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 for the forefront is an emphasis on getting these therapies from bench to bedside without compromising efficacy. For a successful cell therapy establishment, the logistics of storing, processing and transporting cells in clinically viable and functionally intact state is paramount.

Cryopreservation Process

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Osmotic Shock Injury: Addition/Removal of CPA

- Injury due to the addition and removal of cryoprotective agents. Cell-specific characteristics such as biophysical parameters (size, shape, membrane permeability to water and cryoprotectants, osmotically inactive water, osmotic and volumetric tolerance limits) should be considered.

Cooling Injury

- Injury due to an abrupt change in temperature. Cooling rate should be considered, with very slow cooling rates applied to cold shock-sensitive cells.

Chilling Injury

- Injury due to prolonged exposure to cold (but above cryogenic) temperatures. Absolute exposure time is the most critical factor to consider. If cells appear to be chilling sensitive but are tolerant of a cryoprotectant such as DMSO at warmer temperatures, strategies can be employed to perform cryoprotectant additions at or near room temperature and reduce the amount of time “chilled.”

Storage Injury

- Injury due to unwanted thermal fluctuations (transient warming events), cosmic rays and free radical formation. Factors to consider include the glass transition temperature of the cryoprotectant and careful maintenance of the storage temperature at all times. Properly cryopreserved and stored cells are viable indefinitely. Although practically challenging, if all possible a sample should never be removed from cryostorage until it is to be used; otherwise temperature of the sample should be monitored throughout any temporary removal (such as removing a rack of vials or frame of bags). Additional considerations should include the use of closed system containers for storage (in vapor or liquid).

Thawing/warming injury

- Injury associated with warming sample from LN2 storage temperature to above phase change temperature. Potential recrystallization during warming should be considered. If slow cooling is used, a wider range of warming rates is likely acceptable; however, faster warming generally may result in less intracellular recrystallization.

Post-Cryopreservation Processing

- Upon thaw, cells are in a potentially compromised state. Care must be given to appropriately prepare them for use. If a permeable cryoprotectant is used, knowledge of cell-specific osmotic characteristics is important. Cells swell and may lyse upon removal of permeable cryoprotectants and may not survive one-step dilution. If cells are administered directly from thaw without dilution or a washing step, this is effectively a one-step dilution and may result in significant cell loss in vivo.

Two Damage Mechanisms:

- “Solute Effects” Injury
  - Beneficial “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

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- Minimize “Osmotic Injury”
  - Step-wise addition and removal of CPA
  - Choice of CPA – Cell Specific
  - Commonly used – DMSO

- Loss of Intracellular Water
  - Permeant CPA Moves into the Cell

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