# 17 Immunoconjugates

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## 1. INTRODUCTION

Successful anticancer drugs must exploit known or unknown, gross or ever so subtle, differences between normal and malignant cells. The development of immunotoxins is one of the first attempts to develop rationally anticancer drugs that are based on known cellular differences associated with cancer cells. Much immunological evidence had accumulated that transformed cells express tumor-specific antigens. However, it was difficult to generate heterosera with well-defined antitumor reactivity. The isolation in 1967 of an agglutinin from wheat germ that identified a tumor-specific determinant on neoplastic cell surfaces (*1*) marked the first time that a pure molecular species was available for targeting of tumors.

Further probing of cell surfaces with lectins and agglutinins, however, was hampered by the availability of only a small number of lectins with an even smaller number of different binding specificities. This situation changed dramatically with the advent of the monoclonal antibody (MAb) technology (*2*). The potential for generating a nearly unlimited reservoir of reagents each with its own binding specificity for an antigen was rapidly exploited in creating MAbs that bound to novel tumor cell-specific antigens. Although some naked antibodies were used in clinical tests for the treatment of cancer, many immunologists doubted that the humoral part of the immune system would have sufficient cytotoxic potential to eliminate millions of tumor cells. MAb were, therefore, armed with extraneous cytotoxic effector functions and became delivery vehicles that imparted tumor specificity to otherwise nonselective cytotoxic effector molecules.

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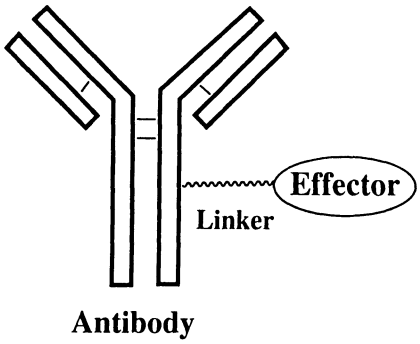


| Effector | Conjugate |
|---|---|
| Radioisotope | Radioimmunoconjugate |
| Toxin | Immunotoxin |
| Cytotoxic Drug | Antibody-Drug Conjugate |

**Fig. 1.** Schematic representation of immunoconjugates.

The covalent binding of an effector molecule to an MAb yields an immunoconjugate (Fig. 1), which is called an immunotoxin, when the effector molecule is a toxin, an antibody–drug conjugate when cytotoxic drugs are used as effectors, and a radioimmunoconjugate in the case of linked radioisotopes. Common to all three methodologies is their reliance on the tumor-specific binding of their MAb component. Therefore, we shall first discuss the generation of "tumor-specific" MAbs and then describe the development and testing of radioimmunopharmaceuticals, of immunotoxins, and of antibody–drug conjugates.

## 2. TUMOR-SPECIFIC ANTIBODIES

The ideal MAb for the generation of immunoconjugates would bind to an antigen exclusively present on the surface of tumor cells, and would further be expressed homogeneously on all tumor cells or at least on all tumor stem cells (the latter, however, is difficult to assay). In addition, the antigen should not be shed from cells, should not be present in the serum of patients, and ideally, for practical medical and commercial reasons, should be present on the tumors of all patients with the same type of cancer.

In the infancy of immunotoxin development, several MAb were claimed to be tumor-specific. However, the development and use of more thorough analytical methods, such as analysis with a fluorescence activated cell sorter (FACS), sensitive immunohistochemical staining techniques using large panels of fresh-frozen tissue sections, and modern biochemical and molecular biological techniques, contributed to today's generally accepted view that most antibodies recognize tumor-associated antigens that are expressed only preferentially on tumors. Some antigens may be found on only a limited number of tissues, whereas others are on only one specific tissue type and are, therefore, tissue-specific. In the best case, some tumor-associated antigens may be expressed only during a particular developmental stage of a certain cell type. Some degree of tumor specificity often presents itself by the overexpression of certain surface antigens on transformed cells, such as *erb*B-2/*HER*-2 on breast tumor cells of a subgroup of patients (*3*), or certain carbohydrate antigens on epidermoid carcinomas (*4*). The only surface antigens that are absolutely tumor-specific are the surface immunoglobulin or idiotype present on the cells of B-cell leukemia and lymphomas, and the clonotypic T-cell receptor on T-cell leukemia and lymphoma cells. Not only are these structures tumor-specific, but individualized, patient-specific

To generate MAb with antihuman tumor reactivity, typically mice or rats were immunized with whole cells or cell membrane preparations from tumor cell lines or from tumor biopsies. The spleens of the immunized animals were then used to generate and select antibody-producing hybridomas. However, many MAbs used in immunoconjugates, in particular, antibodies reactive with hematopoietic cells, were originally developed as research tools to differentiate between various normal cell types and were, therefore, generated by injecting animals with normal human cells, such as the various cell types from blood.

MAb that have the potential to be used in anticancer immunoconjugates are conveniently grouped into those that react with hematopoietic tumors and those that bind to antigens on solid tumors. Because of the rapid renewal of hematopoietic cells and the experience of regeneration of blood cells after bone marrow transplantation, tissue-specific antibodies were widely used in immunoconjugates against leukemias and lymphomas. T-cell malignancies were treated, for example, with conjugates binding to the T-cell markers CD5, CD7, or the IL-2 receptor $\beta$-chain (CD 25); B-cell malignancies with antibody conjugates against the B-cell differentiation antigens CD19, CD20, and CD22; and analogously, myeloid malignancies with conjugates against the myeloid marker CD33 (*6*). Most of these antigens are differentiation antigens that are expressed throughout the ontogeny of a particular cell type starting at the earliest lineage restricted stage to ensure that the conjugates were able to treat the yet unidentified clonogenic tumor cells.

It has been much more difficult to identify cell-surface markers useful for immunoconjugates against solid tumors. The principle of tissue specificity is not as easily applied as in the hematopoietic area, except possibly for tumors of nonessential tissues, where the temporary removal of certain cell populations may be tolerated. In the absence of tumor specificity and tissue specificity, the selection of antigens was largely based on their overexpression on tumor cells relative to normal tissues. For lists of possible candidate surface antigens for immunoconjugate targeting, the reader is referred to two comprehensive reviews (*7,8*).

For the development of highly cytotoxic immunoconjugates that bind to antigens also expressed on some normal tissues, although hopefully at lower levels, it was essential to find animal models for toxicity studies, where similar crossreactivity was observed. Fortunately, many of the antigenic determinants were found to be preserved in nonhuman primates where they were expressed with a similar tissue distribution as in humans. A good example is the data presented for the anti-Le$^y$ antibody in ref. (*4*).

A problem commonly encountered in solid tumors is the heterogeneous expression of an antigen on cells of a given tumor. Although some cells may express large numbers of an antigen on their surface, other cells in the same biopsy sample, equally having a transformed phenotype, may be antigen-negative. If transformation is a clonogenic event, then these different cell populations may represent differentiation stages that are not necessarily all tumorigenic. Heterogeneous expression of an antigen may, therefore, not necessarily disqualify it from being a target for therapeutic immunoconjugates.

If one surveys the known antigenic cell-surface markers for human solid tumors, (*see*, e.g., *7,8*), one is struck by the paucity of such known markers. Also, when antibodies were generated with different tumor tissues or tumor cell lines, often antibodies to the same antigens were generated. For example, when mice were immunized with the breast tumor line MCF-7, MAb B1 and B3 were obtained that reacted with the

metastatic breast adenocarcinoma yielded antibodies BR64 and BR96, both of which also react with the $Le^y$ carbohydrate chain (*9*). These results are a reflection of the limitations of the immunological methodology used to identify these antigens. They probably represent the most immunodominant markers recognized by the murine immune system, and only the screening of much larger panels of hybridomas, a work-intensive and time-consuming undertaking, might allow the discovery of further novel antigens with this technology. This realization, far from being discouraging, predicts that we have barely scratched the surface for the discovery of tumor cell-surface markers for therapeutic targeting, and it has spawned the development of several new methodologies. The most promising techniques might be the phage display of the entire murine or human immunological repertoire and its use in the probing of cell surfaces (*10*), or the searching for interactions on cell surfaces with combinatorial libraries of peptides that carry their genetic information in the form of amplifiable DNA sequences (*11*).

In most patients treated with murine MAb, a prompt human antimurine antibody (HAMA) response was observed, which led to the development of several "humanization" technologies. Humanization is the attempt to give murine antibodies an appearance that is not recognized as foreign by the human immune system while preserving their specificity and binding avidity.

It was well known that heterosera against xenogeneic immunoglobulins largely reacted with the constant region or Fc portion of the molecule, and the first approach at "humanization" was therefore the genetic construction of chimeric antibodies, comprising the murine variable region and the human constant region of IgG (*12*). Most chimeric antibodies displayed much reduced immunogenecity, but a response to the murine Fv portion could ultimately be observed. In reshaped or CDR-grafted antibodies, the murine content was further reduced by grafting the murine complementary determining regions (CDRs) or hypervariable region onto a human variable region framework (*13*). These antibodies were generally found not to be immunogeneic, but it was often difficult to maintain the binding affinity of the parent murine antibodies. Further amino acid changes in the framework region are generally necessary to maintain the original conformations of the CDRs. These changes need to be deduced for each antibody through computer model building, and the ultimate success—preservation of full binding—is often difficult to achieve even with extensive changes that potentially negate the advantage of CDR grafting over chimerization. In the newest approach, called variable domain resurfacing (*14*), the affinity is maintained by retaining the CDRs and the core of the murine variable region framework. Only the surface residues of the murine variable region framework are replaced by those from a human variable region. A simple algorithm predicts the necessary changes in the framework region, and when this method was applied to two murine antibodies, their affinities were unaffected (*14*). This approach assumes that the immunogenecity of murine antibody variable regions is determined by the accessible surface residues only, an assumption not yet tested with globulins, but generally accepted for the antigenecity of proteins (*15,16*).

## 3. RADIOIMMUNOCONJUGATES

Ever since the appreciation of the cytocidal effects of high doses of radiation, oncol-

patients afflicted with cancer. The goal of radiotherapy is to deliver a sufficiently high dose of radiation locally to the tumor in order to sterilize the tumor without causing lethal damage to the surrounding tissues. Successful killing of all tumor cells requires radiation doses of at least 60 gy to be concentrated at the tumor site, which is at the limit of the dose that can be delivered by external beam radiation while sparing normal tissue. Unfortunately, the wide application of external beam radiotherapy, while improving survival, has rarely resulted in cure. The notion that the ability of oncologists to eradicate tumors could be improved by in vivo administration of a radionuclide was first developed using iodine-131 to treat thyroid carcinomas, which concentrate radioiodine from blood resulting in delivery of local tumoricidal doses of 80–300 gy (*17*).

Radioimmunoconjugate therapy, which exploits the availability of specific antibodies that can localize to tumor cells, has been under investigation for a number of years as one way of improving radiotherapy. The hope of radioimmunoconjugate therapy is that targeting of radioactivity by antibodies could overcome two drawbacks of external beam radiotherapy: (1) specific targeting by radiolabeled antibodies should allow more precise delivery of the radiation dose to the tumor with concomitant sparing of a greater amount of the surrounding normal tissue; and (2) radiolabeled antibody will deliver a radiation dose to small undetected areas of tumor or micrometastases.

Radionuclides that are useful for radioimmunoconjugate therapy must emit particles whose energy can be deposited locally, ideally within a radius that encompasses one or a few cells. Furthermore, such radionuclides should have relatively short half-lives, so that radioactivity incorporated into the patient decays within a reasonable period of time, and in addition, they should be isotopes of elements whose chemistry allows them to be readily conjugated to antibodies. Several radioisotopes that may meet these criteria and that have been used in trials of radioimmunoconjugate therapy are shown in Table 1.

Chemically, the radioisotopes shown in Table 1 comprise two groups, the radiometals and radioactive isotopes of iodine. Iodine (and astatine) is generally conjugated directly to tyrosine residues in antibodies simply by mixing the protein with sodium iodide in the presence of an oxidizing agent, such as Chloramine T or related compounds (*20*). The reaction is extremely rapid, even at 0°C, although one must take care to avoid damage to the antibody by excessive oxidation. Alternatively, radioiodine can be conjugated to antibodies using iodinated compounds that allow labeling without exposing the protein to oxidative conditions, and furthermore, allow the possibility of utilizing iodinated compounds that are not subject to enzymatically catalyzed dehalogenation (*21–23*).

The radioactive metals are conjugated to antibodies by the use of chelating agents that are in turn chemically linked to the protein. Although the early chelates have high stability constants, they are kinetically labile, and in vivo, the radiometal readily exchanges into metal-transport proteins, such as transferrin, thereby losing any target specificity. Once lost from a conjugated chelate, a radiometal, such a yttrium-90, can ultimately be deposited in bone, resulting in prolonged irradiation of bone marrow. Recently, chelating agents that "cage" the metal and are far more stable have been developed for diagnostic and therapeutic applications with antibodies (*24,25*). Figure 2 illustrates the structure of two such antibody-conjugated macrocyclic chelators, which are ideal reagents for binding copper-67 and yttrium-90. In vivo studies show

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