SOMATO PUBLICATIONS

ISSN: 2689-8659

Annals of Stem Cell Research

Review Article

Quality Control Standards and Release Criteria for Clinical-Grade Stem Cell Products

Szabo E[1*], Maes E[2] and Bizjak A[3]

[1]Department of Biomedical Engineering, University of Debrecen, Debrecen, Hungary

[2]Department of Molecular Cell Biology, Ghent University, Ghent, Belgium

[3]Department of Biomedical Engineering, University of Ljubljana, Ljubljana, Slovenia

*Address for Correspondence: Eszter Szabo, Department of Biomedical Engineering, University of Debrecen, Debrecen, Hungary; E-mail: eszter.szabo@universityofdebrecen.edu

Received: 11 April 2023; Accepted: 25 July 2023; Published: 7 November 2023; DOI: 10.5281/zenodo.22238016

Citation of this article: Szabo E, Maes E, Bizjak A (2023) Quality Control Standards and Release Criteria for Clinical-Grade Stem Cell Products. Ann Stem Cell Res, 6(1): 01-06.

Copyright: © 2023 Eszter Szabo, et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Background: A cell therapy is released to a patient on a panel of tests, and the panel is largely inherited from criteria written to make research comparable rather than to predict clinical effect.

Objective: To review the release criteria in current use, what each establishes, and where the evidence for them is weakest.

Methods: Narrative review of position statements, validation studies and published release panels from clinical programmes.

Findings: Across six named clinical programmes, viability specifications sit at 70% or above while identity thresholds for the same marker range from 30% to 90%. Cells can meet a viability specification and still have lost immunosuppressive function on thaw. Automated sterility detection flagged all contrived positives within 72 hours against a 14-day compendial incubation, for products whose shelf life is hours.

Conclusions: Identity and viability are cheap and weakly informative. Potency remains the unsolved problem.

Keywords: release criteria; potency assay; sterility testing; cell therapy; quality control

© 2023 Somato Publications. All rights reserved.

Introduction

Every batch of a cell therapy is released on a set of tests, and the set is remarkably consistent between programmes: identity by surface phenotype, viability by dye exclusion, sterility, mycoplasma, endotoxin, and something designated potency. The consistency is not evidence that the tests are right. Most of them were adopted because they were available and are accepted, not because any of them has been shown to predict how a patient will do.

The identity criteria illustrate the problem. The minimal criteria defining multipotent mesenchymal stromal cells were written to let laboratories compare their preparations [1], and the same body has since revisited nomenclature [2] and proposed immunological characterisation as a supplement [3]. None of these documents claims that CD73, CD90 and CD105 positivity distinguishes a lot that will work from one that will not, and the field's own commentary is candid about where that leaves it [4].

This review considers what the standard panel establishes, and what it does not.

Materials and Methods

Records were retrieved from Crossref, Europe PMC and publisher pages for the terms *release criteria*, *potency assay*, *sterility testing*, *cell therapy* and *residual undifferentiated cells*. Priority was given to position statements, to method validation studies, and to publications reporting release panels actually used in clinical programmes. Every bibliographic record was confirmed at source, and quantitative values are reported only where confirmed at source.

Identity and viability

Identity by phenotype is the cheapest test on the panel and the least discriminating. Thresholds are also not standardised: across six clinical natural killer cell programmes, the specification for CD56 positivity ranges from at least 30% to above 90%, while viability is set at 70% or above in all of them [5]. A specification that varies threefold between programmes measuring the same marker is a convention rather than a standard.

Viability is more troubling, because it is the criterion most likely to be believed. Cryopreserved mesenchymal stromal cells display impaired immunosuppressive properties immediately after thaw as a result of a heat-shock response and impaired interferon-γ licensing, with function restored after 24 hours of culture [6]; the effect is context dependent but real [7]. A product can therefore pass a 70% viability specification and be functionally inert at the moment of infusion. The gating test measures membrane integrity, not the property the therapy depends on.

Potency

This is where the field agrees it is stuck. The challenge of defining potency assays and their potential use as release criteria has been set out explicitly [8], an industry survey documented what programmes actually do [9], and the difficulty has been named plainly in a review title [10]. The proposed way forward is a matrix of assays rather than a single readout, since no one surrogate marker captures a multifactorial mechanism [11].

The practical consequence is that most programmes release on something a regulator will accept rather than on something mechanistically true, and this is a stated position rather than an inference.

Where potency is specified, it can be: the same natural killer cell panel gives cytotoxicity specifications of more than 20% lysis at an effector-to-target ratio of 20:1 in one programme, and 32% at 5:1, 39% at 10:1 and 49% at 20:1 in others [5]. These are functional and they are not comparable.

Sterility and adventitious agents

© 2023 Somato Publications. All rights reserved.

Table 1: Release specifications across six clinical natural killer cell programmes, showing the range applied to the same attribute.

Attribute	Specification range in use	Ref.
Viability	70% or above, in all six programmes	[5]
Identity, CD56+	30% / 50% / 70% / 90% depending on programme	[5]
Purity, CD3+	0.2% or less, or a dose limit per kilogram	[5]
Potency, cytotoxicity	20% lysis at 20:1, to 49% at 20:1	[5]
Sterility and mycoplasma	Negative, in all six programmes	[5]
Residual feeder cells	1% or less, or 0.1% or less	[5]

Sterility testing is the clearest example of a specification colliding with the product. The compendial method incubates for 14 days; an autologous cell product may have a shelf life measured in hours. Automated blood culture systems have therefore been validated against compliant methods repeatedly [12] and in apheresis progenitor products [13], and the scale of the underlying contamination problem was characterised early [14].

The quantitative case is now strong. In a recent validation, median time to positivity for the first positive bottle was 13 hours 21 minutes with a maximum of 45 hours 3 minutes, all contrived sets flagged within 72 hours, and the authors proposed shortening incubation from 14 days to 4–7 days [15]. Whether current good manufacturing practice should be reconsidered for minimally manipulated products has been argued directly [16]. Mycoplasma detection by reverse transcription PCR provides a rapid alternative to culture [17].

Real-world contamination rates are not negligible. Across 364 stromal vascular fraction productions in two centres, sterile batches were 95.71% in one cohort and 74.15% in the other ($p < 0.0001$) [18].

Genomic stability and residual undifferentiated cells

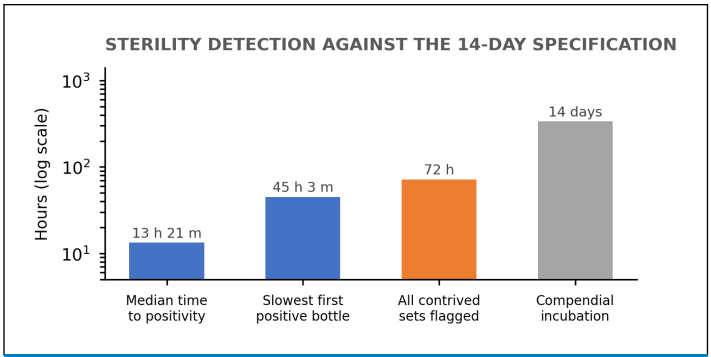

Figure 1: Time to detection of contamination by automated blood culture against the 14-day compendial incubation. All contrived positives flagged within 72 hours, for a product whose shelf life is measured in hours. Data from Snyder et al. [15].

For expanded products, aneuploidy occurs during clinical-grade production without transformation [19], and the consequences of recurrent genetic and epigenetic variants in pluripotent lines have been reviewed by the field's own working group [20].

For products derived from pluripotent cells the critical impurity is the undifferentiated cell, and assays for it have been developed with increasing sensitivity: droplet digital PCR for cardiomyocytes [21] and a multisite-optimised culture assay [22]. Tumorigenicity assessment has its own framework [23]. Preclinical packages for dopaminergic progenitor products illustrate what a full release panel looks like in practice [24,25].

Comparability and specification setting

A manufacturing change requires a demonstration that the product has not changed in a way that matters, and the framework for this was set out for biologics generally [26]; the specific difficulties for stromal cell products, including differences between regulators, have been examined [27]. Where a process has been validated with a defined and reproducible outcome, the specifications follow from the observed distribution [28]. The stromal vascular fraction study did this explicitly, proposing viability at or above 80%, a minimum recovery yield of 1.50×10 viable nucleated cells per ml of adipose tissue, stromal cells at least 20% and leukocytes under 50% —

© 2023 Somato Publications. All rights reserved.

criteria met by 92.9%, 81.3%, 86.8% and 87.4% of 364 batches respectively [18].

That is what specification setting looks like when it is done from data rather than convention.

Discussion

Three conclusions follow. First, identity and viability are inherited convention: cheap, reproducible and weakly related to clinical performance, with the cryopreservation literature showing directly that viability and function can diverge.

Second, potency is the genuine disagreement, and the disagreement is not about which assay to prefer but about whether a single assay can exist for a product whose mechanism is unknown. The assay-matrix proposal is an admission of this rather than a solution to it.

Third, several specifications are inherited from products unlike cell therapies. A 14-day sterility incubation for a product infused within hours is the clearest case, and the validation evidence for shortening it is now substantial.

This is a narrative review without systematic search or risk-of-bias assessment. Several release panels could not be obtained at source and are therefore described rather than tabulated, and specification values are not comparable between programmes.

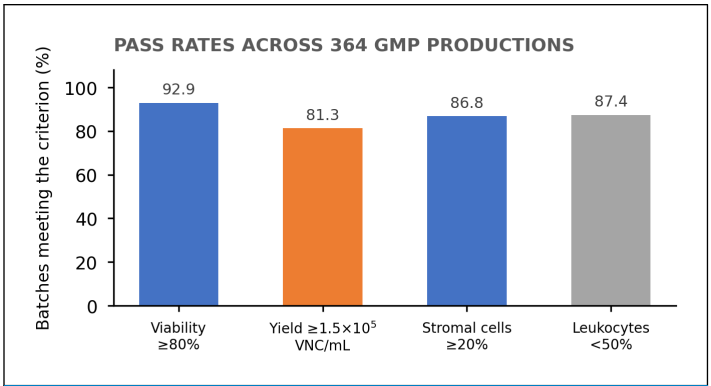

Figure 2: Batches meeting each proposed release criterion across 364 good manufacturing practice productions in two centres. Setting specifications from an observed batch distribution rather than by convention is uncommon and is what this study did. Data from Francois et al. [18].

Conclusion

Release criteria for clinical-grade stem cell products divide into tests that are easy and largely uninformative and tests that would matter but are hard to run. Identity thresholds vary threefold between programmes measuring the same marker; viability gates on membrane integrity while function can be lost and recovered independently; sterility is specified on a timescale the product does not have. The unsolved problem is potency, and it is unsolved because mechanism of action is usually unknown rather than because assay technology is lacking. Programmes should set specifications from their own observed batch distributions, state which criterion each batch failed when one does, and report the potency measure alongside the mechanism it is intended to reflect.

Acknowledgements

The authors thank the library staff who assisted with retrieval of the primary literature reviewed here.

Funding

This review received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Conflict of Interest

© 2023 Somato Publications. All rights reserved.

The authors declare that they have no competing interests relevant to the content of this review.

Ethics Statement

This article reviews previously published studies and contains no new studies with human participants or animals performed by any of the authors. Ethical approval was therefore not required.

References

1. Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F., Krause, D., et al. (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy, 8(4): 315-317.

2. Viswanathan, S., Shi, Y., Galipeau, J., Krampera, M., Leblanc, K., Martin, I., et al. (2019) Mesenchymal stem versus stromal cells: International Society for Cell and Gene Therapy Mesenchymal Stromal Cell committee position statement on nomenclature. Cytotherapy, 21(10): 1019-1024.

3. Krampera, M., Galipeau, J., Shi, Y., Tarte, K., Sensebe, L. (2013) Immunological characterization of multipotent mesenchymal stromal cells - the International Society for Cellular Therapy (ISCT) working proposal. Cytotherapy, 15(9): 1054-1061.

4. Galipeau, J., Sensebe, L. (2018) Mesenchymal stromal cells: clinical challenges and therapeutic opportunities. Cell Stem Cell, 22(6): 824-833.

5. von Werz, V., Szarzynski, A., Zigon-Branc, S., Spadiut, O. (2025) Quality standards for NK cell immunotherapies. Front Bioeng Biotechnol, 13: 1716975.

6. Francois, M., Copland, I.B., Yuan, S., Romieu-Mourez, R., Waller, E.K., Galipeau, J. (2012) Cryopreserved mesenchymal stromal cells display impaired immunosuppressive properties as a result of heat-shock response and impaired interferon-gamma licensing. Cytotherapy, 14(2): 147-152.

7. Burand, A.J., Gramlich, O.W., Brown, A.J., Ankrum, J.A. (2017) Function of cryopreserved mesenchymal stromal cells with and without interferon-gamma prelicensing is context dependent. Stem Cells, 35(5): 1437-1439.

8. Galipeau, J., Krampera, M. (2015) The challenge of defining mesenchymal stromal cell potency assays and their potential use as release criteria. Cytotherapy, 17(2): 125-127.

9. Bravery, C.A., Carmen, J., Fong, T., Oprea, W., Hoogendoorn, K.H., Woda, J., et al. (2013) Potency assay development for cellular therapy products: an ISCT review of the requirements and experiences in the industry. Cytotherapy, 15(1): 9-19.

10. Torrents, S., Grau-Vorster, M., Vives, J. (2023) Potency assays: the bugaboo of stem cell therapy. Adv Exp Med Biol, 1420: 29-38.

11. Chinnadurai, R., Rajan, D., Qayed, M., Arafat, D., Garcia, M., Liu, Y., et al. (2018) Potency analysis of mesenchymal stromal cells using a combinatorial assay matrix approach. Cell Rep, 22(9): 2504-2517.

12. Khuu, H.M., Patel, N., Carter, C.S., Murray, P.R., Read, E.J. (2006) Sterility testing of cell therapy products: parallel comparison of automated methods with a CFR-compliant method. Transfusion, 46(12): 2071-2082.

13. Liu, C., Weber, C., Sempek, D.S., Grossman, B.J., Burnham, C.D. (2013) Sterility testing of apheresis hematopoietic progenitor cell products using an automated blood culture system. Transfusion, 53(11): 2659-2666.

14. Lowder, J.N., Whelton, P. (2003) Microbial contamination of cellular products for hematolymphoid transplantation therapy: assessment of the problem and strategies to minimize the clinical impact. Cytotherapy, 5(5): 377-390.

15. Snyder, M., Lawrence, A., Reese, J., Wald, D., Anderson, N., Ransom, E. (2026) Evaluation of an automated blood culture detection system for sterility testing of cellular and gene therapy products. Microbiol Spectr, 14(4): e02902-25.

16. Fenwick, A.J., Misra, A., Martin, I. (2025) Finding the middle way: rethinking cGMP for sterility testing of cellular therapy products in minimal manipulation settings. J Clin Microbiol,

© 2023 Somato Publications. All rights reserved.

63(12).

17. Peredeltchouk, M., Wilson David, S.A., Bhattacharya, B., Volokhov, D.V., Chizhikov, V. (2010) Detection of mycoplasma contamination in cell substrates using reverse transcription-PCR assays. J Appl Microbiol, 110(1): 54-60.

18. Francois, P., Rusconi, G., Arnaud, L., Mariotta, L., Giraudo, L., Minonzio, G., et al. (2021) Inter-center comparison of good manufacturing practices-compliant stromal vascular fraction and proposal for release acceptance criteria: a review of 364 productions. Stem Cell Res Ther, 12(1): 373.

19. Tarte, K., Gaillard, J., Lataillade, J.J., Fouillard, L., Becker, M., Mossafa, H., et al. (2010) Clinical-grade production of human mesenchymal stromal cells: occurrence of aneuploidy without transformation. Blood, 115(8): 1549-1553.

20. Andrews, P.W., Barbaric, I., Benvenisty, N., Draper, J.S., Ludwig, T., Merkle, F.T., et al. (2022) The consequences of recurrent genetic and epigenetic variants in human pluripotent stem cells. Cell Stem Cell, 29(12): 1624-1636.

21. Kuroda, T., Yasuda, S., Matsuyama, S., Tano, K., Kusakawa, S., Sawa, Y., et al. (2015) Highly sensitive droplet digital PCR method for detection of residual undifferentiated cells in cardiomyocytes derived from human pluripotent stem cells. Regen Ther, 2: 17-23.

22. Watanabe, T., Yasuda, S., Kusakawa, S., Kuroda, T., Furukawa, H., Futamura, M., et al. (2024) Multisite studies for optimization of a highly efficient culture assay used for in vitro detection of residual undifferentiated human pluripotent stem cells intermingled in cell therapy products. Regen Ther, 26: 315-323.

23. Yasuda, S., Sato, Y. (2015) Tumorigenicity assessment of human cell-processed therapeutic products. Biologicals, 43(5): 416-421.

24. Piao, J., Zabierowski, S., Dubose, B.N., Hill, E.J., Navare, M., Claros, N., et al. (2021) Preclinical efficacy and safety of a human embryonic stem cell-derived midbrain dopamine progenitor product, MSK-DA01. Cell Stem Cell, 28(2): 217-229.

25. Doi, D., Magotani, H., Kikuchi, T., Ikeda, M., Hiramatsu, S., Yoshida, K., et al. (2020) Pre-clinical study of induced pluripotent stem cell-derived dopaminergic progenitor cells for Parkinson's disease. Nat Commun, 11(1): 3369.

26. Chirino, A.J., Mire-Sluis, A. (2004) Characterizing biological products and assessing comparability following manufacturing changes. Nat Biotechnol, 22(11): 1383-1391.

27. Tanaka, T., Lee, S.M., Mikami, M., Yokota, K., Takakura, K. (2020) Gaps between Asian regulations for eligibility of human mesenchymal stromal cells as starting materials of cell therapy products and comparability of mesenchymal stromal cell-based products subject to changes in their manufacturing process. Regen Ther, 15: 265-273.

28. Codinach, M., Blanco, M., Ortega, I., Lloret, M., Reales, L., Coca, M.I., et al. (2016) Design and validation of a consistent and reproducible manufacture process for the production of clinical-grade bone marrow-derived multipotent mesenchymal stromal cells. Cytotherapy, 18(9): 1197-1208.

© 2023 Somato Publications. All rights reserved.