

Establishing Patient Centric Quality Standards: Cell & Gene Therapy Case Studies

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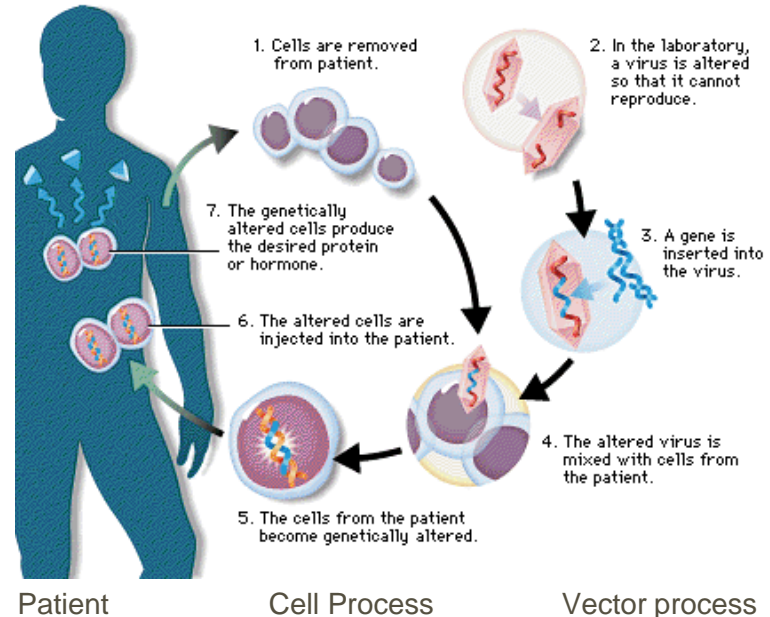
EU CASSS May 11 – 13th

C> patient centric quality standards: setting product-relevant attributes and ranges to ensure patient access



Two C>/ATMP PCQS case studies from an *ex vivo* autologous perspective:

- Vector process: vector potency
 - What is it and how do you measure it?
 - A case study based on a position
- Cell process: vector copy number (VCN)
 - Existing guidance and clinical relevance
 - Vector copies are a key component of dose
 - Safety considerations (clinical risk)
 - How is it measured?
 - Data-based case study
- Conclusion
 - How it relates to establishing PCQSs



Vector potency

A C> PCQS case study based on a position

C> PCQS case study: vector potency



What is vector potency and how do you measure it?

- What is potency: demonstrating **product** function, i.e, Mechanism of Action (MoA)
- Relevance to *ex vivo* autologous therapies, where vector (LVV) is an input material
- Assay formats considered:
 - Translation: assess transgene expression on the surface of a susceptible cell line, which can result from the successful integration of the transgene sequence into the cellular genome
 - A measure of viral infectious titre
 - Based on the assay format, surface recognition of the translated transgene product can only occur in the presence of successfully integrated and transcribed/translated transgene protein – **transgene functionality**
 - Cell signalling: e.g., activate transduced cells and assess downstream signal/activation
 - Indicative of cell product function, not the function of the viral vector
 - Cell function: measure cytotoxicity, or a surrogate of cytotoxicity
 - Again, indicative of cell product function, not the function of the viral vector
 - Others...

C> PCQS case study: vector potency



Experience with this attribute

Testing strategy proposal comprised assessment of vector integration (sequencing) and transgene translation (functionality)

- This strategy was rejected as insufficient

Regulator feedback on this vector testing strategy:

- 1. For release testing of future vector lots, please establish a lot release assay and acceptance criterion for **biological potency**. For example, your **cytotoxicity assay** may be suitable for measuring the potency of your vector.
- 2. [Regulator's] policy has been evolving regarding potency testing of vectors that are used for further manufacturing. I **acknowledge that your infectivity assay is relevant to the potency of the vector**, but nevertheless we believe that it is important to evaluate the potency of the vector using an assay that measures the **full biological function of vector transduced cells**. So please plan to incorporate a potency assay (such as the **cytotoxicity assay** that you have been developing) into the lot release and stability testing for your vector.

Let's review the biology of viral vector integration...

C> PCQS case study: vector potency



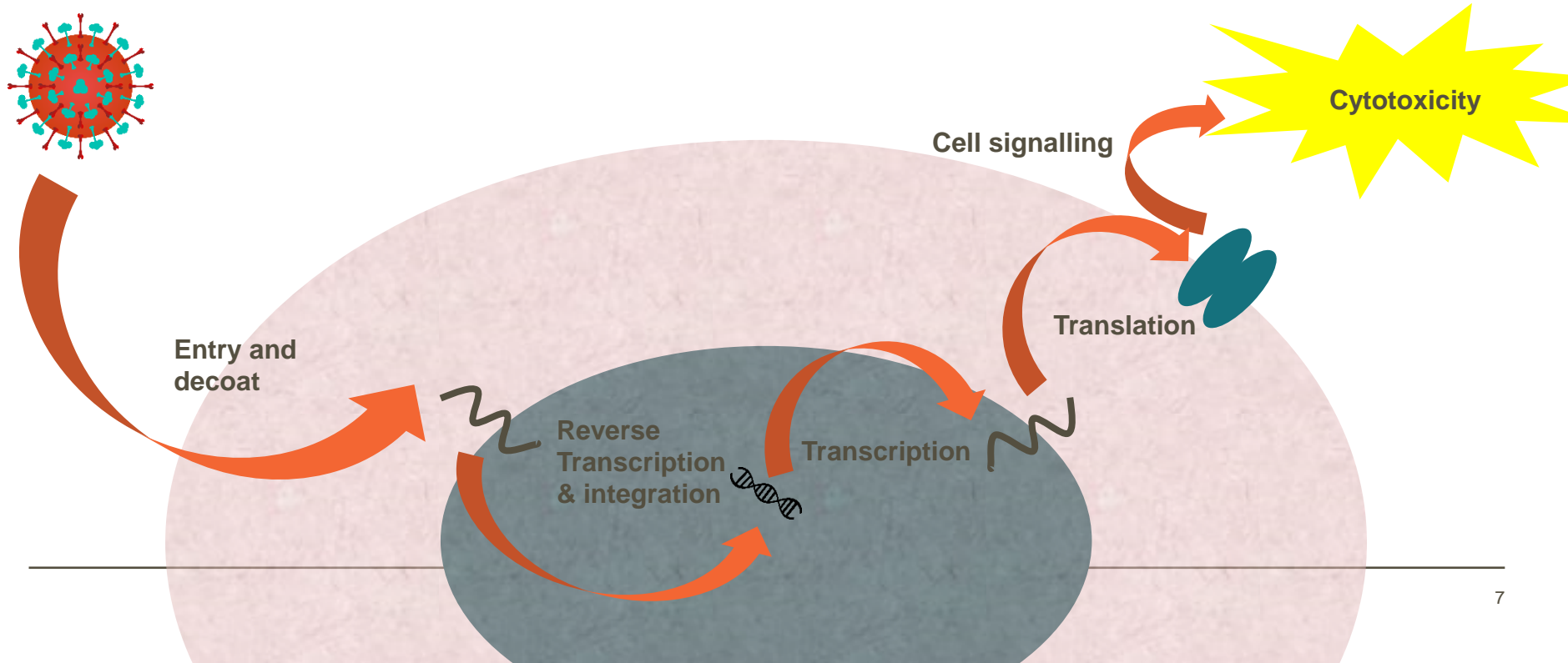
What does viral vector do?

Can	Cannot
1. Gain entry to susceptible cell	
2. Reverse transcribe RNA to DNA	
3. Integrate into cell genome	
	4. Cellular transcription
	5. Cellular translation
	6. Cellular cytotoxicity

C> PCQS case study: vector potency



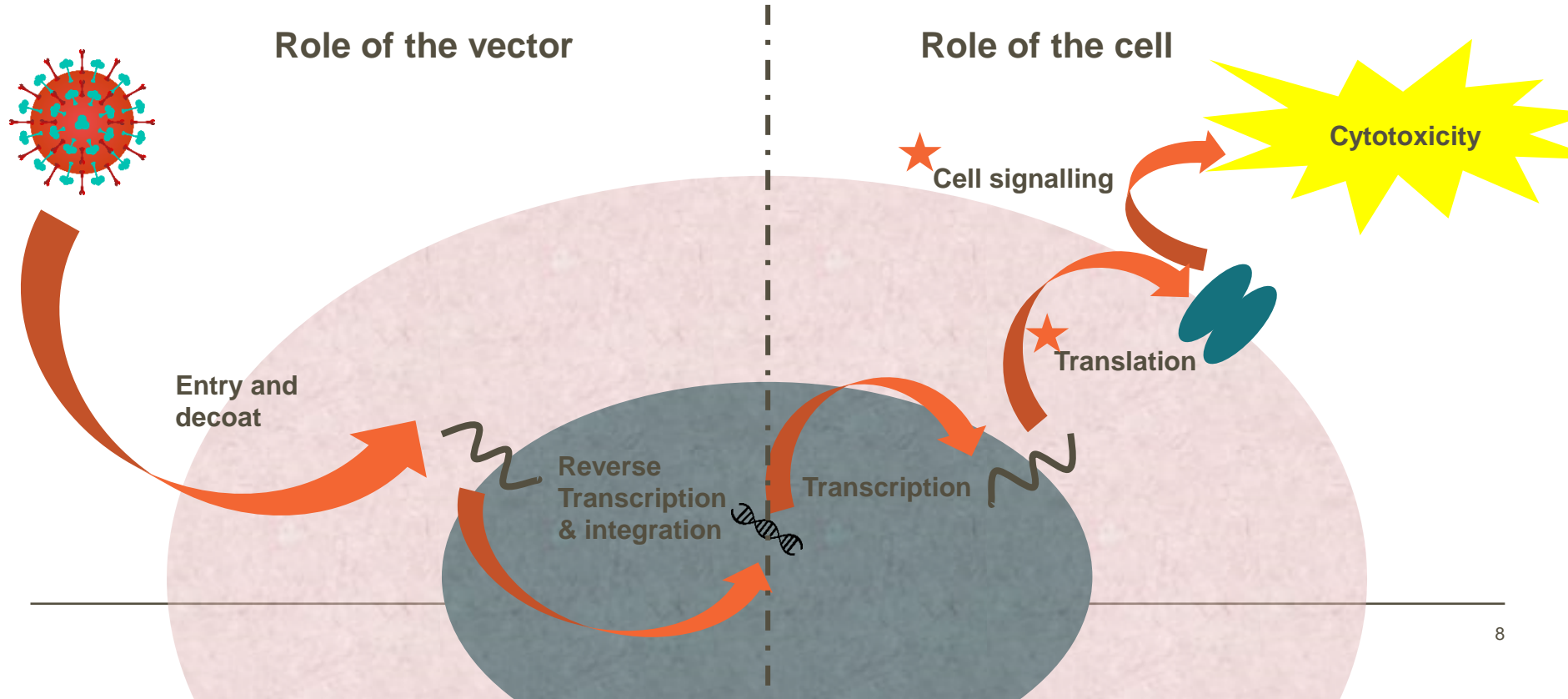
What a viral vector does (and does not)



C> PCQS case study: vector potency



What a viral vector does (and does not)



C> PCQS case study: vector potency



Questions, specifically for *ex vivo* autologous gene therapies

- How does the potency of a viral vector extend past its integration?
 - Events subsequent to transgene integration, that are under the cell's control, are surrogates to the final event that is under vector control
- The regulatory ask is around assessment of transduced cells (the drug product), not the viral vector
 - Vector control strategy already assesses infectivity, characterization of quality
- Define vector potency as events under vector control (i.e., integration), and cell potency as events under cellular control (i.e., cytotoxicity)?

C> PCQS case study: vector potency



What we contend, specifically for *ex vivo* autologous gene therapies

- Applying product attribute testing to an input material to the therapeutic process
- Relevant industry guidance: *Guideline on development and manufacture of lentiviral vectors* CHMP/BWP/2458/03 (2005)
 - “Any preparation of LV should be fully characterised with regard to **transducing activity**, other characteristics relevant to vector particles and the absence of RCL...
 - Important aspects of transducing activity are **integration capacity**, **transgene expression** and **functionality**.
- Sequencing is a direct measure of vector transducing activity
 - Advantages and disadvantages
- Biological function of vector transduced cells is product characterization

VCN

A C> PCQS study about range

C> PCQS case study: VCN



Framed around the clinical risk (patient safety) associated with provirus integration

Industry guidance:

- *Reflection paper on management of clinical risks deriving from insertional mutagenesis*, EMA/CAT/190186/2012 (2013)
 - Factors contributing to the risk of oncogenesis derived from insertional mutagenesis:
 - a) Vector design (including backbone and regulatory elements)
 - b) Insertion profile
 - c) **Vector dose**
 - d) Transgene product
 - e) Target cell population/organ
- *Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products*, EMA/CAT/80183/2014 (2018)
 - “Integration studies should focus, at least, on the following issues, unless justified...genomic stability of the integrated vector over time and persistency of the average vector copy number in the cells.”
- Currently no regulatory requirements for the minimum or maximum integration copy number
 - FDA recommends integration copy number <5 copies per genome
 - EMA/CAT/190186/2012: high vector copy number = risk factor for oncogenesis

C> PCQS case study: VCN



How is it measured?

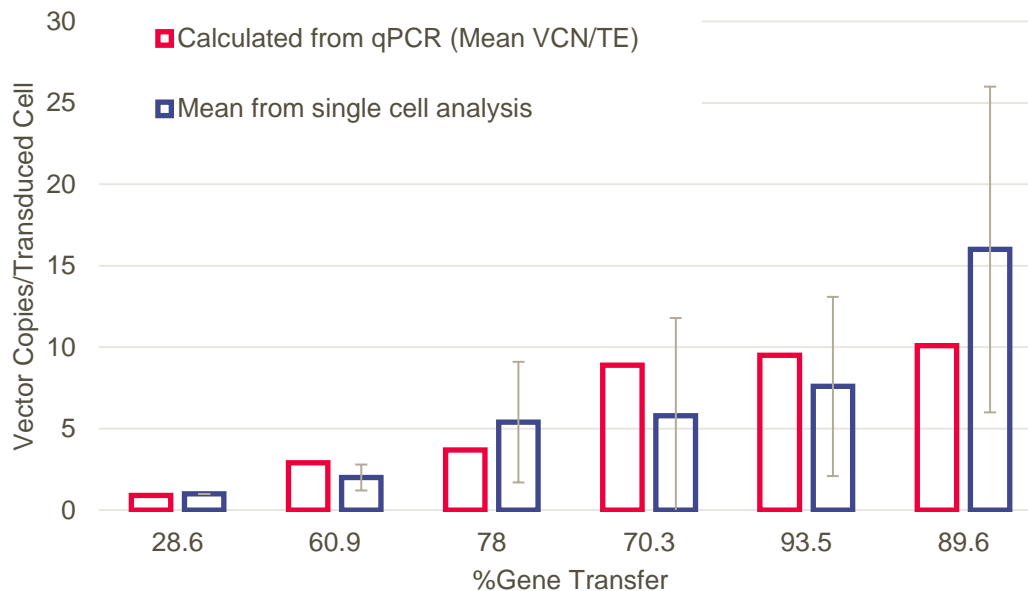
- Commonly duplex qPCR assays: detection of a vector related sequence + detection of cellular genome (housekeeping gene). e.g., $VCN = \text{vector copies}/(\text{housekeeping gene copies}/2)$
- Or simplex qPCR assays: detection of vector related sequence and normalization with the total amount of host cell DNA analysed. e.g., $VCN = (\text{copies}/\text{ng}) * (6 \times 10^{-3} \text{ ng DNA}/\text{cell})$
- For high transduction efficiencies (>90%), $VCN = \text{average vector copies}/\text{transduced cells}$
- For *ex vivo* autologous C>s with < saturating transduction efficiencies, the [DNA] includes non-transduced cells, i.e., $VCN = \text{average vector copies}/\text{total cell population}$.
 - $VCN \neq \text{average vector copies}/\text{transduced cells}$
 - Underestimation of the number of integrations

C> PCQS case study: VCN



The relationship between VCN and transduction efficiency

- VCN increases proportional to transduction efficiency (TE)
- At high TE's, the linear relationship between VCN and TE breaks down
- Data: average vector copies per transduced cells assessed by qPCR and by a single cell analysis approach
 - Single cell analysis: variability of proviral copies scales with increasing TE



Blood. 2003 Dec 1;102(12):3934-7

C> PCQS case study: VCN



Guidance on how to measure VCN - published example

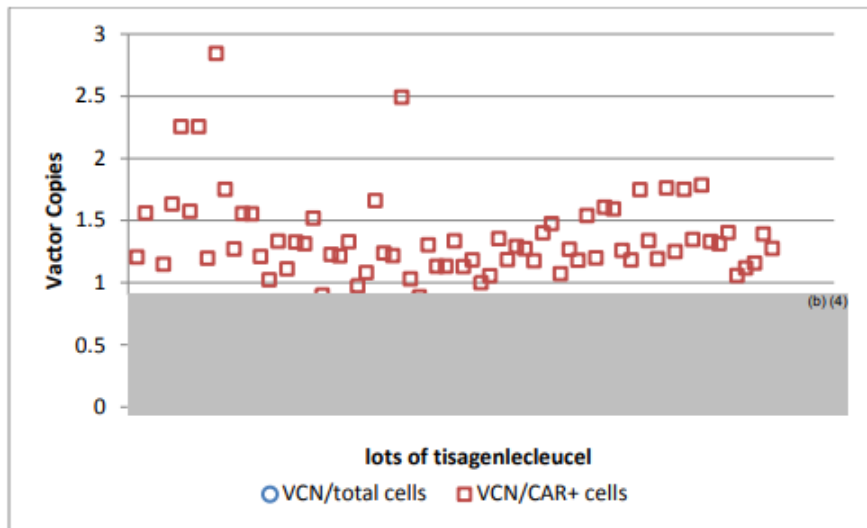


Figure 5. Vector copy number (VCN) for tisagenlecleucel.

Each dot represents one lot of tisagenlecleucel as either VCN/total cells (blue circles) or VCN/transduced cells (red squares) (FDA generated)

BLA 125646 (Tisagenlecleucel/Kymriah)

VCN for tisagenlecleucel is determined by a qPCR assay that measures the average number of integrated copies of the vector-encoded CAR relative to the total number of cells (Figure 5, blue). Calculation of VCN based on the total cells normalizes the measured copy number according to all cells, not just to transduced cells, and therefore uniformly deflates the number of cells with integrated vectors. Therefore, the data were transformed into a measurement of VCN per CAR+ cell (Figure 5, red). Both representations of the VCN data indicate that the average transgene integration into tisagenlecleucel CAR+ T cells is variable.

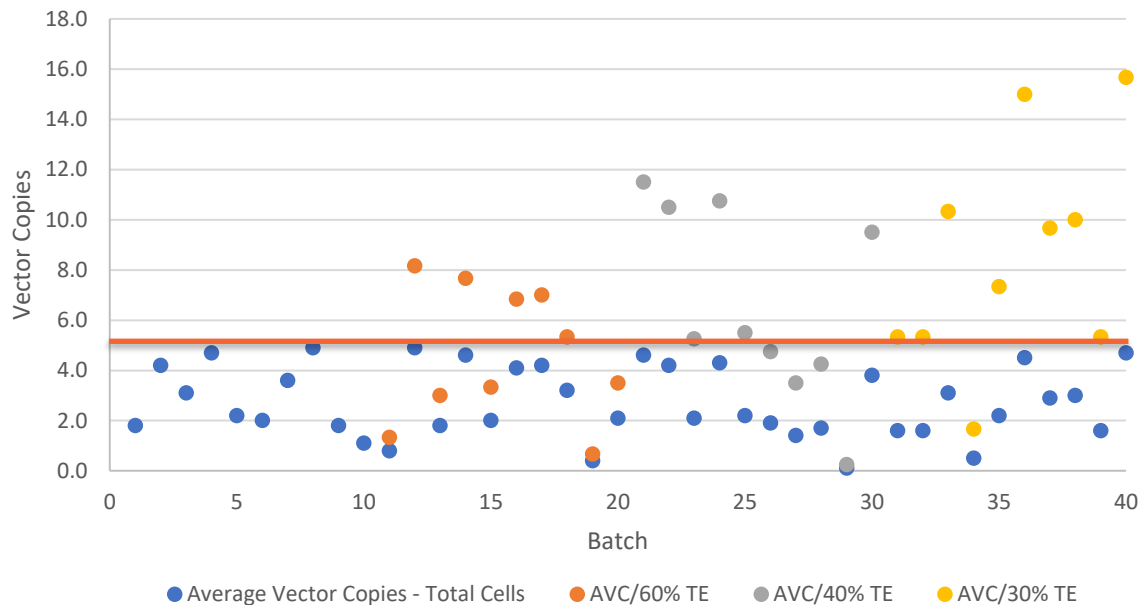
- Average vector copy numbers transformed from total cells to transduced cells using TE
- All lots shown demonstrate < 3 average vector copies per transduced cell
- Standard practice?

<https://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/OncologicDrugsAdvisoryCommittee/UCM566166.pdf>

C> PCQS case study: VCN



Demonstrating patient exposure range with hypothetical *ex vivo* autologous data



- Hypothetical *ex vivo* autologous C> vector copies measured as total cells and transformed to transduced cells (TE)
- Red line = 5 vector copies
- Considering that VCN varies proportional to TE:
 - A 60% TE requires average vector copies/total cells <3 to achieve 5 or fewer vector copies/transduced cell
 - A 30% TE requires average vector copies/total cells <1.5 to achieve 5 or fewer vector copies/transduced cell
- Significant portion of batches will demonstrate >5 average vector copies per transduced cell at clinically relevant Tes
- **Patient variability**
- **Patient access**

Vector potency

- **Patient relevance:** *ex-vivo* autologous gene therapy viral vector \neq product
- Product is transduced patient cells
- Propose: for release, the vector potency method should be a measure of successful integration of transgene (PCR-based)
 - See: *Guideline on Development and Manufacture of Lentiviral Vectors*, CHMP/BWP/2458/03 (2005)
 - “Important aspects of transducing activity are integration capacity, transgene expression and functionality...”

VCN

- **Patient-centric control strategy concept:** using patient exposure to define range acceptance criterion
- Low to no patient safety risk (theoretical risk) in consideration of current vector design and patient monitoring
 - **Patient-centric acceptance criteria:** define based on the observed patient exposure range (risk-based approach)
 - Patient is the product is the biggest variable – TE, and hence VCN, are inherently variable based on patient material
 - Monitoring patient insertion profile
 - SIN vector
 - Transgene product and target cell population

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Thank you!