**Table 10: Rational Setting of Vector Copy Numbers in Genetically Modified Cells**

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**Scope:**

Lentiviral and retroviral vectors are employed frequently to deliver transgenes to patient cells because of their ability to integrate into the cellular genomes. Vector copy number (VCN) methods are widely used to demonstrate control of lentiviral or retroviral transduction of ex vivo cells. Nonspecific integration of lentiviral and retroviral vectors into the cellular genome poses potential safety risks including oncogenesis. Additionally, as the number of integrations increases, the risk for oncogenesis also increases. Accurate and precise measurement of VCN and setting of appropriate limits for VCN are critical for the release of safe genetically modified cell products to patients.

**Questions for Discussion:**

1. What factors contribute to the risk of mutagenesis and oncogenesis?
2. How should risks be assessed when setting VCN ranges?
3. What parameters would you use to determine upper and lower VCN limits?
4. What controls would you have in place to ensure consistent and accurate quantification?
5. What is the CMC strategy for vector copy number methods?
6. What are the risks/benefits of bulk VCN vs single cell VCN analysis?

**Discussion Notes:**

1. What factors contribute to risks
   Multiple factors including due to random nature of integration:
   - integration site, active sites potentially more risky
   - Vector design with certain promoters conveying higher risks
   - Methods of transduction
   - Product cell type, eg. CD34 vs CART
   - Autologous vs allogenic (more patients treated)

2. How risks should be assessed when setting VCN ranges
   Following factors should be considered:
   - Target indication, risk profile of patients
   - Potential insertion sites

- Promoter characteristics
- For autologous: assess per number of batches
- What is the accurate measurement? Is it potentially more meaningful to measure per transduced cell; also better than VCN by genome
- Range set based on experiences, data collected during development, totally of data: vector design, insertion sites, eg. CD34 cells have potentially more insertion sites
- Gather insights from published literature

3. What parameters to determine upper and lower VCN limits
    - Different modality may have different considerations
    - TPP can help determine, whether safety is driver or efficacy is driver

4. What controls to ensure qualification
    - Positive and negative controls, negative controls are critical, in addition to other assay controls
    - Internal controls, such as ddPCR
    - Panel of standards meaningful
        - Reference cell lines with known number of VCNs used as reference standards as assay control, measured by qPCR, post formulation

5. CMC strategy for vector copy number methods?
    - Start with higher number, lower as development progresses
    - Comparable methods throughout development
    - Consider to use Global standards, such as lenti by NIST, Reference controls to qualify methods
    - Consider to use transgene specific primer for qPCR assay if multiple products are manufactured in the same facility, specific primer can be part of ID matrix
        - However, depending on location of primer, you may have different results, recommend using multiple applicons seems make sense, but may not be feasible

6. Risks/benefits of bulk VCN vs single cell VCN
   Single cell more meaningful? How to extrapolate to total population?
    - Single cell can measure heterogeneity of the products, process variability, better accuracy, interpretation,
    - Single cell measurement can monitor profile change
    - However, Challenging to set acceptance criteria with very wide range, so more useful for characterization
    - Can be Used to compare products?

   For bulk, compilation of all data points, trending in the same direction
    - It is a safety measure
    - But can also be used for potency if VCN is important to deliver product efficacy