Antibody to CD137 Activated by Extracellular Adenosine Triphosphate Is Tumor Selective and Broadly Effective In Vivo without Systemic Immune Activation


ABSTRACT

Agonistic antibodies targeting CD137 have been clinically unsuccessful due to systemic toxicity. Because conferring tumor selectivity through tumor-associated antigen limits its clinical use to cancers that highly express such antigens, we exploited extracellular adenosine triphosphate (exATP), which is a hallmark of the tumor microenvironment and highly elevated in solid tumors, as a broadly tumor-selective switch. We generated a novel anti-CD137 switch antibody, STA551, which exerts agonistic activity only in the presence of exATP. STA551 demonstrated potent and broad antitumor efficacy against all mouse and human tumors tested and a wide therapeutic window without systemic immune activation in mice. STA551 was well tolerated even at 150 mg/kg/week in cynomolgus monkeys. These results provide a strong rationale for the clinical testing of STA551 against a broad variety of cancers regardless of antigen expression, and for the further application of this novel platform to other targets in cancer therapy.

SIGNIFICANCE: Reported CD137 agonists suffer from either systemic toxicity or limited efficacy against antigen-specific cancers. STA551, an antibody designed to agonize CD137 only in the presence of extracellular ATP, inhibited tumor growth in a broad variety of cancer models without any systemic toxicity or dependence on antigen expression.

See related commentary by Keenan and Fong, p. 20.

Note: Supplementary data for this article are available at Cancer Discovery Online (http://cancerdiscovery.aacrjournals.org/).


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INTRODUCTION

Over the past decade, mAb therapies delivered significant clinical benefits, including durable responses even in late-stage cancers. Immune checkpoint inhibitors such as anti-CTLA4 and anti–PD-1/PD-L1 antibodies have achieved impressive clinical outcomes and are changing the paradigm of cancer treatment (1, 2).

CD137 is a co-stimulatory receptor in the tumor necrosis factor receptor superfamily that enhances CD28-independent co-stimulation when T-cell receptors recognize antigens, resulting in the proliferation and survival of T cells by upregulating antiapoptotic signaling, and the production of cytokines such as IFNγ (3, 4). The therapeutic potential of agonistic antibody against CD137 was demonstrated in several preclinical models (4, 5). The importance of CD137 signaling in T cells has also been demonstrated by the clinical success of second-generation chimeric antigen receptor T (CAR-T) cells incorporating a CD137 co-stimulatory signaling domain (6, 7).

Several anti-CD137 agonist antibodies have advanced to clinical stages but have never been clinically successful because of the intolerable toxicity caused by systemic immune activation (8). Urelumab (BMS-663513), an IgG4 antibody, caused severe hepatotoxicity in more than 5% of patients enrolled in phase I and II clinical trials (9, 10). In contrast, utomilumab (PF-05082566), an IgG2 antibody, showed fewer grade III–IV adverse effects and no dose-limiting toxicity up to the highest dose evaluated, although it was much less potent (11, 12). This on-target off-tumor toxicity seems to be an inevitable problem for all therapeutic antibodies because they cannot discriminate whether the target antigen is in plasma, normal tissue, or tumor (13).

To overcome the toxicity of anti-CD137 antibody, various bispecific approaches have been developed that induce the CD137 agonistic signal by cross-linking CD137 with tumor-associated antigen (14–16). However, because these bispecific approaches fully rely on expression of specific tumor-associated antigen in the tumor bed to induce CD137 agonistic activity, their clinical use is limited to cancer patients who highly express the antigen. This is in sharp contrast to anti–PD-1/PD-L1 antibody and anti-CTLA4 antibody, which can be used for patients without considering the expression of specific tumor-associated antigen.

Thus, a more broadly applicable approach to effectively and safely target CD137 without relying on specific tumor-associated antigen expression is greatly needed. In addition, this bispecific approach is applicable only to agonist targets whose activity depends on its cross-linking. An engineering
approach that can also be applied to neutralizing or depleting antibody is desired.

Elevated extracellular ATP (exATP) is a hallmark of the tumor microenvironment (TME), accumulating in the range of 100 μmol/L in the TME (17). The abundance of ATP in the TME is caused by the release of millimolar concentration of intracellular ATP by apoptosis and necrosis of cancer cells, and by exocytosis and channel-mediated release (18, 19). Because levels of exATP remain tightly regulated in normal tissues within 10 nmol/L (19, 20), there is a more than 1,000-fold increase of exATP in the TME; this allowed us to exploit exATP as a broadly tumor-selective switch to control the activity of antibodies.

In this study, we developed a novel anti-CD137 agonist switch antibody, STA551, which binds to CD137 only in the presence of ATP and induces strong CD137 agonistic activity selectively within the TME. We demonstrated the antitumor efficacy of STA551 in a broad variety of tumor models without any dependence on tumor-associated antigen expression. Importantly, whereas the conventional anti-CD137 antibody showed both intratumor and systemic T-cell activation, STA551, the exATP switch antibody, showed potent intratumor T-cell activation without affecting normal tissues. A toxicologic study of doses up to 150 mg/kg/week in cynomolgus monkeys supported this finding. This article provides a strong rationale for clinically testing STA551 against a broad variety of cancers, and for the further application of the exATP switch antibody platform to other targets.

RESULTS

Generation of ATP-Dependent Anti-Human CD137 Agonist Antibody STA551

We obtained the lead ATP-dependent anti-CD137 antibody using a phage-displayed synthetic human Fab library...
with a built-in ATP-binding motif, D12 library, followed by optimization (Supplementary Fig. S1A and S1B). The D12 library was constructed in two steps: first, identifying an anti-ATP antibody from a phage-display naïve human Fab library; second, designing and constructing a library with a conserved ATP-binding motif, which has a repertoire that maintains the residues involved in D12 binding to ATP and diversifies other residues in the complementarity-determining region (CDR). The lead ATP-dependent anti-CD137 variable region was isolated using phage panning of the D12 library. ATP metabolite is sandwiched between the antibody and CD137 in the ternary complex, and the CDRs and ATP both interact with CD137. After multiple rounds of optimization, including improving CD137 binding, we identified the STA551 variable region. For the constant region, several mutations were added, including into the CH2 region of human IgG1 to increase Fc gamma receptor IIb (FcγRIIb)-mediated cross-linking of CD137 by FcγRIIb-expressing cells to enhance CD137 agonistic activity (ref. 21; Fig. 1A).

The resulting human IgG1/lambda antibody with optimized variable and constant regions, termed STA551, bound to both recombinant human and cynomolgus monkey CD137 extracellular domains in an ATP-, ADP-, and AMP-dependent manner (Fig. 1B and C; Table 1; Supplementary Fig. S2A–S2F). Binding affinities of STA551 to FcγRs, C1q, and neonatal Fc receptor (FcRn) are summarized (Supplementary Figs. S3A, S3B, and S4; Supplementary Table S1).

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### In Vitro Pharmacologic Profiles of STA551

In previous studies, CD137 agonist antibodies were shown to co-stimulate secretion of cytokines such as IFNγ from human CD8⁺ T cells (3). To evaluate STA551’s ATP-dependent CD137 agonistic activity, we compared it with Ure-hlgG4, a potent conventional CD137 agonist with an urelumab-like Fab having a human IgG4/kappa isotype (Supplementary Tables S2 and S3). IFNγ released from human CD8⁺ T cells increased in a STA551 concentration-dependent manner in the presence of ATP, whereas almost no increase was observed in its absence (Fig. 2A). In contrast, IFNγ levels increased in an Ure-hlgG4 concentration-dependent manner in both the presence and absence of ATP (Fig. 2A). In addition, the levels of IFNγ induced by STA551 were at least as strong as Ure-hlgG4 and significantly higher than Uto-hlgG2, a conventional CD137 agonist with an utomilumab-like Fab having a human IgG2/lambda isotype (Fig. 2A; Supplementary Fig. S5; Supplementary Tables S2–S4).

Next, to further confirm in vitro pharmacologic profiles in a more native setting, we mixed STA551 or Ure-hlgG4 with whole human peripheral blood mononuclear cells (PBMC) from 20 healthy donors in the presence of ATP and evaluated IFNγ levels. STA551 induced even stronger IFNγ production than Ure-hlgG4 among PBMCs (Fig. 2B; Supplementary S6), which include immune suppressor cells and FcγR-expressing native myeloid and B cells (22, 23).

### Antitumor Efficacy of STA551 in Human CD137 Knock-In Mice

We then examined the in vivo antitumor efficacy of STA551 in various immune-competent mouse models. Because STA551 does not bind to murine CD137, we established human CD137 knock-in mice (Supplementary Fig. S7A–S7D). STA551 specifically bound to membranous human CD137 based on flow cytometry using mouse spleen cells (Supplementary Fig. S8A). Furthermore, as the FcγR-binding profile in humans is not recapitated in mice, we generated an engineered constant region of mouse IgG1, MB. Sta-MB, having the same variable region as STA551, but with MB as the constant region, can cross-link human CD137-expressing immune cells with mouse FcγRII-expressing mouse cells to increase agonistic activity in human CD137 knock-in mice (Supplementary Fig. S8B and S8C). MB has a dissociation constant (Kd) against mouse FcγRII-expressing cells comparable to that of STA551 against human FcγRIIb-expressing cells (Supplementary Fig. S9; Supplementary Table S5).

To evaluate the in vivo antitumor efficacy of STA551 against various mouse cell lines, 5 tumors originating from different organs were established in human CD137 knock-in mice and treated with Sta-MB or Ure-MB, which have the same variable region as STA551 or urelumab, but with MB as the constant region (Supplementary Table S2). Sta-MB significantly inhibited tumor growth compared with vehicle in all five models and tended to inhibit it more strongly than Ure-MB in the LLC1/ova/hGPC3 model (Fig. 3A–C). Antitumor efficacy lasted more than 40 days (Supplementary Fig. S10). This clearly demonstrates that

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**Table 1. K_{d} values for the binding of STA551, Ure-hlgG4, and Uto-hlgG2 to human and cynomolgus monkey CD137**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Human CD137</th>
<th>ATP 0 μmol/L</th>
<th>ATP 100 μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>STA551</td>
<td>N.D.</td>
<td>9.82 (±0.13) *10^-9</td>
<td></td>
</tr>
<tr>
<td>Ure-hlgG4</td>
<td>1.66 (±0.03) *10^-8</td>
<td>1.62 (±0.03) *10^-8</td>
<td></td>
</tr>
<tr>
<td>Uto-hlgG2</td>
<td>7.12 (±0.02) *10^-8</td>
<td>7.10 (±0.03) *10^-8</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Cynomolgus monkey CD137</th>
<th>ATP 0 μmol/L</th>
<th>ATP 100 μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>STA551</td>
<td>N.D.</td>
<td>2.51 (±0.03) *10^-8</td>
<td></td>
</tr>
<tr>
<td>Ure-hlgG4</td>
<td>N.D.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uto-hlgG2</td>
<td>13.5 (±0.13) *10^-8</td>
<td>13.0 (±0.10) *10^-8</td>
<td></td>
</tr>
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**Note:** Each antibody was measured in independent experiments both with and without ATP.

**Abbreviation:** N.D., not detectable.

*Mean ± SD (n = 3) of the calculated K_{d} of STA551, Ure-hlgG4, and Uto-hlgG2 binding to human CD137 and cynomolgus monkey CD137 in the absence and presence of 100 μmol/L ATP.
Figure 2. IFNγ release induced by STA551 or Ure-hIgG4 in the absence or presence of ATP. A, Human CD8+ T cells derived from three different donors were cocultured with CHOK1/human FcγRIIb cells and STA551 or Ure-hIgG4 in the absence or presence of 100 μmol/L ATP. IFNγ concentrations were measured by ELISA. Blue circle, STA551 in the absence of ATP; blue triangle, STA551 in the presence of 100 μmol/L ATP; pink circle, Ure-hIgG4 in the absence of ATP; pink triangle, Ure-hIgG4 in the presence of 100 μmol/L ATP. Each point represents mean ± SD (n = 3). *, Statistical differences in IFNγ levels between 1 μg/mL STA551 in the absence of ATP and in the presence of 100 μmol/L ATP (*, P < 0.0001 by t test).

B, Human PBMCs from the fresh blood of 20 healthy donors were incubated with STA551 or Ure-hIgG4 in the presence of 250 μmol/L ATP. IFNγ concentrations were measured by ELISA. The symbols indicate mean of IFNγ levels from different 20 donors. **, Statistical differences in IFNγ levels between the STA551 and Ure-hIgG4 or anti-KLH-hIgG4 and Ure-hIgG4 (**, P < 0.01 by paired t test). FcγRIIb, Fc gamma receptor IIb.
Figure 3. Antitumor efficacy and systemic responses to Sta-MB or Ure-MB in human CD137 knock-in mice. A–C, Human CD137 knock-in mice bearing C1498, E.G7-OVA, and Hepa1-6/hGPC3 (A), LLC1/OVA/hGPC3 (B), and Colon38 (C) were administered vehicle (black/gray), Sta-MB (blue), or Ure-MB (pink). Black dashed lines indicate average tumor volume for vehicle (A, $n = 10$; B, $n = 7$; C, $n = 14$). *, **, Statistical analysis between vehicle and Sta-MB on day 21 (*, $P < 0.01$ by Steel test; B) or day 28 (**, $P < 0.05$ by Steel test; C) or Ure-MB (**, $P < 0.05$ by Steel test). Nonparametric Steel test or parametric Williams test was selected by Bartlett test for assessing tumor volumes of each treatment. †, Euthanasia due to dissemination of tumor cells in the peritoneal cavity; #, euthanasia due to false administration. (continued on next page)
Sta-MB exerts in vivo agonistic activity in mice regardless of any specific tumor-associated antigen expression, with exATP elevated in all the tumors tested. To support this, we analyzed changes in genes related to exATP such as P2rx7, S100a3, and Rpl38 (Supplementary Table S6) in tumors and normal tissues using RNA sequencing (RNA-seq). Their mRNA expression was increased in tumors compared with normal tissues (Supplementary Fig. S11), suggesting elevated exATP in tumors. In addition, tumor cells and tumor-infiltrating lymphocytes (TIL) in these studies expressed CD39, which hydrolyzes ATP to ADP and AMP, and CD73, which hydrolyzes AMP to adenosine (ADO) on the cell surface (Supplementary Fig. S12A–S12E). These expression levels were consistent with TILs from patients (24). Nevertheless, Sta-MB, which binds to CD137 in the presence of ATP, ADP, or AMP, demonstrated potent antitumor efficacy, suggesting that even if CD39 and CD73 are expressed, exATP, exADP, and exAMP remain in the TME at sufficient
levels. Furthermore, Sta-MB inhibited lung metastasis of LLC1/OVA as shown by the weight of metastatic nodules (Supplementary Fig. S13). This suggests that exATP is also enriched in micrometastasis and that Sta-MB was effective therein.

**STA551 Response in Nontumor Tissues in Human CD137 Knock-In Mice**

We investigated the pharmacokinetics of Ure-MB and Sta-MB in non–tumor-bearing wild-type C57BL/6N mice and human CD137 knock-in mice, which systemically express human CD137, to evaluate the effect of systemic CD137 binding on antibody clearance. Ure-MB showed slow clearance in wild-type mice because it does not bind to mouse CD137, and was rapidly eliminated in human CD137 knock-in mice due to systemic CD137-mediated clearance (Fig. 3D). In contrast, Sta-MB showed slow clearance and similar pharmacokinetics in both wild-type and human CD137 knock-in mice, demonstrating that Sta-MB is not subject to systemic CD137-mediated clearance (Fig. 3D). Sta-MB showed faster clearance in human CD137 knock-in mice bearing LLC1/OVA/hGPC3 and Colon38 than in nontumor-bearing mice (Supplementary Fig. S14A and S14B), suggesting that Sta-MB bound to human CD137 within tumors and was eliminated by CD137-mediated clearance there. STA551 was internalized into human CD137-expressing cells (Supplementary Fig. S14C), consistent with reports (25). These pharmacokinetic studies indicate that whereas Ure-MB strongly binds to human CD137 systemically, Sta-MB only does so minimally in blood and normal tissues, places where exATP concentration is reported to be very low; thus, Sta-MB is not expected to exert CD137 agonistic activity systemically.

Next, to confirm that the minimal binding of Sta-MB in nontumor tissues does not induce systemic immune activation in mice, we evaluated its effect in nontumor tissues. Human CD137 knock-in mice bearing LLC1/OVA/hGPC3 or Colon38 tumors were given various doses of Sta-MB or Ure-MB. After the treatment, Ure-MB started to elicit systemic response from 0.093 mg/kg, but Sta-MB showed no clear sign of it even at 7.5 mg/kg. In mice treated with a higher dose of Ure-MB, lymphadenopathy and splenomegaly, characterized by a significant increase of draining lymph node (DLN) and spleen weight, and increased PD-1, KLRG1, or ICOS in CD8+ T cells was increased in tumors after treatment with Sta-MB or Ure-MB according to flow cytometry (Fig. 4D). Together with the increase in CD8+ T cells within tumors.

**Pharmacokinetics and Toxicology of STA551 in Cynomolagus Monkeys**

For the nonclinical pharmacokinetic and toxicology study, cynomolgus monkeys were selected on the basis of species cross-reactivity (Fig. 1B and C; Supplementary S2A–S2F). The plasma concentration–time profiles and pharmacokinetic parameters of STA551 following a single intravenous administration of 0.5, 5, and 50 mg/kg in male cynomolgus monkeys are shown (Supplementary Fig. S16; Supplementary Table S7). Antidrug antibodies were transiently detected in one animal from each 0.5 and 5 mg/kg group of 4, but this did not influence the pharmacokinetic profile (data not shown). The $t_{1/2}$ (elimination half-life) was approximately 14 days independent of the dosage, and other parameters were consistent with typical values for IgG antibodies (26).

The 4-week repeat dose toxicity study in cynomolgus monkeys was conducted using intravenous doses of 5, 30, and 150 mg/kg/week. In this study, STA551 was well tolerated up to 150 mg/kg/week without any abnormalities in general condition, and the highest nonspecific toxic dose was above 150 mg/kg/week in cynomolgus monkeys. In contrast, utomilumab, despite having much weaker agonistic activity, was reported to cause dose-limiting toxicity at doses more than 5 mg/kg/week (27). We also conducted an exploratory 4-week repeat-dose toxicity study of Uto-hlgG2 in cynomolgus monkeys. Uto-hlgG2 elicited systemic toxicity characterized by general condition abnormalities (e.g., decreased food consumption and body weight), hematologic changes (e.g., decrease in neutrophils and platelets), and inflammatory changes in lung and liver even at 30 mg/kg/week.

**STA551 Response in Tumor Tissues in Human CD137 Knock-In Mice**

Because STA551 exerts its activity in a novel exATP-dependent and tumor-selective manner, we examined the mechanism behind its antitumor efficacy. First, we analyzed changes in genes related to CD8+ effector T cells, immune checkpoint, IFNγ, and antigen presentation (Supplementary Table S8) after administration using RNA-seq. Their mRNA expression was increased in mice treated with Sta-MB compared with vehicle in LLC1/OVA/hGPC3 or Colon38 tumors (Fig. 4A and B), suggesting that Sta-MB alters immune status in tumors.

Next, we analyzed changes in expression of the selected immune-related genes in LLC1/OVA/hGPC3 tumors using the nCounter Analysis System by comparing Sta-MB and Ure-MB at various doses. Compared with vehicle, mice treated with Sta-MB showed a significant increase in CD8β1 RNA as a marker of CD8+ T-cell infiltration, granzyme B and perforin as a marker of cytolysis, and IFNγ as a marker of T-cell activation in tumors (Fig. 4C). Sta-MB at 0.83 mg/kg upregulated these molecules to levels similar to 7.5 mg/kg of Ure-MB. Meanwhile, Ure-MB did not upregulate IFNγ even at 7.5 mg/kg compared with vehicle. These results demonstrate that Sta-MB, despite its exATP dependence and tumor selectivity, activated immune cells within tumor tissue more potently than Ure-MB. Consistent with these gene-expression data, the ratio of granzyme B, PD-1, KLRG1, or ICOS in CD8+ T cells was increased in tumors after treatment with Sta-MB or Ure-MB according to flow cytometry (Fig. 4D). Together with the increase in CD8β1 RNA, this indicates an increase of activated CD8+ T cells within tumors.
Figure 4. Analysis of tumors treated with Sta-MB or Ure-MB in human CD137 knock-in mouse model. A and B, mRNA from LLC1/OVA/hGPC3 (A) or Colon38 (B) tumors treated with vehicle, Sta-MB, or Ure-MB were used for RNA sequencing. Each group was tested in triplicate (n = 3). Z scores were calculated using log2-transformed fragments per kilobase per million mapped reads (FPKM) normalized for all samples. C, mRNA expression of CD8b1, granzyme B, IFNγ, and Perforin 1 in LLC1/OVA/hGPC3 tumors were measured by nCounter. The mean and individual values in each group are shown (n = 5). *, **, *** Statistical differences in mRNA levels between vehicle and Sta-MB or Ure-MB (*, P < 0.05; **, P < 0.01; *** P < 0.001 by Dunnett test). Gzmb, granzyme B; [Ifng, IFNγ; Prf1, perforin 1. D, Ratio of Granzyme B, PD-1, KLRG1, and ICOS in CD8+ T cells to whole CD8+ T cells in LLC1/OVA/hGPC3 tumors were calculated by flow cytometry. The mean and individual values in each group are shown (n = 4). *, **, *** Statistical differences in expression levels between vehicle and Sta-MB or Ure-MB (*, P < 0.05; **, P < 0.01; *** P < 0.001 by Dunnett test).
Tumor-Selective Anti-CD137 Antibody Activated by exA TP

Figure 5. Combination efficacy of STA551 with anti–PD-L1 in Colon38. A, Human CD137 knock-in mice bearing Colon38 were administered vehicle (black), Sta-MB (blue), anti-mouse PD-L1 mAb (green), or combination (red; n = 6). Dashed bold lines indicate average tumor volume for vehicle. Tumor volume (mm3) was determined every 3 days. B, Images of Colon38 tumors were stained with anti-CD8, anti-PD-L1, and DAPI (scale bars, 100 μm). C, The number of CD8+ and PD-L1+ cells in tumors was calculated. The mean and individual values in each group are shown (n = 3). Dose categories: Dose 1, Dose 2, Dose 3. Dose 1: Vehicle (black), Sta-MB (blue), PD-L1 Ab (green), Sta-MB + PD-L1 Ab (red). Dose 2: Vehicle (black), Sta-MB (blue), PD-L1 Ab (green), Sta-MB + PD-L1 Ab (red). Dose 3: Vehicle (black), Sta-MB (blue), PD-L1 Ab (green), Sta-MB + PD-L1 Ab (red). E, Human CD137 knock-in mice bearing Colon38 tumors were administered vehicle (black), Sta-MB (blue), Ure-MB (pink), anti-mouse PD-L1 mAb (green), Sta-MB combination (red), or Ure-MB combination (purple; n = 8). **, ***. Statistical differences for multiple-group comparisons on day 32 (*, P < 0.05, ***, P < 0.01 by Tukey test). F, Weights of draining lymph nodes and spleen were measured. The mean and individual values in each group are shown (n = 5). **, ***. Statistical differences in tissue weights for multiple-group comparisons (**, P < 0.01; ***, P < 0.001 by Tukey test). (continued on next page)

Furthermore, we examined the distribution of Sta-MB and Ure-MB in human Colon38 knock-in mice bearing Colon38 tumors. Both Sta-MB and Ure-MB on CD8+ T cells were detected in tumors one day after injection, but in spleen and liver, Sta-MB was markedly lower than Ure-MB (Supplementary Fig. S17A and S17B).

In addition, CD8+ T-cell depletion canceled antitumor efficacy completely. However, depletion of CD4+ T cells, B cells, NK cells, neutrophils, or macrophages did not influence antitumor efficacy in the LLC1/OVA/hGPC3 model (Supplementary Fig. S18). This demonstrates that STA551’s antitumor efficacy was caused by the increase in CD8+ T cells and their higher level of activation and cytolyis, which is also supported by gene-expression analysis (Fig. 4), consistent with previous reports on conventional CD137 agonist antibodies (28).

Efficacy of STA551 in Combination with Anti–PD-L1 Antibody

We next evaluated the in vivo efficacy of Sta-MB in combination with anti–PD-L1. Combination treatment with Sta-MB and anti–PD-L1 mAb completely inhibited Colon38 tumor growth in human Colon38 knock-in mice, whereas monocotherapy with either Sta-MB or anti–PD-L1 mAb showed only moderate efficacy (Fig. 5A). All antibodies were well tolerated in the monocotherapy and combination groups, with mice losing no more than 6.5% body weight and no increase in ALT and AST (Supplementary Fig. S19A and S19B). Analysis of whole-gene expression indicated that combination treatment tended to increase the expression of immune-related gene sets compared with monocotherapies (Fig. 5B; Supplementary Table S8). The combination increased CD8+ T-cell infiltration...
and PD-L1 expression in tumors more than monotherapy, according to IHC (Fig. 5C and D). These results suggest that the combination of Sta-MB with anti–PD-L1 mAb altered the TME to enhance immune activation, leading to synergistic antitumor effects.

Whereas bispecific antibody against human CD137 and human glypican-3 (GPC3), which exerts CD137 agonistic activity only in the presence of human GPC3, demonstrates efficacy in the LLC1/OVA/hGPC3 model (Supplementary Fig. S20), both Sta-MB monotherapy and combination with anti–PD-L1 mAb inhibited tumor growth in human CD137 knock-in mice with LLC1/OVA tumors, which do not express GPC3 (Supplementary Fig. S21A).

We compared the in vivo efficacy and response in nontumor tissues of Sta-MB and Ure-MB when combined with anti–PD-L1 mAb. The Sta-MB combination with anti–PD-L1 mAb showed meaningful potent antitumor efficacy compared with the Ure-MB combination (Fig. 5E), inhibiting tumor

**Figure 5.** (Continued) G–J, Ratio (G) and absolute number (H) of LAG3, PD-1, ICOS, and KLRG1 on CD8+ T cells to whole CD8+ T cells and FOXP3+ Tregs to whole CD4+ T cells in DLNs, spleen (I), and liver (J) were calculated by flow cytometry. The mean and individual values in each group are shown (n = 5). *, **, *** Statistical differences in expression levels for multiple-group comparisons [*P < 0.05; **P < 0.01; ***P < 0.001 by Tukey test]. K, WBC, lymphocyte, and platelet counts in whole blood were analyzed by XT-2000iV. *, **, *** Statistical differences in cell count for multiple-group comparisons [*P < 0.05; **P < 0.01; ***P < 0.001 by Tukey test]. DLN, draining lymph nodes; Tregs, regulatory T cells; WBC, white blood cells.
Tumor-Selective Anti-CD137 Antibody Activated by exATP

CD137 on T cells and human Fcγ exerts exATP-dependent agonistic activity in human cancer (Fig. 6C and D). This result demonstrates that STA551 also where the efficacy of either monotherapy was limited (Fig. 6A). The combination of STA551 with anti-human GPC3/mouse CD3 bispecific agonist against each and every tumor-associated antigen. To test whether efficacy of GPC3-specific TRAB can be enhanced by a CD137 agonist antibody that does not rely on GPC3 expression, this antibody platform provides a novel tumor-selective approach, which is not dependent on the expression of tumor-associated antigens, and we believe the exATP switch is superior to other approaches, such as using low pH and hypoxia, which are also recognized hallmarks of TME that have been widely investigated for use in tumor-specific therapeutics (30, 31). It has been confirmed that healthy tissues and plasma contain very low levels of exATP (10–100 nmol/L; refs. 19, 32), whereas more than 100 μmol/L of exATP was detected in the TME (17). P2X7 is a receptor for exATP with 100 μmol/L Kd and is activated in the TME (33), indicating that exATP is present in the TME at concentrations of more than 100 μmol/L. On the basis of these published reports, we speculate there is more than a 1,000-fold difference in exATP concentration between normal tissues and tumor. Meanwhile, pH in normal tissues and tumor is reported to be 7.2–7.4 and 6.5–6.9, respectively, with a less than 10-fold difference in proton concentration (34, 35), and oxygen levels in arterial blood and the TME are reported to be around 9.5% and 0.3%–4.2%, respectively, with a several-fold difference in oxygen concentration (36, 37). Considering the inefficient distribution of antibodies into solid tumor, a less than 10-fold difference in concentration is clearly not sufficient.

We generated STA551, an ATP-dependent agonist switch antibody to CD137 that does not rely on the expression of a specific tumor-associated antigen. It was designed to bind to CD137 strongly in the presence of 100 μmol/L of ATP, but not in the presence of 1 μmol/L. STA551 inhibited tumor growth in all the mouse models we tested, indicating that exATP concentration is at least 100 μmol/L in the TMEs of a broad range of mouse tumors, which is

**Efficacy of STA551 in Combination with T-cell Redirecting Antibody**

T-cell redirecting antibody (TRAB) is a bispecific antibody targeting tumor-associated antigen and CD3, which activates T cells in an antigen-dependent manner to kill antigen-expressing cancer cells. Because CAR-T cells require CD137 signaling in addition to CD3 signaling for clinical efficacy (6, 7), CD137 agonist antibody would be an ideal combination partner with TRAB. Because TRABs targeting various tumor-associated antigens, such as GPC3, HER2, and CEA, are being tested in the clinic, it is not realistic to develop a unique CD137 bispecific agonist against each and every tumor-associated antigen. To test whether efficacy of GPC3-specific TRAB can be enhanced by a CD137 agonist antibody that does not rely on GPC3 expression, we evaluated the efficacy of STA551 in combination with anti-human GPC3/mouse CD3 (anti-hGPC3/mCD3) bispecific antibody. The combination completely inhibited tumor growth in human CD137 knock-in mice with human GPC3-expressing LLC1 (LLC1/hGPC3) tumors, which are noninflamed tumors (29), whereas the efficacy of either monotherapy was limited (Fig. 6A). The combination also notably increased RNA levels of CD3ε, CD8β, and cytolytic markers including granzyme B and perforin, and IFNγ in tumors (Fig. 6B).

To further evaluate STA551 combined with TRAB in a setting with human cancer cells and immune cells, we used human hematopoietic stem cell (HSC)-engrafted NOD/Shi-scid, IL2RγKO Jic (huNOG) mice expressing human CD137 on T cells and human FcγRIIb on B cells. As TRAB, we used the anti-human GPC3/human CD3 bispecific antibody GPC3-TRAB. The combination of STA551 with GPC3-TRAB strongly inhibited growth of established NCI-H446, which endogenously expresses human GPC3, and dramatically increased the number of human T cells, whereas the efficacy of either monotherapy was limited (Fig. 6C and D). This result demonstrates that STA551 also exerts exATP-dependent agonistic activity in human cancer cells and immune cells.

**DISCUSSION**

Although the importance of CD137 signaling in cancer immunotherapy has been validated both preclinically and clinically (4–7), anti-CD137 agonist antibodies have not progressed beyond early-phase clinical trials. Urelumab was hampered by hepatotoxicity, and utomilumab showed limited efficacy possibly due to its weak agonistic activity (3).

Addressing these issues, recent publications describe several bispecific tumor-targeted CD137 agonists (14–16). However, their therapeutic efficacy relies fully on the expression of tumor-associated antigen, limiting their clinical application to patients who highly express those antigens. Tumor-associated antigens must be highly tumor-specific, and the expression of these rare tumor-associated antigens is often limited to only specific types of cancer. In contrast, the success of anti–PD-L1/anti–PD-L1 antibody is partially attributed to its applicability to broad variety of cancers regardless of antigen expression. In addition, EGFR-targeted CD137 bispecific agonist is rapidly eliminated (16), and FAP-targeted CD137 bispecific agonist requires combination partners like anti–PD-L1 antibody or TRAB to strongly inhibit tumor growth (14). Clearly, a novel anti-CD137 agonist antibody, having strong agonistic activity, long half-life, improved safety profile, and broad applicability without tumor-associated antigen expression, is greatly needed.

Our exATP switch antibody platform provides a novel tumor-selective approach, which is not dependent on the expression of tumor-associated antigens, and we believe the exATP switch is superior to other approaches, such as using low pH and hypoxia, which are also recognized hallmarks of TME that have been widely investigated for use in tumor-specific therapeutics (30, 31). It has been confirmed that healthy tissues and plasma contain very low levels of exATP (10–100 nmol/L; refs. 19, 32), whereas more than 100 μmol/L of exATP was detected in the TME (17). P2X7 is a receptor for exATP with 100 μmol/L Kd and is activated in the TME (33), indicating that exATP is present in the TME at concentrations of more than 100 μmol/L. On the basis of these published reports, we speculate there is more than a 1,000-fold difference in exATP concentration between normal tissues and tumor. Meanwhile, pH in normal tissues and tumor is reported to be 7.2–7.4 and 6.5–6.9, respectively, with a less than 10-fold difference in proton concentration (34, 35), and oxygen levels in arterial blood and the TME are reported to be around 9.5% and 0.3%–4.2%, respectively, with a several-fold difference in oxygen concentration (36, 37). Considering the inefficient distribution of antibodies into solid tumor, a less than 10-fold difference in concentration is clearly not sufficient.

We generated STA551, an ATP-dependent agonist switch antibody to CD137 that does not rely on the expression of a specific tumor-associated antigen. It was designed to bind to CD137 strongly in the presence of 100 μmol/L of ATP, but not in the presence of 1 μmol/L. STA551 inhibited tumor growth in all the mouse models we tested, indicating that exATP concentration is at least 100 μmol/L in the TMEs of a broad range of mouse tumors, which is
consistent with a previous estimation of exATP concentration in the TME (17).

Although CD73 was expressed in those tumors, STA551 activated immune cells and exhibited antitumor efficacy, indicating that a high enough concentration of exATP, exADP, and exAMP remains in the tumors for STA551 to work. This is supported by a previous estimation that exADO stays in the micromolar range in the TME (38). Also, in a recent publication, exATP levels were 100-fold higher in tumor tissues than in adjacent nontumor tissues in human pancreatic ductal adenocarcinoma (PDAC; ref. 39) even though CD73 is expressed in all PDAC (40). This provides more evidence that, even if CD73 is expressed in human tumors, the remaining exATP levels are still high enough for STA551 to achieve efficacy. Furthermore, STA551 did not show CD137-mediated systemic clearance and immune activation, indicating that exATP concentration is less than 1 μmol/L in normal tissues, also consistent with previous reports (19).

Unlike CD137 agonists, which failed to show strong efficacy in most tumor models (14, 41), STA551 monotherapy achieved potent efficacy in various tumor models. We believe this is because STA551 has an engineered IgG1 Fc with enhanced binding to FcγRIIb on the surface of tumor-associated B and...
myeloid cells, allowing enhanced cross-linking and multimerization of CD137, which is important for CD137 signal activation (42). The details on Fc engineering and its biological effect will be published elsewhere.

However, this antitumor efficacy may not be as impressive as that of anti-mouse CD137 antibodies such as 1D8-rat IgG2a (43). This is probably because 1D8-rat IgG2a induces ADCC in mice; therefore it depletes Tregs, which express CD137 (44), leading to strong antitumor efficacy. Sta-MB does not induce ADCC because it does not bind to mouse FcγRIIIA; therefore, it demonstrated antitumor efficacy purely through agonistic activity. In addition, treatment started very early in previous reports, before or within 3 days after transplanting of tumor cell lines (45). Such experiments might represent the prevention effect. Sta-MB was injected after tumors were established; therefore, the results should reflect the therapeutic effect.

In the Colon38 tumor model, 0.093 mg/kg of Ure-MB showed no statistically significant efficacy, but did significantly increase T-cell activation in the liver. This suggests that the therapeutic window of urrelumab is too narrow for efficacy without hepatotoxicity, which is consistent with the clinical results. Even though there are many FcγRIIb-expressing cells in the liver (46) to cross-link STA551, STA551 still did not activate T cells in the liver, even at dosages far higher than required for efficacy. Hence, STA551 has a therapeutic window wide enough to achieve therapeutic efficacy.

STA551 was carefully designed so that nonclinical safety assessment in cynomolgus monkeys can be translated into humans. In both species, STA551 binds with comparable affinity to CD137 in the presence of 100 μmol/L of ATP, whereas the binding is not detectable in the absence of ATP. Affinity to both human and cynomolgus monkey FcγRIIb is also enhanced compared with conventional IgG1. Systemic toxicity of STA551 was significantly less than conventional CD137 agonist antibody, with no elevation of liver enzymes such as ALT, AST, or glutamate dehydrogenase; no activation of immune cells in peripheral blood; and no abnormalities in the histopathologic assessment including lung and liver in cynomolgus monkeys at up to 30 mg/kg. In contrast, all these changes were observed with utomilumab at the same dose. The 14-day half-life of STA551 in cynomolgus monkeys suggests a dosing schedule convenient for patients, such as once every 3 weeks.

One remaining challenge is in detecting the target binding of switch antibody in vivo. This is technically challenging because switch antibodies would dissociate from the antigen during the process of ex vivo analysis, wherein ATP concentration decreases either by enzymatic and spontaneous hydrolysis or by dilution. Thus, observed ex vivo binding may underestimate actual in vivo binding. Given the challenge of quantitatively comparing nonswitch and switch antibody biodistribution, we evaluated pharmacodynamic responses and pharmacokinetics in tumor, DLNs, spleen, and liver, which reflect antibody binding to the target. Although we were able to directly detect the binding of Sta-MB to target CD8+ T cells in tumors, the binding was lower than Ure-MB. Because Sta-MB activated immune cells within tumor tissue more potently than Ure-MB, lower binding of Sta-MB could be due to its dissociation from the antigen during the ex vivo process.

STA551 showed a synergistic antitumor effect in combination with anti–PD-L1 antibody and TRAB by increasing CD8+ T-cell proliferation and/or infiltration and expression of immune-related gene sets in tumors. These combinations could further amplify its antitumor efficacy in noninflamed intermediate/immunosuppressed tumors (47). Importantly, STA551 was effective in combination with these reagents, which could affect the TME (i.e., reducing the exATP concentration by inducing the expression of CD39 that hydrolyzes exATP; ref. 48). Combinations with other reagents such as chemotherapy should be tested in the future.

We have proved preclinically that the exATP switch antibody platform was very effective in expanding the therapeutic window of CD137 agonist antibody. A drug target for which the therapeutic window of conventional antibody is not sufficient could be transformed into a target where no systematic effect was detected even at a dosage much higher than required for maximum efficacy. In the oncology field, there are many drug targets that suffer from on-target off-tumor side effects and cannot be safely made into drugs for patients. We believe that the exATP switch antibody platform can reach those previously undruggable targets. Thus, it could be utilized for antibody–drug conjugates and other approaches where systemic binding may be too toxic, such as with TRABs, CAR-T, and other antibody therapies.

In conclusion, we generated a novel anti-CD137 switch antibody, STA551, that exerts agonistic activity selectively in tumors, in which exATP is elevated more than 1,000-fold compared with normal tissues and plasma, without relying on expression of tumor-associated antigen. STA551 monotherapy demonstrated potent and broad antitumor efficacy against all tumors tested without systemic immune activation. These results strongly support the clinical testing of STA551 for the treatment of a wide variety of solid tumors not restricted by antigen expression. Preclinical evidence that the exATP switch antibody platform expands the therapeutic window warrants its application to other targets stymied on-target toxicity in cancer therapy. STA551 is currently being tested in a phase I clinical study.

METHODS

Study Design

The main objective of our study was to evaluate the antitumor efficacy and safety of STA551 targeting CD137. Antitumor efficacy was assessed in two different tumor-grafted mouse platforms: immunocompetent human CD137 knockout mice and huNOG mice in which CD34+ human HSCs were transplanted to reconstitute human immune cells. Sample size (n = 6–15 per group in human CD137 knockout mouse model and n = 5 per group in huNOG mouse model) was determined on the basis of the consistency of tumor growth observed in preliminary experiments to allow for statistically significant differences in tumor size between the various treatment groups. Animals were randomly assigned to groups based on tumor size so that each group had the same average size. Tumor volumes were calculated according to the following formula: [(length × width)²]/2. All tumor volume data (mean tumor volume with SD) were plotted. Animals were sacrificed at the end of the study. The use of human HSCs...
was approved by an Institutional Review Board. STA551 toxicity was assessed using cynomolgus monkeys. Animals were assigned to each group using a computerized procedure designed to balance body weight equally among groups. The number of animals per group (n = 3 per sex per group) was chosen according to ICH-S4A guidelines for reaching scientific conclusions on safety with consideration for animal welfare. All animal studies described above were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at each facility.

**Cell Lines**

C1498, E.G7-OVA, Hepa1-6, LLC1, and NCI-H446 were purchased from the ATCC. Colon38 was obtained from NIH (Bethesda, MD). GPC3 and/or chicken ovalbumin (OVA)—overexpressing Hepa1-6/hGPC3 (29), LLC1/OVA/hGPC3, LLC1/hGPC3, and LLC1/OVA cells were established by transfecting GPC3 and/or OVA-expressing plasmids into parental cells to enhance the immunogenicity of each tumor, and also to allow us to test the combination of STA551 with a TRAB against GPC3. Human FcRRIb-expressing CHO-K1 cells (CHO-K1/human FcRRIb cells) were purchased from Promega, and CHOK1/human FcRRIb from Thermo Fisher Scientific. Mouse FcRRIb-overexpressing CHO, human FcRRIa-overexpressing CHO, and human FcRRIb-overexpressing CHO were established by transfecting mouse FcRRIb-expressing plasmids, human FcRRIa-overexpressing plasmids, or human FcRRIb-expressing plasmids into parental CHO-DG44 cells. Human CD137-overexpressing CHO was established by transfecting human CD137-expressing plasmids into parental CHO-DG44 cells.

**Measurement of Small Molecule-Dependent Binding and Binding Kinetics against Human or Cynomolgus Monkey CD137**

Binding of anti-CD137 antibodies against recombinant human CD137 extracellular domain (Genbank accession number AAH06196.1) or recombinant cynomolgus monkey CD137 extracellular domain (Genbank accession number ABY47575.1) was assessed in the presence or absence of ATP (Nacalai Tesque), ADP (Nacalai Tesque), AMP (Nacalai Tesque), or ADO (Nacalai Tesque) at 37°C using Biacore T200 instrument (GE Healthcare). Antibody was captured onto a Biacore CM4 sensor surface immobilized with protein G (Merck). Biacore sensorgrams were obtained at 37°C by injecting human CD137 or cynomolgus monkey CD137 in the presence of ATP, ADP, AMP, or ADO at 1,000, 100, 10, 1, and 0 μM/L, over a sensor surface capturing STA551. Kinetics parameters were determined by fitting sensorgrams with 1:1 binding model using Biacore T200 Evaluation Software Version 2.0 (GE Healthcare).

**In Vitro CD8+ T-cell Assay with FcRRIb-Expressing Cells**

All human CD8+ T cells were purchased from Astarte Biologics, Inc. Human CD8+ T cells from healthy donors were stimulated with a fixed concentration of immobilized anti-human CD3ε (1 μg/mL, clone SP34, BD Biosciences) and anti-human CD28 antibodies (5 μg/mL, clone CD28.2, BD Biosciences) for 6 hours, and then variable concentrations of STA551 or Ure-HlgG4 in the absence or presence of 100 μM/L ATP with CHO-K1/human FcRRIb cells were added and incubated for 18 hours in AIM-V medium (Thermo Fisher Scientific) supplied with 5% human serum (Sigma-Aldrich). After incubation, the supernatants were harvested and the IFNγ concentrations in the medium were measured by ELISA.

**In Vitro PBMC Assay**

Human PBMCs were purified from the fresh blood of 20 healthy donors using a conventional ficoll-Paque PLUS gradient (GE Health-care). Human PBMCs were stimulated with a fixed concentration of anti-human CD3ε (0.01 μg/mL) and anti-human CD28 antibodies (5 μg/mL) for 6 hours, and then STA551 or Ure-HlgG4 (5 μg/mL) were added and incubated for 42 hours in the absence or presence of 250 μM/L ATP in AIM-V medium supplied with 5% human serum. After incubation, supernatants were harvested and IFNγ concentrations were measured by ELISA.

**In Vivo Human CD137 Knock-In Mouse Model**

All mouse studies were performed according to IACUC policies. Human CD137 knock-in mice were generated by replacing mouse CD137 with its human counterpart, CD137 (Supplementary Fig. S7A-S7D). The C1498, E.G7-OVA, Hepa1-6/hGPC3, LLC1/OVA/hGPC3, Colon38, LLC1/hGPC3, or LLC1/OVA cells were inoculated subcutaneously into human CD137 knock-in mice. After palpable tumors were established, mice were randomized on the basis of tumor volume and body weight. Subsequently, 2.5 mg/kg of Sta-MB, 7.5 mg/kg of Ure-MB, or vehicle were intravenously administered twice. In the combination models, 10 mg/kg of anti-PD-L1 antibody (clone 10F.9G2, BioXcell) was intraperitoneally administered, and 1 mg/kg of anti-human GPC3/mouse CD3 bispecific antibody and 2.5 mg/kg Sta-MB was intravenously administered. Tumor size was measured twice per week.

**Flow Cytometry Staining**

LLC1/OVA/hGPC3, Colon38, or LLC1/OVA cells were inoculated subcutaneously into the flanks of human CD137 knock-in mice. After the tumors were established, tumors or livers were removed from mice treated with anti-human CD137 antibodies on day 4 after the first dose. Tumors or livers were digested, processed, and enriched into single-cell suspensions with tumor or liver dissociation kit (Miltenyi Biotec) followed by red blood cell lysis. Both digestions were performed according to the manufacturer’s protocol. Single-cell suspensions from DLN and spleen cells were obtained with nylon mesh followed by red blood cell lysis. These suspensions were used for the following experiments. Antibodies to CD3e (clone 145-2C11), CD8α (clone 53-6.7), CD4 (clone RM4-5), CD19 (clone IDB1/CD19), granzyme B (clone QA16A02), ICOS (clone C398.4A), CD279 (clone 29A12), CD11b (clone M1/70), CD39 (clone Duha59), and CD73 (clone TY/11.8) were purchased from BioLegend. Antibodies to CD45 (clone 30-F11), KLRC1 (clone 2F1), LG3 (clone CB7W8), and FOXP3 (clone MF23) were purchased from BD Biosciences. Antibody to ICOS (clone 7E.17G9) and human IgG (H+L) was purchased from Thermo Fisher Scientific. Antibody to CD8 (clone KT15) was purchased from MBL. Antibody to human GPC3 (clone GC33) was generated in Chugai Pharmaceutical. Dead cells were stained with Zombie Aqua Fixable Viability Kit (BioLegend) or Fixable Viability Dye eFluor780 (Thermo Fisher Scientific). These cells were incubated with antibodies for 30 minutes at 4°C with FOXP3/Transcription Factor Staining Buffer Set (Thermo Fisher Scientific) after treatment with mouse Fc receptor blocking reagent (Miltenyi Biotec). Staining was conducted according to the manufacturer’s protocol. Cells were analyzed by FACSLyric (BD Biosciences) or LSRFortessa X-20 (BD Biosciences).

NCI-H446 cells were inoculated subcutaneously into the flanks of huNOG mice. The single-cell suspensions were obtained using the same method as above on day 7. Antibodies to human CD4 (clone RPA-T4), human CD8 (clone SK1), and human CD19 (clone HIB19) were purchased from BioLegend. Antibody to human CD45 (clone HI30), human CD3 (clone UCHT1), and mouse CD45 (clone 30-F11) were purchased from BD Biosciences. Dead cells were stained with Fixable Viability Dye eFluor 780. The staining buffer was the same as above. Cell counts by flow cytometry were determined using CountBright Absolute Counting Beads (Thermo Fisher Scientific). Cells were incubated with antibodies for
30 minutes at 4°C after treatment with both human and mouse Fc receptor blocking reagents (Miltenyi Biotec). Cells were analyzed by LSRFortessa X-20.

Statistical Analysis
Data are presented as means ± SD, means + SD, or means only as stated in the figure legends. Statistically significant differences were determined using specific tests as indicated in the figure legends. *P* < 0.05 was considered statistically significant.

Data and Materials Availability
All data associated with this study are present in the paper or the Supplementary Materials. All materials and the novel mouse strain generated in this study can be made available on request under a material transfer agreement with Chugai Pharmaceutical Co., Ltd. The following restrictions apply to this mouse strain:

(i) Cross-breeding with other mouse strains is prohibited.

(ii) Additional gene manipulations to the mouse strain are prohibited.

(iii) Use of the mouse strain for commercial purposes is prohibited.

(iv) Further transfer of the mouse strain to third parties is prohibited.

Disclosure of Potential Conflicts of Interest
M. Kamata-Sakurai reports personal fees from Chugai Pharmaceutical, Co., Ltd. during the conduct of the study and outside the submitted work; in addition, M. Kamata-Sakurai has a patent for STA551 (patent for substance and patent for use; WO20200323230A1) pending to Chugai Pharmaceutical, Co., Ltd. Y. Narita reports personal fees from Chugai Pharmaceutical, Co., Ltd. during the conduct of the study and outside the submitted work; in addition, Y. Narita has a patent for STA551 (patent for substance and patent for use; WO20200323230A1) pending to Chugai Pharmaceutical, Co., Ltd. Y. Hori reports personal fees from Chugai Pharmaceutical, Co., Ltd. during the conduct of the study and outside the submitted work; in addition, Y. Hori has a patent for STA551 (patent for substance; WO20200323230A1) pending. R. Uchikawa reports personal fees from Chugai Pharmaceutical, Co., Ltd. during the conduct of the study and outside the submitted work; in addition, R. 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