— Minireview —

Side Effects of Molecularly Targeted Drugs and Their Molecular Mechanisms

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Molecularly targeted therapy has become widely used in clinical settings over the last decade. Although it was initially expected that molecularly targeted drugs have fewer side effects, it is becoming increasingly apparent that molecularly targeted drugs have an unanticipated repertoire of side effects. Some side effects are serious, some are manageable, some are rare, and some are frequent. Some may affect individuals with predisposing factors. In this minireview, we briefly summarize how the side effects of molecularly targeted drugs were discovered when new classes of drugs were introduced. We also summarize the clinical characteristics of these side effects focusing on progressive multifocal leukoencephalopathy (PML) associated with the use of an integrin antagonist, cardiotoxicity associated with the use of some tyrosine kinase inhibitors, and hypertension associated with the use of angiogenesis inhibitors. We also review the molecular mechanisms underlying these side effects. Research on the mechanisms underlying these side effects has revealed previously unknown physiological roles of targeted molecules. Awareness and understanding of the side effects of molecularly targeted drugs is important for those working in clinical practice and conducting basic research.

Key words —— side effect, molecularly targeted drug, monoclonal antibody, integrin antagonist, tyrosine kinase inhibitor, angiogenesis inhibitor

INTRODUCTION

The side effects of some conventional drugs are unavoidable. For example, immunosuppressive drugs increase the risk of serious infections. Chemotherapeutic anticancer agents are cytotoxic to all rapidly dividing cells and cause many serious side effects. The advent of molecularly targeted drugs such as tyrosine kinase inhibitors, which are designed to specifically target cancer cells, markedly improved the management of cancers.[1] However, when new classes of molecularly targeted drugs are introduced, the occurrence of unanticipated side effects has not been rare.

A very recent example is the case of using an integrin antagonist. In the treatment of multiple sclerosis, an integrin antagonist, natalizumab, was introduced as a new therapeutic agent,[2] which inhibits the migration of T lymphocytes to the central nervous system (CNS) across the blood-brain barrier. Natalizumab demonstrated a high efficacy and was approved by the United States Food and Drug Administration in 2004, which has not yet been approved in Japan. However, shortly after its approval, it was found that the use of the agent was associated with progressive multifocal leukoencephalopathy (PML),[3–5] a fatal opportunistic infection of CNS. The mechanism is not completely understood as yet.

Although tyrosine kinase inhibitors were considered to affect cancer cells selectively, heart failure became apparent as a side effect in the pivotal efficacy trial of the first anticancer tyrosine kinase inhibitor, trastuzumab. Presently it seems that some, but not all, tyrosine kinase inhibitors affect the survival and function of cardiomyocytes.[6,7] Likewise, although angiogenesis inhibitors were assumed to affect tumor-stimulated endothelial cells selectively, they may also affect the release of vasodilators from endothelial cells; hypertension is a relatively common side effect of angiogenesis inhibitors.[8]

When new classes of molecularly targeted drugs

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are introduced, careful observation and clinical evaluation of side effects are important. Moreover, understanding their molecular mechanisms is important because it may help us identify new side effects and/or find a way to circumvent unwanted side effects in future drug development. Here, we review examples of side effects of molecularly targeted drugs focusing on PML associated with the use of natalizumab (which belongs to a class of integrin antagonists), cardiotoxicity associated with the use of trastuzumab (a tyrosine kinase inhibitor) and imatinib (a small-molecule tyrosine kinase inhibitor), and hypertension associated with the use of bevacizumab (an angiogenesis inhibitor). These drugs are among the initial drugs in their classes and also are the initial drugs whose unexpected side effects were identified. In this mini-review, taking these drugs and side effects as examples, we will discuss how the unexpected side effects became initially known and what is known about their incidences and molecular mechanisms. For other side effects of molecularly targeted drugs, such as cutaneous toxicities[9–11] or interstitial pneumonia[12,13] associated with epidermal growth factor (EGF) receptor inhibitors, thrombotic events and perforations, proteinuria and edema associated with angiogenesis inhibitors,[14] infusion reaction to monoclonal antibodies,[15] or other specific side effects of some drugs such as reversible posterior leukoencephalopathy[16] and altered bone and mineral metabolism,[17,18] readers are referred to the corresponding reviews. Although there is no question about the fact that molecularly targeted therapy has large benefits, it seems that we have much to learn about the safe and wise use of molecularly targeted drugs.

NATALIZUMAB FOR TREATMENT OF MULTIPLE SCLEROSIS

Natalizumab is a recombinant monoclonal antibody that binds to the α_4-subunit of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins on the surface of all leukocytes except neutrophils.[2,19] It is the first α_4 integrin antagonist in a new class of selective adhesion-molecule inhibitors.[20,21] Natalizumab inhibits the α_4 integrin-mediated adhesion of leukocytes to their counter-receptor(s), which include vascular cell adhesion molecule-1 (VCAM-1) expressed on activated vascular endothelial cells and mucosal addressin cell adhesion molecule-1 (MadCAM-1) present on vascular endothelial cells of the gastrointestinal tract. Disruption of molecular interactions would specifically prevent transmigration of leukocytes across the endothelium into an inflamed tissue in diseases such as multiple sclerosis or Crohn's disease, without causing whole-body immunosuppression.

Multiple sclerosis is a demyelinating immune-mediated disease of the brain and spinal cord.[22] The disease is composed of two phases, the relapsing-remitting phase, which lasts for many years, and the treatment-resistant secondary progressive phase. The relapsing-remitting phase is characterized by an intermittent development of an inflammatory demyelinating reaction, which is caused by infiltrating T lymphocytes. In clinical trials of patients with relapsing forms of multiple sclerosis, natalizumab was shown to decrease the rate of clinical relapse and inhibit clinical progression to the secondary phase.[23,24] It was first approved in the United States of America (U.S.A.) in November 2004 on the basis of an expedited review of 1-year results of two clinical trials planned for 2 years.[2]

NATALIZUMAB AND PML

Shortly after the approval, it was found that two patients in the clinical trials, who were treated with natalizumab and interferon beta for 2–2.6 years, developed PML[4,5] (Table 1). It is a lethal opportunistic infection of CNS; one patient died and one patient survived but with a serious disability. Another patient in the clinical trial for Crohn's disease died of PML.[3] The marketing of natalizumab was suspended in February 2005.

PML is caused by the JC polyoma virus (named after the patient from whom the virus was first isolated).[25,26] Apparently, infection by the JC virus occurs asymptomatically in early life and 80–90% of the population are seropositive. The virus latently infects tissues such as those in kidneys and bone marrows (lymphoid tissues). PML is considered to occur following reactivation of the virus in the presence of immunosuppression, but the occurrence is low except among patients with AIDS.

The occurrence of PML was totally unexpected, and how PML occurs in natalizumab-treated patients is as yet not completely understood. However, a reduced surveillance of CNS owing to an attenuated migration of T lymphocytes is likely in-

Table 1. Overview of Examples of Side Effects of Molecularly Targeted Drugs

Drug (Class)	Approval year (Country or Region)	Mechanism (Drug effect)
Natalizumab (Selective adhesion molecule inhibitors; $\alpha_4$integrin antagonist; antibody)	2004 (U.S.A.)[a)] 2006 (E.U.)[b)]	Disruption of $\alpha_4$integrin-mediated interaction between leukocytes and activated vascular endothelial cells, selectively prevents transmigration of T lymphocytes to CNS in patients with multiple sclerosis, without causing general immunosuppression.[20,21,37)]
Trastuzumab (Tyrosine kinase inhibitor; antibody)	1998 (U.S.A.) 2000 (E.U.) 2001 (Japan)	Antibody binding to human epidermal growth factor receptor 2 (ErbB2), which is overexpressed in 20–30% of invasive breast cancers, inhibits signal-transduction pathway for cancer cell proliferation and/or activates antibody-dependent cell-mediated tumor cell lysis.[36)]
Imatinib (Tyrosine kinase inhibitor; small-molecule inhibitor)	2001 (U.S.A.) 2001 (E.U.) 2001 (Japan)	Inhibit dysregulated tyrosine kinase, Bcr-Abl, which is expressed in chronic myelogenous leukemia cells.[1)]
Bevacizumab (Angiogenesis inhibitor; antibody)	2004 (U.S.A.) 2005 (E.U.) 2007 (Japan)	Antibody binding to vascular endothelial growth factor (VEGF) inhibit proliferation of tumor-stimulated endothelial cells.

Drug (Class)	Side effect	Estimated incidence	Mechanism (Side effect)
Natalizumab (Selective adhesion molecule inhibitors; $\alpha_4$integrin antagonist; antibody)	Progressive multifocal leukoencephalopathy[3–5)]	0.01%[d), 30)]	Opportunistic infection of oligodendrocytes by JC polyoma virus owing to reduced immunosurveillance in CNS; however, because there was no increased incidence of other opportunistic CNS infections, additional mechanism is likely involved.[2,27,28)]
Trastuzumab (Tyrosine kinase inhibitor; antibody)	Cardiomyopathy[c), 6,38,39)]	27% concurrent with AC[e), 6,38)] 4.1% with paclitaxel post-AC[f), 39)]	Antibody binding to ErbB2 modulates Bcl protein expression in cardiomyocytes resulting in mitochondrial dysfunction, ATP deficiency, and stress-induced hypertrophy.[34,41–43)]
Imatinib (Tyrosine kinase inhibitor; small-molecule inhibitor)	Cardiomyopathy[c), 7)]	0.8%[g), 47)]	Inhibition of c-Abl in cardiomyocytes activates endoplasmic reticulum stress response pathway, resulting in mitochondrial dysfunction, abnormal mitochondrial morphology and cell death.[7)]
Bevacizumab (Angiogenesis inhibitor; antibody)	Hypertension[8,51,52)]	10–20%[h), 8,51,52)]	Inhibition of release of the vasodilators by endothelial cells.[14)] Decrease in the density of capillaries and arterioles owing to inhibition of new vessel formation, resulting in peripheral vascular resistance.[14)]

a) approved in November 2004 for indication of multiple sclerosis by expedited review process; suspended in February 2005 owing to three cases of progressive multifocal leukoencephalopathy; reintroduced in 2006 under special restricted distribution program; indication extended to Crohn's disease in 2008. *b*) approved in 2006 for indication of multiple sclerosis; application for indication of Crohn's disease denied in 2007. *c*) decrease in left ventricular ejection fraction (LVEF), signs or symptoms of congestive heart failure. *d*) among approximately 3000 patients in clinical trial, mean treatment duration of 18 months, *e*) retrospective analysis, 8% of patients treated with AC alone; 3–7% of patients treated with trastuzumab alone, many of the patients had prior anthracycline treatment, *f*) prospective analysis, 0.8% of those treated with paclitaxel alone post-AC, *g*) retrospective analysis of 1276 patients, *h*) hypertension requiring therapy. Abbreviations: AC, anthracyclines plus cyclophosphamide; CNS, central nervous system; U.S.A., United States of America; E.U. European Union.

volved. The counts of leukocytes in cerebrospinal fluid including $CD4^+$ and $CD8^+$ T lymphocytes are markedly low in natalizumab-treated patients.[27,28)] However, an increase in the incidence of opportunistic infections of CNS other than PML has not been noted in natalizumab-treated patients. Therefore, some other mechanisms are likely involved. In the bone marrow, $\alpha_4\beta_1$ integrin plays roles in adhesion of hematopoietic progenitor cells to stromal cells. It has been proposed that natalizumab may

mobilize B lymphocytes from the bone marrow, and latently infected B lymphocytes may carry the virus to CNS.[29)]

RISK-BENEFIT ANALYSIS OF NATALIZUMAB

After the marketing suspension, the occurrence of PML was thoroughly reviewed. Among 3000 patients treated with natalizumab in clinical trials for multiple sclerosis, Crohn's disease, or rheumatoid arthritis, no other patient was found to have PML; therefore, the estimated incidence of PML among natalizumab-treated patients was 1.0 case per 1000 patients (95 percent confidence interval, 0.2 to 2.8 per 1000 who received a mean of 18-month treatment).[30)] It was also found that PML occurred in patients using natalizumab with interferon beta; therefore, it was considered that the use of interferon beta might have contributed to the development of PML.

In the U.S.A., natalizumab was reintroduced in 2006 as a monotherapy agent for treatment of multiple sclerosis under a restricted distribution program, in which patients were closely monitored for signs and symptoms of PML.[19)] Natalizumab was also approved in the European Union in 2006 for the treatment of highly active relapsing remitting multiple sclerosis as a monotherapy agent.[31)]

Natalizumab was later applied for the treatment of Crohn's disease. In the United State, the indications for natalizumab were expanded to include Crohn's disease.[19)] However, in the European Union, the application of natalizumab for the treatment of Crohn's disease was not approved because it was considered that the benefits of using natalizumab in the treatment of Crohn's disease did not outweigh its risks.[32)] The effect of natalizumab on Crohn's disease was examined in two main studies.[33)] In the first study, the effects of starting treatment with natalizumab and placebo were compared and the response rates (the proportion of patients whose symptoms improved after 10 weeks) were found to be similar. In the second study, patients who responded to the treatment with natalizumab were randomly reassigned to natalizumab or placebo every four weeks until week 36. Continuing natalizumab in the second study resulted in higher sustained response rates.

Because treatment options for multiple sclerosis are limited and natalizumab was shown to have marked efficacy in decreasing the incidence of clinical relapse and inhibiting clinical progression compared with previously available treatment options, the risk-benefit balance of natalizumab was considered to be tolerable with the introduction of measures to minimize risks of PML. However, for Crohn's disease, the clinical efficacy of natalizumab is modest, and the European Union decided that in light of significant concern for the occurrence of PML, which is fatal and has no known treatment, the risk-benefit balance is not sufficiently favorable to approve the expansion of indications.

TYROSINE KINASE INHIBITORS AND CARDIOTOXICITY

Although it was initially assumed that tyrosine kinase inhibitors affect cancer cells that actively express targeted tyrosine kinases and not other types of cell, it has become evident that this simple assumption is not necessarily correct. Notably, some tyrosine kinase inhibitors affect the survival of cardiomyocytes.[34, 35)] Here, we will focus on trastuzumab, which targets human epidermal growth factor receptor type 2 (ErbB2, also known as HER2), and imatinib, which targets nonreceptor Abl kinase (Table 1). The toxicity against the survival and function of cardiomyocytes is not a class effect of tyrosine kinase inhibitors. Those targeting epidermal growth factor receptor (EGFR) have low cardiotoxicity.[35)]

TRASTUZUMAB AND CARDIOTOXICITY

Trastuzumab is a monoclonal antibody that binds to ErbB2, which is overexpressed in 20 to 30% of invasive breast cancers.[36, 37)] It was the first anticancer tyrosine kinase inhibitor and approved in 1998 (2001 in Japan). It was also the first tyrosine kinase inhibitor whose cardiotoxicity was recognized (Table 1). The cardiotoxicity of trastuzumab was found in a pivotal efficacy clinical trial.[6)] The subsequent retrospective evaluation of data from multiple clinical trials (1219 treated patients) has shown that cardiomyopathy characterized by a decreased left ventricular ejection fraction (LVEF) or symptoms of congestive heart failure occurred in 27% of patients treated concurrently

with anthracyclines plus cyclophosphamide (AC) and trastuzumab (39/143), 8% of those treated with AC alone (11/135), and 3–7% of those treated with trastuzumab alone (3/114, 11/213, and 3/46, respectively, in three trials).[38] Most of these patients had received prior anthracycline therapy, which by itself is toxic to cardiomyocytes.

Later, cardiac events, defined as severe congestive heart failure [New York Heart Association (NYHA) class III or IV] or cardiac death, were monitored prospectively in a trial, in which paclitaxel with or without trastuzumab were administered to patients following AC.[39] Among patients with normal post-AC LVEF who proceeded to post-AC treatment, 31 of 850 trastuzumab-treated patients, and 5 of 814 control patients had cardiac events. The estimated cumulative incidence at 3 years was 4.1% for trastuzumab-treated patients, compared with 0.8% for control patients; the difference was 3.3% (95 percent confidence interval, 1.7% to 4.9%). In this trial, when an asymptomatic decrease in LVEF from the baseline did not remain in predefined limits, trastuzumab treatment was discontinued, and 14% of trastuzumab-treated patients discontinued treatment owing to an asymptomatic decrease in LVEF before 52 weeks. The cardiac function of those who discontinued treatment responded to standard medical treatment, and the cardiotoxicity of trastuzumab is considered to be generally reversible.[38–40]

MOLECULAR MECHANISMS UNDERLYING TRASTUZUMAB CARDIOTOXICITY

The above-mentioned finding led to the study of the function of ErbB2 in adult cardiomyocytes, and laboratory data have shown that ErbB2 plays important roles in cardiomyocyte survival and function.[34,35] Cardiomyocytes from conditional mutant mice with cardiac specific deletion of ErbB2 developed dilated cardiomyopathy, including wall thinning and decreased contractility.[41] Because hypertrophy is generally an indicator of stress, it can be interpreted as a consequence of stress caused by an underlying functional deficit due to ErbB2 dysfunction.[42] It is proposed that ErbB2 inhibition by an anti-ErbB2 antibody increases the expression level of the pro-apoptotic Bcl-2 family protein Bcl-xS, while decreasing that of anti-apoptotic Bcl-xL; this leads to mitochondrial translocation and oligomerization of the bcl-associated protein (Bax) and subsequent cytochrome c release.[43] Because an abundant ATP supply is essential for cardiomyocytes to contract, deficits in mitochondrial function and ATP store will result in compromised functions.

IMATINIB AND CARDIOTOXICITY

Imatinib is an inhibitor of Bcr-Abl, a constitutively active form of a nonreceptor tyrosine kinase, c-Abl, which is encoded by the Philadelphia chromosome of chronic myelogenous leukemia.[1,44] It was approved in 2001, and was the first successful small-molecule inhibitor of tyrosine kinase (Table 1). It is an ATP-competitive inhibitor and a relatively specific inhibitor of Abl, but it also inhibits platelet-derived growth factor and c-Kit.[45]

That imatinib may also have cardiotoxicity was reported in 2005.[7] Ten cases of severe congestive failure were reported; all ten patients had normal LVEF prior to treatment, but presented, after a mean of 7.2 ± 5.4 months (range, 1–14 months) of therapy, with heart failure with symptoms corresponding to NYHA class III or IV. Myocardial biopsies of two of these patients showed mitochondrial abnormalities and accumulation of membrane whorls in the sarco- (endo-) plasmic reticulum. Experiments using mice and culture cells showed that imatinib induces activation of the endoplasmic reticulum stress response (also known as the unfolded protein response), which can lead to cell death via several pathways.[7,46] Imatinib activated two distinct pathways—the protein kinase RNA-like endoplasmic reticulum kinase (PERK) pathway and the inositol-requiring protein-1 (IRE-1) pathway, a dual protein kinase and endoribonuclease pathway. The IRE-1 pathway activates Jun N-terminal kinases (JNKs), which then leads to translocation of Bax to mitochondria and cytochrome c release. These responses were shown to be mediated by inhibition of c-Abl.

The incidence of cardiomyopathy in association with imatinib use has not been prospectively assessed; however, it may be low. In a retrospective analysis of 1276 patients participating in clinical trials at an institution, 0.8% was identified as having symptoms that may be attributed to systolic heart failure due to the use of imatinib.[47] Most of these patients had previous medical conditions predispos-

ing them to cardiac failure. Most of the ten patients reported earlier also had predisposing conditions including hypertension, diabetes, and coronary artery disease.[48)]

ANGIOGENESIS INHIBITOR, BEVACIZUMAB, AND HYPERTENSION

Under normal physiological conditions, most endothelial cells are quiescent, and tumor-stimulated endothelial cells have a unique proliferating phenotype.[14)] It was assumed that targeting this phenotype would be specific, and no major side effects would occur. Presently, it seems that the importance of growth factor-induced activation of signalling pathways in endothelial cells for maintaining homeostasis of the body was underestimated. Bevacizumab, a monoclonal antibody that binds to vascular endothelial growth factor (VEGF), was the first drug developed as an angiogenesis inhibitor and was approved in 2004 (2007 in Japan). Gastrointestinal perforations and wound healing complications are now among the well-known side effects described in the boxed warning section of the labeling. However, here, we would like to focus on hypertension, which is a relatively common side effect of angiogenesis inhibitors.[49, 50)]

In the phase 2 and phase 3 clinical trials of bevacizumab, 10–20% of bevacizumab-treated patients experienced Grade 3 hypertension (or that requiring therapy), compared with 0.5–2.3% of placebo-treated patients.[8, 51, 52)] In one study, the median interval of days from the first dose of bevacizumab to the onset of hypertension was 131 days (range, 7 to 316 days).[8)] These episodes of hypertension are manageable with standard oral antihypertensives and usually no discontinuation of treatment is necessary. However, proper monitoring of blood pressure is necessary for patients treated with bevacizumab.

The importance of VEGF for blood pressure regulation was indicated by in vivo experiments.[14)] Endothelial cells promote vasodilation by secreting nitric oxide (NO) and prostacyclin (PGI_2). VEGF induces endothelial cells to release these factors. Therefore, blocking the VEGF activation signalling pathway will decrease the production of these vasodilators. In addition, it has been proposed that a decrease in the density of capillaries and arterioles owing to inhibition of new-vessel formation may also play a role, resulting in peripheral vascular resistance.[14)]

Sorafenib and sunitinib are small-molecule inhibitors that target multiple tyrosine kinases (approved in 2005 and 2006, respectively; both approved in Japan in 2008). Their targets include the VEGF receptor (a receptor tyrosine kinase); therefore, they have an angiogenesis inhibitory effect. Hypertension is one of their side effects.[14, 34)]

CONCLUSIONS AND FUTURE PERSPECTIVES

We have reviewed the clinical characteristics of some examples of the side effects of molecularly targeted drugs and proposed molecular mechanisms underlying such side effects (Table 1). These side effects were not expected, and in some cases, the side effects led to the clarification of previously unknown regulations or roles of targeted molecules. However, information is still limited, and further study is necessary.

In August 2008, two patients with PML were newly identified among those using natalizumab as monotherapy.[53)] Both patients received natalizumab for more than one year; these were among 12000 patients who received natalizumab more than one year worldwide. Therefore, it is not certain yet whether the current assumption that restriction of the use as monotherapy would reduce the risk of PML is appropriate as a risk minimization measure. Because the mechanism by which natalizumab causes PML is not fully understood and because there is no treatment for PML, continuing assessment of the risk of PML will be necessary. Currently, several small-molecule α_4 integrin antagonists are in clinical development.[54)] The risk of PML needs to be carefully monitored for these drugs as well.

Whether molecularly targeted therapy using antibodies and that using small molecules have the same side effects may depend on mechanisms causing the side effects. In the case of cardiotoxicity of trastuzumab, it has been speculated that trastuzumab may trigger a unique intracellular signalling response following its binding to ErbB2.[34)] A dual small-molecule inhibitor of ErbB2 and EGFR has shown minimal cardiotoxicity so far, although definitive safety evaluation must await the completion of ongoing trials. As we have discussed, hypertension caused by angiogenesis inhibitors is

a common side effect of both antibody agents and small-molecule inhibitors.

Currently, nearly 30 tyrosine kinase inhibitors are on the market, and more than one hundred are in clinical trials.[35] There are 518 putative protein kinase genes.[55] Tyrosine kinase inhibitors, particularly small-molecule inhibitors, could target kinases other than those originally intended. As we have discussed, some agents belonging to new groups of molecularly targeted drugs were already found to have hitherto unknown roles in the development of side effects. Therefore, it will not be surprising if more side effects become known as more tyrosine kinase inhibitors are approved for clinical use.

It is likely that as more new molecularly targeted drugs are used clinically, we will learn more about the biology of targeted molecules, which in turn may reveal new pathways for future drug development.

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