CAR T-cell detection scoping review: an essential biomarker in critical need of standardization

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ABSTRACT
The expansion and persistence of chimeric antigen receptor (CAR) T-cells in patients is associated with response, toxicity, and long-term efficacy. As such, the tools used to detect CAR T-cells following infusion are fundamental for optimizing this therapeutic approach. Nevertheless, despite the critical value of this essential biomarker, there is significant variability in CAR T-cell detection methods as well as the frequency and intervals of testing. Furthermore, heterogeneity in the reporting of quantitative data adds layers of complexity that limit intertrial and interconstruct comparisons. We sought to assess the heterogeneity of CAR T-cell expansion and persistence data in a scoping review using the PRISMA-ScR checklist. Focusing on 21 clinical trials from the USA, featuring a Food and Drug Administration-approved CAR T-cell construct or one of its predecessors, 105 manuscripts were screened and 60 were selected for analysis, based on the inclusion of CAR T-cell expansion and persistence data. Across the array of CAR T-cell constructs, flow cytometry and quantitative PCR were identified as the two primary techniques for detecting CAR T-cells. However, despite apparent uniformity in detection techniques, the specific methods used were highly variable. Detection time points and the number of evaluated time points also ranged markedly and quantitative data were often not reported. To evaluate whether subsequent manuscripts from a trial resolved these issues, we analyzed all subsequent manuscripts reporting on the 21 clinical trials, recording all expansion and persistence data. While additional detection techniques—including droplet digital PCR, NanoString, and single-cell RNA sequencing—were reported in follow-up publications, inconsistencies with respect to detection time points and frequency remained, with a significant amount of quantitative data still not readily available. Our findings highlight the critical need to establish universal standards for reporting on CAR T-cell detection, especially in early phase studies. The current reporting of non-interconvertible metrics and limited provision of quantitative data make cross-trial and cross-CAR T-cell construct comparisons extremely challenging. Establishing a standardized approach for collecting and reporting data is urgently needed and would represent a substantial advancement in the ability to improve outcomes for patients receiving CAR T-cell therapies.

INTRODUCTION
The advent of chimeric antigen receptor (CAR) T-cell therapies for treatment of hematological malignancies has brought promising efficacy but this therapy is also associated with a constellation of novel toxicities, including cytokine release syndrome and immune effector cell associated neurotoxicity syndrome.1 Often regarded as a ‘living drug,’2 in vivo CAR T-cell expansion and persistence, measures shown to be associated with response, toxicity and long-term efficacy, result in the CAR construct being a critical biomarker.3 Accordingly, a new field of research—focused on exploring the biology and kinetics of CAR T-cells and correlations with clinical outcomes—has emerged. The close monitoring of CAR T-cell presence and persistence post-infusion is, therefore, incorporated into most early phase CAR T-cell trials. This suggests feasibility for intertrial data comparisons and the potential to evaluate how differences in CAR T-cell expansion across patients and products may correlate with varying response and toxicity profiles.

Methodologies for CAR T-cell detection across clinical trials generally encompass flow cytometry (FC)4,5 and/or quantitative PCR (qPCR) assays as the two primary techniques used for CAR evaluation in peripheral blood, bone marrow (BM), and cerebrospinal fluid (CSF). For example, flow cytometry allows FMC63-based CD19 CAR T-cells to be evaluated via recognition of the single-chain variable fragment region using an anti-human monoclonal antibody against FMC63,6 or via evaluation of a coexpressed safety-tag (e.g., truncated epidermal growth factor receptor).7 These CAR detection methods can be evaluated simultaneously with T-cell markers such as CD3, CD4, or CD8, but are not suitable for evaluation of changes in cell surface CAR expression, which may be variable or modulating in real time. Indeed,
Despite the apparent uniformity, analyses of CAR expression by different techniques and in diverse T-cell subsets can result in differences across groups, confounding intertrial comparability. Regarding qPCR analyses, performed using qualitative or quantitative methods, they too lack cross-trial standardization. Furthermore, in addition to the variability of the CAR incorporated across trials, there is significant heterogeneity in the initial vector copy number and transduction efficiency (TE) in these trials, adding additional layers of complexity that are not appraised in current reports.

Given the critical importance of CAR T-cell monitoring and the variability in metrics used across clinical trials, we sought to evaluate the current landscape of methods used for CAR detection, alongside the cross-trial comparability. To investigate CAR T-cell monitoring and the variability in metrics used across clinical trials, we sought to evaluate the current landscape of methods used for CAR detection, alongside the intervals and frequency of testing, and reporting of these data. This information is indispensable for cross-trial comparisons of patient outcomes.

**METHODS**

To investigate CAR T-cell reporting across trials for hematological malignancies, we conducted a scoping review, using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses for scoping reviews (PRISMA-ScR) checklist (online supplemental appendix table 1). With a primary goal to describe the heterogeneity in CAR T-cell detection, we focused on registration trials leading to US Food and Drug Administration (FDA) approvals for CAR T-cell constructs and included a select few additional trials with either investigational constructs with advanced data or predecessor trials for commercially available constructs. We searched PubMed for all relevant clinical trial manuscripts that met our criteria. Selection of manuscripts was restricted to clinical trials that had a National Clinical Trial number and were primarily conducted in the USA, facilitating review of studies that would fall under the purview of a single regulatory body, particularly as it relates to CAR T-cell detection and oversight. The most recent manuscript included in this review was published on May 17, 2022. Of note, global registration studies which included international enrollment were included in this review, however, in general, the majority of enrolment was in the USA.

All CAR T-cell expansion and persistence data were collected and tracked in a Microsoft Excel spreadsheet; variables included PubMed ID, publication date, clinical trial identifier, CAR construct and antigen, detection technique(s), detection frequency, expansion data, persistence data, and representation of the reported data (ie, table or type of graph). For summary data, only patients who received a CAR T-cell infusion were included and for patients who received multiple infusions, analyses were based only on expansion following the first infusion. Expansion data focused primarily on the first 31 days post-infusion, and data beyond this time point were categorized as persistence data (online supplemental appendix 1).

The first bulk analysis examined the primary publication from the selected 21 clinical trials. When provided, quantitative data for each parameter were collected and the publication was labeled as having reported on the given assessment. Trials received a ‘Y’ (yes) for a unique metric when quantitative data were reported, an ‘I’ when the assessment was included but a quantitative value was ‘indeterminable’, an ‘M’ when the metric was ‘mentioned’ but no data were reported, and an ‘N’ (no) when the metric was not reported. A second bulk analysis analyzed all data for a given trial, compiled from the primary and all subsequent publications. As performed for the primary publication analysis, each metric was annotated as ‘Y’, ‘I’, ‘M’, or ‘N’, as described above.

**RESULTS**

From 105 total screened manuscripts, representing 21 clinical trials, 60 manuscripts—targeting CD19, CD22, and B-cell maturation antigen (BCMA)—were selected based on inclusion of CAR T-cell expansion data (figure 1A,B, online supplemental tables 2 and 3). The overall representations of the CAR constructs shown in figure 1C,D, with figure 1D combining trials with predecessor constructs and their FDA-approved equivalent.

**Reporting of CAR T-cell detection in primary clinical trial publications**

Evaluation of the primary publication from each of the 21 clinical trials assessed, defined as the first publication reporting singular non-pooled trial data, revealed FC and qPCR as the most consistently used CAR T-cell detection techniques. qPCR was used as the sole detection method in 8 of 21 (38.1%) clinical trials, FC was used alone in 1 (4.8%) clinical trial, and both methods were employed together in 10 (47.6%) trials (figure 2A). In addition, only 1 (4.8%) clinical trial relied on droplet digital (dd) PCR to detect CAR T-cells. For overall detection of CAR T-cells post-infusion, only 14 of 21 (66.7%) primary publications reported time points at which CAR T-cells (or CAR construct) were evaluated in blood (figure 2B). Moreover, of those groups that reported time points, evaluation frequency was highly variable. The median number of time points at which CAR T-cells were evaluated was 5 (range 2–12) (figure 2C), with wide variability in timing of detection; intervals between days 4–10, 11–17, and 25–31 were the most frequently employed, suggesting a focus on days 7, 14, and 28 post-infusion (figure 2D).

Across primary publications, a wide variety of parameters relative to CAR T-cell expansion and persistence were evaluated (box 1).

A number of CAR transgene copies were generally derived from qPCR data while percentages or absolute numbers of CAR T-cells were derived from FC analyses. Based on these metrics, 42.9% of primary publications reported concentration of CAR T-cells while 57.1% reported concentration of CAR transgene copies over time (figure 3A,B). However, only 5 of 21 (23.8%)
publications reported on the change in percentage of T-cells expressing CAR over time (figure 3C) and just two trials provided quantitative data regarding the peak percentage of CAR T-cells (figure 3D). Quantitative data for $C_{\text{max}}$, the peak concentration of CAR T-cells or CAR transgene copies, was absent in the majority of studies (14 of 21, 66.7%), even though this parameter was mentioned in all 21 publications, and graphs were included in some publications (figure 3E). For the seven studies that included this parameter, $C_{\text{max}}$ values were provided in transgene copies/µg DNA in six trials and as cells/µL of blood in two trials (figure 3F,G). For $t_{\text{max}}$, the time to peak CAR T-cell expansion, 7 of 21 (33.3%) primary publications failed to provide quantitative summary data (figure 3H). Finally, while area under the curve (AUC) of CAR T-cells or CAR transgene copies over time were a prevalent parameter used to evaluate CAR T-cell expansion, AUC from days 0 to 28 post-infusion (AUC$_{0-28\text{d}}$) was only incorporated in 7 of 21 (33.3%) primary publications (figure 3I and online supplemental figure S1A), reported as either cells/µL PB×days (n=2) or CAR transgene copies/µg DNA (n=5, figure 3J,K). The one parameter that was consistently captured by this scoping review was the site of CAR T-cell detection. All 21 primary publications evaluated CAR T-cells in the peripheral blood. However, only five and four primary publications reported detection of CAR T-cells in BM and CSF, respectively (figure 3L).

In addition to expansion parameters, analysis of CAR T-cell persistence, presented as $t_{\text{last}}$—time to the last quantifiable concentration of CAR T-cells, was inferred in 7 of the 21 (33.3%) primary publications. However, quantitative data for $t_{\text{last}}$ was only provided in three studies (figure 3M). Furthermore, a reporting of $t_{1/2}$—the
half-life of CAR T-cells, together with $C_{\text{last}}$—the last quantifiable concentration of CAR transgene copies, were only included in 1 of 21 primary publications (Figure 3N, O). Collectively, CAR T-cell detection data from these primary publications are highly variable, raising concerns that would preclude intertrial comparisons.

**CAR T-cell detection and reporting in the ensemble of publications resulting from each clinical trial**

Based on extensive heterogeneity in CAR T-cell detection and absence of data points in primary clinical trial publications, we evaluated whether subsequent publications would provide a more robust reporting of CAR T-cell detection metrics. We hypothesized that additional follow-up time would improve reporting of biological correlates, and especially of CAR T-cell persistence and expansion. Indeed, subsequent publications did provide additional data on specific correlates relating to post-infusion toxicities that were not addressed in primary reports.

However, with respect to CAR T-cell analyses, information on detection methodologies used, number of time points analyzed, and interval of testing was generally comparable with primary publications (online supplemental figures S1–S3). Nevertheless, some data points were added: an additional 4 clinical trials provided a quantitative AUC value in their subsequent publications, reaching 11 of 21 clinical trials providing these data (52.4%, online supplemental figure S2I). Peripheral blood CAR T-cell $C_{\text{max}}$ was also more frequently reported in subsequent manuscripts (nine trials) as compared with primary publication (two trials, online supplemental figure S2F). Regrettably, a significant number of parameters remained indeterminable. Seven of 21 (33.3%) clinical trials did not provide quantitative data for $C_{\text{max}}$ (online supplemental figure S2E–G) and 4 of 21 (19.0%) still did not provide quantitative data for $t_{\text{max}}$ (online supplemental figure S2H). While serial assessment of BM is not expected for lymphoma patients, the primary site of CAR T-cell detection in all follow-up studies remained the PB, with only 13 (61.9%) and 8 (38.1%) trials reporting on CAR T-cell assessments in BM and CSF, respectively (online supplemental figure S2O). Furthermore, six additional trials reported detection data from FC for days 18–24; five additional trials reported FC data for days 1–3, 4–10, and 11–17; and three additional trials reported FC data for CAR detection between days 25 and 31 in their subsequent manuscripts (online supplemental figure S3B). With regard to CAR detection using qPCR, five
Box 1 Metrics and parameters used to represent CAR T-cell expansion and persistence in clinical trials

- **AUC**
  - $AUC_{0-28\text{ days}}$ (transgene copies/µg DNA×days).
  - $AUC_{0-28\text{ days}}$ (transgene copies/µL PB×days).
  - $AUC_{0-43\text{ days}}$ (cells/µL blood).
  - $AUC_{0-44\text{ days}}$ (transgene copies/µg DNA×days).
- **Cmax**
  - (transgene copies/µg DNA).
  - (transgene copies/µL PB).
  - (transgene copies/µg DNA).
  - (transgene copies/µL PB).
- **Clast**
  - (transgene copies/µL PB).
  - (transgene copies/µg DNA).
  - (transgene copies/µg DNA×days).
  - (transgene copies/µg DNA×days).
- **tmax** (days).
- **tlast** (days).
- **t1/2** (days).
- **Multilog expansion**.
- **Peak factor change**.
- **AUC fold change**.
- **Peak % of T-cells expressing CAR**.
- **Absolute CAR T-cells at day 28** (cells/µL blood).
- **Peak CAR T-cells circulating in blood** (cells/µL).
- **CD4+:CD8+ CAR T-cell ratio** ($AUC_{0-28\text{ days}}$).
- **CD4+ CAR T-cell ratio** (fold change from infusion product to peak expansion).
- **CAR T-cell counts** (log10 cells/µL of blood).
- **AUC, area under the curve; Clast, last detectable concentration of cell or transgene; Cmax, peak level of transgene or cell; tmax, time to maximum cell or transgene number; t1/2, the half-life associated with the disposition phase slopes of a semilogarithmic concentration-time curve (days) in peripheral blood; tlast, time to last quantifiable concentration following dosing.**

additional trials reported data for days 4–10 and 11–17; four additional trials reported data for days 18–24 and 25–31; and one additional trial reported data for days 1–3 (online supplemental figure S3C).

**DISCUSSION**

Clinical biomarkers relay diagnostic, prognostic, and predictive values. As cellular kinetics of CAR T-cell expansion, contraction, and persistence impact therapeutic efficacy and toxicity, the ability to monitor and facilitate cross-trial comparisons of CAR T-cells is critical to understanding the underlying biology—specifically as regards variability in construct configurations (e.g., hinge, costimulatory domain, and promoter), viral vector used, and overall manufacturing methodologies. These parameters can have a tremendous impact on outcomes. While a universal biomarker for monitoring of living drugs, such as CAR T-cells, has not been identified and optimal methods or techniques used to detect CAR T-cells in early phase studies have not yet been established, this should not preclude some standardization. Indeed, a roadmap outlining a series of recommendations to investigate biomarkers in CAR T-cell therapy specifically indicated the need for harmonized and validated assays across different clinical centers, commercial products, and trials, as an important step in the field. This is especially critical as ongoing efforts strive to optimize CAR T-cell engraftment—in order to improve response and extend remission, while incorporating pre-emptive strategies to mitigate toxicity.

Although the pharmacology of CAR T-cells is an imperative parameter to optimization of this novel therapeutic approach, data on expansion and persistence are generally limited. Accordingly, very little is known regarding variability in the dynamics of CAR T-cell responses across trials, although efforts are being made to develop mathematical models to better capture CAR T-cell pharmacokinetics. To this end, we initially sought to compare CAR T-cell pharmacodynamic data across clinical trials. However, it became apparent that the extensive heterogeneity in methods and techniques used for CAR T-cell detection precluded intertrial comparisons. Thus, we embarked on this scoping review, given the dearth of literature on this topic, to comprehensively describe the current landscape of reporting of CAR T-cell metrics. We report our findings to highlight the critical need to establish universal standards for reporting on CAR T-cell detection, especially in patients on early phase studies.

While FC and molecular assays remain the most used techniques—laying the groundwork for feasibility in facilitating cross-trial comparability—interassay detection methodologies varied tremendously and lacked standardization across trials. Furthermore, there was substantial heterogeneity in metrics used to report on CAR T-cell expansion/persistence, along with variable time points and frequency of testing. Most surprising, in conjunction with use of many different parameters to define expansion, an additional complexity was the failure to consistently provide quantitative data in published reports. With discrepancies in provision of basic parameters such as $C_{\text{max}}$ and $t_{\text{max}}$ along with gaps in other parameters (e.g., methods of lower limits of quantification determination), there is a clear need to establish a standardized manner of reporting data on CAR T-cell detection/expansion and persistence. This is even more important as recent work has reported that the use of surrogate markers, such as the monitoring of B-cell aplasia to designate ‘functional’ CAR T-cell persistence, is limited and poorly predictive of outcomes in the long-term.

In the context of our analysis, the diverse selection of clinical trials ensured inclusivity of different CAR constructs across a range of tumor-associated antigens targets. This type of analysis gains in importance with the increase in CAR T-cell trials targeting novel antigens. While our scoping approach may be limited by variation in the number of manuscripts published for each evaluated trial, our primary focus was on the ‘first’ published manuscript for each trial, thereby mitigating this limitation and serving as a first step toward understanding current gaps. In future research and/or in a future working group designed to develop standardized methods, it will be important to assess why groups chose certain techniques and whether access to equipment/resources contributed...
to the differential utilization of specific technologies. In addition, a global assessment of CAR T-cell detection is a critical next step to understand how detection methodologies vary internationally in order to better align the field.

Given the utility of CAR T-cell monitoring as a key biomarker associated with outcomes, there remains opportunity to unify on establishing a universal framework providing guidance on key metrics, detection time points, and standardized approaches to reporting of biological correlates. With commercialization of CAR T-cells products and a host of investigational products and constructs under study, our findings highlight the critical need to establish a universal standard for reporting on CAR T-cell detection on early phase studies. The current reporting of non-interconvertible metrics and limited provision of quantitative data make cross-trial and cross-CAR T-cell construct comparisons extremely challenging. While unique attributes of CAR constructs may limit utilization of a single detection method, establishing a standardized

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**Figure 3**  Reporting of CAR T-cell expansion and persistence data across primary clinical trial publications: Pie chart depictions of the number of primary clinical trial publications (n=21) reporting CAR transgene concentration over time, CAR T-cell concentration over time, and average peak % of T-cells expressing CAR, all in the peripheral blood (A–D). Pie chart depictions of primary publications reporting a maximum concentration (Cmax) of either CAR T-cells or the CAR transgene in the blood (E–G). Publications reporting the time to maximum concentration of CAR T-cells or CAR transgene copies in blood (tmax) (H). Primary publications reporting any area under the curve (AUC) metric as well as AUC0-28d in cells/µL×days or in CAR transgene copies/µg DNA×days (I–K). Primary publications providing a median time point at which CAR T-cells were no longer detectable in patients (tlast) (L). Publications reporting on the half-life of CAR T-cells in patients (t1/2). Publications reporting the last quantifiable concentration of CAR transgene in the blood (Clast) (N). The sites at which CAR T-cells were evaluated (PB, BM, CSF) in the different publications, either qualitatively or quantitatively, are presented (O). The time points at which CAR T-cells were detected are presented for the primary clinical trial publications (purple bars) as well as for the primary and all subsequent publications of a given clinical trial (blue bars) (P). The number of clinical trials evaluating the indicated metric in the primary publication (purple bars) and in subsequent publications (blue bars) are presented (Q). ‘Indeterminable’ denotes a metric that was reported in a publication but for which no quantitative value was provided. AUC, area under the curve; BM, bone marrow; CAR, chimeric antigen receptor; CSF, cerebrospinal fluid; PB, peripheral blood.
approach for collecting and reporting of data is urgently needed. The development of homogeneous methods and time points would lead to a better understanding of the metrics of CAR T-cell associated toxicities and represent a substantial advance in the ability to improve patient outcomes.

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