A HER2-Targeting Antibody–Drug Conjugate, Trastuzumab Deruxtecan (DS-8201a), Enhances Antitumor Immunity in a Mouse Model

Tomomi Nakayama-Iwata, Chiaki Ishii, Saori Ishida, Yusuke Ogitani, Teiji Wada, and Toshinori Agatsuma

Abstract

Trastuzumab deruxtecan (DS-8201a), a HER2-targeting antibody–drug conjugate with a topoisomerase I inhibitor exatecan derivative (DX-8951 derivative, DXd), has been reported to exert potent antitumor effects in xenograft mouse models and clinical trials. In this study, the immune system-activating ability of DS-8201a was assessed. DS-8201a significantly suppressed tumor growth in an immunocompetent mouse model with human HER2-expressing CT26.WT (CT26.WT/HER2) cells. Cured immunocompetent mice rejected not only rechallenged CT26.WT/HER2 cells, but also CT26.WT-mock cells. Splenocytes from the cured mice responded to both CT26.WT/HER2 and CT26.WT-mock cells. Further analyses revealed that DXd upregulated CD86 expression on bone marrow-derived dendritic cells (DC) in vitro and that DS-8201a increased tumor-infiltrating DCs and upregulated their CD86 expression in vivo. DS-8201a also increased tumor-infiltrating CD8+ T cells and enhanced PD-L1 and MHC class I expression on tumor cells. Furthermore, combination therapy with DS-8201a and anti–PD-1 antibody was more effective than either monotherapy. In conclusion, DS-8201a enhanced antitumor immunity, as evidenced by the increased expression of DC markers, augmented expression of MHC class I in tumor cells, and rejection of rechallenged tumor cells by adaptive immune cells, suggesting that DS-8201a enhanced tumor recognition by T cells. Furthermore, DS-8201a treatment benefited from combination with anti–PD-1 antibody, possibly due to increased T-cell activity and upregulated PD-L1 expression induced by DS-8201a. Mol Cancer Ther; 17(7); 1494–503. ©2018 AACR.

Introduction

Several chemotherapeutic agents are known to induce activation of the immune system (1). Chemotherapeutic agents induce tumor cell death, and an "immunogenic cell death" activates the immune system (1–3). One of the mechanisms of immune activation by chemotherapeutic agents is activation of dendritic cell (DC) function followed by activation of T cells (1, 2, 4). Interestingly, it has been reported that topoisomerase I inhibitors are the agents that stimulate T-cell killing activity (5, 6) and topoisomerase I inhibitors are drawing attention as immunomodulators.

Trastuzumab deruxtecan (DS-8201a) is a HER2-targeting antibody–drug conjugate (ADC), structurally composed of a humanized anti-human HER2 (anti-hHER2) antibody, an enzymatically cleavable peptide-linker, and a topoisomerase I inhibitor, exatecan derivative (DX-8951 derivative, DXd; Supplementary Fig. S1; refs. 7, 8). It has been shown to exhibit an antitumor effect in mouse models (7). Moreover, in a phase I clinical trial, DS-8201a showed antitumor effects in breast, gastric, gastro-esophageal, colorectal, salivary, and non–small cell lung cancer patients (9–11), including ones who had previously been treated with trastuzumab emtansine (T-DM1), an ADC composed of a tubulin polymerization inhibitor, and an anti-hHER2 antibody. Furthermore, DS-8201a was well-tolerated, and the MTD was not reached during dose escalation (0.8–8.0 mg/kg; refs. 9–11). Accordingly, DS-8201a is expected to become a new therapy for HER2-positive tumors. Importantly, athymic nude mouse models had been used for those nonclinical evaluations, and involvement of the immune system in the efficacy of DS-8201a has not yet been elucidated.

Recently, immune checkpoint inhibitors (ICI) have been demonstrated to show remarkable clinical benefits (12–14). Nevertheless, there are many patients who are not sensitive or are refractory to a single agent of ICI, and combination therapies with other drugs are needed (15). Certain chemotherapeutic agents are suggested to show benefits in combination with ICIs in humans (16). This idea is also supported by preclinical mouse studies. Topoisomerase I inhibitors and other chemotherapeutic agents have successfully been combined with ICIs in syngeneic mouse models (4, 5, 17). However, there is a concern that chemotherapeutic agents could induce lymphopenia and thus attenuate the effect of ICIs (2). ADCs could be an ideal alternative option for combination therapy because ADCs are intended to target selectively cancer cells and spare normal cells. T-DM1 and also several ADCs conjugated with tubulysin or pyrrolobenzodiazepine dimer (PBD) showed immune-activating effects and benefit in combination with ICIs in immunocompetent mouse models (18, 19). Moreover, it was reported that a combination of...
brentuximab vedotin, an ADC composed of a tubulin polymerization inhibitor and an anti-human CD30 antibody, and nivo-lumab has synergistic activity in humans (20). To our knowledge, the payloads of ADCs that are currently suggested to be involved in activation of immune system are tubulin inhibitors and a DNA-crosslinking agent, and an immunomodulatory effect of the ADC composed of a topoisomerase I inhibitor has not been reported yet.

In this study, we examined the immunologic effect of DS-8201a and the combination benefit of DS-8201a with anti-PD-1-blocking antibody in a mouse model. Here, we identified a new role of DS-8201a, an immunostimulatory activity that is distinct from the DXd’s cytotoxic activity against tumor cells.

**Materials and Methods**

**Antibodies and compounds**

DS-8201a, DXd, and its parental anti-human HER2 (anti-hHER2) antibody were prepared as previously described (7, 8). Antibodies and compounds used in this study are listed in Table 1.

**Materials and Methods**

**Antibodies and compounds**

DS-8201a, DXd, and its parental anti-human HER2 (anti-hHER2) antibody were prepared as previously described (7, 8).

**Results**

**Cell lines**

Human breast cancer cell line MDA-MB-453 (HTB-131) was purchased from the American Type Culture Collection and was cultured in Leibovitz's L-15 Medium (Thermo Fisher Scientific) supplemented with 10% FBS (Thermo Fisher Scientific) at 37 °C in a free gas exchange with atmospheric air. Human breast cancer cell line KPL-4 was provided from Dr. Kurebayashi at the Kawasaki Medical University (Japan) and was cultured with RPMI1640 medium (Thermo Fisher Scientific) supplemented with 10% FBS at 37 °C and 5% CO2 atmosphere.

**Mouse models, treatments, and analysis of intratumoral cells**

All mouse studies were carried out in an Association for Assessment and Accreditation of Laboratory Animal Care International-approved veterinary research facility and in accordance with the local guidelines of the Institutional Animal Care and Use Committee. Three to 6 mice were housed together in sterilized cages and maintained under specific pathogen-free conditions. The mice were euthanized with CO2 gas when they reached endpoints (tumor volume exceeding 3,000 mm3, 10% reduction of body weight, or clinical signs indicating that mice should be euthanized for ethical reasons). Female BALB/cAnNCrlCrlj (BALB/c) mice, aged 4 weeks to 5 weeks, were purchased from Charles River Laboratories Japan Inc. Mice aged 5 to 6 weeks were inoculated with 5 million CT26.WT-hHER2 cells suspended in saline into the right flank by s.c. injection. Tumor volume was defined as 1/2 × length × width2. When the average volume of tumors reached approximately 100 to 200 mm3, the mice were divided into control and treatment groups based on tumor volumes by using the randomized block method, and treatment was initiated (day 0). DS-8201a (10 mg/kg), anti-hHER2 antibody (10 mg/kg), and anti-PD-1 antibody (5 mg/kg) were administered i.v. at a volume of 10 mL/kg to mice. As a control, ABS buffer (10 mmol/L Acetate Buffer, 5% sorbitol, and pH 5.5) was administered at the same volume as the DS-8201a. DS-8201a and anti-hHER2 antibody were administered on days 0 and 7. Anti-PD-1 antibody was administered on days 0, 3, 7, and 10.

For a rechallenge study, mice whose CT26.WT-hHER2 tumors had been cured by DS-8201a treatment (10 mg/kg, once a week, twice, i.v.) were divided into two groups. The mice were then subcutaneously inoculated with 5 million CT26.WT-mock or CT26.WT-hHER2 cells into the left flank. Naive (never tumor-inoculated) mice were also inoculated with each cell line for comparison.

For flow cytometric analysis of T cells, DCs, and tumor cells, when the average volume of tumors reached approximately 250–400 mm3 (8 days after tumor inoculation), the mice were treated with vehicle or DS-8201a (10 mg/kg, once, i.v.; day 0). The mice were euthanized with CO2, asphyxiation on day 8, and tumors were cut into small pieces and dissociated with the Tumor Dissociation Kit (Miltenyi Biotec) by the gentleMACS Octo Dissociator with Heaters (Miltenyi Biotec). The resultant single cells were blocked with Mouse BD Fc Block reagent (Becton Dickinson) and stained with antibodies against mouse CD3,
hHER2 cells at 37°C, subsequently cocultured with CT26.WT-mock or CT26.WT-hHER2 tumors had been cured by DS-8201a treatment were bearing naïve mice without drug treatment and mice whose CT26.

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The Enzyme-Linked Immunospot (ELISpot) assay

Splenocytes from CT26.WT-mock or CT26.WT-hHER2 tumor-bearing naïve mice without drug treatment and mice whose CT26.

Antitumor effect of DS-8201a in a syngeneic mouse model.

**A**, Expression of exogenously introduced human HER2 gene was confirmed in mouse CT26.

In vitro DC analysis

To obtain bone marrow–derived dendritic cells (BMDC), bone marrow cells from femurs of BALB/c mice were cultured with RPMI 1640 medium supplemented with 10% FBS, 55 μmol/L of 2-mercaptoethanol, 100 U/mL of penicillin, 100 μg/mL of streptomycin, 1 mmol/L sodium pyruvate, 1 mmol/L nonessential amino acid, 2 mmol/L of L-glutamine, and 10 ng/mL of recombinant murine GM-CSF (DC culture medium) for 11 days. The resultant BMDCs were cultured in DC culture medium supplemented with DXd (0.0, 0.0625, 0.125, 0.25, 0.5, and 1.0 μmol/L), or dimethyl sulfoxide as a vehicle control. After 24 hours of cell culture, the cells were harvested, blocked with Mouse BD Fc Block reagent, and stained with antibodies against mouse CD11c, CD45, CD86, and MHC class II.

Statistical analysis

All statistical analyses were performed using SAS System Release 9.02.300 (SAS Institute Inc.). Antitumor effects, rechallenge study, and ELISpot assays were analyzed with a Wilcoxon rank-sum test, and flow cytometric data were analyzed with the Student t test. To analyze efficacy of combination therapy, the Kaplan–Meier analysis followed by the log-rank test was performed for comparisons of the survival curve. Bonferroni correction was applied for multiple comparisons. A P value less than 0.05 was considered to be statistically significant.

Results

Antitumor effect of DS-8201a in an immunocompetent mouse model

Because the parental anti-hHER2 antibody of DS-8201a does not cross-react with mouse HER2, we prepared a mouse cell line that stably expresses the human target gene (22). Human HER2 was expressed on CT26.WT-hHER2 cells at a comparable level to other representative human HER2-positive (around 2–3× determined by Herceptest) cancer cell lines, MDA-MB-453 (25, 26) and KPL-4 (refs. 7, 27; Fig. 1A). The CT26.WT-hHER2 cells were inoculated into BALB/c mice subcutaneously, and tumor growth was confirmed (Fig. 1B). When mice with CT26.WT-hHER2 tumors were i.v. treated with vehicle, 10 mg/kg of DS-8201a, or 10 mg/kg of its parental anti-hHER2 antibody on days 0 and 7, the mean tumor volumes at day 9 of vehicle-treated, DS-8201a-treated, and anti-hHER2 antibody–treated groups were 936, 402, and 969 mm³, respectively (Fig. 1B). Compared with anti-hHER2 antibody, DS-8201a showed a significant antitumor effect (P = 0.0091 at day 9). Importantly, DS-8201a also exerted a significant antitumor effect compared with the isotype control nontargeted ADC (Supplementary Fig. S2). These results indicate that the CT26.WT-hHER2 model can be used for further studies and that the antitumor effect of DS-8201a in this model depends mainly on the payload delivered into tumor by anti-hHER2 antibody and not on the anti-hHER2 antibody itself.

Contribution of DS-8201a to immune memory formation

In a different experiment, a complete response (CR) was seen in 23% (18/80) of CT26.WT-hHER2 (s.c. inoculation)–bearing mice treated with DS-8201a (10 mg/kg, once a week, 2 times, i.v.). These cured mice were rechallenged with CT26.WT-hHER2 or CT26.WT-mock cells (s.c. inoculation). Naïve mice (previously...
not inoculated with tumor cells) were used as a control cohort. CT26.WT-hHER2 cells were completely rejected in the rechallenged mice (Fig. 2A and B). Furthermore, CT26.WT-mock cells were also rejected in the cured mice from the CT26.WT-hHER2 tumor, although to a lesser extent (Fig. 2A and C). We confirmed that the CT26.WT-mock and CT26.WT-hHER2 cells can grow normally in naïve mice (Fig. 2A, D, and E). These results suggest that multiple antigens other than human HER2 were recognized.
by the immune system in mice cured of CT26.WT-hHER2 tumors by DS-8201a. This was also supported by an increase of IFNγ from splenocytes. IFNγ is a cytokine that is produced mainly by activated T cells and natural killer (NK) cells and which has antitumor effects (28, 29). At the endpoint of the rechallenge study, splenocytes were isolated from each mouse. The splenocytes from the naïve mice challenged with CT26.WT-hHER2 without DS-8201a treatment rarely reacted to CT26.WT-hHER2 and CT26.WT-mock cells in terms of IFNγ secretion, whereas the splenocytes from the mice that had been cured from CT26.WT-hHER2 tumor by DS-8201a treatment and rechallenged with CT26.WT-hHER2 cells were significantly activated by not only CT26.WT-hHER2 cells but also CT26.WT-mock cells (Fig. 3A). The results suggest that DS-8201a treatment induced T cells that recognize not only human HER2 but also other antigens of tumor cells. Similarly, the splenocytes from the naïve mice challenged

<table>
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<th>Mice</th>
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<td>-</td>
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<td>CT26.WT-mock</td>
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**Figure 3.** IFNγ secretion by splenocytes from the rechallenged mice. Splenocytes were obtained from rechallenged mice previously cured of CT26.WT-hHER2 tumors by DS-8201a and from naïve mice challenged with CT26.WT-mock or CT26.WT-hHER2 cells. IFNγ secretion was examined by ELISPOT assays. The number of spots was regarded as number of IFNγ-secreting splenocytes. A, Splenocytes from the rechallenged mice with CT26.WT-hHER2 cells were cultured with CT26.WT-Her2 or CT26.WT-mock cells, and immune cell reactivity to tumor cells was determined by IFNγ secretion. B, Splenocytes from the rechallenged mice with CT26.WT-mock cells were cultured with CT26.WT-Her2 or CT26.WT-mock cells, and immune cell reactivity to tumor cells was determined by IFNγ secretion. Graphs show the mean number of IFNγ-secreting splenocytes and standard errors (n = 9). A Wilcoxon rank-sum test was conducted for the comparison between naïve mice and cured mice.
Immunostimulatory Activity of DS-8201a, a HER2-Targeting ADC

with CT26.WT-mock without DS-8201a treatment rarely reacted to CT26.WT-mock and CT26.WT-hHER2 cells in terms of IFNγ secretion, whereas the splenocytes from the mice that had been cured of CT26.WT-hHER2 tumors by DS-8201a treatment and rechallenged with CT26.WT-mock cells were significantly activated by not only CT26.WT-mock cells but also CT26.WT-hHER2 cells compared with cells from the naive mice (Fig. 3B).

**Contribution of adaptive immunity for effect of DS-8201a**

To examine whether adaptive immune system is involved in the antitumor effect of DS-8201a, athymic nude mice with defective T and B cells and robust NK cells (30–32) were s.c. inoculated with CT26.WT-hHER2 cells and treated with DS-8201a (10 mg/kg, once a week, twice, i.v.). In contrast with the immunocompetent mouse model (Fig. 1B), only a slight antitumor effect of DS-8201a was observed in the immunocompromised athymic nude mouse model (Supplementary Fig. S3). Actually, a cell proliferation assay of CT26.WT-hHER2 showed it to be less sensitive to DS-8201a and its payload DXd, compared with human cancer cell line EMT6-hHER2 (Supplementary Fig. S4). These data suggested that even in the tumors less sensitive to cytotoxic activity of DS-8201a, adaptive immunity was activated and played important roles in antitumor effect observed in the immunocompetent mouse model.

Next, intratumoral T cells were examined in the immunocompetent mouse model with CT26.WT-hHER2 (s.c. inoculation). Eight days after DS-8201a (10 mg/kg, once, i.v.) treatment, the ratio of DCs (CD45+CD11c+MHC class II+ cells) to lymphocytes (CD45+CD3+CD8+ cells) and Granzyme B+ CD8+ T cells significantly increased compared with vehicle controls (Fig. 4). The increased CD8+ cells were also observed by immunohistochemical staining (Supplementary Fig. S5). Notably, when CD8+ cells were depleted by an anti-CD8 depletion antibody, the antitumor effect of DS-8201a was negated (Supplementary Fig. S6). These data suggested that adaptive immunity, especially CD8+ T cells, was involved in the antitumor effect.

**Increased expression of DC activation markers by topoisomerase I inhibitor, DXd**

It has been reported that various chemotherapeutic agents, including topoisomerase 1 inhibitors, activate DCs (1, 2, 4, 6, 33). Therefore, DC maturation and activation markers, CD86 and MHC class II, on mouse BMDCs were evaluated to investigate the direct effect of DXd, the payload of DS-8201a, on BMDCs. In the presence of DXd, CD86 and MHC class II expression increased in a concentration-dependent manner 24 hours after culture (Fig. 5A). These results showed that the payload of DS-8201a directly increased DC activation markers and suggested that this mechanism was associated with increased antitumor immunity.

**Upregulation of activation marker levels on intratumoral DCs by DS-8201a**

Because DXd upregulated activation markers of BMDCs in vitro, the effect of DS-8201a on intratumoral DCs was examined in the immunocompetent mouse model with CT26.WT-hHER2 (s.c. inoculation). Eight days after DS-8201a (10 mg/kg, once, i.v.) treatment, the ratio of DCs (CD45+CD11c+MHC class II+ cells) to lymphocytes (CD45+ cells) significantly increased in DS-8201a-treated tumors compared with vehicle controls (Fig. 5B). Moreover, the percentage of CD86+ cells in DCs as well as mean fluorescence intensity (MFI) of CD86 on DCs

**Figure 4.**
Increase of intratumoral CD8+ T cells. Effect of DS-8201a (10 mg/kg, once, i.v.) was examined in the immunocompetent mouse model subcutaneously inoculated with the CT26.WT-hHER2 cells. The expression levels of CD45, CD3, CD8, CD4, and Granzyme B+ cells were also observed by flow cytometry after the treatment. A, Percentages of CD4+ T cells (CD45+CD3+CD4+ cells) among live cells. B, Percentages of CD8+ T cells (CD45+CD3+CD8+ cells) among live cells. C, Percentages of Granzyme B+ cells among CD8+ T cells. D, Percentages of Granzyme B+ CD8+ T cells among live cells. The open circles show individual data, and the black squares show mean with standard errors (n = 8). The Student t test was conducted for the comparison between the DS-8201a-treated and vehicle-treated groups.
significantly increased in DS-8201a-treated tumors compared with those of vehicle control (Fig. 5C and D). Based on these results, we confirmed that DS-8201a activates DCs in vivo. This is a new role of DS-8201a that is distinct from its previously observed cytotoxic activity (7).

**Increased expression of immune-associated molecules on tumor cells by DS-8201a**

To examine whether DS-8201a modulates immune-associated markers on tumor cells, PD-L1 and MHC class I expression levels were determined in the immunocompetent mouse model with CT26.WT-hHER2 (s.c. inoculation). Eight days after DS-8201a treatment (10 mg/kg, once, i.v.), MFI of PD-L1 and MHC class I on hHER2+ cells was higher in DS-8201a-treated tumors than in vehicle controls (Fig. 5E and F). These results are consistent with previous reports of certain chemotherapeutic agents upregulating PD-L1 and MHC class I expression on tumor cells (8, 14). Further experiments in vitro suggested that DXd did not obviously change PD-L1 expression on CT26.WT-hHER2 cells (unpublished observation). In contrast, DS-8201a directly increased MHC class I expression on CT26.WT-hHER2 cells in vitro (Supplementary Fig. S7A); the effect was comparable at lower dose and even more...
ICIs, especially anti-PD-1 blocking antibody. In vivo effect of DS-8201a (10 mg/kg, once a week, twice, i.v.) combined with anti-PD-1 antibody (5 mg/kg, twice a week, 2 cycles, i.v.) was compared with each agent individually in the immunocompetent mouse model s.c. inoculated with CT26.WT-hHER2 cells. Compounds were administered at the time points indicated by arrows. Survival was analyzed by the Kaplan-Meier method, and groups were compared with the log-rank test (n = 20). Vehicle-treated group was compared with DS-8201a-treated group and anti-PD-1 antibody-treated group (P = 0.0001 and P = 0.0000, respectively). DS-8201a and anti-PD-1 antibody–treated group was also compared with the DS-8201a–treated group and the anti-PD-1 antibody–treated group (P = 0.0006 and P < 0.0001, respectively).

In vivo combination effect of DS-8201a with an anti–PD-1 antibody

Whereas DS-8201a demonstrated immune-modulating activity, the expression of PD-L1, an immune-inhibiting molecule, increased during DS-8201a treatment. Therefore, the combination of DS-8201a with an anti–PD-1 antibody was examined in the immunocompetent mouse model with CT26.WT-hHER2 (s.c. inoculation). When mice were treated with vehicle, DS-8201a (10 mg/kg, once a week, 2 times, i.v.), anti–PD-1 antibody (5 mg/kg, twice a week, 2 cycles, i.v.), or a combination of the two, the survival rates at day 38 were 9%, 20%, 20%, and 80%, respectively (Fig. 6). DS-8201a or anti–PD-1 antibody monotherapy extended the overall survival time (vs. vehicle, P = 0.0001 and P = 0.0010, respectively), and the combination of DS-8201a and anti–PD-1 antibody further increased the overall survival time compared with each monotherapy (P = 0.0006 vs. DS-8201a, P = 0.0001 vs. anti–PD-1 antibody). The number of mice that achieved a CR with vehicle, DS-8201a, anti–PD-1 antibody, and combination of DS-8201a and anti–PD-1 antibody were 0, 4, 2, and 13 out of 20, respectively. These results suggest that the PD-1/PD-L1 pathway inhibits activation of the immune system by DS-8201a. Thus, the anti–PD-1 antibody can block the PD-1/PD-L1 inhibitory signal and increase the efficacy of DS-8201a. Therefore, the benefit of combination therapy with DS-8201a and anti–PD-1 antibody is strongly supported. This combination therapy is expected to be a new effective treatment for HER2-positive tumors.

Discussion

A variety of preclinical studies have been conducted using immunocompromised mouse models (xenograft mouse models) to see the direct effect of agents on human-derived tumor cells. In such experiments, the importance of the immune system, especially T and B cells, tended to be overlooked, as their functions and/or numbers are impaired in the models. In our studies, we examined the immunologic effect of DS-8201a by using an immunocompetent mouse model and identified a novel role of DS-8201a. DS-8201a activated the mouse immune system and attenuated tumor growth. The importance of the immune system for the antitumor effect of DS-8201a was supported by the increased expression of MHC class I in tumor cells, the increased expression of DC activation markers, the increase of tumor–infiltrating CD8+ T cells, the negation of DS-8201a antitumor effect by CD8+ cell depletion or in athymic nude mice, and the rejection of rechallenged tumor cells in the immunocompetent mouse model.

The parental anti-hHER2 antibody and isotype control non–targeted ADC did not show the obvious antitumor effect in the model. Therefore, the antitumor effect of DS-8201a is primarily dependent on the payload delivered into HER2-expressing tumors by the antibody. Since it has been reported that anti-ErbB2 antibody itself can promote antitumor immunity and shows combination benefit with anti–PD-1 antibody in a mouse model, a larger DS-8201a effect would be expected if tumors are HER2 signal dependent (35). Remarkably, the mice whose CT26.WT-hHER2 tumors had been cured by DS-8201a treatment rejected not only rechallenged CT26.WT-hHER2 but also CT26.WT-mock cells, suggesting that the antigens were not limited to human HER2 but also to other CT26.WT cell–derived molecules. Multiple recognition of tumor antigens by the immune system was also suggested by the experiments where splenocytes from the CT26.WT-hHER2–rejected mice were reactive even against CT26.WT-mock cells. Previous evidence also suggests the spreading of antigens by chemotherapeutic agents or antibodies (15), and the mode of action of DS-8201a is possibly associated with antigen spreading where tumor cells are killed by the payload, and tumor antigens are recognized by the DS-8201a–elicited immune response. Notably, although direct antitumor effect of DS-8201a on CT26.WT-hHER2 cells was not substantial in the cultured cells (Supplementary Fig. S4) and the nude mouse model (Supplementary Fig. S3), immune cells recognized the tumor antigens (Fig. 3) and mediated antitumor effect in the immunocompetent mouse model (Fig. 1B; Supplementary Fig. S6). These results support the idea that small fraction of tumor cell death could be enough to induce immune reaction and subsequent immune-mediated antitumor activity in case of DS-8201a. This idea is also supported by another type of payload (19).

The payload of DS-8201a has 10 times more potent topoisomerase I inhibitory activity than SN-38 (7), and this could lead to more immunologic cell death than treatment with SN-38 and other existing topoisomerase I inhibitors. In addition, DS-8201a...
works selectively at the tumor site; therefore, it kills tumor cells with minimal damage to immune cells compared with traditional systemic treatment with chemotherapeutic agents, possibly leading to efficient activation of antitumor immunity via release of tumor cell antigens (2, 36). Therefore, DS-8201a can be considered a potent immune stimulator, and further analyses to show whether DS-8201a induces immunologic cell death are needed.

Mechanisms underlying the increased immunity and the immunologic memory effect would rely on a series of processes including cell death, activation of DCs, T-cell activation, and possibly others. DS-8201a upregulated the expression of DC maturation and activation markers both in vitro and in vivo and increased the intratumoral DC population in vivo. A recent report shows that a topoisomerase I inhibitor activates DCs via cancer cells (6). These results indicated that topoisomerase I inhibitors including DXd were potentially associated with DC activation. In addition, DXd directly upregulated MHC class I expression on tumor cells in vitro, and DS-8201a enhanced MHC class I expression on tumor cells in vivo. The increased MHC class I expression possibly contributed to the increased immune activity and immunologic memory formation. Recently, three different topoisomerase I inhibitors (camptothecin, topotecan, irinotecan) have been shown to enhance tumor-killing activity of T cells (5). Our results in this study also support this idea. Although the antitumor effect of DS-8201a was significant, the effect was not sufficient to show CR, as only approximately 20% of tumor-bearing mice with DS-8201a achieved CR in the CT26 WT-hHER2 model. The reason could be that DS-8201a also upregulated tumor PD-L1 expression as the consequence of T-cell activation. Therefore, we combined DS-8201a and anti–PD-1 antibody, and the combination increased the antitumor effect.

ICIs show remarkable antitumor effects in patients with various cancers (12–14). However, only a limited number of patients respond to ICIs (15). Therefore, new treatments for poor responders are needed. One solution to this problem is combination therapy. Combination benefits of HER2-targeting therapy with ICIs have been shown in mouse models (18, 35) and are currently being evaluated in clinical trials (37). For combination with ICIs, ADCs are considered to be better than chemotherapeutic agents because ADCs work selectively at the tumor site with reduced side effect to other organs. T-DM1 has shown immunomodulatory activity and benefits in combination with ICs in vivo, and DM1, a component of T-DM1, activates DCs in vitro (18).

In this study, DS-8201a, a HER2-targeting ADC with a topoisomerase I inhibitor, showed an antitumor effect with immunostimulatory activity in a mouse model. DS-8201a also benefited from combination with anti–PD-1 antibody, as evidenced by the following results: (1) DS-8201a increased MHC class I expression on tumor cells, in addition to their cytotoxic activity, (2) DS-8201a upregulated DC activation markers, (3) DS-8201a activated an adaptive immune response via (1) and (2), followed by PD-L1 expression, and (4) the combination of DS-8201a and anti–PD-1 antibody showed higher antitumor effect. Further, DXd-based ADC technology is widely capable of being applied to various antibodies. Theoretically, other DXd-based ADCs with the same payload, but different antibodies, would show similar immunostimulatory activity. The results of this study provide preliminary evidence for the potential role of DXd-based ADCs and ICIs. Further studies including clinical studies are needed to better understand the role of this combination in cancers.

Disclosure of Potential Conflicts of Interest
T.N. Iwata, C. Ishii, S. Ishida, and T. Wada have ownership interest (including patents) in a patent application. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions
Conception and design: T.N. Iwata, T. Wada, T. Agatsuma
Development of methodology: T.N. Iwata
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T.N. Iwata, C. Ishii, S. Ishida
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T.N. Iwata
Writing, review, and/or revision of the manuscript: T.N. Iwata, Y. Ogitani, T. Wada, T. Agatsuma
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Y. Ogitani
Study supervision: Y. Ogitani, T. Wada, T. Agatsuma

Acknowledgments
This study was funded by Daiichi Sankyo Co., Ltd. The authors would like to thank Yuki Abe, Kyoyoshi Sugihara, Masaya Ishizaki, Kyoko Harikae, Masato Hata, Takafumi Otsuka, Jun Harada, Hirokazu Ishikawa, Megumi Minami, Yoshinori Kashimoto, and other colleagues in the facility for critical review, comments, and materials.

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Received August 9, 2017; revised December 7, 2017; accepted April 17, 2018; published first April 27, 2018.

References


Molecular Cancer Therapeutics

A HER2-Targeting Antibody–Drug Conjugate, Trastuzumab Deruxtecan (DS-8201a), Enhances Antitumor Immunity in a Mouse Model

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