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POTENCY ASSAYS for CELL THERAPY PRODUCTS

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YOUR HEALTH AND SAFETY... OUR PRIORITY.

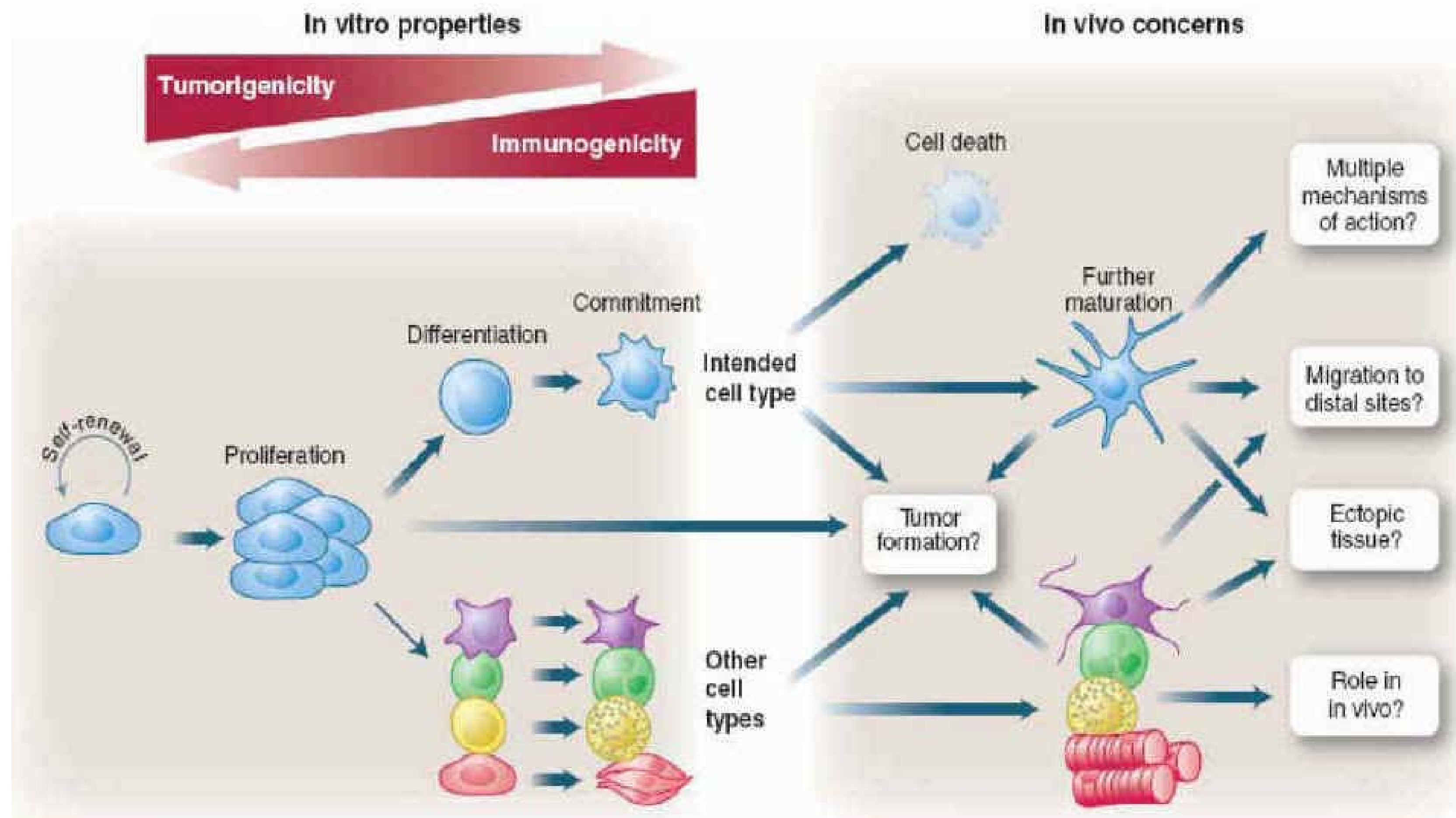
Presentation Outline

- Issues and challenges for cell therapy products (CTPs)
- General expectations and specific issues regarding potency
- "Case studies" for release specifications, including potency

Scientific & Regulatory Issues for Cellular Therapy

- Aseptic processing and inability to sterilise
  - Importance of qualification programmes for all components
- Product distribution and short dating periods
  - Additional use of in-process sterility testing and rapid microbial detection test on final product
- Cellular viability
- Product tracking and segregation
  - Many autologous products (patient sample = lot)

Cell Therapies – Additional Challenges



D.W Fink Science 2009: 324:1662

Some Technical Issues Identified During Submission Review by Health Canada

- Some reagents only available as research grade with questionable safety profiles
- Use of cell viability assays that distinguish between live and dead cells but not between live and apoptotic cells
- Challenges with specifications for impurities when effectiveness of cell therapy products could be attributable to more than one cell type
- Challenges with potency assays especially in cases where the mechanism of action is unclear
- Variability in analytical method used for characterization
- Lack of reference standards

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Specifications

General reference ICH Q6B

(cells outside scope but principles may apply)

- Specifications are the quality standards that confirm the quality of products and other materials used in the production of a product

- Specifications should be appropriate to the stage of product development

- The product specification should be in place and validated prior to licensure

Specifications – ICH Q6B re Potency
(cells outside scope but principles may apply)

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.

Specifications – ICH Q6B re Potency
(cells outside scope but principles may apply)

- Potency (expressed in units) is the quantitative measure of biological activity based on the attribute of the product which is linked to the relevant biological properties
- Mimicking the biological activity in the clinical situation is not always necessary. A correlation between the expected clinical response and the activity in the biological assay should be established in pharmacodynamic or clinical studies.
- The results of biological assays should be expressed in units of activity calibrated against an international or national reference standard, when available and appropriate for the assay utilised. Where no such reference standard exists, a characterised in-house reference material should be established and assay results of production lots reported as in-house units.

Specifications

Potency

- Product-specific or class-specific test
- Ideally measures quantitative biological activity
- Could be qualitative if linked to a quantitative analytical assay

Other specifications can be "supportive"

- Viability: – usually >70% for CTPs
- Cell #/dose: – minimum dose (cells)

Potency

§ 21 CFR 600.3(s)

- “The specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result”

§ 21 CFR 610.10

- “Tests for potency shall consist of either in vitro or in vivo tests, or both, which have been specifically designed for each product so as to indicate its potency in a manner adequate to satisfy the interpretation of potency given by the definition in § 600.3(s) of this chapter”

Potency

Potency measurement is especially important for complex products such as CTPs

- Essential aspect of the QC system for DS & DP
- Performed to help assure identity, purity, potency (strength), and stability of products used at all phases of clinical study (as well as for licensed products)
- Needed to meet requirements of product conformance testing, crucial to comparability

General Considerations for Potency (1)

N.B. Regulators may allow considerable flexibility in determining appropriate measurement(s) of potency (case-by-case for cutting-edge products).

Potency methods should:

- represent the product's MoA, relevant therapeutic activity, or intended biological effect, and/or other relevant product attributes (unless not possible:– MoA unknown; extremely complex/cannot be adequately characterized)
- be demonstrated to measure activity/strength/potency of all active ingredients
- provide quantitative test results for product release (could use quantitative physical assay that correlates with and is used in conjunction with a qualitative biological assay)
- be available in time for lot-release

General Considerations for Potency (2)

Potency methods should:

- be demonstrated to contribute to the prediction of clinical efficacy for each lot (understood that other analyses contribute to a summation of evidence)
- meet predefined data acceptance/rejection criteria ("system suitability")
- include appropriate reference materials/standards &/or controls
- undergo validation to establish accuracy, sensitivity, specificity, precision & robustness
- be demonstrated to be stability-indicating, and to show lot-to-lot consistency

More than one bioassay may be necessary

Special Challenges for CT Products (1)

- Complex M(s)oA which may not be fully characterized or understood. Can have multiple effector functions, perhaps requiring multiple steps

- In vivo fate can be complex and make design and correlation with MoA difficult or impossible: e.g. cells may need to differentiate and/or migrate. (Nevertheless, the selected potency markers should be present in potent cells but missing in sub-potent or undifferentiated cells)

- The possible need for multiple (or a matrix of) potency assays compounds the challenges of individual assays

Special Challenges for CT Products (2)

- Limited lot size & limited material for testing
- Inherent variability of CT starting materials (donors, cell line)
- Limited stability (e.g. related to cell viability)
- Lack of appropriate reference materials (e.g. because autologous cells)
- Multiple active ingredients (e.g. multiple cell lines combined)

- Cellular products often cannot be cryopreserved and must be administered soon after harvest. Their testing, shipping and administration procedures must be rapid to ensure product quality, potency and integrity

Analytical methods for CTP potency testing

- Used when technical limitations make a suitable bioassay not feasible.
- Assays based on immunochemical, molecular, or biochemical characteristics of the product can also be used to demonstrate potency
  - should directly reflect, or be correlated with a biological activity.

Use of Biomarkers

- Avoid simple cell-identity markers that may not change under conditions that affect cell function (and so unsuitable for potency).
- Identify biomarkers that correlate with, e.g., cell survival and in vitro differentiation.
  - can detect unacceptable behavior of cultured cells
  - detect functional cells in a complex mixture
- Potentially identified through genomic and/or proteomic techniques

Some Conclusions on Potency for CT Products

- A typical potency assay dose response curve approach of comparing sample to standard may not work for CT products. A bioassay more indicative of potency might be a matrix of biomarkers turned on and off in a specific pattern (qualitative). (With an analytical method?)
- Early in development it is critical that a sponsor demonstrate assay ability to reject sub-potent material (use purposefully degraded or stressed material). This is critical to support clinical trials.
- Validation may come late in development with wide variability allowed
- Develop a clear rationale for linking MoA to basis of potency assay.

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"Case Studies" for CTP Potency Testing & Specifications

Provenge

- Autologous CD54+ Ag presenting cells (APC) "primed" with tumour Ag.
- Cells obtained via leukapheresis; selection/sorting for CD54+ cells; exposure to rh-fusion protein (tumour Ag (prostatic acid phosphatase) and GM-CSF).
- Product is a liquid cell suspension (so there are shelf-life issues).

Prochymal

- Allogeneic (unmatched donor), phenotypically selected, adult hMSC.
- Cells from bone marrow aspirates; in vitro expansion and "phenotypic sculpting".
- Product is a frozen cell suspension in a bag for re-suspension and infusion.

Dendritic cells (many products in development)

- Autologous, Ag- or RNA-loaded, Ag processing mature dendritic cells.
- Leukapheresis-derived (usually) monocytes are selected for immature dendritic cells that are loaded with Ag or RNA then "phenotypically sculpted" to become mature antigen processing dendritic cells.
- Products are provided as cell suspensions, some liquid and others frozen.

Provenge Release Specifications

From FDA Summary Basis for Regulatory Action

Final Product Testing			
Identity	Identity	Final Product	--b(4)-----------
Potency	CD54 upregulation	Final Product/--b(4)----------	--b(4)-----------
	Number of CD54+ cells	Final Product	--b(4)-----------
Purity	--b(4)---------	Final Product	--b(4)-----------
Safety	Endotoxin content	Final Product	--b(4)-----------
	Microbial contamination	Final Product	--b(4)-----------
	Sterility (--b(4)------)	Final Product	--b(4)-----------

Code b(4) indicates information was redacted (as proprietary)

Prochymal Mechanisms

Prochymal functional properties beneficial for GVHD treatment include:

- Homing to sites of injury/inflammation
- Immunomodulation: suppression of T-lymphocytes at injury/inflammation sites
- Anti-inflammatory activity: inhibition of pro-inflammatory cytokines (TNF-α and IFN-γ)
- Tissue repair

Potency Markers Selected for Screening

Marker	Justification for marker selection
Prostaglandin E2 (PGE_2)	PGE_2 suppresses immune response. MSCs produce PGE_2, and PGE_2 mediates MSC-induced immunosuppressive and anti-inflammatory effects *in vitro.*
Indoleamine 2,3-dioxygenase (IDO) enzyme activity	IDO is an enzyme inducible by pro-inflammatory cytokines such as IFN-γ and TNF-α. IDO inhibits immune response via depletion of tryptophan, an amino acid that is essential for immune cell activation. IDO enzyme mediates MSC-induced immunosuppression *in vitro.*
Tumor Necrosis Factor-α (TNF-α)	TNF-α is a pro-inflammatory cytokine playing an important role in GVHD. MSCs inhibit TNF-α secretion by immune cells *in vitro*.
Interferon-γ (IFN-γ)	IFN-γ is a cytokine secreted by Th1 cells that are involved in GVHD development. MSCs can inhibit secretion of IFN-γ that is beneficial for GVHD treatment
Tumor Necrosis Factor-α Receptor (TNFR)	TNFR is expressed on MSCs. TNFα is present in organs targeted by GVHD. TNF-α via TNFR up-regulates secretion of PGE_2, induces expression of IDO and stimulates MSC migration *in vitro*. TNFR is a mediator of MSC biological activities.

From A. Danilkovitch, Osiris, 2006

Prochymal "Key Characteristics"

There are 100 × 10^6 hMSCs in each frozen product bag upon thaw.

hMSC Key Characteristics

Macroscopic Appearance	Opaque, off-white to pale amber in color, absence of cell clumps and particulate matter, intact package integrity.
Cellular Viability	≥ 70%
Identity (Cell Surface Markers)	
Positive Markers	CD105 (Endoglin), CD166 (Activated Leukocyte Adhesion Molecule)
Negative Marker	CD45 (Leukocyte common antigen)
Potency	TNF RI Expression ≥ 108 pg/mL Inhibition of IL-2Rα Expression ≥ 30% inhibition

The inactive ingredients (excipients) are 10% dimethyl sulphoxide, 5% human serum albumin solution and up to 15 mL of Plasma-Lyte A.

Assays for Dendritic Cell Products (1)

Table 3. Assays for functional evaluation of dendritic cell products

Test	Assay	Assay results
DC maturation status	Flow cytometry for surface expression of CD83 and CCR7	Upregulated expression of CD83, CCR7 on >50% of DCs
	Microscopic evaluation of endocytosis of latex beads or FITC-labeled dextran particles	More-efficient uptake of beads by iDCs than by mDCs
	Flow cytometry for the mannose receptor (CD206) expression	High levels of expression on iDCs relative to mDCs
APM component expression	Flow cytometry for intracytoplasmic staining for APM expression levels using antibodies specific for APM components	Normal levels defined by comparisons to DCs generated in parallel from normal donors

From T.L. Whiteside, BioPharm Int'l March 1, 2008

Assays for Dendritic Cell Products (2)

Cells shipped cryopreserved for thaw and administration

Table 3. Assays for functional evaluation of dendritic cell products*

Test	Assay	Assay results
Potency	IL-12p70 production: two-step co-culture assay	The ability of mDCs to produce high levels of IL-12p70
	MLR-type assays: T-cell activation and proliferation in response to alloantigens expressed by DCs	Efficient proliferation of T cells in response to alloantigens
	Single-cell assays: antigen-specific T-cell activation ELISPOT Cytokine flow cytometry Tetramer frequency	An increased frequency of antigen-specific T cells in the population relative to cultures established in parallel with DCs not pulsed with the antigen or DCs pulsed with an irrelevant antigen; HLA-compatible DC needed
	Costimulatory bioassay: ability of DCs to co-stimulate T cell proliferation	Expression levels of co-stimulatory molecules on DCs is related to their ability to activate T cells
	Flow cytometry after DC staining	Upregulated expression of ICAM (CD54) on >50% of DCs

*A selected list of functional assays is presented. None of the assays has been formally validated, although many are standardized to ensure assay reliability. APM = antigen processing machinery; MLR = mixed leukocyte reaction

From T.L. Whiteside, BioPharm Int'l March 1, 2008

Assays for Dendritic Cell Products (3)

Table 4. Rationale for selecting IL-12 production assay to measure potency of dendritic cells

- IL-12p70 production is a characteristic feature of matured DCs (mDCs)
- *In vitro*, it can define lot-to-lot consistency of DC products and their quality based on function
- It is readily applicable to a high-throughput clinical platform (product monitoring)
- It is specific (antibody-based) and sensitive (in a low pg range)
- *In vivo*, it may prove to be a good correlate of efficacy of DC products and their clinical usefulness as vaccines based on our preliminary correlations

From T.L. Whiteside, BioPharm Int'l March 1, 2008

Potency "matrix" approach for protein biologics

- Typical biologics can move from a complicated and highly variable *in vivo* assay to multi-assay approach
- One example is change from the polycythaemic mice assay for erythropoietin to two complementary pharmacopoieal potency assays:
  - Use of an erythropoietin-responsive cell line (similar to UT-7) in a cell-based proliferation assay
  - Separate and measure isoforms reflecting sialic acid residues (e.g., using capillary electrophoresis)

Potential Potency Assay Matrix for CT Product
(perhaps for DCs or TILs)

- Viable cell number
- Target-specific cytotoxicity and/or cytokine release
- Surrogate biomarker (functional)
  - Phenotype expression or factor release that correlates with function
- Biological activity such as antigen presentation

In Summary

- Sponsors should start efforts to measure potency early in development
  - Proof of concept; collect data and learn; correlation with/rationale for MoA; ability to reject lots
- Potency assay should evolve/upgrade in mid- to late-stage development
  - Contribute to the prediction of clinical efficacy for each lot
- Matrix approach to assays is an option
  - (Licensed CTPs feature multiple potency assays)
- Validate for licensure

References and Reading Material

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