Molecular Target Therapy in Hematological Malignancy: Front-runners and Prototypes of Small Molecule and Antibody Therapy

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Molecular-targeted drugs were first introduced for the treatment of hematological malignancies. Herein, the success stories of small molecule-targeted drugs, such as imatinib for the treatment of chronic myeloid leukemia and the tumor-specific antibody rituximab for the treatment of CD20-positive lymphoma, will be introduced. The introduction of imatinib and rituximab has changed the mortality rates associated with chronic myeloid leukemia and CD20-positive lymphoma, respectively. In particular, the therapeutic outcomes of imatinib treatment have been so good that clinical trials to assess the feasibility of treatment discontinuation after remission are ongoing. Methods for developing new anti-cancer agents have changed, and structure-based chemical compounds are now screened in silico. Second- and third-generation anti-cancer agents have already been successfully identified, and resistance mechanisms have been explained based on the interaction of these chemical structures with their target molecules. In the area of antibody development, the introduction of humanized antibody has been successful, and the use of antibody carriers has resulted in the development of potent second-line antibody drugs. The discovery of new target surface markers is also being reported, and trials for both chimeric and humanized antibodies against these molecules are ongoing. Through these efforts, disease mortality rates have begun to decline. We are facing a brilliant future in which we can strive to eliminate cancer-related mortality.

Key words: chemo-hematopoietic – hematol-leukemia/lymphoma – targeted therapy

INTRODUCTION

Molecular-targeted drugs were first introduced for the treatment of hematological malignancies. In this mini-review, the contribution of hematologists to the development of molecular-targeted drugs, including small molecule-targeted drugs and tumor-specific antibodies, will be introduced, so that this knowledge can be shared with oncologists specializing in other types of malignancies.

DEVELOPMENT OF IMATINIB THERAPY AGAINST CHRONIC MYELOGENOUS LEUKEMIA

Imatinib, which is used for the treatment of chronic myeloid leukemia (CML), was the very first molecular-targeted drug to be developed successfully (1). The molecular target of imatinib is the abl gene, which is activated in nearly all cases of CML because CML itself is defined by the presence of bcr–abl gene fusion derived from the translocation of chromosomes (2), otherwise known as the Philadelphia chromosome. Consequently, the fused bcr–abl gene is activated and expressed in all patients with CML. The fused gene works as a constitutively activated form of the abl gene and induces downstream signals. Therefore, the suppression of these signals by inhibitors was expected to exert a suppressive effect on growth and survival signals, and the development of such inhibitors was anticipated to suppress abl gene activity completely in normal cells. The advantage of this situation was that even if the normal abl gene was suppressed, it would not affect other cellular functions or cell survival. This line of reasoning was demonstrated in an experiment using knockout mice (3). The resulting mice

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were normal with minimal bone changes, presumably because of the redundancy of tyrosine kinases and the bypassing effects of other kinases.

Imatinib was originally developed to block the suppression of PDGFα and the differentiation of 12-O-tetradecanoylphorbol 13-acetate (TPA), and not as a drug for the treatment of CML. Thus, imatinib was intended for the treatment of tumors in which the PDGF receptor was abundantly expressed, such as gliomas (4). The commercial name for imatinib, ‘Gleevec’, reflects this initial intended use for the treatment of gliomas. However, the compound was also found to exert an inhibitory effect against the abl gene, which led to a switch in the direction of drug development toward the treatment of CML (5).

This switch in the direction of drug development was a great success. The reported 7-year overall survival rate of CML patients treated with this drug was 86% (1). This significantly better survival outcome reflected a sustained remission throughout the observation period. The occurrence of events such as blast transformation and the need to discontinue treatment decreased toward the end of the protocol study. In the clinical trial examining the use of imatinib for the treatment of CML with the longest follow-up period to date, the IRIS study, the incidence of events [loss of complete hematological response, loss of major cytogenetic response, development of accelerated phase (AP)/blastic phase (BC) and death from any cause] occurring during each post-treatment year steadily decreased, with recorded incidences in the first, second, third, fourth, fifth and sixth years of 3.3, 7.5, 4.8, 1.7, 0.8 and 0.3%, respectively (6). Furthermore, the rates for the development of AP/BC during the same follow-up period were 1.5, 2.8, 1.6, 0.9, 0.5 and 0%, respectively (6).

The depth of remission can be evaluated by the quantity of fusion gene expression, and the number of patients achieving complete molecular response (CMR), defined as the absence of fusion gene as detected using nested polymerase chain reaction (PCR), has been shown to increase even after 4 and 5 years of therapy (7). Together with the decrease in the AP/BC rate, the above observation has led to the idea that imatinib therapy may be a curative therapy for CML.

Mahon et al. reported the occurrence of long-term remission after the discontinuation of imatinib therapy in CML patients (8). They identified 50 cases of CML in which a CMR had been achieved and followed these cases after the discontinuation of therapy. They reported that 15 of these patients were still in remission at 2 years after the discontinuation of therapy. The outcome was particularly remarkable among the patients who had received interferon a. This report prompted the start of a study to examine the feasibility of treatment discontinuation.

In many countries, clinical trials to demonstrate the feasibility of the discontinuation of imatinib treatment after remission have been ongoing for many years. However, the eligibility criteria vary among the studies. Goh et al. (9) recruited 26 patients that had achieved not only a CMR, but also exhibited major molecular remission and conducted a retrospective review of the effect. The results were disappointing: almost all the cases showed an increase in the expression level of the bcr/abl gene. However, all the patients again achieved the same expression level as observed at the start of discontinuation once treatment was resumed, resulting in the imatinib treatment being ‘saved.’

The Japan Adult Leukemia Study Group (JALSG) and our group are also planning a trial to examine the feasibility of discontinuing imatinib treatment in cases with molecular remission. The eligibility criteria would include cases with documented complete remission at a minimum of two time points. In the planned protocol, imatinib would be incrementally discontinued: 1 month of discontinuation would be followed by 3 months of treatment, followed by 2 months of discontinuation and 2 months of treatment and so on. The end-point would be the complete discontinuation of treatment, and re-induction would be undertaken if an increase in the bcr/abl level was subsequently observed. The institutions involved in the study will be limited to certain subsets of JALSG facilities, and careful molecular monitoring will be performed.

The success of imatinib therapy has led to a change in clinical practice. For example, treatment with imatinib has resulted in an annual decrease in the number of patients requiring transplantations. This outcome is remarkable, since prior to the use of imatinib, transplantation was the only way to cure patients with CML. Younger patients were previously recommended to receive allogeneic stem cell transplantations as soon as suitable HLA-compatible donors could be found; this practice has now changed, and the current practice is to recommend transplantation only if/when oral chemotherapy including imatinib therapy fails.

The success obtained with imatinib has also brought the molecular monitoring of CML into practice. Using molecular methods, the disease status of CML can be precisely monitored (10). The remission level must be closely monitored to evaluate the depth of remission. The results of such assessments can then be used in decisions regarding treatment strategies, with treatment re-initiated if an increase in the expression level of the bcr/abl gene is observed using PCR.

The success of imatinib has also changed the manner in which pharmaceutical companies develop new agents (11). Imatinib was the first molecular-targeted drug with a known mechanism of efficacy. Three-dimensional structural analysis can be used to reveal the contact of a drug with its target molecule. Better structural fitting can lead to the discovery of improved drugs. Using this method, a new drug, nilotinib, was discovered for the treatment of CML; nilotinib has a precise and specific contact with the abl protein. Recently the results of a head-to-head comparison of this second-generation abl inhibitor with imatinib in a phase III trial were reported, and the primary endpoint of molecular response at 1 year was better than that obtained with imatinib (12).

The mechanism of imatinib resistance has been intensively studied, and the discovery of a mutation in the abl gene has led to the discovery of yet another drug, dasatinib. This drug is effective against the mutated abl gene, as it binds to the
alternative form of the abl gene, namely, the inactive src-kinase-like form. The resistance induced by the mutation of the abl gene can be overcome using this new agent, which binds to the abl gene more precisely, although the spectrum of abl mutations may vary. With these alternative agents, nilotinib and dasatinib, patients with CML can successfully achieve a second long-lasting molecular remission even after imatinib-resistant clones have emerged (13).

Recently, CML stem cells have been identified and have been shown to be resistant to imatinib therapy. A mechanism for maintaining leukemic stem cells has also been proposed. In the proposed model, FOXP3 activity, which is up-regulated for maintaining leukemic stem cells has also been proposed. This testing for predicting response (15). As the inhibition of a kinase is determined by the concentration of the agent, the concentration of the drug must be greater than the target level to be effective, and achieving the target concentration is directly related to the effect of the drug. The situation is comparable to that of antibiotic therapy for the treatment of bacterial infections, in which it is necessary to keep the drug concentration above the sensitivity level.

The serum levels of imatinib in Japanese patients were rather high, compared with previously reported levels, because the body surface area (BSA) and the body weight of Japanese subjects are less than the values of populations in the USA and the UK. The results of a phase II study in Japan were recently published, and the actual dose used was approximately 300 mg, which is less than the previously reported dose of 400 mg. Nonetheless, the reported molecular effect was the same. In the phase II JALSG CML-202 study, the estimated complete cytogenetic response rate in 489 patients at 48 months was 92%, consistent with the results of the IRIS study, in which the rate was also 92% (Table 2). Therefore, the observation of the same effect at a lower drug dosage prompted us to measure the serum concentration of the drug, and the serum levels in the Japanese patients were comparable to those observed in populations in the USA and UK (16).

These results clearly show the importance of drug concentration monitoring in the era of molecular-targeted drugs. The serum levels of imatinib were previously reported to be unrelated to the BSA, body weight or age, and they appear to vary among individuals (17): as the serum level of imatinib is directly related to the treatment efficacy, serum concentrations should be monitored to ensure that they exceed the minimal tumor suppressive concentrations (18).

From a marketing viewpoint, the number of patients with CML is too small to launch a profitable drug discovery project. Following the success of imatinib, however, the development of cytostatic drugs has emerged as a new trend in drug development. With cytostatic drugs, even if the number of patients is small, drug development may proceed since such drugs will be used over prolonged periods. This approach has led to an enormous number of ‘maintenance’ drugs, such as drugs for maintaining the remission of acute myelogenous leukemia and malignant lymphoma, for the induction of myelodysplastic syndrome, and for prolonging remission after the resection of solid cancers. Some of these drugs are oral medications that have never before been pursued (19). The effectiveness of these drugs must be confirmed in phase III randomized clinical trials.

### DEVELOPMENT OF DRUGS TARGETING CD20-EXPRESSING MALIGNANT TUMORS

Follicular lymphoma (FL) is the most common subtype of indolent lymphoma. With the mass production of monoclonal antibodies on a commercial scale, remarkable progress has been made in the treatment of this disease. In 1997, the US Federal Drug Administration (FDA) approved rituximab for the treatment of CD20-positive B cell malignancy; if the

<table>
<thead>
<tr>
<th>Targeted kinase</th>
<th>Drug</th>
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<tbody>
<tr>
<td>Bcr-Abl</td>
<td>Adaphostin, AZD0530, CNS-9,1 NNO-406, SKI606 (bosutinib)</td>
</tr>
<tr>
<td>VEGF</td>
<td>AEE778, AG01376, sorafenib, CHIR258, PTK787, sunitinib</td>
</tr>
<tr>
<td>mTOR</td>
<td>RAD001 (everolimus), CCI-779 (temsirolimus), AP23573</td>
</tr>
<tr>
<td>Raf, Mek, Erk</td>
<td>AAL881, BAY 43-9006, levostatin, PD98059</td>
</tr>
<tr>
<td>Src</td>
<td>AP23464, dasatinib, PD166326, SU6656, AZD0530</td>
</tr>
<tr>
<td>Aurora kinase</td>
<td>MK0457, Perifosine</td>
</tr>
<tr>
<td>Akt, PI3K</td>
<td>IGF-1R</td>
</tr>
<tr>
<td>Jak2, Stat 3</td>
<td>AEE541, ADW742, tyrophostins</td>
</tr>
<tr>
<td>MAPK</td>
<td>AG490, WP-1066</td>
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**Table 1. New kinase inhibitors under development for the treatment of CML**

**Table 2. Results of the JALSG CML202 study**

<table>
<thead>
<tr>
<th>Response</th>
<th>JALSG-CML202 (%)</th>
<th>IRIS (%)</th>
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<tbody>
<tr>
<td>MCR</td>
<td>98</td>
<td>98</td>
</tr>
<tr>
<td>CCR</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>MMR</td>
<td>55</td>
<td>70</td>
</tr>
</tbody>
</table>

CCR, complete cytogenetic response; CML, chronic myeloid leukemia; JALSG, Japan Adult Leukemia Study Group; MCR, major cytogenetic response; MMR, major molecular response.
aim of treatment for FL is assumed to be prolonging the disease-free survival period, this antibody is an ideal tool, considering its low toxicity.

Rituximab was subsequently approved for the treatment of diffuse large B-cell lymphoma by the FDA in 2002 and by the Japanese government in 2003. The survival rate of patients with this disease has been prolonged by the addition of rituximab to cyclophosphamide, hydroxydaunorubicin, vincristine and prednisone (CHOP) therapy. The success of anti-CD20 monoclonal antibody therapy has prompted efforts to identify new target molecules and to increase the killing activity of antibodies, and a new generation of antibody is now being developed.

The anti-CD20 antibody rituximab exerts its killing activity via three mechanisms: complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), and the induction of apoptosis (20). Resistance to this agent is also generated through resistance to these three mechanisms: low ADCC activity, low CDC activity and resistance to apoptosis. In addition, pharmacokinetic factors, such as decreased serum concentrations, must also be considered.

ADCC is triggered by the binding of the Fc receptor and immunoreceptor tyrosine-based activation and phosphorylation (21). A polymorphism at the 158th amino acid residue is involved in the first step of this binding: if the amino acid residue is Val, the binding is weaker than when the residue is Phe. Thus, polymorphisms might also be an important factor determining individual sensitivities.

### NEWER-GENERATION ANTI-CD20 ANTIBODIES (Table 3)

One of the newer generations of anti-CD20 antibodies that is expected to be developed is ofatumumab (also known as HuMax-CD20; Genmab) (22). The antibody is a completely humanized IgG1 that recognizes different epitopes from that recognized by rituximab. Preclinical data show a stronger binding activity than that of rituximab, and the drug is anticipated to be effective against chronic lymphocytic leukemia (CLL), as higher doses of antibodies are required to overcome relative resistance. Recently, the FDA approved its use for the treatment of CLL in patients who are resistant to fludarabine and alemtuzumab (23).

The recognition site of ocrelizumab is the same as that of murine 2H7 monoclonal antibody. The advantage of ocrelizumab, however, is that infusion reactions are rare, and this agent can be injected subcutaneously for the treatment of autoimmune diseases (24). A phase I/II study has been completed (25), and a phase III trial targeting non-malignant disease is now ongoing (26).

Veltuzumab (Immu-106, hA20) is another humanized antibody that recognizes the same epitope recognized by ocrelizumab. The humanized part is the same as that of epratuzumab, an anti-CD22 humanized antibody manufactured by the same company. Veltuzumab is expected to offer a lower frequency/severity of infusion reactions (27,28). A phase II study has been completed (29).

The Fc portion has been engineered to achieve a high efficacy, even in patients with a low Fcγ binding affinity. AME-133 was developed to work even in cases with the low affinity-type FcyRIIa, and a clinical trial is ongoing (30).

Another antibody, obinutuzumab (formerly known as GA101), recognizes a different epitope. The variable portion is that of the murine B1 antibody, and the remaining part is humanized. The Fc portion is bound to sugar chains, and is, therefore, more flexible and fits better at the hinge portion. The binding capacity of this formulation to FcγRIIIa is 50 times more potent than that of the current antibody (31,32). Thus, the development of anti-CD20 antibodies has been bi-directional: the newer agents are less toxic (ocrelizumab and veltuzumab) and more effective (ofatumumab, AME-133, and GA101).

Antibodies work additively when combined with radioisotopes and toxins. Two types of radioimmunotherapy and one type of immunotoxin therapy have been developed for this purpose. Ibritumomab tiuxetan (Zevalin®) was approved by the FDA in 2002 for the treatment of relapsed or refractory
FL, low grade non-Hodgkin lymphoma (NHL) and transformed NHL. The manufacturing of this drug was approved in Japan in January 2008 (33). The murine monoclonal antibody 2B8 is conjugated with $^{90}$Y through the chelator tiuxetan (MX-DTPA). For this agent, a murine monoclonal antibody is used instead of a chimeric or humanized antibody because multiple injections are rarely needed.

The cytotoxic activity of radiation is mainly achieved by beta emission, and the selection of the radionucluear material has a direct bearing on the antitumor effect. If the half-life is too long, protection is difficult, meanwhile, if the half-life is too short, the radiation treatment can be difficult to perform in a timely manner. In this sense, $^{90}$Y is an ideal radionuclide, with an energy intensity of 2.3 MeV, a range of 5 mm and a half-time of 64 h, also, $^{90}$Y is a pure beta emitter.

$^{131}$I-tositumomab (Bexxar®) (34) was approved by the FDA in 2003. This agent also recognizes a novel epitope of CD20. The radioactive material is not conjugated through linkers, but the tyrosine residues of the antibody are iodinated. To protect the thyroid gland from the radiation, the patient is given iodine in advance. Compared with ibritumomab tiuxetan, the radiation dose to the kidney is higher, whereas the dose to the liver is lower. Despite approval in the USA, the company is not preparing for a clinical trial of this agent to file for approval in Japan.

The above-mentioned successes have also induced the development of antibodies against other surface target molecules. The loss of CD20 has been reported to be a mechanism of resistance to antibody therapy (35). In such cases, therapy targeting other surface molecules would be more effective.

CD22 is an adhesion molecule that is rapidly internalized after binding. Its activity is related to the activation of B cells, modifying antigen signaling. The anti-CD22 antibody epratuzumab is a modified and humanized form of the murine mLL2 antibody. Its efficacy has been demonstrated not only against low-grade lymphomas, but also against aggressive lymphomas, although with lesser efficacy (36). Rituximab combined with this antibody was more effective than rituximab alone in patients with relapsed lymphoma (37).

Inotuzumab ozogamicin (CMC-544) also recognizes CD22, and this antibody is linked to a toxin, calicheamicin. Once the antibody binds to CD22, it is internalized and cleaved within the cell. The process after internalization is the same as that of Mylotarg, an anti-CD33 antibody that is also combined with calicheamicin. A Japanese phase I/II study on this agent has been completed (38).

**NEW AGENTS UNDER CONSIDERATION**

Table 4 lists the molecular-targeted drugs that are being developed for the treatment of hematological malignancies. The targets of these drugs are not limited to B cells. Alemtuzumab was originally used to treat T cell suppression in allogeneic transplantation settings. Some of these drugs have already been used for the treatment of solid cancers. Bevacizumab for VEGF has been widely used to treat colon cancer and is being examined in a phase III trial to compare the effect of its addition to R-CHOP therapy with that of R-CHOP therapy alone.

Hematological malignancies include acute leukemia and multiple myeloma. Treatment advances for this disease have been achieved using new agents. Among them, a humanized anti-CD33 antibody conjugated with calicheamicin, gemtuzumab ozogamicin, has been developed for use against CD33-positive acute myelogenous leukemia (39). Other drugs being investigated for the treatment of leukemia are shown in Table 5. The introduction of the proteasome inhibitor bortezomib has changed the survival outcome of multiple myeloma (40).

**INTRODUCTION OF BIOMARKERS**

The use of these drugs for hematological malignancies requires biomarkers for identifying populations that can benefit from these drugs to be established. Identifying patients who are likely to be sensitive to the newer agents is problematic. In 1991, when the efficacy of tretinoin (an all-trans retinoic acid, ATRA, which is a retinoic acid alfa...
derivative) was reported, the detection of the gene fusion of PML/RARA was used as a diagnostic test (41). If the fusion was found to exist in an individual, the patient was almost guaranteed to be sensitive to tretinoin. This test was performed using a chromosomal analysis or reverse transcription PCR (RT–PCR), and, more recently, using fluorescent in situ hybridization. The Philadelphia chromosome, which is the hallmark of CML and acute lymphoblastic leukemia, is the target of imatinib therapy. CD20 positivity is also an indicator of the efficacy of rituximab. Thus, the introduction of molecular-targeted drugs has produced changes in the approval process for diagnostic tests: approval for an assay system for molecular markers and the corresponding molecular-targeted drugs should be sought simultaneously.

In Japan, however, the approval process for molecular diagnostic tests is slow, creating a serious issue for the introduction of newer agents. In Japan, if the test is approved by the Government, the cost is covered by the national health insurance program; however, the extent of coverage is far less than the global standard, and the actual cost is not fully covered. Some hospitals send the invoice to the government insurer for the diagnostic test as a pre-determined cost. The nature of this system leads to hesitation in filing with the Japanese regulatory agent for the approval of such tests.

Even if the test is not covered by health insurance, hospitals can pay for the cost. However, the cost is not for clinical use, and the cost should be covered by the insurer. In this situation, combined payment by the insurer and an extra-insurance payment is not allowed. Thus, new molecular-targeted drugs are generally approved without the approval of the corresponding biomarker test. The approval process for molecular-targeted drugs and corresponding biomarker tests should be simultaneous, otherwise patients will not benefit from the new treatments. In fact, the FDA is planning a newer simultaneous approval process for biomarkers and drugs (42).

CONCLUDING REMARKS

In the field of molecular-targeted therapy, hematological malignancies are the front-runners for drug development. Figure 1 shows the annual changes in the mortality rate of CML, adjusted for age. The first down-slope represents the change in the mortality rate that occurred during the introduction of allogeneic bone marrow transplantation. The second decline, starting in 1999, clearly reflects the introduction of imatinib therapy. The mortality rate has been reduced by half and continues to decline. This figure can be interpreted as depicting the triumph of molecular-targeted therapy.

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Conflict of interest statement.

None declared.

References


![Figure 1](http://www.seer.cancer.gov/)


