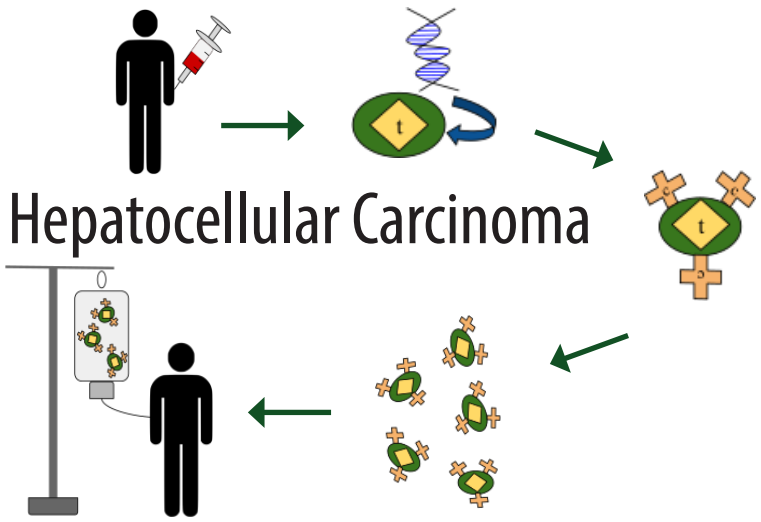
NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

DisCARding HCC:

CAR T-Cell Therapy Against Hepatocellular Carcinoma



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Part I – Some Bad News

Steven, a 65-year-old white male, spent much of his younger years creating extremely unhealthy drinking habits beginning with daily consumption of alcohol from the age of 35. This alcohol abuse led to a diagnosis of liver cirrhosis at age 62. This acted as a harsh wake-up call and Steven quit consuming alcohol and started maintaining a healthier lifestyle, including regular exercise.

Although he had mended his ways and had quit drinking alcohol, it was unfortunately too late. Due to the damage caused from the cirrhosis, at age 65 he was diagnosed with hepatocellular carcinoma (HCC), also known as liver cancer. Steven's oncologist, Dr. Vasudha Ranganathan, had determined that the cancer was inoperable because Steven was not healthy enough to endure surgery. Since the cancer had spread throughout his liver, if the cancer was surgically removed, Steven would not have enough healthy liver tissue left because of the damage from the cirrhosis. Therefore, Steven would need a liver transplant if an alternative form of treatment was not available. Other treatment options included chemotherapy, radiation therapy, and immunotherapy. Since on average there was a five-year survival rate after diagnosis, a treatment option needed to be chosen swiftly.

Questions

1. What is liver cirrhosis? How does excessive alcohol consumption lead to this condition?

2. What is HCC and what are its most common causes?

3. What are the symptoms of HCC?

4. How is HCC diagnosed? Why are patients with HCC primarily diagnosed in the later stages of cancer development?

5. Provide a brief description of the treatments for HCC that were mentioned above:
   a. *Chemotherapy:*

   b. *Radiation therapy:*

   c. *Immunotherapy:*

Part II – Some Good News

While Steven followed the steps to enter the organ transplantation waiting list, Dr. Vasudha sent tumor samples to be analyzed for molecular markers. The cancerous cells were analyzed using flow cytometry, which uses lasers and fluorescent molecules to characterize chemical and physical properties of cells. This technique was used to determine that the CD133 antigen, a known molecular biomarker for liver cancer, was found on the surface of 45% of the cells from Steven's tumor. Dr. Vasudha was very pleased because very few cell types have CD133 on their surface, and within those cell populations, only 0.5–2% of the cells were $CD133^+$. This meant that Steven was eligible for a form of treatment involving the body's natural immune response. She explained to him that the presence of this antigen meant that they could take advantage of chimeric antigen receptor T-cell (CAR T-cell) therapy, where an enhanced bodily immune response combined with cancer stem cell theory is used as a therapeutic strategy. Cancer stem cell theory refers to the concept that stem cells, continuously dividing cells which can differentiate to make other cell types, exist within a tumor and are responsible for its sustenance and metastasis.

Questions

1. What are molecular biomarkers and how are they useful in cancer treatment?

2. What is cancer stem cell theory?

3. Dr. Vasudha mentioned that CD133 antigens are present on the cell surface. What is CD133 and what is its connection with cancer stem cell theory?

4. Why is CD133 a good cell surface antigen target for HCC?

5. What is the normal function of T cells in the body? What do we mean when we say that T cells are "specific"?

6. T-cell receptors are proteins located on the surface of T cells that directly interact with antigens. T-cell receptor proteins have different domains, or regions, which have distinct functions. There is an intracellular domain, a transmembrane domain, and an extracellular domain. Which specific region of a T-cell receptor binds to antigens? Is this part of the T-cell receptor located inside or outside of the T-cell?

7. Describe CAR T-cell therapy using your own words.

8. Why do you think that Dr. Vasudha is suggesting CAR T-cell therapy for Steven instead of chemotherapy or radiation? Is there an advantage that immunotherapy has over the alternatives?

9. How do you think having an identifiable cell-surface antigen, such as CD133, on the surface of cancer cells help the efficacy of CAR T-cell therapy?

Part III – CAR T-Cell Therapy

Steven was curious and a little concerned about CAR T-cell therapy. He had never heard of it before and wanted to know more prior to changing his treatment plan. Steven asked the questions below. Answer his questions as if you were Dr. Vasudha.

Questions

1. *How would I receive CAR T-cell therapy?*
   Include step-by-step points that address such issues like whether Steven would be connected to a machine, whether his blood would be drawn, etc. List the steps in the appropriate order. The webpage "CAR T-cell Therapy" from the Cleveland Clinic may be helpful: https://my.clevelandclinic.org/health/treatments/17726-car-t-cell-therapy.

2. *Since CD133 is present on some normal stem cells, will the CAR T-cell therapy destroy my healthy cells along with the cancerous cells? Do I need to worry about this as a potential side effect?*
   To answer these questions, see "The Role of CD133 in Cancer: A Concise Review" by P.M. Glumac and A.M. LeBeau (2018) in *Clinical and Translational Medicine* 7(1), https://doi.org/10.1186/s40169-018-0198-1.

3. *Will I need to return for routine sessions like patients who get chemotherapy or radiation therapy do? Why or why not?*

After Dr. Vasudha answered these questions, Steven felt less worried and he was ready to undergo the treatment. Before he could have his own T-cells converted into CAR T-cells, Dr. Vasudha needed to properly design the structure of the chimeric antigen receptor that would end up on the surface of Steven's T cells so that the most effective immune response would be stimulated. Upon further research, she found that there are four different generations of CAR T-cells. Each one is created by transferring a gene containing the information allowing the T cells to produce CARs into the patient's T-cell genome. After insertion, these natural T-cells start producing the CAR protein encoded by the inserted gene, transforming them into CAR T-cells.

Questions

To answer the following questions, refer to "Engineering CAR-T Cells" by C. Zhang et al. (2017) in *Biomarker Research* 5, https://doi.org/10.1186/s40364-017-0102-y.

4. Dr. Vasudha determined that the fourth generation would be the most suitable CAR T-cell generation for Steven's treatment. This is because the fourth generation CARs include an IL-12 portion. IL-12 is a type of protein called a cytokine which is released when the immune system encounters something foreign to the body. It then regulates inflammation and acts as a signal in the immune system. The addition of the IL-12 portion in the CAR significantly enhances the immune response by the CAR T-cells.
   a. Recall the central dogma of molecular biology. What is the connection between a nucleic acid sequence found in DNA and the protein that it encodes?

   b. Different gene segments encode different domains found within a single protein. What segments would the gene that encodes the CAR contain, i.e., what regions would the gene be comprised of? The blank template below represents the gene that encodes the CAR. Label the different parts of the template with the different segments found in the fourth generation CAR gene. *Hint:* there are four different coding regions; see Zhang et al. (2017). Remember from Part II (Question 7) that T cell receptors have portions inside the cell, outside the cell, and embedded in the cell membrane. The labels found below the blank template correspond to the ultimate cellular location of the protein domains encoded by each gene segment. Two of the gene segments code for the intracellular domain, one for the transmembrane domain, and one for the extracellular domain.

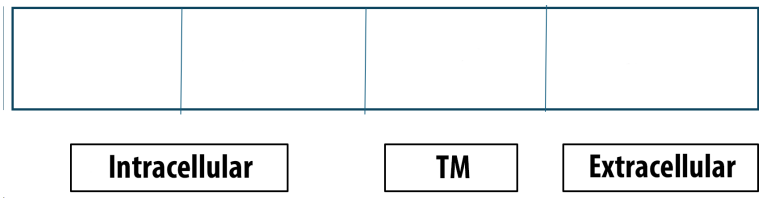

Figure 1. Blank template representing the gene that encodes the CAR.

   c. Briefly describe the function(s) of each CAR domain encoded by the four different gene segments.

5. The gene now needs to be transferred to the patient's original T-cells so it can insert itself into the donor DNA to be expressed. There are two types of transfer methods used to accomplish this. Compare and contrast the different types of transfers and determine which would be the best option.

6. After transcription and translation of the above-mentioned gene, what structure would the resulting CAR have? Draw a diagram of what the CAR protein would look like.

Part IV – Victory

A year after treatment, Steven's cancer was completely eradicated according to the regular screenings. Table 1 displays the reduction in tumor size with treatment progression.

Table 1. Steven's tumor size over time.

Time Point	*Number of tumors*	*Average size of tumor (at least one)*
Post 3 months	4	5.4 cm
Post 6 months	3	3.8 cm
Post 9 months	2	2.5 cm
1 year	0	0

For the first two years after treatment, he needed a PET scan every three months, and then every six months for the next three years before it could be officially declared that the cancer was unlikely to reappear. Dr. Vasudha was extremely happy with the results and assured him that these screenings were precautionary and that it was unlikely the cancer would resurface. However, Dr. Vasudha strongly recommended Steven adhere to his lifestyle changes and continue to abstain from drinking alcohol. Steven acknowledged the dangers of excessive alcohol consumption and agreed to commit to this healthier lifestyle choice.

Questions

1. Why does Dr. Vasudha think the cancer is not likely to come back? Would this be true for all forms of HCCs? Can you think of a situation in which the cancer might come back?

2. While CAR T-cell therapy yielded good results for Steven, some challenges still exist with this technique. Name a few of these challenges.