


## RESEARCH PAPER

# Evaluation and use of an anti-cynomolgus monkey CD79b surrogate antibody–drug conjugate to enable clinical development of polatuzumab vedotin

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**Background and Purpose:** Polatuzumab vedotin is an antibody–drug conjugate (ADC) being developed for non-Hodgkin's lymphoma. It contains a humanized anti-CD79b IgG1 monoclonal antibody linked to monomethyl auristatin E (MMAE), an anti-mitotic agent. Polatuzumab vedotin binds to human CD79b only. Therefore, a surrogate ADC that binds to cynomolgus monkey CD79b was used to determine CD79b-mediated pharmacological effects in the monkey and to enable first-in-human clinical trials.

**Experimental Approach:** Polatuzumab vedotin, the surrogate ADC, and the corresponding antibodies were evaluated in different assays in vitro and in animals. In vitro assessments included binding to peripheral blood mononuclear cells from different species, binding to a human and monkey CD79b-expressing cell line, binding to human Fcγ receptors, and stability in plasma across species. In vivo, ADCs were assessed for anti-tumour activity in mice, pharmacokinetics/pharmacodynamics in monkeys, and toxicity in rats and monkeys.

**Key Results:** Polatuzumab vedotin and surrogate ADC bind with similar affinity to human and cynomolgus monkey B cells, respectively. Comparable in vitro plasma stability, in vivo anti-tumour activity, and mouse pharmacokinetics were also observed between the surrogate ADC and polatuzumab vedotin. In monkeys, only the surrogate ADC showed B-cell depletion and B-cell-mediated drug disposition, but both ADCs showed similar MMAE-driven myelotoxicity, as expected.

**Abbreviations:** acMMAE, antibody-conjugated MMAE; ADC, antibody–drug conjugate; CL, clearance; DAR, drug-to-antibody ratio; MMAE, monomethyl auristatin E; NHL, non-Hodgkin's lymphoma; PBMCs, peripheral blood mononuclear cells; PD, pharmacodynamics; PK, pharmacokinetics; SCID, severe combined immunodeficiency; TK, toxicokinetics

\*Dongwei Li and Donna Lee contributed equally to the research.

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**Conclusions and Implications:** The suitability of the surrogate ADC for evaluation of CD79b-dependent pharmacology was demonstrated, and anti-tumour activity, pharmacokinetics/pharmacodynamics, and toxicity data with both ADCs supported the entry of polatuzumab vedotin into clinical trials.

## 1 | INTRODUCTION

Non-Hodgkin's lymphoma (NHL) is the most common haematological malignancy in adults. Despite improvements in clinical outcomes of patients, approximately half of patients with aggressive NHLs are refractory to or relapse following available standard of care therapies (Campo et al., 2011; Fisher, Miller, & O'Connor, 2004; Hennessy, Hanrahan, & Daly, 2004). Thus, there is a large unmet need for new treatments. Antibody–drug conjugates (ADCs) represent a class of therapeutics (Beck, Goetsch, Dumontet, & Corvalia, 2017) that aim to deliver chemotherapy selectively to cancer cells using tumour-targeting monoclonal antibodies (mAbs). **Gemtuzumab ozogamicin**, **ado-trastuzumab emtansine**, **brentuximab vedotin**, and **inotuzumab ozogamicin** are ADCs that have been approved for cancer therapy (Damle & Frost, 2003; Diamantis & Banerji, 2016; Piccaluga et al., 2011). **Polatuzumab vedotin** is an anti-CD79b ADC being developed for the treatment of NHL (NCT01290549, 2017; Palanca-Wessels et al., 2015). The structure and mechanism of action of polatuzumab vedotin are described in Figure 1 (Caculitan et al., 2017; Doronina et al., 2003; Sutherland et al., 2006). **CD79b** is a surface antigen whose expression is restricted to pre-B and mature B cells. CD79b is expressed on nearly all major subtypes of **B-cell-derived NHL** (Dornan et al., 2009; Polson et al., 2009). Antibodies that bind to CD79b rapidly internalize and traffic to the lysosomal compartment, making CD79b a suitable tumour antigen for targeted delivery of cytotoxic agents (Polson et al., 2007; Zheng et al., 2009).

A challenge for the non-clinical development of polatuzumab vedotin was that this ADC only binds to CD79b on B cells of humans but not on those of mouse, rat, or cynomolgus monkey, resulting in the lack of a pharmacologically relevant non-clinical species to evaluate antigen-dependent pharmacokinetics (PK), pharmacology, and safety prior to first-in-human trials (ICH S6, 2011 and ICH S9, 2009). In such cases, alternative approaches to the non-clinical assessment of antibody-based therapeutics can include the use of surrogate molecules, surrogate animal models, or in vitro pharmacological systems (ICH S6, 2011 and ICH S6 addendum, 2012).

Non-human primates are considered an appropriate species for non-clinical development of most antibody-based therapeutics because of their biological similarity to humans as shown by target expression, distribution, and pharmacological activity (Deng et al., 2011). Anti-murine surrogate antibodies are often used in the development of antibody-based therapeutics (Bussiere et al., 2009). However, the mouse is not considered an appropriate species for toxicological assessments of auristatin/dolastatin-containing ADCs because mice are not sensitive to the effects of these cytotoxic agents

### What is already known

- Polatuzumab vedotin binds human CD79b, but it does not bind CD79b of most non-clinical species.
- There were no relevant non-clinical species to evaluate the pharmacological effects of polatuzumab vedotin.

### What this study adds

- Non-clinical studies with a surrogate ADC provided relevant safety information to enable first-in-human trials.
- The principles for alternative testing approaches could potentially be applied to other biopharmaceuticals.

### What is the clinical significance

- The use of the surrogate ADC provided an understanding of CD79b-dependent pharmacological activity in patients.
- Clinical adverse effects were predicted from the non-clinical pharmacology and toxicity assessments.

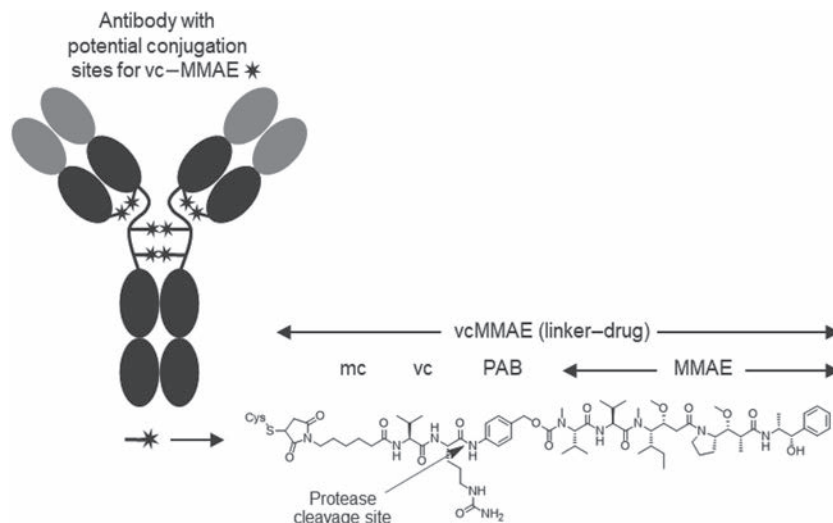
(Mirsalis et al., 1999). In order to assess the pharmacology and toxicity of polatuzumab vedotin, we developed a surrogate ADC that binds to cynomolgus monkey CD79b at an epitope similar to where polatuzumab vedotin binds to human CD79b (Zheng et al., 2009). A comprehensive evaluation of the surrogate ADC was performed to demonstrate its suitability. Studies with both ADCs provided relevant antigen-dependent and -independent pharmacology, PK, and safety information to support entry of polatuzumab vedotin into clinical trials.

## 2 | METHODS

### 2.1 | Animal welfare and ethical statement

All animal care and experimental protocols were in compliance with Testing Facility Standard Operating Procedures (SOPs) that adhere to regulations outlined in the USDA Animal Welfare Act (9 CFR, Parts 1, 2, and 3) and the conditions specified in the Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Research, National Research Council, 1996). The study protocols were approved by the Testing Facility Institutional Animal Care and Use Committee

**FIGURE 1** Chemical structure of polatuzumab vedotin and the surrogate antibody–drug conjugate (ADC). Polatuzumab vedotin consists of a humanized IgG1 antibody specific for human CD79b and is chemically conjugated to monomethyl auristatin E (MMAE) with a highly stable peptide linker (maleimidocaproyl [mc]–valine–citrulline [vc]–p-aminobenzoyloxycarbonyl [PAB]) that is selectively cleaved by lysosomal enzymes after internalization of the ADC. MMAE is a synthetic analogue of the natural product dolastatin. The intrachain cysteine disulfide bonds of the antibody are reduced, and the linker–MMAE is conjugated to the cysteines. With this method of conjugation, MMAE is coupled to an even number of cysteines (2, 4, 6, or 8). The surrogate ADC has a similar structure to polatuzumab vedotin except it contains a chimeric IgG1 antibody specific for monkey CD79b. Polatuzumab vedotin and the surrogate ADC are a mixture of the mAb linked with 0, 2, 4, 6, or 8 vc–MMAE molecules with an average of 3.7 vc–MMAE molecules per antibody for polatuzumab vedotin and an average of 3.5 vc–MMAE molecules per antibody for the surrogate ADC. The mechanism of action involves ADC binding to CD79b, followed by its internalization, cleavage of the linker by lysosomal enzymes, and release of MMAE, a cytotoxin. MMAE binds to tubulin and disrupts the microtubule network, resulting in inhibition of cell division and cell death



prior to dose administration. Studies were conducted following the SOP applicable to the Contract Research Organization (Covance, Inc. [RRID:SCR\_001224], for the rat study, or Charles River Laboratories International, Inc. [RRID:SCR\_00379], for the cynomolgus monkey study). Animal studies are reported in compliance with the ARRIVE guidelines (Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010) and with the recommendations made by the *British Journal of Pharmacology*. All animals were purpose bred and experimentally naïve at the outset of the studies. Prior to study assignment, animals were quarantined, maintained, and monitored for good health in accordance to testing facility SOP and/or as mandated by the Centers for Disease Control and Prevention, Atlanta, Georgia. Environmental control in the animal rooms were set to maintain a temperature of 18–29°C (64–84°F), a relative humidity of 30–70%, minimum fresh air changes of 10 per hour, and a light/dark cycle of 12:12 hr. All animals were housed individually in stainless-steel cages but were allowed to comingle to provide psychological enrichment, with the exception of telemetry animals. Prior to dosing, animals were allowed to acclimate in their housing for a minimum of 2 weeks. Certified primate diet was provided daily and supplemented with fruits or vegetables 2–3 times a week as part of the Testing Facility's environmental enrichment

programme. Water was provided ad libitum to each animal via automatic watering devices.

Cynomolgus monkeys were killed under deep, unrecoverable anaesthesia induced by ketamine and nembtal, or equivalent, followed by exsanguination. Rats were anaesthetized with sodium pentobarbital, and samples were collected for toxicokinetics (TK) and anti-drug antibody analysis, exsanguinated, and necropsied.

## 2.2 | Generation of antibodies and ADCs

The antibody for polatuzumab vedotin was generated and then humanized, and the antibody for surrogate ADC was generated as a mouse/human chimeric antibody (Diamantis & Banerji, 2016; Gorovits et al., 2013; Polson et al., 2009). ADCs were made as described previously (Gorovits et al., 2013). Briefly, ADCs were prepared by incubating the maleimide drug derivative with the partially reduced antibodies for 1 hr at 4°C. After quenching the reaction with excess *N*-acetyl-cysteine to react with any free linker–drug, the conjugated antibody was purified. Conjugation conditions were chosen to achieve an average drug-to-antibody ratio (DAR) of approximately 3.5. ADC protein concentrations were calculated using absorbance at 280 nm (320-nm

reference) and the molar extinction coefficient of the antibody. The average DARs were calculated from the integrated areas of the DAR species resolved by hydrophobic interaction chromatography on an analytical column (TSK butyl-NPR 4.6 mm, 10 cm, and 2.5 mm, Tosoh Bioscience).

## 2.3 | Cell lines

The NHL cell lines WSU-DLCL2 (DSMZ Cat# ACC-575, RRID:CVCL\_1902), BJAB (DSMZ Cat# ACC-757, RRID:CVCL\_5711), and BJAB.PD.cyCD79b.E3 were obtained from the Genentech (RRID:SCR\_003997) cell line repository and maintained in RPMI-1640 supplemented with 10% FBS (Sigma) and 2-mM L-glutamine. Each cell line was authenticated by short tandem repeat profiling using the Promega PowerPlex 16 System (Promega Corporation, Madison, WI [RRID:SCR\_006724]) and compared with external short tandem repeat profiles of cell lines to determine cell line ancestry.

## 2.4 | Flow cytometry of peripheral blood

Human, cynomolgus monkey, and rat peripheral blood mononuclear cells (PBMCs) were isolated from whole blood using a BD Vacutainer® CPT™ according to the manufacture's protocol. Mouse PBMCs were isolated from whole blood by ACK lysis buffer treatment to lyse red blood cells followed by centrifugation to recover PBMCs. Human, cynomolgus monkey, rat, and mouse PBMCs were incubated with 10 µg·ml<sup>-1</sup> of polatuzumab vedotin, surrogate ADC, or a human IgG isotype control labelled with Zenon Alexa Fluor 647 (Thermo Fisher Scientific). Samples were acquired on FACSCalibur flow cytometer (BD Biosciences [BD FACSCalibur Flow Cytometry System, RRID:SCR\_000401]). Data were analysed using FlowJo (RRID:SCR\_008520), and the geometric mean fluorescent intensity was calculated. A murine anti-human CD20 antibody (clone 2H7, BD Biosciences Cat# 555623, RRID:AB\_395989) was used as a B-cell marker for both human and cynomolgus monkey PBMCs. A murine anti-rat CD45RA (clone OX33, BD Biosciences Cat# 551402, RRID:AB\_394186) and a rat anti-mouse CD45R (clone RA3-6B2, BD Biosciences Cat# 553090, RRID:AB\_394620) were used as B-cell markers for rat and mouse PBMCs, respectively.

## 2.5 | Binding affinity with human and cynomolgus monkey CD79b-expressing BJAB cell lines by equilibrium binding analysis

The binding affinity of polatuzumab vedotin, the surrogate ADC, and their corresponding unconjugated antibodies to human and cynomolgus monkey CD79b was determined by equilibrium binding analysis using transfected BJAB cells, a human Burkitt's lymphoma-derived B-cell line that expresses both human and cynomolgus monkey CD79b (Zheng et al., 2009), in a radio-ligand competition cell-binding assay. The transfected BJAB cells were incubated for 2 hr at room temperature with a constant concentration of <sup>125</sup>I-labelled antibody and a dilution series of the non-labelled antibody. After incubation,

the competition reactions were transferred to a filter bottom plate and washed. Filters containing <sup>125</sup>I-labelled antibody bound to cells were counted using a gamma counter. Data were analysed using the non-linear regression method of Munson and Rodbard (1980) to determine the binding affinity of the antibody.

## 2.6 | Binding activity to human Fcγ receptors

The Fcγ receptor-binding activities of polatuzumab vedotin, the unconjugated clinical antibody (the anti-human CD79b mAb used for polatuzumab vedotin conjugation), the surrogate ADC, and the unconjugated surrogate antibody (the anti-cynomolgus monkey CD79b mAb used for surrogate ADC conjugation) were measured by ELISA using a panel of recombinant human Fcγ receptors (IA, IIA-H131, IIA-R131, IIB, IIIA-F158, and IIIA-V158) as previously described (Chung et al., 2012). For measuring binding to human FcγRII and FcγRIII, test antibodies were preincubated with anti-human κ light chain F(ab')<sub>2</sub> to form a complex to increase binding avidity. Briefly, test antibodies were serially diluted and then added to plates coated with recombinant human FcγRs. Bound antibodies were detected using goat F(ab')<sub>2</sub>-anti-human, F(ab')<sub>2</sub>-HRP, and tetramethylbenzidine (TMB). Dose-response binding curves were generated by plotting the mean absorbance values from duplicates of sample dilutions against the sample concentrations and fitted with a four-parameter model using SoftMax Pro (Molecular Devices [SoftMax Pro Data Acquisition and Analysis Software, RRID:SCR\_014240]). For comparison, the EC<sub>50</sub> value of the reference molecule was set at 1, and the relative activity of each sample was calculated as follows:

$$\text{Relative Activity} = \frac{\text{Reference EC}_{50}}{\text{Sample EC}_{50}}$$

## 2.7 | In vitro plasma stability studies

Freshly frozen human, cynomolgus monkey, rat, and mouse plasma (lithium-heparin; Bioreclamation, Inc. [RRID:SCR\_004728]) were thawed, centrifuged at 228× g (Beckman CS-6R centrifuge) for 5 min at 4°C followed by filtration through a 0.22-µm filter (Pall Co.) into sterile polypropylene tubes and kept on ice until used. ADC stock solutions were added to plasma at a final concentration of 100 µg·ml<sup>-1</sup>. ADC vehicle with 0.5% BSA was used as a control matrix. Aliquots of 100 µl from each mixture were transferred into sialated microcentrifuge tubes and incubated at 37°C in a CO<sub>2</sub> incubator with gentle rotation, to maintain the plasma pH levels close to the physiological pH of 7.2 throughout the incubation period. To stop the reaction, samples were first transferred to dry ice at predetermined time points (0.01, 8, 24, 48, and 96 hr) and then stored in a -80°C freezer. The sample at 0.01 hr was collected within the first minute after adding the conjugate to the plasma.

## 2.8 | Efficacy studies in human tumour xenograft mouse models

All animal studies were conducted in compliance with National Institutes of Health (Bethesda, MD) guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee at Genentech, Inc. Human tumour cells ( $20 \times 10^6$  cells in 0.2-ml HBSS; Hyclone) were inoculated subcutaneously into the flanks of female CB17 severe combined immunodeficient (SCID) mice (Charles River Laboratories [IMSR Cat# CRL:236, RRID:IMSR\_CRL:236]). When mean tumour size reached about  $150 \text{ mm}^3$ , the mice were divided into groups of 8 to 10 with the same mean tumour size and dosed i.v. via the tail vein with ADCs or unconjugated antibodies. Treatment information was not blinded during tumour measurement. Tumours were measured in two dimensions (length and width) using calipers, and tumour volume was calculated using the formula: Tumour size ( $\text{mm}^3$ ) =  $0.5 \times (\text{length} \times \text{width} \times \text{width})$ . The results were plotted as mean tumour volume  $\pm$  SEM of each group over time. Partial response was defined as a tumour regression of  $>50\%$  but  $<100\%$  of the initial tumour volume, and complete response was defined as 100% tumour remission (i.e., no detectable tumour) on any given day during the study.

## 2.9 | PK study in SCID mice

The PK study in SCID mice was approved by the Institutional Animal Care and Use Committee at Genentech, Inc. Female SCID mice received a single i.v. dose of  $5 \text{ mg}\cdot\text{kg}^{-1}$  of either polatuzumab vedotin or surrogate ADC and their corresponding unconjugated antibodies, via the tail vein ( $n = 20$  per group). Due to the limited blood volume in mice, serial samples from the same mouse for all time points were not feasible. Instead, blood samples were collected via retro-orbital bleeds conducted on alternate eyes, and the terminal blood sample was collected via cardiac stick from each animal in each dosing group at the following time points: pre-dose; 5 min; 1, 6, and 24 hr; and 2, 3, 7, 10, 14, 21, and 28 days post-dose, and processed to collect plasma. Three blood samples were taken from each mouse, and there were four mice per time point. Plasma concentration–time data were used to estimate relevant PK parameters.

## 2.10 | Multiple dose toxicity and TK study in Sprague–Dawley rats

This study was performed under GLP compliance at Covance Laboratory (Madison, WI). Male and female Sprague–Dawley rats (15 per sex per toxicity group; nine per sex per TK group [RGD Cat# 10395233, RRID:RGD\_10395233]) were administered with i.v. doses of vehicle, 2, 6, or  $10 \text{ mg}\cdot\text{kg}^{-1}$  of polatuzumab vedotin once weekly (Q1W) for four dose cycles (on Study Days 1, 8, 15, and 22). A weekly dosing regimen was used to ensure that the ADC exposures were adequate to detect any target-independent and/or MMAE-driven adverse findings.

The reversibility, persistence, or delayed occurrence of any effects was assessed during the 6-week recovery period. Blood was collected pre-dose and at selected time points throughout the study for analyses of haematology, serum chemistry, coagulation, and TK. Additionally, functional observational battery and motor activity assessments were conducted to determine impact on neurobehavioral systems. At scheduled terminal and recovery necropsies, tissues were grossly examined, collected, preserved, and processed for histological assessments.

## 2.11 | Multiple dose toxicity and TK study in cynomolgus monkeys

This study was performed under GLP compliance at Charles River Laboratories (Reno, NV). Experimentally naïve male and female cynomolgus monkeys ( $N = 5$  per sex per group) were given an i.v. injection of vehicle, 1, 3, or  $5 \text{ mg}\cdot\text{kg}^{-1}$  of polatuzumab vedotin or 3 or  $5 \text{ mg}\cdot\text{kg}^{-1}$  of surrogate ADC once every 3 weeks (Q3W) for a total of four dose cycles (on Study Days 1, 22, 43, and 64). The Q3W i.v. dosing regimen was used to support the intended treatment regimen in patients. A terminal necropsy was conducted 1 week after the last dose (Day 71) to assess adverse effects associated with repeated polatuzumab vedotin and surrogate ADC administration and after a 9-week recovery period to assess reversibility of toxicity and the pharmacodynamic response of decrease in B cells mediated through CD79b. The length of this recovery period was selected based on confirmation of repletion of circulating B cells to  $\geq 40\%$  of baseline values by 9 weeks post-dose. Blood was collected pre-study and at selected time points throughout the study for analyses of haematology, serum chemistry, coagulation, TK, anti-drug antibodies, and measurement of circulating lymphocyte populations by flow cytometry. In addition, ophthalmic and physical examinations were conducted during the pre-dose phase and at the end of the first and last dose cycles. Safety pharmacology assessment was also conducted as a part of this study and included cardiovascular (ECG and haemodynamic endpoints in surgically implanted telemetry animals), respiratory, and neurobehavioral endpoints. At scheduled terminal and recovery necropsies, tissues were grossly examined, collected, preserved, and processed for histological assessments.

## 2.12 | Immunophenotyping of circulating lymphocyte populations by flow cytometry

Immunophenotyping analyses were conducted as part of the multiple dose toxicity study in monkeys. Flow cytometric assays were used to determine the percentage of lymphocyte-gated  $\text{CD3}^+$  T cells,  $\text{CD3}^+\text{CD4}^+$  T helper cells,  $\text{CD3}^+\text{CD8}^+$  cytotoxic T cells,  $\text{CD3}^-\text{CD20}^-$  NK cells, and  $\text{CD20}^+$  B cells in peripheral blood at Pre-dose Study Days  $-14$ ,  $-7$ , and 1 and at Post-dose Study Days 2, 8, 22, 29, 43, 50, 64, 71, 85, 99, 113, 120, and 127. Samples were analysed according to Charles River Laboratory SOP. In brief, peripheral blood samples were collected from each animal and aliquoted into 96-well

plates containing saturating concentration of one or more of the fluorescently conjugated anti-CD3, -CD4, -CD8, and -CD20 antibody or the respective isotype controls. Samples were mixed and allowed to be incubated at room temperature in the dark for 30 min followed by 10 min of incubation in red blood cell lysis solution. Samples were then centrifuged and washed once with CRL (Charles River Laboratories) flow cytometry staining buffer followed by immediate acquisition on the BD FACSCanto™ II Flow Cytometer (BD Biosciences [RRID: SCR\_013311]). Data were analysed using BD FACS DIVA® 6.0 Software (BD Biosciences). Relative abundance of each cellular subset was calculated by multiplying the percentage of the individual gated lymphocyte subset by absolute lymphocyte counts (determined by the standard haematological analysis) of that animal at that time point. The magnitude or percentage of change from baseline (%BL) was calculated by taking absolute subset counts and dividing that by average of given subset at baseline (average of Day -14, Day -7, and Day 1 pre-dose)  $\times 100$ . Data were expressed as group mean  $\pm$  SD.

### 2.13 | Bioanalysis of serum and plasma samples from PK and toxicity studies

Total antibody and antibody-conjugated MMAE (acMMAE) were measured to characterize the non-clinical PK for polatuzumab vedotin and the surrogate ADC (Gorovits et al., 2013).

Mouse plasma concentrations of total antibody (including fully conjugated, partially deconjugated, and fully deconjugated either anti-human CD79b antibodies or anti-cynomolgus monkey CD79b antibodies) and unconjugated antibody following a single administration of polatuzumab vedotin or surrogate ADC, and their corresponding unconjugated antibodies, respectively, were analysed by a bridging ELISA method (Kozak et al., 2013). The ELISA utilized goat anti-human IgG-HRP and biotinylated 15-mer peptide, targeting the human or cynomolgus monkey CD79b extracellular domain, to capture either the anti-human or anti-cynomolgus monkey CD79b antibodies, respectively. HRP-conjugated goat anti-human IgG antibody and biotin-labelled extracellular domain peptide were diluted in sample buffer and premixed. An equal volume of this mixture was then combined with serially diluted ADC-containing samples. The complexes were then captured onto NeutrAvidin-coated plates (Thermo Scientific, Rockford, IL), and colour development was initiated using TMB peroxidase substrate (Moss, Inc., Pasadena, MD). Absorbance was measured at 450 nm against a reference wavelength of 620 nm using a microplate reader (Molecular Devices, Sunnyvale, CA, or Titertek, Huntsville, AL). The concentration of the total antibody in the samples was extrapolated from a four-parameter fit of the unfractionated ADC standard curve. The minimum reportable value for this assay was 15.6 ng·ml<sup>-1</sup>.

Mouse plasma concentrations of acMMAE were quantified by LC-MS-MS. The acMMAE assay measured the concentration of MMAE conjugated to the ADC. Polatuzumab vedotin, surrogate ADC, and trastuzumab-vc-monomethyl auristatin F (ADC Internal Standard)

were captured from plasma samples using a MabSelect Protein A resin (GE Healthcare, Piscataway, NJ [RRID:SCR\_000004]). Samples were washed extensively to remove any non-specifically bound proteins, followed by enzymic cleavage of the linker to release both MMAE (analyte) and monomethyl auristatin F (internal standard). After elution with 70% ethanol, samples (containing free MMAE and monomethyl auristatin F) were dried, reconstituted, and extracted by protein precipitation. The supernatant (containing MMAE and monomethyl auristatin F) was then analysed using LC-MS-MS. For in vivo sample analysis, the assay had a lower limit of quantification of 0.00195 nM (0.140 ng·ml<sup>-1</sup>). A similar assay was used to quantify acMMAE concentrations in the in vitro plasma stability study and had a lower limit of quantification of 0.781 nM (0.561 ng·ml<sup>-1</sup>).

Cynomolgus monkey serum concentrations of total antibody (both conjugated and unconjugated either anti-human CD79b antibodies or anti-cynomolgus monkey CD79b antibodies) following repeat dose of polatuzumab vedotin or surrogate ADC were analysed using validated ELISAs. Two 21-amino acid peptides that correspond to the anti-CD79b antibody epitopes of human CD79b and cynomolgus monkey CD79b were synthesized and utilized as capture reagents. Microtitre plates were coated with either human or cynomolgus monkey CD79b peptide in PBS for 16–72 hr at 4°C. After blocking with the assay buffer, serum samples diluted to a minimum of 1/100 were added and incubated at room temperature (RT) for 2 hr. After washing, biotinylated sheep anti-human IgG ([Bethyl Cat# A80-155B, RRID: AB\_10749191], Bethyl Laboratories, Montgomery, TX [Bethyl, RRID: SCR\_013554]) was added and incubated at RT for 1 hr. Following this incubation and washing, Amdex streptavidin-conjugated HRP (GE Healthcare; Milwaukee, WI) was added and incubated at RT for 1 hr followed by the addition of TMB. Absorbance was measured at 450 nm against a reference wavelength of 620 or 630 nm using a microplate reader (Biotech, Winooski, VT, or Tecan, San Jose, CA). The serum total antibody concentrations were calculated by fitting the data with a four-parameter model using Watson LIMS version 7.2.0.04 (Thermo Fisher, Waltham, MA). The minimum reportable value was 150 and 60 ng·ml<sup>-1</sup> for polatuzumab vedotin total antibody and surrogate ADC total antibody, respectively. A similar non-validated assay was used to quantify total antibody concentrations in the in vitro plasma stability study; the minimum reportable value was 100 and 75 ng·ml<sup>-1</sup> for polatuzumab vedotin total antibody and surrogate ADC total antibody, respectively.

### 2.14 | PK data analysis and statistics

Mouse plasma concentration–time data for a naïve pool of animals from all groups were used to estimate PK parameters using WinNonlin (version 5.2.1; Certara, Mountain View, CA). A two-compartment model with i.v. bolus input was used to describe the observed data, and the following PK parameters were reported: observed maximum serum concentration ( $C_{max}$ ), total drug exposure defined as area under the serum concentration–time curve extrapolated to infinity ( $AUC_{inf}$ ), clearance (CL), volume of distribution of the central compartment ( $V_1$ ), volume of distribution at steady state ( $V_{ss}$ ), half-life of the alpha

phase ( $t_{1/2\alpha}$ ), and half-life of the elimination phase ( $t_{1/2\beta}$ ). As expected, the inter-group variability in estimation of PK parameters was observed to be large due to the staggered sampling from the different mice and different time points.

Cynomolgus monkey serum concentration–time profiles were used to estimate TK parameters using WinNonlin (version 5.2.1; Pharsight Corporation, Mountain View, CA). For TK data calculations, Study Day 1 (in-life dosing day) was converted to TK Day 0 to indicate the start of dosing. All time points after the in-life dosing day are calculated as study day minus 1. A non-compartment model with i.v. bolus input was used to describe the observed data, and the following TK parameters were reported: observed maximum serum concentration ( $C_{\max}$ ) after the first dose and the fourth dose, area under the concentration–time curve from TK Day 0 to TK Day 21 ( $AUC_{0-21}$ ), the total exposure over the first dosing interval, area under the concentration–time curve from TK Day 63 to TK Day 84 ( $AUC_{63-84}$ ), and the total exposure over the last or fourth dosing interval. Each animal was analysed separately, and the results for each dose group were summarized as mean  $\pm$  SD.

Rat serum concentration–time profiles were used to estimate TK parameters using WinNonlin (version 5.2.1; Pharsight Corporation, Mountain View, CA). A non-compartmental model with i.v. bolus input was used to describe the observed data, and the following TK parameters were reported: observed maximum serum concentration ( $C_{\max}$ ) after the first dose and the fourth dose, area under the concentration–time curve from TK Day 0 to TK Day 7 ( $AUC_{0-7}$ ), and area under the concentration–time curve from TK Day 21 to TK Day 28 ( $AUC_{21-28}$ ). Each animal was analysed separately, and the results for each dose group were summarized as mean  $\pm$  SD. The data and statistical analysis comply with the recommendations of the *British Journal of Pharmacology* on experimental design and analysis in pharmacology.

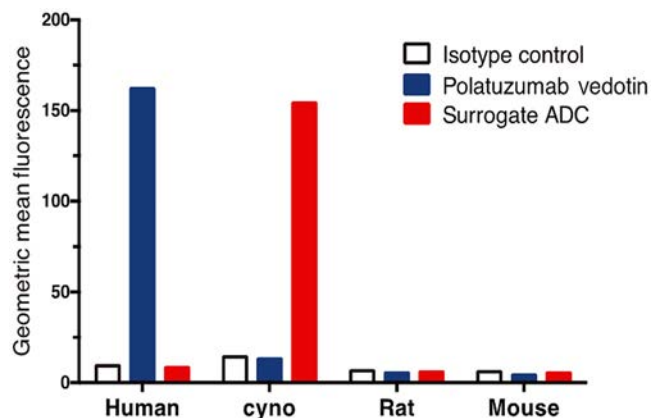
## 2.15 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander et al., 2017).

## 3 | RESULTS

### 3.1 | Binding of polatuzumab vedotin and the surrogate ADC to mouse, rat, cynomolgus monkey, and human B cells

Despite only a three-amino acid difference in the epitope for CD79b between human and cynomolgus monkey, polatuzumab vedotin binds only human CD79b (Zheng et al., 2009). Here, we evaluated the binding of polatuzumab vedotin and surrogate ADC to mouse, rat, cynomolgus monkey, and human B cells, the only cell type that



**FIGURE 2** Species-specific binding of polatuzumab vedotin and the surrogate antibody–drug conjugate (ADC) to B cells. Polatuzumab vedotin, surrogate ADC, or isotype control (Hu anti-HER2 4D5), labelled with Zenon Alexa Fluor 647, were incubated with human, cynomolgus monkey, rat, and mouse PBMCs, isolated from whole blood. To allow for gating on CD20-positive B cells (human and cynomolgus monkey, isotype control), CD45RA-positive B cells (rat), or CD45R-positive B cells (mouse), the cells were co-stained with PE murine anti-human CD20 antibody, PE murine anti-rat CD45RA antibody, or PE rat anti-mouse CD45R antibody, respectively. After washing, cells were fixed with paraformaldehyde/PBS, and flow cytometry was performed on a BD FACSCalibur (BD FACSCalibur Flow Cytometry System, RRID:SCR\_000401). The geometric mean fluorescence intensity, indicative of the extent of binding, was recorded for each sample, and the data were analysed using FlowJo v8.4.5 software

expresses CD79b, by flow cytometry using PBMCs. Polatuzumab vedotin and surrogate ADC demonstrated binding exclusively to the B-cell (CD20<sup>+</sup>) subpopulation of the human PBMCs and cynomolgus monkey PBMCs, respectively (Figure 2). Neither ADC showed binding to the B-cell (CD45RA<sup>+</sup>) subpopulation of the mouse or rat PBMCs.

### 3.2 | Binding affinity of polatuzumab vedotin, the surrogate ADC, and their respective antibodies to human and cynomolgus monkey CD79b-expressing BJAB cells

The binding affinities of polatuzumab vedotin, clinical antibody, surrogate ADC, and the surrogate antibody to human and cynomolgus monkey CD79b were determined by radio-labelled, equilibrium binding assays using CD79b-transfected BJAB cells. Polatuzumab vedotin demonstrated high binding affinity to human CD79b (Table 1). The binding affinity of polatuzumab vedotin appeared similar to that of the unconjugated clinical antibody confirming that the antibody maintains antigen binding integrity and affinity after conjugation. Similarly, the surrogate ADC and the unconjugated surrogate antibody bound to cynomolgus monkey CD79b with high affinities (Table 1). Furthermore, the binding affinity of surrogate ADC to cynomolgus monkey CD79b was similar to that of polatuzumab vedotin to human CD79b.

**TABLE 1** Comparison of the properties of polatuzumab vedotin, the surrogate ADC, and their corresponding unconjugated antibodies

Properties	Polatuzumab vedotin	Surrogate ADC
Target antigen	Human CD79b	Cynomolgus monkey CD79b
Antibody	Unconjugated clinical antibody Humanized IgG1 monoclonal antibody	Unconjugated surrogate antibody Chimeric mouse–human IgG1 monoclonal antibody
	Affinity ( $K_D$ ): $1.33 \pm 0.14$ nM	Affinity ( $K_D$ ): $1.21 \pm 0.10$ nM
Structure	Anti-human CD79b humanized IgG1–vc–MMAE	Anti-cynomolgus monkey CD79b chimeric IgG1–vc–MMAE
Mean DAR <sup>a</sup>	3.7	3.5
Epitope <sup>b</sup>	<b>AR</b> SEDRYRNP <b>KGS</b>	<b>AK</b> SEDLYPNP <b>KGS</b>
Affinity ( $K_D$ )	$1.83 \pm 0.26$ nM	$1.51 \pm 0.25$ nM

Note. Each value represents the average of three independent experiments  $\pm$  the SD of those measurements.

Abbreviations: ADC, antibody–drug conjugate; DAR, drug-to-antibody ratio; vc–MMAE, MC–vc–PAB–MMAE.

<sup>a</sup>DAR represents the vc–MMAE-to-antibody ratios for polatuzumab vedotin and the surrogate ADC lots used in the non-clinical studies.

<sup>b</sup>Peptide sequence used in competition experiments (Zheng et al., 2009); sequence difference is indicated in bold.

### 3.3 | Binding activity of polatuzumab vedotin, the surrogate ADC, and their respective antibodies to human Fc $\gamma$ R<sub>s</sub>

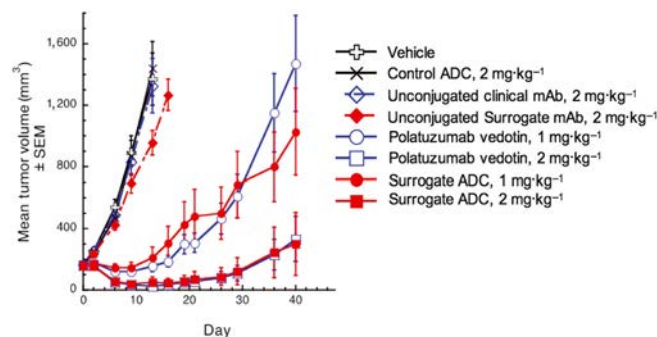
Binding to human Fc $\gamma$ R<sub>s</sub> was assessed by ELISA. Polatuzumab vedotin, unconjugated clinical antibody, surrogate ADC, and the unconjugated surrogate antibody showed comparable binding to human Fc $\gamma$  receptors: Fc $\gamma$ RI<sub>A</sub>, two allotypes of Fc $\gamma$ RII<sub>A</sub> (H131 and R131), Fc $\gamma$ RII<sub>B</sub>, and two allotypes of Fc $\gamma$ RIII<sub>A</sub> (F158 and V158; Table S1). Similar binding between the ADCs and their corresponding unconjugated antibodies demonstrated that binding to Fc $\gamma$ R<sub>s</sub> was not compromised by the conjugation process. Polatuzumab vedotin demonstrated similar binding to Fc $\gamma$ RI<sub>A</sub> as compared with the surrogate ADC and two-fold to threefold reduced binding activity to the rest of the receptors tested (Table S1).

### 3.4 | In vitro linker–drug stability of polatuzumab vedotin and surrogate ADC in human and animal plasma

To evaluate the stability of the linker in the ADC molecule and to evaluate any species differences, in vitro plasma stability studies were conducted. A comparable decrease across species was observed in the total antibody concentration following incubation of polatuzumab vedotin or surrogate ADC in plasma over time. Most of the decrease in concentration occurred during the first 24 hr of the study, with a more gradual decrease for the remainder of the 96 hr (Figure S1a,b). The acMMAE concentrations, which are a measure of the amount of MMAE conjugated to polatuzumab vedotin or surrogate ADC, decreased gradually over the 96-hr study in all species tested (Figure S1c,d). The proportion of acMMAE, relative to time 0.01 hr, decreased to approximately 60% at 96 hr for all species. Overall, the in vitro plasma stability with polatuzumab vedotin and surrogate ADC were comparable across species and between the two ADCs.

### 3.5 | Efficacy of polatuzumab vedotin and the surrogate ADC against human Burkitt's lymphoma xenograft in SCID mice

To compare the anti-tumour activity of the surrogate ADC and polatuzumab vedotin, a tumour xenograft model of human Burkitt's lymphoma (BJAB-PD.cyCD79b.E3) expressing similar amounts of surface cynomolgus monkey and human CD79b was employed (Zheng et al., 2009). Polatuzumab vedotin and the surrogate ADC showed comparable inhibition of tumour growth (Figure 3). The unconjugated clinical antibody, unconjugated surrogate antibody, and the non-binding control ADC administered at  $2 \text{ mg}\cdot\text{kg}^{-1}$  had minimal effects on tumour growth, demonstrating that Fc $\gamma$ R-mediated effector functions (e.g., antibody-dependent cell-mediated cytotoxicity) are not the primary mechanism of action of both ADCs. Therefore, comparable tumour growth inhibition by both ADCs was thought to be driven by



**FIGURE 3** Growth inhibition of BJAB-PD.cyCD79b.E3 xenograft tumours in response to single doses of polatuzumab vedotin, the clinical antibody, surrogate antibody–drug conjugate (ADC), surrogate antibody, or non-binding control ADC. Female CB17 SCID mice were subcutaneously implanted with  $20 \times 10^6$  tumour cells and administered with a single i.v. dose (Day 0) of vehicle, antibodies, or ADCs at 1 or  $2 \text{ mg}\cdot\text{kg}^{-1}$  when the average tumour size reached  $162 \text{ mm}^3$ . Mean tumour volumes ( $\pm$  SEM) of each group ( $n = 10$ ) are plotted over time (days post-dose)



antigen binding, ADC internalization, and release of the cytotoxic MMAE.

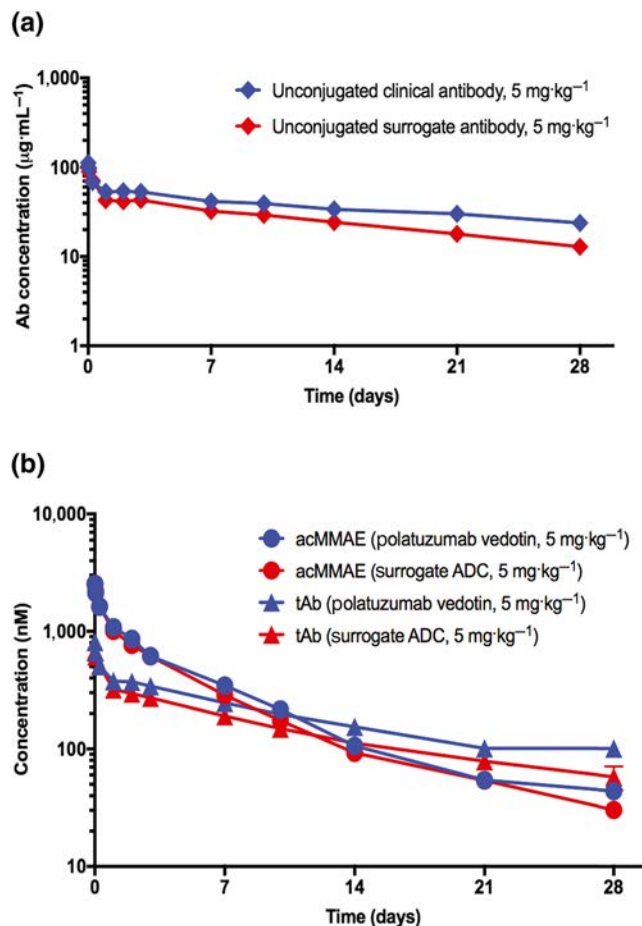
### 3.6 | Dose-dependent anti-tumour activity of polatuzumab vedotin against human diffuse large B-cell lymphoma and human Burkitt's lymphoma in SCID mice

The anti-tumour activity of polatuzumab vedotin at multiple dose levels was evaluated in a tumour xenograft model of human diffuse large B-cell lymphoma WSU-DLCL2, which only expresses human CD79b. Mice with a tumour volume range of 101–197 mm<sup>3</sup> were treated with a single i.v. dose of vehicle; polatuzumab vedotin at 0.3, 1, 3, 6, or 12 mg·kg<sup>-1</sup>; unconjugated clinical antibody at 12 mg·kg<sup>-1</sup>; or a non-binding control ADC at 12 mg·kg<sup>-1</sup>. Polatuzumab vedotin showed dose-dependent tumour growth inhibition compared with the vehicle group when administered at ≥1 mg·kg<sup>-1</sup> (Figure S2a). The corresponding unconjugated clinical antibody and the non-binding control ADC had minimal effects on tumour growth.

Similarly, the anti-tumour activity of polatuzumab vedotin at multiple dose levels was evaluated in another human CD79b-expressing xenograft model, BJAB. Mice with a tumour volume range of 105–177 mm<sup>3</sup> were administered with single i.v. doses of vehicle; polatuzumab vedotin at 0.1, 0.5, 1, 2, or 4 mg·kg<sup>-1</sup>; the unconjugated clinical antibody at 4 mg·kg<sup>-1</sup>; or a non-binding control ADC at 4 mg·kg<sup>-1</sup>. Polatuzumab vedotin showed dose-dependent inhibitory activity when administered at ≥0.5 mg·kg<sup>-1</sup> compared with vehicle (Figure S2b). Neither the unconjugated clinical antibody nor the non-binding control ADC administered at 4 mg·kg<sup>-1</sup> had any substantial inhibitory activity on tumour growth. Both xenograft studies further confirmed that efficacy observed with polatuzumab vedotin is driven by the cytotoxic effect of MMAE upon internalization of the ADC by CD79b-expressing tumour cells (Polson et al., 2007), indicating that unconjugated antibody-mediated immune effector functions do not play an essential role in the anti-tumour efficacy.

### 3.7 | PK of polatuzumab vedotin, the surrogate ADC, and their respective unconjugated antibodies in SCID mice

Polatuzumab vedotin and the surrogate ADC do not bind to mouse CD79b. Therefore, the antigen-independent PK of polatuzumab vedotin, the surrogate ADC, and their corresponding unconjugated antibodies were characterized and compared in SCID mice. The PK profiles of both unconjugated antibodies following a single 5 mg·kg<sup>-1</sup> i.v. dose and total antibody following a single i.v. dose of either polatuzumab vedotin or the surrogate ADC at 5 mg·kg<sup>-1</sup> are shown in Figure 4; PK parameters are summarized in Table S2. Overall, PK profiles of the two unconjugated antibodies were comparable and characterized by a short distribution phase and a long elimination phase (Figure 4a). The PK of total antibody following administration of the ADCs was also similar between the two ADCs



**FIGURE 4** Pharmacokinetics of unconjugated clinical antibody (Ab), unconjugated surrogate Ab, polatuzumab vedotin, and surrogate Ab-drug conjugate (ADC) in female severe combined immunodeficiency mice. (a) Group mean ( $\pm$  SD) serum Ab concentration ( $\mu\text{g}\cdot\text{mL}^{-1}$ )–time profiles following i.v. administration of 5 mg·kg<sup>-1</sup> clinical Ab and surrogate Ab. (b) Total Ab (tAb) and plasma Ab-conjugated MMAE (acMMAE) concentration (nM)–time profiles following i.v. administration of 5 mg·kg<sup>-1</sup> of polatuzumab vedotin or surrogate ADC in severe combined immunodeficiency mice

and characterized by a slow CL and a long elimination half-life of 10–12 days. The PK profile was similar to that typically seen with most mAbs (Deng et al., 2011). Additionally, acMMAE plasma concentrations following administration of polatuzumab vedotin or the surrogate ADC were comparable between the two ADCs (Figure 4b). acMMAE plasma concentrations decreased over time in a manner similar to the total antibody concentrations for both ADCs, indicating that MMAE is not rapidly released from polatuzumab vedotin or the surrogate ADC in mice and, as expected, the PK of the ADC is driven by the antibody component of the ADC.

### 3.8 | Toxicity and TK of polatuzumab vedotin in rats

The antigen-independent toxicity profile of polatuzumab vedotin was assessed in rat, a non-binding species. Polatuzumab vedotin administration was generally well tolerated up to 6 and 10 mg·kg<sup>-1</sup>

**TABLE 2** Safety findings in cynomolgus monkeys and rats treated with polatuzumab vedotin or surrogate ADC

Dose schedule	Monkey Q3W × 4 (9-week recovery)					Rat Q1W × 4 (6-week recovery)		
	5 M/5 F					15 M/15 F		
Ns per group	Polatuzumab vedotin		Surrogate ADC			Polatuzumab vedotin		
	Dose (mg·kg <sup>-1</sup> )							
Target organ	1	3	5	3	5	2	6	10
Bone marrow -Decreases in red cell mass, reticulocytes, neutrophils, and lymphocytes -Hypocellularity of bone marrow	-	+	+	+	+	-	+	+
Thymus -Decreased lymphocyte cellularity in thymic cortex	-	-	-	-	-	-	+	+
Liver -Increased serum liver transaminases -Multifocal hepatic necrosis; increased mitosis/apoptosis of hepatocytes and sinusoidal cells	-	-	-	-	-	-	+	+
Lung -Alveolar macrophage infiltrates -Hyperplasia/hypertrophy of type II pneumocytes	-	-	-	-	-	-	+	+
Skin -Increased mitosis/apoptosis	-	-	-	-	-	-	+	+
Testicles -Small soft testes -Seminiferous tubule degeneration; abnormal lumen contents in the epididymis	-	-	-	-	-	+	+	+
Test Article-related mortality	-	-	-	-	1 M	-	-	1 M
	HNSTD = 3 mg·kg <sup>-1</sup>					STD <sub>10</sub> = 10 mg·kg <sup>-1</sup>		

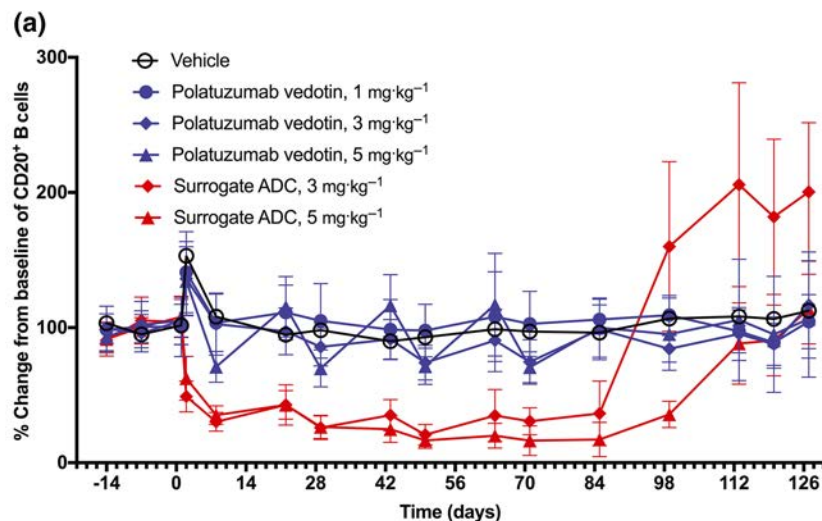
Note. All toxicity findings were dose dependent in nature and reversible by the end of the recovery period, with the exception of findings in the testicles. Abbreviations: ADC, antibody–drug conjugate; F, female; HNSTD, highest non-severely toxic dose; M, male; Ns, number of animals; Q1W, once weekly; Q3W, once every 3 weeks; STD, severely toxic dose.

in males and females, respectively, when it was given at 2, 6, or 10 mg·kg<sup>-1</sup> weekly for four doses. One of 15 males given 10 mg·kg<sup>-1</sup> of polatuzumab vedotin was killed in moribund condition, with significant bone marrow toxicity. The main toxicology findings at 6 and 10 mg·kg<sup>-1</sup> dose levels were bone marrow toxicity, thymic lymphoid depletion, and hepatobiliary toxicity (Table 2). Reproductive toxicity was limited to male testes and epididymitis. Additionally, in the lung, alveolar macrophage infiltrates accompanying hyperplasia/hypertrophy of type II pneumocytes were identified. Nevertheless, the histological findings were completely reversible after a 6-week recovery period, with the exception of residual effects in the liver of one 10 mg·kg<sup>-1</sup> female, in the lungs of one 10 mg·kg<sup>-1</sup> male, and the testicular and epididymal toxicity observed at all doses in male rats. These histological findings were consistent with the pharmacological effects of MMAE resulting in mitotic arrest, particularly in tissues with a high background mitotic rate.

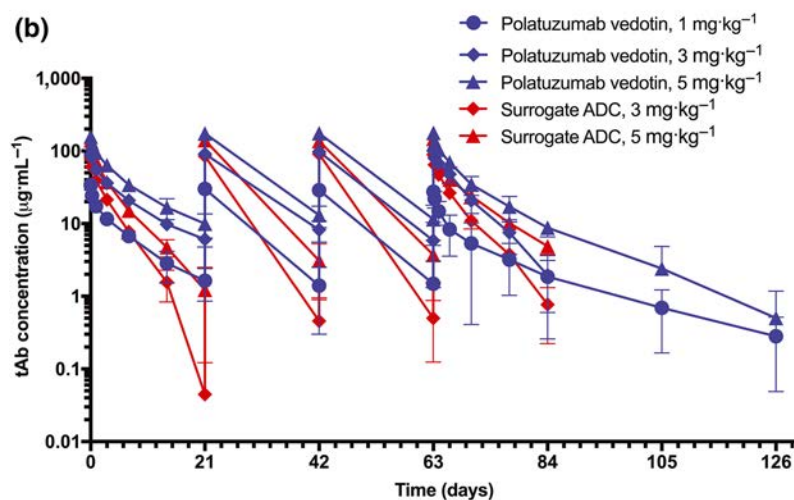
As predicted for a non-binding species, the exposure of total antibody following i.v. administration of polatuzumab vedotin was dose proportional with moderate accumulation observed after repeated weekly doses (Table S3). The severely toxic dose in 10% of animals (STD<sub>10</sub>) was determined to be 10 mg·kg<sup>-1</sup> based on adverse effects on haematopoiesis and the bone marrow.

### 3.9 | Pharmacodynamics, TK, and toxicity of surrogate ADC and polatuzumab vedotin in cynomolgus monkeys

To confirm the antigen-independent toxicity profiles of polatuzumab vedotin observed in rats and to determine the PK/pharmacodynamics (PD) and safety from targeting CD79b, both polatuzumab vedotin and the surrogate ADC were assessed in cynomolgus monkeys. Polatuzumab vedotin was administered i.v. every 3 weeks for four doses at 1, 3, or 5 mg·kg<sup>-1</sup>, and the surrogate ADC was also administered i.v. at 3 or 5 mg·kg<sup>-1</sup> with the same dosing regimen. Peripheral blood CD20<sup>+</sup> B cells decreased only in animals treated with the surrogate ADC. During the recovery phase, the estimated CD20<sup>+</sup> B cells recovered to >40% of baseline levels between Days 134 and 148 (Figure 5a). As expected, treatment of cynomolgus monkeys with polatuzumab vedotin or vehicle did not show any decrease in peripheral CD20<sup>+</sup> B cells. Consistent with the observed peripheral B-cell depletion, TK was linear and slightly non-linear for the polatuzumab vedotin and surrogate ADC, respectively (Figure 5b). The slightly non-linear TK observed with the surrogate ADC is due to the saturable B-cell-mediated CL of the ADC (Mager, 2006). No accumulation was observed with polatuzumab vedotin. The accumulation ratio



**FIGURE 5** Depletion of CD20<sup>+</sup> B cells and systemic exposure of total antibody following administration of polatuzumab vedotin or surrogate antibody-drug conjugate (ADC). (a) Peripheral blood CD20<sup>+</sup> B cells in animals administered with 1, 3, or 5 mg·kg<sup>-1</sup> of polatuzumab vedotin or 3 or 5 mg·kg<sup>-1</sup> of surrogate ADC. Presented data are group mean absolute counts expressed as a percentage of baseline (%BL) at each time point. Baseline was calculated as the average value obtained at two pre-dose time points (shown). Error bars represent SEM. The arrow denotes administration of the test articles. (b) Total antibody (mean ± SD) concentration–time profiles following i.v. administration of 1, 3, or 5 mg·kg<sup>-1</sup> of polatuzumab vedotin or 3 or 5 mg·kg<sup>-1</sup> of surrogate ADC in cynomolgus monkeys



(AUC<sub>63–84</sub>/AUC<sub>0–21</sub>) for the surrogate ADC ranged from 1.22 to 1.52 (Table S4). Although immunogenicity was observed in both ADC-treated groups, it did not appear to affect the TK exposures (Figure 5b and Table S4).

Doses of up to 5 mg·kg<sup>-1</sup> of polatuzumab vedotin and 3 mg·kg<sup>-1</sup> of surrogate ADC were well tolerated in cynomolgus monkeys with no adverse effects on body weight or clinical evidence of toxicity, including an absence of cardiovascular, respiratory, or neurological effects, as part of safety pharmacology assessments (Table 2). One male monkey administered 5 mg·kg<sup>-1</sup> of surrogate ADC died shortly after the third dose due to bacterial endocarditis secondary to test article-induced myelosuppression and, in particular, neutropenia. The most prominent changes in clinical pathology observed with administration of both 5 mg·kg<sup>-1</sup> of polatuzumab vedotin and 3 or 5 mg·kg<sup>-1</sup> of surrogate ADC were minimal decreases in mature red blood cell mass parameters, reticulocytes, and lymphocytes. Monocytes and neutrophils were also decreased at 5 mg·kg<sup>-1</sup> of surrogate ADC. These haematological changes correlated with bone marrow cell depletion microscopically and were reversible after a 9-week recovery period

(Table 2). No changes in serum chemistry attributed to the administration of polatuzumab vedotin were identified.

Overall, toxicological findings were similar between the two ADCs, indicating that the findings were mainly antigen independent and driven by MMAE, consistent with what had been observed for other acMMAE containing ADCs (Saber & Leighton, 2015). The highest non-severely toxic dose of 3 mg·kg<sup>-1</sup> in monkeys was determined based on the tolerability of the surrogate ADC.

## 4 | DISCUSSION

Unlike low MW pharmaceuticals, biotechnology-derived antibody-based therapeutic agents are designed to be highly specific to human proteins. The high degree of species specificity observed with human biotherapeutic agents, combined with species differences in biology, can present certain challenges in the identification of a pharmacologically relevant non-clinical species for toxicity assessment that is meaningful to humans. Surrogate molecules are typically considered when

the clinical candidate is pharmacologically active only in humans and/or chimpanzee or is active in other species, but the presence of anti-drug antibodies can affect drug exposure, thereby limiting the ability to conduct a thorough toxicity evaluation (Bornstein, Klakamp, Andrews, Boyle, & Tabrizi, 2009; Bussiere et al., 2009). Some examples of successfully marketed biotherapeutic agents where mouse surrogate molecules were used to evaluate non-clinical safety in longer term general toxicity and developmental and reproductive toxicity studies include [efalizumab](#), [infliximab](#), and IFN- $\gamma$  (Bussiere et al., 2009; Clarke et al., 2004; Green & Terrell, 1992; Treacy, 2000).

In the case of polatuzumab vedotin, a three-amino acid difference in the CD79b between human and cynomolgus monkey conferred selective binding of the clinical antibody to human CD79b only. Because CD79b is a signalling component of the B-cell receptor complex and was a novel target to be evaluated in patients for the first time, it was important to evaluate CD79b-mediated *in vivo* pharmacology and safety prior to entry into humans. Therefore, an anti-cynomolgus monkey CD79b surrogate antibody that binds to a similar epitope as the clinical antibody was generated (Zheng et al., 2009). The choice of using a cynomolgus monkey surrogate instead of a mouse surrogate was based on the following considerations: (a) The close homology between non-human primate and human CD79b is likely to provide a better understanding and translation of CD79b and B cell receptor pharmacology, compared with data from rodents. The cynomolgus monkey was, thus, considered a relevant species to capture any immunological effects such as B-cell depletion or signalling following anti-CD79b ADC administration. (b) Non-clinical studies with polatuzumab vedotin demonstrated that cynomolgus monkey was a more sensitive species to assess MMAE-driven toxicities, compared with rodents. (c) The cynomolgus monkey has been demonstrated to be the animal model of choice to appropriately predict non-clinical PK to human PK of antibody-based therapeutic agents (Deng et al., 2011). (d) Due to limitations in blood sampling in mice, the PK/PD relationship or PD effects on targeted normal B cells cannot be evaluated longitudinally and is more feasible in cynomolgus monkeys.

A major challenge in using a surrogate is to demonstrate its suitability to assess the pharmacological effects of the clinical molecule. As a first step, it is important to show that the two molecules are comparable from a structural perspective. The surrogate ADC had similar linker-drug (vc-MMAE) as polatuzumab vedotin and similar average number of MMAEs conjugated to the antibody or drug-to-antibody ratios (Table 1). Although, the surrogate antibody differed from the clinical antibody in being a chimeric construct with a similar human IgG1 backbone but non-humanized (mouse) complementarity-determining regions, we did not anticipate that these characteristics would affect the PK/PD and toxicity evaluation in cynomolgus monkeys. This was supported by the fact that the surrogate ADC binds to monkey CD79b with a affinity similar to that of polatuzumab vedotin binding to human CD79b (Table 1). In addition, both antibodies bind to similar epitopes and have a similar human Fc region.

Rigorous *in vitro* and *in vivo* assessments further demonstrated that the surrogate ADC resembles polatuzumab vedotin. The surrogate ADC showed comparable *in vitro* plasma stability, *in vivo* anti-

tumour activity, and *in vivo* PK in mice as polatuzumab vedotin. The surrogate ADC also showed similar binding activity to human Fc $\gamma$ RIIA with approximately twofold to threefold increase in binding towards human Fc $\gamma$ RIIA (H131 and R131), Fc $\gamma$ RIIB, and Fc $\gamma$ RIIIA (F158 and V158), as compared with polatuzumab vedotin. Although Fc $\gamma$ R-mediated effector functions, such as antibody-dependent cell-mediated cytotoxicity, are not the primary mechanism of action of polatuzumab vedotin, the ability of the surrogate ADC to bind to Fc $\gamma$ Rs enabled evaluation of potential Fc $\gamma$ R-mediated pharmacology and/or toxicity in a species (monkey) that is biologically relevant to humans. The *in vitro* and *in vivo* evaluations presented here demonstrated the similarity of the surrogate ADC to polatuzumab vedotin, further enabling the use of the surrogate ADC to assess the antigen-dependent pharmacology, toxicology, and PK/PD activities in cynomolgus monkeys.

As cynomolgus monkey is the binding species for the surrogate ADC but not for polatuzumab vedotin, peripheral blood CD20<sup>+</sup> B cells were decreased only in the surrogate ADC-treated animals. Consistent with this observation, animals that were treated with surrogate ADC showed faster CL of the ADC and complete histological absence of splenic lymphoid follicular germinal centres at Day 71 (7 days after the last dose) and with the return of follicular germinal centres in spleen, after a 9-week recovery period. These data were previously reported by Fuh et al. (2017) and suggest that *in vivo* depletion of CD20<sup>+</sup> B cells by the surrogate ADC is not limited to circulating B cells but is also observed in solid B-cell-associated lymphoid tissues. These results validated the anticipated pharmacological effects of the surrogate ADC in both blood and lymphoid tissues and also demonstrated the specificity of our antibody in targeting only B cells and not other lymphocytic subsets. Additionally, no evidence of toxicity resulting from cytokine release was observed in monkeys upon binding of the surrogate ADC to CD79b, and there were no adverse events related to cytokine release syndrome reported in phase I study with polatuzumab vedotin (Palanca-Wessels et al., 2015), providing both non-clinical and clinical evidence that induction of B cell receptor signalling did not result in catastrophic cytokine release.

As part of the investigational new drug application to enable first-in-human dosing trials, the surrogate ADC was tested in cynomolgus monkeys to evaluate both antigen-dependent and -independent effects on toxicity, PK, and PD. Additionally, antigen-independent as well as MMAE-mediated effects of polatuzumab vedotin were assessed in both cynomolgus monkeys and rats. This approach has been employed by other pharmaceutical companies developing ADC therapeutic agents and has been accepted by the Food and Drug Administration. The predominant findings associated with administration of polatuzumab vedotin and the surrogate ADC included reversible bone marrow toxicity and associated haematological effects in both rats and monkeys, which were considered MMAE related and antigen independent. In rats, we evaluated polatuzumab vedotin but not the surrogate ADC. Previously, we tested a slightly different structural variant of polatuzumab vedotin and the corresponding monkey surrogate ADC in rats. We found that the toxicities were driven only by MMAE, as expected, and not the linker or antibody component

of the ADC. To enable entry of polatuzumab vedotin into clinical trials, we concluded that testing of the surrogate ADC in rat was not beneficial to inform the first-in-human study. We also wanted to minimize the use of animals in the spirit of 3Rs—replace, reduce, and refine (Prescott & Lidster, 2017). In cynomolgus monkeys, as expected, target (B cell)-mediated CL was observed with the surrogate ADC but not with polatuzumab vedotin (Mager, 2006). Thus, at the same dose level, the systemic exposure of total antibody following i.v. administration of polatuzumab vedotin was 1.2- to 1.4-fold higher than that of the surrogate ADC in monkeys. However, this difference in exposure was not associated with any differences in toxicity between polatuzumab vedotin and the surrogate ADC in monkeys, confirming that the bone marrow toxicity observed with both ADCs was driven mainly by the antigen-independent effects of MMAE (Saber & Leighton, 2015).

The highest non-severely toxic dose was determined to be 3 mg·kg<sup>-1</sup> based on the tolerability of the surrogate ADC in monkeys and enabled a phase I starting dose of 0.1 mg·kg<sup>-1</sup>, which provided a safety factor of 30, based on body weight-normalized dose calculations. Polatuzumab vedotin has been administered to patients with NHL in several phase I, II, and III trials to date, with an acceptable safety and tolerability profile. Neutropenia has been identified as one of the treatment-emergent adverse effects of polatuzumab vedotin, which was expected on the basis of non-clinical toxicity assessments and is clinically monitorable and manageable (Palanca-Wessels et al., 2015).

In summary, the clinical development plan of polatuzumab vedotin took into consideration the implication of CD79b as a signalling component of the B cell receptor and CD79b as a novel target for oncology therapeutic development. The use of a surrogate antibody in this development plan provided evaluation into the biological and potential toxicological impact of the binding of ADC to CD79b and B-cell depletion in a non-clinical species. While the development of a surrogate ADC entails ensuring comparability and toxicity evaluation of both the surrogate ADC and the clinical candidate, identifying and understanding the relevant risks of the surrogate molecule is crucial to avoid over-/under-representation of the risks associated with the clinical candidate. Although this study highlights the utility of a surrogate in assessing PK/PD, pharmacology, and safety of an ADC, the principles for alternative testing approaches could potentially be applied to other biopharmaceuticals.

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## AUTHOR CONTRIBUTIONS

All of the listed authors contributed to the design, research, drafting, and final approval of the work. They each agree to be accountable for all aspects of the work.

## CONFLICT OF INTEREST

All authors are current or past employees of Genentech, Inc., a member of the Roche Group.

## DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the *BJP* guidelines for [Design & Analysis](#) and [Animal Experimentation](#) and as recommended by funding agencies, publishers, and other organizations engaged with supporting research.

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