Guidance for working with viral vectors reclassified from DNIR to PC2 NLRD

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Contained Dealings Evaluation Section
Which vectors?

Two categories

Replication-defective viral vectors:

- **able** to transduce human cells
  --> risk to humans, especially lab workers

- **not able** to transduce human cells
  --> risk to animals, not lab workers
Not able to transduce human cells

### Transgene Classification

<table>
<thead>
<tr>
<th>Transgene</th>
<th>Classification</th>
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</thead>
<tbody>
<tr>
<td>&quot;low risk&quot;</td>
<td>Stays PC2 NLRD</td>
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<tr>
<td>pathogenic determinant</td>
<td>Stays PC2 NLRD</td>
</tr>
<tr>
<td>oncogenic</td>
<td>DNIR --&gt; PC2 NLRD</td>
</tr>
<tr>
<td>immuno-modulatory</td>
<td>DNIR --&gt; PC2 NLRD</td>
</tr>
<tr>
<td>toxin</td>
<td>Stays DNIR</td>
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</tbody>
</table>

- **In vivo use**
- Retroviral or non-retroviral
- Transgene is oncogenic or immuno-modulatory

Examples: vectors based on animal viruses unable to infect humans, or with modified envelope protein
LV with animal-specific envelope protein
Not able to transduce human cells

Risks are to animals/environment, not lab workers

Ensure containment

- Work practices as per PC2 certification guidelines
- Correct PPE (gowns and gloves)
- Transport, storage and disposal as per Regulator’s guidelines
Able to transduce human cells

Retroviral vectors

Lentiviral vectors

HIV-1 genome
Retro/lentiviral vectors will be PC2 NLRD if:

- all structural genes removed from vector
- self-inactivating OR only gag, pol, env & rev
- any gene in vitro, low risk genes in vivo

Able to transduce human cells

Brings regulation of lentiviral vectors into line with similar retroviral vectors

Extra genes don’t increase risk posed by basic genome
Risks posed by lentiviral vectors

Risks are to humans, especially lab workers

Recombination
formation of replication-competent virus during virion production

Transduction & integration into genome while working with the vector
Risks are to humans, especially lab workers.

- Infection with RCLs formed during packaging
- Effect of transgene, e.g., oncogene
- Remobilisation of vector genome
- Insertional mutagenesis
Minimising the potential for adverse outcomes

Technology

safety features designed into vector

- Removal of all viral genes from gene transfer plasmid
- Multiple packaging plasmids
- Minimal sequence overlap
- Self-inactivation
Minimising the potential for adverse outcomes

Technology

- Removal of all viral genes from gene transfer plasmid
- Multiple packaging plasmids
- Minimal sequence overlap
- Self-inactivation

Work practices

- Exposure
- Infection/transduction
- Potential for adverse outcome
Routes of exposure leading to transduction

ASK: how can the viral vector enter the body in a way that allows cell transduction?

For lentiviral vectors:

- across mucous membranes
- deleted image (eyes)
- deleted image (needlestick injury)
- deleted image (broken glass)
- direct entry into blood stream
Work practices for minimising exposure

Minimum requirements based on PC2 containment:

- PPE (gowns, gloves)
- Contain aerosols
  e.g. in biosafety cabinet

PLUS
## Suggested additional work practices

<table>
<thead>
<tr>
<th>Protect eyes</th>
<th>Wear safety glasses</th>
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</thead>
<tbody>
<tr>
<td>Prevent blood contact</td>
<td>Avoid use of <strong>sharps</strong> or <strong>glass</strong> equipment for <em>in vitro</em> work</td>
</tr>
<tr>
<td></td>
<td>Cover broken skin with waterproof dressing</td>
</tr>
<tr>
<td></td>
<td><em>In vivo</em>: consider type of animal, tendency to bite, effective restraint/sedation?, use of sharps/glass</td>
</tr>
<tr>
<td>Protect others in shared spaces</td>
<td>Decontaminate shared work areas and equipment after use</td>
</tr>
<tr>
<td></td>
<td>Ensure <strong>others</strong> in same work area aware of the GMO and associated risks</td>
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<tr>
<td>Minimise opportunities for recombination</td>
<td>Don’t use in same BSC as other vector systems, or replication-competent viruses</td>
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</table>
Summary

The IBC assessment of a proposed NLRD takes into account:

- **The GMO** - properties of the viral vector relevant to risk (e.g. characteristics of parent virus, safety features, transgene)

- Appropriateness of the **facility** (dedicated vs shared space)

- **Appropriate work practices**

- **Appropriate training** (includes understanding viral vectors and associated risks)
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Questions?