Supplemental Data

Supplemental Materials and Methods

Reagents

Synthesis of [^{3}H]T-DXd

T-DXd and *N*-succinimidyl propionate [propionate-2,3-^{3}H] ([^{3}H]NSP; American Radiolabeled Chemicals Inc., St. Louis, MO) were mixed at a 1:1 ratio in 0.05 M borate buffer (pH 8.5) and incubated for 1 h; this was followed by the addition of ethanolamine (1 M) to terminate the reaction. After 15 min, the sample was applied to a PD Minitrap G-25 column (GE Healthcare UK Ltd., Buckinghamshire, UK), which was equilibrated with 10 mM acetate buffer and 5% sorbitol, pH 5.5 (ABS; Nacalai Tesque, Inc., Kyoto, Japan).

Measurement methods of radiochemical purities in [^{3}H]T-DXd

The [^{3}H]-labelled T-DXd ([^{3}H]T-DXd) was fractionated and pooled, and the purity was measured by radio-HPLC. The HPLC apparatus was an Acquity Ultra Performance LC (UPLC) system (Waters Corp., Milford, MA) with a TSKgel SuperSW3000 column (300 × 4.6 mm I.D., 4 μm, Tosoh Corp., Tokyo, Japan) and TSKguardcolumn SuperSW (35 × 4.6 mm I.D., Tosoh Corp.) as the guard column. The mobile phase was phosphate-

buffered saline (PBS) and had a constant flow rate of 0.35 mL/min; an analysis time of 30 min was used. The radio-HPLC apparatus was a UPLC system equipped with a radiomatic 625TR (PerkinElmer, Inc., Waltham, MA).

Sample preparation and measurements

Ligand binding assay for quantification of T-DXd and total Ab

This study employed the Gyrolab wizard method 200-3W-002-A (1% photomultiplier tube). The reagents for the total Ab measurement were 350 nM biotinylated anti-T-DXd idiotype mAb in PBS, with 0.1% polysorbate 20 (v/v) as the capture reagent, and 10 nM Alexa Fluor® 647 AffiniPure Goat Anti-Human IgG (Fcγ fragment specific) (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) in Rexxip F (Gyros Protein Technologies AB, Uppsala, Sweden) as the detector reagent. The capture reagent for the T-DXd measurement was the same as that for the total Ab measurement and the detector reagent was 10 nM DyLight® labelled anti-DXd mAb in Rexxip F. The samples were diluted to 1% plasma using Rexxip HN (Gyros Protein Technologies AB). The standard curve was fitted using a 5-parameter logistic curve fit by the Gyrolab Evaluator Software (Version 3.3.9.175, Gyros Protein Technologies AB).

Liquid chromatography-mass spectrometry (LC-MS) with a fluorescence detector for catabolic profiling of T-DXd in tumours

The tumours were weighed and then homogenised in three times their volume of PBS. Acetone (4 mL) was added to approximately 1 mL of the homogenate with 15 μL formic acid for extraction and deproteination, and the resulting pooled samples were then centrifuged at 2900 ×*g* for 10 min at 4°C. The supernatants were concentrated using a centrifugal evaporator, and the concentrates were loaded into solid-phase extraction (SPE) cartridges (Oasis HLB, 200 mg/6 mL, Waters Corp.). The SPE cartridges were first preconditioned with acetonitrile and then with 0.1% formic acid in purified water (v/v). After loading, the cartridges were washed with 0.1% formic acid in purified water (v/v, 2 mL), and the extracted substances were eluted using 0.1% formic acid and 90% acetonitrile in purified water (v/v/v, 3 mL). The eluted fractions were concentrated using a centrifugal evaporator until completely dry. The obtained residues were dissolved in 0.1% formic acid and 20% acetonitrile in purified water (v/v/v, 300 uL) and centrifuged at 2900 ×*g* for 10 min at 4°C. The supernatants were collected and filtered (18000 ×*g*, 5 min, 5°C) through a hydrophilic polyvinylidene difluoride membrane (Ultrafree-MC filter devices, 0.22 μm, Merck Millipore, Billerica, MA) and then injected into an LC-MS system equipped with a fluorescence detector (excitation at 375 nm and emission at

445 nm). The HPLC apparatus was an Acquity Ultra Performance LC (UPLC) system (Waters Corp.), and the column was an Acquity UPLC BEH C18 (100 mm × 2.1 mm I.D., S-1.7 μm, Waters Corp.) maintained at 40°C. The mobile phases were (A) 0.1% formic acid in purified water and (B) 0.1% formic acid in acetonitrile. The composition of B was initially 5% for 1 min, increased linearly to 40% at 15 min and 95% at 16 min, and was maintained at 95% until 18 min at a constant flow rate of 0.5 mL/min. Finally, the composition was decreased to 5% B, and the column was equilibrated for 1.9 min before the next injection. The MS apparatus was LTQ Orbitrap XL (Thermo Fisher Scientific Inc., Waltham, MA) and used electrospray ionisation (ESI) in positive mode.

Radio-detected high-performance liquid chromatography (radio-HPLC) and LC-MS for excretion study of [^{14}C]-labelled T-DXd

Faecal samples collected between 6 and 24 h were homogenised, extracted, and deproteinised twice with four times volumes of acetonitrile. After centrifugation, the two series of supernatants were pooled and concentrated using a centrifugal evaporator and then diluted in purified water. Next, appropriate volumes of urine or the concentrated faecal samples were loaded into SPE cartridges (Aqusis PLS-3 200 mg/6 mL, GL Sciences, Inc., Tokyo, Japan) and successively eluted with 20%, 40%, 60%, 80%, and

90% acetonitrile (v/v, 5 mL each). As more than 95% of the loaded radioactivity was recovered in the 20% and 40% fractions, these fractions were collected and pooled. The pooled samples were concentrated using a centrifugal evaporator and freeze-dried. Finally, the freeze-dried samples were diluted to the appropriate concentrations (volumes; faecal samples: 400 uL, urinary samples: 200 uL) in 20% acetonitrile (v/v). These pretreated samples were injected onto radio-HPLC and LC-MS systems for analyses. The HPLC apparatus was an Acquity Ultra Performance LC (UPLC) system (Waters Corp.) with an Inertsil ODS-3 HP column (150 × 4.6 mm I.D., S-3 μm, GL Sciences Inc.) maintained at 60°C. The mobile phases were (A) 0.1% formic acid in purified water and (B) 0.1% formic acid in acetonitrile. The composition of B was initially maintained at 5% for 1 min, increased linearly to 55% at 20 min and 95% at 23 min, and was maintained at 95% until 25 min at a constant flow rate of 1 mL/min. Finally, the composition was decreased to 5% B, and the column was equilibrated for 4.9 min before the next injection. The radio-HPLC apparatus was a UPLC system equipped with a radiomatic 625TR (PerkinElmer, Inc.). LC-MS utilised the above mentioned UPLC system and an LTQ Orbitrap XL (Thermo Fisher Scientific Inc.) using ESI in positive mode.

Table S1. Cumulative excretion of radioactivity (% dose) in urine and faeces for up to 2 weeks after a single intravenous administration of 10 mg/kg [^{14}C]T-DXd to mice (Mean of N = 2).

Time (h)	Cumulative excretion of radioactivity (% dose)		
	Urine	Faeces	Urine + Faeces
0-6	0.59	0.60	1.19
0-24	3.52	22.51	26.03
0-168	6.45	69.61	76.06
0-336	6.65	89.02	95.67