Off the shelf cellular therapeutics: Factors to consider during cryopreservation and storage of human cells for clinical use

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Overview:
The field of cellular therapeutics has immense potential, affording an exciting array of applications in unmet medical needs. At the forefront is an emphasis on getting these therapies from bench to bedside without compromising efficacy. For a successful cellular therapy program, it is essential to extend the shelf-life of these therapies beyond shipping “fresh” at ambient or chilled temperatures for “just in time” infusion. Cryopreservation is an attractive option because of major advantages such as storing and retaining patient samples in case of a relapse, banking large quantities of allogeneic cells and retaining testing samples for leukocyte antigen typing and matching. However, cryopreservation is only useful if cells can be reanimated to physiological life with negligible loss of viability and functionality. Also critical is the logistics of storing, processing and transporting cells in clinically appropriate packaging systems and storage devices. Rationalized approaches to develop commercial-scale stem cell therapies require efficient cryopreservation system that provides the ability to inventory standardized products for later on-demand distribution and use, as well as a method that is scientifically sound and optimized for the cell of interest. While many commercial cell therapy establishments do employ good manufacturing methods, scientific optimization of cell specific cryopreservation methods can be overlooked.

- **Osmotic Shock Injury**: Addition /Removal of CPA
  - **Addition of CPA**
  - **Exposure to CPA**
  - **Loss of Intracellular Water**
  - **Permanently CPA Moves into the Cell**
  - **Minimize “Osmotic Injury”**
  - **Step-wise addition and removal of CPA**
  - **Choice of CPA - Cell Specific**
  - **Commonly used - DMSO**

- **Cooling Injury**
  - **Phase change Temperature**
  - **Ice Crystals**
  - **Rapid, Optimal, Slow**

- **Thawing/Warming Injury**
  - **Major Player: Recrystallization Injury**: This phenomenon occurs when innocuous extra- or intracellular ice formed during freezing melts and coalesces into larger, more damaging crystals during a temperature excursion or suboptimal warming procedures (typically slow warming).
  - **Rapid Thawing is Optimal**

Cryopreservation Process

- **Addition of Cryoprotectants (CPA)**
- **Freezing**
- **Storage**
- **Thawing/Rewarming**
- **Post-Cryopreservation Processing**

**Two Damage Mechanisms:**
- **“Solute Effects” Injury**: Becoming a “prune” by extended exposure to highly concentrated salt solutions - Referred as
  - “Intracellular Ice Formation” Injury
  - Ice crystals within the cells tend to “poke” and “rupture” them