Review

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Cell therapy products: focus on issues with manufacturing and quality control of chimeric antigen receptor T-cell therapies

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Abstract

Recent accelerated approvals of Chimeric Antigen Receptor T-cell (CAR-T) therapies targeting refractory haematological malignancies underscore the potential for this novel technology platform to provide new therapeutic options for oncology areas with high unmet medical needs. However, these powerful 'living drugs' are markedly different to conventional small molecule and biologic therapies on several levels. The highly complex nature and varied composition of CAR-T based products still requires considerable investigation to resolve the best approaches to ensure reproducible and cost-effective manufacture, clinical development, and application. This review will focus on key issues for manufacturing and quality control of these exciting new therapeutic modalities, preceded by a brief description of CAR principals and clinical development considerations.

Keywords: cancer; oncology; immunology; cell therapy; viral vector; release testing

INTRODUCTION

Chimeric Antigen Receptor T-cell (CAR-T) therapies are novel cancer treatments comprising *ex vivo* expanded T-cells redirected to tumour cell surface expressed B-cell epitopes by antibody-like fusion proteins.[1–3] CAR expressing transgenes are integrated into the genomes of patient or donor-derived T-cells during the manufacturing process; predominantly using recombinant retroviral vectors, although non-viral approaches are also relevant.[4] Pharmacodynamic effects are human leukocyte antigen (HLA) unrestricted and there is no requirement for antigen presentation or T-cell priming necessary for an endogenous or vaccine initiated anti-tumour T-cell response.[5] CAR technology offers a mechanism for immunotherapeutic destruction of tumours with poor intrinsic T-cell immunogenicity due to low mutational loads, immune-editing or mutation of HLA molecules as an immunological escape pathway.[6] Therefore, these novel therapies can be used to treat tumour phenotypes insensitive to immune checkpoint inhibiting monoclonal antibodies (mAbs) and represent an important armamentarium to the rapidly expanding toolbox of immuno-oncology treatment options.[7] CAR-T applications represent a spectrum of therapies and this is an important consideration when developing quality control and safety strategies as discussed in this article.

Molecular biology

CAR mediated tumour targeting is typically achieved with an extracellular binding moiety; usually a single-chain variable fragment (scFv) comprising cloned variable regions of light and heavy chains from a suitable mouse monoclonal antibody (Fig. 1). Accumulated data suggest humanised.[8] or fully human[9] scFv may incur less risk of anti-drug immunogenicity with resultant benefits in terms of clinical activity[10] and safety.[11] Enhanced binding affinity may also improve activity, particularly for low density targets[12] but risks increased off-tumour immunopathology. A flexible protein, often comprising sequences derived from CD8α or immunoglobulin Fc domains, links the antigen binding moiety to transmembrane and intracellular signalling domains.[13,14] As CAR-Ts operate independently of antigen presenting cells, and potentially in tumour microenvironments replete with coinhibitory signals, costimulatory inputs must be genetically hardwired.[1–3] Initial CAR approaches relied solely on CD3ζ immunoreceptor tyrosine-based

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activation motifs for T-cell activation following target ligation,[15] but fared poorly in clinic. Later generations, such as the CAR-T therapies in trials today, augment CD3ζ moieties with CD28 and/or 4-1BB costimulatory domains.[2,7] Optimisation of extracellular targeting moieties and intracellular signalling components is an active and important area of applied research.[16,17]

Development

Regulatory environment

CAR-T therapies are considered advanced therapy medicinal products (ATMPs) in Europe, and more specifically gene therapy medicinal products (GTMPs), per regulation (EC) No 1394/2007.[18] Despite a centralised marketing authorisation procedure, individual European member states have slightly different requirements for trial applications; for example, some authorities regard CAR-T therapies as Genetically Modified Organisms (GMO) which necessitate additional environmental risk assessment.[19] Further harmonisation of European clinical trials regulations is set to come into force in 2019.[20] In the United States, CAR-T therapies are regulated under the Public Health Service Act (section 351); necessitating pre-marketing approval via conventional clinical trial pathways.[21] The United States Food and Drug Administration (FDA) and European Regulatory Agency (EMA) have published several important guidance documents for cell and gene therapy development, although none pertain specifically to CAR-T products.[19–22]

Marketed CAR-T therapies

Two Biologics License Applications, both for CD19 targeting CAR-T therapies, were recently approved by the FDA[23,24] Kymriah (tisagenlecleucel) received approval for treatment of relapsed or refractory B-cell acute lymphoblastic leukaemia based on the results of the pivotal open-label, multicentre single-arm Phase II ELIANA trial.[25] The product confers an impressive 70–90% complete response rate in this patient population. A Biologics License Application has also been submitted for treatment of relapsed or refractory diffuse large B-cell lymphoma patients who are ineligible for autologous stem cell transplant, and Kymriah is currently under accelerated review in Europe.[26] Yescarta (axicabtagene ciloleucel), an experimental CD19 targeted CAR-T based treatment for aggressive non-Hodgkin lymphoma, also received accelerated FDA approval, based on very promising phase 2 data from the ZUMA-1 trial.[27,28] Phase 3 trials were not required for marketing authorisation approval, highlighting a progressive regulatory process in the States based on preliminary endpoints with respect to ground-breaking treatments for conditions with high unmet needs. Such approvals are conditional to detailed post-marketing monitoring and confirmatory clinical trials to ascertain mortality, morbidity, and efficacy compared with standard-of-care.[29]

CAR-T therapies targeting other haematologic tumour associated antigens are also in late stage clinical development; primarily because CD19 is not expressed on all cancers of interest, or may be lost as an escape mechanism[2,30] bb2121, a CAR-T therapy targeting B-cell maturation antigen (BCMA), has been granted breakthrough designation and PRIME eligibility in the USA and Europe for treatment of patients with relapsed/refractory multiple myeloma.[31] LCAR-B28M, an anti-BCMA CAR-T therapy developed in China, also has encouraging clinical activity in this patient population.[32,33]

Preclinical assessment

Preclinical stage-gates include verification of CAR-T specificity and potency; predominantly using *in vitro* systems. Animal based experimentation is usually limited to assessment of CAR-T function in immunocompromised mouse models. Conventional preclinical PK and toxicology studies, of the type required to inform and support first-time-in-human assessment of small molecule therapeutics, have limited usefulness due to species specificity. Arguably, there is a need for new and improved preclinical models to provide translationally relevant information on candidate safety and efficacy.[34–36] Improved models to understand and predict adverse reactions, such as cytokine-release syndrome (CRS), a frequent clinical complication of CAR-T therapy,[37–40] would be particularly welcomed.[34,41]

MANUFACTURING

Backdrop and challenges

There is a desire to increase patient access to CAR-T therapies through increased manufacturing capacity and reduced cost of goods.[42,43] Development of new CAR-T therapeutics for solid tumour types is also a priority.[44,45] Good Manufacturing Practice (GMP) compliant manufacture of CAR-T therapies is considerably more challenging than many other biological medicines, primarily due to increased complexity and variability of the cellular component and criticality of the vector mediated genetic engineering step.[43–46] Published information for αCD19 CART-T therapies indicate up to 10% of manufacturing runs routinely fail.[47,48] This is an important consideration for setting tractable product specifications that ensure quality and allow comparability within clinical trials and manufacturing process optimisations.[21] Final product quality is ideally linked with measurable molecular and cellular characteristics related to clinical activity. Understanding mechanism of action is therefore fundamentally important to define critical product quality attributes such as potency. Without this information, assigning a quality target profile for manufacturing process and materials optimisation is challenging. Elucidating the mechanistic principals underpinning αCD19 CAR-T activity has been difficult because multifactorial and potentially interrelated product and patient-specific factors are likely responsible for activity *in vivo*.[22] Preclinical models have shortcomings and the cost and limited availability of the treatment further narrow scope to generate sufficiently powered clinical biomarker data sets to provide this information. European regulators recently published guidelines on GMP for ATMPs in which some of these issues are specifically addressed.[49] The guidance document outlines a risk-based assessment approach and communicates that the level of detail required concerning some elements of ATMP chemistry, manufacturing and control will increase incrementally with product progression through developmental phases. Nonetheless, regulatory complexity and manufacturing challenges represent a significant 'energy hump' for translation of laboratory-scale experiments into scalable processes for pivotal clinical trials and market readiness.

Process and optimisation

Notwithstanding some of these complexities, the overall manufacturing scheme is broadly similar for each patient (Fig. 2); involving CAR transgene insertion *ex vivo,* then large-scale T-cell expansion and end-of-process formulation.[42,43,46] Ancillary components, e.g. cytokines, media, viral vectors, antibody-coated magnetic beads, must have a certificate of analysis and meet GMP acceptance criteria.[50] Due to the 'just-in-time' nature of CAR-T product manufacture and potentially narrow window of opportunity for patient therapy, supply chain interruptions are highly impactful. Increased

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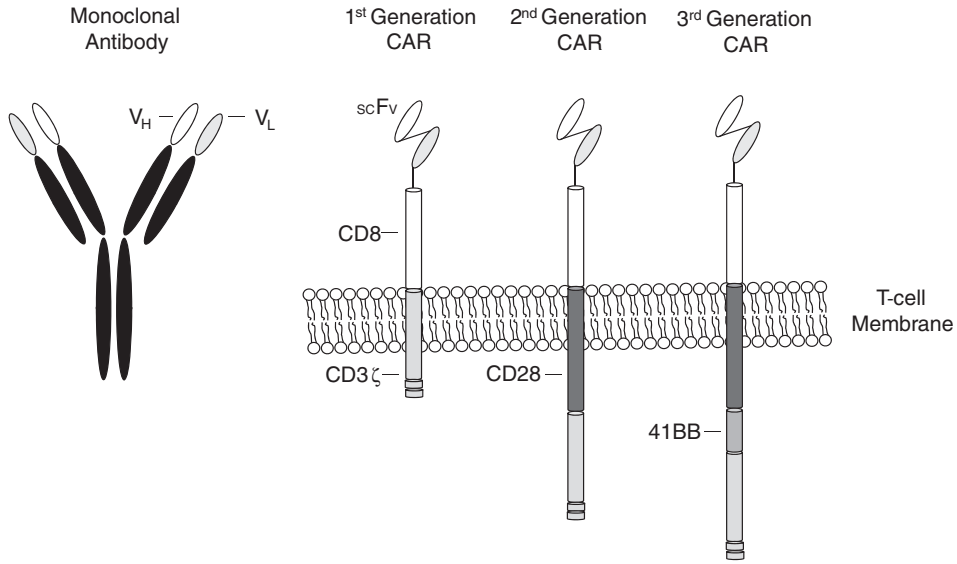

Figure 1. Typical CAR structure and evolution of intracellular signalling domains.

supply chain resilience and interoperability for Good Manufacturing Practice (GMP) quality vectors, cytokines, antibodies and other critical reagents will help manufacturing organisations. Provision of suitable cell lines, assays, standards, and reference materials to support this is now recognised as an important objective at our establishment and elsewhere.[51,52]

Cellular starting materials

Most CAR-T manufacturing approaches utilise autologous T-cells derived from the patient by leukapheresis.[53,54] Invariably, the apheresis is a complex, heterogeneous, and variable starting material, making it difficult to precisely define and control process reproducibility[55]: Cancer patients may have elevated numbers of circulating tumour cells and be heavily pre-treated with immunomodulating pharmacological agents, resulting in atypical circulating immune-cell profiles and functionality. The apheresis product may contain these elements in sufficient concentrations to impact the manufacturing process.[56] Understanding cellular starting material profiles linked to manufacturing success or failure is an important objective for manufacturers. Application of contemporary multicolour flow cytometry to monitor immune-cell profiles from start throughout CAR-T manufacture is one strategy.[57,58] The option to use more generic and standardised cellular starting materials would have several advantages; with significant scope to reduce manufacturing costs, improve reproducibility and widen patient access.

The arrival of gene editing technologies means allogenic sourcing may become more routine, as these techniques can be applied to disrupt, and even substitute, genes encoding potential alloantigens.[59–62] Gene editing approaches may also improve reproducibility and potency. A recent paper described targeted delivery of CAR to TRAC locus, thereby placing CAR expression under control of the endogenous T-cell promotor and abolishing T-cell receptor expression. This avoided tonic CAR signalling and prevented T-cell exhaustion, resulting in markedly higher persistence and reduced variability at much lower doses.[63] Others have eliminated inhibitory receptors, e.g. programmed death 1 (PD-1).[64,65] Gene editing will likely become a key enabling technology when appropriately applied to CAR-T therapy manufacture. Understanding the potential for, and implication of, off-target effects should now be an imperative to guide technical strategy and regulation.[66] Bioinformatics and whole-genome sequencing are fundamentally important tools that should be deployed to investigate and control this; complementing and even substituting for animal studies.

Recent development of experimental pluripotent stem cell derived T-cell substrates offers another potential way forward in this area.[59,67] Despite the appeal of this concept, concerns about genomic unpredictability and associated risks of tumorigenicity remain incompletely resolved at this stage.[68] Efforts to understand transcriptomic profiles associated with 'safe' induced pluripotency, combined with improved methodologies for controlled transgene insertion, may advance the exciting possibility of 'synthetic' T-cells.[69–71] However, technical and safety challenges entailing autologous cell sourcing is likely to remain the mainstay of CAR-T based treatments, at least in the short to medium term.

T-cell selection and activation

Delivering safe and effective cell therapies is contingent on understanding and specifying specific cell populations linked to target product profiles. T-cell activation and expansion can be achieved using magnetic beads or polymers coated with anti-CD3 and CD28 mAbs referred to as artificial antigen presenting cells.[42,43] While mediating efficient T-cell expansion, protracted stimulation via these signalling pathways can drive differentiation and ultimately senescence: several studies have shown that less differentiated central memory (CD45RO+, CD62L+, CD95+) and stem (CD45RA+, CD62L+, CD95+) memory T-cell subsets are superior in terms of metabolic profile, persistence and efficacy.[72–74] Procedures to enrich or promote these cell populations during CAR-T manufacture are likely to improve patient outcomes. Recent work suggests that co-culture with IL-7 and IL-15 during the T-cell expansion phase may help achieve this.[75,76]

Integration of CAR-expressing transgene

Gamma retroviral vectors were the first transgene integration strategy used in CAR-T manufacture, and benefit from high transduction efficiency and readily available and scalable packaging strategies.[43] Lentiviral vectors have the advantage that they

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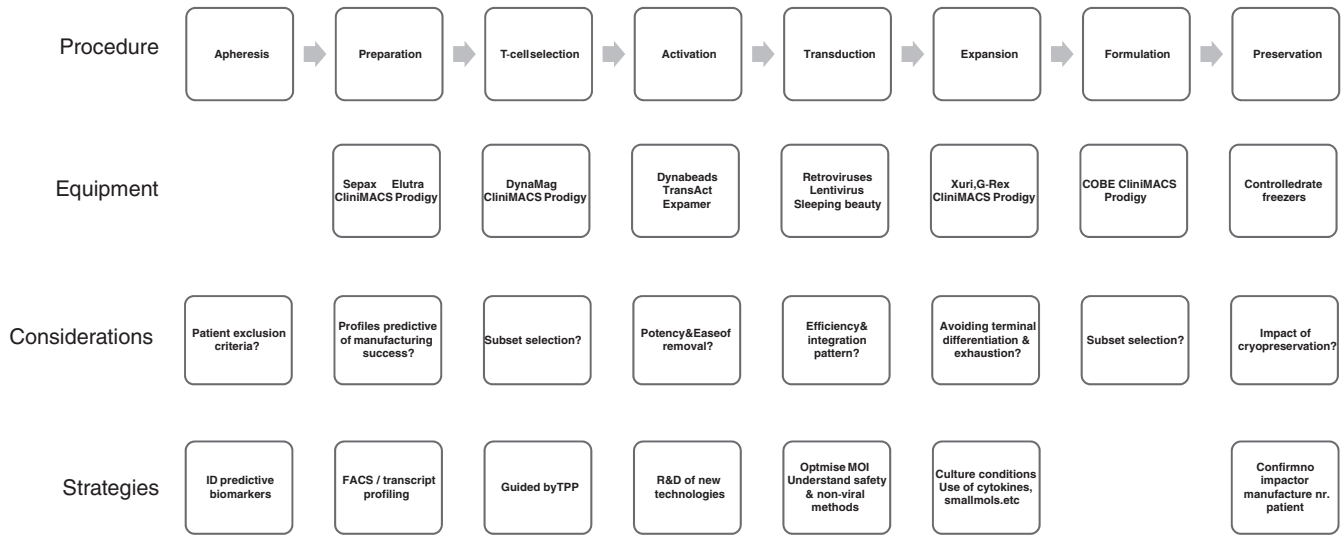

Figure 2. Overview of CAR-T therapy manufacturing process, including some examples of commercially available equipment. Potential factors influencing each procedural stage and strategies to understand/influence each step are also listed (lower rows).

efficiently transduce non-dividing and dividing cells, although scaled production is challenging but not insurmountable.[46] Issues primarily relate to Vesicular Stomatitis G protein-mediated cell fusion, and yield limitations of the multi-plasmid transfection approach.[77] Construction of stable packaging cell lines, such as the prototypes described by Sanber *et al.*,[78] could improve Lentivirus vector manufacturing capacity. Lentiviruses may also be favoured due to lower genotoxic potential in contrast to gamma retroviruses.[79,80] Available evidence indicates that gamma retroviral-mediated integration favours transcriptional start sites, whereas lentivirus shows no increased propensity for this over other sites.[81,82] Nevertheless, the theoretical risk of genotoxicity remains and is thought to be amplified with increased numbers of transgene insertions per cell.[83] Development of vectors targeting genomic safe harbours and other targeted integration methods will further de-risk this.[84]

GMP compliant viral vector production is very expensive and there is currently manufacturing under-capacity.[85] Cheaper and more accessible alternatives to viral vector-based transduction would be highly beneficial. Transposon based technology, such as Sleeping Beauty, has been used successfully in CAR-T manufacture.[4,86] Transposition is accomplished by co-electroporation of plasmid-based vectors comprising the transposon and a transposase. The transposase binds to inverted terminal repeats either side of the transposon encoded transgene, resulting in random genomic integration.[87] Whether CAR-T products manufactured with non-viral approaches will be equally safe and effective as those produced by viral vector mediated transgene integration remains to be confirmed by readouts from ongoing clinical studies.

Expansion and final formulation

After a defined period of cell expansion, CAR-Ts are washed and concentrated. Unselected T-cell subsets were historically used to manufacture CAR-T products. Recent data infers improved efficacy when both CD4 and CD8 CAR-T are infused[88] and some researchers have moved towards more manipulated cellular subset ratios.[89–91] Reproducible and dose related CAR T-cell expansion, with pronounced anti-tumour effects even at low doses, were a notable feature of these trials. Antibody-coated magnetic bead approaches are one way to enrich and define T-cell subsets during manufacture.[92]

As CAR-T manufacture may occur at production facilities which are geographically disparate from patient treatment centres, product cryopreservation in an infusible medium is usually employed to facilitate storage and shipment prior to thawing and administration. Cryopreservation also enables de-pressurised release testing. Despite logistical drivers, it is prudent to consider its necessity and the potential effect on CAR-T activity.[93,94] A decentralised manufacturing approach (see operations management) may circumvent the requirement for freeze–thaw procedures in some instances but also brings the challenge of demonstrating comparability of product released from multi-site production centres. Further research to understand and optimise CAR-T preservation may help better inform these decisions[95] and T-cells have been identified as a key model system for development of new approaches to preservation of cell therapies.

Operations management

Recent approvals for CAR-T therapy products marketed by biopharmaceutical companies[23,24] reflects traction and optimism concerning scalability of manufacture for complex ATMPs.[96] Manufacturing has evolved from a predominantly investigator-led institutionalised activity utilising generic equipment and infrastructure, to more automated and closed system device-based processes. Essentially, two operational 'models' exist for cost-effective commercial scale manufacture going forward[42,46] (i) A flow process approach, of the type commonly employed for manufacturing mass produced commodities. In the context of CAR-T therapy manufacture, a patient's cells enter a qualified 'production line' segmented into the various aforementioned manufacturing process steps. Each step necessitates bespoke infrastructure, requires highly trained operators, and rigorous line clearance protocols are necessary that guarantee the integrity of each personalised

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product. Cell expansion, which occurs over days to weeks, would necessarily take place in physically demarked units prior to end-of process formulation. This model lends itself to a centralised manufacturing approach, with cellular starting materials and final, likely cryopreserved, products shipped back and forth between disparate patient treatment centres. (ii) Device centric. In this set-up, a dedicated device is committed to manufacture of a patients' CAR-T product, more-or-less, in entirety. Multiple independently operating devices can be housed together and monitored by a relatively small workforce who implement pre-planned remedial actions in the event of systems failures. This approach is flexible and potentially more cost-effective due to lower staffing levels and clean room stringency. It lends itself to a more de-centralised approach, perhaps geographically co-located with specialised patient treatment centres and dedicated analytical testing capabilities. Irrespective of operational model system employed, robust and reliable data management and good distribution practice is critical to ensure custody of patient-specific tissues and therapeutic products.[18–22]

IN-PROCESS CONTROL AND RELEASE TESTING

Manufacturing genetically modified cellular therapies entails extensive in-process and quality control testing.[21,43,96,97] Release tests are critical to confirm identity, purity, safety and potency of manufactured medicinal products (Table 1). Quality control testing is particularly onerous for autologous CAR-T therapies as each individualised product 'lot' must be tested.[109] Timeliness is critical to avoid CAR-T product degradation prior to infusion or cryopreservation. Testing often involves complex assays which may not lend themselves to automation or high-throughput modus operandi (see below). Manufacturers highlight a lack of suitable standards, reference materials and performance controls to ensure reproducibility and interoperability of testing.[51,52] Critical knowledge gaps remain concerning the molecular and cellular characteristics associated with clinical efficacy and safety.[96,97] Application of contemporary multi-parameter and agnostic biomarker strategies, of the types applied to other areas of immuno-oncology,[99,108] should help identify more relevant critical quality attributes linked to clinical activity. Similarly, as long-term clinical safety experience increases, it may be possible to refine or redact certain tests such as genetic stability, subject to regulatory approval.[29]

Safety

Levels of endotoxin, mycoplasma, superfluous ancillary components and CD3 negative impurities carried over from the apheresis must be within tightly defined conformance limits.[43,96] Microbial safety is a significant concern for CAR-T products and cellular therapeutics in general, as the manufacturing processes have much less defined conditions than conventional parenteral drugs.[102] Ensuring the sterility of source materials can be problematic and final product sterilisation is not applicable. Conventional methods of sterility testing may be less sensitive for cell-based products; for example, sterility of a sample may not ensure sterility of the whole infusion product and standard sterility test protocols, such as microbiological growth media inoculation may not detect all potential contaminants. In principal, vanishingly low residual bacterial burdens could expand during storage and shipping. Novel approaches for growth-based microbiological control as well as new methods for rapid bacterial detection are warranted[103] and guidance for rapid microbial testing of cell therapy preparations has been developed by EDQM.

Current requirements are that master cell banks, end of production cells, vector concentrates, and *ex vivo* transduced T-cell are scrutinised for replicative virus; although there is no evidence to date that third generation Lentivirus constructs can attain replication competency in any infused T-cell products tested.[105,106] Information concerning integrated vector copies per genome, integration profile, and integration sites is also requested.[110,111] Work-up and availability of WHO standards comprising deeply characterised cell lines with defined vector copy numbers and insertion loci[104] will be highly advantageous to ensure manufacturing quality – and useful to control long-term clinical safety studies.

Purity and identity

FACS analysis is the current method of choice to evaluate phenotypic signatures and CAR expression as a measure of purity and identity.[96] Making available suitable antibodies and standardised preparations representing defined cellular phenotypes is now a priority to improve measurement standardisation across cytometers and analytical laboratories.[51,52] The Biotherapeutics Division at the National Institute for Biological Standards and Control (NIBSC) has produced CE-marked fluorochrome labelled T-cell subsets and is working up similar materials to support cytometer based measurement across life-sciences.[98]

Although extremely valuable for routine characterisation, fluorescence activated cell sorting (FACS) analysis is limited to a relatively small number of parameters that are selected based on prior knowledge and reagent availability. Mass cytometry and other advanced multiplexing technologies are powerful tools that can now be applied to identify additional phenotypic markers that could be monitored to improve manufacturing and quality control processes.[112] By extension, it is logical that massively parallel technologies have utility in this space. Single-cell RNA sequencing (scRNA-seq) is an emerging technology that can be used for unbiased molecular characterisation of distinct T-cell subsets within heterogeneous immune cell populations.[113] scRNA-seq could be applied to CAR-T manufacturing and mechanism of action studies to inform standardisation. Specifically, RNA-seq could be used to identify transcriptome signatures correlative with the target product profile, namely proliferation, persistency, anti-tumour effector function and safety.[114,115]

Potency tests

Potency is an important parameter used to confirm consistency, stability and quality between lots, according to cGMP guidelines. Assessment usually involves one or more bioassays which measure some facet of biological activity intrinsically linked to the products mechanism of action.[43,97,109] Potency testing for αCD19 CAR-T therapies has primarily been achieved by measuring cytolytic activity against target-bearing cell lines or IFN-γ secretion following co-incubation of CAR-T with CD19-expressing cell lines, i.e. short-term effector functionality presumed linked to anti-tumour activity *in vivo*.[97] However, bioassays of this type give an averaged readout for the entire effector cell population and do not consider full potential diversity of T-cell functions. Availability of multiplexed, single-cell approaches will greatly assist pre-infusion assessments of cellular immunotherapies, and these are now emerging.[100]

The relevancy of readouts based on short-term effector functions to overall CAR-T potency is also questionable. Kunkele

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Table 1. Considerations for commonly employed CAR-T product release tests

Quality	Conventional test	Issues/shortcomings	Remedial approach/refinements	References
Purity	% T-cells	Effect of other cell types and carry over from manufacture. Cell based standards	Research by deep profiling and correlation to clinical outcome. Development of FACS standards and reference materials	56,58,96,98
	% CAR + cells	Optimal subset profile often unknown. Effect of CAR + Treg. Exhaustion status	Research by deep profiling of final product and correlation to clinical outcome. Subset enrichment. Gene editing techniques	62,88,99–101
	Ancillary residuals		Manufacturing process optimisation	50
	Tumour contaminants		Manufacturing process optimisation	54
Identity	% CAR + cells	See above	See above	See above
Safety	Sterility			43,96,102,103
	Mycoplasma			43,96
	Endotoxin			43,96
	Transgene copy number	Safe limits unknown. Lack of standardised assays	Research of insertional mutagenesis and genomic safe-havens. Develop standards and reference materials with defined copy number per genome	84,104
	Replication competent viral vector	No evidence of replication competency when contemporary vector designs used	Long-term follow-up within clinical trials. Undertake research to inform regulation	66,105,106
Potency	CTL activity vs target bearing line	Suitability of target-bearing lines. Relevance of short-term lytic activity to CAR-T MOA in clinic	Develop 'low background' lines and 3D approaches. Identify biomarkers connected to clinical activity and use these to develop improved assays	37,97,100,107
	IFN-g recall response	Suitability of target-bearing lines. Results are 'summation' of effect from heterogeneous population. Other cytokines likely important	See above Consider new assays monitoring single cells. Use multiplex technologies	97,99,100,108

et al.[107] demonstrated that CAR-T constructs that generated the highest activity in assays measuring specific lysis and cytokine secretion exhibited attenuated anti-tumour potency *in vivo*. Consistent with this, an increasing weight of clinical biomarker data suggest improved outcomes are associated with infusion of αCD19 CAR-Ts with enhanced potential for expansion (Cmax) and persistence (AUC) post-infusion.[116,117] Immunological dogma and phenotypic and transcriptomic profiling data support the thesis that less differentiated central memory (CD45RO+, CD62L+, CD95+) and pluripotent stem cell (CD45RA+, CD62L+, CD95+) memory T-cell subsets may be optimal in this regard.[73–75] Fraietta *et al.*[101] investigated biomarkers in 41 Chronic Lymphoid Leukaemia (CLL) patients treated with Kymriah. Durable remissions were associated with transcriptomic signatures of early memory T-cells, while T-cells from non-responding patients were enriched in genes belonging to known pathways of terminal differentiation and exhaustion. Accordingly, FACS showed the frequency of CD27 + CD45RO- cells in the CD8+ T-cell population correlated significantly with complete and durable responses to this therapy. Non-responders had higher levels of T-cell exhaustion markers on the infused CAR-T-cells and reduced CD27 expression. The combined assessment of PD1 and CD27 expression on infused CD8+ cells served to accurately predict clinical response and, as such, may represent useful measurement parameters for αCD19 CAR-T product quality control. Proliferation assays[75] may also augment cytokine release or killing assessments.

CONCLUSIONS

CAR-T therapies to date have provided impressive objective response rates in several refractory haematological cancers but represent a complex range of different therapeutic tools which require careful evaluation for monitoring their safety and efficacy. Recent approvals for CAR-T therapy products marketed by biopharmaceutical companies reflects traction and optimism concerning scalability of manufacture for complex cell therapies.[42,43,46] While two CAR-based products have been approved based on available knowledge and processes, wider patient access and development of CAR-T therapies for more common cancer types is a priority.[6,45,48] The development of closed and more automated production units has been a major advancement for manufacturing, but GMP compliant manufacture is inherently challenging due to the nascent regulatory environment, heterogenic nature of the cellular component and criticality of the vector-mediated genetic engineering and T-cell expansion steps.[21,22,29,43,46] Release testing for each lot is onerous and setting manufacturing success criteria is complicated in many instances because of an incomplete understanding of product mechanism of action.[51,97,109] Standardisation is inherently difficult, but should be an essential component; if appropriately designed.[51,52,97]

The wider applicability of learnings garnered from successful αCD19 CAR-T approaches to clinical development of CAR-T therapies for solid tumours remains unclear at this stage.[118] Likely different target product profiles will be necessary to

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tackle non-liquid cancers.[45,48,119] Application of contemporary multi-parameter and agnostic biomarker strategies, of the types applied to other areas of immuno-oncology,[99,108] should improve linkage of CAR-T product critical quality attributes to biological effector functions associated with positive clinical outcomes for carcinomas. Similarly, clinical data can now be correlated with deeply profiled infusion material to inform quality target product profiles. It is logical, therefore, to interoperate groups developing product quality control assays with clinical biomarker groups.[97] This may also inform patient selection criteria or underpin development of companion diagnostics, a key objective considering the potential cost of CAR-T therapies and ATMPs in general.[120] Lastly, gene editing is likely to become increasingly utilised within CAR-T therapy manufacture.[121] Understanding the potential for, and implication of, off-target effects should be an imperative to guide manufacturing strategy and quality control.[66]

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