Novel Bispecific Antibody Construct for Pretargeted Radioimmunotherapy

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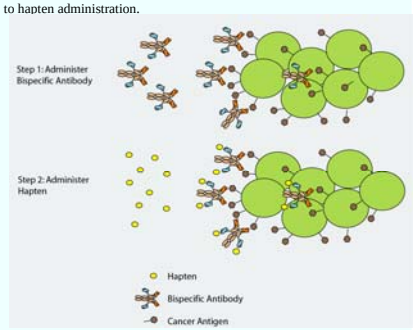
Background

Pretargeted radioimmunotherapy decouples the pharmacokinetics of antibody delivery and radionuclide delivery. In pretargeting, the antibody is a bispecific molecule with specificity for both a cancer antigen and a radioactive hapten. The bispecific molecule is introduced first and accumulates at the site of cancerous cells. Subsequently, a small, quickly cleared radionuclide is administered and the interaction between the antibody and radionuclide allows the antibody to secure the radioactive molecule at the site of the cancer.

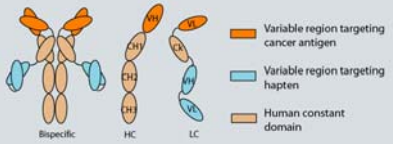
In pretargeted radioimmunotherapy, one goal is to maximize the amount of bispecific antibody localized to the site of the tumor prior to hapten administration.



Bispecific Antibody Construct Design Criteria

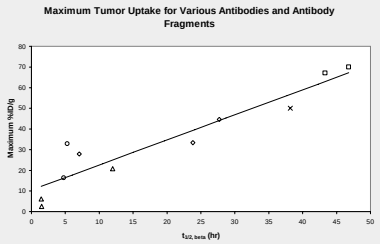
Requirements and ensuing design parameters

1) Slow blood clearance for increased tumor uptake
   - IgG-like design with Fc domain
   - scFv attached to C terminus of the light chain as opposed to the C terminus of the heavy chain where it may interfere with Fc receptor binding
2) Minimal immunogenicity
   - Human Fc and human heavy and light constant domains
3) Stability
   - scFv attached to the C terminus of the light chain with a $(Gly_4Ser)_2$ linker to minimize steric interference
4) Simultaneous binding to both cancer antigen and hapten
   - scFv attached to C terminus of light chain instead of N terminus
5) Modular design to easily synthesize bispecific constructs with different specificities
   - Recombinant platform with strategic restriction sites to easily cut and paste scFvs that bind other cancer antigens and haptens



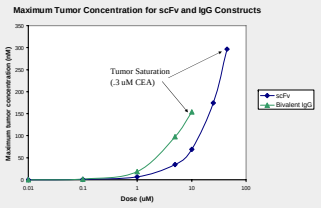
Slower Antibody Clearance Results in Higher Tumor Uptake

Experimental Data from Literature



Maximum reported tumor uptake (%ID/g) for various antibodies and antibody fragments with varying blood clearance. Data is from published xenograft mouse studies: diamonds - Kenanova et al.[1], circles – Hu et al.[2], squares – Williams et al.[3], triangles – Milenic et al.[4], x – Lee et al.[5].

Mathematical Model Data



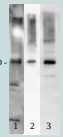
	Diffusion Rate (m^2/s)	Void Fraction	Capillary Permeability (m/s)	$t_{1/2,beta}$ (hr)
scFv	$80x10^{-12}$	.3	$5x10^{-9}$	5.32
IgG	$14x10^{-12}$	.1	$3x10^{-9}$	86.92

A cylindrical tumor model[6] was used to simulate maximum tumor uptake for IgGs and scFvs for various initial doses and human parameters. Even though scFvs demonstrate increased diffusion, void fraction, and capillary permeability, IgGs exhibit a 2-3 fold increase in tumor uptake due to its significantly slower blood clearance. scFv and IgG parameters are given in the above table. All other parameters were held constant during the simulations.

Model Construct Secreted from Yeast

A model construct of the bispecific antibody design was synthesized using the high affinity CEA binding scFv Sm3e[7] and an scFv designed from the DOTA binding antibody 2D12.5[8]. This bispecific antibody, coined Sm2D, was secreted in *Saccharomyces cerevisiae* from two plasmids, one containing the sequence for the light chain and the other the sequence for the heavy chain.

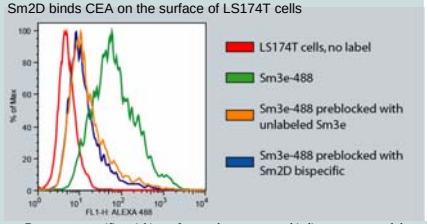
Western blot analysis confirms the secretion of both heavy and light chains



Western blot analysis of yeast supernatant under reducing SDS-PAGE conditions, detecting heavy chain (lane 1) and light chain N terminal FLAG epitope (lane 2) and C terminal cmyc epitope (lane 3).

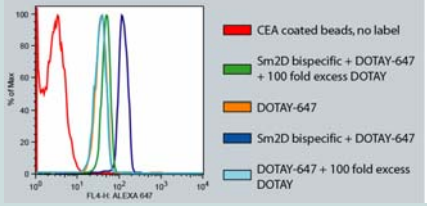
Flow cytometry analysis confirms the secretion of functional protein

Sm2D binds CEA on the surface of LS174T cells



Due to nonspecific sticking of secondary reagents, binding was assayed by competition. Cells were labeled with CEA binding Alexa-488-Sm3e with and without preblocking with Sm2D and unlabeled Sm3e as a control.

Sm2D binds CEA and DOTA simultaneously



Due to nonspecific sticking of secondary reagents on cells, CEA coated magnetic Dynal beads (Invitrogen) were used. CEA beads were labeled with Sm2D followed by Alexa-647-DOTA-Y with and without an excess of unlabeled DOTA-Y to show specificity of DOTA binding.

Conclusions and Future Work

A novel, tetravalent IgG-like bispecific antibody construct with specificity for carcinoembyonic antigen and the metal chelate DOTA was designed and synthesized. The bispecific antibody was secreted from *Saccharomyces cerevisiae* and the product retains binding specificity to both target antigens.

Future work includes secreting the bispecific antibody from mammalian cells to eliminate any yeast mannosylation of the protein (mannose residues may alter the pharmacokinetics of the antibody). After purification, the *in vivo* pharmacokinetics, biodistribution, and stability of the bispecific antibody will be studied.

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