MOLECULAR CANCER THERAPEUTICS | LARGE MOLECULE THERAPEUTICS

Novel Auristatins with High Bystander and Cytotoxic Activities in Drug Efflux–positive Tumor Models
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ABSTRACT

Auristatins, a class of clinically validated anti-tubulin agents utilized as payloads in antibody–drug conjugates, are generally classified by their membrane permeability and the extent of cytotoxic bystander activity on neighboring cells after targeted delivery. The drugs typically fall within two categories: membrane permeable monomethyl auristatin E–type molecules with high bystander activities and susceptibility to efflux pumps, or charged and less permeable monomethyl auristatin F (MMAF) analogs with low bystander activities and resistance to efflux pumps. Herein, we report the development of novel auristatins that combine the attributes of each class by having both bystander activity and cytotoxicity on multidrug-resistant (MDR) cell lines. Structure-based design focused on the hydrophobic functionalization of the C-terminal N-methylevaine of the MMAF scaffold to increase cell permeability. The resulting structure–activity relationships of the new auristatins demonstrate that optimization of hydrophobicity and structure can lead to highly active free drugs and antibody–drug conjugates with in vivo bystander activities.

Introduction

With the recent approval by the FDA of belantamab mafodotin (BLENREP), there are now a total of nine antibody–drug conjugates (ADCs) on the market: brentuximab vedotin (ADCETRIS), ado-trastuzumab emtansine (KADCYLA), inotuzumab ozogamicin (BESPONSA), gemtuzumab ozogamicin (MYLOTARG), polatuzumab vedotin (POLIVY), enfortumab vedotin (PADCEV), sacituzumab govettecan (TRODELVY), and trastuzumab deruxtecan (BLENREP), there are now a total of nine antibody therapeutic index of ADCs, and in contrast to conventional chemotherapy, can be dosed systemically. This has led to a new generation of potent delivery of highly potent payloads, some of which are too toxic to be dosed systemically. This has led to a new generation of potent microtubule-disrupting and DNA-damaging agents including auristatins (11, 12), maytansinoids (13), tubulysins (14), pyrrolobenzodiazepines (15), calicheamicins (16), and camptothecins (17), among others (18). The ability to spare normal, healthy tissues with very limited target antigen is critical for optimizing the therapeutic index of ADCs, and in contrast to conventional chemotherapy, can be controlled (19).

Although release of highly potent payload inside targeted tumor cells is the main mechanism of antitumor activity of ADCs, the activities can extend to surrounding antigen-negative cells through bystander effects, which can have an impact on both conjugate efficacy and tolerability (20). Bystander activity occurs when the drug released from the ADC inside the antigen-positive cell upon internalization and degradation diffuses outside the target cell and is taken up by the neighboring antigen-negative cells or tissues. How much an ADC mediates bystander killing depends largely on the ability of the released cytotoxic payload to cross cellular membranes, which often correlates with its hydrophobicity. For example, part of the remarkable clinical response to brentuximab vedotin may be attributable to bystander killing effects of the released drug (21).

The capacity to eradicate antigen-negative tumor cells could be critical to achieve maximum antitumor activity in tumors where target heterogeneity is common (22). However, this feature may be less important in cancers with homogenous antigen expression where drug can be delivered to most cancer cells by an ADC. Moreover, it could be detrimental whether the potent drug released inside targeted tumor cells can escape those cells, diffuse outside the tumor, and be taken up by neighboring healthy tissues leading to their damage and increased systemic toxicity (23). This emerging picture of the importance of bystander killing indicates that tuning the level of bystander activity based on tumor type and tumor-specific antigen expression pattern may be an important parameter for the design of improved ADCs. Recent studies aimed at understanding how properties of various components of antibody–drug conjugates affect bystander killing indicate that physicochemical characteristics of the released cytotoxic payload such as membrane permeability and the ability to diffuse to surrounding cells are critical (21).

Auristatins are a highly potent class of anti-tubulin drugs that provide the opportunity to study how physicochemical properties of the released payload impact ADC efficacy and tolerability. Both the N- and C-terminal positions of the drug tolerate significant structural modifications while maintaining potency when delivered in targeted form. The best-known member of the family, monomethyl auristatin E (MMAE; 1, Fig. 1), is membrane permeable and a highly potent free drug in cancer cell proliferation assays (24). Furthermore, MMAE ADCs have confirmed bystander killing in vivo (21). Alternatively, replacement of its C-terminal norephedrine with phenylalanine introduces a negatively charged carboxylate group into the drug and provides monomethyl auristatin F (MMAF; 2, Fig. 1), which has significantly reduced (~1,000 fold) cytotoxic activity as a free drug (25). Such a reduction in potency is attributed to the reduced lipophilicity and impaired intracellular access of the compound (26).
Figure 1.
Peptide-based tubulin inhibitors of the vinca alkaloid pocket classiﬁed by their ADC bystander killing effects and activities on MDR⁻ cell lines.

Corresponding MMAF conjugates, while still highly potent, were conﬁrmed to release active species with reduced membrane permeability and bystander killing (21). In addition, MMAF is highly amenable to functionalization at the N-terminus, and modiﬁcation of the N-methyl valine has been validated by multiple clinical stage ADCs. These include the recently approved BLENREP and clinical candidate ARX788, which use the noncleavable drug linkers maleimidocaproyl-MMAF (5) and amberstatin-269 (27). Multiple reviews summarizing structure–activity relationship of structural modiﬁcations of auristatins, as well as updates on ongoing clinical trials with auristatin-based ADCs, have been published previously (28, 29).

Taking advantage of these properties of auristatins, we decided to use the MMAF backbone to generate a series of N-terminus alkyl-MMAF derivatives to progressively increase hydrophobicity and consequently membrane permeability. We found that we can modulate bystander killing by properly tuning the hydrophobicity at this position while preserving other desirable properties, such as high potency and ability to overcome multidrug resistance (MDR; ref. 30). Despite their long history, auristatins with the combination of these properties have not been reported previously (31), in contrast to other anti-tubulin agents such as tubulysin M and taltobulin (Fig. 1). Lead alkyl-MMAF ADCs described here extend auristatin technology by imparting activity on MDR⁻ cells, in vivo bystander killing, and improved tolerability of corresponding conjugates compared with previously reported molecules within the class.

Materials and Methods

Chemicals

Free drugs and drug linkers were prepared by solution phase or solid phase peptide synthesis as described previously (32). Detailed experimental procedures for all compounds are provided in the Supplementary Methods. All compounds were formulated in DMSO at 10 mmol/L and stored at −20°C.

In vitro cytotoxicity assays

In vitro potency was assessed on multiple cancer cell lines: HL60 [acute myeloid leukemia (AML)], HL60/RV (AML), SK-MEL-28 (melanoma), A2058 (melanoma), IGR-37 (melanoma), Colo-853 (colorectal cancer), Karpas299 [anaplastic large-cell lymphoma (ALCL)], Karpas-35R (ALCL), 786-O [renal cell carcinoma (RCC)], A498 (RCC), and L428 (Hodgkin lymphoma). All cell lines were authenticated by short tandem repeat proﬁling at IDEXX Bioresearch and cultured for no more than 2 months after resuscitation. Cells cultured in log-phase growth were seeded for 24 hours in 96-well plates containing 150-μL RPMI1640 supplemented with 20% FBS. Serial dilutions of ADCs in cell culture media were prepared at 4 × working concentrations, and 50 μL of each dilution was added to the 96-well plates. Following addition of test articles, cells were incubated with test articles for 4 days at 37°C. After 96 hours, growth inhibition was assessed by CellTiter-Glo (Promega) and luminescence was measured on a plate reader. The EC₅₀ value, determined in triplicate, is deﬁned here as the concentration that results in half-maximal growth inhibition over the course of the titration curve.

Crystallography

Tubulin was crystallized as described previously (33). Auristatin compound data were collected at beamline 4.2.2 at the Advanced Light Source (Lawrence Berkeley Labs). Images were indexed and processed using XDS, and structure solution by the difference Fourier age (34). Model building was carried out iteratively using the Coot software. Data collection and reﬁnement statistics are given in Supplementary Table S1. The atomic coordinates have been deposited in the Protein Data Bank, www.rcsb.org (PDB ID code 7JFR).

Preparation of ADCs

All antibodies used for this study were the IgG1 isotype. The oCD30 antibody is clone cAC10/brentuximab (35) and the oCD70 antibody is clone h1F6 (36). “IgG” denotes a human mAb with no known binding epitope. Partial reduction of antibody interchain disulﬁdes to an average of four thiols per antibody was achieved by incubation with 2.2–3 equivalents of tris(2-carboxyethyl)-phosphine for 60 minutes at 37°C. Reduced mAb was then buffer exchanged into PBS + 2 mmol/L EDTA using a NAP-5 desalting column (GE Healthcare). Partial reduction was conﬁrmed by reversed-phase chromatography (PLRP 3 μm, Agilent) by analyzing a small aliquot conjugated to maleimidocaproyl-valine-citrulline-monomethylauristatin E (29). Conjugation was carried out at room temperature by adding 1.15 molar equivalents of drug linker per reduced cysteine. After 20 minutes, the extent of conjugation was...
assessed by evaluating the mass increase of antibody chains using reversed-phased UPLC (PLRP 3 μm, Agilent) coupled with mass spectrometric detection (Waters Xevo G2-S QTOF). Additional drug linker was added when needed. Once all available cysteines were occupied with drug, excess drug linker was removed by incubation with activated charcoal followed by buffer exchange into PBS using a NAP-5 desalting column. Aggregation was assessed by size exclusion chromatography. Drug loading at interchain disulfide was confirmed by comparing predicted and observed changes in the molecular weights of heavy and light chains using PLRP-mass spectrometry analysis and confirming absence of underloaded species. Final ADC solutions were sterile filtered through a 0.22-μm centrifugal filter, and concentration was determined by UV-visible (Vis) spectroscopy.

**Animal studies**

All experiments were conducted in concordance with the Institutional Animal Care and Use Committee in a facility fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

**In vivo xenograft efficacy models**

Efficacy experiments were conducted in two human xenograft models: 786-O (CD70+/MDR+ RCC) and Karpas299/Karpas-35R (CD30+/CD30+/MDR− ALCI), an antigen heterogeneous model of bystander activity. Tumor cells, as a suspension, were implanted subcutaneously in immunocompromised SCID mice (Envigo). Upon tumor engraftment, mice were randomized to study groups (n = 5) once the average tumor volume reached approximately 100 mm3. The ADCs were dosed by intraperitoneal injection at indicated times. Tumor volume as a function of time was determined using the formula (L x W^2)/2. Animals were euthanized when tumor volumes reached 1,000 mm^3. Complete response (CR) is defined here as absence of measurable mass at tumor site during an experiment.

**Toxicology experiments**

In vivo rat toxicity studies were done in naïve female Sprague Dawley rats (Envigo). Studies had three animals/group/timepoint and included a vehicle control group (1× PBS pH 7.4). Animals were administered a single dose of 5, 10, or 15 mg/kg IgG-28, or 5, 10, or 30 mg/kg IgG-25 (DAR = 4) via intravenous injection. Blood was collected 4 days post dose under isoflurane anesthesia via the jugular vein for hematology analysis on the Sysmex XT-2000iV. At 8 days postdose, blood was collected from anesthetized animals from the caudal vena cava for clinical chemistry analysis on a Beckman Coulter AU680.

**Results**

**Design and synthesis of N-alkylated MMAF analogs**

MMAF was chosen as a parental scaffold because of its high biochemical potency (33), yet the hydrophilic, zwittieronic nature of the molecule results in low membrane permeability and low potency as a free drug (25, 32). However, when delivered intracellularly, MMAF provides highly potent ADCs. In addition, MMAF-based ADCs are active on MDR+ cell lines, and this feature is correlated to the hydrophilic nature and C-terminal carboxylate of the auristatin free drug. Earlier, it had been shown that auristatins can tolerate steric bulk at the N-terminus without losing its tubulin-binding activity. To increase hydrophobicity of the molecule and potentially improve its bystander killing, a library of N-alkylated MMAF derivatives was synthesized. The substituents chosen for this library were a series of C1 to C12 branched and unbranched aliphatic groups (compounds 5–16, 18–20, Fig. 2A) The progressive homologation of the alkyl group was intended to incrementally increase the hydrophobicity of the MMAF free drug to improve its passive diffusion across cellular membranes. We hoped to utilize this approach to observe and take advantage of any trends in structure–activity relationships between drug hydrophobicity and cytotoxicity. In addition, a C-terminal β-homophenylalanine derivative 17 was synthesized with a branched C9-alkyl chain.

Preparation of the library by Fmoc solid-phase peptide synthesis provided the common MMAF intermediate. Afterward, the resin was split, and the secondary N-methylvaline amine was reductively alkylated by the appropriate aldehyde and NaBH(OAc)_3 to afford the final products (see Supplementary Information file). Alkylation at the N-terminal position maintained the zwitterionic state of the molecule and avoided modification to the core structure of the auristatin. A similar resin-based synthesis was used to append a protease cleavable dipeptide and a maleimide moiety to the C-terminal phenylalanine to generate the drug-linker compounds.

**In vitro potency of N-alkylated MMAF analogs on HL60 cell line**

To evaluate new payloads for bystander activity, we used in vitro potency as a surrogate for passive membrane permeability and bystander activity (37). The compounds were first tested on the acute promyelocytic leukemia HL60 cell line, which has an MDR phenotype that expresses low levels of P-glycoprotein (P-gp; ref. 38). Consistent with previous reports, MMAE demonstrated excellent activity with a half-maximal inhibitory concentration (IC50) of 0.1 nmol/L, whereas MMAF was nearly 1,000-fold less active (Fig. 2B). All the N-alkylated MMAF library compounds demonstrated improved cytotoxicity over the parental molecule, presumably due to increased cellular membrane permeability. Free drug activity of the alkylated MMAFs on the MDR+ HL60 cell line demonstrated a clear trend between alkyl chain length and cytotoxicity (Fig. 2C). As the alkyl chain length and hydrophobicity increased, the IC50 values decreased. Beginning with C8-substituted auristatin 9, all compounds show single-digit nmol/L activity. Even the sterically bulky analogs 18 and 19 display single-digit nmol/L activity (1.8 nmol/L and 1.4 nmol/L, Fig. 2B). These results indicate that passive membrane permeability is the limiting factor of MMAF activity in the absence of efflux pumps.

**In vitro potency of N-alkylated MMAF analogs on MDR+ cells**

To test the activity of the compounds on MDR+ cells, we utilized the HL60/RV cell line, which is a P-gp-overexpressing variant of the parental HL60 cell line (39). Treatment with MMAE on HL60/RV showed dramatically reduced activity in comparison with MDR−HL60, while MMAF treatment on both cell lines had similar potency (Fig. 2B and C). In the HL60/RV cell line, the incremental increase in the size of the N-alkyl substitutions on MMAF resulted in two opposing trends. The first trend demonstrates improved activity with increasing alkyl chain length, similar to results with HL60. This trend begins with MMAF and continues until the C6-derived compounds 11 and 12 (IC50 41 nmol/L and 21 nmol/L, Fig. 2B). Additional increases in the alkyl group size did not lead to lower IC50 values on the MDR+cell lines, and activity was diminished in some analogs (compounds 13–20, Fig. 2B). The ratio between the MDR− and MDR+ cell lines showed that alkyl substitutions between C1 and C6 generally maintained activity regardless of P-gp expression (Fig. 2C). Alkyl substitutions of larger size led to a growing discrepancy in activity between HL60 and HL60/RV. Such differences in activity between the two...
cell lines treated with the same free drug are presumably due to variations in the susceptibility of the compound to efflux.

**In vitro evaluation of tert-butyl carbamate MMAF analogs**

In addition to purely hydrocarbon functionalization of the N-methylvaline, we examined a series of alkyl tert-butyl carbamate derivatives of MMAF. The Boc-carbamate was attached to alkyl homologs C2–C5. These compounds were tested on four solid tumor lines (SK-MEL-28, A2058, IGR-37, and Colo-853). While all the compounds displayed low nmol/L cytotoxicity, the activity varied by the length of the alkyl tether (Fig. 3). In this case, compound 23 bearing a tert-butyl carbamate and butyl tether was the most active compound, whereas shorter or longer alkyl groups resulted in lower potency.

**Crystallographic analysis of N-alkylated MMAF**

X-ray crystallography was used to assess the binding mode of the alkylated MMAF analogs. For that purpose, a crystal structure of N-alkylated auristatin compound 17 was solved (Fig. 4). In this case, the modification of the C-terminal position is well tolerated and had similar IC_{50} values (HL60 = 0.4 nmol/L, HL60/RV = 25.8 nmol/L; Fig. 2B) to compounds with similar hydrophobicity. Analog 17 bound to the same site as other auristatins (33, 40). In addition, compound 17 maintained the same contacts with tubulin as MMAE, including the strong interaction between the N-methylvaline with Asp197. The nonpolar tert-butyl alkyl group stretched out to a hydrophobic cleft adjacent to Asp197 (41). Overlay of the crystal structure with MMAE and vinblastine shows the sterically bulky alkyl group occupying the space typically occupied by vinca alkaloids.

**In vitro evaluation of ADCs**

Selected MMAF analogs that demonstrated excellent free drug activity were tested as ADCs. Free drugs were linked at the C-terminus to a protease-cleavable glutamic acid-lysine dipeptide, and the drug linker was capped with a propionic acid-derived maleimide for cysteine conjugation (Fig. 5A). Similar dipeptide linkers were previously shown to be stable and deliver active auristatin payloads including auristatin F (32, 42). The C-terminal dipeptide bears two negatively charged carboxylic acids to mitigate any potential aggregation issues. The purified drug linkers were conjugated to cysteines from partially reduced interchain disulfides of cCD70 or cCD30 antibodies to furnish average 4-load (DAR = 4) ADCs.

The ADCs were tested for cytotoxicity on CD70⁺ cell lines, including MDR⁺ 786-O RCC and L428 Hodgkin lymphoma cells (32, 42). Despite high levels of P-gp expression, those cell lines were found to be sensitive to the conjugates (Fig. 5B). Thus, the N-alkyl cCD70-25 and
CD70-26 auristatin F conjugates have cytotoxicities comparable with CD70-28 MMAF conjugate across all cell lines. When delivered inside cells, MMAF derivatives maintain their potency against the target cells and the ability to overcome efflux pumps. In contrast, the CD70-29 MMAE conjugate is inactive against those cell lines.

**In vivo evaluation of ADCs**

Conjugates of the carbamate auristatin 25 were evaluated in tumor xenograft models in mice. For the MDR⁺ model, we used a CD70⁺ 786-O xenograft model in SCID mice. CD70⁻25 was dosed at 0.5 and 1.5 mg/kg and compared with similar doses of CD70⁻28 (Fig. 5C). All untreated animals had tumor grow outs within 50 days postdose. Both doses of CD70⁻25 and CD70⁻28 had similar growth delays and number of CRs, showing the MDR⁺ activity of CD70⁻25 was maintained.

To investigate in vivo bystander activity, we utilized an admixed tumor model using Karpas299 CD30⁺ cells and Karpas-35R CD30⁻/C0 cell lines (21). In this model, CD30⁻29 dosed once at 3 mg/kg resulted in significant tumor growth delay and four of five CRs (Fig. 5D). On the other hand, CD30⁻28 resulted in minimal tumor growth delay due to the lack of bystander activity on antigen-negative tumor cells. Here the CD30⁻25 showed an improvement over MMAF with more sustained tumor suppression and two of five CRs. The combined results of the efficacy studies demonstrate that optimization of hydrophobicity can maintain the MDR⁺ efficacy of auristatin F payloads and increase bystander activity to improve efficacy on heterogeneous tumors.

**Toxicology**

The toxicologic effects of increased permeability and bystander activity was examined in Sprague Dawley rats using noncross-reactive ADCs (IgG). At a single dose of 5 and 10 mg/kg, the toxicity of the IgG:25 was less severe compared with IgG:28 (Fig. 6), with lower reductions of platelets and lymphocytes 4 days postdose and less of an increase in the liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) 8 days postdose.

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**Figure 3.** Evaluation of the tether length and in vitro activity. Chemical structures and activities of free drugs tested on solid tumor cell lines. Cells were assessed by CellTiter-Glo following a 96-hour incubation with test article. IC₅₀ values are expressed in nmol/L. CRC, colorectal cancer.

**Figure 4.** Crystal structures of tubulin inhibitors bound to the vinca site. A, Vinblastine (PDB ID code 5J2T). B, MMAE (PDB ID code 5IYZ). C, Auristatin 17 (PDB ID code 7JFR).
The MTD of conjugate 25 was double that of the MMAF conjugate, 30 mg/kg versus 15 mg/kg, respectively (Fig. 6). When evaluated in combination with the xenograft data, this work demonstrates that more hydrophobic ADCs can be similarly effective but better tolerated in rodent models, suggesting an improvement in therapeutic index with this strategy.

**Discussion**

The investigations reported here focused on designing auristatins with easily tunable bystander activities, while preserving high potency and favorable MDR properties of corresponding ADCs. The choice of MMAF as the parental compound was due to two considerations. The first was its low membrane permeability and known tolerability for N-terminal modifications (25, 31), a feature that enables changes to the overall physicochemical properties without modification to the core residues of the auristatin and its intrinsic potency. The second consideration was the resistance to P-gp efflux, which is a valuable property of MMAF that we would like to retain in the newly designed analogues.

The standard measurement of ADC bystander activity involves making a panel of conjugates, and measuring their activities using *in vitro* (37) or *in vivo* (21) coculture systems. However, the current models were developed in MDR⁺ cell lines and do not account for the effect of efflux pumps on cytotoxicity. Therefore, we first utilized *in vitro* cell-based cytotoxicity assay with MDR⁺ and MDR⁻ variants of the same cell line to evaluate new analogs, based on the hypothesis that the bystander activity of the ADC payload should correlate with the potency of the free drug. The initial testing was done in the MDR⁻ HL60 cell line. We observed increased cytotoxicity of analogs as the length of the alkyl chain was increased (Fig. 2B). The IC₅₀ values plateaued to the single digits, and at no point did increased steric bulk or hydrophobicity of the substituent lead to decreased activity.

To gain further understanding surrounding why the activities were maintained with such large modifications, we obtained a crystal structure of compound 17 bound to tubulin (Fig. 4). The results confirmed that 17 binds to the same site as previously reported for auristatins and maintains similar hydrogen bond contacts (33, 40, 43). Superimposition of 17 with vinblastine and MMAF shows that the alkyl chain overlaps with the vindoline moiety of vinblastine.

We then compared the activity of the C1-C12 -alkyl-MMAF compounds on the MDR⁺ HL60/RV cell line. This line was originally developed by the isolation of HL60 clones that became resistant to vinblastine and MMAF shows that the 17 binds to the same site as previously reported for auristatins and maintains similar hydrogen bond contacts (33, 40, 43). Superimposition of 17 with vinblastine and MMAF shows that the alkyl chain overlaps with the vindoline moiety of vinblastine.

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of MDR16 containing the more hydrophobic drug was less active than conjugate cCD70-26 with analog 12 bearing the shorter alkyl substituent (Fig. 2B) on two of three cell lines with the most significant difference on 786-O cells which are highly MDR+. This is different from the free drug data where compound 16 was more potent than compound 12 on HL60 cells (Fig. 2B). Conjugate cCD70-25 with heteroatom-containing analog 23 had similar potency to cCD70-26 following the pattern of free drug data (Fig. 3).

In the *in vivo* experiments, the cCD70-25 conjugate was comparable with cCD70-28 in a mouse xenograft model of human 786-O renal cell carcinoma (Fig. 5C). At both dose levels tested, tumor regressions were obtained, including four of five complete tumor regressions at 1.5 mg/kg. This confirmed the ability of the novel payload to overcome MDR+ phenotype. In addition, the cCD30 conjugate of drug linker 25 was highly active in the admixed CD30+ and CD30+ tumor model Karpas299/Karpas-35R, where bystander activity is required for pronounced efficacy (21). The effects were much greater than cCD70-28, but somewhat less than cCD70-29, a conjugate with excellent bystander activity but susceptibility to MDR drug efflux mechanisms (Fig. 5D; ref. 45). Complete regressions were observed in two of five animals, a clear indication the antigen-negative tumor cells were killed via bystander effects from the alkyl-MMAF payload. mAb-25 ADCs provide a combination of properties that have not been previously observed with auristatins. An additional benefit is improved tolerability compared with IgG-28 in rats, where there was reduced thrombocytopenia and liver toxicity, the main dose-limiting toxicities observed for MMAF-based conjugates (5). By utilizing
A uristatins with High Bystander and Cytotoxic Activities

P.N. Moquist reports a patent for WO2019164987 pending to Seattle Genetics, Inc. R.P. Lyon reports personal fees from Seattle Genetics outside the submitted work. S.O. Doronina reports a patent for Hydrophobic auristatin F compounds and conjugates thereof WO2019164987 A1 pending. No disclosures were reported by the other authors.

Authors’ Contributions
P.N. Moquist: Conceptualization, supervision, investigation, methodology, writing—original draft, project administration, writing—review and editing. T.D. Bovec: Conceptualization, investigation, methodology. A.B. Weight: Conceptualization, formal analysis, investigation, methodology. J.A. Mitchell: Investigation, methodology. J.B. Miyamoto: Investigation. M.L. Mason: Investigation. K.K. Emmerton: Investigation. N. Stevens: Investigation. C. Balasubramanian: Investigation. J.K. Simmons: Investigation, project administration, writing—review and editing. R.P. Lyon: Supervision, project administration. P.D. Senter: Supervision, project administration, writing—review and editing. S.O. Doronina: Conceptualization, supervision, investigation, methodology, writing—original draft, project administration, writing—review and editing.

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Disclosures


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