitate [5].

5) The lysate was centrifuged at 15,000 x g for 30 min at 4°C to pellet the white precipitate [9]. After centrifugation, the supernatant should be clear, containing plasmid DNA [24].
6) The clarified lysate was transferred to a QIAfilter cartridge to remove residual particulates, leaving a clear liquid with only plasmid [4]. Incubate at room temperature for 10 min [2].
7) Add 5 ml of Buffer BB and mix by inverting 6 times [36]. This is to anchor the plasmid to the filter while impurities wash through.
8) Add 0.7 ml Buffer ETR, then 0.7 ml Buffer PE. This is to clean the DNA, as the buffer helps remove cell contaminants present on the plasmid [21].
9) Place the QIAGEN Plasmid Plus Maxi spin column into a 2ml collection tube, and centrifuge the column at 10,000 x g for 1 min. This removes leftover alcohol from the buffer, which could interfere with using the DNA later [22].
10) To elute the DNA, 400 µL Buffer EB/Water was added to the spin column, waited 1 minute, and centrifuged for 1 minute [5, 6] (As shown in Figure 6) (As shown in Figure 7).

Figure 6. White precipitate formed when bacterial cell component clump together.

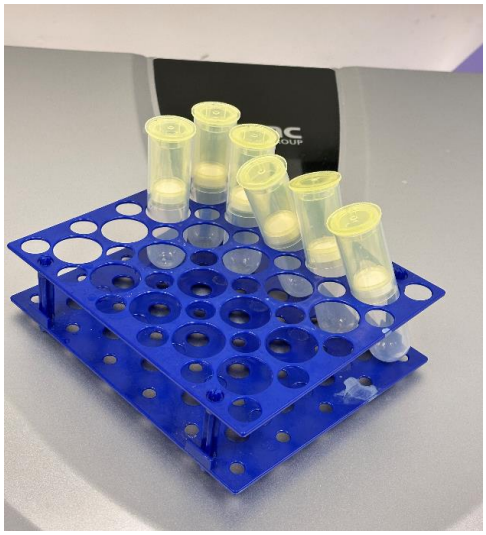

Figure 7. Qlarified lysate was transferred to a QIAfilter cartridge for filtration.

3.2.5. Lentivirus Transduction

The lentiviral vectors carrying the CAR gene were produced using HEK293T cells, which are an easily transfectable human embryonic kidney cell line; they are chosen due to their ability to grow continuously in cell culture [37]. The MOI is 10.

1) Activate T cell by CD3/CD28 dynabeads for 2 days, which is a superparamagnetic polymer beads coated with ligands or specific antibodies [8].
2) Approximately 1x10^7^ cells were placed per 10 cm culture dish to reach a 70-80% confluency; this ensures there were enough cells to make high amounts of virus, but not too many that they become crowded and unhealthy [20].
3) Transfection was performed using polyethyleneimine-mediated delivery [40]. PEI is a nonviral transfection reagent; as it's a cationic polymer, it would bind to DNA via electrostatic forces, which condense the negatively charged DNA into positively charged particles [16, 34]. The positively charged PEI-DNA complexes would bind to the anionic cell membrane and be brought into the HEK293T cell via endocytosis [20].
4) The HEK293T cells were transfected with 3 plasmids: a transfer plasmid containing the CAR gene that was previously designed, a psPAX2 packaging plasmid providing the structural and enzymatic proteins required for viral assembly, and pMD2.G, which coats lentiviral particles, enabling it to infect a broad range of cells [28]. The 3 plasmid amounts were calculated according to an excel spreadsheet to achieve a final DNA concentration of around 1.05 μg/mL and PEI:DNA ratio of 5:5:1
5) After 8 hours of transfection, the culture medium was replaced with fresh DMEM containing 10% FBS to remove excess transfection reagent and maintain cell vitality [19].
6) Virus-containing supernatant was harvested at both 48 hours and 72 hours post-transfection [10]. The harvested supernatant was clarified by low-speed centrifugation (2000 x g for 10 minutes) to remove cell debris [28].
7) The cleared supernatant was then concentrated by ultracentrifugation (20,000 x g for 2 hours at 4°C), after which the viral pellet was resuspended in a reduced volume of DMEM and incubated overnight at 4°C with gentle shaking [22]. The purpose is to achieve higher concentration [13, 38].
8) The concentrated lentiviral stock was stored at -80°C
9) Once a concentrated lentivirus stock was obtained, it was added to the 293T cell line [21]. Polybrene is added to reduce the electrostatic repulsion between the virus and cell membrane, which facilitates viral entry [18].
10) 48 hours later, the transduction efficiency, as measured by mCherry fluorescence and CAR+ expression, was evaluated by flow cytometry [1] (As shown in Figure 8).

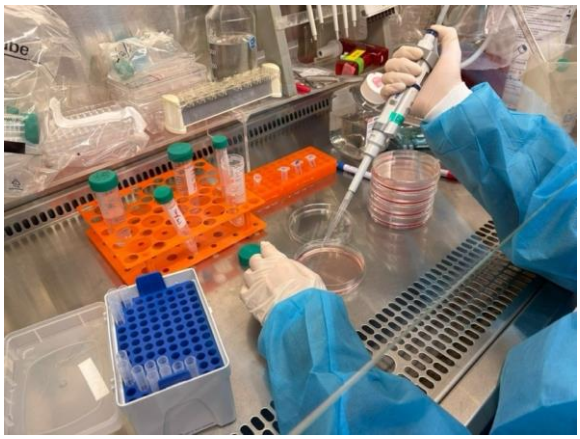

Figure 8. Sabella conducting lentivirus transduction.

4. Result

4.1 Defining and Measuring Transduction Efficiency via Flow Cytometry

The primary experimental endpoint, transduction efficiency, is defined as the percentage of viable primary T cells expressing both CAR and the co-translational mCherry reporter, measured via flow cytometry 48 hours after lentiviral transfection. Flow cytometry is a laser-based technique used to analyze the CAR+ expression of single T cells as they pass through a laser beam in a fluid stream [20]. The T cells containing mCherry are fluorescently tagged and become excited when passing through the laser beam, emitting light of different wavelengths, which is detected by a monitor near the beam. These signals are then converted into electronic signals that can be analyzed by a computer. The fluorescence and visible light scattering profile of the individual cells are used to determine whether they are expressing the CAR gene [7] (As shown in Figure 9).

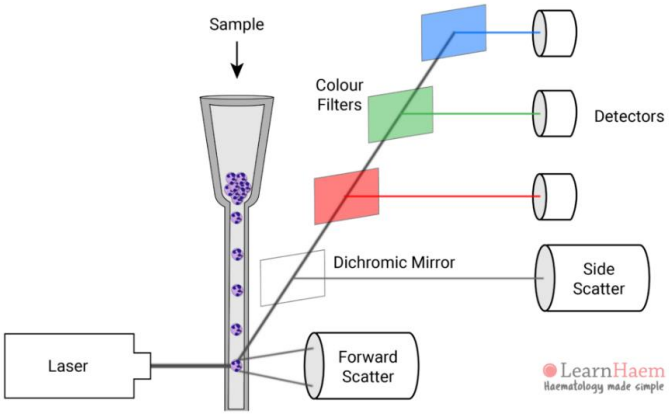

Figure 9. CAR T cell Detection by Flow Cytometry.

The core experimental purpose of determining how CAR-T therapy's efficacy can translate to clinical value for patients with refractory hematological malignancies and how optimized manufacturing can help close the current access gap is addressed in this dataset [32]. The observed transduction efficiency for the anti-CD19 CAR-T cells is 59.9%. This result significantly exceeds the common release criterion of greater than 15% and also places it within the higher end of clinical benchmarks of 30-70% (As shown in Figure 10).

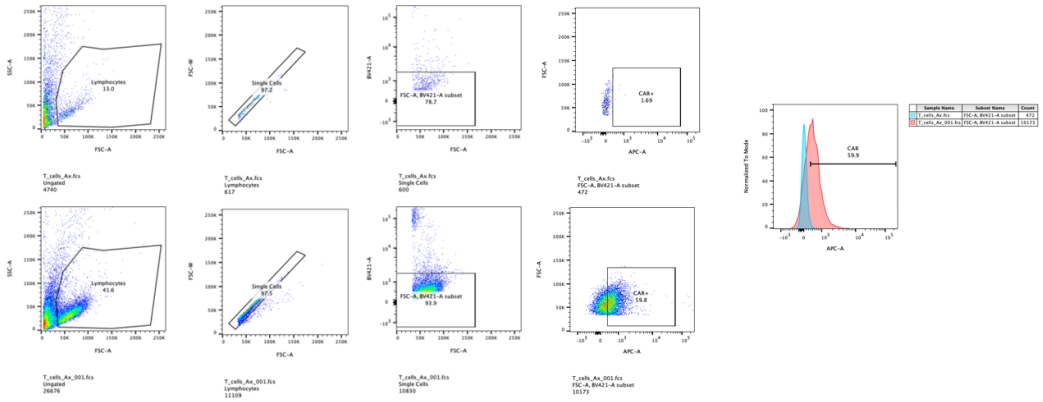

Figure 10. Flow Cytometry Analysis of CAR-mCherry Expression.

According to data presented in a clinical trial, reducing the waiting time by two months results in a 14% increase in treatment efficacy, along with a 3.3% increase in survival gains per treated patient [4, 27]. These findings underscore the critical impact of timely access and highlight the need to shorten the manufacturing timeline, which acts as

the primary bottleneck for patient access, causing prolonged waits and restricted available slots [9].

A product in which 59.9% of cells are CAR+ means that for every one million expanded T cells, approximately 600,000 are therapeutically active CAR-T cells. This requires fewer cell doublings to reach the clinical target dose requirement of 50-100 million CAR+ cells, allowing manufacturers to shorten culture periods. A minimum of one million cells must be acquired from patients for CAR T-cell therapy. A highly efficient architecture could enable successful production from a small initial blood sample, reducing manufacturing time to reach the target dosages ranging from 50 to 100 million CAR+ T cells [34]. A higher initial transduction yield, when accompanied by vigorous proliferation, significantly reduces the culture time necessary to achieve the goal dose from the available sample [15, 16]. This directly compresses the manufacturing window, with the goal of reducing patient wait times, which is crucial in cancer treatment.

In the context of Hong Kong SAR, China, where capacity constraints, limited production slots, and high-cost manufacturing already exclude many candidates, demonstrating a construct that delivers near clinical-grade transduction efficiency from markedly fewer T cells could reduce both the starting blood volume and the duration of ex vivo culture required, addressing the driving question of how optimized manufacturing can help close the current access gap of CAR-T therapy [21, 34].

4.2 Modelling

To contextualize biological efficiency within clinical manufacturing, a growth model was designed comparing the CAR construct with the standard manufacturing construct. The model is based on estimates of T cell kinetics in humans, which provide knowledge of T-cell proliferation rates, indicating that T-cell proliferation rates are highly dynamic, with activated T cells capable of dividing as quickly as once every 4 to 6 hours during peak expansion, resulting in a 10- to 100-fold increase in population within 3 to 7 days. The relative time for reaching the target dose number of 100 million CAR T cells is based on primary T cell culture and expansion, stating that T cell expansion occurs in an average time frame of 10-14 days. The starting T cell number of one million, which is the minimum amount required from patients, is used as the starting point for both models [24] (As shown in Figure 11).

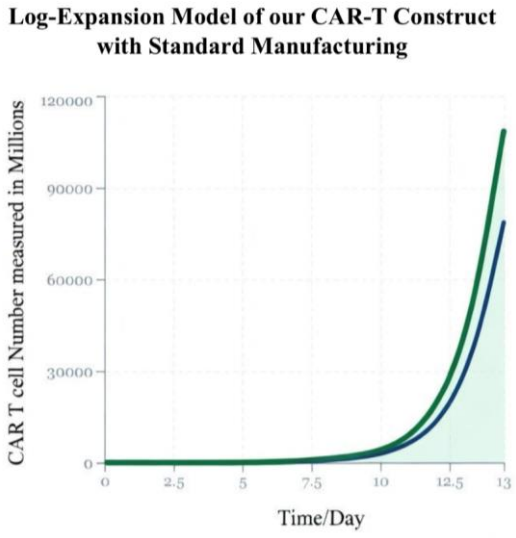

Figure 11. Log-Expansion Model of our CAR-T construct with Standard Manufacturing.

The cell numbers were modeled by the mathematical equation $\mathbf{N(t) = N_0 \times n \times 2^{(t/g)}}$
In which:

- N_0: 10^6 Input (starting cell number)

- H: Efficiency
- η: Transduction efficiency
- G: Doubling
- T: Day Elapsed

This model is extremely useful, as it allows visualization of how many days sooner a patient can be infused with CAR-T cells. Increasing the transduction efficiency from 25% to 60% results in a 2.4-fold expansion of the therapeutic CAR-T cell pool, as seen by the increased height of the green curve compared to the dashed curve between days 8 and 15. Assuming log-phase proliferation rates of 2.4 doublings per day, this translates to a 3-5 day reduction in ex vivo culture time. Consequently, the CAR T construct results in a faster rate to reach the target therapeutic number of CAR-T cells, which reduces the vein-to-vein time and wait times for patients. This is especially important when dealing with rapidly progressing relapsed disease. Additionally, with a higher yield of CAR-T cells, the cost of production per dose would also be reduced, making it more accessible to patients [29].

Based on research presented at an annual meeting, only about 40% of heavily pretreated relapsed or refractory multiple myeloma patients on the waiting list received the therapy within a year, with one-quarter of patients dying while waiting for the therapy [9]. The main problem is the lack of available slots due to limited production of the treatment [16].

In this context, the research represents a paradigm shift in scalable manufacturing, transforming CAR-T from a slot-starved therapy into a more widely available treatment for patients with refractory hematologic malignancies. Ultimately, it redefines equity in access [40]. This innovative CAR architecture ensures fewer patients die from waitlist mortality by optimizing every leukapheresis dollar and facility time, bringing the region closer to becoming a leader in the deployment of optimized CAR-T cell therapy.

5. Extended Analysis

A landmark case from a clinical team at a prominent medical institution in collaboration with a major hospital in March 2025 demonstrates the remarkable potential of CAR T-cell therapy beyond its established indications, successfully treating a myeloma patient with this therapy without severe complications [10]. This hospital was the first in the region to offer CAR T-cell therapy in 2021; the clinical team has performed it on over 60 patients with different blood cancers, constituting the largest cohort of patients treated with CAR-T cells in the area. The existing program offering CAR-T cell therapy aims to treat 5-10 myeloma patients annually. A leading expert stated that BCMA CAR-T cell therapy will have a significant impact on myeloma management in the region, as it stands as one of the most effective salvage strategies for myeloma. It can provide a life-saving option to patients who do not respond to standard therapy or experience a relapse after autologous bone marrow transplantation.

Multiple myeloma is a type of blood cancer that occurs when a patient's bone marrow produces certain blood cells (plasma cells) that do not function properly [24]. It is important to note that myeloma cannot usually be cured, but it can be managed with treatment [37]. Patients with myeloma often suffer from bone pain, shortness of breath, muscle weakness, or headaches [23, 32].

A patient, a 73-year-old man with multiple myeloma, was first diagnosed with stage 1 myeloma in 2010. Initially, in 2014, he received standard treatment and underwent hematopoietic stem cell transplantation. However, in 2016, the myeloma relapsed, indicating the cancer had returned after a period of improvement, often due to a niche group of myeloma cells not eliminated by the first treatment beginning to multiply again [35]. The patient underwent multiple treatments, but unfortunately, there was no response [21].

By 2024, the patient had tried all available treatment options; he experienced severe pain and bowel incontinence and required catheters in his bladder and kidney to pass urine [17]. He was unable to sit or walk [4]. Subsequently, on 31 December 2024, he underwent CAR-T cell therapy infusion. Subsequent evaluation in late January to early February 2025 confirmed he had achieved a complete response of the myeloma tumor; no cancer was detected, as shown by a CT scan. According to the latest follow-up in May 2025, he was completely recovered; he regained full independence, with complete resolution of his prior symptoms.

This success opens new possibilities and hopes for patients who have not responded to traditional treatments, having the potential to help a wide range of patients in the future [28]. The collective findings from both global researchers and the regional case study underscore the potential of CAR-T therapy to fundamentally alter the treatment paradigm for blood cancers in the region [19].

6. Economic and Accessibility

The statement that prescribing the best drug is ineffective if patients cannot afford or access it highlights a critical issue in cancer treatment. Financial toxicity significantly affects patients' well-being, adding to their physical and mental exhaustion from the disease [29, 40]. Research indicates that over a quarter of cancer patients face medical expenses beyond their financial capacity [21].

The story of an 8-year-old diagnosed with Acute Lymphoblastic Leukemia and his mother illustrates this systemic issue in Hong Kong SAR, China. As the sole provider and caregiver, the mother faced an overwhelming financial burden for her child's treatment. Her income was already stretched thin by daily and medical needs. For example, a targeted cancer therapy drug costs approximately 50,000 HKD for a jar of 90 capsules, with the child taking three capsules daily, depleting the jar in about a month. Combined with other medications and frequent hospital visits, the total expense for medication alone reached around 1,000,000 HKD [34]. This ongoing financial strain, exacerbated by repeated hospital visits and costly medication, highlights the profound disruption to family stability caused by long-term cancer management [7].

Although the initial cost of CAR T therapy is high, ranging from 2,882,000 to 4,128,000 HKD, excluding hospital fees and side effect treatments, it offers a potentially curative, one-time treatment. This can be more economically efficient than a lifetime of chronic care [23]. When conventional treatments like chemotherapy and radiotherapy fail, patients often face relapses, requiring increasingly intensive and costly therapies with diminishing returns on quality of life and survival rates. For patients who have exhausted other options, a single, definitive therapeutic intervention can be more economically viable than the continuous escalating expenses of chronic disease management and repeated hospitalizations.

7. Future Prospects—In Vivo CAR-T Cell Therapy

Although ex vivo CAR-T cell therapy has demonstrated solid efficacy in treating various blood cancers, it faces significant challenges related to manufacturing processes and high costs, limiting its accessibility to a broader range of patients [5, 9]. Only about 20% of eligible patients receive the treatment, primarily due to the high costs involved, which can range from approximately 2,882,000 to 4,128,000 HKD for a single dose [23].

To address these challenges, the in vivo CAR-T cell method has been developed recently [37].

In vivo CAR-T cell engineering involves the direct manufacturing of CAR-T cells within the patient's body using delivery vehicles and carriers [9]. This approach aims to eliminate the need for complex ex vivo cell processing, achieve faster turnaround times, and improve clonal performance [4].

In vivo CAR therapies have the potential to be an off-the-shelf solution, ready for use at the time of patient referral [3].

At least seven biotechnology companies are developing in vivo therapy technology and advancing it into clinical trials and preliminary tests [1]. A clinical-stage biotechnology engineering company has reported that in early 2025, the German regulatory agency approved the expansion of the first-in-human Phase 1 clinical trial evaluating a specific treatment for B-cell malignancies to Europe. This trial is the first to assess the safety of an intravenous infusion of in vivo CAR gene therapy in adults with refractory or relapsing B-cell malignancies, potentially paving the way for future treatments and studies.

In preclinical studies for in vivo CAR-T therapy, B-cell malignancies, which are cancers developing from B-lymphocytes, were completely eradicated in both small and large animal models without the use of chemotherapy [16]. Additionally, there were no signs of cytokine release syndrome or neurotoxicity [12, 15].

8. Conclusion

Through laboratory experimentation, modeling, and literature-based clinical evaluations, the findings collectively confirm the hypothesis that CAR-T therapy holds substantial translational potential for Hong Kong SAR, China's healthcare system. The potential for success was validated by the in vitro experiment of the EF1a-driven CD19-targeted CAR construct to optimize manufacturing, which achieved a transduction efficiency of 59.9%. When paired with global and local clinical outcomes, these results collectively affirm CAR-T therapy's ability to induce durable remission rates, improve post-treatment quality of life, and yield long-term cost efficiencies when compared to prolonged hospitalization. The therapy's clinical application in Hong Kong SAR, China, as shown in the case study, underscores the therapy's potential to alter treatment paradigms, though challenges like manufacturing costs and toxicities remain.

Several limitations and potential sources of error of our study must be acknowledged.

1) The in vitro results may not fully reflect the transduction efficiency of patient-derived T cells, as the experiment's laboratory scale may be different from the manufacturing procedures needed to produce clinical-grade CAR-T cells.
2) The model is based on global data as no specific data or research paper exists for Hong Kong SAR, China. Local experimental results may differ from what is hypothesized in the model.
3) Human models are not used, and the experiment is not tested in person, so the effectiveness remains unknown.
4) The study of real-world accessibility may have been hindered by the lack of data on long-term outcomes and patient outcomes unique to Hong Kong SAR, China's healthcare paradigm.

In conclusion, by prioritizing manufacturing optimization first, a significant bottleneck is transformed into a life-saving accelerator, ensuring that patients who might otherwise not survive during cell production experience the long-lasting remissions that have revolutionized blood cancer treatment globally. This study not only demonstrates technical feasibility but also paves the way toward standardized, accessible CAR-T therapy as a significant advancement in Hong Kong SAR, China's healthcare system.

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