

Bioassays for Cell and Gene Therapy Products: A Canadian Regulatory Perspective and Experience

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YOUR HEALTH AND SAFETY... OUR PRIORITY.





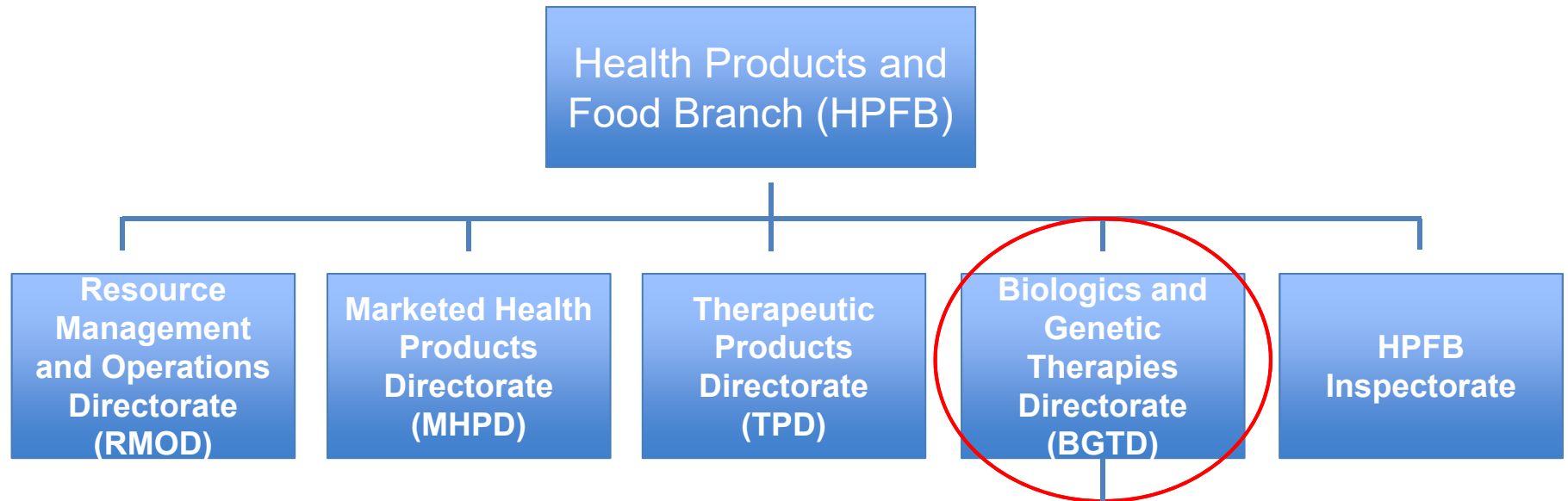
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- The opinions expressed in this presentation are those of the reviewer and do not necessarily reflect an official Health Canada position

Outline

- Health Canada organization
- Regulation of Gene and Cell Therapy Products
- Regulations with respect to potency for Cell and Gene Therapy (C>) products
- Guidance Documents followed by Health Canada
- Health Canada C> Potency assay expectations
- Types of products and associated regulatory concerns for each
- Case Studies
- Resources available

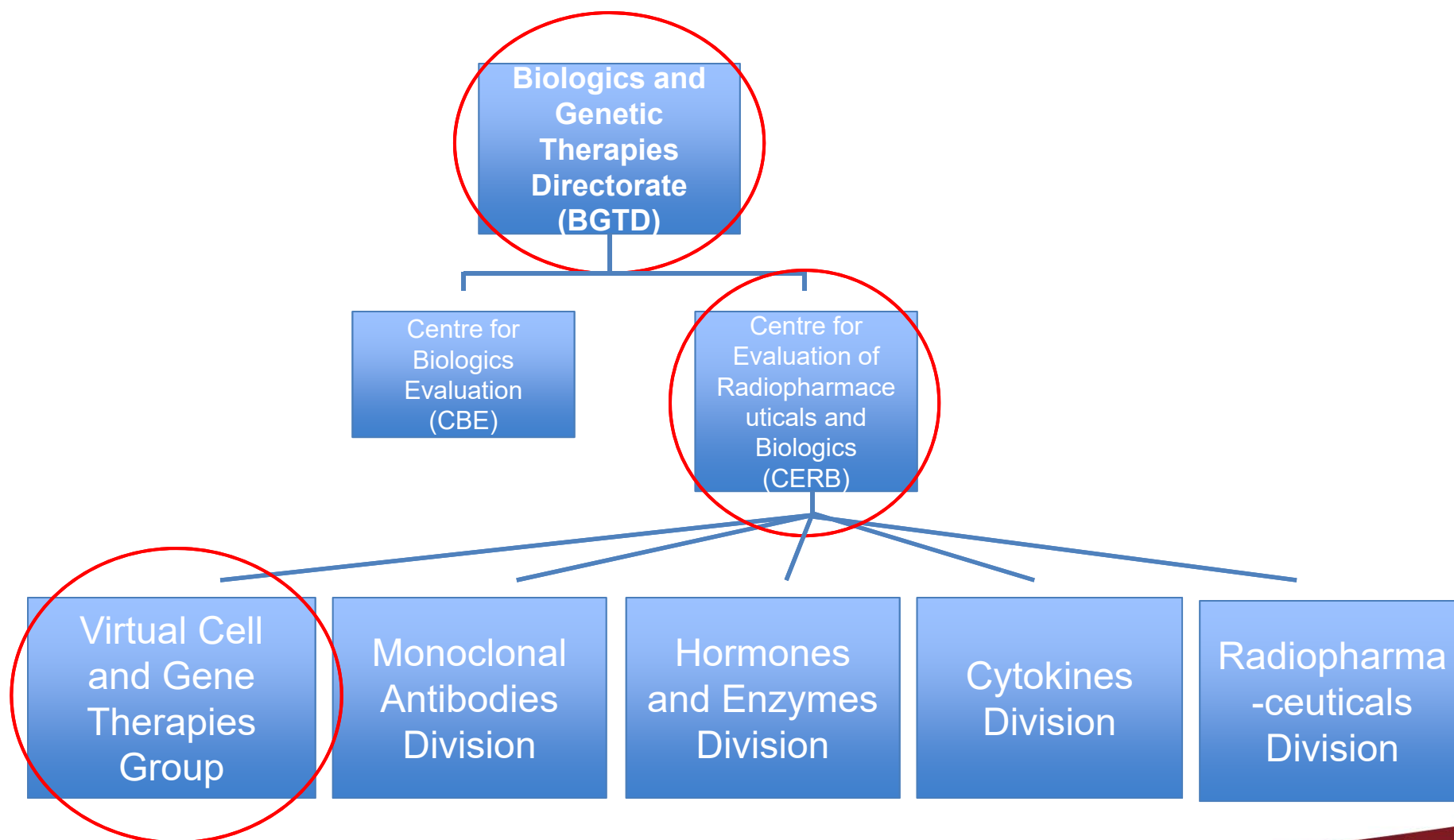
Where does BGTD fit in?



Mission:

BGTD works to maximize the quality, safety and efficacy of biological and radiopharmaceutical products in Canada.

Where do Cell and Gene Therapies go within BGTD (for Quality Review)?



Regulation of Gene and Cell Therapy Products in Canada

- Gene Therapy:
 - Transfer and expression of an exogenous gene compensating for a missing or non-functional endogenous gene including by the following means:
 - a) Nucleic acid (DNA or RNA) delivered directly or by viral vector resulting in expression of RNA (mRNA, miRNA or siRNA) and in some cases the translation of protein (directly *in vivo* or via *ex vivo* transduction and re-introduction of cells)
 - b) Modification of genes (expression or repair) without transfer of genetic material
 - Direct treatment of cells *in vivo* or *ex vivo* with regulatory RNA or protein that bind DNA is not considered gene therapy but a therapeutic use of nucleic acids and proteins
 - c) Oncolytic viruses for treatment of cancer

Regulation of Gene and Cell Therapy Products in Canada (cont'd)

- Cell and Gene Therapeutic Products
 - Regulated as Biologics, in Schedule D (Biologic Drugs) of the Canadian Food and Drug Regulations
 - Gene therapies are better captured by Schedule D than cell therapies
 - Safety of Human Cells, Tissues, and Organs Regulations for Transplantation Regulations
- Cell therapies meet the definition of a drug as defined by Food and Drugs Act
 - Food and Drugs regulations are widely applicable to Cell Therapies
 - Assisted Human Reproduction Act: embryonic stem cells

Canadian Food and Drug Regulations

- A.01.002 “These Regulations, where applicable, prescribe the standards of composition, strength, **potency**, purity, quality or other property of the article of food or drug to which they refer.”

Importance of Potency Assays

- Provides assurance of consistent manufacture of product
 - A requirement for Product release
- Essential for comparability of product following manufacturing changes
- Essential for evaluation of product stability and establishment of shelf life
- Required to establish compatibility of product with product contact materials
- Potency assay program is invaluable to the development of C> products

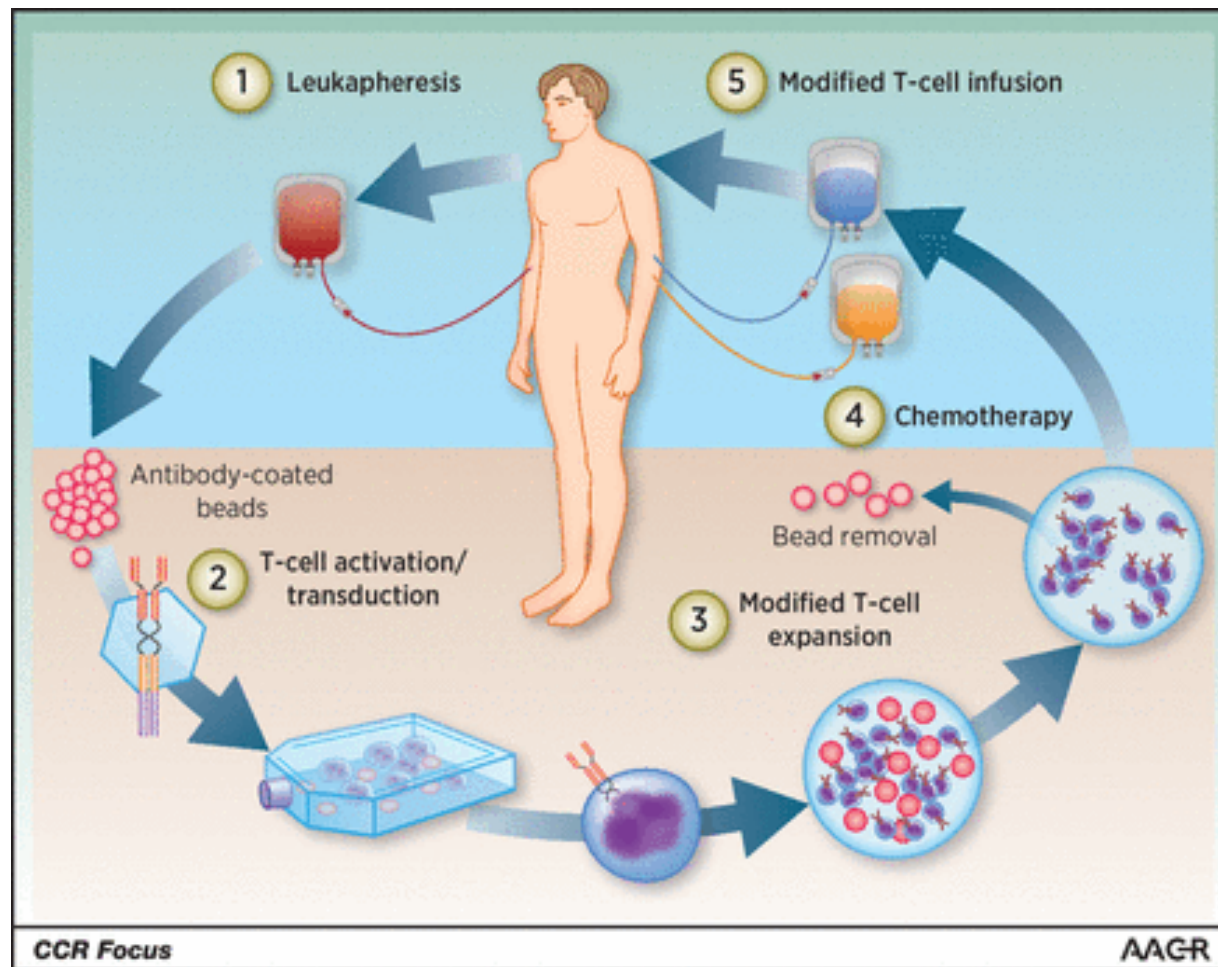
ICH Quality Guidelines

- Although scope may exclude CGT's, many of the principles can and should be applied
 - Eg. Comparability, stability, viral clearance etc.
- ICH Q6b – (Specifications) - Biological Activity
- “A valid biological assay to measure the biological activity should be provided by the manufacturer. Examples of procedures used to measure biological activity include:”
 - Animal-based biological assays, which measure an organism's biological response to the product;
 - Cell culture-based biological assays, which measure biochemical or physiological response at the cellular level;
 - Biochemical assays, which measure biological activities such as enzymatic reaction rates or biological responses induced by immunological interactions.
- ICH Q5c – (Quality of Biotechnological Products) - Stability Testing
- ICH Q5e - Comparability
 - Potency a central CQA evaluated in product stability and comparability studies
- FDA Guidance for Industry: Potency Tests for Cellular and Gene Therapy Products

Potency Assays for Cell and Gene Therapy Products – Mechanism of Action

- C> products often have multiple MoAs (eg. CAR T-cells: tumor cell killing, cytokine release, CAR T-cell proliferation), some may be unknown
- Ideally potency assay should capture the most relevant MoA
- Potency assay need not capture every MoA, only key MoA(s)
- Where assay variability is problematic, a matrix approach may be an acceptable strategy (capturing two or more surrogate measures)
- Potency assay should be quantitative and expressed in units of activity calibrated against an international or national reference standard, or against an in-house reference where no other standard exists

CAR T-Cell Therapeutic Products

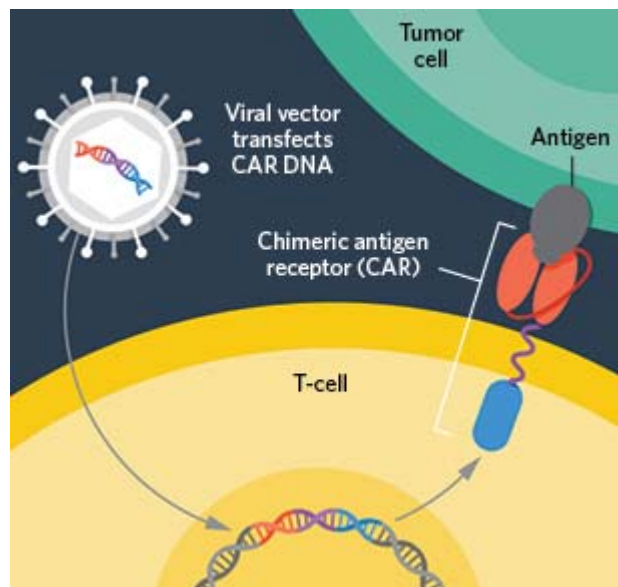


CAR T-Cell based Potency Assay Challenges

- Gold standard is cytotoxicity (thought to be the main mechanism of action)
- Limitations of measuring cytotoxicity *in vitro*
 - HLA matched target cells (Tg T-cell receptors)
 - Length of time required for cell based assay
 - Amount of product required
 - In vitro assays may still not be predictive of efficacy in vivo
- Surrogate markers that correlate with clinical efficacy
 - CD8+/CD4+
 - The presence of these two cell types is predictive of clinical efficacy
 - Pro-inflammatory cytokine production (ie. IFN- γ)
 - Disadvantages include CD8+ IFN- γ producing cells not always cytotoxic
 - Cytokine production independent of other functions like migration/homing, engagement with target cell, cytotoxic functions like secretion of

Potency Assays for CAR T-cells

CAR T-cell Products



Determinants of Potency

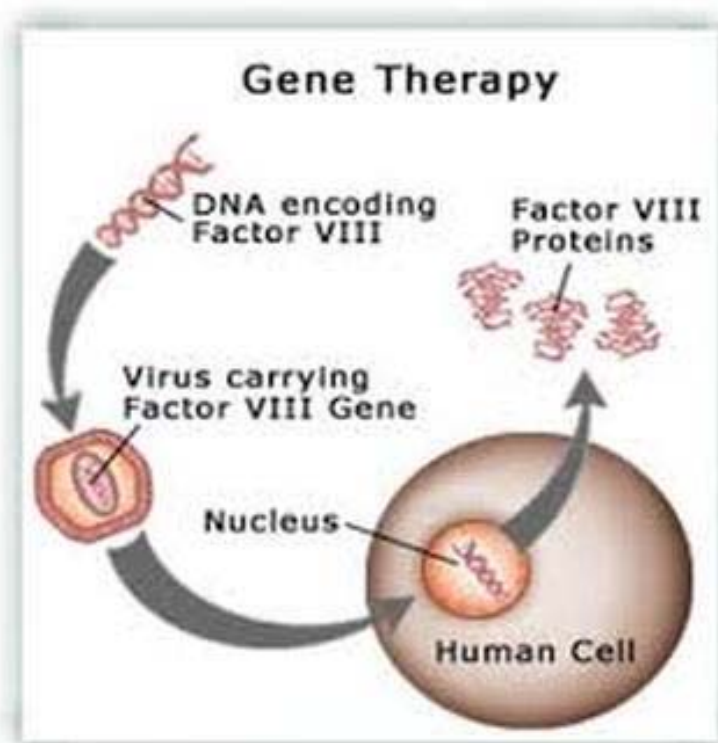
- Composition of Product
 - CD8+, CD4+, NK, B-cells
- Mechanism of Action
 - cytotoxicity → cell based assay
 - surrogates → biochemical assay
 - markers
 - cytokines
- Possible to have more than one potency assay (matrix)

Challenges:

- no appropriate reference standard

Potency Assays for Cell and Gene Therapy Products – Types of Products

Viral vector Gene Therapy Products

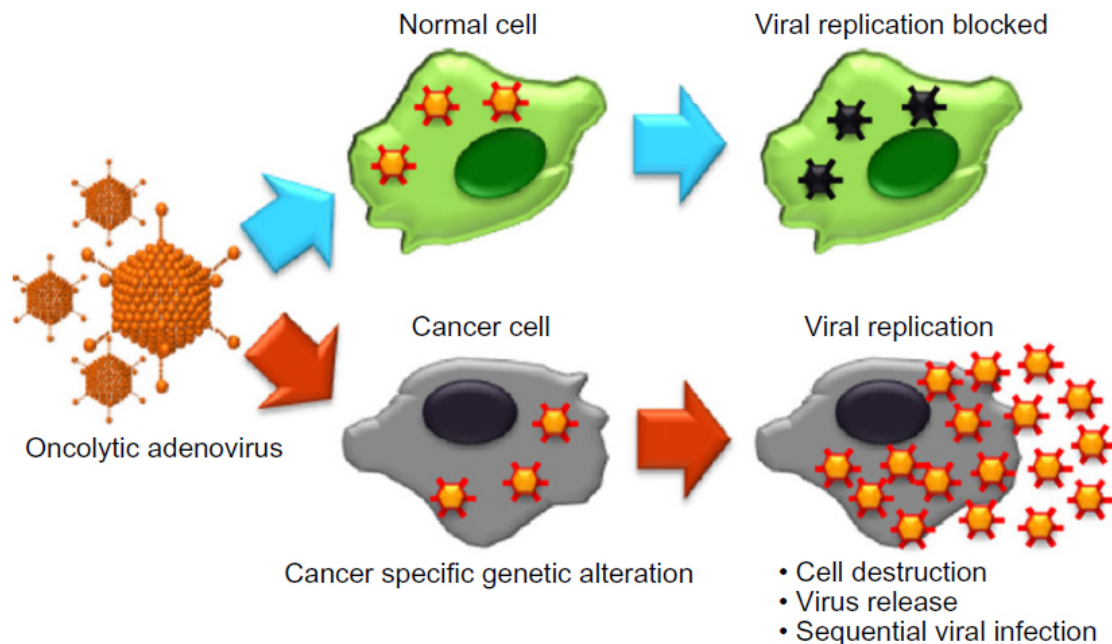


Determinants of Potency

- Infection of target cell types in vivo
- Function of transgene

Potency Assays for Cell and Gene Therapy Products – Types of Products

Oncolytic Virus Products



Determinants of Potency related to MoA

- Infection of target cells
- Destruction of tumor cell
- Progeny virus (amplifying therapeutic)
- Immune system role

Potency Assay Considerations During Development

- Early phase Clinical trials
 - Patient safety is main concern
 - Potency Assay less developed, linked to pre-clinical understanding of product
 - Acceptance criteria may not yet be established
- Later phases (pivotal trials)
 - More stringent requirements
 - Improved Potency assay, more reflective of *in vivo* mechanism of action and predictive of clinical efficacy
 - Acceptance criteria established
 - Manufacturing consistency critical to link trials
- Marketing Application
 - Potency assay validated
- Potency assays evaluated on a case by case basis

Case study 1

- AAV mediated delivery of a therapeutic transgene
- Potency assay measured production of transgene
- AAV manufacturing change resulted in the presence a proportion of empty capsids
- Sponsor claimed empty capsids contribute to product efficacy by sequestering neutralizing antibodies away from viable capsids
- Therefore, a bioassay could be developed to substantiate this claimed MoA as each batch will vary in terms of empty capsid content

Case Study 2

- CAR T-cell product
- Drug Product specifications:
 - min % transduced cells (flow cytometry)
 - fg IFN- γ per transformed cell (ELISA- a range with limits)
 - minimum fold-induction of IFN- γ production following co-culture with TA expressing cells vs non-related TA expressing cells.
- In vitro co-culture IFN- γ release assay (ELISA)
- Effector cell: CAR T-cell product
- Target cell: tumor target cell expressing tumor antigen or unrelated tumor antigen

	Target tumor cell	Target tumor cell expressing unrelated TA
Transduced CAR T-cell	++++	+

Examples of Developmental Potency Assays

Product type	Developmental Phase	Potency assay	Assay Principle
HSC	I-II	Identity (CD34+, CD45+)	Flow cytometry detecting cells expressing markers
AAV gene therapy	I/II	In vitro infection of Huh-7 cells (release test) In vivo using animal model (mice)	In vitro- detected by Western blot (% expression relative to ref std) In vivo-efficacy – disappearance of deficiency marker in urine, liver expression of Tg
AAV gene therapy	I/IIa	Genome copy # In vivo test for reduction in disease marker in ko mice	PCR based Allows detection in changes in potency of vector on stability
Oncolytic virus	I	Virus titre cytokine production cytokine activity	Plaque assay ELISA In vitro cell based assay
Autologous dendritic cells	III	Cell surface activation marker expression Cytokine production	Flow cytometry ELISA

Summary and Conclusions

- Cell and Gene Therapy Products are evolving at a rapid pace and Marketing applications are increasing
- Cell and Gene Therapy products can present unique regulatory challenges with respect to potency assays
- Development of potency assay at earliest possible stages is encouraged
- Scientific advances will push development of Cell and Gene Therapy potency assays
- Early engagement with Health Canada is encouraged

Harmonization

- Health Canada embraces harmonization of regulations with respect to Cell and Gene Therapeutic Products
- Contributor to international harmonization efforts through ICH
- Welcome discussion if our position differs significantly from other regulatory authorities

Relevant Guidance Documents

- Health Canada Guidance Document: Preparation of Clinical Trial Applications for use of Cell Therapy Products in Humans
- EMEA/CHMP guideline on potency testing of cell-based immunotherapy medicinal products for the treatment of cancer (CHMP/BWP/271475/06)
- EMEA/CHMP guideline on human cell-based medicinal products (EMA/CHMP410869/2006)
- FDA Guidance for Industry: Potency Tests for Cellular and Gene Therapy Products

Health Canada

- We welcome regulatory questions via pre-CTA meetings or pre-NDS meetings in-person or via teleconference
- Contact Office of Regulatory Affairs

Office of Regulatory Affairs

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Thank you for your attention!