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Process Systems Engineering in Precision Medicine: Opportunities in Autologous CAR-T Therapy

B. Wayne Bequette[1,2]

[1]Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, New York, USA | [2]Center for Engineering and Precision Medicine (CEPM), New York, New York, USA

Correspondence: B. Wayne Bequette (bequette@rpi.edu)

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ABSTRACT

Autologous chimeric antigen receptor (CAR)-T therapies have given hope to many cancer patients whose other lines of treatment have failed. Unfortunately, limited manufacturing capability has resulted in many patients dying while on a waitlist. Similarly, since clinical trial treatments are personalized, it is difficult to treat many patients simultaneously, resulting in longer clinical trials. Therapeutic production often takes over 4 weeks, so a product failure means that a patient may need to wait another month for treatment, putting them at severe risk for disease progression. The labor-intensive manufacturing process has led to therapeutic costs of roughly $500,000 per treatment, which can be reduced by better automation and shorter manufacturing times. The goals of this article are to review CAR-T therapeutics development, manufacturing, and treatment, and to encourage the development of data analytics-based multi-scale decision support tools for all humans "in the loop." A systems approach is needed since prior treatments and current state of health (including the immune system and microbiota), initial cell quality, manufacturing failure, bridging and lymphodepletion therapy before infusion, and supply chain management, all impact treatment success. Continuous updates as more patient data are made available can lead to better treatment recommendations and outcomes.

1 | Introduction and Motivation

Often cancer patients have been treated with several lines of chemotherapy, radiation, or immunotherapy, but the cancer has progressed or returned after a period of remission. For some of these patients, chimeric antigen receptor (CAR)-T therapy, based on genetically programming immune system T-cells to target specific tumor antigens, has provided a "last chance" for survival. A notable example is Emily Whitehead, who in 2012, at the age of six, had relapsed after treatment for acute lymphoblastic leukemia (ALL). Given just days to live, she was the first patient to enroll and be treated in a Phase 1 trial for CAR T-cell therapy in pediatric patients with ALL (CHOP 2022); after 10 years one could argue that she has been "cured" [1]. The product she was given, Kymriah, was approved by the FDA in 2017.

The autologous CAR-T "vein-to-vein" manufacturing process includes the steps shown in Figure 1 beginning with leukapheresis to separate the immune cells from the patient's blood. Not shown is a quality control (QC) step before the infusion of product CAR-T cells, which are further expanded in the patient. If centralized manufacturing is used, the patient's immune cells are cryopreserved and shipped to the manufacturing site. The therapeutic product is also cryopreserved and shipped back to the hospital for infusion into the patient, after thawing and other preparation steps. Thus, centralized manufacturing involves at least 2 days of delay and additional manual handling. Current vein-to-vein time is typically 3–4 weeks. The traditional focus of chemical and biological engineers involves the sequence of separations-reactions-separations shown in Figure 1 with a distinction of the patient serving as the final bioreactor. A broader

Practical Application

Autologous CAR-T therapies have given hope to many cancer patients whose other lines of treatment have failed, but limited manufacturing capability has resulted in many patients dying while on a waitlist. This paper encourages the development of decision support tools that integrate data from lab studies, clinical trials, and *real-world* treatment, including the immune system and microbiota, for enhanced treatment decision-making. Continuous updates as more patient data are made available can lead to better treatment recommendations and outcomes.

systems view is shown in Figure 2, where there are many people and entities involved in the diagnosis and treatment process.

The initial diagnosis may be many months (or years) after a tumor has started growing and based on observations during an annual physical or other medical visit and perhaps after several rounds of testing. Referrals for different treatment regimens are often dependent on the type and location of hospital or clinic, background of the clinicians, recommendations of the tumor board, and insurance coverage. In some cases, it may be desirable to skip traditional treatment and go directly to advanced treatment (such as CAR-T). An individual's immune system and current state of cancer affect the quality of cells and the leukapheresis product, which is the "feedstock" for the biomanufacturing process. During the 3–4 weeks taken by the biomanufacturing process, it is likely that the patient will need bridging therapy (BT, chemotherapy, radiation, and surgery) followed by lymphodepleting chemotherapy to prepare the immune system for infusion of the CAR-T product. Although these cells are growing in vivo by roughly three orders of magnitude, there are often side effects such as cytokine release syndrome (CRS) or immune effector cell-associated neurotoxicity syndrome (ICANS), requiring that the patient be monitored for 4 weeks. All FDA-approved CAR-T products require monitoring for 15 years postinfusion.

The FDA has approved seven CAR-T therapies for the treatment of blood cancers [3], with five targeting the CD19 antigen and two targeting the BCMA antigen, as summarized in Table 1; links to FDA-related documents are in the Appendix. In this paper, we will refer to the trade names for the seven FDA-approved therapeutics. Although CAR-T has saved many lives, including Emily Whitehead, roughly 50% of patients receiving CAR-T are not successfully treated or relapse.

This article focuses on autologous CAR-T treatment of blood-based cancers because there is patient outcome data for the first FDA-approved products. Our contribution to the many CAR-T review articles is to provide a process systems perspective to better integrate data, modeling (including machine learning), decision-making, and automation to improve patient outcomes. In Section 2, we provide background on the challenges of CAR-T therapy, and in Section 3, we review efforts in each of the components of CAR-T manufacturing and delivery. In Section 4, we propose systems-related approaches that can be developed to improve patient outcomes. Two colleagues provide their perspectives in Section 5.

2 | Background

Hernández-López et al. [4] review cancer immunotherapies, including adoptive cell therapy, which includes CAR-T. CAR designs have evolved from the first-generation [5], with all seven FDA-approved therapies based on the second generation (Figure 3a), where the single chain fragment variable (scFv) is designed to bind to a specific antigen on the tumor, and the costimulatory domains are designed for improved "expansion" (growth of CAR-T cells). The specific design for Tisagenlecleucel/Kymriah, where scFv FMC63 binds to the antigen CD19 on the tumor cell surface, is shown in Figure 3b. McLaughlin et al. [6] review the first six approved CAR-T products, including their targets and costimulatory domains. They provide a comprehensive assessment of design variables that can be used throughout drug discovery and development using a model-informed drug development (MIDD) approach.

Sadelain et al. [7] and June and Sadelain [8] provide historical perspectives and outline challenges, while Lamprecht and Dansereau [9] present a tutorial of CAR-T focused on implications for practice and provide education resources for nurses and patients. Colina et al. [10] describe how specific changes in manufacturing steps, such as media composition and isolation or enrichment of specific T-cell subsets, can help address some of challenges with CAR-T cell efficacy. Abou-El-Enein et al. [11], Ceja et al., [12], and Agliardi et al. [13] provide reviews with a focus on rapid manufacturing for improved therapeutics efficacy. Dias et al. [14] provide a comprehensive bench-to-bedside view of nine critical steps from antigen/binder selection to trial initiation and product manufacture.

All FDA-approved CAR-T therapeutics are for hematological (liquid) tumors, which only involve about 10% of cancers [15, 16]. There are significant efforts to develop CAR-T therapies for solid tumors (e.g., NCT04503278 to treat CLDN6-positive relapsed or refractory advanced solid tumors).

Current supply and manufacturing technologies cannot meet the demand for CAR-T therapeutics. Tomtishen [17] estimates that only 4000 doses of CAR T-cell therapy were manufactured in 2021, while Gordon [18] suggests that more than 34,400 doses of the first six approved CAR-T products have been delivered since 2017. Consider CAR-T cell therapies that are FDA-approved for patients with triple-refractory multiple myeloma (MM) who have received more than four lines of standard therapies. Kourelis et al. [19, 20] found that the median time on the waiting list for idecabtagene vicleucel (Abecma) was 6 months with only 25% of patients receiving CAR-T eventually. Since the median survival of this group is less than 12 months, it can be assumed that many of the 75% of patients not receiving CAR-T died.

Manufacturing challenges for CAR-T are perhaps exacerbated in clinical trials. Chen et al. [21] estimated the value of reducing wait times for CAR-T treatment using data from the JULIET trial for Kymriah and reported that of 167 patients included in the analysis, 52 patients did not receive CAR-T for reasons including death and dropouts due to manufacturing delay. Indeed, we are motivated by experience with the BNT211 trial for CLDN6 CAR-T cell therapy for relapsed/refractory solid tumors, which was

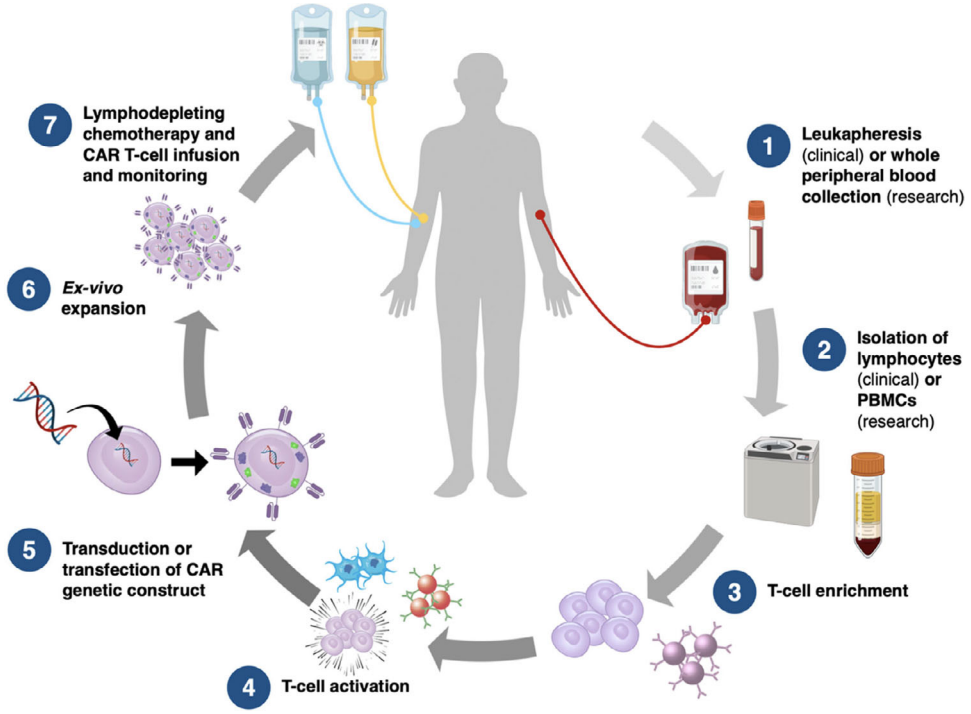


FIGURE 1 | Engineered T-cell manufacturing (Ramesh et al. [2]).

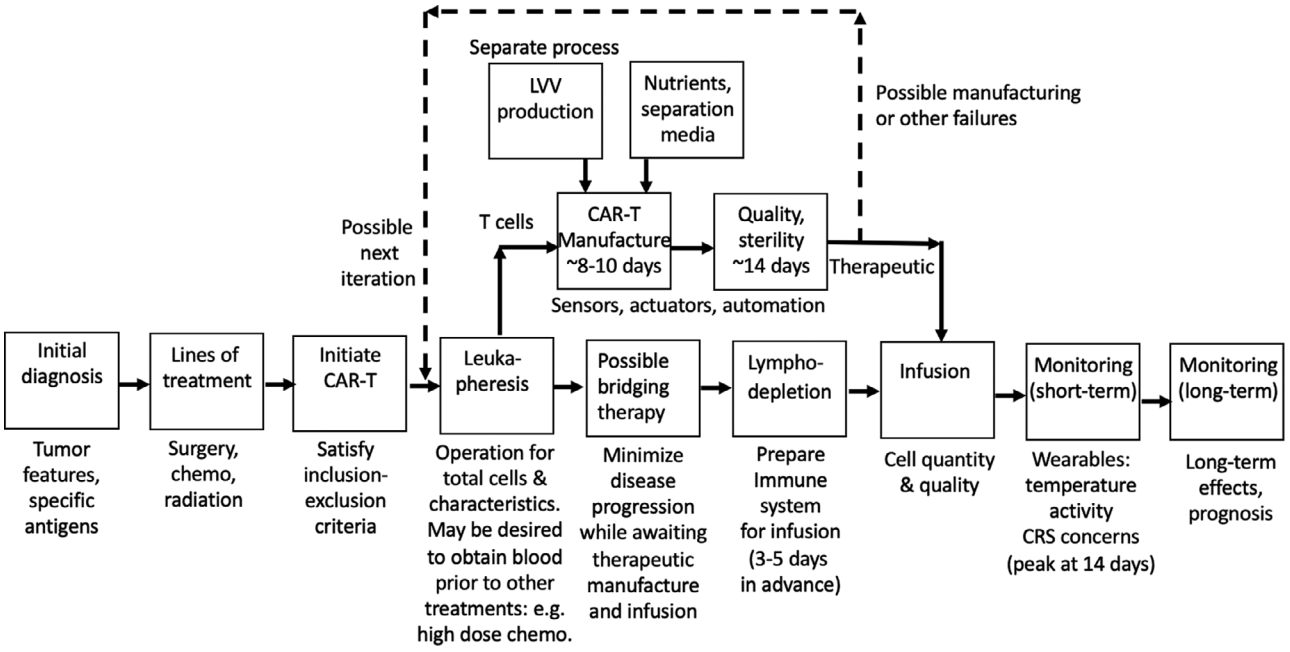


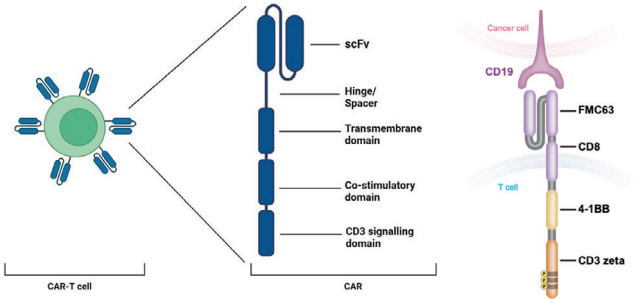
FIGURE 2 | Chimeric antigen receptor (CAR)-T therapy is usually initiated after several lines of prior treatment have failed to maintain the patient cancer-free. Systems engineering techniques (including data analytics, machine learning, modeling, optimization, and control) can be applied on multiple scales, from large-scale trials and treatment results to shorter time-scale manufacturing operation of bioreactors and separations processes.

delayed while changing from a manual to automated process, pushing back patient enrollment by over 6 months [22].

Arcangeli et al. [23] state that ~10%–20% of therapeutic failures are due to hurdles in the manufacturing process, and cite Zhang et al. [24], who note that an average of 10%–20% of patients who undergo leukapheresis do not receive CAR T-cell therapy due to failure of production and/or disease progression during the therapeutic cell production. Wang et al. [25] suggest that the best quality therapy may not lead to the best outcome for an individual patient, and it may be better to treat an individual more quickly with a therapeutic that does not strictly meet release criteria.

TABLE 1 | FDA-approved CAR-T products.

Brand name Company name Generic name Abbrev. name	Target antigen/ antibody	Hinge/ transmembrane	Costimulatory domains	Vector/promoter	Targeted cancers	Dose	Cost
Kymriah Novartis Tisagenlecleucel Tisa-cel	CD19 Mouse FMC63	CD8α/CD8α	4-1BB + CD3γ	Lentiviral EF1α	R/R CAYA B-ALL	$0.5–5 \times 10^6$ cells/kg, <50 kg $10–250 \times 10^6$ cells, >50 kg	$373,000
Yescarta Kite Axicabragene ciloleucel Axi-cel	CD19 Mouse FMC63	CD28/CD28	CD28 + CD3γ	Gammaretroviral LTR	R/R LBCL	2×10^6 cells/kg max 200×10^6 cells	$373,000
Tecartus Kite Brexucabtagene autoleucel Brexu-cell	CD19 Mouse FMC63	CD28/CD28	CD28 + CD3γ	Gammaretroviral LTR	R/R MCL	2×10^6 cells/kg max 200×10^6 cells	$373,000
Breyanzi Juno, BMS Lisocabtagene maraleucel Liso-cel	CD19 Mouse FMC63	IgG4/CD28	4-1BB + CD3γ	Lentiviral EF1α	R/R LBCL	$50–100 \times 10^6$ cells/kg, 1:1 ratio of CD4 and CD8 cells	$410,300
Abecma Bluebird, BMS Idecabtagene vicleucel Ide-cel	BMCA dual camel single-domain antibodies	CD8α/CD8α	4-1BB + CD3γ	Lentiviral EF1α	R/R MM	$300–460 \times 10^6$ cells	$419,500
Carvykti J&J, Legend Ciltacabtagene autoleucel Cilta-cel	BCMA dual camel single-domain antibodies	CD8α/CD8α	4-1BB + CD3γ	Lentiviral EF1α	R/R MM	$0.5–1 \times 10^6$ cells/kg max 100×10^6 cells	$465,000
Aucatzyl Autolus Obecabtagene Autoleucel Obe-cel	CD19 CAT	CD8-derived	4-1BB + CD3γ	Lentiviral	R/R ALL	10×10^6 cells (Day 1), 400×10^6 cells (Day 10)	$525,000



a. Generic (McLaughlin et al., 2023) b. Tisagenlecleucel/Kymriah (Tyagarajan et al., 2020)

FIGURE 3 | Chimeric antigen receptor (CAR)-T cell and components of a second-generation CAR.

NASEM [26] provided a comprehensive overview and detailed assessment of applying systems thinking to regenerative medicine, and NASEM [27] outlined opportunities to increase availability and reduce costs of regenerative therapies by

- investing in manufacturing models and regenerative medicine therapies that expand patient access,
- automating and standardizing much of the manufacturing and CMC processes,
- employing decentralized manufacturing models for autologous therapies,
- developing ML and artificial intelligence (AI) algorithms to control manufacturing and QC,
- continuing to develop regulatory guidance for manufacturers of regenerative medicine therapies.

The focus of this article is on autologous CAR-T therapy. We refer the reader to Caldwell et al. [28] and Lonez and Breman [29] for allogenic therapy status and challenges.

3 | Discovery/Development, Manufacturing, and Treatment: State of the Art and Challenges

Carini and Seyhan [30] provide a perspective on the potential use of AI in drug development and clinical practice, including the use of Chatbots and large language models (LLMs), while highlighting concerns and limitations in applying AI. Verkouter et al. [31] and Jacobs et al. [32] develop a data management framework for personalized medicine which is adaptable and can process hundreds of parameters from multiple sites, remains in sync at multiple locations, has real-time syncing to analytics, and is compliant with international privacy legislation. An example use case involved data integration from a multicenter clinical trial.

3.1 | Immune System

B-cell lymphocytes produce antibodies in response to antigens. T-cells are largely composed of helper cells (with a CD4 receptor) and cytotoxic cells (with a CD8 receptor). The CD8+ T-cells kill cells infected with viruses and bacteria and tumor cells. There are several "hallmarks of cancer" that include the ability of tumor cells to "evade" the immune system [33–35]. The focus of this review is on blood (often called "liquid") cancers (leukemia, MM, non-Hodgkin lymphoma, and Hodgkin lymphoma) that represent 10% of all cancers, with the rest being solid tumors.

T-cells differentiate into different subsets, which can lead to "exhaustion" allowing tumors to effectively escape and grow. Baessler and Vignali [36] provide a comprehensive review of T-cell exhaustion, including methods to circumvent exhaustion. Zhuang et al. [37] provide a comprehensive assessment of T-cell subsets in healthy volunteers compared with kidney transplant recipients, using flow cytometry.

3.2 | CAR-T Design and Synthesis

Prybutok et al. [38] present an agent-based modeling approach to predict T-cell quality and impact on treatment outcomes. They provide a comprehensive review of CAR-T design choices and parameters, including intracellular costimulatory domains, tuning the affinity of receptor–antigen interactions, CAR T-cell dose, and CD4+:CD8+ CAR T-cell ratio. They suggest that their approach could ultimately enable identification of promising treatment strategies to accelerate solid tumor CAR T-cell design-build-test cycles. Razavi et al. [39] review how CAR T-cell gene constructs combine a tumor-targeting device coupled to the T-cell receptor zeta chain domain with different signaling domains and suggest that the next generation of CARs can be improved by regulating their metabolism. Zhu et al. [40], and Gumber and Wang [41] note that CAR-T design, such as selection of the costimulatory domain, affects T-cell exhaustion.

3.3 | Diagnosis and Treatment Referrals

For MM and lymphoma, the median time from initial symptoms to initial treatment is 5 months [42]. It is important that patients and families be informed enough to play a role in treatment decisions. McConville et al. [43], in an article targeting nurses, discuss the importance of educating patients and families undergoing CD19-targeted CAR T-cell therapy. Haslam et al. [44] note that less than 20% of patients are likely to receive therapies for which they are eligible, referring to DeMartino et al. [45] who suggest that to maintain the recent trend of cancer drug spending, the 2018 cancer drug approvals need to be used in fewer than 20% of eligible patients. Hoffmann et al. [46] discuss barriers to receiving

CAR-T therapy and provide recommendations for a collaboration between referring physicians and academic centers. Community physicians may not fully appreciate CAR-T treatment options and patients may not travel to a treatment center or are concerned about expense or postinfusion toxicity. Pasetto et al. [47] present a Bayesian-based approach to handle uncertainty, and consider updated information, to enable tumor boards to make better treatment recommendations. Kourelis et al. [20] recognize that CAR-T treatment availability remains limited and present a survey of 20 treatment centers on the role of ethical considerations in resource allocation. Benzinger et al. [48] review AI methods for ethical decision making, with a major concern being that AI might replicate existing decision-making biases.

3.4 | Prescreening

Baguet et al. [49] cite the growing need to identify early risk factors for unsuccessful manufacturing production or treatment and provide a review of possible predictive factors. They recommend that prescreening of patients should include disease condition, previous treatments, and apheresis product quality. Indeed, they suggest that identifying predictive factors in peripheral blood would help in selecting patients before apheresis to avoid treatment delay and the manufacturing of the costly treatments. Levstek et al. [50] provide a comprehensive review of predictive biomarkers and suggest that a more integrative approach is needed to better predict treatment outcomes.

3.5 | The Microbiome

Stein-Thoeringer et al. [51] found that patients who had taken strong, broad-spectrum antibiotics before receiving CAR-T cell therapy had less microbiome diversity and poorer outcomes to treatment. They also found that the bacterium *Bacteroides eggerthii* was associated with a higher chance of developing a response to treatment, while *Bacteroides stercoris* was associated with patients not achieving a response. Matson et al. [52] review the relationship between the host microbiota and cancer and anti-tumor immune response, with implications for cancer therapy. Asokan et al. [53] provide a comprehensive review of the effect of the microbiome on CAR-T treatment outcomes and suggest that the potentially confounding nature of antibiotic usage upon microbiota, as related to CAR-T therapy, should be further studied. Smith et al. [54] study the effects of the microbiome on axi-cel (Yescarta), tisa-cel (Kymriah), and 19-28z at Memorial Sloan Kettering and Penn and find that exposure to piperacillin/tazobactam, meropenem, or imipenem in the 4 weeks before CD19 CAR T-cells was associated with worse survival and increased toxicity in patients with ALL and NHL. Based on preclinical models and ALL patients treated with Kymriah, Uribe-Herranz et al. [55] suggest that CAR-T therapy outcomes can be improved by using vancomycin to modulate the gut microbiota.

3.6 | Leukapheresis

Bonig et al. [56] provide a detailed presentation of the screening requirements before an apheresis collection and discuss how to design an apheresis collection; the two common platforms, Amicus and Spectra Optia, have differing collection characteristics. Wang et al. [57] discuss how one type of cell (CD14+) in the leukapheresis product could predict problems with therapeutic production. By reducing the concentration of CD14+ to less than 40% they could maximize production success. Donor-to-donor variability is discussed by Liu et al. [58], who suggest multi-task learning, Bayesian latent variable modeling, and representation learning for finding donor-specific models to improve monitoring and control in manufacturing. Zhang and Larson et al. [59] classify variability into three categories: patient leukapheresis material, manufacturing process, and QC testing and propose patient-specific product specifications. Elavia et al. [60] found that the composition of leukocytes used to manufacture CAR-T cells can affect cell expansion and the composition of CAR-T cell products. Korell et al. [61] found that patients undergoing NHL or ALL CAR-T therapy with axi-cel (Yescarta), tisa-cel (Kymriah), or HD-CAR-1 (under clinical investigation) had a higher rate of remission if their leukapheresis products were like those of healthy donors.

Qayed et al. [62] provide a comprehensive review and guidance for leukapheresis based on their experience with Kymriah. They propose that future studies evaluating the phenotype of T-cells in peripheral blood before leukapheresis and understanding the immuno-modulatory effects of chemotherapy on T-cells could be useful to optimize leukapheresis.

Better et al. [63], in an Abecma study, note that the median absolute lymphocyte count of the apheresis material was 0.7×10^9/L, with a wide range of $0.1–2.9 \times 10^9$/L; similarly, the CD3 count ranged from 3% to 97% of the apheresis product, but greater than 90% in the final therapeutic product. Traynor et al. [64] found that therapeutically relevant doses of CAR-T cells could be successfully manufactured from whole blood.

3.7 | Cell Washing

Anticoagulants added during the apheresis process, red blood cells and platelets are contaminants which are usually removed in a washing step [65]. Li et al. [66] review the historical origin and technologies behind each wash-and-concentrate device, and their relative advantages and disadvantages in cell therapy applications. Kunitskaya and Piret [67] find that in concentrated cell washing, the cells are frequently exposed to reduced oxygen, temperature, pH, and nutrient levels. Average cell recovery at room temperature for 3 h was 91%, and they recommended operation at lower temperatures if longer periods of time were needed. After expansion, there is also a cell washing and concentration step before cryopreservation.

3.8 | Cryogenic Freeze/Thaw

It is often necessary to freeze the leukapheresis product before further processing, particularly when centralized manufacturing is used. Tyagarajan et al. [68] compare leukapheresis product (Kymriah) stored either at 2–8 or 10–25°C. Cells stored at 10–25°C showed reduced viability as early as 24 h and deteriorated progressively during the 72-h assessment period, whereas cells stored

at 2–8°C remained viable throughout the assessment period. On the other hand, Brezinger-Dayan et al. [69] found that frozen products still exhibit high in vitro potency, and cryopreservation did not seem to affect the clinical outcome. Jandova et al. [70] review cryogenic processing and suggest possible substitution of DMSO with DMSO-free cryopreservation medium, the use of outer metal cassettes during storage and transport, the strict control of temperature records during transport and storage, and the use of continuous evaluation of the post-thaw viability, recovery, and sterility of the starting material.

3.9 | Selection/Enrichment

Following apheresis, a cell selection or enrichment step may be used to further isolate target cells, since contaminating red blood cells, monocytes, and neutrophils in the starting material may adversely affect T cell expansion in culture as well as final CAR-T cell product characteristics [71]. Methods can be broadly characterized as either eliminating contaminating cells or specifically selecting for T-cells. GMP-compliant cell elimination techniques include density gradient separation, counter-flow elutriation, and flask adherence techniques, while cell selection techniques include various antibody-fluorochrome conjugates and antibody-magnetic bead conjugates (including dynabeads). The difference between the Kite products Yescarta and Tecartus is that Tecartus has an enrichment step while Yescarta does not. Noaks et al. [72], in a study with healthy donors, found that removal of monocytes from leukapheresis improved the level of activation two-fold. Breyanzi comprises two different cell products—made separately from CD4+ and CD8+ starting cell populations—that are mixed before administration [73].

3.10 | T-Cell Activation

Wang and Rivière [74] summarize several techniques for T-cell activation, including by soluble antibodies, expamers, antibody-coated beads, and artificial antigen-presenting cells (AAPCs). Dynabeads (Thermofisher) are paramagnetic beads that are used for both selection and activation of CD3+ T-cells; they must be removed at the end of the culture before formulation. TransAct CD3/CD28 beads are polymeric nano-beads that are bio-degradable and can be largely washed out during formulation, therefore do not need to be removed at the end of the process. These T-cell activation reagents are currently the most widely used for the activation of T-cells in CAR-T cell manufacturing [65].

Stock et al. [75] note that the predominant T-cell activation strategy in recent studies is the use of anti-CD3/anti-CD28 antibody-coated magnetic beads, followed by monoclonal antibodies. Zhang et al. [76] show that accommodating individual differences in the donor cells by controlling the precise level of stimulation during T-cell activation directly impacts the functional attributes of CAR-T cell products, including level of exhaustion. Gargett et al. [77] compare three methods of activation and find that magnetic selection of CD4+ and CD8+ CAR-T cells before culturing results in more central memory phenotype with better cytolytic activity, but with slower kinetics.

3.11 | Lentiviral Vector (LVV) Manufacturing

A separate manufacturing process is used to supply the viral vectors with the encoded genetic information to "program" the T-cells for action against the tumor cells (see [78], for a review). The production of lentiviral vectors using good manufacturing practice (GMP) remains a challenge [79]. Wang et al. [80] show that a fixed-bed reactor can produce enough vector to transduce 500 patients.

Transfiguracion et al. [81] propose ion exchange chromatography for in-line process monitoring of lentivrial vector processing. Ran et al. [82] note that using an alternative to a lentivirus could reduce costs substantially. Perry et al. [83] study three different envelop proteins and bioprocessing conditions, such as pump flow and temperatures. Their results contradict a common misconception that lentiviral vectors are susceptible to shear or high salt concentration in ion exchange chromatography.

Comisel et al. [84] perform a detailed economic analysis of the type of reactor and volume (10-layer, rocking reactor, hollow fiber, stirred tank, and fixed bed), LV concentration, and number of doses/year, and find that costs per dose vary by more than an order of magnitude—from $38,000/dose (10-layer) to $1200/dose (2000 L stirred tank at 10,000 doses/year).

3.12 | Transduction

Prommersberger et al. [85] provide a detailed protocol for the transduction of T-cells using anti-CD3/CD28 Dynabeads and a lentiviral vector. Watanabe et al. [86] note that ex vivo culture conditions could significantly impact CAR T-cell functionality. Ghassemi et al. [87] propose rapid manufacturing by transduction of nonactivated T-cells. Lentiviral transduction is used for Kymriah, Breyanzi, Abecma, Carvykti, and Aucatyzl, while retroviral transduction is used for Yescarta and Tecartus [88].

Jin et al. [89] studied transfection temperatures of 4, 25, 32, and 37°C and found that infection at 32°C produces CAR-T cells with the greatest proportion of naive cells and the lowest expression of immune checkpoints. They also note that Novartis and Bristol Myers Squibb (BMS) use lentiviruses to infect at 37 and 32°C respectively to produce CAR-T cells, and Kite uses a 32°C infection with gamma-retrovirus to infect T-cells.

3.13 | Bioreactors: Expansion

Vormittag et al. [65] in a comprehensive review of CAR-T manufacturing find that rocking motion bioreactors are the major expansion reactor. Garcia-Aponte et al. [90] provide an extensive overview of bioreactor types and systems (G-rex, Prodigy) where they have been implemented and suggest that cellular metabolic profiles could provide additional phenotypic information to expand preferred cell subpopulations. Baradez et al. [91] use a single use bioreactor for an autologous cell therapy process, with pH, temperature, and dissolved oxygen sensors, and detail how Raman spectroscopy measurements were correlated with off-line measurements.

Stock et al. [75] discuss the effect of culturing conditions during ex vivo T-cell expansion, different T-cell activation strategies, cytokine supplementation, and specific pathway inhibition for the differentiation blockade, as well as CD8+/CD4+ ratio. Colina et al. [10] describe the strategies used to selectively expand specific phenotypic subsets.

Atanasova et al. [92] perform a detailed fluid mechanics analysis of the CliniMax Prodigy CentriCult bioreactor, which operates by spinning an outer cylinder intermittently. They suggest that their results could be used to change the periodic operation to improve mixing performance during the cell expansion step in CAR-T therapy manufacturing.

Costariol et al. [93] note that there have been unsubstantiated concerns that T-cells grow better under static conditions or that the potential fluid dynamic forces in a stirred-tank bioreactor system would impair the growth and/or function of T-cells. They demonstrate that T-cells can be cultured in stirred-tank bioreactors, and that higher impeller agitation speeds yield higher cell densities with no adverse impact on cell quality. Baudequin et al. [94] provide an overview of types/classes of bioreactors, focused on T-cell expansion.

Zhu et al. [40] note that longer expansion time typically leads to higher T-cell exhaustion. The addition of cytokines such as IL4, IL7, and IL21 supports the stemness of CAR T-cells and restrains the exhaustion [95]. Jenkins et al. [96] outline culture strategies that focus on boosting the metabolic program of CAR-T cells as a mechanism to overcome the immunosuppressive tumor microenvironment (TME). For example, supplementing the culture media with L-arginine results in metabolic activity leading to the formation of central memory-like T-cells, with enhanced survival and improved anti-tumor activity. Geltink et al. [97] showed that glucose restriction during CAR-T cell expansion was a potential strategy to enhance the efficacy of CAR-T cell therapy. Chen et al. [98] develop a novel two-chamber perfusion reactor resulting in the enrichment of central memory T-cells compared with the static cultures. Egan et al. [99] use on and off-line data to continuously update the dynamic model of a perfusion bioreactor and include "soft sensor" estimates of variables not directly measured. This approach enables an estimate of the optimal harvest time for any particular patient.

3.14 | Earlier Treatment

Generally, the FDA has approved the use of the therapeutics after two lines of treatment have failed. Locke et al. [100] report a Phase 3 randomized trial (ZUMA-7) of axi-cel (Yescarta) versus standard chemoimmunotherapy for large B-cell lymphoma (LBCL) for second-line treatment. Axi-cel (Yescarta) therapy led to significant improvements, compared with standard care, in event-free survival and response. Further, Filosto et al. [101] note that, in apheresis material and final product, a naive T-cell phenotype (CCR7+CD45RA+) expressing CD27 and CD28 was associated with improved response durability, event-free survival, progression-free survival (PFS), and a lower number of prior therapies. Soon thereafter, Kite [102] announced FDA approval of axi-cel (Yescarta) for the first-line treatment of adult patients with newly diagnosed, high-risk LBCL who have a positive positron emission tomography (PET) scan after two cycles of first-line chemoimmunotherapy.

3.15 | Rapid Manufacturing

A major reason that CAR-T therapy fails is the differentiation of T-cells from central memory to effector memory phenotypes over time, leading to exhaustion, so there is an incentive to decrease manufacturing times.

Yang et al. [103] develop a rapid manufacturing process, reporting that CD19 F-CAR-T cells demonstrated excellent proliferation with a younger cellular phenotype, less exhaustion, and more effective tumor elimination compared to conventional CAR-T cells in a preclinical study. In a Phase I study, F-CAR-T cells were successfully manufactured and infused in all 25 enrolled pediatric and adult patients with B-ALL.

Dickinson et al. [104] present 2-day manufacturing results for YTB323 (Rapcabtagene autoleucel), which expresses the same validated CAR as Kymriah with enhanced clinical activity at a 25-fold lower dose. Shah et al. [105] provide an update recommending the approach for Phase II/III studies. Costa et al. [106] used the NEX-T platform to produce BMS-986354, an investigational BCMA-targeted CAR T-cell product, with a less differentiated, more potent cellular drug product than orva-cel (orva-cel development has been discontinued by BMS). At low doses, it had a favorable safety profile and efficacy durable responses in patients with RRMM. Chen and Liu [107] provide a perspective on rapid manufacturing, noting several therapeutics under investigation, including YTB323.

3.16 | Quality Control and Product Release Testing

Although the manufacturing process may take less than 2 weeks, the QC testing and release by the Quality Assurance (QA) unit typically takes 2–3 weeks [105]. Mohammed et al. [108] propose a rapid (4-day) sterility testing procedure based on detecting fluorescently labeled microorganisms. To negate the additional delays due to QC/QA testing of the therapeutic product, Boyer et al. [109] propose initial certification and release based on analysis of microbiologic tests performed on samples during the production process, rather than from the final product.

Chong et al. [110] present results for Kymriah and found no statistically significant association between cell product viability and complete remission rate for either ALL or DLBCL, so they suggested that the required viability be reduced from 80% to 70%. Similarly, Dulobdas et al. [111] in a retrospective review of 981 LBCL patients found that overall survival (OS) and PFS were not significantly different for patients infused with out of specification product.

3.17 | Bridging Therapy (BT)

Bhaskar et al. [112] note that BT (chemotherapy, immunotherapy, radiation, and steroids) can be very important when manufacturing and other problems lead to substantial delays between

enrollment and therapeutic infusion. They provide a detailed literature review and suggest decision-making for BT. Jain et al. [113] use data from the multicenter US Lymphoma CAR-T Consortium and suggest that BT may be safe without a significant impact on long-term survival for patients who require disease stabilization during the manufacturing period. There was no clear advantage to using radiation-based BT over nonradiation-based BT.

3.18 | Lymphodepletion

Lickefett et al. [114] perform a detailed meta-analysis of the use of lymphodepletion (LD) in CAR-T therapy, reporting 171 studies. They noted that the main purposes of LD are (1) the reduction of endogenous lymphocytes to prepare a niche for engraftment of CAR-T infusions, (2) the reduction of tumor cells to avoid rapid exhaustion of CAR-T, and (3) preparation of the microenvironment to ensure long-term survival of CAR-T. The most common agents are the combination of fludarabine and cyclophosphamide. Hirayama et al. [115], in a study involving patients with aggressive non-Hodgkin lymphoma treated with CD19 CAR-T cells, found that PFS was superior in patients who received high-intensity LD and achieved a favorable cytokine profile compared with those who received the same intensity of LD without achieving a favorable cytokine profile.

Fabrizio et al. [116] in a retrospective analysis of the data from 152 pediatric patients taking Kymriah for relapsed/refractory B-cell ALL found the optimal fludarabine exposure for cyclophosphamide/fludarabine lymphodepleting chemotherapy. Patients with a lower exposure had a 2.5-fold higher risk of relapse and twofold higher risk of relapse/loss of B-cell aplasia. They suggest testing personalized PK-directed dosing to achieve optimal fludarabine exposure in prospective trials, with a goal of reducing disease relapse after CAR T-cell therapy.

3.19 | Infusion and Pharmacokinetics/Pharmacodynamics

Singh et al. [117] use in-vitro data to construct a multi-scale pharmacokinetic–pharmacodynamic model and to characterize the relationship among CAR-affinity, antigen abundance, tumor cell depletion, and CAR-T cell expansion; in vivo xenograft mouse models were used to establish an in vitro and in vivo correlation. Because of biological variability, Joslyn et al. [118] present a method to capture the distinct cellular kinetics of each patient in response to distinct dose amounts and compositions of TCR-engineered T-cells.

Kirouac et al. [119] provide an overview of the pharmacology of CAR-T cells, noting that dose fractionation, where the infusion is provided as a series of two or three doses over a time-period, can reduce the risk of toxicity. For example, in a study of ARI0002h, a humanised, BCMA-directed CAR-T-cell therapy, patients received an initial fractionated infusion on Days 0, 3, and 7 and a booster dose at least 100 days after the first infusion [120]. Frigault et al. [121] review 18 studies where dose fractionation was used and suggest that administering dose fractions over 2–3 days instead of a single dose infusion may mitigate the incidence and/or severity of CAR-T cell toxicity, including CRS and neurotoxicity, especially in patients with high tumor burden and for CAR-T cell therapies requiring higher doses for efficacy. The most recently FDA-approved therapeutic, Aucatzyl, is delivered in a split-dose, with the second dose at around 10 days [122].

Ong et al. [123] review efficacy and dosing amounts for the first six FDA-approved therapies. For example, Yescarta is delivered in 68 mL bags with a dose of 2×10^6 cells/kg and a maximum dose of 2×10^8 cells. Rotte et al. [124] provide a comprehensive review of clinical studies involving the effect of dose magnitude on response and toxicity for the first six FDA-approved CAR-T therapies. Sheih et al. [125] find that clonal diversity of CAR-T cells is highest in the infusion products and declines following infusion.

3.20 | Postinfusion Toxicity and Wearable Technology

Banerjee et al. [126] discuss the toxicity risks of CAR-T therapy and provide a review of digital health tools that can be used to detect toxicity-related problems. A major concern is CRS, with fevers as a hallmark indication typically occurring within 1–2 weeks after treatment. Many patients remain hospitalized, although a significant number could be outpatient, particularly if *wearables* are used to help detect problems like CRS. Bogatu et al. [127] use machine learning algorithms to identify CRS based on specific cytokine peak concentrations and evidence from previous clinical studies.

Cox et al. [128] present a wearables (continuously transmitting pulse, respiratory rate, oxygen saturation, skin, and axillary temp), a tablet, and a BP cuff (readings 3×/day) involving patients within 15–30 min of an infusion facility. Romon et al. [129] review home care for stem cell transplant and CAR-T patients but suggest that further research is required to prove the advantages of at-home/outpatient management over in-patient treatment. Peterson et al. [130] reported a study with a continuous-wear biometric sensor involving ten patients for up to 90 days following allogeneic bone marrow transplant and up to 30 days following CAR T-cell therapy or autologous bone marrow transplant, engaging with a chatbot by SMS text for health status checks and symptom reporting. Brown et al. [131] reviewed nine studies involving different wearable device brands for varying lengths of time but were concerned about bias in all but two of the studies.

Ceballos et al. [132] note that Yescarta, Kymriah, and Breyanzi have revealed incidence rates of CRS of around 92%, 60%, and 42%, respectively. Fischer et al. [133], in an Abecma study, show that in vivo expansion of CD8 is essential for durable remission in RRMM patients. An increased fraction of CD8 at day of leukapheresis in combination with successful LD positively influences the outcome, and patients at risk for higher-grade CRS can be identified before LD. Acosta-Medina et al. [134] also find that patients at risk for toxicity can be identified before LD, based on EASIX and modified EASIX scores.

3.21 | Combination Therapies and Time-Dependent Considerations

Dabas and Danda [135] suggest that combining immune checkpoint inhibitors with CAR-T may prevent T-cell exhaustion and

refer to Grosser et al. [136] and Miller et al. [137]. Uslu et al. [138] review CAR-T combination therapies, including immune checkpoint-targeting antibodies, small molecules, and radiation. They provide examples of clinical trials testing different agents to (i) enhance efficacy of, and (ii) decrease toxicities associated with, CAR-T therapeutics. Zemek et al. [139], although not studying CAR-T, discuss immune checkpoint therapy (ICT) and suggest that combinations of ICT with other treatment modalities should consider time-dependent immunological events. Zhang et al. [140] provide an overview of how metabolism can be changed during each stage of the manufacturing process.

Bachiller et al. [141] developed a dual CAR-T (ARI0003) based on their academic anti-CD19 (ARI0001) and anti-BCMA (ARI0002h) CAR-T cells and test it in preclinical studies. A Phase I clinical trial (CARTD-BG-01, NCT06097455) has been initiated to evaluate the safety and efficacy of ARI0003 in NHL. Larson et al. [142] report a Phase I clinical trial studying autologous naive and memory T-cells engineered to express a bispecific anti-CD19/CD20 CAR for patients with relapsed/refractory non-Hodgkin lymphoma.

3.22 | Centralized Versus Distributed Manufacturing

Ran et al. [82] note that decentralized production can yield a product in 12–14 days, compared to the current 3–4 weeks with centralized manufacturing, since cryopreservation and shipping are not needed. Zhang et al. [24] produced CAR T-cells in as short as 8 days, demonstrating the feasibility of successful decentralized CAR-T-cell production in a University Hospital for Phase I clinical trials. Elsallab and Maus [143] provide a comprehensive review of manufacturing protocols and clinical studies of autologous CAR-T cell therapy products and provide a strong argument for distributed manufacturing to meet the demand for these therapeutics.

Bonnot et al. [144] estimate that CAR-T therapeutic costs can be reduced from \$214–288K to \$40–100K by using automated decentralized manufacturing. James [145] presents three different manufacturing strategies, from fully manual and a Class B cleanroom environment to fully closed and automated in a Class C cleanroom environment. He proposes an intermediate solution, where incubators are centralized and requiring less Class B space.

3.23 | Automation

Lopes et al. [146] state that labor represents approximately 50% of manufacturing costs since most of the processing steps involve manual operation. McCoy et al. [147] compare four levels of automation for producing CAR-T products, noting that the initial capital costs increase with each level, but a much higher throughput is obtained with more automation. Several papers have proposed the use of advanced analytics, monitoring and control strategies, but they are not yet state of the art. Hort et al. [148] provide a vision for how a "smart hospital" will have a highly automated manufacturing process, with less manual intervention than current technology. Hort et al. [149] refer to the AIDPATH (Artificial Intelligence-driven, Decentralized Production for Advanced Therapies in the Hospital) project to develop and implement Industry 4.0 concepts in CAR-T manufacturing. They stress the importance of device manufacturers making their products open for connectivity. Bäckel et al. [150] also refer to the AIDPATH project, which aims to develop an open platform to produce CAR-T cells using flexible automation and the integration of AI. They note that predicting patient side-effects after treatment has been a large focus of AI research. It important to estimate and update parameters to update "digital twin" models during manufacturing, as presented by Aghamiri and Amin [151].

3.24 | Automated Manufacturing Platforms

Several companies, including Miltenyi, Apel et al. [152], Lonza [15, 153], Sartiorius, ThermoFisher Scientific (Gibco), Cytiva, and others, have developed platforms for manufacturing cell therapies, but limited quality measurements and control are available. Syed et al. [154] provide a comprehensive review, with photos of the manufacturing platforms available, including CliniMACS Prodigy, Cocoon, ekko acoustic cell processing, Xuri cell expansion, Quantum cell expansion, G-Rex bioreactor, CAR-TXpress platform, Gibco Cell Therapy Systems, and others. Shah et al. [105] review centralized and decentralized manufacturing models, as well as the major manufacturing platforms, and provide a nice summary of the first six approved CAR-T products. They note that at least 3 academic institutions have started producing in-house products: Case Western Reserve University Hospital (CWRU/UH), Medical College of Wisconsin (MCW) in Milwaukee, and Stanford, all using the CliniMACS platform and a lentiviral vector. Illic and Liovic [155] refer to the recently developed, fully enclosed, Cellares Shuttle, which can process 16 batches in parallel [156].

Song et al. [157] compare four expansion platforms (CliniMACS Prodigy, Xuri W25 rocking platform, G-Rex gas-permeable bioreactor, and static bag culture) and find differences in T-cell differentiation states. The Prodigy resulted in a lower total cell expansion but significant enrichment in naïve/stem central memory-like cells compared to bag and G-Rex. They suggest that low oxygen during expansion may lead to less differentiated product.

3.25 | Process Analytical Technologies (PAT)

Piscopo et al. [158] highlight the importance of interdisciplinary collaborations in the manufacturing of CAR-T cells. They propose PAT combined with Model Predictive Control (MPC) to improve bioreactor cell yields. Marshall et al. [159] explore the gap between in-process product characterization (phenotype, cell stress, and cell cycle) and properties monitored that are largely inferential in nature (e.g. dO_2, pH, glucose consumption, or cell mass). Odeh-Couvertier et al. [160] summarize objectives of PAT and use several machine learning algorithms to identify a set of critical process parameters and critical quality attributes from multiomics data, measured from time-dependent cultures, to predict end-of-manufacturing product quality. Wang and Bowles-Welch et al. [161] provide an assessment of the state of the art of PAT in cell manufacturing and suggest that high-throughput and

high-dimensional analyses, machine learning, and novel sensor technologies can lead to improved product quality.

Caldwell et al. [162] demonstrate the use of feedback control as part of a next-generation bioprocess for patient-specific cell therapies, by applying culture control of secreted TGF-β1 (which has an inhibitory effect) over a 12-day period, using a fed-batch bioreactor. Aifuwa [163] notes the limited number of in-line sensors currently available and suggests that viable cell concentration (VCC) or total cell number would be very beneficial for monitoring and control. Liu et al. [164] develop a low cost, disposable, sensor to measure cell viable cell count in real-time. The impedance-based sensor accuracy is demonstrated using a cell culture and calibration based on cell image analysis.

Blache et al. [165] present flow cytometry for use in leukapheresis product analysis, manufacturing, final therapeutic, as well as patient immunological monitoring after infusion. They also refer to Mues et al. [166], who discuss their use of assays for real-time monitoring in manufacturing, as part of the CliniMACS Prodigy. Lazarski and Hanley [167] review flow cytometry applications in cell and gene therapy, while Isaacson et al. [168] use flow cytometry to assess cell type composition, cell viability, and expression of T-cell activation markers throughout the manufacturing process. Yang et al. [169] review how multidimensional omics data can be used in a wide-range of CAR-T applications, from predicting T-cell exhaustion to analyzing the microbiome based on fecal samples.

Hewitt et al. [170] note that current closed, automated, and scalable cell therapy manufacturing platforms have limited ability to offer real-time culture data beyond pH and dissolved oxygen or implement feedback-driven automation. They discuss the power of Raman spectroscopy but note that the cost and ongoing algorithm optimization make it unsuitable for autologous cell therapy manufacturing.

Williams et al. [171] develop a PAT-based system to monitor and improve the metabolic activity of an HEK293T LVV production process by changing the pH setpoint with time. Nettleton et al. [172] as part of the ADIPATH project, use an Aglaris perfusion reactor to demonstrate the combined use of multiple sensors and AI-techniques to improve bioreactor performance in series of six batch experiments.

Tong et al. [173] have developed a deep learning image analysis technique using pre-treatment CT and PET/CT images to predict lesion-level responses in patients with lymphomas treated with CAR-T. They note that these approaches may provide new information to predict which patients will (or not) respond to treatment in advance of initiating therapy. Fröse et al. [174], in mice studies, image CAR-T cells with a labeled antigen and find that the antigen does not affect CD19 functionality, while predicting early mortality risk.

3.26 | Supply Chain Management, Simulation, and Decision-Making

Papathanasiou et al. [175] provide an overview of the resource task network, and a vision of how the supply chain will change with increasing product demand. Wang et al. [176] present a supply chain simulation framework (AuCTsim) for autologous CAR-T manufacturing and present three scenarios: (i) centralized production, (ii) regional manufacturing hubs, and (iii) point of care production. There are several challenges compared to classic supply chain problems because a patient both provides a critical feedstock and consumes the final product. Tseng et al. [177) propose the SimPAC framework, which is more dynamic than AuCTsim and includes the effect of changes in the patient condition. A limit to these current frameworks is that they do not have an appropriate level of "granularity," since they do not incorporate detailed dynamic bioreactor models in the "manufacturing agent," for example.

3.27 | Clinical Trial Versus "Real-World" Performance

Locke et al. [178] present a timeline of the rapid clinical development of axi-cel (Yescarta) from bench to bedside. Yassine et al. [179] review patients treated with Yescarta and Kymriah in the real-world setting show comparable results to those reported in clinical trials in terms of efficacy and toxicities. Pasquini et al. [180] report that Kymriah in the real-world setting demonstrates outcomes with similar efficacy and improved safety as those seen in the pivotal trials. Westin et al. [181] summarize the JULIET (Kymriah), ZUMA-1 (Yescarta), and TRANSCEND (Breyanzi) trials and report that patients treated with commercial products were older than those in the clinical trials.

Bachy et al. [182] compare real-world results for Kymriah and Yescarta, for DLBC and find that Yescarta had the best overall response rate/complete response rate (ORR/CRR), PFS, and OS. They also note that Kymriah is a 4-1BB costimulatory domain-based second-generation CAR T, whereas Yescarta is CD28 based. This paper also refers to real-world studies by Jacobson et al. [183] (Yescarta), Nastoupil et al. [184] (Yescarta), Sesques et al. [185] (B-cell lymphoma), and Vercellino et al. [186].

Cuffel et al. [187] studied 30 patients treated with tisa-cel (Kymriah) and found that the proportions of CD4 and CD8 naïve/stem cell memory T-cells ($T_{N/SCM}$) in the apheresis starting material are critical for the quality of the CAR T-cell product. A lower ratio of CD4 $T_{N/SCM}$ to CD8 $T_{N/SCM}$ in the apheresis starting material was associated with a higher PFS. Awasthi et al. [188] note that Kymriah (tisa-cel) is available in 34 countries and at more than 430 certified treatment centers, and thus far, it has been shipped for more than 7000 patients in clinical trials and the real-world setting. Lambert et al. [189] noted that the outcomes of patients with lymphoma treated with tisa-cel (Kymriah) in real life appeared somewhat improved, even significantly when compared to the BELINDA trial. Faramand et al. [190] found that CRP and ferritin levels were independent risk factors for predicting the risk of outcomes associated with axi-cel (Yescarta) therapy.

Jacobson et al. [183] note that ZUMA-1 (axi-cel, Yescarta) and JULIET (tisa-cel, Kymriah) have been criticized for including highly selected patients. Oluwole et al. [191] also compare axi-cel (Yescarta) and tisa-cel (Kymriah) in large B-cell lymphoma and conclude that axi-cel (Yescarta) may have better efficacy than tisa-

cel (Kymriah) based on a matching-adjusted indirect comparison (MAIC) analysis using data from the two pivotal trials of these therapies, ZUMA-1 and JULIET, respectively. Zhang et al. [192] also considered the same research question but determined that such an ITC was infeasible owing to irreconcilable differences between the ZUMA-1 and JULIET trials. They note that ZUMA-1 did not allow BT.

Jacobson et al. [193] performed a review of real-world outcomes and found that axi-cel (Yescarta) had a significantly shorter median time from apheresis to CAR-T infusion than tisa-cel (Kymriah). They also found that, despite including broader patient populations, the real-world effectiveness and safety of both axi-cel (Yescarta) and tisa-cel (Kymriah) were consistent with data from the pivotal clinical trials. Further, they noted the lack of available real-world data for liso-cel (Breyanzi), due to its more recent regulatory approval. Stefanski et al. [194] in a study of 180 people receiving Kymriah report that higher doses led to better outcomes, yet did not increase toxicity.

Hansen et al. [195] and Dima et al. [196] find that the safety and efficacy of ide-cel (Abecma) in patients with RRMM in the SOC setting were comparable with those in the phase II pivotal KarMMa trial despite most patients (75%) not meeting trial eligibility criteria. Hansen et al. found a 6% manufacturing failure rate. Sanoyan et al. [197] report on the first 16 consecutive RRMM patients treated with ide-cel (Abecma) at the University Hospital of Bern and find that the median time from lymphapheresis to ide-cel (Abecma) infusion for the whole cohort was 7 (7–11) weeks. Ahmed et al. [198], in an Abecma study at the University of Kansas, found that 60% of patients on a waitlist were able to secure a production slot, with a median time from consult to collection of 38 days and median time from collection to infusion of 42 days. The median OS time for those not receiving treatment was 9 months.

Raj et al. [199] study 327 patients with LBCL treated with commercial CD19-CAR-T and find that combining modalities in a multimodal framework improves predictive accuracy. They reduced a large dataset to 141 variables across five modalities representing host, tumor, and CAR-T product features. Particularly important features included (1) TCR signaling pathway expression in the infusion product, (2) tumor sphericity, and (3) FOXO1 mutations.

3.28 | Cost-Benefit and Availability

Gaijra et al. (2022) review the barriers to CAR-T Therapy for the first five FDA approved products, including manufacturing, clinical outcomes, reimbursement and a cost-benefit analysis and conclude that the positive out-comes of CAR-T therapy outweigh the barriers and justify its continued use and development. Similarly, Cliff et al. [200] suggest price negotiation, innovative manufacturing approaches, and alternative payment models to reduce costs. A challenge of CAR-T therapy is that patients have been required to have at least two lines of prior therapy, so their immune system may be more compromised than if they received CAR-T as a first-line treatment. As noted earlier, Kite recently received FDA approval of axi-cel (Yescarta) for the first-line treatment of adult patients with newly diagnosed, high-risk LBCL. Gye et al. [201] performed a cost-benefit analysis of young patients receiving Kymriah.

Khang et al. [202] suggest that the per-patient cost can be reduced from \$170–\$220K to \$42–\$68K through several manufacturing-related changes. Much of these savings are labor costs and largely a function of reduced expansion times and using point-of-care (decentralized) manufacturing. Although biosimilars might be able to reduce costs, Canter et al. [203] discuss the many limitations to developing biosimilars for cell and gene therapy.

3.29 | Decision Support Systems

Nafees et al. [204] focus on clinical decision support (CDS) systems for oncology, including clinical practice guidelines (CPGs), computerized prescriber order entry (CPOE), patient reported outcomes (PROs), and integration with electronic health records (EHRs), but note a growing gap between the development of advanced CDS tools (e.g., AI-based approaches) and real-world implementation with evaluation of these tools. Niraula et al. [205] discuss human-AI interaction and note that some clinicians may disregard AI recommendations due to skepticism or if deemed harmful or suboptimal.

3.30 | Solid Tumors

The seven FDA approved CAR-T therapies are for hematological malignancies (liquid tumors). Guzman et al. [206] and Swanton et al. [207] review the challenges of applying CAR-T to solid tumors, including the hostile TME and tumor heterogeneity. Albelda [208] notes that CAR-T therapies for solid tumors have not been as successful as for B-cell malignancies and suggests that the use of pluripotent stem cells, cotargeting multiple mechanisms of immune evasion, employing multiple costimulatory domains, and CAR ligand-targeting vaccines. He also suggests using multiple doses of short-lived CAR-T cells to preempt exhaustion and maintain a functional effector pool. The NexTGen grand challenge (https://www.cancergrandchallenges.org/nextgen), focusing on childhood cancers, requires a multiscale approach that includes the heterogeneity of the TME and limited accessibility.

Solid tumors often have highly expressed antigens that are also expressed at low levels in normal tissues. Further, solid tumors have antigen heterogeneity between patients with the same cancer. Thus, a major challenge is designing a CAR-T cell for an “ideal” target antigen in solid tumors. For germ cell tumors, it appears that Claudin-6 (CLDN6) is an ideal target because, while active after birth, CLDN6 is not prevalent in adults. Good preliminary results applying CAR-T, including an anti-tumor mRNA vaccine, have been obtained by Mackensen et al. [22], as further discussed by Chehrazi-Raffle et al. [209]. Tumor-associated macrophages (TAM) are abundant cell types in many solid tumors and typically exert protumor effects. Sánchez-Paulete et al. [210] report a strategy for TAM depletion in mouse solid tumor models using CAR T-cells targeting the macrophage marker F4/80.

4 | Challenges and Opportunities

The previous sections have largely reviewed prior work in the many aspects of CAR-T discovery, manufacturing, and treatment. Here, we provide a perspective on how to improve outcomes, reduce costs and treat more patients by using/developing process systems engineering techniques. The integration of data from lab studies, clinical trials, and *real-world* treatment and including the immune system and microbiota are all very important in enhanced treatment decision-making.

4.1 | Process Systems Engineering and Smart Manufacturing

Process systems engineering and smart manufacturing (Industry 4.0) technology can play important roles in CAR-T manufacturing and treatment. Pistikopoulos et al. [211] summarize that "Process Systems Engineering (PSE) is the scientific discipline of integrating scales and components describing the behavior of a physicochemical system, via mathematical modeling, data analytics, design, optimization, and control." More broadly PSE includes biological/biochemical/biopharmaceutical systems. The Clean Energy Smart Manufacturing Innovation Institute (CESMII) [212] notes "Smart Manufacturing is the information-driven, event-driven, efficient and collaborative orchestration of business, physical and digital processes within plants, factories, and across the entire value chain."

4.2 | Spatial and Time Scales

Classically, process systems engineers are involved at many spatial and time scales and collaborate with a variety of disciplines in product development, process design, manufacturing site selection, coordinating plant construction and start-up, plant operation, planning and scheduling, and product formulation. In pharmaceutical applications, there is awareness of the direct impact of the product on the health and welfare of the final consumer, but process engineers are not involved in consumer follow-up. For autologous CAR-T therapy for cancer the most important "human in the loop" supplies the raw material, consumes the therapeutic product and grows that product by several orders of magnitude! And that therapeutic product is judged by the ability to safely bring the patient into remission. Thus, process systems engineers should be involved in using patient data to improve models and future treatment recommendations. Further, these models and recommendations need to be updated as more data from clinical trials and treatments are accumulated.

4.3 | Data Availability and Model Development

In manufacturing industries, it is not unusual for a company to have many data silos, with different formats and protocols between manufacturing plants and even within the same plant. Analyzing data to develop models for decision-making often requires a substantial data cleaning effort. Smart manufacturing platforms have enabled manufacturers to move from these independent silos to integrated systems for improved decision-making throughout the enterprise. Given the wide-range and different types of data for everyone treated, information integration is more difficult in personalized medicine applications. Although the microbiome is known to be important in cancer development, progression, and treatment outcomes, it is not a routine clinical measurement. There is a need for it to be included in future clinical trials and in real-world administration of therapeutics; and studies are needed to understand the effect of proactive treatments such as diet and fecal transplants.

4.4 | Vein-to-Vein Manufacturing

Many of the current CAR-T manufacturing steps are manual and performed in a strict clean room environment, but there has been a move toward closed systems with more automation and less restrictive clean room requirements. Novel process designs are likely to benefit from a "plug and play" arrangement enabling different unit operations to be arranged even if from different manufacturers. Although bioreactors for the expansion step, for example, should have dissolved oxygen, temperature, and pH control, ideally these setpoints would be changed based on cytokine and T-cell phenotype measurements, clearly requiring a modeling effort. Hybrid models that combine a physical basis with machine learning techniques may be successful here; this includes the use of causal inference [213] and causal graphical models [214].

Although current FDA approvals are based on specific time-based manufacturing protocols, it is desirable to use real-time measurements to complete steps when product specifications are met, and model-based control provides an ideal framework for this. Also, some patients will benefit from therapeutics that do not rigorously meet all the specifications. Although currently approved therapeutics have an 8–12-day manufacturing time, this concept is also valid for rapid manufacturing products under development.

Finally, it is important that manufacturing automation be considered at the earliest stages, before planning for Phase 1 clinical trials. This will become easier as more platforms are available and can be used in University Hospitals before transitioning to centralized facilities (if that mode of production is desired). Model-based process control can then be iteratively improved (adapted) as more data and experience are collected through the trial phases and as more *real-world* treatment data is obtained.

4.5 | Hierarchical Decision-Making

Classic manufacturing decision-making largely occurs on different spatial and time scales. A petroleum company may make major market-based decisions on a quarterly basis (preparing for different seasonal specifications and demand), while individual refinery operating decisions are made on much more frequent time scales. Unusual weather, natural and other disasters, will change the original optimum feed and product scheduling.

Decision-making in autologous CAR-T is also hierarchical but with high-level decisions based on a single patient. Ideally, when a patient is diagnosed (which itself may require an iteration with several tests) with a particular type of cancer, a decision

tool should enable the clinician (or tumor board, etc.) to make an initial recommendation for a course of treatment (standard protocols or more advanced). This, by nature, is probabilistic and would be updated as more outcome data (based on demographics, tumor burden, etc.) is accumulated. Further, the treatment center communicates with the insurance provider and manufacturer, which both should have access to up-to-date data and treatment outcomes for different patient populations. The most important decision-maker is the patient (or care provider) who will largely make a decision based on their comfort level with the clinical staff. Depending on the background of the patient, the staff must be able to distill a lot of information, including risks, success rates of alternative treatments, concerns about treatment costs, and logistics of treatment (travel, etc.) in a way that helps patients make a decision.

Smart manufacturing enables Walmart, for example, to know their supply chain status when ordering a product for any individual store. Similarly, for a CAR-T therapeutic, the oncologist may recommend one product over another (e.g. Abecma vs. Carvykt for MM) based on expected "vein to vein" time. Once a therapeutic product is selected and the patient is scheduled for treatment, the schedule may need to be updated due to changes in QA review time, lot-specific release testing or in the event of a manufacturing failure, patient death, or updated decision not to treat.

4.6 | Novel Artificial Intelligence and Machine Learning Requirements

The challenges and opportunities identified above will require continued development of novel ML/AI techniques that are multiple-model (fundamental, statistical, and machine learning) and multiple-modal (text, images, and numerical data) in nature. With individual patient data containing different types of test results and values, with some data missing or uncertain, different forms of prior treatment, etc., novel LLMs and related techniques will be needed. Perhaps more than typical manufacturing applications, the explainability of decision recommendations will be extremely important. Further, given that patient lives are at stake and resources are constrained, decisions should be based on ethics.

5 | Commentary by Dr. Daniel W. Lee and Dr. Drew N. Kelner

I asked colleagues in oncology and biomanufacturing to provide brief comments on topics related to this paper.

Daniel W. Lee[1]: CAR T-cell therapies are paradigm changing treatments for relapsed and/or refractory B-cell malignancies and MM, particularly in children, where a single dose can be curative in the multiply relapsed setting. Still, many die of their disease while awaiting manufacturing of autologous products prompting the field to invest in off-the-shelf, third-party derived cell products. These are by definition severely alloreactive and so additional genetic manipulations of the cells are required in addition to transduction with the CAR. Even then, products are still fraught with alloreactivity risk and are exponentially more expensive to manufacture. Others have moved toward point-of-care manufacturing in fully enclosed cell culture systems, where autologous CAR T products can be made where the patient is located. Although this can be faster than the 6–8 weeks generally needed for off-site commercial manufacturing, product manufacturing and release is still lengthy. Therefore, the field is in dire need of refined manufacturing and product release approaches that, ideally, could reduce time to infusion to a few days or less. In addition, as numerous factors impact whether a treatment will be successful (e.g., CAR design is often serendipitous, each T-cell product behaves differently in manufacturing and in vivo, host immune systems react differently, tumors incorporate different resistance mechanisms, etc.) incorporating AI approaches to better predict improved CAR efficacy and reduced CAR toxicity by integrating a host of patient, T-cell, immune, and tumor factors has the potential to further personalize this therapy. Doing so would dramatically increase access and improve responses across the world to these proven, life-saving therapies.

Drew N. Kelner[2]: Four decades ago, at the dawn of the biopharmaceutical era, developing a reproducible and reliable process to consistently manufacture biologicals that meet the specifications to ensure product safety and efficacy was one of the greatest challenges in the industry. In the early years of the recombinant DNA revolution, the goal of achieving process consistency by gathering process knowledge during development and scale-up that we take for granted today did not exist and had to be built up over time, from the ground up. Over the past few decades, methods to obtain process control have included extensive design of experiment-based studies to define the process design space accompanied by rapid improvements in process analytics, both in the laboratory and on the manufacturing floor (Process Analytical Technology, aka PAT).

Today, the emerging modality of cell therapy faces a challenge similar to that faced by the earlier generation of biologicals that included growth factors, monoclonal antibodies, and other therapeutic proteins. In both cases, high manufacturing costs need to be modulated over time by improvements in technology and process consistency so we can achieve (to borrow the Amgen mantra) "Every patient, every time." This is especially critical in autologous cell therapy, where the precious raw material cannot easily be replaced for a "do-over" like we might conduct with a traditional biological product in the event of process failure. To once again steal a phrase (from NASA), in cell therapy, "failure is not an option."

This is where the strategies detailed in this paper play a critical role. Given the criticality of delivering high-quality CAR-T cells to each patient in an expeditious manner, measures to improve the efficiency, speed, and consistency of the production process will help lower costs and reduce the time from vein to vein. This work, only in its early stages, will over time improve CAR-T manufacturing success and hopefully improve both outcomes and access to these life-saving medications.

6 | Dedication

This paper is dedicated to the memory of my son, Brendan Fahy Bequette, who was a filmmaker in New York City. He was

diagnosed with a rare mediastinal germ cell tumor in 2020, at Age 24, and went through many lines of chemo- and immunotherapy. His last hope was a clinical trial for a novel CAR-T treatment for solid tumors. Unfortunately, the trial was delayed by over 6 months while the sponsor automated the process, and Brendan died during this period. Thus, I was motivated to enter the cell and gene manufacturing field to reduce the number of people that die while awaiting treatment.

I appreciate the efforts of his oncologists Dr. David Shaffer (Albany Medical College) and Dr. Darren Feldman (Memorial Sloan Kettering Cancer Center, MSKCC), and particularly the way that Dr. Feldman kept us "in the loop" in discussing treatment options. I also appreciate the care given Brendan by the amazing nurses at the MSKCC Sidney Kimmel Center.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing is not applicable to this article, as no new data were created or analyzed in this study.

Endnotes

[1] Daniel W. Lee, MD. Director Pediatric Stem Cell Transplant & Cellular TherapyDivision of Pediatric Hematology/Oncology. Department of PediatricsUniversity of Virginia Comprehensive Cancer Center, University of Virginia School of Medicine

[2] Drew N. Kelner, PhD. Author of Amazon #1 Bestseller "Taming Cancer" and President, Shenandoah Biotechnology Consulting, LLC offering consulting services in biopharmaceutical process and product development, quality, and regulatory affairs.

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Appendix

FDA-related documents for the seven approved CAR-T therapeutics

Abecma:

https://www.fda.gov/vaccines-blood-biologics/abecma-idecabtagene-vicleucel

https://www.fda.gov/media/147055/download

https://www.abecma.com

Aucatzyl:

https://www.fda.gov/vaccines-blood-biologics/aucatzyl

https://www.fda.gov/media/183463/download

https://www.aucatzyl.com

Breyanzi:

https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/breyanzi-lisocabtagene-maraleucel

https://www.fda.gov/media/145711/download

https://www.breyanzi.com

Carvykti:

https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/carvykti

https://www.fda.gov/media/156560/download

https://www.carvyktihcp.com

Kymriah:

https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/kymriah

https://www.fda.gov/media/107296/download

https://us.kymriah.com

Tecartus:

https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/tecartus

https://www.fda.gov/media/140409/download

https://www.tecartushcp.com

Yescarta:

https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/yescarta

https://www.fda.gov/media/108377/download

https://www.yescartahcp.com/2l-large-b-cell-lymphoma