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Innovations

ImmunoGen Inc.

Immunoconjugates in Cancer Therapy: If at First You Don't Succeed, Try, Try Again

Monoclonal antibodies, or any class of immunoglobulin in the body, are not designed to kill cells. Cytotoxic "killer" T cells play that role. However, as a tool to fight cancer, monoclonal antibodies have been widely exploited to target tumor cells. Dr. Walter Blättler, Executive Vice President of ImmunoGen Inc., points out, "Since an antibody is not very well equipped to kill aberrant cells, one can give it a mechanism to kill." By conjugating monoclonal antibodies to any of a variety of cytotoxic drugs, tumor cells can be specifically targeted for destruction, thereby circumventing the indiscriminate destruction of healthy cells and tissues.

A directed targeting approach to cancer therapy has been around for over 20 years, and research has steadily progressed despite many obstacles. Since its inception in 1981, ImmunoGen Inc. has focused on achieving more effective and better-tolerated therapies for the treatment of cancer. The company's trials, tribulations, and successes over the last 22 years make for a good story of scientific discovery, fortitude, and resolve.

In the early 1980s, ImmunoGen worked with the chemotherapy agent doxorubicin crosslinked to monoclonal antibodies specific for cancer cells. Doxorubicin binds to DNA and inhibits the essential enzyme topoisomerase II, resulting in a block in DNA replication and ultimately cell death. Within one year of intensive analysis, ImmunoGen determined that doxorubicin was not sufficiently potent when linked to monoclonal antibodies to reach and effectively destroy a tumor. This project was subsequently abandoned; however, other companies have pursued doxorubicin, which has been widely used as a chemotherapy drug and is most commonly known by the brand name Adriamycin. Adriamycin destroys dividing cells and thus has some effect on cancer cells; however, it also has a widespread and indiscriminate effect on the patient's entire body. The side effects of this drug are drastic and include nausea, vomiting, and exudative gastritis (inflammation of the stomach lining).

Since doxorubicin proved to be an unattractive drug candidate, ImmunoGen shifted its focus from chemical anticancer agents to the more potent ribosome-inactivating protein toxins. Ribosome-inactivating proteins (RIPs) are N-glycosidases, which catalytically and irreversibly inactivate eukaryotic ribosomes. These proteins can be extremely cytotoxic: one molecule can be enough to kill a cell. RIPs therefore have great potential for the treatment of cancer. There are two kinds of toxic RIPs, type I and type II. Type I RIPs, such as gelonin, consist of one catalytically active "A" peptide chain, whereas type II toxins, such as ricin, the main toxic protein in the castor bean plant *Ricinus communis*, are composed of an "A" peptide and a distinct "B" chain. It was known that the A peptide contained the catalytic moiety, while the B chain encoded the binding site for galactose residues which are abundant at cell surfaces. To avoid the indiscriminate cell surface binding of the ricin B chain, ImmunoGen designed an antibody conjugated to the catalytic ricin A chain alone. Unfortunately, this conjugate did not kill cells efficiently. Consequently, it was discovered that both the cell surface binding domain and the machinery to introduce the A chain into the cell were both encoded in the ricin B chain, leading ImmunoGen's researchers to design a variant of ricin (bR). bR contains intact A and B ricin chains; however, through covalent modification of the B chain, the nonspecific cell surface binding site is abrogated. bR therefore allows for transport of the highly cytotoxic ricin A chain into selected cells (the selectivity is determined by the associated antibody).

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"From a scientific point of view, the ideal cell marker would be expressed only on tumor tissues but not on other tissues. But that does not exist. Instead, there are markers expressed (at high levels) in tumor tissues and at much lower levels in normal tissues or (at higher levels) on tissues which are not so important," explains Blättler. ImmunoGen conjugated bR to a number of cancer cell marker antibodies: anti-CD6 (chronic B-lymphocytic leukemias), anti-CD33 (acute myeloid leukemia [AML] cells), anti-CD38 (acute lymphocytic leukemia [ALL] and AML), anti-N901 (small cell lung carcinoma [SCLC]). and anti-B4 (B lineage leukemia, B cell non-Hodgkin's lymphoma and AIDS-related non-Hodgkin's lymphoma [ARL]). The various antibody blocked-ricin immunoconjugates had varying levels of efficacy, with anti-B4-bR reaching phase III clinical trials. In preclinical studies, the bR conjugates were administered in immune-compromised animals and patients. Under these suppressive conditions, the bR conjugates proved to be efficient agents for relatively specific destruction of cancer cells. However, in the majority of cancer patients who are not immunosuppressed, the ricin protein conjugated to any antibody was highly immunogenic. Hence, ImmunoGen's pursuit of an anticancer conjugate linked to ricin ended.

In parallel with the ricin conjugate program, ImmunoGen was generating type I RIP conjugates, primarily using gelonin and pokeweed antiviral protein (PAP). However, these

plant toxin immunoconjugates were not effective cell killers, since the conjugated antibody could not efficiently transport the toxin into cells, and the gelonin and PAP toxins could not access the cancer cells that they were intended to destroy.

In the early 1990s, a crucial observation was made before abandoning the unsuccessful antibody-toxin conjugate program. Researchers reported that in humans the immunoconjugates induced a severe immune response directed against the protein toxin and the mouse monoclonal antibody. The latter type is known as a human anti-mouse antibody (HAMA) response. As a means of clearing this obstacle, ImmunoGen began to develop means to "humanize" monoclonal antibodies. However, ImmunoGen was not alone in its quest to engineer monoclonal antibodies with crucial human-like traits. Several laboratories recognized the clinical limitations of murine antibodies, and consequently there are now several different strategies for generating human or human-like antibodies.

An early approach for antibody humanization focused on reducing the immunogenicity of murine IgGs by incorporating key residues characteristic of human antibodies, resulting in approximately 75% human chimeric antibodies. When tested in immunosuppressed patients, the chimeric antibodies had limited immunogenicity but were still highly immunogenic in patients with normal immune systems.

A more advanced tactic was explored to transfer the antigen specificity and binding avidity of murine monoclonal antibodies to a human antibody: the murine complementarity-determining regions or CDRs (the antibody domain that binds the antigen) were grafted onto a human variable region framework. However, CDR grafting can require additional amino acid changes within the human framework to preserve proper binding affinities, and such changes in the human variable region can produce troublesome antigenic epitopes.

ImmunoGen learned from these mistakes and embraced a novel knowledge-based approach termed variable domain resurfacing that uses computer modeling to identify surface residues within the variable region of the murine antibody. Surface residues are replaced with amino acids from the corresponding location in the human antibody variable region. If the substitution results in reduced binding affinity, the residues are "back-mutated" to the murine state to reinstate the high-affinity binding associated with the original murine antibody. High-affinity humanized murine variable regions are then joined with constant regions from human antibodies to generate stable hybrids. The premise behind a "resurfacing" approach stems from the origin of immunogenicity, which is directly correlated with the nature of surface residues of proteins. In fact, surfaces carry most if not all of the antigenic potential of the protein.

Antibody resurfacing had been established for several years by the time the protein toxin conjugate studies were finally ended. With their pipeline of potential drugs wiped out and the humanized antibody in hand, ImmunoGen regrouped and pushed forward. From previous studies on cytotoxic "nonprotein" compounds, ImmunoGen reevaluated the molecules reported to have the highest cytotoxicity. Maytansine, an antimicrotubule agent, was at the top of the potency list. The anticancer properties of Maytansine had been investigated by the National Cancer Institute in the 1970s, and during testing in phase I and II clinical trials maytansine treatment resulted in complete and partial regression in a few patients with different forms of cancer. However, its severe toxic effects precluded further study at that time.

ImmunoGen believed the extremely potent properties of maytansine to be ideal for creating tumor-specific antibody-drug conjugates. To this end, four molecules of a chemical derivative of maytansine, DM1, which is 3- to 10-fold more potent than maytansine, were conjugated to the humanized monoclonal antibody huC242. Anti-huC242 binds specifically to a glycoform of the Muc1 carbohydrate that is highly expressed in most pancreatic, biliary, and colorectal cancers as well as in a large proportion of nonsmall cell lung (40%) gastric (55%), uterine (45%), and bladder (40%) cancers. Fortunately, Muc1 is minimally expressed in normal tissues and cells. In addition to the low global toxicity of the conjugate, an advantage of the structure is the free sulfhydryl group of DM1 that enabled stable disulfide linkage to the huC242 antibody. The disulfide linkage stabilizes DM1 in the bloodstream, rendering it inactive. But upon recognition of Muc1 by the huC242 antibody, the entire complex is internalized and DM1 is released in the cell by proteolytic cleavage of the DM1-huC242 disulfide bond.

ImmunoGen named their antibody-drug conjugates tumor-activated prodrugs (TAPs) since the conjugates were not toxic to cells lacking the target antigen. The DM1-Muc1 conjugate, referred to as anti-CanAg-DM1 TAP, is presently undergoing phase II clinical trials, and trials have also begun with other DM1 conjugates. BB-10901 TAP (huN901-DM1) targets CD56, which is expressed on small cell lung carcinoma (SCLC) and tumors of neuroendocrine origin. There are also DM1 TAP conjugates targeting prostate-specific membrane antigen (PSMA) for the treatment of prostate cancer and CD44v6-expressing solid tumors. An anti-CD33-DM1 conjugate that targets AML cells is currently in preclinical development.

With their pipeline full and flowing, it's no wonder that ImmunoGen recently made a deal with Aventis. Aventis, France's largest drug maker, will pay ImmunoGen $12 million upfront and $50 million to fund research over the next three years, allowing ImmunoGen to continue and expand its research. However, the company cannot rest on its laurels just yet; one cannot truly predict the final efficacy of these prodrugs prior to phase III trials. As Blättler points out, "These diseases are very heterogenous. In AML, there isn't just one tumor lesion, there are many. One never knows exactly why one drug will be effective." It seems that ImmunoGen's history of dedicated cancer research will help them clear further hurdles on their journey toward effective cancer therapies.

***Chemistry & Biology* invites your comments on this topic. Please write to the editors at chembiol@cell.com.**

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