Creating New Tools to Fight an Old Foe: Cancer Immunotherapy with Alpaca Antibodies

Presented by: Dr. Craig M. Story, Gordon College
Talk Outline

• What is cancer?
  • Cancer myths

• What is cancer immunotherapy?
  • It’s a little complicated

• Some new tools for cancer immunotherapy.
  • The part having to do with alpaca antibodies.

• Cancer and the Christian faith.
Cancer – things to know

• Cancer is not new

• Term refers to non-benign growths. Benign tumor is not typically called “cancer.”

Cancer – things to know

• Most forms of cancer arise spontaneously (90-95%) while others result from inheriting a genetic predisposition (5-10%)

https://garlandandpendant.com/category/retinoblastoma/
Cancer – things to know

• Cancer gets worse over time. Decades usually.

From Vogelstein, 1990
Development of metastatic colon cancer takes years
Cancer – things to know

• Diet probably has little to do with cancer compared to other factors

  Alcohol
  Antioxidants
  Artificial Sweeteners
  Garlic
  Tea
  Vitamin D

“RCTs show no conclusive protection or causation”

#10: Prayer and Building Peace

A joyful heart is good medicine, but a broken spirit dries up the bones.
Proverbs 17:22
Cancer myths abound

• “Sharks don’t get cancer”

Contagious cancer found in clams and mussels

As bad as cancer is in humans, at least it’s not contagious. The same can’t be said for clams, mussels, and other marine bivalves. According to a new study, published online today in *Nature*, **these creatures can suffer from a form of cancer similar to leukemia that appears to be transmitted through the water** and can pass not only between members of one species, but even between two different ones. Genetic analyses
Malignant Transformation of *Hymenolepis nana* in a Human Host

Atis Muehlenbachs, M.D., Ph.D., Julu Bhatnagar, Ph.D., Carlos A. Agudelo, M.D., Alicia Hidron, M.D., Mark L. Eberhard, Ph.D., Blaine A. Mathison, B.S.M.(A.S.C.P), Michael A. Frace, Ph.D., Akira Ito, Ph.D., Maureen G. Metcalfe, M.S., Dominique C. Rollin, M.D., Govinda S. Visvesvara, Ph.D., Cau D. Pham, Ph.D., Tara L. Jones, Ph.D., Patricia W. Greer, M.T., Alejandro Vélez Hoyos, M.D., Peter D. Olson, Ph.D., Lucy R. Diazgranados, M.D., and Sherif R. Zaki, M.D., Ph.D.

Cancer is a genetic disease

Normal cell you are born with

Cancer cell that takes over your body
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Normal cell you are born with

Genetics or environment? BOTH
One mutation or several? SEVERAL
What genes? MANY
What Pathways? NOT AS MANY

Cancer cell that takes over your body
Strategies to combat cancer typically fail due to selection and genetic/epigenetic instability.

“New and improved” cancer is the result. The tumor is now resistant to the drug...
1970’s

“War on Cancer” declared

Result: failure

Lack of knowledge and tools

2010’s

“Breakthrough of the Year, 2013”

The other “C-word” is being uttered

Avalanche of knowledge and tools

Genetic knowledge, cheap DNA sequencing
(Single cell DNA sequencing)

Transgenic mice

Cancer-specific cell lines

Ability to “order a gene” online

New insights:

Why does chemo kill cancer cells?

And… Alpaca antibodies (of all things)
Video... then, how did it work?

This is the Emily Whitehead story... the result of Carl June and colleagues’ work using CAR-T cell therapy. U. Penn.

Link: https://www.youtube.com/watch?v=UE-E-gpUCJ0
RESULTS
A total of 30 children and adults received CTL019. Complete remission was achieved in 27 patients (90%), including 2 patients with blinatumomab-refractory disease and 15 who had undergone stem-cell transplantation. CTL019 cells proliferated in vivo and were detectable in the blood, bone marrow, and cerebrospinal fluid of patients who had a response.
Strategy for Whitehead’s blood cancer

Once chemo fails... there was not much that could be done. But now... Adoptive Cell Transfer

1. Remove patient’s T cells
2. Introduce a new a “man made” gene into these cells
   a. The new protein, a chimaera causes the T cell to “recognize” B-cells and kill them
   b. “Serial Killer” cells... one T cell kills many tumor cells throughout the body
3. Restore normal immune system once the cancer cells are gone. Or use antibody replacement to make up for the lack of antibody-producing cells.

Here, one is using the immune system itself to fight a specific tumor.
“Checkpoint Blockade”

Here, an antibody binds CTLA-4 and blocks the inhibitory signal molecule: the T cell is re-activated.
Summarizes the complexities of this, as well as the different approaches.

Cancer Vaccines
Provenge vs prostate feed antigens to dendritic cells so-so results

Adoptive T cell Therapy
CAR-T cells
good results on leukemia
bi-specific T-engagers

Antibody therapies
anti-CTLA4

Nature “Cancer immunotherapy comes of age”
Mellman, Coukos, Dranoff. Dec 2011
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Solid tumors present more challenges to all kinds of cancer therapy, including immunotherapy.
New Tools in Cancer Research
Conventional Antibody (mouse, human)

- two chains: Light, heavy
- binding site made up of 6 loops
- cloning requires cloning two separate genes
- not easy to produce in bacteria

Camelid Antibody (alpaca, camel)

- single chain (HC only)
- binding domain is one polypeptide large loop and additional S-S bond
- easier to clone and select
- can be expressed in bacteria
The Immune System is Regulated by a Series of Positive and Negative Signals

Checkpoint inhibitors—antibodies that block normal T cell inhibitory interactions—are effective

anti-CTLA-4

Robert et al. NEJM 2015

anti-PD1

Hodi et al. NEJM 2010
Cancer Immunotherapy—Future Challenges

• Monitoring/predicting anti-tumor immune responses: avoid side effects
  • Distinguish response from progression
  • match therapy to patient/tumor

• Combination therapies: improved efficacy
  • anti-CTLA-4/PD-1
  • Cytokines
  • Fusion proteins
The camelid antibody response

Traditional antibody
±150-170 kD

Heavy-chain only antibody
±100 kD

VHH
±12-15 kD
Single domain “nanobodies”

readily modified and expressed in bacteria

no need for glycosylation

folds independent of disulfide bond

deep tissue penetration

rapidly cleared after injection

no FcR or complement engagement

no cross-linking

Adapted from Wesolowski, 2009
Nanobodies are well tolerated in patients

anti-vWF to block aggregation with platelets was effective and did not generate a significant anti-nanobody response
Generation of nanobodies against PD-L1

Multiplexed immunization → Isolation of lymphocytes → Amplification of VHH sequences and subclone into phagemid vector → Generation of phage library → Phage display → Expression of specific VHH in bacteria

Alpaca blood donation
$^{18}\text{F-B3 immunoPET}$

anti-PD-L1 nanobody (B3) concentrates in the tumor microenvironment.

WT B3 + B16 tumor
Nanobodies are readily modified as fusion proteins
Benefits of B3-IL2 therapy extends to a mouse pancreatic cancer model

Stephanie Dougan, DFCI
Future Directions

• Other cytokine fusions
  • IFN\(\gamma\) and GM-CSF (undergoing testing now)
  • IL-15, IL-10, and enhanced versions of IL-2

• Combination reagents using additional checkpoint inhibitors
  • There is a growing list of candidates
  • Combinations of these may have synergistic effects
Faith Aspects

What about death?

Death before the Fall? → Re-examination of how to interpret “The Fall”
Faith Aspects

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Death before the Fall?  →  Re-examination of how to interpret “The Fall”

Yet... Jesus wept at the premature loss of his close friend, Lazarus
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- conceptually, knowing “how cells work” it seems unlikely that cells, in principle, can avoid cancer
- I suggest that cancer is a necessary by-product of cell biology
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Physical death (on a large scale) is necessary for evolution to happen
The Hope (which I cannot explain)

• SOMEHOW God will overcome death
• Plenty of scripture and biblical teaching, quotes from Christ seem to indicate we will “Be with him.” Resurrection is “a thing.”
• I don’t worry too much about trying to explain this logically.
• To me, the person of Jesus outweighs these doubts. I believe he (God) does provide real strength and power for living right now.

These last slides are important. Other kinds of questions answered by faith are still important questions.

But remember they are not scientific questions.

No amount of science will answer them, yet the science does raise questions that may cause us to re-examine our theology from time to time. This is a healthy thing.
For further reading

Sid Mukherjee’s book: “The Emperor of All Maladies: A Biography of Cancer”

For those that want to read a review of cancer with more scientific detail:

Hanahan and Weinberg and Hallmarks of Cancer: The Next Generation (Cell, March 4, 2011)
Why does traditional chemo kill cancer cells?
    some fast-dividing tumors are chemo-resistant
    some slow-dividing tumors are chemo-sensitive.
    (Therefore, the reason is not only about killing fast-dividing cells)

What is it?
    All cells are ”set” at a certain distance from going over the “cliff” of PCD
    Embryonic-like cells, as well as blood-precursor cells are close to the cliff 
    require a little abuse and “poof” they undergo PCD  
    this sensitivity to PCD of blood cells sets the dosing limit for chemo
    Tumors that are farther from the cliff are more resistant
    The distance from the cliff can be directly measured by titrating in peptides