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## Targeting tumor cells

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Several recent scientific and technical developments have made it possible to postulate the use of the 'magic bullet' concept; that is, the identification of specific antigens present on tumor cells that can be targeted either by therapeutic antibodies or by small molecules. The use of monoclonal antibodies in cancer, in particular, has moved beyond the proof-of-concept stage, and many such antibodies are presently being tested in the clinic. Several antibodies have been successfully developed and are now in use against various cancers, and we can expect many more to become available in the next few years. The use and development of these new therapeutics represent significant opportunities but also new challenges.

### Addresses

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### Abbreviations

**ADC** antibody–drug conjugate  
**CML** chronic myelogenous leukemia  
**Her-2** human epidermal growth factor receptor 2  
**SCF** stem cell factor

### Introduction

The advent of human or humanized antibodies has revolutionized the therapeutic use of monoclonal antibodies in humans [1]. There was a long period between the original reports of Milstein *et al.* [2] documenting the development of monoclonal antibodies and the breakthroughs in humanization [3<sup>•</sup>] or in the production of fully human antibodies [4<sup>•</sup>], using genetically altered mice or other technologies, which have led to the increased attention that these possibilities offer for the production of therapeutic antibodies. Some of the successes in the use of therapeutic antibodies are in autoimmune disease — for example, the use of infliximab (Remicade<sup>®</sup>, Centocor) for treating Crohn's disease and rheumatoid arthritis [5] — but by far the most popular therapeutic area for the use of monoclonal antibodies has been in cancer. Here, we will review recent progress in this area.

A key development has been the extensive use of gene arrays that has allowed the identification of cell-surface molecules specifically expressed in tumor cells. A pioneering step in this area was the development of Herceptin<sup>®</sup> (see below), an antibody that targets the breast antigen, human epidermal growth factor receptor 2 (Her-2) [6<sup>•</sup>,7]. Importantly, this antibody also has another key characteristic: it is able to act on its own (a so-called 'naked antibody') without the need for conjugation to other toxic molecules. Her-2, moreover, also exhibits another common feature of therapeutic tumor targets: it is present only in a subset of breast cancer patients [7]. The latter feature is more likely to be the rule rather than the exception because at the molecular level, cancer is heterogeneous [8<sup>•</sup>]. While this heterogeneity will eventually aid us in establishing better diagnoses and treatments for patients, in the meantime it is likely to result in further work necessary to define such subgroups of patients. In turn, the identification of these disease subtypes will improve the diagnosis and treatment of cancer patients. However, for the time being, it is necessary to keep this heterogeneity in mind when considering highly specific new therapeutics. Furthermore, recent advances point to an important role for chemokines, integrins and other adhesion molecules in metastasis. Understanding the molecular mechanisms that regulate metastasis is also critical in order to identify future targets of importance in cancer. Here, we will review both the advances and successes in this area, key research questions and some of the challenges that lie ahead.

### The case for small molecules: Imatinib mesylate

Imatinib mesylate (STI-571/Gleevec<sup>®</sup> [US]/Glivec<sup>®</sup> [non-US], Novartis) is a small-molecule inhibitor of three tyrosine kinases, Bcr-Abl, c-kit and the platelet-derived growth factor receptor (PDGFR). c-Abl expression is linked to the Philadelphia chromosome [9], which is present in chronic myelogenous leukemia (CML) [10]. c-kit is the receptor for the cytokine stem cell factor (SCF) [11], or kit ligand. The expression of c-kit on cells usually means that SCF can act either as a growth factor (on its own) or as a growth cofactor (i.e. it potentiates the growth of the cell to other growth factors) [11]. c-kit was originally characterized as a growth cofactor present on many immature hemopoietic cells, usually before lineage decisions were decided [11]. It could be argued, therefore, that it qualifies as a developmental antigen, and as such it is not surprising that it is expressed on some cancer cells. One of these is a variety of stomach cancer of stromal origin [12]. Accordingly, Imatinib has been shown to be highly effective in both CML and the stromal cell

variety of stomach cancer. Recently, another report has found both c-kit and SCF expressed in Ewing's sarcoma, a form of pediatric bone marrow cancer [13]. It is therefore likely that Imatinib will also be effective for this indication. This is an excellent example of what we should consider to be the norm for the applications of these new class of tumor-targeting drugs: that they will be effective for a variety of indications, but more likely will cross the classical tumor types and will be useful against subsets of different diseases. This reflects the molecular heterogeneity of cancer discussed above.

Imatinib also exemplifies the future limitations for these new class of highly specific cancer drugs. In some patients, Imatinib-resistant forms of CML have arisen following treatment. Interestingly, analysis of these resistant tumors indicates that they now have mutations in the c-Abl receptor that are no longer able to bind Imatinib [14••]. It can be postulated that these CML variants were selected following treatment with Imatinib. Unfortunately, these variants might be more aggressive than the original tumor, and patients that relapse typically have a poor prognosis [15]. This situation is reminiscent of the early use of antibiotics in bacterial diseases, where the use of a highly effective antibiotic often resulted in the appearance of resistant strains. We could anticipate that in the future CML may be optimally treated with two drugs, the original Imatinib and another one, yet to be developed, that would target the appearance of the Imatinib-resistant tumor variants. Fortunately, the mutations that result in resistance appear to be limited and relatively conserved [14••].

This analogy of the current status of cancer treatment with bacterial infections is very appropriate. In both cases, the pathology is caused by the growth and colonization of various organs by an 'alien' cell, in one case a prokaryote, and in another a human cell that has lost its normal growth controls. The finding that certain molecules (i.e. antibiotics) could be effective in the elimination of bacterial infections promoted enormous interest in this area. We are now in a similar stage in the treatment of cancer, and we also have at our disposal new molecular tools that allow us to identify specific tumor antigens that can be targeted using either antibodies or small molecules. To carry the analogy further, we can expect varying levels of effectiveness in these newly developed drugs.

### The case for antibodies: Herceptin<sup>®</sup>, Rituxan<sup>®</sup>

While Imatinib is one of the best examples of a specific small molecule targeting a cancer, much more interest has been generated for the use of antibodies that target specific molecules expressed by tumor cells. The reason for this increased attention is that there are now several examples of effective antibodies to treat various cancers. Two such examples are Herceptin<sup>®</sup> (Trastuzumab,

Genentech) and Rituxan<sup>®</sup> (Rituximib; IDEC Pharmaceuticals Corporation). Herceptin<sup>®</sup> targets the Her-2 antigen expressed by a subset of breast cancer patients; Rituxan<sup>®</sup> is an anti-CD20 antibody effective against non-Hodgkin's lymphoma [6•]. Both antibodies have developed large markets and represent significant improvements in the treatment of these diseases; however, they remain the first examples of a trend that will accelerate as many more such antibodies are developed.

In reviewing the process of anti-cancer antibody development, it is first necessary to identify the specific expression of a particular antigen in tumor cells. This can be done using gene arrays or proteomics. The availability of the human genome has made this possible, and there exist many reports already in the literature that document many such cancer-specific antigens [16]. Specificity in this case may be crucial, although not necessarily obligatory. For example, Her-2 is also expressed in heart tissue [17]. Nevertheless, complete or nearly complete specificity is clearly desirable. Second, the ideal target in question should be a cell-surface antigen. Finally, an ideal target is one with a critical function for the cancer cell. In the case of Her-2, the antibody (Herceptin<sup>®</sup>) induces apoptosis of the tumor cells [6•]. Other critical functions for the tumor cell include growth (an example is c-kit-SCF interaction discussed above for Imatinib). Clearly, finding a target with all of these characteristics would be highly desirable, because it allows for the development of therapeutic 'naked' (i.e. unconjugated) antibodies. Such targets exist but are not easy to find.

### Antibody-drug conjugates

Cell-surface molecules that exhibit high specificity for tumor cells are easier to find [16]. Often, little is known about the function of these highly specific cell-surface molecules; for this reason, the development of antibodies against these targets might not result in the impairment of tumor progression or survival. The specificity of these molecules instead can be used to turn these antibodies into a highly specific way of delivering toxins. Popular toxins that have been used in this way include radio-nuclides or toxins such as ibritumomab tiuxetan (Zevalin, IDEC Pharmaceuticals Corporation) or gemtuzumab ozogamycin (Mylotarg<sup>®</sup>, Wyeth Ayerst) [6•]. Often, however, the development of an antibody-drug conjugate (ADC) improves an existing therapeutic antibody, as is the case for Bexxar<sup>®</sup> (Tositumomab, Corixa/GlaxoSmith-Kline) [18]. Unfortunately, the use of toxins or radio-nuclides in these antibodies complicates the preclinical and clinical testing necessary to gain approval. For the latter reason, many companies developing antibodies prefer naked antibodies. However, since we now know of many more tumor-specific antigens rather than tumor-specific functional targets, ADCs are likely to expand in the medium term. Consequently, the possibility of developing effective ADCs for cancer treatment has spurred

much interest in the development of better toxins for use with antibodies. All of these developments suggest that we are likely to see significant success in this area in the near future.

### Other issues in the development of tumor therapeutics

There are several issues that bear great importance in the development and use of specific cancer therapeutics. Some of these are the discussed below.

#### The heterogeneous nature of cancer

We touched on this issue briefly above, but it deserves further comment. Cancer today is mostly defined on an organ-specific basis. As such, many companies express most interest in targeting cancers with 'big markets'. These include breast, lung, colorectal and prostate cancers — defined as 'big' markets on the basis of the number of patients that suffer from these diseases. However, as exemplified by our discussion above on Herceptin<sup>®</sup>, the specific targeting of tumor cells will invariably define subsets of patients in most cancers. In other words, it is very difficult to find a specific target molecule present in most patients of a particular cancer. Most of them are expressed in subsets of patients, and some of these will also represent subsets of the disease, as defined by pathological and clinical findings, sometimes including prognosis. Already, some reports have documented that gene-array analyses can define subsets of, for example, breast cancer based on the aggressiveness of the disease [19<sup>••</sup>].

#### The goal line is survival benefit

Often, there is confusion defining 'success' in the treatment of cancer. In some of the examples reviewed above, success is clear; in many patients, Imatinib results in the disappearance of the leukemic cells. In many other cases, however, success is not defined in such clear terms. For example, in many cancer cases, the primary tumor is not really a big concern in the treatment, because part of the treatment will include surgery to remove it. What most often kills a patient is not the primary tumor but its metastases; and our understanding of this phenomenon is still very poor. Perhaps the most noteworthy realization is that metastasis is governed not only by mechanical but also molecular factors [20]. Until recently, the notion that mechanical factors determined the metastatic destination of tumor cells was a widely held concept. More recently, however, various molecular families have been shown to be involved in this process, including chemokines [21<sup>••</sup>], integrins [22<sup>••</sup>] and metalloproteases [23<sup>••</sup>]. This is important because it opens the possibility of using naked antibodies against some of these targets to influence the course of metastasis. Furthermore, to gain regulatory approval, a new drug usually does not have to show a positive influence on the growth of the primary tumor but instead a survival benefit for the patient. This means controlling metastasis, and more importantly, controlling

metastasis to a critical organ that determines the cause of death for a particular cancer.

#### Identifying the cause of death

From the discussion above, it becomes clear that it is important to understand the natural course of a given cancer in order correctly to design an experiment to identify targets that will influence that particular cancer. For example, in breast cancer, tumor cells metastasize preferentially to lymph nodes, lung, liver, bone marrow and, in some patients, to the brain. Except for the latter patient cohort (i.e. those with brain metastasis), most breast cancer patients probably die because their lung function eventually becomes impaired by the tumor growing there [24]. It follows that effective therapeutics in breast cancer should target cell-surface molecules specifically expressed by lung metastasis of breast cancer. This raises a very important question: is the profiling of primary breast cancer tumors an accurate reflection of the disease and its metastases? In other words, is the gene expression of a primary tumor the same as its metastases? There is little information on this question so far [25<sup>••</sup>], and even if that becomes documented for a particular cancer, we may not be able to extrapolate to others.

This is an area ripe for future research. In any case, this example implies that any program for the development of a particular target in breast cancer (usually identified from the analysis of primary tumors) would be wise to explore the expression of this target in lung metastasis (or, for that matter, brain metastasis) of breast cancer.

#### Other metastasis questions

As cancer advances in patients, they are likely to have metastasis in many different organs. This raises the question of whether all metastases exhibit similar gene expression. We would predict that this will not be the case. The tumor cells are going to encounter different microenvironments in different sites. For example, lymph nodes should be rich in cytokines and other factors, which are well known to influence gene expression. Therefore, even if we identify different gene-expression programs in metastases, we do not know if these changes are associated with the capacity of tumor cells to metastasize to that particular organ; nor whether the differential gene expression simply reflects the ability of the microenvironment to influence the gene expression of tumor cells.

A related question is whether a particular gene target will be expressed in all metastases present in a particular patient. If the target is efficiently expressed in, let's say, 12 out of 18 metastases, our test drug may show important benefit, although it might not ultimately save the patient. This becomes a situation like the one discussed above for Imatinib. However, this situation might not be critical for the success of such a drug candidate,

because, as discussed, it might still have a significant survival benefit. Using the same case as an example, if in breast cancer the lung metastases express the target antigen then it is likely that the drug will have a significant survival benefit. Conversely, under the same example, if the lymph node and not the lung metastasis express the target, then it is doubtful that the survival benefit will be significant.

### Influence of chemotherapy

It is unlikely that any new therapeutic will, at least initially, be used alone in the treatment of a cancer patient. Most patients will still undergo the currently approved treatment regimen that may include radiation and/or chemotherapy. It therefore becomes important to verify that the expression of the tumor target on the cancer cells (metastases?) is still present following these treatments.

### Conclusions

There is little doubt that we are entering a period of exponential growth in the development of highly specific and effective anti-cancer drugs. Most of these initially are going to be antibodies, although, as the example of Imatinib shows, small molecules will also be successful. The latter class of drugs has the limitation of being likely to target only certain molecular families that are more amenable for small-molecule discovery (i.e. G-protein-coupled receptors, ion channels, etc.). Antibodies will preferentially target cancer-specific cell-surface molecules.

Although identifying tumor specific molecules has become easier, owing to new gene-array technologies, it is still difficult to identify molecules with critical functions for the tumor cell that will result in cell death when that function is interrupted. For this reason, ADCs will also become popular. Two critical aspects to keep in mind when developing cancer drugs are the heterogeneous nature of most cancers at the molecular level, and the role of metastasis in the development of the disease. Both of these represent very fertile areas for future research. We have reason to be very optimistic that the next few years will witness the availability of many new highly effective drugs with limited side effects. This should translate into significant enhancement of patient survival. Even if most treatments do not represent a real 'cure', a more realistic goal might be to turn many cancers from acute to chronic diseases, with enhanced quality of life. The current time can be compared with the onset of antibiotic development that transformed the treatment of many bacterial infectious diseases.

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