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Commentary

The very stable lentiviral vector

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Lentiviral vectors (LVs) have emerged within the last 20 years as a leading gene therapy vehicle.[1] Although LVs are favored for their ability to permanently deliver a desired payload to nearly any human target cell, clinical-grade production of LVs is difficult and expensive, often with year-long lead times. This stifles the innovation of important new therapies for patients. One way to mitigate this production burden is to extend the expiration date of LVs. In this issue of *Molecular Therapy Methods and Clinical Development*, Jadlowski et al. provide compelling data for just that.[2] Located at the Center for Cellular Immunotherapies (CCI), the innovation center for many novel gene therapy products including tisagenlecleucel (Kymriah), the authors were uniquely positioned to evaluate the stability of 13 clinical-grade LVs produced during the past decade. The data plainly show that when stored properly, vector remains potent for at least 8 years post-production. This novel dataset is relevant to clinical LV suppliers generally and could support arguments for extended expiry dates and reduced early stability studies, which otherwise would deplete valuable vector stocks. While a broad variety of products were included in the analysis, the method of LV production and purification, the freezing media, and the storage temperature were consistent across the lots analyzed. Therefore, differences in these aspects should be acknowledged when relating this report to other clinical LV products, as the impact of these variables on LV stability is not fully understood.

Lentiviruses are a type of retrovirus that cause slowly progressing diseases. Useful for their ability to stably transfer genetic material to target cells independently of cell-cycle status, LVs are subject to cell-intrinsic species-specific restrictions. Therefore, LVs derived from human immunodeficiency virus (HIV) were optimal for broad clinical application.[3] An opportunity to apply HIV-derived LV in humans first came in the setting of HIV gene therapy, where the biosafety risk was perceived more favorably, and the first patient was dosed in 2001.[4] Today, there are 6 marketed products using LVs, and they are all cellular therapy products for monogenic disorders or cancer immunotherapy.

Despite the advancement to commercial use, LV is still a relatively new modality, with a narrowing but meaningful level of variability in production and purification methods and outcomes. Transient transfection continues to be a dominant production method rather than the use of stable and well-characterized packaging or producer cell lines. However, this is expected to evolve in the coming years with continuous advancements in producer lines. Nevertheless, today, the supply chain for LVs continues to be expensive, with long lead times due to production complexities. In addition, the resulting potency of each vector lot is highly variable, with some production lots only yielding enough vector for a small number of patients. These aspects can be prohibitive to efficient and cost-effective early development of novel cell and gene therapies that require initial clinical proof of concept to justify continued investment. In addition, in accordance with ICH guideline Q1A(R2), stability testing is required for new drug substances and products, which depletes valuable clinical supply. Extending the clinical LV use runway and minimizing the depletion of early clinical LV lots for the purpose of early stability testing would be beneficial.

The authors of this study are from the CCI at the University of Pennsylvania, which is one of only a handful of academic sites in the world with such extensive experience across multiple clinical LV lots. Previously this team published valuable observations supporting the safety of integrating vectors across multiple vector lots and cell production lots and in patients.[5] These data were applicable to the broader field, providing key supporting data to allow for case-by-case removal of the requirement for cell-based testing for replication-competent lentivirus in cellular products (a time-intensive and costly analytic assay). Now, this same team has mined their data once more for the benefit of the field, reporting compelling data from a retrospective study, which illustrates the durable stability of LVs, independent of transgene, when stored properly.

To do this, Jadlowski et al. monitored the stability of 13 lots of LV produced between 2014 and 2020. All but one lot was manufactured by the same facility; similar production methods, storage buffer, and storage conditions were used across the 13 lots. Basic stability was measured by vector titration in a SupT1 cell-based assay using flow cytometry for surface expression of the transgene. All lots represented individual products. No meaningful loss in titer was observed across lots ranging from 3 to 8 years post-initial manufacture. The consistency in stability across 13 different products strengthens the relevance of the dataset to clinical LVs more broadly. The argument for long expiration dates would be further strengthened with similar data from alternate suppliers, preferably ones with different production protocols and/or freezing medias.

When used as drug substance/critical starting material (rather than a final drug product), as LV is in nearly all cases today, independent stability testing of the clinical LV lot is of modest utility in early development. Transduction efficiency is measured as a release criterion on cell products, and potency drift could be monitored over time as a surrogate for LV stability testing. If this were accepted by regulators, it would reduce depletion of precious LV stocks in early clinical development. As a demonstration of this, the authors also evaluated expression of the transgene across all cellular products

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produced with all but two LV lots (either not used for cell manufacturing or not assessable due to a unique manufacturing process). These data are especially useful because they provide insight into the level of variability in transduction efficiency arising from donor differences and thus provide some sense of how potency drift could be assessed over time using release data from cellular production runs.

The authors also evaluated vector product potency across lots, in cases where the payload redirected T cell activity through receptor expression (11 of 13 lots). The FDA requires a functional potency assay for vector, and the authors took a typical approach, measuring interferon-γ expression upon exposure of transduced cells to targets expressing the ligand to the engineered receptor but not against target cells without the ligand. In all cases, the activity of the engineered receptor was confirmed. These potency assays are qualitative and do not have established thresholds or acceptability criteria. While these data are required by the regulators, and thus in this context are relevant in the paper, the data themselves are of limited objective value. The most relevant measure of vector potency is titer, which is measured by receptor expression on the transduced cells. Since the sequence of the vector does not change during gene transfer, there is no rationale for an alteration of receptor function that would require testing, and the receptor function would have been assessed in preclinical studies and *in vitro* biological function tests. Thus, for cases where vector is a drug substance/critical starting material, it would be logical for regulators to accept the vector titration assay as the potency test. Notably, clinical cell products manufactured using vector that was 36 months or later from manufacture (shown to be stable by the titration data in this report) resulted in complete or partial disease remission in 12 of 16 patients, which is the definitive measure of potency.

In biopharmaceuticals, the implementation of platform processes transformed the supply of therapeutic antibodies through predictable outcomes and supported advancement of the field.[6] The adoption of the best platform was forged through collaboration and data sharing. The hope is that LV production will experience a similar evolution and that this will support accelerated innovation, such as *in vivo* gene delivery, reduced production and product costs, and increased patient access.[7,8] The work by Jadlowski et al.. offers an example of how this can be done in the LV field.

DECLARATION OF INTERESTS

G.K.B. is an employee of Cabaletta Bio, a member of the board of directors of Instil Bio, and a member of the scientific advisory board of Immatics. C.-C.C. is an employee of Cabaletta Bio.

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