Bispecific T-Cell Engaging Antibodies for Cancer Therapy
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1. Briefly describe the protein structure of a BiTE antibody? What two antigens does it recognize?

   The BiTE antibody can be described as two scFv fragments that have been linked together with an additional linker, besides the one used to link the $V_L$ and $V_H$ domains in scFVs.

   One antigen is CD3, which are the signaling chains on the TCR.

   The other antigen is found on tumor cells.

2. Describe how you might construct a gene that would express a BiTE antibody. What genetic material would you have to begin with, and how would you manipulate that material?

   You would have to isolate either the cDNA or genomic for both chains of an anti CD3 antibody and the cDNA or genomic DNA for the antibody against the tumor antigen. Either source of DNA is fine since we are only interested in the V exons, each of which codes for a single domain. You would then have to link up the domains in this way: $V_L$ - Gly-Ser linker - $V_H$ - Gly-Ser linker - $V_L$ - Gly-Ser linker - $V_H$ where the first to domain could be from the anti CD3 antibody and the next two V segments from the tumor antigen antibody.

3. Although T$_{CTL}$ may be effective at killing cancer cells early on in the disease, what escape mechanisms ultimately allow the tumor to avoid killing by T-cells.

   Change of tumor antigens presented on class I MHC.

   Suppression of T$_{CTL}$ response

   Defect in antigen presentation

   **Note that there is positive selection for cells which escape surveillance by T$_{CTL}$.**

4. In what way do BiTE antibodies circumvent one viral escape mechanisms.

   They do not rely on antigen presentation of viral antigens on MHC I.

5. Which protein is required at a lower dose for effective treatment, BiTE antibodies or conventional IgG?

   Steady state levels of BiTE Ab in the serum are 1ng/ml, 1000 fold lower than 10 μg/ml required for antibodies.

6. Why is it necessary to infuse the BiTE antibodies into the patient?

   The half-life of the BiTE antibody is very short, compared to intact IgG.

7. Why do BiTE antibodies only appear to stimulate memory T$_C$ cells and not naive T$_C$ cells.

   Naïve cells require co-stimulation by the binding of b7 to CD28 on the T$_C$ cell.

   Memory cells do not. The BiTE Ab does not engage CD28 so naïve cells remain unstimulated. In addition, the high density of clusters of antigen-BiTE-CD3 complexes may also drive stimulation.

8. Why do BiTE antibodies only activate T$_C$ cells in close proximity/contact to their target, as opposed to all memory T$_{C}$ cells, even though all T$_{C}$ cells would have CD3.

   The clustering of antigen-BiTE-CD3 complexes is required to activate the memory T cells. This clustering would only occur as a result of a cell-to-cell interaction, i.e. the BiTE antigens on the target cell would induce clustering of the CD3 on the Tc cell.

9. Why would BiTE antibodies be more effective at fighting solid tumors than treatment with IgG?

   They can penetrate tumors more easily than the larger IgGs.
10. When BiTE antibodies were first envisioned, it was thought to be better to link together two Fab fragments, one recognizing CD3 and the other the tumor antigen.
   i) The Fab-based BiTE antibodies are generally more stable than the Fv based ones. Why?
   ii) The origin of the anti-CD3 and anti-tumor antigen antibodies that were used to generate the BiTE-Fab and BiTE-Fv molecules were murine (mouse) hybridomas. Both the bi-specific Fab and Fv molecules, caused some patients to develop antibodies against these proteins. How could you modify the protein to reduce this side-effect?

   i) Additional stability would be provided by the interaction between the constant domains on the light and heavy (CH1) chains.
   ii) First attempts were to make chimeric antibodies, where the mouse V-domains were fused (using recombinant DNA) to human constant regions. Later, just the hypervariable regions from the mouse antibody were “transplanted” into the human V-domains. Most recently, there are ways to generate human hybridomas, which avoid the problem entirely.