Must Should May

MITreg Checklist

1) Cells at the start of procedure

a) Essential information about the donor

i) Species and strain

- Species
- Strain (if applicable)

ii) Characteristics of the organism

- Health
- Age
- Treatment/Environment
- Individual identifier number
- Source of purchase (if applicable)

b) Source of cell material

- Organ, tissue, fluid or blood product
  - Source (if applicable)
- Quantity (volume, size or weight)
- Anti-coagulant (if applicable)
- If using cryopreserved sample:
  - Method and duration of storage
  - Initial cell counts
- Ethical committee approval/ written informed consent

c) Cell separation process

i) Cell handling and labelling

- Cell extraction method
- Tissue conditions between tissue retrieval and cell separation
  - Duration
  - Temperature
  - Container
  - Fluid
- Cell labelling
  - Buffers and reagents (incl. source)
  - Cell suspension volume and concentration
  - Incubation temperature and duration
  - Washing steps

ii) Cell separation equipment and process

- Methodology
- Equipment
- Presence of target cells in starting material described

Must Should May

d) Phenotype

For any of the below, indicate the percentage of cells displaying the characteristic (if known)

i) Cell surface and intracellular markers

- Molecules measured (using CD names)
- Details of reagents used and source (incl. mAb clone, fluorochrome)
- Methodology
- Stimulus and time of stimulation (if applicable)
- Gating strategy to determine positive cells

ii) Secreted molecules

- Molecules measured
- Details of reagents used (incl. mAb clone, conjugate) and source
- Methodology
- Cell density/ml of medium and type of tissue culture plate
- Time point of supernatant collection
- Stimulus and time of stimulation (if applicable)

iii) Epigenetic modifications

- Epigenetic modification relevant to the characteristics

iv) Specificity

- Specificity of the cells (polyclonal or antigen-specific)
- Methodology used to obtain specificity
- Methodology used to confirm specificity

e) Cell numbers

i) Absolute cell number

- Total number of cells at the end of the isolation process
  - Methodology

ii) Viability

- Percentage of viable cells
  - Methodology

2. Expansion/Differentiation

a) Pre-culture conditions

- Storage conditions
  - Fluid
  - Type of container
  - Temperature
  - Fresh or thawed
- Storage time

Must Should May

b) Culture conditions

i) Cell number

The total number of cells put into culture

ii) Cell concentration

The number of cells per ml of medium at start of culture

iii) Culture medium

Type(s) of medium

Source(s)

Additives (excluding agents to maintain/induce Tregs)

Refreshment of the medium

iv) Culture container

Type of container

Size

Manufacturer

Cell culture volume per container or well

Total number of containers or wells

v) Culture environment

Temperature and CO2 concentration

Use of pre-warmed medium

Equipment

c) Differentiation/tolerization protocol

Name of cytokine(s) or other agent(s) used

Concentrations

Time-point(s) added to cell culture

Total length of the culture period

Rounds of stimulation

Number of cell splitting

d) Stimulus

Polyclonal/antigen-specific/allo-antigen

Stimulus (agent and/or accessory cell)

Source

Concentration

Time point(s) added to culture

Restimulation conditions (if applicable)

e) Storage

Storage time

Storage conditions

If fresh

Fluid

Container

Temperature

If cryopreserved

Must Should May

Freezing/thawing process
Freezing medium
Cell recovery & viability after thawing
Time point at which cells are stored if different to the end of the culture process

3. Cells after expansion/differentiation

a) Phenotype

For any of the below, indicate the percentage of cells displaying the characteristic (if known)
Stability of the phenotype (if tested)
Phenotype tested on fresh or thawed cells

i) Cell surface and intracellular markers

Molecules measured (using CD names)
Details of reagents used and source
Methodology
Stimulus and time of stimulation (if applicable)
Gating strategy to determine positive cells

ii) Secreted molecules

Molecules measured
Details of reagents used and source
Methodology
Cell density/ml of medium and type of tissue culture plate
Time point of supernatant collection
Stimulus and time of stimulation (if applicable)

iii) Epigenetic modifications

Epigenetic modification relevant to the characteristics

b) Functional assay

Response of the cells to a defined stimulus
Behaviour of other biological entities after exposure to the cells
If using accessory cells, describe phenotype and source

c) Cell numbers

i) Absolute cell number

Total number of cells at the end of the isolation process
Methodology

ii) Viability

Percentage of viable cells
Methodology

Must Should May

d) Dosing

- Dose of cells transferred into organism (if applicable)
- Vehicle (solvent/medium) and intermediate components (for clinical trials only)

e) Quality control (for clinical trial only)

- Specificity
- Purity
- Sterility
- Potency

4. About the protocol

a) Regulatory authority

- External authority that approved the protocol
- Does protocol follow GMP?

b) Purpose

- The disorder for which the cell treatment has been manufactured

c) Relationship between the source organism for the cells and the target organism

- Allogeneic/Autologous/ Xenogeneic/Syngeneic

d) Contact details

Name and contact information of the corresponding author(s)

e) Citation

Acknowledge the MITREG reporting guidelines