

Antibody-Based Therapy and Detection of Solid Tumors

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Monoclonal antibodies (MAbs) are becoming commonly used for the detection and treatment of cancer. The FDA has licensed unconjugated MAbs targeting growth factor receptors, such as EGFR and HER2, which are overexpressed in many solid malignancies, and the CD31 antigen, which is ubiquitous in non-Hodgkin's lymphoma. Immunoconjugates consisting of MAbs and radioisotopes, drugs or toxins have also been licensed or are in advanced clinical trials. However, antibody-conjugates that are based on intact MAbs can be limited by the physical characteristics of the MAb. Immunoglobulin molecules have evolved to recognize and clear foreign pathogens and as such, exhibit prolonged retention in circulation. This can lead to unacceptable bone marrow exposures when radioisotopes are conjugated to the MAb. The large size



of the intact MAbs also limits their ability to diffuse from vasculature into tumor. A major focus of our research is to manipulate the structure of MAbs in order to optimize their targeting properties for desired applications. For radioimmunotherapy and PET imaging, we are creating small, engineered antibody fragments such as diabodies and pairing them with radioisotopes with compatible properties. Diabodies are small, 52 kDa noncovalent single-chain Fv dimers composed of the variable (antigen-binding) domains of antibodies. Our lead molecule, the C6.5 diabody, exhibits excellent tumor penetration properties, rapid systemic clearance and highly specific tumor targeting. We have found that this molecule can be effectively used to target alpha and beta particle emitting radioisotopes for tumor therapy and positron-emitting radioisotopes for tumor detection. We have also focused on generating antibodies that directly affect the growth of tumor cells by altering signal transduction through the members of the Epidermal Growth Factor Receptor Family (e.g., with bispecific antibodies) or mimicking the Müllerian Inhibiting Substance – a ligand that can trigger the death of ovarian cancer cells.

Radioimmunotherapy. Adams, Robinson, Shaller, in collaboration with Brechbiel,^b Waldmann,^b Marks,^c Baklanov,^d Marchenkov^e

While MAbs have been identified that are capable of directly inhibiting the growth of tumors, the combination of antibodies and radiation is often quite effective. The highly specific targeting that MAbs are capable of provides an effective platform for the delivery of conjugated radioisotopes to tumors. We are working with collaborators at the National Cancer

Institute and the Kurchatov Institute to develop and test strategies to target alpha-emitting radioisotopes to solid tumors. We have found that 52 kDa diabody molecules can be effectively used to target Astatine-211, a radioisotope with a 7-hour half-life, to HER2/neu (c-erbB-2) overexpressing tumors. Due to the diabody's small size, it falls below the threshold for first pass renal elimination (~65 kDa), facilitating its rapid clearance via the kidneys. This leads to faster elimination

from the circulation and reduced myelotoxicity. A second approach under development is known as pretargeted radioimmunotherapy. This method utilizes bispecific single-chain Fv (bs-scFv) molecules that target both a tumor antigen and a radiolabeled peptide ligand. Unlabeled bs-scFv is administered and allowed to localize in the tumor. After 24 hours the majority of the bs-scFv has cleared from the circulation and radiolabeled peptide ligand is administered that is only retained where the bs-scFv has pre-localized. This strategy is expected to be most effective when used with short-lived radioisotopes like Bismuth-213. By using human scFv phage display libraries as the source of these antibody-based molecules, they are unlikely to elicit an immune response when ultimately administered to patients.

Antibody heterodimers. Adams, Hodge, Robinson, Simmons, Yuan, in collaboration with Marks^c

The antibodies that have proven to be most effective in cancer therapy are typically those that perturb signal transduction. Signal transduction in the Epidermal Growth Factor Receptor (EGFR) family (EGFR, HER2, HER3, HER4) involves ligand binding and subsequent heterodimerization of two members. We hypothesized that an antibody-based strategy that interferes with heterodimerization of EGFR family members could block mitogenic signaling and inhibit tumor growth. To test this theory, we have created bispecific single-chain Fv (bs-scFv) molecules that bind to pairs of members of the EGFR family. The extracellular domains of all four EGFR family members were expressed and scFv clones that specifically bind to each were isolated from a naive human scFv phage display library. These scFv were used to create bs-scFv fusion proteins that bind to HER2 and HER3 or HER2 and HER4. The lead molecules have been found to alter signal transduction through the PI-3 kinase/AKT pathway that is triggered by the binding of heregulin. As the bs-scFv are capable of specifically targeting human tumor xenografts overexpressing these pairs of antigens that are growing in immunodeficient mice, it is expected that they may elicit a similar effect *in vivo*. These results suggest that bs-scFv targeting EGFR family members may be effective for the detection and treatment of cancer.

Developing anti-MISIIR immunotherapeutic agents for ovarian cancer. Adams, Simmons, Yuan, in collaboration with Weiner,[§] Marasco^f

The Müllerian Inhibiting Substance Type II Receptor (MISIIR) plays a critical role in the regression of the Müllerian ducts, the anlagen of the uterus, fallopian tubes and vagina in males during fetal development (1). MISIIR has been found to be expressed on the surface of human ovarian cancer cells. The observation that cells apoptose in response to treatment with the Müllerian inhibiting substance (MIS) ligand has led to the proposed development of MIS as a therapeutic agent for the treatment of ovarian cancer. We postulated that antibody-based molecules directed against the ligand-binding site on MISIIR could exhibit a similar cytotoxic effect with a significantly prolonged bioavailability. Additionally, anti-MISIIR antibodies could be used as vehicles to selectively target agents to ovarian tumors.

We have expressed recombinant MISIIR extracellular domain (ECD) and have used it as a target to isolate (pan) anti-MISIIR human scFv molecules from a large non-immune phage display library. The resulting anti-MISIIR antibodies have been found to bind specifically to cells that express MISIIR and are currently being developed as vehicles to deliver cytotoxic agents to cancer cells. We are also continuing to attempt to identify agonistic anti-MISIIR antibodies that mimic the activity of the MIS ligand.

Developing novel imaging agents for immuno-PET-based detection of cancer. Adams, Robinson, Shaller, Doss, in collaboration with Weiner,[§] Gonzalez-Trotter,^g Brogan^g

Antibody fragments exhibit very rapid tumor targeting and systemic elimination. These properties make them ideal vehicles for the delivery of imaging radioisotopes to tumors. We have a number of imaging projects at various stages in development including using both directly conjugated antibody fragments and pretargeted antibody-based constructs. We are currently initiating the process to produce clinical-grade C6.5 diabody for use in an antibody-based positron emission tomography (PET) [Immuno PET] clinical trial. It is our expectation that this approach may be useful for the detection and staging of tumors as well as determining the response of disease to treatment.

Developing antibody-nanoparticle conjugates for the detection and treatment of cancer.

Adams, Shaller, Smith, in collaboration with Luzzi^h

One of the limitations of radioimmunotherapy using radioisotopes directly conjugating to antibodies is that each antibody can only carry at most a few radioactive atoms. To address this, we have developed radioactive nanoparticles

that are 2–5 nm in diameter and each contain 100–500 radioactive rhenium isotopes. Targeted radioactive nanoparticles can each deliver multiple hits to a targeted tumor, significantly increasing the efficiency and efficacy of RAIT. These radioactive nanoparticles will be conjugated to anti-tumor scFv and peptide molecules to confer tumor-targeting specificity.

Publications

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