Anti-Lymphocyte Antigen 6 Complex, Locus E-Seco-Cyclopropabenzindol-4-One-Dimer Antibody-Drug Conjugate That Forms Adduct with α 1-Microglobulin Demonstrates Slower Systemic Antibody Clearance and Reduced Tumor Distribution in Animals^S

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ABSTRACT

Anti-Ly6E-seco-cyclopropabenzindol-4-one dimer antibody-drug conjugate (ADC) has been reported to form an adduct with α 1-microglobulin (A1M) in animal plasma, but with unknown impact on ADC PK and tissue distribution. In this study, we compared the PK and tissue distribution of anti-Ly6E ADC with unconjugated anti-Ly6E mAb in rodents and monkeys. For PK studies, animals received an intravenous administration of anti-Ly6E ADC or unconjugated anti-Ly6E mAb. Plasma samples were analyzed for total antibody (Tab) levels and A1M adduct formation. PK parameters were generated from dose-normalized plasma concentrations. Tissue distribution was determined in tumor-bearing mice after a single intravenous dosing of radiolabeled ADC or mAb. Tissue radioactivity levels were analyzed using a gamma counter. The impact of A1M adduct formation on target cell binding was assessed in an in vitro cell binding assay. The results show that ADC Tab clearance was slower than that of mAb in mice and rats but faster than mAb in monkeys. Correspondingly, the formation of A1M adduct appeared to be faster and higher in mice, followed by rats, and slowest in

monkeys. Although ADC tended to show an overall lower distribution to normal tissues, it had a strikingly reduced distribution to tumors compared with mAb, likely due to A1M adduct formation interfering with target binding, as demonstrated by the in vitro cell binding assay. Together, these data 1) demonstrate that anti-Ly6E ADC that forms A1M adduct had slower systemic clearance with strikingly reduced tumor distribution and 2) highlight the importance of selecting an appropriate linker-drug for successful ADC development.

SIGNIFICANCE STATEMENT

Anti-lymphocyte antigen 6 complex, locus E, ADC with seco-cyclopropabenzindol-4-one-dimer payload formed adduct with A1M, which led to a decrease in systemic clearance but also attenuated tumor distribution. These findings demonstrate the importance of selecting an appropriate linker-drug for ADC development and also highlight the value of a mechanistic understanding of ADC bio-transformation, which could provide insight into ADC molecule design, optimization, and selection.

Introduction

Tremendous advances have been made in cancer therapeutics in the past decade. Molecule classes such as checkpoint inhibitors against programmed cell death protein 1 [e.g., Keytruda (anti-programmed cell death protein 1 or anti-PD-1, pembrolizumab) and Tecentriq (anti-programmed death-ligand 1 or anti-PDL-1, atezolizumab)] (Jin et al., 2011; Garon et al., 2019), chimeric antigen receptors-T-cell (e.g., Kymriah, tisagenlecleucel against CD19) (Miliotou and Papadopoulou, 2018; Seimetz et al., 2019), and antibody-drug conjugates

(ADCs) (e.g., Kadcyla, ado-trastuzumab emtansine) (Diamantis and Banerji, 2016; Abdollahpour-Alitappeh et al., 2019) have recently been marketed. These new therapeutics with improved specificity to tumor targets have dramatically improved the quality of life in patients (Inthagard et al., 2019). However, there is still a need to better characterize the behavior of these molecules, as the number of the targets being investigated is expanding. ADCs in particular, with their multiple variables, such as antibody, linker, payload, and site of conjugation, have very different physical and biologic properties arising from different combinations of these variables (Abdollahpour-Alitappeh et al., 2019; Birrer et al., 2019).

An ADC combines the specificity from its monoclonal antibody (mAb) and potency from its payload, as the payload by itself is usually too toxic for direct administration. By combining both antibody and

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ABBREVIATIONS: ADC, antibody-drug conjugate; A1M, α 1-microglobulin; AUC_{INF}, area under the concentration-time curve extrapolated to infinity; CBI, cyclopropabenzindol-4-one; DOTA, 1,4,7,10-tetraazacyclododecane-N,N',N'' tetraacetic acid; CL, clearance; DOTA gD, glycoprotein-D; %ID, percentage of injected dose; Ly6E, lymphocyte antigen 6 complex, locus E; mAb, monoclonal antibody; PK, pharmacokinetics; SCID, severe combined immunodeficient; SEC-HPLC, size exclusion column with high-performance liquid chromatography; Tab, total antibody (could include DAR2, DAR1, and DAR0 species for ADCs in this study); V_{SS}, volume of distribution at steady state.

Fig. 1. Anti-Ly6E ADC [drug-to-antibody ratio (DAR) of 2] with the structure of the linker payload [*seco*-cyclopropabenzindol-4-one (*seco*-CBI) dimer]. After the payload loses its phosphate group by the phosphatase in circulation, it rearranges molecularly into the biologically active form of CBI-dimer, where the A1M or other plasma proteins alkylate to the conjugated CBI-dimer through its electrophilic moieties forming the adduct (Su et al., 2019). GSH: Glutathione.

payload via a linker, the antibody acts as a guide for the payload to its intended target. Numerous reports have been published showing that manipulating these variables alters the PK and efficacy of the molecules (Frigerio and Kyle, 2017; Ohri et al., 2018; Su et al., 2018). However, as both antibody and payload are chemical entities, they have the potential to interact with endogenous proteins, as such resulting in biotransformation/modification of the molecules and alteration of PK and activity (Sousa et al., 2008; Palleria et al., 2013; Su et al., 2018). Although this type of biotransformation/modification is often seen in small-molecule drug development, it can also occur for large molecules on a case-by-case basis. It has not been extensively reported in the development of ADC, which contains both large and small molecules (Lu et al., 2013).

Lymphocyte antigen 6 (Ly6) complex, locus E, (Ly6E) is a member of the lymphostromal cell membrane Ly6 superfamily protein. Ly6E has been shown at elevated expression level in patients with breast, lung, bladder, brain, gastric, and skin cancers and is positively correlated with poor overall survival rate (Asundi et al., 2015; AlHossiny et al., 2016). Although the exact mechanism of biologic function and clinical significance of Ly6E is largely unknown, overexpression of Ly6E seems to promote cancer cell growth and metastasis. Because Ly6E is overexpressed in multiple cancer indications, it is an ideal candidate for targeted tumor delivery by an ADC. The payload of our ADC is a cyclopropabenzindol-4-one (CBI)-containing derivative, *seco*-CBI-dimer (henceforth CBI-dimer) (Fig. 1). Briefly, the phosphate prodrugs present in the *seco*-CBI-dimer-containing payload are quickly removed by plasma/blood phosphates while the conjugate circulates in vivo to produce (via molecular rearrangement) the biologically active CBI-dimer entity. After its ADC-mediated delivery to targeted cells, the released CBI-dimer payload alkylates DNA and leads to tumor apoptosis (Parrish et al., 2003). The anti-Ly6E antibody and the *seco*-CBI-dimer payload are linked by a maleimide moiety via a peptidomimetic, protease-cleavable linker.

Anti-Ly6E-CBI-dimer ADC was previously shown to form adducts with glutathione and especially with α 1-microglobulin (A1M) in animal plasma (Su et al., 2019). The A1M alkylates to the conjugated CBI-dimer through its electrophilic moieties, forming the adduct at the same site where the DNA alkylates, which slightly reduces the potency of the ADC both in vitro and possibly in vivo, as one of the two conjugated CBI-dimers loses its activity to cross-link with DNA (Fig. 1). The extent of this adduct formation differs among species; for example, it is much faster in rodents than cynomolgus monkey, which could be attributed to the phosphatase activity and A1M level (Su et al., 2019). However, there is little data on whether the adduct formation would alter the PK and distribution of the anti-Ly6E ADC. Therefore, in this paper, we have

compared the PK between anti-Ly6E ADC (forming A1M adduct) and its unconjugated monoclonal antibody (no A1M adduct formation) in rodents and monkeys. In addition, we have further compared the tissue distribution of anti-Ly6E ADC with its unconjugated antibody in tumor-bearing mice. Here, we report that the A1M adduct formation of ADC appeared to influence its PK. More importantly, the ADC-A1M adduct formation greatly attenuated tumor tissue distribution, likely by reducing target binding to tumor cells.

Materials and Methods

Reagents

All unconjugated and conjugated antibodies used for in vitro and in vivo PK, efficacy, and safety studies presented here were generated at Genentech, Inc. (South San Francisco, CA). The unconjugated antibody (anti-Ly6E mAb) is a humanized monoclonal IgG1 antibody that binds to the Ly6E human receptor. Anti-Ly6E mAb was humanized from its parental hybridoma clone, which was generated by immunizing BALB/c mice with purified LY6E protein. A control nonbinding antibody targeting the glycoprotein-D epitope of herpes simplex virus, anti-glycoprotein-D IgG1 antibody (anti-gD mAb), was produced and humanized at Genentech, Inc. Mouse plasma used in in vitro preincubation was purchased from BioIVT (catalog number MSEPLLIHP; Westbury, NY).

ADC Conjugation

Anti-Ly6E ADC corresponds to anti-Ly6E mAb carrying two molecules of a cytotoxic drug (a DNA-damaging agent), also referred to as payload. The payload is a *seco*-CBI-dimer conjugated to the antibody via a cleavable disulfide-linker (Fig. 1). The linker-drug has been described in a previous publication (as linker-drug 10) (Su et al., 2019). Anti-Ly6E ADC uses THIOMAB antibody technology, resulting in the conjugation of two drug molecules per antibody to engineered cysteine residues (Junutula et al., 2008a,b).

Radiolabeling

Anti-Ly6E ADC and anti-Ly6E mAb were both radiolabeled with ^{125}I (nonresidualizing) or ^{111}In with 1,4,7,10-tetraazacyclododecane- N,N',N'',N''' -tetraacetic acid (DOTA) (residualizing) (Chizzonite et al., 1991; Lombana et al., 2019). [^{125}I]Anti-Ly6E ADC was spiked into mouse plasma and incubated at 37°C for 4 days to form A1M adduct in vitro, and [^{125}I]anti-Ly6E mAb was treated similarly as control. All radiolabeled materials were analyzed on an Agilent (Foster City, CA) high-performance liquid chromatography system 1100 series with a Phenomenex (Torrance, CA) Yarra S-3000 size exclusion column, 5- μm particle size, 7.8 $\times$ 3000 mm (SEC-HPLC), at isocratic flow rate of 0.5 ml/min of PBS at pH 7.4 for 30 minutes. Radioactivity from the SEC-HPLC was detected by an in-line Raytest gamma detector (Elysia s.a., Angleur, Belgium). SEC-HPLC chromatogram profiles were used to confirm the formation of A1M adduct.

In Vivo Studies

All in vivo PK studies in rodents were approved by the Institutional Animal Care and Use Committee at Genentech, Inc., and were conducted in compliance with the regulations of the Association for Assessment and Accreditation of Laboratory Animal Care.

PK Studies of Anti-Ly6E mAb and Anti-Ly6E ADC in Mice

To evaluate the PK of anti-Ly6E mAb and anti-Ly6E ADC in mice, 60 female naïve CB17 severe combined immunodeficient (SCID) mice (6–8 weeks old) were obtained from Charles River Laboratories, Inc. (Hollister, CA). Animals received either a single intravenous dose of 1 mg/kg of anti-Ly6E ADC or anti-Ly6E mAb via tail vein injection ($N = 30/\text{group}$). Blood samples were collected from three mice in each dosing group at each of the following time points: 10 minutes; 1 and 6 hours; and 1, 2, 3, 7, 10, 14, and 21 days. The sample collection was done via retro-orbital bleeds or cardiac puncture. Samples were processed to collect plasma and measure anti-Ly6E ADC or anti-Ly6E mAb antibody concentrations. Additionally, for the group receiving anti-Ly6E ADC, samples were analyzed using the affinity capture assay to detect changes in the antibody molecular weight.

PK Studies of Anti-Ly6E mAb and Anti-Ly6E ADC in Rats

To evaluate the PK of anti-Ly6E ADC and anti-Ly6E mAb in rats, nine female naïve Sprague-Dawley rats were obtained from Charles River Laboratories, Inc., and split into two groups. Four rats received a single intravenous dose of anti-Ly6E mAb at 5 mg/kg, and five rats got a single intravenous dose of anti-Ly6E ADC at 2 mg/kg (lower ADC dose level used because of the potential toxicity, but this was not expected to interfere with PK in nonbinding species) via the jugular vein cannula followed with a saline flush to clear the cannula. Blood samples were collected from each rat at each of the following time points: 10 minutes; 1 and 6 hours; and 1, 3, 7, 10, 14, and 21 days. Rat blood samples were processed to collect plasma and measure total antibody level and changes in molecular weight using the affinity capture assay.

PK Studies of Anti-Ly6E mAb and Anti-Ly6E ADC in Cynomolgus Monkeys

These monkey studies were performed at Charles River Laboratories (Reno, NV) at different times. For the first study, five cynomolgus monkeys were split into two groups, and anti-Ly6E mAb was given as a single intravenous bolus administration at 0.3 ($N = 2$) and 3 mg/kg ($N = 3$), respectively. Blood samples were collected from the monkeys in each dosing group at each of the following time points: 15 minutes; 4 and 12 hours; and 1, 3, 4, 7, 10, 14, 21, 28, 35, and 42 days. Monkey blood samples were processed to collect serum and measure anti-Ly6E mAb antibody level. For the second monkey study, a total of 10 monkeys were given multiple intravenous bolus administrations of anti-Ly6E ADC at 2, 4,

and 8 mg/kg every 3 weeks for four cycles ($N = 3$ for 2 mg/kg, $N = 6$ for 4 mg/kg, and $N = 1$ for 8 mg/kg, respectively). Blood samples were collected at the following time points after the first dose: predose and 15 minutes and 1, 3, 7, 14, 21 days postdose. From the second to fourth cycles, blood samples were collected at the following time points: 15 minutes and 1, 3, 7, 14, 21 days postdose. Blood samples were processed to collect plasma and used to measure anti-Ly6E ADC total antibody level and changes in molecular weight using the affinity capture assay. PK parameters were generated only using the first cycle total antibody concentration data for the anti-Ly6E ADC.

Tissue Distribution Study with Radiolabeled ADC and mAb in Tumor-Bearing SCID Mice

Eighty female naïve C.B-17 SCID-beige mice (Charles River Laboratory) were inoculated with 5×10^6 HCC1937X1 cells (a derivative from HCC1937 from American Type Culture Collection or ATCC) suspended in 0.1 ml of Hanks' balanced salt solution with matrigel in the thoracic mammary fat pad area. When the xenograft tumor reached the size of 200–300 mm³, radiolabeled anti-Ly6E ADC or anti-Ly6E mAb (5 μCi of each radioprobe) was singly dosed intravenously at two different levels: radiolabeled tracer alone (at 0.05 mg/kg) or tracer + 0.4 mg/kg unlabeled materials (which sustain static tumor). For the tracer-only groups, whole blood and tissues were collected at 1 and 6 hours and 1, 3, and 7 days postdose ($n = 5$ for each time point). Similarly, for the tracer + 0.4 mg/kg unlabeled material groups, whole blood and tissues were collected at 1 and 3 days postdose ($n = 5$ for each time point). Whole blood samples were processed for plasma and cell pellet. The collected tissues included tumor, liver, lungs, kidneys, heart, spleen, stomach, small intestine, large intestine, fat pad, and skin. After tissue collection, the tissues were rinsed with PBS (pH 7.4), blotted dry, and weighed. All samples were analyzed for total radioactivity using a Perkin Elmer Wizard² gamma counter (Waltham, MA), and radioactivity data were calculated as a percentage of injected dose (%ID) normalized by volume/weight (%ID/ml or %ID/g). Selected plasma samples were analyzed by the SEC-HPLC method as described in the *Radiolabeling* section. SEC-HPLC chromatogram profiles were compared across time points.

Ex Vivo Formation of A1M-ADC Adduct and In Vitro Cell Binding Assay

Anti-Ly6E ADC-A1M adduct was preformed by incubating ¹²⁵I-radiolabeled anti-Ly6E ADC (namely, as preformed A1M-ADC) in mouse plasma at 37°C for 4 days. As control, [¹²⁵I]anti-Ly6E mAb was also incubated under the same condition (namely, as preincubated mAb). A1M adduct formation was characterized using SEC-HPLC showing about 50% (calculated as “area under the curve”) as A1M-ADC, whereas no A1M adduct formed for mAb (Supplemental Fig. 1).

For cell binding assay, HCC1937X1 cells (high Ly6E expression) or DOV13 cells (low Ly6E expression) were plated on a six-well cell culture plate (costar) at $\sim 0.5 \times 10^6$ cells per well in Dulbecco's modified Eagle's medium growth media with penicillin-streptomycin at 37°C with 5% CO₂ overnight, allowing cells to attach to the plates. Then, the growth media was replaced with 1 ml of Dulbecco's modified Eagle's medium without antibiotic for 1 hour before adding radiolabeled molecules, including preformed A1M-ADC adduct, preincubated mAb, anti-Ly6E ADC (no preincubation with plasma), anti-Ly6E mAb (no preincubation with plasma), or a nontargeted anti-gD mAb at $\sim 0.15 \mu\text{g}/\text{ml}$ (equivalent to $\sim 0.1 \mu\text{Ci}$ per well) in 50 μl of mouse plasma. The cells were then incubated at 37°C with 5% CO₂ for 3 hours. At the end of the incubation, the growth media was removed and the cells were washed with ice-cold PBS three times. Then, the trypsinized cells were collected and analyzed for radioactivity on the gamma counter. The radioactivity was then converted as a percentage of total radioactivity added to the cells per well.

Bioanalysis of Plasma Samples

Anti-Ly6E mAb and Anti-Ly6E ADC Total Antibody Assay. To determine anti-Ly6E ADC total antibody concentrations (in rodents and cynomolgus monkeys), as well as the anti-Ly6E mAb (in mice and monkeys), a specific peptide-based Liquid Chromatography-Tandem Mass Spectrometry quantitative assay was used. Samples were enriched from mouse plasma via affinity capture using streptavidin magnetic beads coupled with biotinylated anti-human IgG antibody and then subjected to “on-bead” proteolysis with trypsin. A representative

Fig. 2. Mean ($\pm$ S.D.) antibody concentration-time profiles of anti-Ly6E ADC and anti-Ly6E mAb after a single intravenous administration in female CB17 SCID mice ($N = 3$).

TABLE 1
Mean ($\pm$ S.D.) noncompartmental PK parameters of anti-Ly6E mAb and anti-Ly6E ADC in CB17 SCID mice and Sprague-Dawley rats

Species	Treatment Dose, <i>N</i>	$C_{\max}$ /Dose ($\mu\text{g/ml}/(\text{mg/kg})$)	$AUC_{\text{INF}}/\text{Dose}$ ($\text{day}\cdot\mu\text{g/ml}/(\text{mg/kg})$)	$AUC_{\text{Extrap_PRED}} (\%)$	CL (ml/d per kilogram)	V_{ss} (ml/kg)	$t_{1/2}$ (day)
Mouse ^a	anti-Ly6E mAb 1 mg/kg, <i>n</i> = 3	24.0	244	36.6	4.12	85.6	14.6
Mouse ^a	anti-Ly6E ADC 1 mg/kg, <i>n</i> = 3	26.1	300	31.4	3.33	59.7	11.7
Rat	anti-Ly6E mAb 5 mg/kg, <i>n</i> = 4	25.2 ± 2.03	161 ± 63.2	14.7 ± 13.6	6.97 ± 2.60	79.6 ± 19.1	10.8 ± 5.94
Rat	anti-Ly6E ADC 2 mg/kg, <i>n</i> = 5	28.2 ± 2.52	194 ± 84.3	22.0 ± 11.5	5.87 ± 1.88	68.5 ± 9.63	9.51 ± 4.02

$AUC_{\text{Extrap_PRED}}$, percent of area under the concentration-time curve extrapolated from the last time point; $t_{1/2}$, terminal half-life.

^aParameters were calculated using naïve pooling approach.

signature tryptic peptide selected from the complementarity-determining region of Ly6E was identified as the surrogate for quantification of the antibody. The lower limit of quantitation was 1 $\mu\text{g/ml}$ in the Liquid Chromatography-Tandem Mass Spectrometry assay. Similarly, to determine anti-Ly6E mAb in rats, a bridging ELISA technique (capturing via sheep anti-human IgG followed by detection using a sheep anti human IgG conjugated to horseradish peroxidases) was employed, and the lower limit of quantitation was 0.02 $\mu\text{g/ml}$.

Affinity Capture LC-MS Assay. An affinity capture LC-MS assay was used to detect changes in molecular weight of the antibody, as described by Su et al. (2019). Briefly, the biotinylated Ly6E receptor extracellular domain immobilized on streptavidin-coated magnetic beads was used to specifically capture various antibody species. The captured ADC was then eluted from the beads and injected onto a reversed phased liquid chromatography coupled to a quadrupole time-of-flight mass spectrometer operated in the positive electrospray ionization mode. This hybrid LC-MS method, which is not quantitative, compared the ratio of the total ion current from the anti-Ly6E ADC and anti-Ly6E ADC adduct.

Pharmacokinetic and Statistical Analysis. Total antibody plasma concentration-time profiles for anti-Ly6E mAb and anti-Ly6E ADC were used to estimate the following PK parameters in mouse, rat, and cynomolgus monkey using noncompartmental analysis in Phoenix 1.4 (WinNonlin PK software version 6.4; Certara):

$AUC_{\text{INF}}^{\text{TAB}}/\text{Dose}$: area under the total antibody concentration-time curve extrapolated to infinity normalized by dose.

$C_{\max}^{\text{TAB}}/\text{Dose}$: observed total antibody maximum serum concentration.

CL^{TAB} : total antibody clearance.

V_{ss}^{TAB} : total antibody volume of distribution at steady state.

$t_{1/2}^{\text{TAB}}$: total antibody terminal half-life.

A naïve pooled approach was used in the analysis of mouse studies to provide one estimate for each dose group. For the analysis of rat and monkey data, each

animal was analyzed separately, and results for each dose group were summarized as means $\pm$ S.D. Because of the nature and study design of the PK studies, no formal statistical analysis was performed to determine the significance of the difference in PK exposure of anti-Ly6E mAb and anti-Ly6E ADC, as these studies were not powered for statistical analysis. Instead, only a post hoc exploratory statistical analysis was done for PK parameters from rats and monkeys to compare the $C_{\max}/\text{dose}$ and $AUC_{\text{INF}}/\text{dose}$ between the anti-Ly6E ADC and anti-Ly6E mAb, but the data were not reported in the manuscript because of the limited study power.

Results

Mouse PK. The PK of anti-Ly6E mAb and anti-Ly6E ADC was examined in CB17 SCID mice (nonbinding species). The total antibody plasma concentration-time profiles of anti-Ly6E mAb and anti-Ly6E ADC after a single intravenous bolus dose in CB17 SCID mice at 1 mg/kg are shown in Fig. 2, and the corresponding PK parameters are summarized in Table 1. Measured values of the dosing solutions were within the acceptable range ($\pm 20\%$); therefore, nominal doses were used for the PK analysis. Both anti-Ly6E mAb and anti-Ly6E ADC exhibited biexponential disposition with comparable values of dose-normalized $C_{\max}$ [24.0 and 26.1 ($\mu\text{g/ml}/(\text{mg/kg})$) for anti-Ly6E mAb and anti-Ly6E ADC, respectively]. The estimated values for the dose-normalized AUC_{INF} appeared to be higher for the anti-Ly6E ADC versus the mAb, with values of 300 versus 244 ($\text{day}\cdot\mu\text{g/ml}/(\text{mg/kg})$), respectively; however, a high fraction ($>30\%$) of the total area was extrapolated to compute these values, so they must be interpreted with caution. Accordingly, the clearance values for the anti-Ly6E ADC versus the mAb were estimated as 3.33 and 4.12 (ml/d per kilogram), consistent with the apparent higher exposure of the

Fig. 3. Mean ($\pm$ S.D.) antibody concentration-time profiles (dose-normalized) of anti-Ly6E ADC and anti-Ly6E mAb after a single intravenous administration (2 mg/kg for ADC and 5 mg/kg for mAb) in Sprague-Dawley rats (*N* = 4 for ADC and *N* = 5 for mAb).

Fig. 4. Mean ($\pm$ S.D.) antibody concentration-time profiles (dose-normalized) of anti-Ly6E ADC from first cycle after multiple intravenous doses (every 3 weeks) and anti-Ly6E mAb after a single intravenous dose in cynomolgus monkeys. Anti-Ly6E ADC was dosed at 2, 4, and 8 mg/kg, and anti-Ly6E mAb was dosed at 0.3 and 3 mg/kg (for ADC, *N* = 3, 6, and 1 for 2, 4, and 8 mg/kg respectively; for mAb, *N* = 2 and 3 for 0.3 and 3 mg/kg).

TABLE 2
Mean ($\pm$ S.D.) noncompartmental PK parameters cynomolgus monkey anti-Ly6E ADC and anti-Ly6E mAb

Treatment	$C_{\max}$ ($\mu\text{g/ml}$)	$C_{\max}/\text{Dose}$ ($\text{kg} \cdot \mu\text{g}/\text{ml}/\text{mg}$)	$\text{AUC}_{\text{INF}}/\text{Dose}$ ($\text{day} \cdot \mu\text{g}/\text{ml}/(\text{mg}/\text{kg})$)	% $\text{AUC}_{\text{Extrap-PRED}}$ (%)	CL (ml/d per kilogram)	V_{ss} (ml/kg)	$t_{1/2}$ (day)
Anti-Ly6E ADC (2 mg/kg, $n = 3$) ^a	52.0 $\pm$ 12.2	26.0 $\pm$ 6.11	177 $\pm$ 35.7	16.0 $\pm$ 3.92	5.78 $\pm$ 1.10	61.3 $\pm$ 12.3	8.91 $\pm$ 0.907
Anti-Ly6E ADC (4 mg/kg, $n = 6$) ^a	98.3 $\pm$ 20.6	26.0 $\pm$ 3.58	205 $\pm$ 43.3	20.7 $\pm$ 8.04	6.82 $\pm$ 1.13	62.6 $\pm$ 5.46	7.25 $\pm$ 1.02
Anti-Ly6E ADC (8 mg/kg, $n = 1$) ^{a,b}	191	23.9	157	18.1	6.38	74.2	9.13
Anti-Ly6E mAb (0.3 mg/kg, $n = 2$) ^b	9.31	31.0	222	7.92	4.51	71.2	11.9
Anti-Ly6E mAb (3 mg/kg, $n = 3$)	104 $\pm$ 11.7	34.6 $\pm$ 3.91	256 $\pm$ 14.0	7.53 $\pm$ 2.12	3.92 $\pm$ 0.221	60.2 $\pm$ 8.56	11.6 $\pm$ 1.18

$\text{AUC}_{\text{Extrap-PRED}}$, percent of area under the concentration-time curve extrapolated from the last time point; $t_{1/2}$, terminal half-life.

^aParameters based on first cycle only.

^bS.D. was not calculated as $n < 3$.

ADC versus the mAb. Differences in the calculated V_{ss} values [85.5 (ml/kg) for anti-Ly6E mAb versus 59.7 (ml/kg) for the anti-Ly6E ADC] suggest that anti-Ly6E mAb has a higher volume of distribution than anti-Ly6E ADC.

Rat PK. The PK of anti-Ly6E mAb and anti-Ly6E ADC were also examined in a second nonbinding species Sprague-Dawley rats. Total antibody plasma concentration–time profiles after the administration of a single intravenous bolus of 2 mg/kg of anti-Ly6E mAb and 5 mg/kg of anti-Ly6E ADC (dose-normalized to 5 mg/kg) are shown in Fig. 3, and the corresponding PK parameters are summarized in Table 1. Both molecules show biexponential PK similar to that in mice. Values of the dose-normalized $C_{\max}$ [25.2 vs. 28.2 ($\mu\text{g/ml}/(\text{mg}/\text{kg})$) for the mAb and the ADC, respectively] and dose-normalized AUC_{INF} [161 vs. 194 $\text{day} \cdot (\mu\text{g/ml})/(\text{mg}/\text{kg})$ for the mAb and the ADC, respectively] are comparable for both test articles. The estimated clearance values were 6.97 (ml/d per kilogram) for anti-Ly6E mAb and 5.87 (ml/d per kilogram) for anti-Ly6E ADC. Although this study was not powered for statistical analysis, a post hoc exploratory statistical analysis was done on rat PK parameters comparing the $C_{\max}/\text{dose}$ and $\text{AUC}_{\text{INF}}/\text{dose}$ between the anti-Ly6E ADC and anti-Ly6E mAb. The results were not statistically significant ($P > 0.05$) in rats (data not shown).

Monkey PK. After a single intravenous administration of 0.3 or 3 mg/kg of anti-Ly6E mAb, dose-normalized AUC_{INF} values were 222 and $256 \pm 14.0 \text{ day} \cdot (\mu\text{g/ml})/(\text{mg}/\text{kg})$, respectively, suggesting roughly dose-proportional PK behavior within the dose range tested. The mean clearance values of anti-Ly6E mAb were 4.51 and 3.92 (ml/d per kilogram), respectively. The PK parameters derived for anti-Ly6E mAb were as expected for a human IgG1 mAb in cynomolgus monkeys and consistent with other typical IgG1 mAbs developed by Genentech, Inc. (Deng et al., 2011).

Anti-Ly6E ADC is expected to bind to cynomolgus monkey Ly6E, since conjugation of payload at K149C site is not anticipated to alter antibody-target interaction (Leipold et al., 2018). Anti-Ly6E ADC shows a comparable dose-normalized $C_{\max}$ [26.0, 26.0, and 23.9 ($\mu\text{g/ml}/(\text{mg}/\text{kg})$) and dose-normalized AUC_{INF} [177, 205, and 157 $\text{day} \cdot (\mu\text{g/ml})/(\text{mg}/\text{kg})$] across the dose range tested, suggesting a linear PK behavior across this dose range (at 2, 4, and 8 mg/kg). The respective mean clearance estimates were 5.78, 6.82, and 6.38 (ml/d per kilogram) at 2, 4, and 8 mg/kg dose, respectively. All dosing solutions were within the acceptable range ($\pm 20\%$). The dose-normalized total antibody PK profiles after the administration of anti-Ly6E ADC (first cycle) and anti-Ly6E mAb to cynomolgus monkeys are shown in Fig. 4. Individual dose group's concentration-time profiles for monkey are shown in Supplemental Fig. 2. The corresponding noncompartmental PK parameters are summarized in Table 2. Post hoc exploratory statistical analysis was also done for monkey PK parameters comparing the $C_{\max}/\text{dose}$ and $\text{AUC}_{\text{INF}}/\text{dose}$ between the anti-Ly6E ADC and anti-Ly6E mAb. The results were modestly statistically significant ($0.01 < P < 0.05$) in monkeys (data not shown).

Different Rate and Extent of A1M Adduct Formation Across Species In Vivo. ADCs carrying a CBI-dimer payload (e.g., anti-Ly6E ADC) are known to undergo major biotransformations via payload-protein adduct formation, resulting in attenuation of ADC activity (Su et al., 2019). We have analyzed plasma samples from mice, rats, and monkeys after anti-Ly6E ADC administration to detect formation of the A1M adduct by using an Mass Spectrometry-affinity capture assay and explore whether the appearance of the A1M adduct is associated with changes in PK and/or biodistribution of the molecule. In CB17 SCID mouse, adduct (+24.2 kDa) was detected as early as 1 hour after dosing and became dominant by 24 hours (Fig. 5A). Similarly, affinity capture assay also showed the formation of A1M adduct (+23.3 kDa) at 6 hours postdosing and became the dominant species on day 7 postdosing. One of the rats receiving anti-Ly6E ADC dosing showed much higher

Fig. 5. Analysis of anti-Ly6E ADC-A1M adduct formation in mouse and monkey plasma using affinity capture LC-MS analysis of representative plasma sample. (A) DAR distribution and A1M adduct formation of anti-Ly6E ADC in mouse plasma. A1M adduct (+24.2 kDa) was detected in mouse plasma 1 hour after dosing and became dominant by 24 hours; no significant deconjugation (maleimide exchange) was observed. (B) Analysis of drug-to-antibody ratio (DAR) distribution and A1M adduct formation of anti-Ly6E ADC in monkey plasma. A1M adduct (+~26 kDa) was detected in monkey plasma at 24, 72, and 168 hours after dosing at both dosing levels (2 and 4 mg/kg). RT, Retention Time.

exposure than the other rats; coincidentally, this rat also showed higher extent of A1M adduct. In contrast, in cynomolgus monkeys, A1M adduct (+25.9 kDa) was detected at 24 hours postdosing at 2 mg/kg and 72 hours after dosing at 4 mg/kg. It took around 7 days for A1M adduct to become the dominant species after anti-Ly6E ADC administration (Fig. 5B). Taken together, these data suggest that A1M adduct formation occurs most rapidly with higher extent in mice followed by rats as compared with that in monkeys. Thus, the rate and extent of A1M adduct formation appeared to inversely correlate with ADC Tab clearance.

Anti-Ly6E ADC Trends To Have Reduced Tissue Distribution with Striking Reduction to Tumors in Mice. After dosing of radiolabeled ADC in tumor-bearing mice, plasma radioactivity levels were higher than that in mice dosed with radiolabeled mAb, consistent with PK study in mice. SEC-HPLC analysis revealed that the retention

time of main peak shifted to the left for ADC samples, but not for mAb samples (Fig. 6), indicating the formation of A1M adduct with ADC, as seen in previous analysis.

Measurement of tissue radioactivity demonstrated that anti-Ly6E ADC tended to have overall lower tissue radioactivity levels than that for anti-Ly6E mAb throughout the study course, although the difference was not significant (Fig. 7). At 3 days after dosing, liver, kidneys, and spleen (nontargeted tissues) have a total ^{111}In radioactivity level of 21.5%ID/g for anti-Ly6E ADC versus 30.7%ID/g for anti-Ly6E mAb. However, anti-Ly6E ADC showed a striking reduction to tumors in comparison with mAb. As shown in Fig. 6A, tumor radioactivity levels in mice dosed with radiolabeled ADC were significantly lower than that seen in mice dosed with radiolabeled mAb over the study course of 7 days. On day 1 postdosing, the ^{111}In radioactivity in tumor for animals

Fig. 5. Continued.

dosed with anti-Ly6E ADC was $10.0\% \pm 1.95\% \text{ID/g}$ versus $17.3\% \pm 1.10\% \text{ID/g}$ for animals dosed with radiolabeled anti-Ly6E mAb. On day 3 postdosing, the tumor radioactivity from the anti-Ly6E ADC group was around $9.90 (\pm 2.12) \% \text{ID/g}$ as compared with $20.4 (\pm 3.78) \% \text{ID/g}$ in tumor from animals dosed with anti-Ly6E mAb (Fig. 7). The trend continued to the end of the study course.

To further understand the extent of molecule internalization and catabolism, we have analyzed the difference between ^{111}In and ^{125}I radioprobes. Although both probes can be used to assess the tissue

distribution, only ^{125}I can be released back into the extracellular space after the intracellular degradation of antibody, whereas ^{111}In residualized inside cells, as the DOTA cannot cross the cell membrane. Side-by-side comparison of the radioactivity from these two probes in different tissues not only enabled us to monitor the tissues distribution but also helped evaluate the site where internalization and catabolism occurred. In our results, there was little difference between ^{125}I and ^{111}In radioactivity in tumor tissues dosed with anti-Ly6E ADC (Δ of 1.36% and 2.41%ID/g for 1 day and 3 days postdosing, respectively),

Fig. 6. Analysis of A1M adduct formation after dosing radiolabeled anti-Ly6E ADC or anti-Ly6E mAb using SEC-HPLC with in-line radio detector. (A) Baseline profiles of ^{125}I -radiolabeled anti-Ly6E ADC (blue) and anti-Ly6E mAb (red) dosing materials showing the same retention time. (B) At 1 hour postdose, retention time of the main peak shifted to the left for anti-Ly6E ADC plasma, whereas it remained the same for anti-Ly6E mAb, indicating the formation of A1M adduct in anti-Ly6E ADC plasma (only representative plasma samples analyzed).

Fig. 7. Biodistribution of radiolabeled anti-Ly6E ADC and anti-Ly6E mAb in normal and tumor tissues after a single intravenous dosing of radiolabeled anti-Ly6E ADC or anti-Ly6E mAb in tumor-bearing mice. The graph is presented as %ID per gram of tissue or %ID per gram of blood. The solid bars represent the data by the nonresidualized radioactivity of ^{125}I , whereas the hollow bars represent the data by the residualized radioactivity of ^{111}In . Data are represented as means $\pm$ S.D. ($N = 5$). (A) Distribution profiles at 1 day postdosing radiolabeled anti-Ly6E ADC or anti-Ly6E mAb, in which there was a significant reduced distribution to the tumor comparing the two molecules ($*P < 0.01$). (B) Distribution profiles at 3 days postdosing radiolabeled anti-Ly6E ADC or anti-Ly6E mAb. Radioactivity in tumor for anti-Ly6E ADC was much more attenuated as compared with that of anti-Ly6E mAb, suggesting that the adduct formation resulted in a reduced internalization of ADC in tumor. ($**P < 0.001$).

whereas there was a much greater difference between the two radioprophes in tumor dosed with anti-Ly6E mAb (Δ of 6.34% and 9.26%ID/g for 1 and 3 days postdosing, respectively). This result indicated that little anti-Ly6E ADC was internalized into the tumors as compared with anti-Ly6E mAb (Fig. 7). Consistently, codosing with an excess amount of unlabeled ADC materials did not appear to impact tumor distribution, indicating that there was no displacement of specific target binding; therefore, formation of A1M adduct alters the specific tumor distribution.

Anti-Ly6E ADC A1M Adduct Showed Reduced Target Binding in Tumor Cells In Vitro. To further understand whether A1M adduct formation would interfere with cell binding, A1M adduct of ADC was preformed as described in *Materials and Methods* and then incubated in a cell line expressing either high (HCC1937X1) or low (DOV13) levels of Ly6E antigen. As shown in Fig. 8, the total radioactivity in cells treated with control anti-Ly6E ADC (no preincubation with plasma, thus no A1M adduct formation) was similar to that of cells treated with anti-Ly6E mAb (also no preincubation with plasma and no A1M adduct formation) ($0.760\% \pm 0.0281\%$ for anti-Ly6E mAb vs. $0.708\% \pm 0.0744\%$ for anti-Ly6E ADC in HCC1937X1 and $0.313\% \pm 0.0514\%$ for anti-Ly6E mAb vs. $0.334\% \pm 0.0469\%$ for anti-Ly6E ADC in DOV13) after 3 hours of incubation. However, cellular radioactivity after treatment with preformed A1M-ADC group (preincubated with plasma to form A1M adduct) was

significantly lower ($P < 0.01$) than preincubated mAb group (anti-Ly6E mAb that was preincubated in plasma) in both cell lines after 3 hours of incubation ($0.602\% \pm 0.0876\%$ for preincubated mAb vs. $0.437\% \pm 0.0201\%$ for preformed A1M-ADC in HCC1937X1 and $0.350\% \pm 0.0718\%$ for preincubated mAb vs. $0.197\% \pm 0.0473\%$ for preformed A1M-ADC in DOV13) (Fig. 8). As expected, the cellular radioactivity in the HCC1937X1 (higher Ly6E expression) cell line was higher than DOV13 (lower Ly6E expression) for anti-Ly6E mAb under both conditions (i.e., with or without preincubation with plasma). The nontargeted antibody, anti-gD, had the lowest radioactivity binding and uptake in both cell lines.

Discussion

The PK of ADCs has been previously described in multiple publications (Hamblett et al., 2004; Boswell et al., 2011; Lin et al., 2013; Kamath and Iyer, 2015; Leipold et al., 2018). In general, the systemic clearance of ADCs tends to be faster than that of the unconjugated mAbs, attributed to the “impact of conjugation” (Boswell et al., 2011). High hydrophobicity, emerging pockets of increased electrostatic, or altered the neonatal crystallizable fragment receptor (FcRn) binding upon conjugation of the payload all could potentially cause higher CL of ADC (Boswell et al., 2011; Kamath and Iyer, 2015). However, the impact of conjugation on ADC clearance is expected to differ in magnitude with different types of payload, linker, and conjugation technologies. In addition, ADC may undergo biotransformation, such as interacting with plasma protein, which may further complicate the impact on ADC clearance.

Anti-Ly6E-*seco*-CBI-dimer ADC was previously reported to form an adduct with A1M in animal plasma in circulation once the phosphate group was removed by phosphatase and the payload rearranged molecularly to become biologically active (Su et al., 2019). The binding of A1M to one of the CBI-dimers appeared to attenuate ADC’s activity in an in vitro assay, likely due to reduced DNA alkylation, as such attenuating its activity. However, the impact of A1M adduct formation on ADC PK and tissue distribution is not fully understood, which may also affect ADC activity and its developability.

In the current study, we have compared the PK of anti-Ly6E ADC Tab (forming A1M adduct) with anti-Ly6E mAb (no A1M adduct formation) in mice, rats (both nonbinding species), and cynomolgus monkeys (binding species). The clearance of ADC Tab was slower than that of mAb in mice [3.33 vs. 4.12 (ml/d per kilogram) for ADC and mAb, respectively] and rats [5.87 ± 1.88 vs. 6.97 ± 2.60 (ml/d per kilogram) for ADC and mAb, respectively] (Table 1). Unlike mice and rats, the clearance for ADC in cynomolgus monkey was faster than that of mAb at both 2 and 4 mg/kg [5.78 ± 1.10 and 6.82 ± 1.13 (ml/d per kilogram), respectively]. In contrast, the clearance of mAb at 3 mg/kg in monkeys was 3.92 ± 0.221 (ml/d per kilogram), which was slower than that of either 2 or 4 mg/kg of ADC in monkeys (Table 2).

We hypothesized that the rate and extent of A1M adduct formation may differ between rodents and monkeys, leading to the different ADC clearance. As such, we compared the relative adduct formation rate and extent in plasma from mice, rats, and monkeys using the affinity capture assay. The results revealed that the rate of A1M adduct formation inversely correlated with the clearance of anti-Ly6E ADC. The A1M adduct formation was most rapid in mouse plasma. It became dominant (greater than 80% of ion intensity observed by affinity-liquid chromatography tandem mass spectrometry with +24 kDa) within 24 hours after dosing. In contrast to mice, the formation of A1M adduct in monkeys was much slower and took 7 days to become dominant (greater than 80% of ion intensity with +26 kDa). This is consistent with the results reported by Su et al. (2019) for anti-CD22-*seco*-CBI-dimer ADC (with same linker payload that also forms A1M adduct). These data indicated

Fig. 8. In vitro cell binding and uptake of radiolabeled anti-Ly6E ADC A1M adduct (preformed by preincubation with mouse plasma), anti-Ly6E ADC control (no A1M adduct formation), and anti-Ly6E mAb in tumor cell lines. HCC1937X1 (high Ly6E expression) and DOV13 (low Ly6E expression) cell lines were cultured and treated with the respective radiolabeled material as described in the *Materials and Methods*. (A) Radioactivity levels in HCC1937X1 cell line as percentage of added radioactivity dose at 3 hours postincubation. Data are represented as individual points $\pm$ S.D. ($N = 5$); (B) radioactivity levels in DOV13 cell line as percentage of added radioactivity dose at 3 hours postincubation. Data are represented as individual points $\pm$ S.D. ($N = 3$). In both cell lines, the comparison of cell binding and uptake of anti-Ly6E mAb to anti-Ly6E ADC without preincubation in plasma was not significant; however, the cell uptake for preformed A1M-ADC was significantly lower than the preincubated mAb ($P < 0.01$). * indicates p value ≤ 0.05 , significant difference; ns: no significant difference. Black circle: anti-Ly6E mAb; Black square: anti-Ly6E ADC; Black triangle: preincubated anti-Ly6E mAb; Reversed black triangle: preformed A1M-ADC adducts; Semi-black circle: anti-gD mAb control.

that the A1M adduct formation could reduce the clearance of anti-Ly6E ADC, particularly in rodents that have a higher level and rapid formation of A1M adduct in plasma after intravenous dosing. Presently, it is unknown why the rate and extent of A1M adduct formation with ADC are different between rodents and monkeys. A1M is known to be highly heterogeneous, with different molecule size and abundance between rodent and monkey (Akerström, 1985), which may contribute to the discrepancy in A1M-ADC adduct formation between rodent and monkey. These data also bring up a challenge in translating the PK for this ADC across animal species.

In general, the complex formation of biologics is expected to enhance its clearance because of an increase in the molecular size that would trigger the uptake and degradation of high molecular complexes by the reticuloendothelial system. However, here we have observed a paradoxical effect of complex formation on clearance. Since tissue disposition is known to play a key role in driving systemic clearance of antibody, we have further assessed whether the A1M adduct formation would alter the normal tissue (nontarget mediated) and tumor (target-specific mediated) distribution of anti-Ly6E ADC in a tumor-bearing mouse model by comparing with the tissue disposition profiles of unconjugated mAb. After administration of [^{125}I] and [^{111}In]anti-Ly6E ADC or [^{125}I] and [^{111}In]anti-Ly6E mAb into mice bearing high Ly6E expression tumor xenograft model, the plasma radioactivity levels in mice dosed with anti-Ly6E ADC were higher than that from mice dosed with anti-Ly6E mAb, consistent with PK results in mice. SEC-HPLC analysis confirmed that

A1M adduct was only seen in plasma samples dosed with anti-Ly6E ADC but not in samples dosed with anti-Ly6E mAb. Analysis of tissue radioactivity demonstrated that, overall, the tissue radioactivity levels in mice dosed with anti-Ly6E ADC tended to be lower than that detected in mice dosed with anti-Ly6E mAb (Fig. 7B). Although no individual tissue showed statistical difference, the overall totality of the differences from all tissues may explain, at least in part, the lower systemic clearance of this ADC, implying that the A1M adduct formation of ADC may impact nontarget-mediated tissue uptake. It is currently unknown how the A1M adduct formation alters the nonspecific tissue uptake. One possibility may be due to the adduct formation changing the physical-chemical properties of ADC, such as charge or hydrophobicity, which needs to be further investigated.

To understand whether A1M adduct formation would impact specific target-mediated tissue distribution, we have further determined the tumor distribution in a tumor-bearing mouse model. To our surprise, anti-Ly6E ADC showed a strikingly reduced tumor tissue distribution as compared with unconjugated mAb. As shown in Fig. 6, the [^{111}In] radioactivity amount detected in tumors from mice dosed with anti-Ly6E ADC after 1 day was about 2-fold lower than that seen in the tumors from mice dosed with anti-Ly6E mAb. This profile is in accordance with the formation of A1M adduct in plasma from mice dosed with anti-Ly6E ADC. In addition, both residualizing [^{111}In] and nonresidualizing [^{125}I] probes equally showed a low uptake of radioactivity into tumor tissues. The intact molecules are represented by the nonresidualized probe,

iodine-125, since this probe would be eliminated from circulation once molecules get internalized and catabolized by the lysosomes, whereas the residualizing probe, DOTA-indium-111, would represent both the intact molecules and any catabolized molecule. Also, codosing with an excess amount of unlabeled ADC did not appear to alter tumor radioactivity. Together, these data indicate that the A1M adduct formation of ADC resulted in little specific distribution or internalization into tumor tissues, even though systemic exposure is higher.

We hypothesized that the A1M adduct formation with ADC may interfere with the target antigen binding. To test this hypothesis, the uptake of preformed [¹²⁵I]A1M-ADC adduct and [¹²⁵I]anti-Ly6E mAb was assessed in a cell line that expresses either high Ly6E (HCC1937X1) or low Ly6E expression (DOV13). Although the uptake of nonincubated [¹²⁵I]anti-Ly6E ADC (i.e., no A1M adduct formation) and [¹²⁵I]anti-Ly6E mAb was similar, the preformed A1M-ADC showed a much lower uptake than that of preincubated mAb (also preincubated in plasma). The reduction of A1M-ADC uptake was more pronounced in high-Ly6E-expressing HCC1937X1 cell line than the low-Ly6E-expressing DOV13 cell line, which further supports that the A1M adduct formation could interfere with the target binding of anti-Ly6E ADC. This is consistent with the in vivo data that showed little internalization, as both residualizing ¹¹¹In and nonresidualizing ¹²⁵I probes had similar lower distribution to tumors. These data demonstrate that the A1M adduct formation of anti-Ly6E ADC could reduce the tumor distribution, likely through interfering with antigen target binding/recognition. One possibility is that A1M adduct formation with anti-Ly6E ADC may change the steric hindrance of the binding site, leading to masking the binding site on the complementarity-determining region, as such blocking the antigen binding. Further study will be needed to elucidate the exact molecular mechanism on how A1M adduct formation interferes with antigen binding. Previous study (Su et al., 2019) showed that an anti-CD22 mAb conjugated with the same CBI-dimer payload also formed an adduct with A1M. Therefore, the A1M adduct formation appears to be independent of antibody target rather than a linker-drug-specific issue. Limited in vitro data indicated that the *seco*-CBI-dimer ADCs are likely to form adduct with A1M if administered into human (Su et al., 2019). Thus, a similar impact on ADC PK and tumor distribution is expected. Unfortunately, this ADC was not moved forward to humans.

In conclusion, we have investigated the impact of A1M adduct formation on PK and tissue distribution of anti-Ly6E-*seco*-dimer ADC in rodents and monkeys. Our data demonstrated that A1M adduct formation of anti-Ly6E ADC could reduce the total antibody clearance dependent on A1M adduct formation rate and extent. Although increasing the systemic exposure, A1M adduct formation showed a striking reduction in tumor distribution, probably due to interfering with target antigen binding. These findings highlight the importance of selecting/optimizing the linker-drug structure for ADC molecules given its complexity and emphasize the value of conducting mechanistic understanding of ADC biotransformation in early stages of ADC development.

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Authorship Contributions

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