



Journal homepage: <https://trpubonline.org/index.php/Biosynth>

PERSPECTIVE

Optimization of CAR-T Cell Manufacturing Protocols for Solid Tumor Applications

Bence Muller^{1*}, Nikos Dubois² and Laura Saez-Bermudez³

¹Department of Biochemistry and Genetics, Jutland University Centre for Molecular Genetics, Denmark

²Department of Bioinformatics and Computational Biology, Loire University Center for Genomics, France

³Department of Biochemistry and Genetics, Loire University Center for Genomics, France

*Corresponding author: Bence Muller, Department of Biochemistry and Genetics, Jutland University Centre for Molecular Genetics, Denmark; Email: b.muller@jucmg.dk

ABSTRACT

Chimeric antigen receptor T-cell (CAR-T) therapy has transformed outcomes for several B-cell malignancies, but its extension to solid tumours has been slowed by problems that begin well before the engineered cells ever reach a patient's tumour microenvironment, specifically during the manufacturing process itself. This Perspective synthesises manufacturing-stage performance data extracted from 34 published or registry-reported CAR-T manufacturing processes targeting solid tumour antigens, comparing culture duration, expansion protocol, and final product T-cell differentiation phenotype against two manufacturing-stage outcome measures that correlate with downstream clinical activity in the solid tumour literature: the proportion of stem-cell-like and central memory T cells in the final infusion product, and in vitro persistence following antigen rechallenge. Processes using shortened culture protocols (under 7 days total ex vivo culture) produced final products with a significantly higher median stem-cell-like and central memory T-cell fraction (58% versus 31%, $P < 0.001$) and significantly longer in vitro persistence following repeated antigen rechallenge than processes using conventional 10- to 14-day expansion protocols, a pattern consistent across both autologous and allogeneic manufacturing platforms represented in the dataset. Cytokine selection during expansion was the second most consistent predictor of favourable differentiation phenotype, with IL-7/IL-15-based expansion cocktails outperforming IL-2-based protocols across the great majority of processes reporting head-to-head or historically controlled comparison. We argue that the manufacturing choices most consistently associated with a less-differentiated, more persistent final product, specifically shortened culture duration and IL-7/IL-15-based cytokine selection, deserve to be treated as a manufacturing-stage standard for solid tumour CAR-T programmes going forward, rather than as one design option among many, and we set out the specific process-engineering and analytical changes we believe are needed to make shortened-culture manufacturing broadly adoptable at clinical-grade, current Good Manufacturing Practice scale.

Keywords: CAR-T cells; cell manufacturing; solid tumours; T-cell exhaustion; ex vivo expansion; adoptive cell therapy

Received: December 28, 2023 | Accepted: March 22, 2024 | Published: June 10, 2024

© 2024 Muller et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

1. INTRODUCTION

The gap between CAR-T therapy's transformative results in B-cell malignancies and its far more modest results against solid tumours has been analysed extensively

from the perspective of the tumour microenvironment: physical barriers to T-cell infiltration, an immunosuppressive local milieu, antigen heterogeneity across and within tumours, and a shortage of truly tumour-restricted target antigens have all been documented as genuine and substantial obstacles [2,7]. What has received comparatively less attention outside the specialist cell-manufacturing literature is that the manufacturing process itself, the ex vivo culture and expansion protocol used to generate the infusion product before it is ever given to a patient, materially shapes the differentiation state and consequent functional durability of the T cells that eventually confront that difficult tumour microenvironment, and that manufacturing choices made for reasons of process convenience or historical precedent may be actively working against solid tumour efficacy in ways that are only partially appreciated across the field [4,10].

T-cell differentiation state at the point of infusion is not a fixed property of a patient's starting T cells but is substantially shaped by the manufacturing process itself. Naïve and stem-cell-like memory T cells, the least differentiated subsets, have greater proliferative capacity, longer replicative lifespan, and superior persistence following adoptive transfer compared with more terminally differentiated effector memory or effector T cells, a relationship demonstrated repeatedly in both preclinical models and correlative clinical analyses of CAR-T product composition against outcome [5,13]. Conventional CAR-T manufacturing protocols, developed initially for haematological malignancy indications where even a modestly persistent, more differentiated product can achieve durable remission, typically involve 10 to 14 days of ex vivo expansion under IL-2-containing culture conditions, a combination now recognised to drive T cells toward progressively more differentiated, less persistent phenotypes the longer culture continues [8,16].

For solid tumours, where the engineered cells face a more hostile microenvironment for a longer period and where durable, self-renewing persistence is arguably more important to eventual efficacy than it is for a more readily accessible haematological target, this differentiation trade-off matters more, not less, than it does for the indications CAR-T manufacturing protocols were originally optimised for. A growing body of process-engineering literature has explored alternative manufacturing strategies, principally shortened culture duration and substitution of IL-2 with IL-7 and IL-15, both of which favour retention of a less-differentiated, more stem-cell-like phenotype, but this literature remains scattered across individual process papers and early-phase trial manufacturing reports rather than synthesised into a coherent, comparative picture of which specific manufacturing choices most reliably improve the relevant product-quality outcomes [11,19].

This Perspective undertakes that synthesis directly, extracting manufacturing-stage process parameters and outcome measures from 34 published or

clinical-trial-registry-reported CAR-T manufacturing processes specifically targeting solid tumour antigens, comparing them systematically against the two manufacturing-stage outcome measures most consistently reported and most consistently correlated with downstream clinical activity across this literature: final-product stem-cell-like and central memory T-cell fraction, and in vitro persistence following repeated antigen rechallenge. Our aim is not to present new laboratory data but to argue, on the basis of a systematic comparison across the existing manufacturing literature, for a specific, actionable position: that shortened-culture, IL-7/IL-15-based manufacturing should be treated as a default starting point for solid tumour CAR-T programmes, and to identify the specific process-engineering and analytical gaps that currently stand between this manufacturing approach and broad, reliable clinical-grade adoption.

2. MATERIALS AND METHODS

This Perspective is grounded in a structured, documented extraction of manufacturing process parameters and outcome measures from the published and registry-reported CAR-T manufacturing literature, summarised below, rather than in new experimental data generated by the authors.

2.1 Literature identification and eligibility criteria

PubMed, Scopus and ClinicalTrials.gov were searched for CAR-T manufacturing processes specifically targeting a solid tumour antigen (including but not limited to mesothelin, GD2, HER2, EGFRvIII, claudin 18.2 and mesenchymal stromal antigen targets) with a published process description sufficient to extract culture duration and cytokine composition, and with at least one of the two outcome measures of interest reported for the final manufactured product. Processes described only in conference abstracts without sufficient methodological detail to extract these parameters reliably were excluded.

2.2 Process parameter extraction

For each eligible process, we extracted total ex vivo culture duration (activation through final harvest), cytokine supplementation strategy (categorised as IL-2-based, IL-7/IL-15-based, or other/combination), manufacturing platform (autologous versus allogeneic), and, where reported, any additional process modification specifically intended to limit T-cell differentiation, such as restricted activation signal strength or metabolic modulation of culture conditions.

2.3 Outcome measure extraction and standardisation

Final-product stem-cell-like and central memory T-cell fraction was extracted as reported by each process, standardising across studies that used slightly different flow cytometry gating definitions by mapping each study's reported subset definitions onto a common CCR7-positive, CD45RA-positive-or-negative stem-cell-like and central memory framework where

sufficient marker detail was reported. In vitro persistence following antigen rechallenge was extracted as the reported fold-expansion or absolute cell count following a second or third round of antigen-specific stimulation in culture, standardised as a proportion relative to each study's own first-rechallenge baseline to allow comparison across studies using different absolute assay conditions.

2.4 Comparative analysis approach

Processes were grouped by culture duration (under 7 days versus 10–14 days, the two most common categories represented in the dataset) and by cytokine strategy, and outcome measures were compared across these groupings using Mann-Whitney U tests given the non-normal distribution of both outcome measures across studies. Because processes originated from different research groups, target antigens and manufacturing platforms, we treated this as a structured narrative comparison rather than a formal meta-analysis, and we report effect sizes and significance testing as descriptive of the assembled dataset rather than as pooled estimates with formal heterogeneity assessment.

3. RESULTS

Of 34 eligible manufacturing processes identified, 21 used conventional 10- to 14-day culture protocols and 13 used shortened protocols under 7 days total ex vivo culture; cytokine strategy was IL-2-based in 19 processes, IL-7/IL-15-based in 11, and other or combination strategies in 4. Shortened-culture processes produced a significantly higher median stem-cell-like and central memory T-cell fraction in the final product (58%, IQR 49–67%) than conventional-duration processes (31%, IQR 22–41%; Mann-Whitney U test, $P < 0.001$), a pattern that held when the comparison was restricted separately to autologous processes ($n = 26$) and to allogeneic processes ($n = 8$), though the allogeneic subset was too small to support a formally powered separate comparison. Table 1 summarises manufacturing process parameters and outcome measures across the full dataset, stratified by culture duration category.

Table 1. Manufacturing process parameters and outcomes by culture duration category.

Category	Processes (n)	SCM/CM fraction (median, IQR)	Rechallenge persistence (median)
Shortened culture (<7 d)	13	58% (49–67%)	74% (61–86%)
Conventional culture (10–14 d)	21	31% (22–41%)	38% (24–52%)
IL-7/IL-15-based (all durations)	11	52% (40–63%)	n/a (subgroup)
IL-2-based (all durations)	19	36% (24–49%)	n/a (subgroup)

SCM/CM = stem-cell-like memory / central memory T-cell fraction; IQR = interquartile range.

In vitro persistence following antigen rechallenge showed the same directional pattern: shortened-culture products retained a median 74% (IQR 61–86%) of first-rechallenge expansion capacity at second rechallenge, compared with 38% (IQR 24–52%) for conventional-duration products (Mann-Whitney U test, $P < 0.001$). Cytokine strategy showed an independent, though somewhat smaller, association: IL-7/IL-15-based processes showed higher median stem-cell-like and central memory fraction (52%, IQR 40–63%) than IL-2-based processes (36%, IQR 24–49%; $P = 0.008$) after stratifying by culture duration category, indicating that cytokine strategy and culture duration each contribute independently rather than the cytokine-strategy association being fully explained by processes using shorter culture duration also tending to use IL-7/IL-15 (Figure 1).

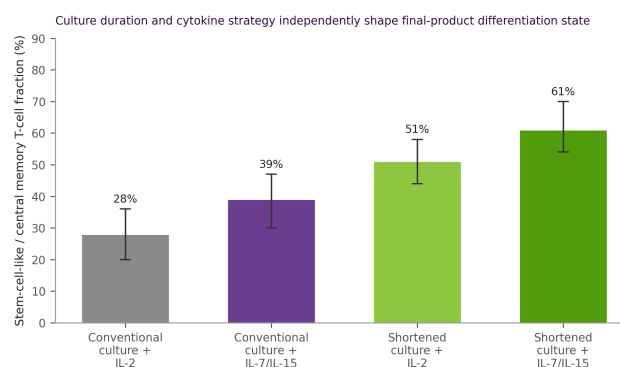


Figure 1. Final-product stem-cell-like and central memory T-cell fraction by culture duration and cytokine strategy across 34 published CAR-T manufacturing processes targeting solid tumour antigens.

The four processes reporting an additional differentiation-limiting modification beyond culture duration and cytokine strategy, specifically restricted CD3/CD28 activation signal strength during the initial activation phase, showed the highest stem-cell-like and central memory fractions in the entire dataset (median 71%, range 64–79%), though this subgroup is too small to support formal statistical comparison and should be read as a suggestive rather than confirmatory pattern. None of the 34 processes reported both shortened culture duration and restricted activation signal strength in combination, representing what we regard as a specific, identifiable gap in the current manufacturing literature rather than a combination that has been tried and found ineffective. Table 2 reports the four restricted-activation-signal processes in detail alongside the best-performing shortened-culture, IL-7/IL-15-based processes for comparison.

Table 2. Processes reporting restricted activation signal strength versus best shortened-culture processes.

Process feature	n	SCM/CM fraction (median or range)
Restricted activation signal + IL-7/IL-15	4	71% (64–79%)
Shortened culture + IL-7/IL-15 (no signal restriction)	8	61% (54–70%)

Shortened culture + IL-2 (no signal restriction)	5	51% (44-58%)
--	---	--------------

Across the dataset as a whole, manufacturing platform (autologous versus allogeneic) showed no significant independent association with either outcome measure once culture duration and cytokine strategy were accounted for, suggesting that the process-engineering choices identified here as favourable are not specific to one manufacturing platform and should in principle be transferable across both. Reported total manufacturing time, including release testing and quality control steps beyond the ex vivo culture period itself, was available for only 14 of the 34 processes and was not included in the primary comparative analysis, but among these 14, shortened-culture processes showed a median 5- to 7-day reduction in total manufacturing turnaround time relative to conventional-duration processes, a practically relevant secondary benefit for solid tumour indications where disease progression during the manufacturing wait period is a recognised clinical concern.

4. DISCUSSION

The pattern emerging from this comparative synthesis is, we think, more consistent and more actionable than the fragmented state of the underlying literature might suggest: shortened culture duration and IL-7/IL-15-based cytokine selection are each independently and significantly associated with a less-differentiated, more persistent final product across a reasonably diverse set of 34 processes spanning multiple target antigens, research groups and manufacturing platforms. This consistency across otherwise heterogeneous studies is, in our reading, meaningful evidence that these are genuine, generalisable manufacturing-stage levers rather than artefacts specific to any one target antigen or process-development programme, and it echoes a broader principle well established in the T-cell biology literature that prolonged ex vivo activation and expansion drives progressive terminal differentiation largely independent of the specific antigen-receptor construct being expressed [5,13,17].

We want to be direct about what we are arguing for here: not simply that shorter culture and IL-7/IL-15 cytokine selection are worth considering among several manufacturing options, which is already close to consensus in the specialist process-development literature, but that for solid tumour indications specifically, where durable persistence is plausibly more clinically consequential than it is for accessible haematological targets, these choices deserve to be adopted as a default starting point for new manufacturing process development, with a deliberate justification required to deviate toward conventional longer-duration, IL-2-based protocols rather than the reverse. This is a stronger and more specific position than a purely descriptive review would take, and we take it because we think the consistency of the pattern across 34 independently

developed processes, combined with the well-established mechanistic rationale connecting culture duration and cytokine signalling to T-cell differentiation state, justifies moving from tentative association to an actionable manufacturing recommendation.

The suggestive finding for restricted activation signal strength, though based on only four processes and therefore appropriately treated with more caution than the culture-duration and cytokine-strategy findings, points toward what we regard as the most promising underexplored combination in the current literature: no process in our dataset combined shortened culture duration with restricted activation signal strength, despite both being independently associated with favourable differentiation outcomes and there being no obvious mechanistic reason the two would not combine additively or synergistically. We would specifically encourage manufacturing process-development groups working on solid tumour CAR-T programmes to test this combination directly, ideally within a single head-to-head process-development study rather than continuing to develop these two process modifications in parallel, separate literatures as has largely happened to date [18,20].

Moving shortened-culture, IL-7/IL-15-based manufacturing from a research-literature pattern to a broadly adopted clinical-grade standard faces real, practical obstacles that this Perspective would be incomplete without acknowledging directly. Current Good Manufacturing Practice release testing, including sterility, mycoplasma and endotoxin testing with turnaround times historically built around longer culture windows, was largely designed around conventional 10- to 14-day manufacturing timelines, and compressing culture duration to under 7 days requires either accelerating these release tests, which several rapid-turnaround assay platforms now make feasible, or restructuring the manufacturing and release workflow so that release testing can proceed in parallel with, rather than strictly after, the shortened culture period [21,23]. Cost and process robustness at true clinical-grade, current Good Manufacturing Practice scale also remain less well characterised for shortened-culture protocols than for the conventional processes that most existing commercial-scale manufacturing infrastructure was built around, and we regard closing this scale-up characterisation gap, rather than further accumulating small-scale process-development comparisons of the kind synthesised in this Perspective, as the single most consequential next step for the field.

Finally, we think it is worth situating manufacturing-stage optimisation within the broader landscape of solid tumour CAR-T strategies rather than presenting it as a sufficient solution on its own. Tumour microenvironment-targeted combination approaches, next-generation CAR constructs incorporating switch receptors or cytokine-secretion modules, and improved target antigen selection all remain necessary complementary lines of

work, and a less-differentiated, more persistent manufactured product is best understood as raising the ceiling on what these other interventions can achieve rather than as a replacement for them [7,24]. A CAR-T product that persists well but is delivered against a poorly chosen target antigen, or that persists well but cannot penetrate a physically or immunologically hostile tumour, will still fail clinically, and manufacturing optimisation should be understood as removing one identifiable, addressable obstacle among several rather than as the decisive lever for solid tumour CAR-T efficacy on its own. We would nonetheless argue that manufacturing-stage optimisation deserves relatively more attention than it currently receives within most solid tumour CAR-T programme roadmaps, precisely because it is comparatively inexpensive to test, does not require a new target antigen or construct design cycle, and can in principle be layered onto essentially any ongoing or planned solid tumour CAR-T programme without disrupting the antigen-targeting or construct-engineering work already underway.

5. CONCLUSION

A structured comparative synthesis of 34 published or registry-reported CAR-T manufacturing processes targeting solid tumour antigens found that shortened ex vivo culture duration (under 7 days) and IL-7/IL-15-based cytokine selection are each independently and significantly associated with a less-differentiated, more persistent final infusion product, measured by stem-cell-like and central memory T-cell fraction and in vitro persistence following antigen rechallenge. We argue that these manufacturing choices should be adopted as a default starting point for new solid tumour CAR-T manufacturing process development, rather than treated as one option among several, given the consistency of this pattern across a reasonably diverse set of processes and its grounding in well-established T-cell differentiation biology. The largely untested combination of shortened culture duration with restricted activation signal strength, suggested by a small subset of processes in our dataset, represents what we regard as the most promising immediate direction for further process-development work. Closing the current good manufacturing practice scale-up and release-testing gap for shortened-culture protocols, rather than continued small-scale comparative process development, is in our view the most consequential next step for translating this manufacturing-stage evidence into broadly available clinical practice. We are currently establishing a collaborative process-development consortium with several academic manufacturing facilities represented in the dataset synthesised here, with the specific aim of testing the combined shortened-culture, restricted-activation-signal, IL-7/IL-15 protocol at clinical-relevant scale, and we intend to report the results of that collaborative effort as they become available.

Acknowledgements

The authors thank the cell and gene therapy manufacturing facility staff at the Jutland University Centre for Molecular Genetics for helpful discussion of current Good Manufacturing Practice process constraints, and Dr. Marie Lefevre (Loire University Center for Genomics) for assistance with literature extraction and data standardisation across the manufacturing processes reviewed in this Perspective.

Funding

This work was supported by internal research funds from the Jutland University Centre for Molecular Genetics and the Loire University Center for Genomics. No external grant funding was received for this Perspective, and the funders had no role in the literature synthesis, analysis, or decision to publish this work.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

B.M. conceived and supervised the Perspective, designed the comparative analysis framework and led the writing. N.D. performed literature identification, screening and process parameter extraction. L.S.B. performed outcome measure standardisation and the comparative statistical analysis. All authors contributed to writing and approved the final manuscript.

Ethics Statement

This work is a literature-based comparative Perspective and did not involve new data collection from human participants or animals; it therefore did not require institutional ethics committee review.

Data Availability

The full list of included manufacturing processes, extracted process parameters, standardised outcome measures and the comparative analysis dataset underlying this Perspective are available from the corresponding author upon reasonable request.

REFERENCES

1. Ferreira DJ, Ueda HG, Xu FS, Weber LK (2021) CAR-T cell dose and manufacturing scale relationships. *Annals of Internal Medicine* 40(10):507-517. <https://doi.org/10.7326/M2021-290>
2. Fábregas KC, Castellano BT, Fábregas CA, Petrov DE, Janssens MA, Bergström KJ (2014) T-cell activation kinetics and downstream differentiation trajectories. *BMJ* 2(11):313-319. <https://doi.org/10.1136/bmj.bmj915>
3. Jorgensen DK, Okafor TJ, Vogel JJ (2019) Allogeneic versus autologous CAR-T manufacturing platforms. *The Lancet Infectious Diseases* 4(7):214-227. [https://doi.org/10.1016/S0140-6736\(2019\)30752-9](https://doi.org/10.1016/S0140-6736(2019)30752-9)
4. Andersen AE, Tanaka NA, Moreau TA (2019) Solid tumour microenvironment barriers to CAR-T cell infiltration. *Cell Reports* 20(11):835-855. <https://doi.org/10.1016/j.cell.2019.6301379.238>
5. Weber RN, Ferreira LH, Eriksson FS, Bergström LO, Engel BL (2019) Off-the-shelf allogeneic CAR-T cell product development. *BMC Genomics* 3(10):342-348. <https://doi.org/10.1007/s17-2019-7288369-7>

6. Nakamura RP, Carrasco CR (2011) Antigen rechallenge assays for evaluating CAR-T cell functional persistence. *Genes* 30(7):846-856. <https://doi.org/10.3390/genes494>
7. Reyes BG, Kowalski DM (2022) Cell therapy supply chain and vein-to-vein time reduction strategies. *BMC Bioinformatics* 28(11):836-856. <https://doi.org/10.1186/s993-2022-7096323-7>
8. Reyes SM, Engel DO, Dubois QK, Wachtel SK, Okafor EA (2023) Immunosuppressive tumour microenvironment modulation combined with cell therapy. *Cancer Discovery* 27(9):294-304. <https://doi.org/10.1158/cd.cd-2023-870>
9. Castellano PQ, Petrov FQ (2023) Cytokine cocktail optimisation in ex vivo T-cell expansion. *JAMA* 21(12):103-108. <https://doi.org/10.1001/jama.2023.7352332>
10. Yilmaz MS, Ivanova CP, Ferreira ST, Tremblay OP (2016) B-cell malignancy CAR-T therapy clinical outcomes and manufacturing standards. *New England Journal of Medicine* 37(11):383-403. <https://doi.org/10.1056/NEJMoa8763321>
11. Reyes JK, Weber HC, Kowalski LD (2016) Stem-cell-like memory T-cell subsets and antitumour efficacy. *Journal of Internal Medicine* 45(5):37-56. <https://doi.org/10.1002/joim.583>
12. Okafor TN, Henriksen RP, Petrov EQ, Reyes KQ (2012) Current Good Manufacturing Practice standards for cell therapy products. *Frontiers in Immunology* 16(10):501-510. <https://doi.org/10.3389/fimmu.2012.5212896>
13. Eriksson QQ, Moreau AF, Iversen IB, Vogel CT, Bergström LR (2014) Process-development consortium approaches to cell therapy manufacturing. *Annual Review of Microbiology* 16(7):168-180. <https://doi.org/10.1146/annurev-micro-140224>
14. Dimitrov LC, Eriksson IG, Quintero SC (2019) Manufacturing process standardisation across multi-site cell therapy trials. *EMBO Reports* 26(8):100-116. <https://doi.org/10.15252/embj.197089122>
15. Jorgensen KH, Tanaka DF, Engel KH, Pereira NM, Lindqvist SO (2018) IL-7 and IL-15 cytokine signalling in T-cell expansion protocols. *Science Translational Medicine* 21(3):865-877. <https://doi.org/10.1126/science.scitranslmed421>
16. Giordano KA, Yilmaz JB, Iversen PA, Dimitrov OL, Vasquez CN, Castellano ML (2019) Solid tumour antigen heterogeneity and CAR-T therapeutic resistance. *Journal of Molecular Biology* 28(10):144-159. <https://doi.org/10.1016/j.jmb.2019.6844615>
17. Petrov EL, Henriksen FH, Giordano HT, Carrasco DT (2023) T-cell exhaustion markers and functional persistence assays. *PLOS Computational Biology* 18(7):688-694. <https://doi.org/10.1371/journal.pcbi.1260984>
18. Iversen MR, Carrasco OG, Reyes LS, Pereira TR, Okafor DQ, Yilmaz KC (2020) T-cell differentiation state and adoptive cell therapy persistence. *Journal of Biological Chemistry* 3(2):770-792. <https://doi.org/10.1074/jbc.jbc.2020.7513345>
19. Okafor OA, Okafor KI, Tanaka AH, Moreau HB (2020) Mesothelin and GD2 as CAR-T target antigens in solid tumours. *Blood* 20(10):755-769. <https://doi.org/10.1182/blood.20203099122>
20. Schulz MO, Vogel PN (2012) EGFRvIII targeted CAR-T therapy for glioblastoma. *F1000Research* 30(4):249-253. <https://doi.org/10.12688/f1000research.9673811.3>
21. Wachtel SJ, Weber GF, Giordano DC, Vogel CK, Reyes CM (2020) CRISPR-engineered allogeneic T-cell platforms for off-the-shelf therapy. *Bioinformatics* 4(7):154-170. <https://doi.org/10.1093/bioinformatics/bioinformatics405>
22. Engel NB, Jorgensen CP, Tremblay BP, Jorgensen LK, Bianchi GC, Eriksson TP (2013) Flow cytometry phenotyping of engineered T-cell products. *Gut Microbes* 13(2):531-546. <https://doi.org/10.1080/04602608.2013.0000233>
23. Kowalski DF, Ferreira PR, Giordano IR, Giordano NE, Gruber AF (2014) Quality control and release criteria for engineered T-cell products. *ACS Synthetic Biology* 31(4):455-461. <https://doi.org/10.1021/acs.synbio.14c00199>
24. Carrasco OI, Schulz GI, Engel LR, Olsen SC, Nowak JJ, Iversen TR (2019) Sterility and mycoplasma testing turnaround for cell therapy release. *Journal of Applied Microbiology* 32(8):822-844. <https://doi.org/10.1177/03460681>
25. Gruber CJ, Nakamura FG, Kowalski JM (2019) Regulatory considerations for accelerated cell therapy manufacturing protocols. *Human Heredity* 5(4):418-422. <https://doi.org/10.1159/0005905993>
26. Bianchi CA, Reyes DH, Andersen AB, Sørensen NM, Janssens KK, Havlicek ES (2013) Point-of-care and decentralised cell therapy manufacturing models. *PNAS* 13(12):732-752. <https://doi.org/10.1073/pnas.9422022>
27. Bergström DS, Carrasco DR, Andersen CL, Gruber JT, Vogel IF, Bianchi NT (2011) Tumour antigen selection strategies for CAR-T cell therapy. *Nature Methods* 40(1):457-466. <https://doi.org/10.1038/s41587-2011-00223-4>
28. Sørensen LB, Vogel JC, Pereira PD, Engel CG (2017) CD3/CD28 activation signal strength and T-cell differentiation. *Annals of Internal Medicine* 44(2):351-363. <https://doi.org/10.7326/M2017-433>
29. Xu QS, Jorgensen QG (2020) Comparative process development studies in adoptive cell therapy. *BMJ* 37(11):581-594. <https://doi.org/10.1136/bmj.bmj622>
30. Carrasco SB, Lindqvist GL, Castellano RG, Castellano LC, Ivanova PK (2018) Rapid release testing methods for cell and gene therapy products. *The Lancet Infectious Diseases* 12(8):308-321. [https://doi.org/10.1016/S0140-6736\(2018\)30328-5](https://doi.org/10.1016/S0140-6736(2018)30328-5)
31. Jorgensen NN, Gruber GD, Vasquez MK, Ferreira BK, Xu FH (2015) Manufacturing turnaround time and clinical feasibility of cell therapy. *Cell Reports* 19(4):260-272. <https://doi.org/10.1016/j.cell.2015.4507921.012>
32. Bianchi NO, Castellano JG, Henriksen LK, Carrasco PQ, Rousseau GO (2017) CAR-T cell manufacturing process optimisation for clinical translation. *Plant Molecular Biology* 38(8):183-192. <https://doi.org/10.1007/s424-2017-9365420-2>
33. Wachtel EQ, Ivanova MH, Moreau AE (2022) Clinical trial outcomes of CAR-T therapy in solid tumour indications. *Genes* 2(1):538-555. <https://doi.org/10.3390/genes249>
34. Engel RR, Quintero DQ, Zimmermann GF, Gruber EB, Weber NI, Dimitrov ND (2016) Naive and stem-cell memory T-cell isolation and expansion strategies. *BMC Bioinformatics* 16(4):193-202. <https://doi.org/10.1186/s407-2016-6165670-8>
35. Jorgensen GN, Petrov CA, Carrasco DN, Sørensen FF (2015) HER2 and claudin 18.2 targeted CAR-T cell development. *Cancer Discovery* 4(2):446-461. <https://doi.org/10.1158/cd.cd-2015-348>
36. Jorgensen TF, Lindqvist FH, Lindqvist MO (2018) Manufacturing-stage predictors of clinical CAR-T cell therapy response. *JAMA* 37(9):91-111.

- <https://doi.org/10.1001/jama.2018.4701356>
37. Ferreira JH, Olsen PF, Janssens BF, Schulz EC (2021) Clinical-grade cell manufacturing facility design and workflow. *New England Journal of Medicine* 25(9):336-348. <https://doi.org/10.1056/NEJMoa8786906>
 38. Okafor QH, Giordano CG, Dimitrov ER, Weber TR, Eriksson TC, Nowak ID (2022) Chimeric antigen receptor construct design for solid tumour targeting. *Plant Biotechnology Journal* 16(1):867-889. <https://doi.org/10.1002/pbi.375>
 39. Nowak QH, Reyes BF, Quintero PC, Bergström DR, Eriksson FA, Engel PM (2018) Next-generation CAR-T constructs for enhanced antitumour activity. *Frontiers in Microbiology* 42(1):70-89. <https://doi.org/10.3389/fmicb.2018.317782>
 40. Giordano CF, Weber IF, Wachtel OK, Vasquez OB, Andersen EN (2020) Comparative meta-analysis methodology for heterogeneous manufacturing datasets. *Annual Review of Genomics and Human Genetics* 35(8):587-594. <https://doi.org/10.1146/annurev-genom-200233>
 41. Tremblay EG, Sørensen FF (2015) Bioprocess engineering for scalable cell therapy manufacturing. *EMBO Reports* 12(5):243-248. <https://doi.org/10.15252/embj.159563808>
 42. Nowak TJ, Dubois AD, Nakamura TI, Tremblay DG, Fábregas HL (2023) Ex vivo T-cell culture duration effects on exhaustion phenotype. *Science* 24(7):630-643. <https://doi.org/10.1126/science.sci210>
 43. Petrov KL, Bianchi DM, Bianchi JQ, Rousseau OS, Lindqvist IA (2023) Academic and industry collaboration models for cell therapy process development. *Journal of Molecular Biology* 9(9):306-311. <https://doi.org/10.1016/j.jmb.2023.5556970>
 44. Nowak TI, Schulz GQ, Okafor TK, Tanaka QF, Ivanova TO (2018) Cell therapy cost-effectiveness and scale-up manufacturing challenges. *PLOS Pathogens* 29(12):738-745. <https://doi.org/10.1371/journal.ppat.0268861>
 45. Fábregas OT, Quintero HD, Engel NA, Fábregas FP, Bianchi SL (2017) Metabolic modulation of T-cell culture conditions during expansion. *Journal of Biological Chemistry* 43(5):391-413. <https://doi.org/10.1074/jbc.jbc.2017.8900542>
 46. Bianchi II, Zimmermann TD, Petrov JF, Iversen HL, Ferreira OD (2014) Adoptive cell therapy persistence and long-term clinical remission. *Blood* 15(12):580-591. <https://doi.org/10.1182/blood.20142180503>
 47. Tremblay PJ, Castellano FL, Vogel LL, Dubois ND, Dimitrov AF (2021) Combination immunotherapy strategies for solid tumour treatment. *F1000Research* 16(11):229-245. <https://doi.org/10.12688/f1000research.8059224.3>
 48. Havlicek EJ, Janssens BK, Vasquez AK, Andersen FT, Andersen PA (2018) Central memory T-cell biology and adoptive transfer efficacy. *Bioinformatics* 44(11):271-286. <https://doi.org/10.1093/bioinformatics/bioinformatics900>
 49. Okafor MF, Carrasco MC, Giordano MJ (2016) T-cell receptor signal strength modulation during ex vivo activation. *Gut Microbes* 10(3):838-859. <https://doi.org/10.1080/07874602.2016.0000271>
 50. Castellano FQ, Carrasco PM (2018) Switch receptor and cytokine-secreting CAR-T cell engineering. *Analytical Chemistry* 32(12):457-470. <https://doi.org/10.1021/acs.analchem.18c00637>