Use of Chimeric Antigen Receptors as a Novel Cancer Immunotherapy Approach
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Abstract

- Immunotherapy consists of the use of immunologic principles against tumours. Unlike chemotherapy or radiotherapy, immunotherapy has the potential to induce such a dynamic immune response that can kill tumour cells for an extended period of time.
- The use of chimeric antigen receptors (CARs) in lymphocytes is an emerging immunotherapy approach. These receptors bind to tumour-specific antigens, promoting the activation of the host’s immune cells, which results in a specific immune response against tumour cells.

Materials and Methods

- Search of scientific literature in PubMed: reviews and recent papers about CARs and their use in pre-clinical and clinical trials were selected.
- Use of Immunology books: reading of the chapters concerning T-cell activation, antigen receptors, immune responses and autoimmunity.

Introduction to CARs

- **Structure:**
  - **Antigen-binding extracellular domain:** binding site of the antibody that targets the cognate antigen.
  - **Trans-membrane domain**
  - **Signal-activating intracellular domain:**
    - Stimulatory molecule: CD3 ζ chain
    - Co-stimulatory molecule: CD28, 4-1BB…
  - **Stimulatory molecule:**
    - CD3 ζ chain
    - CD28
  - **Co-stimulatory molecule(s):**
    - Name, only, or more of CD27, CD79, ICOS, 4-1BB, OX40

Use of CARs in different tumours

<table>
<thead>
<tr>
<th>Targeted antigen</th>
<th>Disease/hallmark of cancer</th>
<th>CAR generation</th>
<th>Used co-stimulatory molecule(s)</th>
<th>Stage of trial</th>
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<tr>
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<td>CD28 or 4-1BB</td>
<td>Clinical</td>
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<td>ALL</td>
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<td>VEGF2</td>
<td>Tumour angiogenesis</td>
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<td>CD28 and 4-1BB</td>
<td>Phase 1</td>
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</table>

Table provided by the author.

Ex vivo methods and genetic engineering

**Steps for lymphocyte modification:**

1. **Apheresis:** separation of T-cells from the rest of blood cell types.
2. **Internalization of CAR constructs into T-cells:**
   - a. Electroporation
   - b. Viral vectors: most used strategy
   - c. Transposon-based systems
3. **Expansion of T-cells**
   - Activating signals: Anti-CD3 activating monoclonal antibody
   - Supporting medium: IL-2 containing medium
4. **Transfusion of an optimal number of modified T-cells back to the patient.**

Future approaches and main conclusions

**Future approaches:**

- Considerations for the improvement of CARs in terms of efficacy increase and toxicity decrease:
  - It is extremely important to use tumour-restricted antigens in order to decrease toxicity and off-target effects.
  - Suggested measurements to decrease the likelihood of generating autoimmune reactions:
    - Splitting the modified T-cell doses.
    - Introducing suicidal genes into the CARs construct. These genes get activated in highly toxic environments, promoting the apoptosis of the modified T-cells.
  - Economic and logistic costs: the modification of T-cells is performed for each individual’s T-cells. The use of allogeneic lymphocytes is an alternative to this strategy.

**Main conclusions:**

- B-cell malignancies are the cancer types in which the use of CARs has provided the best outcomes and CD19 is the most successful target.
- The success of this approach relies on the development of modified T-cells able to overcome the hostile tumour microenvironment and to promote efficient and accurate cognate immune responses.

References

- Only relevant references are cited below.