CART CELLS: A PROMISING IMMUNOTHERAPY FOR CHRONIC LYMPHOCYTIC LEUKEMIA

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INTRODUCTION

There are clear evidences about the essential role of the immune system in the control of cancer. An effective response needs some steps collectively known as "**Cancer-Immunity Cycle**". However, tumors have immune system evasion mechanisms that can break this cycle. Thus, it is interesting to think about the possibility of act on the immune system to develop an effective anticancer response. In cancer treatment, immunotherapy is a promising field that has undergone a renaissance in recent years.

Chronic lymphocytic leukemia (CLL) is the most common hematologic disease in Western Hemisphere and remains incurable, like most hematological malignancies. This leukemia is characterized by gradual accumulation of mature B-lymphocytes that co-express CD5, CD23 and **CD19**. CD19 is an exclusive antigen of B cells, which is an important advantage for therapy.

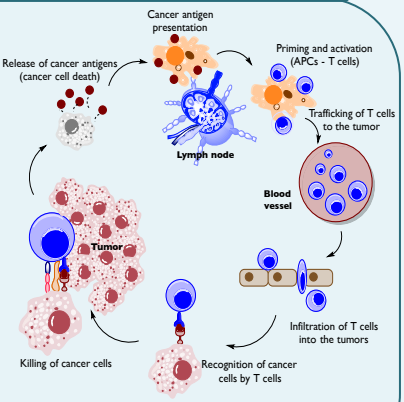


Figure 1: Cancer Immunity cycle. The cycle can be divided into seven steps, starting with the release of antigens from the cancer cell and ending with the killing of cancer cells. Abbreviations: APC, antigen presenting cells; CTLs, cytotoxic T lymphocytes. Adapted from reference [1].

CHIMERIC ANTIGEN RECEPTOR (CAR)

CARs have a single-chain antibody fragment (scFv), expressed in tandem with signaling elements derived from the T cell receptor (essentially CD3 ζ) and co-stimulatory domains such as 4-IBB and CD28.

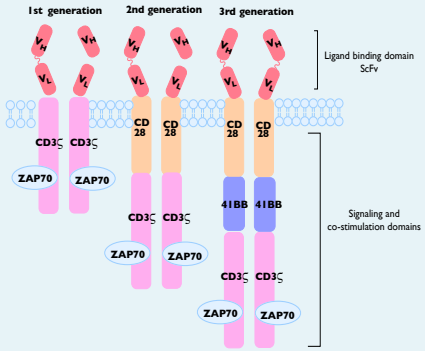


Figure 2: Schematic structure of chimeric antigen receptors (CARs). The structures of first-, second- and third-generation CARs are shown.

IMMUNOTHERAPY: ADOPTIVE CELL TRANSFER WITH CART-19 CELLS

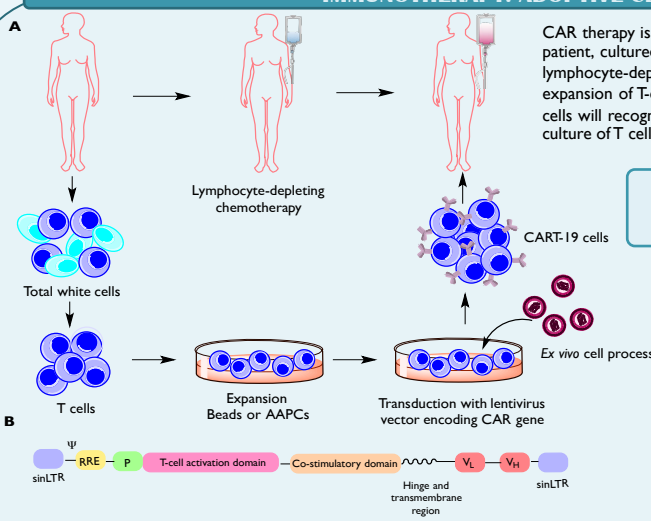
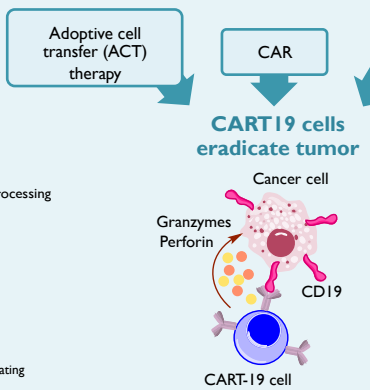


Figure 3: (A) Scheme of ACT procedure. (B) Scheme of lentiviral vector. Abbreviations: sinLTR, self-inactivating long terminal repeat; RRE, rev responsive element; P, promoter; Ψ, packaging signal.

CAR therapy is similar to an autologous bone marrow transplantation procedure. T cells are collected from the patient, cultured and genetically modified using **lentiviral vectors**. During this process, the patient receives lymphocyte-depleting chemotherapy in order to create an environment that supports the homeostatic expansion of T-cells and improves their effector function. Then, CART-19 cells are infused to the patient. These cells will recognize **CD19+ tumor cells** (without the need for antigen presentation by HLA), and kill them. In culture of T cells, CD28, CD137 (4-IBB) and CD3 co-stimulation can improve the replicative capacity.



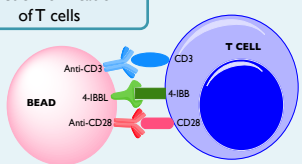


Figure 4: Scheme of artificial antigen presenting cells (AAPCs) or beads for T-cell stimulation in culture.

CLINICAL TRIALS USING CART-CELLS

POSITIVE ASPECTS

Evidence of immune response: Cytokine increase + CART cells infiltration

Specific immune response against CD19 → Leukemia cells elimination

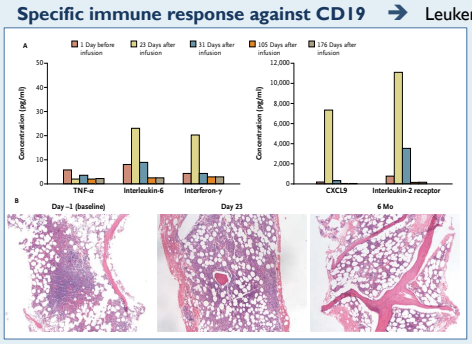


Figure 5: (A) Induction of the immune response in bone marrow. The cytokines TNF-α, interleukin-6, interferon-γ, chemokine CXCL9, and soluble interleukin-2 receptor were measured in supernatant fluids of marrow aspirates at various days before and after CART-19 cell infusion. The increases in levels of interleukin-6, interferon-γ, CXCL9, and soluble interleukin-2 receptor coincided with the tumor lysis syndrome (5a), peak chimeric antigen receptor T-cell infiltration, and eradication of leukemic infiltrate (B). (B) Bone marrow biopsy specimens for 3 days after chemotherapy and 23 days and 6 months after CART19-cell infusion (hematoxylin and eosin). Reference [8].

NEGATIVE ASPECTS

Tumor lysis syndrome
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Disappears with treatment

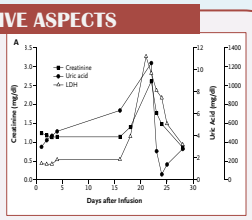

Figure 6A: Serum creatinine, uric acid, and lactate dehydrogenase (LDH) levels from day 1 to day 28 after the CART-19 cell infusion. Reference [8].

Absence of normal B cells

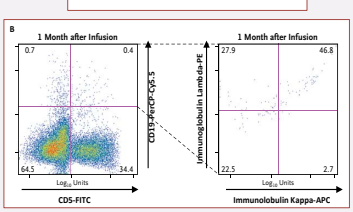

Figure 6B: Flow-cytometric analysis of bone marrow aspirates on day 31 after infusion shows CD5+ T cells were present, and no normal or malignant B cells were detected.. Reference [8].

FUTURE DIRECTIONS

- Optimal tumor antigens and factors associated with expansion and persistence in vivo.
- Non-viral gene transfer technologies → minimal T cells manipulation *ex vivo*.
- Combinatorial strategies: ACT and agents that impact on tumor biology → high response
- Issues about scale, automation, commercialization and intellectual property.

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Cellular therapy, still immature, will revolutionize cancer treatment.

CONCLUSIONS

Immunotherapy for cancer has undergone a renaissance in recent years. Genetic modification can provide to lymphocytes specificity against tumors and thus overcome evasion mechanisms. The usage of CART-19 cells is a promising example with many encouraging results. Although there is much room for improvement, the immunotherapy based on engineered T cells can be a big part of the future for cancer treatment.

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[1] Chen S, Mellman I. Oncology Meets Immunology: The Cancer-Immunity cycle. *Immunity* 2013; **39**: 1-10. [2] Kindt TJ, Goldsby RA, Osborne BA. Inmunología de Kuby. 6ª ed. México: McGraw Hill, 2007: 525-545. [3] Ruella M, Kalos M. Adoptive Immunotherapy for Cancer. *Immunological Reviews*, 2013 [4] Rozovski U, Hazan-Havely I, Keating M, Estrov Z. Personalized medicine in CLL: Current status and future perspectives. Cancer *Letters*, 2013. [5] Husebekk A, Fellowes V, Read EJ, Williams J, Petrus MJ, Gress RE, Fowler DH. Selection and expansion of T cells from untreated patients with CLL: source of cells for immune reconstitution? *Cytotherapy*, 2000. **2**:187-193. [6] Barret DM, Singh N, Porter DL, Grupp SA, June CH. Chimeric Antigen Receptor Therapy for Cancer. *Annual Review of Medicine*, 2014. **65**: 10.1-10.15. [7] Liu L, Sun M, Wang Z. Adoptive T-cell therapy of B-cell malignancies: conventional and physiological chimeric antigen receptors. *Cancer Letters*, 2013. **316**: 1-5. [8] Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric Antigen Receptor-Modified T Cells in Chronic Lymphoid Leukemia. *The New England Journal of Medicine*, 2011. **365**: 725-733.