MultipleMyelomaHub

Patients eligible for transplant

EBMT 2019 | Targeting NKG2D ligands in patients with multiple myeloma

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In the search for new therapies, CAR-T cells are showing promising results for multiple myeloma (MM), however, cytokine release syndrome is a well-known toxicity that can be life-threatening. To avoid these toxicities CAR-NK based approaches are being investigated to achieve better clinical responses.

On 27 March 2019, Oral Session 20 (OS20) took place at the 45th Annual Meeting of the European Society for Blood and Marrow Transplantation (EBMT), Frankfurt, Germany. During this session, Alejandra Leivas, from the Hospital Universitario 12 de Octubre, Spanish National Cancer Center, Madrid, Spain, presented results regarding the efficacy of transduced primary natural killer cells expressing NKG2D-CAR to target MM cells.

The primary objective of the study was to establish NKG2D-CAR NK (CAR-NK) cells for patients with MM, and to compare their anti-tumor activity to untransduced activated and expanded natural killer cells (NKs) and $CD45RA^-$ T cells +/- the NKG2D-CAR construct.

Study design

- NKs were produced by co-culture of peripheral blood mononuclear cells (PBMCs) with the previously irradiated CSTX002 cell line.
- $CD45RA^-$T cells were attained by depletion with CD45RA magnetic beads and subsequent culture.
- Cells were transduced using an NKG2D-CAR with signaling domains of 4-1BB and CD3z
- Flow cytometry was used to evaluate the phenotypic gene expression of NKG2D-CAR
- Assays performed:
- *In vitro*
  - Europium-TDA (EuTDA) release assays (2–4 hours): cytotoxic activity
  - Anti-tumor activity studied against MM U266 cells
  - Methylcellulose culture: assess activity against clonogenic tumor cells
- *In vivo* studies were carried out on NOD scid gamma (NSG) mice given:
- Day 1: $5.10^6$of U266-luc MM cells
- Day 4: 10^6 either CAR-NK or NKs

Key findings

In vitro

- Primary NKAE cells could be efficiently transduced with an NKG2D-CAR
- NKs were found to have a significantly higher cytotoxicity than CD45RA$^-$ T cells from the same patient.
- CAR-NK had 10% higher anti-tumoral activity than NKs resulting in almost complete destruction of U-266 MM cells
- However, when CD45RA$^-$ T cells were transduced with NKG2D-CAR, minimal improvement in anti-tumoral activity was seen (5.8%)

Cell type	***In vitro* cytotoxicity (%)**
NKAEs	86.6 ± 13.9
CD45RA$^-$ T cells	16.7 ± 13.6
CAR-NK	96.4 ± 19
CD45RA$^-$ T cells transduced with NKG2D-CAR	22.5 ± 10.6

- Transduction of NK cells with an NKG2D-CAR produced no change in the immunophenotype
- CAR-NKs destroyed 71.2% ± 2.5% of clonogenic tumor cells at a ratio of 8:1
- NKAEs reached 56.5% ± 2.6% at a ratio of 32:1
- No activity against autologous lung, colon or PBMCs was observed

In vivo

- NKs and CAR-NKs were both effective in abrogating MM growth in the mouse model
- CAR-NK showed higher efficiency 14 days after the injection of tumor cells than NKs
- 42 days after tumor cell injection, only animals in the CAR-NK treatment arm remained free of disease
- At day 130, only CAR-NK injected mice were left alive

Conclusion

Dr. Leivas concluded that it is possible to modify NK cells and CD45RA$^-$ T cells from patients with MM to express NKG2D-CAR. The data collected by the team identified that CD45RA$^-$ T cells, *in vitro,* even when transduced, were not effective against MM. Dr. Leivas highlighted that CAR-NK were successful at destroying MM cells *in vitro* and *in vivo* and therefore provide a foundation for the future development of NKG2D-CAR NK based cell therapy.

Reference

Leivas. A. *et al.* NKG2D-CAR transduced primary natural killer cells efficiently target multiple myeloma cells. Abstract OS20-7: 45th EBMT Annual Meeting, Frankfurt, Germany